The Physiology of Vegetable Crops

2nd Edition

H.C. Wien and H. Stützel

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Preface

This book on the physiology of vegetable crops is focused on the activities and functions of vegetables, defined as herbaceous plants that are harvested for edible parts that can be consumed fresh or with little preparation. Physiology deals with the growth and development processes of these plants, and while this book is focused primarily on the organ and whole-plant level, brief mention of cellular and genetic events is made for some crops.

Enhanced health consciousness, growing knowledge about the benefits of vegetable consumption, and the desire for greater food diversity have resulted in increased vegetable consumption all over the world and are important drivers of innovations in vegetable production and science. Furthermore, the increase in world population has pointed out the need to increase production and consumption of fruits and vegetables. A suggested annual increase of 8% in fruit and vegetable production worldwide would require a major emphasis on vegetable research, exploring ways of augmenting yields and expanding into new production areas.

In recent years, the study of physiology has deepened to include a focus on cellular and molecular mechanisms, and revealing the role of genes in function and activity. Translating these findings to the plant and crop level is a big challenge to the discipline of vegetable crop physiology, and will require good communication links between laboratory and field scientists.

The initial chapters of the book cover processes common to most vegetables, such as the germination of seeds and the processes governing development of plants from small seeds, in preparation for transplanting into their final growth environment. The rapid advances in understanding of the induction of flowers among herbaceous plants is then summarized, followed by consideration of the major abiotic stress factors facing vegetable plants. The development of models of crop growth among vegetables has also made recent advances, and is contributing to our understanding of how plants function. A chapter on the mechanisms by which growth of different plant parts is regulated among vegetables is followed by a dozen chapters describing the physiology of 21 major vegetable species. For each, the taxonomic location in the plant kingdom is described, followed by the crop's life course from seedling to harvest stage, and the role of environmental factors on plant ontogeny. Since vegetables are grown for their harvested products, factors affecting product quality during growth are also emphasized.

The fact that many parts of the world are becoming marginalized for crop production with the advent of increased occurrence of higher temperatures and droughts has been frequently documented. While much research is under way to address these challenges among vegetable crops (see Chapter 4), translating the results of genetic and cellular studies into effects in production situations

has lagged behind. We encourage collaboration of production-focused scientists with fundamental researchers to use the new knowledge for practical good.

The great relief felt by Wien when the first edition of this book appeared in 1997 was followed by pleasant surprise that copies continued to be sold over 20 years. Invitations by Ms. Rachael Russell of CABI, to produce a second edition, were not seriously entertained until after Wien's retirement from Cornell in 2015. Fortunately, Prof. Hartmut Stützel agreed to share the load of writing and editing, and a total of 29 authors spent many hours of dedicated work sharing their knowledge and summarizing the current state of science in their respective fields. Wien and Stützel are immensely grateful for their dedication and diligence. The work could also not have been accomplished without the internet and the availability of electronic conferencing facilities, which enabled us to access the world scientific literature from our desks at home, and to resolve such issues as the failure of three authors to produce their promised chapters. The timely assistance of Ms. Rebecca Stubbs at CABI, Ms. Russell's successor, has also been invaluable, and we are grateful for her help. We also thank the production staff at CABI, led by Ms. Lauren Davies and Mr. James Bishop, who engaged in the final steps of getting this book launched.

1 Seed Storage, Germination, Quality, and Enhancements

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Before vegetables are harvested, before their growth and development, even before the seedling becomes photosynthetically competent, the source of the vegetable—the seed—holds major keys to final product yield. To fully comprehend the complexities of vegetable crop growth and development, we must understand seed physiology related to storage, germination, quality, and enhancements. The challenge is to present an in-depth knowledge of vegetable seeds that covers considerable diversity with respect to botanical classification, seed size, and composition. This chapter and book encompasses 33 common vegetable crop seeds from ten plant families (Table 1.1). This diverse group of plants makes a comprehensive overview of vegetable seeds difficult to achieve, especially since there is little scientific literature on seed physiology of many small-seeded crops of minor economic importance. Due to these limitations, we enlist several approaches to develop a coherent picture of vegetable seeds. Relative differences between seeds are shown with regard to seed size and composition, storage longevity, seed and seedling morphology, temperature requirements for germination, and the use of reserve materials during emergence. More generalized information is presented on factors and events associated with storage, germination, and aging. Finally, the process of aging and other aspects of seed physiology and technology are illustrated using specific crop examples.

The focus of this chapter is on postharvest aspects of seeds; the period of seed development and production stages are not addressed. We start with storage of seeds with low moisture content as most vegetable crop seeds have unique characteristics that permit them to withstand desiccation (Leopold and Vertucci, 1986). Desiccation tolerance is essential for long-term survival and allows for a time interval between seed production and crop production. Most vegetable seeds imbibe water readily and, provided with a suitable environment, will germinate and resume active growth. The seed uses its reserve materials following germination and then becomes an active photosynthetic seedling, fixing its own carbon and producing energy.

Seed quality is a broad term and encompasses several attributes of seeds including the germination and seedling performance. In this chapter, certain physiological and biochemical processes associated with loss of seed quality are described, and symptoms of seed aging are presented at physiological and whole-plant levels. The sowing environment has a direct effect on germination and stand establishment, and under severe stress, seedling performance is seriously impaired. To ensure stand establishment, high quality seeds are needed for transplant production and for direct seeding. Seed performance is improved by seed enhancements to achieve maximum emergence when sown in suboptimal conditions. In addition, seed-coating technologies are used to improve

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Table 1.1. Botanical and common names (Maynard and Hochmuth, 2007). Thousand seed weight (TSW), percent oil and protein content from representative seed samples (Royal Botanic Gardens Kew, 2017, except where noted).

Class, family, *genus*, *species*	Common name	TSW (g)	Oil (%)	Protein (%)
Monocotyledons				
Alliaceae (onion family)				
Allium ampeloprasum L. *Porrum group*	Leek	2.26	15‡	27‡
Allium cepa L. *Cepa group*	Onion	3.32	19	–
Liliaceae (lily family)				
Asparagus officinalis L.	Asparagus	24.1	15‡	16‡
Poaceae (grass family)				
Zea mays L. subsp. *Mays*	Corn, sweet	227	6‡	12‡
Diocotyledons				
Apiaceae (carrot family)				
Apium graveolens L. var. *dulce* (Mil.) Pers.	Celery	0.30	29	19
Daucus carota L. subsp. *Sativus* (Hoffm.) Arcang.	Carrot	1.00	19	24
Pastinaca sativa L.	Parsnip	3.00	33	19
Petroselinum crispum (Mill.) Nym. Var. *crispum*	Parsley	1.70	27	20
Asteraceae (sunflower family)				
Lactuca sativa L. *var. capitala* L.	Lettuce	1.00	38	29
Brassicaceae (mustard family)				
Brassica napus L. var. *napobrassica* (L.) Reichb.	Rutabaga	3.30	42	25
Brassica oleracea L. var. *acephala* DC.	Kale, collards	2.30	26	34
Brassica oleracea L. var. *botrytis* L.	Cauliflower	3.15	32**	–
Brassica oleracea L. var. *capitata* L.	Cabbage	3.15	35**	28‡
Brassica oleracea L. var. *gemmifera* Zenk.	Brussels sprouts	3.15	34**	–
Brassica oleracea L. var. *italica* Plenck.	Broccoli	3.15	31**	–
Brassica rapa L. var. (DC.) Metzg. *Rapa*	Turnip	2.10	39	25
Raphanus sativus L. *Radicula group*	Radish	19.0	41	31
Chenopodiaceae (goosefoot family)				
Beta vulgaris L. *Crassa group*	Beet, garden	12.9	5.1	13
Spinacia oleracea L.	Spinach	6.80	5.3	20
Cucurbitaceae (gourd family)				
Citrullus lanatus (Thunb.) Matsum & Nakai	Watermelon	83.0	20	18
Cucumis melo L. Reticulatus *group*	Muskmelon	28.8	36	36
Cucumis sativus L.	Cucumber	16.3	32	28
Cucurbita maxima Duchesne	Pumpkin, w. squash	241	48	39
Cucurbita moschata Duchesne	Squash, butternut	88	48	40
Cucurbita pepo L.	Squash, summer	145	47	39
Fabaceae (pea family)				
Phaseolus coccineus L.	Bean, runner	1066	2.0	24
Phaseolus lunatus L.	Bean, lima	456	1.0	24
Phaseolus vulgaris L.	Bean, snap bean	307	1.1	28
Pisum sativum L. ssp. *Sativum*	Pea, garden	168	1.2	24
Solanaceae (nightshade family)				
Capsicum annuum L. *Grossum group*	Pepper, bell	7.09	28†	–
Capsicum frutescens L.	Pepper, tabasco	5.00	20	18
Solanum lycopersicon Mill. *(formerly Lycopersicon esculentum* Mill.*)*	Tomato	1.97	20	–
Solanum melongena L.	Eggplant	3.50	27*	–

*Kaymak, 2014.
†Jarret *et al*., 2013.
‡Taylor, 1997.
**West *et al*., 2004.

precision placement of seeds for sowing, and to provide a delivery system for compounds and agents to both protect and enhance seed and plant performance.

Composition and Water Status in Seeds

The composition of seeds is important to many aspects of seed physiology, and the percent oil or lipid content, and percent protein, are provided for the 33 common vegetable crop seeds in Table 1.1. Seed size expressed as thousand seed weight (TSW) was calculated; small-seeded vegetables have a TSW of < 10 g, while large-seeded vegetable crops have TSW > 100 g. Intermediate or medium-sized seeds range from 10 to 100 g TSW. The relationship of TSW and percent seed oil content is shown in Fig. 1.1.

There are two groups of seeds based on their storage reserves: starch and lipid storing. Seeds with less than 10% oil are starch-storing seeds, and the starch content of snap bean, pea and sweet corn is 42%, 48%, and 53%, respectively (cited by Taylor, 1997). Table beet seeds contain starch, which is stored in the perisperm (see "Seed and seedling morphology" section). All small-seeded crops (TSW < 10g) are oil-storing seeds with > 18% oil. Though large-sized seeds generally store starch, the large-seeded cucurbits are exceptions with nearly half of their weight as lipids. All seeds contain proteins, and values range between 12% and 40% (Table 1.1).

Seeds in storage are said to be in a dry condition; however, "dry" is a relative term and does not mean that water is absent from seeds. Water is present in seed tissue, and the status of water is related to many aspects of seed physiology, including seed longevity. First, the concentration of water is measured and expressed in meaningful units.

Standardized gravimetric methods to determine the moisture content of seeds were published by the Association of Official Seed Analysts (Elias *et al.*, 2018). Other primary and secondary methods were described and reviewed by Grabe (1989). Seed moisture content calculated on a wet or fresh weight (fw) basis is commonly used in seed testing and in commerce. The following formula is used to determine the percentage of seed moisture content on a fresh weight basis:

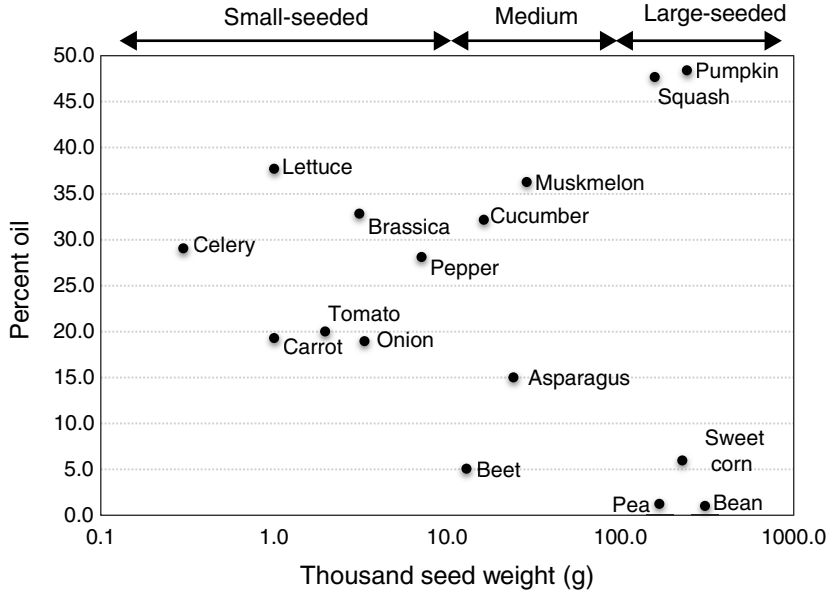

Fig. 1.1. The relationship of seed size expressed as Thousand Seed Weight (TSW) in grams in relationship to percent seed oil content. Seeds are grouped as small-seeded, medium and large-seeded with <10, 10–100 and >100 g TSW, respectively. Data from Table 1.1

$$\% \text{ moisture, fw basis} = [\text{weight of water (dry weight of seed + weight of water)}^{-1}] \times 100$$

The seed moisture content expressed on a fresh weight basis provides a direct assessment of the concentration of water in any quantity of seed. For example, 100 grams of seeds with 10% moisture content contains 10 grams of water and 90 grams of seed dry weight. Another unit to express seed moisture content is g H_2O g^{-1} fresh or dry weight.

The seed moisture content comes into equilibrium with the relative humidity of the air. The relationship between the equilibrium moisture content and relative humidity reveals a negative sigmoidal-shaped curve known as a moisture isotherm (Iglesias and Chirife, 1982). This relationship is determined by placing seeds in a range of known relative humidity conditions produced in closed containers with the use of saturated salt solutions (Taylor *et al.*, 1992). We prepared a number of saturated salt solutions and desiccants to achieve a range of humidity levels from *c.* 0% to 89% in equilibrated snap beans (*Phaseolus vulgaris*) and broccoli (*Brassica oleracea* italica group) seeds. Three regions or zones of water binding are observed: Zone I, < 20% RH; Zone II, 25–65% RH; and Zone III, > 70% RH (Fig. 1.2).

The curves are similar for both kinds of seeds; however, the equilibrium moisture content at a given relative humidity is always greater for snap beans compared to broccoli. Differences between these two species are attributed to seed composition, in particular lipid content, as lipids have little affinity for water. Thus, seeds with high lipid content have lower equilibrium moisture content at a given relative humidity than seeds with low lipid content. In our isotherm, the lipid content for the snap bean and broccoli seeds was 1% and 31%, respectively (Table 1.1).

Another method used to quantify the water status of seeds, which is not affected by seed composition, is to measure the water activity (a_w). Water activity is defined as the ratio of the vapor pressure of water in a seed to the vapor pressure of pure water at the same temperature (Bourne, 1991). Water activity is measured by determining the equilibrium relative humidity of the seed's headspace in a closed container and is expressed as a decimal. For example, a seed (any seed) equilibrated to 50% relative humidity has a water activity of 0.50. Measuring water activity of pelleted seeds (described in the "Seed enhancements" section) was the only nondestructive method to accurately measure the water status of coated seeds (Taylor *et al.*, 1997).

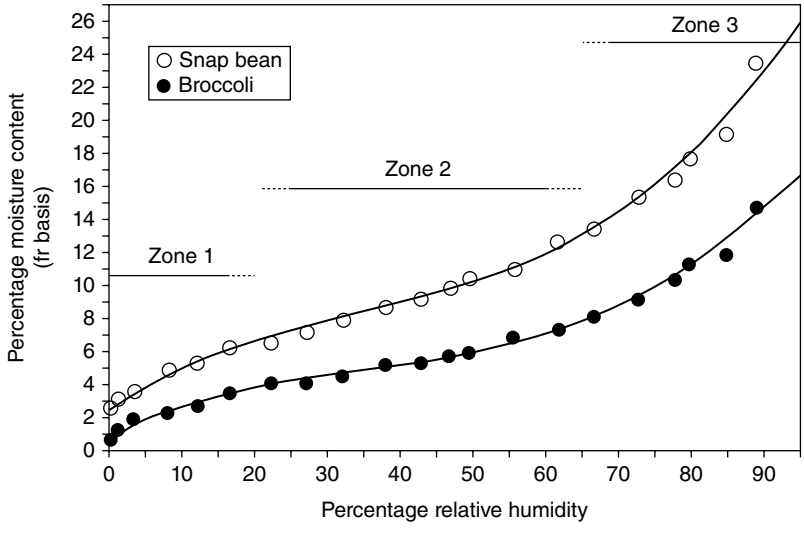

Fig. 1.2. Moisture isotherms for snap bean and broccoli seeds with oil contents of 1.0 and 31 percent, respectively. Figure from Taylor, 1997.

Storage: Moisture Content and Temperature

Two major environmental factors that influence seed storage are seed moisture content and temperature. Moisture content, as previously shown, is determined by the storage relative humidity and by seed characteristics, largely lipid content. The concentration of water in the seed tissue directly affects the rate of aging at a given temperature. The moisture isotherm reveals three zones or types (see Fig. 1.2) in which the water binding to seed tissues differ. Type I water is bound tightly and water interacts very strongly with charged groups of proteins (Vertucci, 1993). Type II water is less tightly bound and condenses over the hydrophilic sites of macromolecules (Leopold and Vertucci, 1989). Type III water is bound with negligible energy and forms bridges over hydrophobic moieties (Vertucci, 1993). The status of water in the seed tissue governs the kind of reactions (enzymatic or non-enzymatic) that occurs in storage (Vertucci, 1993). In practice, vegetable seeds are stored in Zone II (Fig. 1.2), and the recommended seed moisture content for vegetable crop seeds stored in hermetically sealed containers is listed in Table 1.2.

Temperature has a direct influence on longevity, and the rate of deterioration increases as temperature increases at a given relative humidity. Seeds are stored over a wide range of temperatures depending on the particular needs and available conditions. Seeds for most applications are stored above 0°C; however, for long-term preservation, seeds are stored below 0°C. Seeds with water contents in Types I or II are not injured since the water is non-freezable in the seed tissue. Long-term germplasm preservation has taken advantage of this ability to withstand freezing injury as seeds are stored above liquid nitrogen in the vapor phase at −150 to −180°C (Roos, 1989).

The need to keep seeds cool and dry for long-term preservation has been known for centuries. In the 1960s, "Harrington's Rules of Thumb" were developed as guidelines for storage (cited by Justice and Bass, 1978). The first two rules relate the influence of moisture content and temperature independently on longevity. The life of the seed is halved by a 1% increase in seed moisture, and this rule applies when seed moisture content ranges from 5% to 14%. The life of the seed is halved by a 5°C increase in storage temperature, and this applies to storage between 0 and 50°C. The most widely quoted rule combines both temperature and relative humidity to storage: "The sum of the temperature in °F and the percentage of relative humidity should not exceed 100."

Since the 1960s, mathematical equations have been developed to model seed aging for a

Table 1.2. Recommended seed moisture content for vegetable crop seeds in hermetically sealed containers. Minimum percentage germination standards for seeds in interstate commerce in the United States. Data from Federal Seed Act (USDA, 2017).

Vegetable	Seed moisture (%)	Germination (%)	Vegetable	Seed moisture (%)	Germination (%)
Asparagus	—	70	Lettuce	5.5	80
Bean, garden	7.0	70	Melon	6.0	75
Bean, lima	7.0	70	Onion	6.5	70
Beet	7.5	65	Parsley	6.5	60
Broccoli	5.0	75	Parsnip	6.0	60
Brussel sprouts	5.0	70	Pea	7.0	80
Cabbage	5.0	75	Pepper	4.5	55
Carrot	7.0	55	Pumpkin	6.0	75
Cauliflower	5.0	75	Radish	5.0	75
Celery	7.0	55	Rutabaga	5.0	75
Corn, sweet	8.0	75	Spinach	8.0	60
Cucumber	6.0	80	Squash	6.0	75
Eggplant	6.0	60	Tomato	5.5	75
Kale	5.0	75	Turnip	5.0	80
Leek	6.5	60	Watermelon	6.5	70

particular species maintained at a given condition of temperature and moisture content (fw basis), known as the Ellis and Roberts' equations (cited by Priestley, 1986). These equations take into consideration the initial percent germination of the seed lot and will predict the germination after a specified period of time in storage. The general equation for modeling the loss of germination in storage is as follows (Ellis *et al.* 1982):

$$v = K_i - \frac{p}{10^{[K_E - (C_w \times \log m) - (C_H \times t) - (C_q \times t^2)]}}$$

where v represents the probit of the percentage germination after a storage period of p days; K_i is the probit of the initial germination for the seed lot; K_E, C_w, C_H and C_q are species-specific constants; m is the seed moisture content (expressed on a fresh weight basis); and t is the storage temperature (°C). The species-specific constants for onion are: K_E = 6.975; C_w = 3.470; C_H = 0.040; and C_q = 0.000428 (Ellis and Roberts, 1981). We used this equation to plot onion seed aging curves (Taylor, 1997). For illustration, the influence of temperature, moisture content and initial viability were studied as variables for aging of onion seeds. Increasing temperature or moisture content independently had a profound effect on aging rates (Figs 1.3a,b).

The curves generally reveal a sigmoidal shape, especially when the initial germination is high, while the initial plateau phase is not observed with seed lots with low initial germination (Fig. 1.3c). Additional information on the seed viability equation and viability constants is available at the Royal Botanic Gardens, Kew (2017).

Species Differences in Storability

Although the storage environment is important, differences exist among species held under the same conditions. Results from a number of earlier storage studies on different species of seeds including vegetable seeds were summarized (Justice and Bass, 1978; Priestley, 1986). Attempts were made to relate seed longevity to other aspects of seeds such as composition. Many seeds with high lipid content are short-lived; however, tomato seed with 20% lipid content is a notable exception (Priestley, 1986). At this time, the physiological basis for differences in longevity between species is not well understood.

Long-term seed storage experiments were initiated in the past, and data from these studies were compiled and analyzed. As shown earlier, a sigmoidal relationship is revealed with a loss of germination in time. The sigmoidal curve is then transformed by probit analysis to reveal a linear relationship and from these data the P50 is calculated, defined as the period of time in years for viability reduced by half. The first set of P50 values were derived from tests of seeds held in open storage conditions in the temperate zone throughout the world (Priestley *et al.*, 1985). The second set of P50 values were obtained from seeds stored in semi-controlled conditions in the United States, and the initial samples were obtained from the US Department of Agriculture's Horticultural Field Station in Cheyenne, Wyoming (Roos and Davidson, 1992). This collection was moved to the National Seed Storage Laboratory in Fort Collins, Colorado, in 1962 and stored at 5°C and <40% relative humidity. In about 1977, most of the samples were transferred to sealed moisture-proof bags at −18°C and maintained under those conditions.

The P50 for the study conducted under open storage conditions ranged from 3 to 25 years, and seeds of asparagus, celery, parsley and parsnip were short lived, while tomato and pea were long lived (Table 1.3).

The P50 for the second study, in which the latter portion of the study was performed under controlled conditions, ranged from 29 to 130 years. In the second study, onion and pepper were short lived and okra was long lived. A significant linear relationship (r = 0.68*) was found between the P50s for the first and second study in which data was available for the same species. The regression equation revealed that the P50 values were approximately four times greater for the second study, compared to the first study. For example, the P50 of onion, the shortest-lived seed in the second study, was greater than tomato, the longest-lived seed in the first study. In conclusion, even though differences exist in the longevity among species, the major factor influencing storage life is the storage environment of temperature and relative humidity, which directly affects seed moisture content.

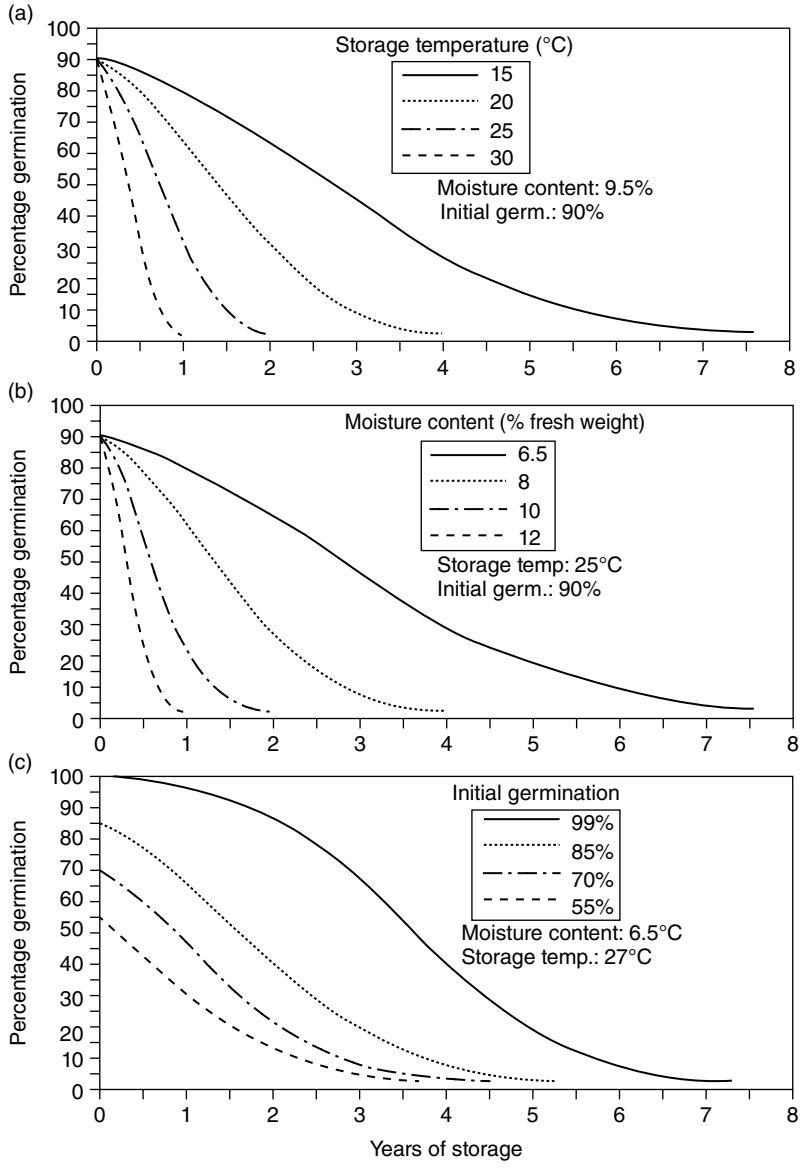

Fig. 1.3. The influence of temperature (a), moisture content (b) and initial germination (c) on onion seed ageing. Ageing curves were developed from the equation and constants of Ellis and Roberts (1981). Figures from Taylor, 1997

Germination and Seedling Growth

Germination is the transition period between the resting and the growth stages of the plant, and germination is considered completed at the time of visible radicle emergence (Bewley *et al*., 2013). In this discussion, post-germination events are included to the point when the seed becomes a functional seedling. Definitions are needed to describe the resting seed condition with respect to environmental conditions favorable to support growth. Seeds in storage with low moisture content are in a state of quiescence, defined as the absence of growth because of environmental

Table 1.3. The half-viability periods (P50) in years for different vegetable seeds.

Seed	Genus species	P50*	P50†
Asparagus	*Asparagus officinalis*	3.92	—
Bean, lima	*Phaseolus lunatus*	13.12	—
Bean, runner	*Phaseolus coccineus*	7.99	—
Bean, snap	*Phaseolus vulgaris*	15.97	46
Beet	*Beta vulgaris*	16.51	43
Cabbage	*Brassica oleracea*	7.15	—
Carrot	*Daucus carota*	6.63	35
Celery	*Apium graveolens*	4.11	—
Corn, sweet	*Zea mays*	9.6	65
Cucumber	*Cucumis sativus*	4.92	45
Eggplant	*Solanum melongena*	—	54
Leek	*Allium porrum*	5.30	—
Lettuce	*Lactuca sativa*	6.42	—
Muskmelon	*Cucumis melo*	—	61
Okra	*Abelmoschus esculentus*	—	125
Onion	*Allium cepa*	5.43	29
Parsley	*Petroselinum crispum*	3.41	—
Parsnip	*Pastinaco sativa*	4.04	—
Pea	*Pisum sativum*	15.86	130
Pepper	*Capsicum annuum*	—	27
Radish	*Raphanus sativus*	13.82	—
Spinach	*Spinacia oleracea*	12.76	37
Tomato	*Lycopersicon esculentum*	24.52	124
Watermelon	*Citrullus lanatus*	—	43

*P50 values obtained from open storage conditions (adapted from Priestley *et al*., 1985).
†P50 values obtained from semi-controlled storage conditions (adapted from Roos and Davidson, 1992). Original samples from open storage in Cheyenne, Wyoming, were later transferred to the National Seed Storage Laboratory in 1962 and stored at 5°C and < 40% RH. In *c.* 1977, most samples were then stored at −18°C.

conditions that do not favor growth (Copeland and McDonald, 2001). The environmental factors needed to overcome this state of arrested development are water, oxygen, and a suitable temperature. Seed dormancy, in contrast, is a physical or physiological condition of a living seed that prevents germination even in the presence of otherwise favorable environmental conditions (Copeland and McDonald, 2001). For additional information on germination and dormancy, the reader is referred to books devoted to the subject area of seed physiology (e.g. Khan, 1977, 1982; Benech-Arnold and Sanchez, 2004; Bradford and Nonogaki, 2007).

Seed and seedling morphology

The botanical term *seed* refers to the mature ovule from the mother plant that contains the embryonic plant or embryo, and the integuments that become the seed coat and additional storage tissue such as the endosperm (Copeland and McDonald, 2001). Many *seeds* are enclosed in remnants of the fruit and are not technically true seeds. We will take a broader interpretation and consider the dispersal units or propagules from the mother plant including dry indehiscent fruits as seeds. The embryo morphology is generally similar within a plant family; however, vegetable seeds include both monocots and dicots, and each class contains several plant families (Table 1.1). Seeds that contain reserve materials in a well-developed endosperm are called *endospermic*. If they contain most of their reserve materials in the cotyledons (cotyledons are embryonic tissue), they are called *non-endospermic* (Black *et al*., 2006). After the completion of germination, the embryo develops the root and shoot system of the seedling. Seedlings are categorized into two groups depending on the orientation of the cotyledons with regards to the soil or growing media. Seedlings in which the cotyledons are raised above the soil by the expansion of the

hypocotyl are termed *epigeal*. Those seedlings, in which the hypocotyl does not elongate appreciably, and results in the cotyledons remaining in the soil, are termed *hypogeal* (Black *et al.*, 2006). The cotyledons often become photosynthetic in epigeous seedlings, while expansion of the epicotyl or mesocotyl is responsible for shoot development in hypogeous seedlings.

The following discussion will group selected vegetable seeds by their seed and seedling morphology (Table 1.4 and Fig. 1.4).

Sweet corn and asparagus have reserves in the endosperm and exhibit hypogeal seedling growth. The sweet corn seed is a caryopsis in which the pericarp is tightly fused to the seed coat (Copeland and McDonald, 2001), and the embryo is oriented in a lateral position as is typical of the grasses (Martin, 1946). The endosperm is non-living in sweet corn, and the scutellum, which is a part of the embryo, is considered analogous to the cotyledon. Many endospermic seeds, such as asparagus, have live endosperm tissue. The endosperm is considered living if there is a positive test with the vital stain 2,3,5-triphenyl tetrazolium chloride (AOSA, SCST, 2010). The solanaceous and umbelliferous crops and genus *Allium* seeds all contain linear embryos embedded in a living endosperm with epigeal germination. Though the embryo is considered linear, it is commonly found coiled, as in the case of tomato and pepper (Martin, 1946). In this group, carrot and celery are examples of schizocarps that have two fused carpels separating at maturity to form one-seeded mericarps (Copeland and McDonald, 2001). Beet and spinach have embryos that surround non-living nutritive tissue, and the nutritive tissue is a well-developed perisperm rather than endosperm (Hayward, 1938; Heydecker and Orphanos, 1968). Beet seed is a fruit and in many cultivars a *seed ball* is formed by the aggregation of two or three flowers to produce a multigermed propagule (Hayward, 1938). Non-endospermic seeds may contain remnants of endosperm that is adjacent to the testa; however, little reserve material is present. The endosperm is specialized in lettuce with a two-layer envelope surrounding the embryo that serves as a semi-permeable barrier to solute diffusion (Hill and Taylor, 1989). Non-endospermic seeds can exhibit hypogeal germination (found in pea and runner bean) or epigeal germination (represented by seeds from four different families). The bent embryo orientation is found in the large-seeded legumes and brassicas and is termed the *jackknife* position of the embryonic axis with the cotyledons. Lettuce and the cucurbits have spatulate embryos, and lettuce is an achene where the ribs on the seed surface are formed by the pericarp (Hayward, 1938). Definitions of anatomical parts of seeds and other seed terms throughout this chapter are found in *The Encyclopedia of Seeds* (Black *et al.*, 2006).

Water

Germination begins with water uptake. This essential process is explained by classical water relations, and this section is summarized from Bewley *et al.*, (2013). A more detailed explanation of water relations in seeds is found in

Table 1.4. The embryo orientation, the presence of a well-developed endosperm for storage of reserve materials and cotyledon orientation after germination of different vegetable seeds (Taylor 1997).

Seed kind	Embryo orientation	Location of reserves	Cotyledon orientation
Sweet corn	Lateral*	Endospermic	Hypogeal
Asparagus	Linear†	Endospermic	Hypogeal
Tomato, pepper, eggplant, onion, carrot, celery	Linear†	Endospermic	Epigeal
Beet and spinach	Peripheral‡	Endospermic	Epigeal
Pea and runner bean	Bent	Non-endospermic**	Hypogeal
Snap bean and *Brassicas*	Bent	Non-endospermic	Epigeal
Lettuce and cucurbits	Spatulate	Non-endospermic	Epigeal

*Embryo partially surrounded by non-living tissue.
†Embryo surrounded by living nutritive tissue.
‡Embryo surrounds non-living nutritive tissue.
**Non-endospermic seeds have little or no nutritive tissue as endosperm.

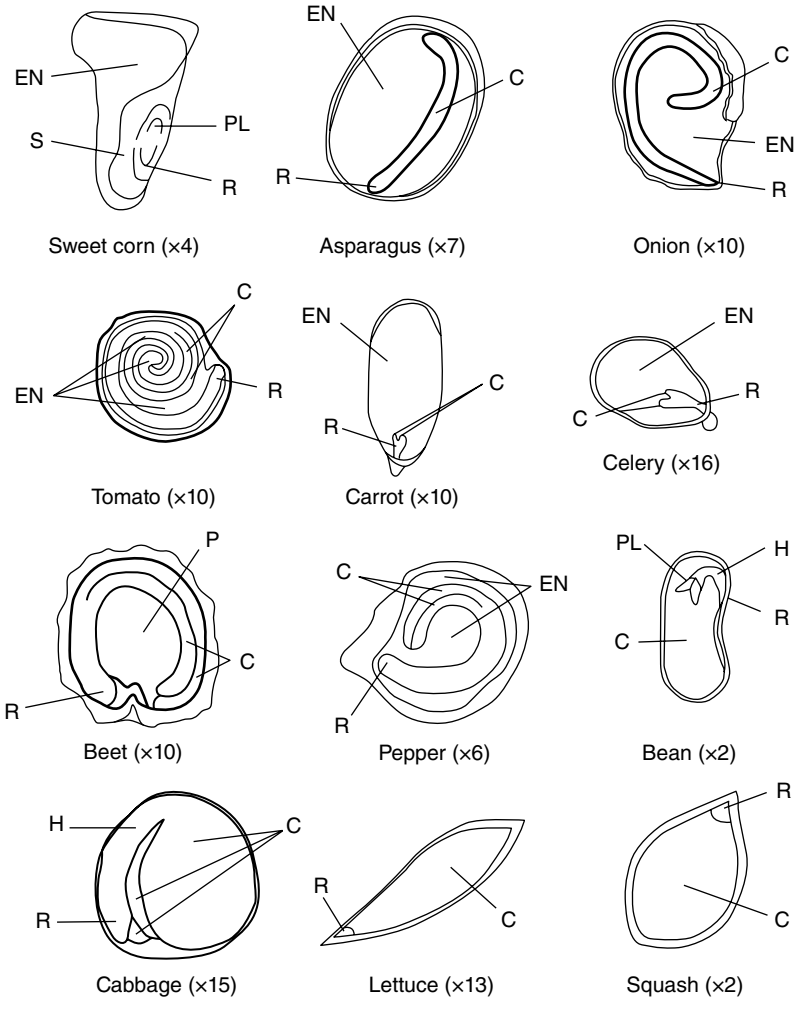

Fig. 1.4. Internal morphology of selected vegetable seeds (courtesy of D.H. Paine). EN, endosperm; S, scutellum; PL, plumule; R, radicle; C, cotyledon; P, perisperm; H, hypocotyl, (Figure from Taylor, 1997).

Koller and Hadas (1982). Water potential is an expression of the energy status of water. Water moves in a passive manner from a high to a low potential. The water potential of pure water is 0, and a decrease in water potential (less water available) is denoted by more negative values. The components of the water potential are the algebraic sum of the matric, osmotic, and pressure potentials. The matric or suction potential refers to the ability of the matrices in cells to bind water and is a negative value. The osmotic potential refers to the contribution of dissolved solutes to decrease water potential and is also a negative value. The pressure potential is a positive value and occurs when water enters the cells and creates an internal positive force on the cell walls. Water potential measurements can also be made on seeds in storage, and the water activity, described previously, is related to the water potential in a log-linear relationship (Taylor *et al.*, 1992).

To illustrate the process of water uptake in vegetable seeds, we conducted water uptake investigations by imbibing cabbage and tomato seeds on moistened blotters maintained at 25°C in the dark (Fig. 1.5).

Water uptake in seeds reveals a triphasic pattern with an initial rapid uptake phase, followed by a lag phase and then a second increase in moisture content. The generalized illustration of the triphasic pattern of water uptake is shown in the later section on "Seed Enhancements" (Fig. 1.9). Phase I is known as imbibition and is a physical process that occurs in both living and dead seeds (Bewley *et al.*, 2013). Seeds of most vegetable crops take up water readily, and, in our study, seeds were fully imbibed in a period of 4–8 h (Fig. 1.5). The rapid water uptake is attributed to the negative matric potential of the seed, which is caused by cell wall components and protein (Leopold, 1983). Swelling occurs during imbibition due to the expansion of hydrophilic compounds such as proteins, cellulose, pectic substances, and mucilage (Mayer and Poljakoff-Mayber, 1982). The rate of water uptake is influenced by a number of factors including temperature, initial moisture content, seed composition, and morphology. Seed–soil contact is another important factor; however, water vapor and not liquid water may be the principle source of water for seeds imbibed in unsaturated soils (Wuest, 2007). Some vegetable seeds, such as okra, do not imbibe water readily due to impermeable seed coverings (Anderson *et al.*, 1953). A lag in the imbibition time was shown in snap bean seeds with the semi-hard seed characteristic only when the initial seed moisture content was low (Taylor and Dickson, 1987). During the second phase (lag phase), there is little uptake of water. The matric potential is negligible during this period, and the osmotic and pressure potentials regulate the total water potential. During this phase, enzymes and membranes are functional in the fully hydrated cells as the seed advances to the completion of germination. The duration of Phase II is dependent on species and is influenced by environmental conditions (see "Water and temperature stress" sections to follow), and in our example, tomato has a longer lag period than cabbage even though tomato had greater moisture content after imbibition than cabbage (Fig. 1.5). Phase III of water uptake commences with visible germination (see arrows on Fig. 1.5) in which the seed coat is ruptured by the emerging radicle that forms the root system of the plant. Radicle growth is caused by cell elongation, and is then followed by shoot growth. Greater uptake of water is caused by a further decrease in osmotic potential caused by degradation of reserve materials into osmotically active smaller molecules (discussed under "Mobilization of reserves") and elongation of seedling tissue. The increase in the moisture content is much slower in Phase III (seedling growth) than

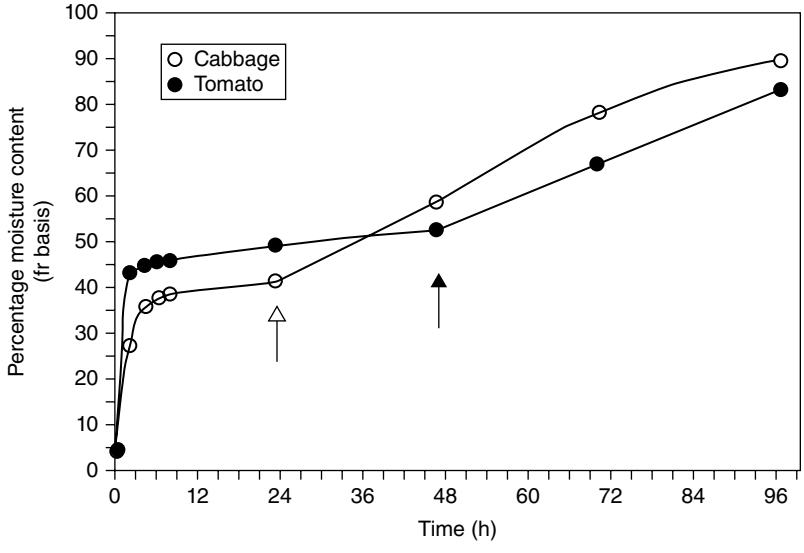

Fig. 1.5. Triphasic water uptake curves for imbibing cabbage and tomato seeds (Figure from Taylor, 1997). Phase I (imbibition) occurs from 0–6 h, Phase II (lag phase) from *c*. 6 to 24 h in cabbage and 6 to 48 h in tomato, and Phase III (seedling growth) occurs after visible germination as indicated by the arrow for each species.

the physical process of water uptake in Phase I (Fig. 1.5). In Phase III, the seed becomes a seedling and also loses its ability to withstand desiccation. Therefore, drying seeds after visible germination will result in death of the radicle, while drying seeds during Phase I or II is not injurious to the viability of the seed.

Oxygen

Oxygen is necessary for germination and is the substrate required for respiration to produce energy in the form of adenosine triphosphate (ATP). Seeds with low moisture content exhibit negligible gas exchange, and under a static environment, respiration is not appreciable until Type III water binding in which the water activity is greater than 0.9 (Vertucci and Roos, 1990). Germination is a dynamic process, and gas exchange increases rapidly as seeds imbibe water. The different phases of water uptake (previously described) are used to describe respiration patterns. In Phase I, there is a rapid increase in respiration which is attributed to the activation of existing enzymes. For example, in peas the respiration rate was shown to increase linearly with the degree of swelling (Kolloffel, 1967). In Phase II, a lag phase in oxygen uptake is observed in many large-seeded species and was attributed to the relative impermeability of the seed coat or seed coverings to gas diffusion since removal of the seed coat reduced this lag (Mayer and Poljakoff-Mayber, 1982). In Phase III, visible germination results in piercing of the seed coat, allowing ample oxygen for a second increase in the respiration rate. Also, there is an increase in the number of mitochondria resulting in greater total respiratory activity. Finally, Phase IV was described only for storage tissue, such as the cotyledons, in which there is a decline in respiration rate attributed to the exhaustion of reserve materials.

Mobilization of reserves

Germination was previously described in three phases with respect to water uptake and gas exchange. The source of substrates for respiration and/or growth is different before and after radicle emergence. Readily available substrates are needed for the early phases of germination since mobilization of reserve materials to produce smaller molecules does not occur until Phase III. Dry seeds store sugars that are a source of soluble carbohydrates needed for respiration during Phases I and II. Sucrose is commonly found in dry seeds, and other oligosaccharides such as raffinose and stachyose may also be present (Amuti and Pollard, 1977).

Mobilization of reserves is a post-radicle emergence event and was studied at the subcellular as well as the biochemical levels. Early studies were performed with germinating seeds in time-course experiments by removing samples, dissecting seed and seedling parts and weighing each component. There was a general loss of weight in storage tissue such as the cotyledons or endosperm with an increase in weight of roots and shoots (Mayer and Poljakoff-Mayber, 1982). These studies illustrated trends for utilization of reserves in germinating seed and provided the foundation for more detailed biochemical studies. Unfortunately, our understanding of biochemical events associated with mobilization of reserves in vegetable seeds is fragmentary. Most information is available on agronomic crops and especially those seeds that are also used for human consumption. The following section will briefly outline biochemical events associated with the catabolism of the reserve compounds: starch, lipids, proteins and phosphorus (Bewley *et al.* 2013). An expanded description of starch, lipids, and proteins is found in Black and Bewley (2000).

Starch is the common form of storage carbohydrate and is found in the cotyledons of beans and endosperm of sweet corn. Starch occurs in starch grains and is found as a straight chain polymer of glucose called *amylose* or the branched polymer, *amylopectin* (Copeland and McDonald, 2001). Starch is enzymatically degraded by amylase and other enzymes to form monomeric units of glucose. Glucose is respired or converted to the disaccharide sucrose since sucrose is the form that is transported to the growing regions of the seedling.

Seeds have unique biochemistry to produce and utilize lipids and to store oil as a reserve material in oil bodies within the cells. Noteworthy, no other part of the vegetable plant produces and stores lipids as a reserve material. Many small-seeded crops store lipids (Fig. 1.1) since

lipids are more chemically reduced than starch, and have higher energy content. The lipid is converted to a form that is transported from the storage tissue to the growing points. Lipid degradation occurs by unique biochemical pathways and also utilizes a specialized organelle, the glyoxysome (Bewley *et al*., 2013). Glyoxysomes are either present in dry seeds and enlarge during germination or are formed by *de novo* synthesis. The integration of the biochemical pathways with the organelles results in a process known as *gluconeogenesis* or *making new sugar*. Briefly, lipids are degraded by lipases to produce three free fatty acids and glycerol. Breakdown products from fatty acids are utilized ultimately to form sucrose and transported as described for starch.

Proteins are ubiquitous in seeds (see Table 1.1) and occur as enzymes or storage proteins. The storage proteins are found in protein bodies and degraded by proteinases to form different amino acids. The fate of the released amino acids is complex, and amino acids can be converted to other amino acids or transported to the growing points. Organic acids can also be formed from amino acids and later respired. Phosphorus is stored in seeds in an organic form called *phytic acid* or *phytin*. Phytic acid or myo-inositol hexaphosphate occurs as a salt and can contain potassium, magnesium, and calcium as well as the minor elements iron, manganese, and copper. Phytase releases phosphate, which is used for synthesis of nucleic acids, ATP, and phospholipids for membrane synthesis. Macro- and micronutrients are also released and are used for cell growth and development.

In conclusion, reserve materials in seeds provide a source of carbon in the form of sugars, nitrogen in the form of amino acids, and phosphorus in the form of phosphate along with other elements. Seeds are dependent on these stored reserve materials to support initial growth and development of the seedling. After seedling emergence and subsequent growth, the seedling becomes self-supporting by producing its own carbon in photosynthesis and through the uptake of other nutrients via the root system.

Seed Quality

Seed quality encompasses many parameters of a seed lot, but this discussion will focus on the robustness of germination and seedling growth potential. One method used to estimate seed quality is the standard germination test that is conducted under ideal environmental conditions in the laboratory (AOSA, 2017a; ISTA, 2017). The criterion for germination used by physiologists is radicle emergence; however, the seed analyst extends this interpretation by classification of seedlings as either normal or abnormal. Abnormal seedlings are those seedlings with an impaired root and/or shoot development or other seedling defects (AOSA, 2017b). Only normal seedlings are considered when reporting the actual germination of a seed lot and the percent normal seedlings is determined following standardized time and test conditions (AOSA, 2017a; ISTA, 2017). The standard germination test is the only test accepted for commercial labeling, and the minimum germination standards are presented for vegetable seeds in the United States (Table 1.2). However, in commerce, the germination is generally much higher to ensure high quality seeds for growers.

The term *seed vigor* is one component of seed quality, and the following definition was adopted by the AOSA (Baalbaki *et al*., 2009): "Seed vigor comprises those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions." Seed vigor differs from germination in that vigor emphasizes the germination rate (rapid and uniform) and the application of the results to forecast field emergence rather than laboratory performance. Seed vigor is generally associated with a higher level of plant performance, and during the early phases of seed aging, a reduction in seed vigor occurs prior to a reduction in germination (Baalbaki *et al*., 2009).

The effect of seed aging is considered on a population or sample basis as well as on a single seed basis. The decline in the germination of a seed lot maintained under the same environmental conditions reveals a negative sigmoidal pattern with time (Fig. 1.3). This curve indicates that all seeds do not die at the same time and that, in general, most seed lots are composed of a mixture of viable and non-viable seeds. The sigmoidal pattern is most evident from lots with an initial high germination (Fig. 1.3c), and these lots are most desirable from a horticultural perspective since

they can withstand stress or aging before showing a marked reduction in germination.

On a single seed basis, seeds placed in a favorable environment will either germinate or fail to germinate. This categorical judgment is inadequate since a number of events occur to a viable seed before it is rendered non-viable. These events during aging also support the concept of a loss of vigor prior to a loss in germination. Different schemes or models were developed to illustrate changes associated with the loss of viability, and a proposed sequence of changes in seeds during aging was illustrated by Taylor (2003a) and is shown in Fig. 1.6.

Merits of this scheme are that both whole plant and physiological (biochemical and biophysical) responses are presented and the model does provide a frame that ranks the relative sensitivity of whole plant to physiological aging. In addition, events are ranked as early stage, or most sensitive to aging, to late stage events until the seed is rendered nonviable with the loss of cell viability. A review of other events associated with seed aging and another model of seed aging based only on biochemical and physiological changes associated with the loss of viability was described by Priestley (1986).

Whole plant responses

A primary interest in horticulture is to determine the consequence of seed aging on the whole-seed and whole-plant levels. The germination rate is the most sensitive index of seed quality at the whole-seed level (Fig. 1.6). However, germination rate is not recorded in a standard germination test; only the total or final germination is recorded. Actually, the only step of the germination process that is accurately measured is the onset of Phase III (visible germination). Therefore, the time to radicle emergence provides quantitative information on the relative vigor of a seed lot. To illustrate this effect, lettuce seed was aged for a short period by first increasing the moisture content to 20% (fw basis) and then incubating the seeds at 40°C for 24 hours (Tomas, 1990). This procedure was adapted from the controlled deterioration test developed as a method to assess seed vigor (Powell *et al.*, 1984). The time to radicle emergence was recorded using time-sequence photography and recording germination at two-hour intervals. Aged seeds germinated approximately six hours later than non-aged seeds and produced a greater percentage of necrotic seedlings (Fig. 1.7).

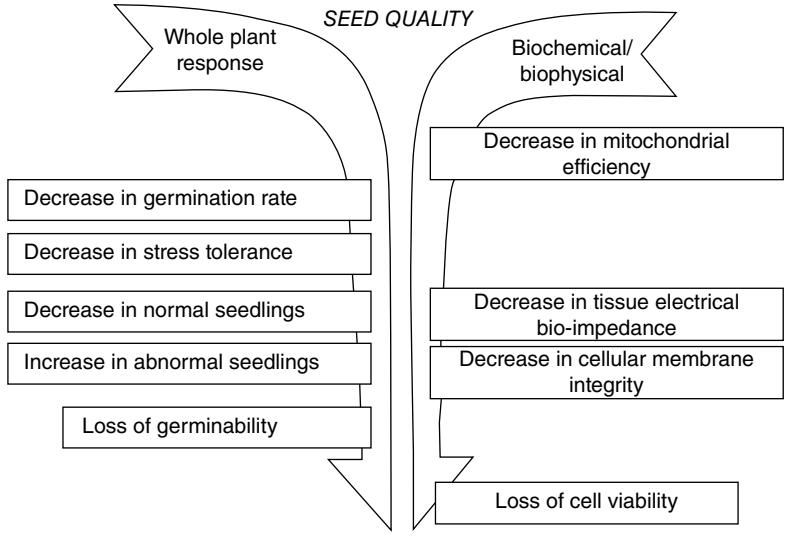

Fig. 1.6. The relative sensitivity of selected whole-plant and physiological events during ageing in storage. Reprinted with permission from Taylor (2003a).

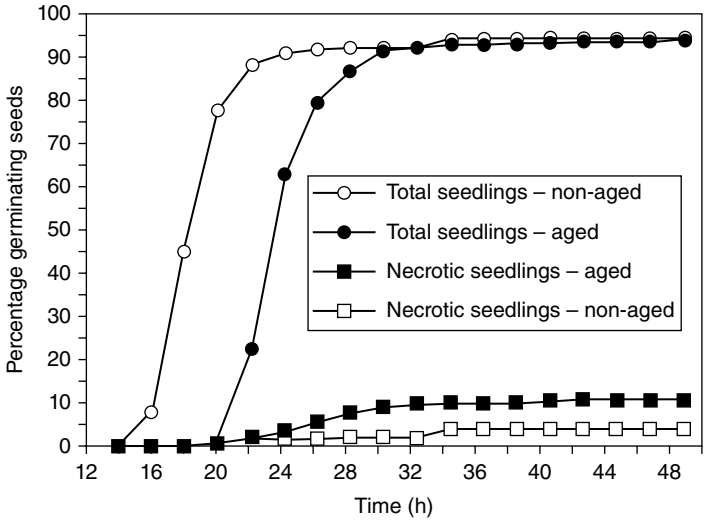

Fig. 1.7. The influence of ageing on germination rate of lettuce seeds including the presence of necrotic seedlings (adapted from Tomas, 1990, Figure from Taylor, 1997).

Necrotic seedlings are symptomatic of a disorder associated with aging in lettuce seeds called *physiological necrosis* (Tomas *et al.*, 1992) and are classified as abnormal seedlings (AOSA, 2017b). These data reveal that a mild aging treatment decreases the germination rate and increases the incidence of abnormal seedlings; however, the total percentage of seeds to germinate was not changed.

A decrease in the stress tolerance of a seed lot is a more sensitive indicator of seed quality than the standard germination test (Fig. 1.6). Stress tests are the most common type of vigor tests used by seed testing laboratories and by the seed industry. One of the most common tests is rapid aging under controlled conditions followed by a standard germination test. Methods used for rapid aging are termed "accelerated aging," "controlled deterioration," and "saturated salt aging," and the test methods are described in detail in Baalbaki *et al.* (2009). The basis of all three rapid aging techniques is to impose a controlled harsh storage environment for a specified short duration (generally several days), and then a standard germination is conducted after the aging regime. The test results are used to rank seed lot performance and estimate seed storage potential.

Another commonly used method to assess stress tolerance (Fig 1.6) imposes cold, wet soil conditions for a given period of time and then the seeds are transferred to warmer conditions for the completion of germination. This test is commonly referred to as the "cold test" (Baalbaki *et al.*, 2009), and is routinely used on sweet corn and adopted for other vegetable crops. The cold test imposes two environmental stresses: low temperature and wet soil conditions. The soil media used depends on the test conditions, and a commercially available sand is commonly used as a reproducible medium. Another test medium is a field soil from the same geographic region as the seed testing laboratory. Other variables to consider are the presence of indigenous soil pathogens; for example, using non-sterilized field soil. Despite these many factors, the test variables are standardized for a reproducible procedure.

Field emergence is of major horticultural importance for direct seeded crops since seeds are sown to achieve a desired plant population for optimal harvest efficiency. Seed vigor was related to field emergence (Roberts, 1972; Heydecker and Coolbear, 1977) with poor seed quality resulting in poor stand and reduced yield. Although the effect of plant population on yield was frequently studied and reviewed (Wiley and Heath, 1969), another more interesting question arises: does a reduction in seed vigor affect yield, if plant population is not a factor? Data from many separate

studies were summarized to address this question, and the following information was adapted from the review by Tekrony and Egli (1991). Crops were grouped based on the time of harvest of the harvestable product, either as vegetative, early reproductive or fully reproductive stages. Sources of seed quality differences were obtained by aging. In general, crops harvested in the vegetative stage showed the greatest response to aging with loss of vigor resulting in reduced uniformity of seedling emergence. Fairly consistent beneficial responses from sowing high quality seeds were also measured on crops that were harvested in the early reproductive stage. Crops harvested at full maturity, including most agronomic crops, generally did not have a positive yield response to seed vigor (Tekrony and Egli, 1991). Dry beans had a variable response to vigor. In conclusion, seed vigor is important for yield potential in many vegetable crops since most of these crops are harvested in the vegetative or early reproductive stages.

Physiological aspects

Respiration and energy synthesis pathways are vital to high vigor seed, and both processes are associated with early events during seed aging (Fig. 1.6). The mitochondrial membrane is required for respiration and electron transport (Goodwin and Mercer, 1983). Cytochrome C dissociates from the inner mitochondrial membrane during normal seed maturation drying, and seed aging impairs the re-association of cytochrome C (cited by Rutzke *et al.*, 2008). The production of ethanol, a by-product of respiration, was shown to increase under aerobic conditions as seeds age, while under anaerobic conditions, ethanol production decreases. A test was developed that employs the ratio of these two measurements, that provided a sensitive index of seed quality (Taylor *et al.*, 1999). The ratio of anaerobic to aerobic (ANA) ethanol production was more sensitive than a loss in either the percent germination or germination rate (Rutzke *et al.*, 2008).

Measuring electrical bio-impedance is a biophysical method adapted from medical diagnostics for investigating seed quality (Repo *et al.*, 2002). Snap bean seeds were first hydrated to desired seed moisture content and then small electrodes were inserted into the cotyledons. Seeds were exposed to a small alternating current, and the capacitive and resistive components of the seed tissue were measured to reveal an electrical impedance spectrum. Changes in the spectra were observed as seeds aged, and the sensitivity of the test results was comparable to a decrease in normal seedlings in a standard germination (Fig 1.6). The advantage is that the entire bio-impedance test was conducted in 24 hours compared to seven days for the standard germination.

Cell membrane integrity is tested directly or indirectly as a means to predict seed quality (Fig. 1.6). Certain vital stains are used in seed testing and provides a direct method to assess cellular integrity (Overaa, 1984). Indigo carmine, a cell membrane permeability stain, is excluded from living cells and is not injurious to subsequent embryo growth. Measuring leakage of compounds during the early stages of germination provides an indirect test of cellular integrity. Leakage tests are commonly performed on intact seeds and compounds such as electrolytes, sugars, amino acids, phenolic compounds, and others are measured from the imbibing solution (Priestley, 1986). Leakage tests have found application in seed quality testing, and methods to assess seed vigor by measuring conductivity are described (Baalbaki *et al.*, 2009). The major advantage of this method is that it is performed in a relatively short period of time and the data is objective. Unfortunately, there are serious limitations for the wide-scale use of leakage tests since seeds of many species possess an inner semi-permeable seed coat layer that restricts leakage of solutes and electrolytes (Beresniewicz *et al.*, 1995b). For example, seeds of onion and leek contain a semi-permeable layer composed of cutin, while tomato and pepper have a suberized layer (Beresniewicz *et al.*, 1995a). Therefore, measuring leakage of vegetable crop seeds is limited to large-seeded vegetable crops including pea and *Phaseolus* spp.

The loss of cell viability is the final step for the loss of seed viability (Fig. 1.6). All biochemical pathways require enzymes to catalyze reactions within the cell. The relationship of enzyme activity with seed aging was studied, and many enzymes remained active after all viability is lost; however, the dehydrogenases are one group of enzymes that were directly related with a loss of

cell viability (MacLeod, 1952). Dehydrogenase enzymes are found in several steps in respiratory pathways and catalyze oxidation-reduction reactions (Goodwin and Mercer, 1983). As previously discussed, respiration rate increases rapidly during imbibition, and maximal respiration is needed for the completion of germination. Therefore, dehydrogenase activity is determined during Phase II germination, a period of active metabolism. The most common method to measure dehydrogenase activity is with the vital stain 2,3,5-triphenyl tetrazolium chloride (TTC or TZ). Tetrazolium salts in the oxidized form are colorless and water-soluble and are reduced by dehydrogenase enzymes to the water insoluble red stain, formazan (Copeland and McDonald, 2001). Tetrazolium salts were first used in the 1940s, and the TZ test is still the most widely used test for seed viability (AOSA, SCST, 2010). Limitations are that the test is subjective and staining patterns are difficult to interpret, resulting in inaccurate assessment of viability. Other vital stains are used in seed testing (Overaa, 1984) and provides an alternative to the TZ method.

Sowing Environment

The soil environment is finally considered as it can greatly influence germination and seedling establishment of vegetable crops. In practice, direct seeding is often performed early in the growing season in soil conditions that are less than optimal for a particular species. Various abiotic stresses include water, temperature and physical impedance, and biotic stresses such as soil pathogens, insects, and other predators, are present from the time of sowing to seedling establishment. The following discussion will briefly review the role of the three major abiotic stresses and describe the sensitive phase for each.

Water stress

Water is essential for germination; however, water stress may occur as either an excess or deficit in the field for direct seeded crops or in the greenhouse for transplant production. In the case of water excess, oxygen is limiting for the completion of germination or seedling growth, because Phases II and III in germination are sensitive to oxygen deficiencies. Seeds of different species were subjected to anoxia by soaking in water, and germination was recorded after 72 hours (Crawford, 1977). Of the vegetable seeds tested, lettuce was tolerant to soaking, while pea was most sensitive, and it was shown that sensitivity to soaking was associated with a large production of the fermentation product, ethanol. Seeds are generally sown initially under favorable soil moisture conditions and then rain or irrigation can create a flooding condition. A period of low oxygen (hypoxia) can condition germinating seedlings so that seedling roots can survive for a longer period of time in a subsequent anaerobic condition compared to those that were not conditioned (Hole *et al.*, 1992).

Seeds are commonly sown at a shallow depth, and the soil may dry to a water potential below that necessary for the completion of germination. Due to their tremendous matric potential, seeds can imbibe or at least partially imbibe water even in dry soils and may enter Phase II germination (Bewley *et al.*, 2013). However, due to the dry conditions the seed cannot achieve sufficient moisture content to complete germination. Phase III is associated with cell elongation and later cell division, and these processes are most sensitive to water stress in growing tissue (Hsiao, 1973). Higher water potential (more water available) is needed for the initiation of cell elongation than for the maintenance of radicle growth after visible germination (reviewed by Hegarty, 1978). This indicates that a threshold moisture level must be achieved before the seed will complete germination (Phase III). Since seeds are desiccation-tolerant in Phases I and II, the inability to complete germination under water deficits would help to ensure survival under these stressful environments.

Temperature stress

Temperature regulates all aspects of biology including the germination of seeds. The cardinal temperatures of germination (minimum, optimum, and maximum) were summarized for vegetable crops seeds (Maynard and Hochmuth, 2007). Though temperature does affect the time for full imbibition, temperature primarily influences the germination rate by regulating the

duration of the lag phase or Phase II. Germination is predicted by incorporating a heat sum in degree days (S) and the minimum temperature for germination (T_{min}) (Bierhuizen and Wagenvoort, 1974). Seeds were germinated in a range of temperatures from 3–17 or 3–25˚C for fruit vegetables, and daily counts recorded. The S and T_{min} were calculated, and shown for selected vegetable crop seeds (Table 1.5) and the predictive equations were highly correlated over the temperature range tested. In general, the warm season crops have a higher T_{min} than the cool season crops, while the value for S varies with species.

Germination at low temperatures was investigated on sugary (su), sugary enhancer (se), and shrunken-2 (sh_2) sweet corn genotypes over a range of temperatures from 11.1–30.0˚C (Hassell *et al.*, 2003). Achieving 75% germination within seven days was the criterion for successful germination at each temperature for nine varieties of each genotype. The nine varieties examined were commercially released over a period of 89, 16 and 10 years for su, se and sh_2 genotypes, respectively. The mean number of days for 75% germination averaged over all temperatures tested was 3.6, 3.9 and 4.4 days for su, se and sh_2 genotypes, respectively. Varietal differences were measured within each genotype for low-temperature germination; however, there were no trends for improved cold tolerance with release date. Therefore, based on the scope of this study, plant breeding objectives have not included low temperature germination.

Temperature stress may occur, and temperatures may be suboptimal or supraoptimal for a particular species. The effect of elevated temperatures on lettuce seed germination is presented in Chapter 14. Seeds of many warm season crops are negatively influenced by low temperature, and this physiological disorder is known as chilling injury (reviewed by Herner, 1990; Bedi and Basra, 1993). There are two groups of chilling sensitive seeds:

> (i) those that are injured during Phase III germination such as the solanaceous crops and the cucurbits; and (ii) those seeds that are susceptible during Phase I and lima beans are very sensitive, while snap bean and sweet corn are sensitive (Bedi and Basra, 1993). In the second group, damage occurs during hydration of the seed tissue and is referred to as imbibitional chilling injury. Seeds become more susceptible to this type of injury as the initial seed moisture content decreases. Seed coat permeability and integrity are also important factors as seeds that imbibe water rapidly; especially seeds with cracked seed coats are more prone to imbibitional chilling injury.
>
> (Taylor *et al.*, 1992)

Table 1.5. Minimum germination temperature (T_{min}) and heat sum (S) in degree days for seedling emergence, and the applicable temperature (T) range for germination of various vegetables. Crops are ranked within groups by heat sum (S) in degree days (adapted with permission from Bierhuizen and Wagenvoort 1974).

Group	Crop	Genus species	T_{min} (°C)	S (degree days)	T (°C)
Leaf vegetables and *Brassica* crops	Lettuce	*Lactuca sativa*	3.5	71	6–21
	Turnip	*B. campestris var. rapa*	1.4	97	3–17
	Kale	*B. oleracea var. acephala*	1.2	103	3–17
	Red cabbage	*B. oleracea var. purpurea*	1.3	104	3–17
	White cabbage	*B. oleracea var. capitata*	1.0	106	3–17
	Brussels sprouts	*B. oleracea var. gemmitera*	1.1	108	3–17
	Spinach	*Spinacea oleracea*	0.1	111	3–17
	Cauliflower	*B. oleracea var. botrytis*	1.3	112	3–17
	Leek	*Allium porrum*	1.7	222	3–17
	Celery	*Apium graveolens*	4.6	237	9–17
	Parsley	*Petroselinum crispum*	0.0	268	3–17
Fruit vegetables	Tomato	*Solanum lycopersicon*	8.7	88	13–25
	Eggplant	*Solanum melongena*	12.1	93	15–25
	Gherkin (cucumber)	*Cucumis sativus*	12.1	108	15–25
	Melon	*Cucumis melo*	12.2	108	15–25

To better understand the role of seed moisture content and temperature on imbibitional chilling injury, two cultivars (chilling sensitive and tolerant) of snap bean seeds were adjusted to a range of moisture contents from 5% to 25% (dw basis) and germinated at 20°C or 5°C (Wolk *et al.*, 1989). Those seeds that were germinated at 5°C were transferred to 20°C after 24 hours. Critical seed moisture content was determined for each treatment that marked the onset of imbibitional chilling injury and was termed the breakpoint. Only seeds with moisture contents below the breakpoint showed a reduction in germination. At 20°C, the moisture content breakpoints for the chilling sensitive and tolerant cultivars were 15% and 11%, respectively. When seeds were tested at 5°C, the breakpoints were 19% and 16% for the sensitive and tolerant cultivar, respectively. Thus, at either temperature, the breakpoint moisture content was always greater for the sensitive cultivar, compared to the tolerant cultivar. Decreasing the temperature from 20°C to 5°C shifted the breakpoint to a higher level for each cultivar; however, the deleterious effects of imbibing seeds at 5°C were totally overcome by the elevated moisture content. Below the breakpoint for all treatments, there was an average 4.6% decrease in germination for each 1% decrease in moisture content. In conclusion, imbibitional chilling injury is influenced by the interaction of environmental and seed factors. The initial seed moisture content is the primary factor that determines the incidence of imbibitional chilling for a particular seed lot, while temperature has a moderating effect.

Physical impedance

The soil can act as a physical barrier to seedling emergence and may decrease or even prevent seedling establishment especially under conditions of soil crusting (Goyal *et al.*, 1980). Germinating seedlings must produce sufficient force to overcome this barrier. The sensitive period to this type of stress is late in Phase III when these seeds have already completed radicle emergence. There are several factors that influence the emergence ability of a seedling including the speed of germination and morphological characteristics (Inouye *et al.*, 1979). A faster emerging seedling has a better chance of escaping the physical barrier of a soil crust since it may emerge before soil crusting occurs. Two primary factors that determine the rate of emergence are soil temperature and species (Table 1.6). Considering a particular crop, seed quality may play a role because the rate of germination is influenced by vigor. Seedling morphology is also important (Table 1.4), since seeds with hypogeal germination have a smaller cross-sectional area to penetrate the soil than those with epigeal germination that must also pull the cotyledons through the soil.

Seedling emergence forces were quantified for a number of vegetable seeds and are shown in Table 1.6 (Taylor and Ten Broeck, 1988).

A positive relationship ($r = 0.98^{**}$) was determined between seed weight and force generated for eight species tested, and among small, medium, and large snap bean seeds of the same lot. The pressure (force per unit cross-sectional area of emerging hypocotyl or cotyledon in onion) was positively correlated ($r = 0.85^{*}$) with time to achieve maximum force. Seedlings with the ability to continue to generate forces may have a better chance of establishment than those that produced pressure for a short time. The energy content is related to seed composition as seeds generally store either starch or lipid (Table 1.1, Fig. 1.1). The energy content of the starch storing snap beans yielded 17 kJ g^{-1} in comparison to the lipid containing seeds that ranged from 22 to 26 kJ g^{-1} of seed. The use of reserves varied by seed type, but does provide some relative information on the efficiency of reserve mobilization. The study on seed size in snap bean revealed that small seeds contained fewer reserves than large seeds; however, the small seeds were more efficient in utilization of their reserves in seedling emergence forces (Taylor and Ten Broeck, 1988). A subsequent study measured emergence forces from snap bean seeds subjected to imbibitional chilling injury (Taylor *et al.*, 1992). The seeds subjected to chilling conditions produced less force per seedling and required a longer period of time to generate the maximum force, indicating that the injury sustained during imbibition reduced subsequent seedling growth potential.

Seed Enhancements

The last section of this chapter turns from the theme of seed biology to seed technology. *Seed enhancements* are defined as post-harvest methods

Table 1.6. Seedling emergence force characteristics and energy contents of vegetable crop seeds. Crops are ranked by maximum seedling emergence forces generated (reprinted with permission from Taylor and Ten Broek 1988).

Crop	Seed wt. (mg)	Maximum force (mN)*	Time to achieve maximum force (h)	Pressure exerted (kPa)†	Energy content (J seed^{-1})‡	Use of reserves (N kJ^{-1})***
Snap bean	268.0	3400 ± 360**	21 ± 1	234	4554 ± 12	0.75
Radish	10.0	558 ± 88	19 ± 8	317	231 ± 1	2.40
Cucumber	31.4	241 ± 49	9 ± 3	63	801 ± 1	0.30
Cabbage	4.20	157 ± 24	11 ± 2	241	111 ± 1	1.41
Onion	4.04	83 ± 11	19 ± 4	259	90.4 ±0.2	0.92
Tomato	2.90	44 ± 5	10 ± 2	96	71.8 ± 0.1	0.61
Carrot	1.00	35 ± 9	5 ± 1	117	23.8 ± 0.1	1.47
Lettuce	1.07	29 ± 6	7 ± 2	89	27.3 ± 0.1	1.06
Beets	–	26 ± 6	4 ± 2	62	–	–

*Maximum force achieved per seedling in millinewtons.
†Pressure exerted in kilopascals.
‡Energy content per seed in joules.
**Mean ± standard error.
***The ability of a seed to produce seedling emergence forces with respect to stored energy reserves

that improve germination or seedling growth, or facilitate the delivery of seeds and other materials required at the time of sowing (Taylor *et al.*, 1998). Therefore, single or multiple technologies are used on a particular vegetable crop seed from the time of harvest to sowing (Halmer, 2003). An overview of these technologies performed on commercial seed lots was adapted from Halmer (2000) (Fig. 1.8).

Seed conditioning is a physical method employed to remove contaminants from the seed lot including weed seed, other crop seeds, and inert matter, and seed conditioning principles and practices are described by Harmond *et al.* (1968) and Copeland and McDonald (2001). *Functional Treatment—Enhancement* includes a number of hydration methods under the heading of priming, such as *steep*, *soak*, and *pre-germinate*, and our discussion will focus on moisturization and priming. Active ingredients and materials are applied by seed treatment and coating technologies that are plant protectants, biostimulants, micronutrients, and inoculants. Finally, the need for high quality seeds is a prerequisite for the application of value-added seed enhancements (Kaufman, 1991), and seed testing is needed before and after an enhancement procedure to insure that high seed quality is preserved (Halmer, 2000). To place seed enhancements in context with seed testing (Fig. 1.8), the cornerstone of seed labeling in commerce is the standard germination and purity tests. A purity test includes the examination for weed seed, other crop seeds, and inert matter (AOSA, 2017a). One goal of the seed quality assurance by the seed industry is to provide a seed lot of pure seed. Seed health is testing for the presence of seed-borne pathogens. The importance of vigor was discussed in the earlier "Seed quality" section. Finally, storability is important as seed priming can negatively impact the storage life of seeds.

Hydration treatments

The importance of water in seeds is described previously in relation to storage, germination, and seedling establishment. Two hydration methods to improve germination and seedling establishment especially under stressful conditions, namely moisturization and priming, are presented. Moisturization was developed for large-seeded legumes, in particular snap beans; however, the technique has potential for other leguminous vegetable seeds. Priming is performed on many small-seeded vegetable seeds, and this method was adapted for a wide range of species.

The importance of seed moisture content on the resistance of bean seeds to imbibitional chilling injury was discussed (see "Temperature stress" section). Moisturization improved field

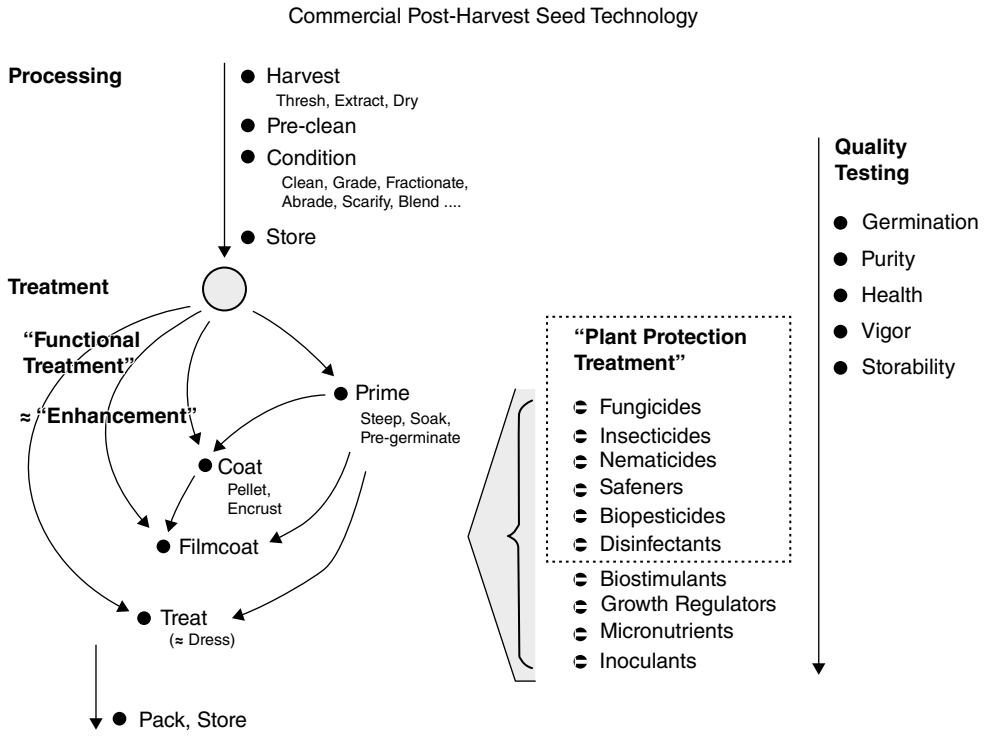

Fig. 1.8. The interrelationships of seed processing technologies used in commercial practice. Processing involves various techniques according to seed type. Optional treatments are used singly or in permutation according to seed type and market needs. Reprinted with permission and adapted from Halmer (2000).

emergence of early plantings, especially if the soil was wetted immediately after sowing (Wilson and Trawatha, 1991; Taylor *et al.*, 1992). The percentage of initial moisture content can affect germination of the same bean seed lot in the laboratory, and low moisture content samples had lower germination results than high moisture samples (Pollock and Manalo, 1970). Another benefit of moisturization is increased resistance to mechanical damage as moist seeds are less brittle (Bay *et al.*, 1995). However, moisturization at too high a level is deleterious, as the rate of aging in storage will increase dramatically. The moisture content of bean seeds is adjusted prior to packaging, and seeds are then stored in this condition. In practice, seed moisture content is adjusted to the upper level of Region 2, which corresponds to a seed moisture content of 12–13% (fw basis) or an a_w from 0.6 to 0.65 (Fig. 1.2). Seeds are moisturized by passing humidified air through the seed mass to increase the seed moisture content or by incubation with a moist solid media (Wilson and Trawatha, 1991).

Seed priming is a general term that refers to several different techniques used to hydrate seeds under controlled conditions, but preventing the completion of germination (Phase III). During priming, seeds are able to imbibe or partially imbibe water and achieve elevated seed moisture content usually in Phase II (lag phase) germination (Fig. 1.9).

Seeds are kept in this condition for a period of time that may range from less than one day to several weeks (Taylor and Harman, 1990). Priming temperatures range from 10°C to 35°C, but 15°C to 20°C is most commonly used (Bradford, 1986). Since seeds have not completed germination, they remain desiccation-tolerant and are dried for long-term storage. All priming techniques rely on the controlled uptake of water to achieve a critical

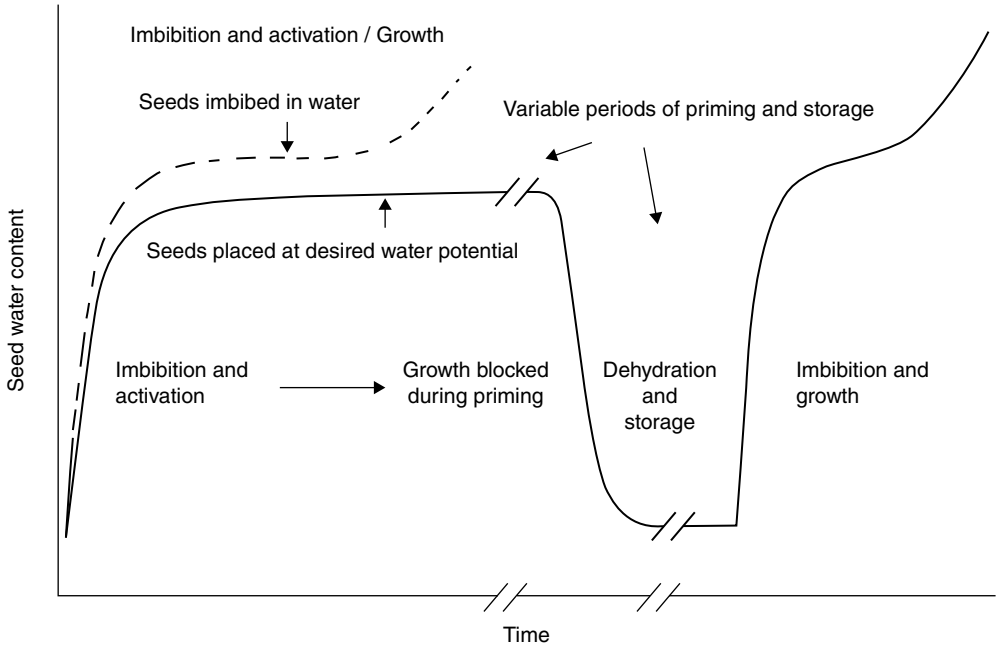

Fig. 1.9. Seed priming with respect to seed water content in comparison to imbibition in water. After priming period, seeds may be dried, stored and the transported to the grower. When seeds are sown, the lag period is reduced improving the germination rate and uniformity. Reprinted with permission and adapted from Bradford and Bewley (2003).

moisture content that will activate metabolic activity in a controlled environment. There are a considerable number of terms used in literature and in the industry to describe these methods, and definitions of *enhancement method* are found in *The Encyclopedia of Seeds* (Black *et al.*, 2006).

There are three priming techniques employed to advance the germination of seeds. The most studied technique utilizes aqueous solutions as the priming medium. In large-scale priming, a ratio of approximately ten parts priming solution to one part seeds is used (Nienow *et al.*, 1991). Therefore, due to the large reservoir of priming solution, the water uptake by seeds is regulated by the water potential of the solution, which varies by species and ranges from −0.5 to −2.0 MPa (Khan *et al.*, 1980/81). Many compounds were used to achieve a solution of known water potential and include inorganic salts such as NaCl, KNO_3, K_3PO_4, KH_2PO_4, and $MgSO_4$, low molecular weight organic compounds such as glycerol and mannitol and large molecular weight polymers such as polyethylene glycol (PEG) (Khan, 1992). The 8000 molecular weight PEG is widely used to regulate water potential, and formulas were developed to calculate the water potential of a solution of known concentration and temperature (Michel, 1983). Since gas diffusion is limited in solution, aeration is needed during the priming process. A number of terms used to describe this technique include *liquid priming*, *osmotic conditioning*, and *osmoconditioning*. The terms *osmo-* or *osmotic* are misleading when PEG is used since the water potential of PEG solutions is controlled primarily by matric forces (Steuter *et al.*, 1981).

Two other proprietary priming techniques, solid matrix priming (Eastin *et al.*, 1993) and drum priming (Rouse cited by Gray, 1994), have also gained attention as an alternative to liquid priming. In solid matrix priming (SMP), seeds are mixed with a solid particulate material and water (Taylor *et al.*, 1988). Seeds, due to their negative matric potentials, are able to imbibe

water from the solid material. Several materials was used in this process including leondardite shale, diatomaceous silica, exfoliated vermiculite, and expanded calcined clay (Khan, 1992). The amount of solid carrier required for a particular species depends on its water-holding capacity, and was reported from 2 to 0.2 times the weight of the seed (Taylor *et al.*, 1988; Khan, 1992). The amount of water needed to achieve equilibrium water potential conducive for priming is determined on an empirical basis as described for liquid priming. Leondardite shale is a material that regulates water potential by its osmotic potential (Taylor *et al.*, 1988). Diatomaceous silica materials regulate water potential by their matric properties, and their use in priming was termed *matriconditioning* (Khan, 1992). Drum priming was developed in the UK and involves hydration by misting seeds with water during a one- or two-day period in a revolving drum (Gray, 1994). The level of hydration is controlled for each species, and tumbling ensures uniformity of moisture distribution.

The benefits of priming by different techniques were documented for many vegetable seeds and were reviewed by Heydecker and Coolbear (1977), Bradford (1986), Khan (1992) and Parera and Cantliffe (1994). The subcellular basis for priming was reviewed by Varier *et al.* (2010). In general, priming hastens the rate of germination and seedling emergence, especially under suboptimal temperatures for germination. In the case of lettuce, priming ameliorates the deleterious effect of high temperatures causing thermoinhibition and thermodormancy. Priming may predispose the seed to aging, and primed lettuce aged faster than nonprimed seeds, especially those aged with a high relative humidity as used in the saturated salt aging test (Hill *et al.*, 2007).

Coating technologies

Seed coating technologies have evolved with time and are used for a different purposes to improve agricultural productivity and reduce environmental hazards. Early emphasis was placed on the development of coatings for small and irregularly shaped seeds to facilitate precision placement during sowing (Tonkin, 1979). Coatings were later developed to act as a delivery system for a number of materials required at time of sowing (Scott, 1989; Taylor and Harman, 1990). Coating technologies were further refined to reduce worker exposure to seed treatments during handling (Robani, 1994). Halmer (2000) and Taylor (2003b) reviewed seed coating technologies.

Several coating technologies are commercially used on different vegetable crops including seed treatment or dressing, film coating, encrusting, and pelleting (Fig. 1.8). The amount of material applied or weight gain differs for each coating technology (Taylor, 2003b). A conventional liquid treatment or dressing uses a small amount of water to uniformly apply the active ingredient over the seed surface and from seed-to-seed. Generally, less than 1% of the seed weight is applied by this method. The weight increase from film coating ranges from 0.5% to 5.0%. The weight increase varies with vegetable crop seeds, or more importantly seed shape to make a spherical pellet, and ranges from 2 to +50 fold (200 to +5,000% increase). An intermediate or mini pellet is termed *encrusting*, and ranges from 0.2 to 2.0 fold (20 to 200%).

Seed pelleting consists of the application of solid particles that act as a filler with a binder or adhesive to form a more or less spherically shaped dispersal unit (Fig. 1.10).

Pelleting is routinely performed by the seed industry on high-value, small-seeded vegetable seeds. In this process, seeds are generally pelleted on a batch basis in a coating pan or tumbling drum. Pellets are sized during and at the end of the process and then dried. The materials and techniques used are proprietary; however, a number of ingredients were listed in the literature (Halmer, 1988; Scott, 1989; Taylor and Harman, 1990). Seeds are tumbled with repeated applications of the coating filler material followed by intermittent spraying of seeds with water to activate the binder and result in the formation of the pellet around each seed. Encrusting, like pelleting, employs an application of a finely ground, solid particulate material and water or aqueous binder solution.

Film coating of seeds is a more recent development than pelleting and is derived from techniques originally developed for the pharmaceutical industry (Porter and Bruno, 1991). Film coating consists of spraying a solution or suspension of film-forming polymer onto a mass of

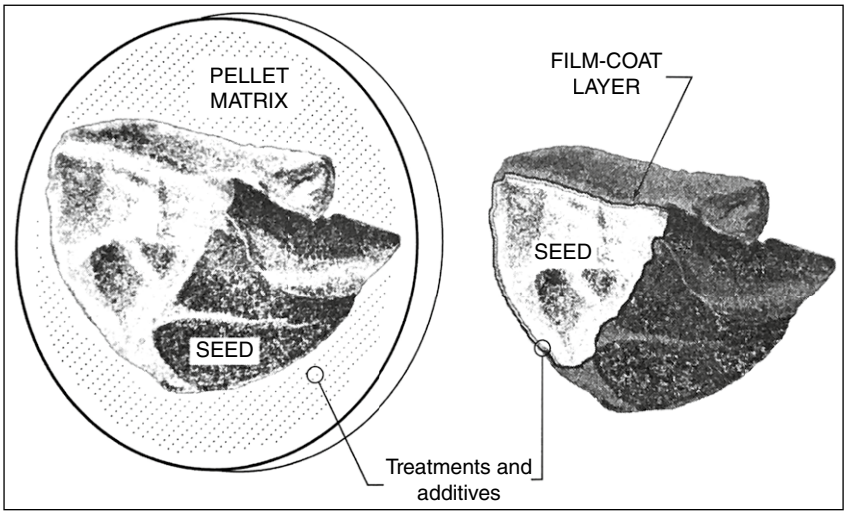

Fig. 1.10. Illustration of a pelleted (left) ad film coated (right) onion seed. Both pellet matrix and film coating layer can serve as a delivery system for active ingredients or other materials. Reprinted with permission from Taylor (2003b).

seeds to achieve a uniform deposition of materials (Fig. 1.10). A number of film-forming polymers and pigments are used (Halmer, 1988; Robani, 1994). Coating pans described for pelleting are used; however, in contrast to the wet operation of pelleting, the aqueous film-forming formulation is dried immediately after spraying to avoid agglomeration. Perforated pans are used to allow for rapid drying, and continuous flow methods were developed (Robani, 1994; Halmer, 2000). The benefits of film coating include uniform placement of seed treatment chemicals onto seeds, essentially a dust-free environment, and enhanced appearance due to the addition of pigments (Robani, 1994).

Seed treatments are categorized into different groups based on their mode of action or properties including plant protection, growth enhancement (Fig. 1.8) or environmental stress reduction. Plant protection by chemical seed treatments is performed on a global scale on vegetable and field crops. Seed treatment insecticide usage has increased dramatically over the past 20 years for below-ground insect pests (Nault *et al.*, 2006), and systemic seed treatment insecticides for above ground insects (Kuhar *et al.*, 2009). Seed treatments eliminated the need for in-furrow treatments, and resulted in a 90% reduction in pesticide usage (Taylor *et al.*, 2001).

Seed treatments are used to eradicate internal seed-borne pathogens, but to be effective the organic compound must first diffuse through the seed coat to the embryo. The physical/chemical nature of systemic compounds was investigated on uptake into the embryo of several vegetable crop seeds (Salanenka and Taylor, 2011). Seed coat permeability to systemic compounds was grouped into three categories: (i) permeable: snap bean; (ii) selective permeable: tomato, pepper and onion; and (iii) non-permeable: cucumber and lettuce. Selective permeable allowed nonionic compounds to diffuse, while ionic compounds were blocked. The semipermeable layer as the innermost layer of the seed coat restricted solute leakage from tomato, pepper, and onion seeds (Beresniewicz *et al.*, 1995b) and are also responsible for blocking ionic (charged) molecules from entering seeds. Information on seed coat permeability is used as a criterion for selecting effective compounds to target pathogens in the embryo.

There is a growing need for organic seed treatments, as synthetic chemical seed treatments are

not permitted for certified organic vegetable production. An organically approved insecticide, Spinosad, was effective in managing below-ground insects in onion (Nault *et al.*, 2006). Selected beneficial fungi and bacteria are effective as biological control agents for management of soil-borne pathogens. The coating environment can be tailored to enhance the biocontrol organism by adjustment of the pH and addition of food bases (Taylor and Harman, 1990). Selected biological treatments can ameliorate abiotic stress in the soil environment. Biological treatments partially negated the environmental stress of soil salinity, and the most effective biological treatments increased the K^+/Na^+ ratio, which was positively correlated with plant growth (Yildirim *et al.*, 2006). Seed treatments can enhance plant growth and serve as *Biostimulants* (du Jardin, 2015). Plant proteins were investigated as biostimulants, and seed coatings containing soy-flour enhanced broccoli seedling and plant growth (Amirkhani *et al.*, 2016). Collectively, film coating, encrusting and pelleting can apply high loading rates of active materials onto seeds compared to conventional liquid treatments (Taylor, 2003b), and the coating technologies are delivery systems for single or multiple active agents that may serve as a protectant, enhancement or stress alleviator.

Concluding Remarks

An understanding of vegetable seeds is an important first step for the subsequent study of vegetable physiology and culture. Vegetable seeds are a diverse group of edible plants with respect to botanical classification, morphology, and composition, and this diversity impedes rapid progress in our understanding of vegetable seed physiology. This diversity, including different market needs and seed costs, also dictates the choice of seed enhancements employed for each crop, variety, and seed lot.

The high value of the harvested vegetable product increases the demand for seeds of high quality and maximum performance. The goal is for each seed sown to develop into a usable transplant or productive plant in the field. To achieve this goal, a seed lot must have complete, uniform and rapid germination and seedling emergence. This goal is seldom achieved due to one or a combination of factors including small seed size, slow germination rate, low inherent seed quality, and sensitivity to environmental stresses at the time of sowing. Unlike most agronomic crops, most vegetable seeds are not directly consumed, and have not been selected for large seed size. In the case of sweet corn—a crop selected for its high sugar content for consumption—the increased sugar content in mature seed can negatively impact seed quality potential and increases susceptibility to seed- and soil-borne pathogens.

To overcome some of the seed quality and slow germination challenges, seed companies routinely conduct seed conditioning to clean and upgrade seed quality. Seed enhancements, such as priming, can have a beneficial effect on seedling emergence of small-seeded vegetable crop seeds under environmental stress, but may accelerate aging and thus decrease seed quality after storage. Therefore, the potential risk/benefit of each seed enhancement requires consideration. Seed coating technologies provide a delivery system of active ingredients and agents required at time of sowing. Seed treatment and coating technologies are used on both small- and large-seeded crops.

Continued effort is needed by seed biologists to develop the knowledge of physiological mechanisms associated with seed aging, and the factors limiting seed performance. Some of these basic studies are performed with vegetable species, and others are adapted from agronomic crops and model plant species. Seed quality criteria should be incorporated into breeding and selection programs in the development of improved cultivars. In conclusion, by integrating several approaches and disciplines, seed quality of vegetable crops can be improved and will ultimately benefit vegetable growers.

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2 Transplanting

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In most vegetable production systems, transplanting with containerized transplants or plugs is the most common practice used to maximize stand establishment, shorten the growing period, and enhance earliness in production. Transplanting also allows better planning to time harvests for specific markets and extension of the harvest season for late-maturing crops. The milestones and foundation of the present-day transplant technology were set up back in 1925 through transplant studies outlined in the doctoral dissertation by W.E. Loomis of Cornell University (Cantliffe, 2009). This was followed by the invention of reusable styrofoam trays with inverted pyramid cells known as Speedling® flats in Florida (Todd, 1972). From then, the plug concept and technology grew up almost exponentially worldwide. Now transplants are grown to specific growers' requirements based on species, cell volume, plant height, and post-transplant environmental conditions, whether in open field or under protected structures. Transplanting is a standard crop establishment method for several vegetable species (Fig. 2.1) including sweet and pungent pepper (*Capsicum annuum* L.), fresh market and processing tomato (*Solanum lycopersicon* L.), eggplant (*Solanum melongena* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), cucumber (*Cucumis sativus*), celery (*Apium graveolens*), squash (*Cucurbita pepo*), globe artichoke (*Cynara cardunculus* var. *scolymus*), lettuce (*Lactuca sativa*), fennel (*Foeniculum vulgare*), leek (*Allium ampeloprasum*), and cruciferous species (*Brassica oleracea*) such as broccoli, cauliflower, cabbage, and Brussels sprouts. In the United States, most large commercial transplant nurseries are located in warmer climatic regions (Florida, Georgia, California, Texas) with less costs of heating and lighting, but more cost of transportation to distant northern markets and more challenges to control high temperatures since most are passively ventilated. Conversely, the main challenges for nurseries in North America are the high cost of heating and lighting. The history of the transplant industry and components of the technology required to produce transplants were earlier reviewed by Styer and Koranski (1997) and Cantliffe (2009). This chapter will update research conducted during the last two decades and expand on factors affecting transplant quality, stress adaptation, physiology, and performance previously contained in the first edition of *The Physiology of Vegetable Crops* edited by Wien (1997). Here additional emphasis is given to transplant stress adaptation, water management, and growth modulation by plant growth regulators and light quality. A brief section introduces grafting as a specialized transplant production method which is growing rapidly worldwide.

Transplant Quality

A high quality containerized vegetable transplant is defined as one that is compact, with thick non-elongated stem, and a well-developed and balanced root and shoot system (Fig. 2.2) that

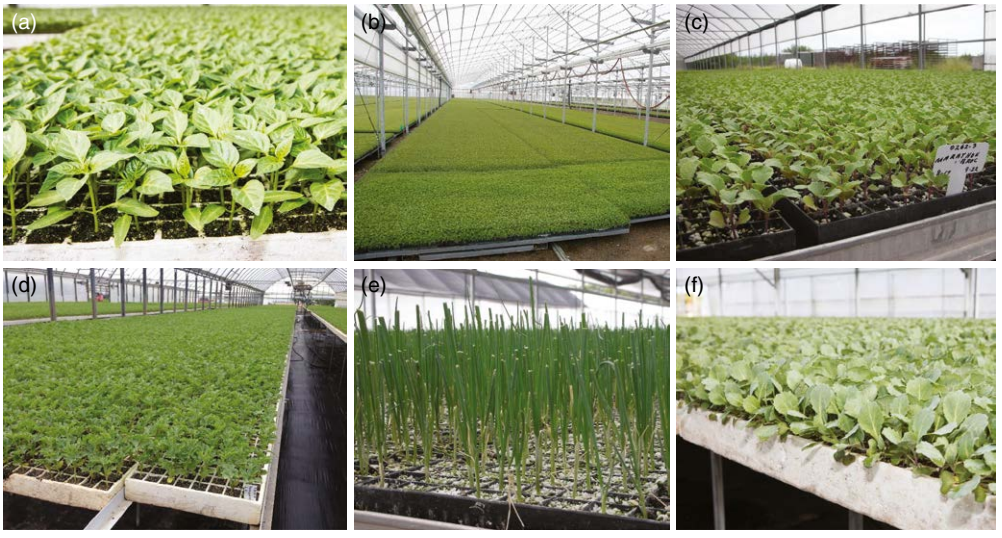

Fig. 2.1. Containerized vegetable transplants: (a) bell pepper, (b) tomato, (c) broccoli, (d) watermelon, (e) leek, and (f) cabbage.

Fig. 2.2. High quality containerized transplants with balanced root and shoot system: (a) watermelon, (b) lettuce, (c) artichoke, and (d) tomato.

has the capacity for a rapid leaf area development and root regeneration upon transplanting under diverse climatic, environmental, and outdoor field conditions. During transplanting, seedlings are pulled from the tray, causing mechanical root pruning and loss of root hairs, disturbing the initial root:shoot morpho-physiological balance. Once in the field, transplants will undergo a short period of field acclimation or "recovery phase" before they can resume shoot growth. Therefore, morphological and physiological adaptation leading to enhanced resource (water, nutrients) capture efficiency by roots is important for successful stand establishment and crop performance. However, despite the initial losses of root tips and root hairs, plants are plastic and able to compensate during the recovery phase with no lasting effect on yield as reported in tomato and lettuce transplants (Bar-Tal *et al.*, 1994; Kerbiriou *et al.*, 2013). The importance of improving root development during the nursery period and to enhance seedling performance in the field has been earlier reviewed by Leskovar and Stoffella (1995). Monitoring moisture and nutrient content, particularly nitrogen of the growing medium, is critically important for producing high quality transplants in nursery operations. Ideally, irrigation management strategies should lead to a gradual growth with minimum stress imposed. Fertigation (simultaneous application of water and nutrients) can also significantly impact growth, development and final transplant quality as reported in several vegetable species, including lettuce (Soundy *et al.*, 2005) and artichoke (Leskovar and Othman, 2016). In the future, for automatic transplanters with vision system technology, leaf area will be a key indicator of transplant quality and necessary to select healthy plants in high- to low-density trays. Tong *et al.* (2013) has evaluated a machine vision system and developed algorithms that segment overlapped leaves from neighboring cells and calculated leaf area with an accuracy greater than 95% in distinguishing "bad" from "good" seedlings of tomato, pepper, eggplant, and cucumber. Ultimately the morphology of vegetable seedlings is species-specific, with unique growth developmental patterns in leaf area expansion, shoot elongation, root development, and time required to reach transplant maturity.

Factors Regulating Growth in Containers

Container cell size

Vegetable transplant nurseries select trays containing from 72 to 800 cells per standard tray size (67 x 34 cm, 0.23 m^2). It is widely accepted that container cell size or volume have a direct effect on root and shoot growth, dry matter partitioning, stand establishment, reproductive development, and early yield. A limited media-cell volume imposes root growth restriction turning on internal root sensing mechanisms that affect plant responses, even under optimum conditions of water, nutrient, and oxygen supply. The underlying morphological responses and physiological mechanisms associated with root volume confinement on seedling growing in close proximity in multi-celled trays is complex, and not a single factor can be isolated due the interaction of crop species and even cultivars, environmental conditions, physical/chemical properties of substrate (media), and management strategies used in nurseries. Controlled studies comparing root-restricted (small cell volume) versus unrestricted tomato and cucumber plants have shown low root respiration rates (Peterson *et al.*, 1991), low leaf net assimilation rates, and greater specific leaf weight (Hameed *et al.*, 1987), and less transport of starch from leaves at night on root-restricted plants (Robbins and Pharr, 1988). The growth rate of vegetable seedlings grown in containers tends to be proportional to cell volume (Marr and Jirak, 1990) with larger cells resulting in bigger and older transplants (Romano *et al.*, 2003). Despite the advantages of a large cell volume, transplant nurseries seek to optimize production space by increasing plants per unit area (NeSmith and Duval, 1998). Multi-celled trays with small individual cell volume (e.g. 2 to 3 cm^3) allow raising transplants in less time at a reduced cost of production due to an increase in space efficiency. However this practice will produce a smaller transplant which may reduce early yield as reported in tomato (Wien, 1997). Research conducted in lettuce transplants grown in cells from 2 to 40 cm^3 showed a two-week harvest delay for transplants grown in 2 cm^3 cell volume (Nicola and Cantliffe, 1996). Conversely, transplants grown in large cell

volume were shown to increase early yields as reported in tomato, pepper, onion, and watermelon (Weston, 1986, 1988; Leskovar and Vavrina, 1999; Graham *et al.*, 2000).

The transplant growth response to container cell size varies with species, and often mixed results on growth, earliness and total yield have been reported (NeSmith and Duval, 1998). Tomato transplants produced in larger cell volumes enhanced earliness and high yield (Knavel, 1965; Liptay *et al.*, 1981). Muskmelons grown in larger cell volumes produced greater early yields but similar total marketable yield, fruit weight, and number, when compared to those produced in small cell volume (Walters *et al.*, 2005). Similarly, in bell pepper, early yield was proportional to cell volume, while total yields were unaffected by cell size (Weston, 1988). In this species it is known that cell volume affects root growth (De Grazia *et al.*, 2002) and that root restriction has been associated with early flowering (NeSmith *et al.*, 1992). Therefore it is recommended to transplant peppers before flowering if early yield is expected, otherwise it might result in reduced vegetative growth rate and final yield (Nicklow and Minges, 1962).

Similarly, in asparagus containerized transplants increase shoot, root, and total dry weight in response to large cell volume, with an increase in the number of shoots once established in the field (Nicola and Basoccu, 2000). In this species, the combination of deep cells (10 cm) and large cell volume (186 cm^3) improved shoot and root growth, respectively (Dufault and Waters Jr, 1984; Nicola and Basoccu, 2000). In allium species, manipulating transplant size during the nursery stage could optimize the time of bulbing and final bulb size. For example, small leek transplants should onset earlier bulbing and high quality bulbs at favorable light and temperature conditions; whereas bigger transplants should mature later and increase bulb size (Gray and Steckel, 1993). Onion transplants are typically grown in trays with small cell volume, which can also accommodate multiple seeds per cell if a nursery target is to maximize space and reduce cost per plant. For example, Leskovar *et al.* (2004) evaluated a production system using one, two, and three onion seedlings per cell (228 cells, 10 cm^3) in the tray, and concluded that two and three seeds per cell were the best when targeting small to medium bulb size (50–70 mm diameter category), which is the preferred size for Brazilian markets. Conversely a recent study conducted in Texas concluded that transplants produced from one plant per cell (392 cell tray, 14 cm^3) were best to obtain jumbo and colossal bulb sizes as compared to transplants produced from three plants per cell (Macias-Leon and Leskovar, 2019). In another study with non-pungent jalapeno cultivars, transplants produced with two plants per cell had a 25% higher yield compared to those with one plant per cell (Russo, 2003).

Transplant age

Extending the seedling growing time at the nursery will produce a larger transplant size especially when using a large cell volume. There is a direct correlation between cell volume, transplant age, and final size, and ultimately the overall transplant quality. Early field studies comparing growth and yield responses of tomato transplants differing in age showed that early yield increased linearly from three to five weeks, but decreased for six-week-old when grown in sandy soils under Florida conditions (Leskovar *et al.*, 1991). Conversely, 21-day-old tomato transplants (two-true leaf stage) grown in 200 cell tray (24 cm^3) had higher early yield than 35- and 46-day-old plants when transplanted in sawdust bags and grown hydroponically in a temperature control tunnel (Maboko and Du Plooy, 2014). In onions, an early study by Herison *et al.* (1993) reported that 10–12-week-old transplants growing in 4.6 cm^3 cells produced higher yields of bulbs ≥ 76 mm diameter. In another onion study, use of younger 8–10-week-old transplants grown in small cell volume (4 cm^3) was suggested as a viable method of stand establishment, but survival or bulb size could be reduced under stress conditions when compared to 10–12-week-old transplants grown in large cell volume (7.1 cm^3) (Leskovar and Vavrina, 1999). In cucurbit species, early production is often important in determining profitability. In muskmelons grown in 102 and 281 cm^3 cell volumes, leaf number, plant height, and leaf area increased linearly as transplant age increased from one to four-weeks-old; however best early yield and total season yield were reported for two-week-old transplants grown in 281 and 102 cm^3, respectively

(Walters *et al*., 2005). In summer squash, NeSmith (1993) suggested a target age of 21 days. In endive (*Cichorium endivia* L.), best growth and development was reported with 30–35-day-old transplants grown in 128 cells (40 cm^3) as compared to 42 days-old grown in 200 (16 cm^3) or 288 (12 cm^3) cells (Reghin *et al*., 2007). It is important to note that young transplants typically have smaller root systems, and when pulling plugs they may lose media resulting in an increase of transplant shock during establishment. Another important consideration for nurseries is the length of time transplants can be held in containers without negative effects on subsequent growth and yield. Under adverse weather and field conditions at the time of transplanting, vegetable growers often delay plantings, reducing the potential growth and yield due to extreme root growth restriction, decrease in the relative growth rates, excessive shoot growth with spindly stems and overall reduced transplant quality. In summer squash, a 21-day-old transplant may have at least a 10-day window of flexibility before additional aging negatively affects yield (NeSmith, 1993). In broccoli transplants grown in 21–24 cm^3, holding the plants for 14 days beyond the optimal planting date differentially affected root growth, shoot growth, head quality, and reduced yields by 18% (Damato and Trotta, 2000). Conversely, when lettuce transplants were grown in organic peat blocks and transplanted in the field as over-developed seedlings (seven- to nine-leaf stage, with roots emerged out of the block and mechanically removed at transplanting) or under-developed (three-leaf stage with no visible roots emerged out of the block except for the taproot), the latter resulted in slower growth with smaller plants that matured later, while the former adapted rapidly to restore the initial shoot:root ratio without impacting the final yield (Kerbiriou *et al*., 2013). Growers need an ideal transplant size to minimize damage during shipping and transplanting operations and to enable a successful establishment in the field. Recent research addressing growth holding agents to increase the marketability of transplants explored the potential of plant growth regulators, such as ABA and uniconazole, applied at transplant maturity to transiently suppress growth of bell pepper and tomato transplants (Agehara and Leskovar, 2015, 2017). Details of those studies will be presented later in the section "Control of transplant size."

Irrigation management

Water management is one of the most intensive activities in a transplant production system. Adjusting irrigation and fertilization inputs during the growing season has a direct effect on the morphology, growth pattern, assimilate partitioning, and physiological conditioning of seedlings, and ultimately the final transplant quality and crop performance after field transplanting. Irrigation frequency is more critical when growing transplants in small cell root volume (10 cm^3 or less), requiring three or more times of irrigation per day to remain turgid, especially on sunny and hot days. Most of the current information available on irrigation is limited for vegetable transplants. Four factors to consider for proper irrigation management include water quality, water availability, irrigation scheduling, and irrigation methods.

Water quality

The quality of the irrigation water in the root medium has a direct impact on the pH of the growing media and the nutrient availability. Properties that determine water quality for transplant irrigation are: alkalinity, electrical conductivity (EC), sodium absorption ratio (SAR), and elemental toxicities.

Water alkalinity should range from 40 to 80 mg L^{-1} (ppm) in order to maintain the pH stability of the root medium; otherwise if levels are greater than 80 ppm it can cause an increase in pH (Styer and Koranski, 1997). Critical levels of alkalinity depend on the time required for transplant development, cell size, and crop tolerance limit. When using alkaline water, cumulative quantity of carbonates and bicarbonates increases over time causing an upsurge in pH. The management of alkalinity in the growing media is challenging due to the small cell volume used for vegetable transplants. Generally, irrigation water with pH of 5 to 7 is desirable for producing healthy transplants (Boyhan and Granberry, 2010). Crops sensitive to high pH conditions are also less tolerant to water alkalinity. If the pH starts rising, it should be managed using acidifying fertilizers containing ammonium (NH_4) up to 20% or neutralizing acids added to the irrigation water such as nitric, phosphoric, sulfuric, and citric acid (Biernbaum and Versluys, 1998).

Electrical conductivity is the measure of all soluble salts present in the irrigation water. The upper acceptable EC limit for young transplants is 0.75 dS m^{-1} (Nelson, 2003), however values depend on the species. For fertigation, a typical range of EC used for vegetable seedlings may be between 1.5 and 2.5 dS m^{-1}. Excess application of fertilizers or high level of salts in the irrigation water leads to a buildup of salts in the root medium, limiting water uptake through osmotic effects. As salt concentration in the root medium exceeds the solute concentration in the root cells, water stops moving into roots and plants may start wilting even though enough water is present in the root medium. Sodium absorption ratio (SAR) of the irrigation water is defined as the ratio of the sodium (Na^+) to the combination of calcium (Ca^{+2}) and magnesium (Mg^{+2}) ions. High concentration of Na^+ replaces Ca^{+2} and Mg^{+2} ions which are responsible for stable aggregates. The mechanism is that Na^+ disperses soil particles, leading to a destruction of air-filled pores, reducing infiltration and causing the media to hold more water, and consequently oxygen concentration is reduced, which in turn results in poor root growth. The SAR of the irrigation water should be less than 2.0 and sodium ion concentration should be less than 40 ppm (Styer, 1996). Higher values of SAR and/or sodium limit the availability of calcium and magnesium. Certain elements generally found in irrigation water can be phytotoxic at very low concentration. Boron and fluoride are the two most common elements found in excessive amounts when they reach levels greater than 0.5 and 0.75 ppm, respectively. Excess of calcium (40–100 ppm) and magnesium (30–50 ppm) with carbonates may also cause plugging of the spray nozzles and may settle as residue on transplant leaves (Biernbaum and Versluys, 1998).

Water availability in transplants

In containerized transplants, the available water is more important than the total water retention capacity of the root medium. Available water is the amount of water a transplant can extract between the time an irrigation event ends (representing the total retention capacity of the medium in the transplant cells) and the time of the next irrigation (before wilting starts). Container capacity (comparable to field capacity) is the total amount of water present in the container after excess amount of water has been drained after saturation. Seedlings require optimum levels of oxygen and water to be maintained throughout the growing period (Boyhan and Granberry, 2010); however, balancing aeration and water retention is challenging. In an ideal root medium, 90% of the total cell volume should be occupied by pore space, with 65 and 25% of this pore space occupied by water and air, respectively (de Boodt and Verdonck, 1972). Factors affecting air, water and solid particle distribution in the container medium are porosity of the root media, particle size, cell volume and cell depth.

Total pore space present in a root medium is directly influenced by the bulk density of the medium. There is an inverse relationship between the pore space and bulk density; for example in soilless root media, 7 to 15% is solid space and 93 to 85% of the container volume is occupied by pore space, respectively (Argo, 1998). Pore space in the root media comprises two types of pores, capillary (< 3 mm) and non-capillary (> 3 mm). Capillary pores retain most of the available water after drainage, whereas, non-capillary pores hold very small amounts of water as a thin lining of water along the edges of the pores, which are important for providing aeration to the roots. Particle size of the media also influences porosity of the medium and indirectly the ratio of air and water present in the medium after drainage. If the particle size is very small (< 0.01 mm), capillary diameter also becomes too narrow which increases the water tension to such a level that it becomes unavailable to transplant roots. Further, smaller particles come so close that water films adhering to the surfaces unite and leave no space for gas exchange. As a result roots do not get sufficient oxygen for respiration and the carbon dioxide produced may remain in the medium and consequently slow down root growth.

Particle size and pore diameter determines the force by which the water is held by the media particles (moisture tension expressed in positive numbers). The proportion of the stored water in the root medium that is easily available to the plants is generally held at 1 to 5 kPa. The proportion of water held at 5 to 10 kPa is termed as water buffering capacity (de Boodt and Verdonck, 1972), while the water held at more

than 30 kPa moisture tension is unavailable to the plants (Milks *et al*., 1989). Cell depth and volume characteristics also affect the proportion of air and water in the root zone of containerized vegetable transplants. For example, coarse media in deeper cells may improve aeration but create a problem of reduced water holding capacity. Generally, the plug volume ranges from 2 cm^3 (800 plug tray) to 25 cm^3 (128 plug tray). The depth of a plug cell has also an impact on air porosity, since deeper cells have a large percentage of air porosity (Fonteno, 1989). As the height of the water column increases, there is an increase in gravitational drainage. In shallower cells, the gravitational pull is limited by adhesive forces of the water being held in the medium.

Irrigation scheduling

In the nursery, automatic seeding is done in trays filled with pre-moistened growth media, which are then kept in a germination chamber at high relative humidity and above 21°C. The medium should be moist enough to allow uniform radicle emergence of the seeds, but preventing excess moisture that reduces the oxygen levels in the growing media. For example, seedless or triploid (3x) watermelon seeds are hard to germinate under high moisture conditions, while seeded or diploids (2x) watermelons are less sensitive to high or low moisture levels (Grange *et al*., 2000). After radical emergence, watering is decreased to promote root growth, with irrigation applied when 50 to 75% of the available water has been lost. Under hot and dry weather conditions transplants are usually irrigated two to three times a day or even more when using small cell volumes. Irrigation is scheduled during the early part of the day so that foliage becomes dry before night, otherwise remaining moisture on the leaves may encourage the development of diseases. Growers learn by experience to schedule irrigation depending upon container size, type of media, growth stage, and prevailing weather conditions. One strategy used in the nursery to harden off and increase tolerance to drought stress is to gradually reduce medium moisture levels as the seedlings advance towards maturity. This practice is commonly used to increase the survival rate and reduce transplant shock after field transplanting, especially when harsh field conditions are expected.

Irrigation methods

Vegetable transplants are irrigated with either overhead irrigation or subirrigation (floatation) through the ebb and flow system. Above overhead irrigation, also known as boom watering or rail system, delivers water to particular areas or sides of the greenhouse, while keeping others dry. The plug trays are supported in benches by a T-rail system or suspended by a set of wire railings (Fig. 2.3). Due to simplicity and cost, it is the predominant irrigation method used by the vegetable industry (Leskovar, 1998). This system, if properly maintained, applies water and fertilizer uniformly, and saves labor. In addition, it can better control salt accumulation in the container because excess water can leach out salts and prevent buildup in the growing medium; however, nutrient deficiency and low water use efficiency are still concerns.

The subirrigation system was developed by Speedling Inc. (Todd, 1988) as an alternative to overhead irrigation, and has been used initially to grow bell pepper and tomato transplants in Florida and California (Fig. 2.4). It utilizes recycled stored or collected water, and generally uses less water, fertilizer, and pesticide as compared with the traditional overhead systems. In subirrigation water moves up through capillarity action until plugs are saturated. The major advantage is in disease control as it keeps leaves dry, and in the reduction of potential groundwater contaminants. A report indicates an 85% reduction in water use, 50% in fertilizer use, and 50 to 60% in pesticide use (Thomas, 1992) as compared to overhead irrigation. However, the subirrigation system could increase water-borne diseases such as *Phytophthora* sp. and *Pythium* sp., thus requiring chlorination of the recycled water/nutrient solution with chlorine rate up to 20 $mg{\cdot}L^{-1}$ as reported for the production of five-week-old tomato transplants (Saha *et al*., 2011). Subirrigation proved to be an effective system to produce uniform and high-quality transplants in several species. For example, tomato transplants grown with subirrigation had a better root system with more lateral roots and higher root:shoot ratio than those grown with overhead irrigation (Leskovar *et al*., 1994); while subirrigated pepper transplants had more laterals but less basal roots than those grown under the overhead system (Leskovar and

Fig. 2.3. Overhead irrigation system for greenhouse vegetable transplant production. Watering nozzle arms attached to a semi-automatic assembly are suspended on trellis in the center of the greenhouse. Production system with a T-rail benches (top) and wire railings (bottom) for tomato transplants.

Cantliffe, 1993). Similarly, jalapeño pepper transplants grown with subirrigation maintained a uniform lateral root development and promoted hardiness (Leskovar and Heineman, 1994). Muskmelon transplants fertigated through a subirrigation system had better adaptation to hot and dry weather conditions, showing a trend of increased yield when compared with those fertigated through overhead irrigation (Franco and Leskovar, 2002). In lettuce, subirrigation promoted basal root number, and leaf, root, and total dry mass, resulting in improved transplant quality as compared to those grown under overhead irrigation (Nicola *et al*., 2004). A most recent study comparing overhead and subirrigation systems in artichoke transplants growth did not reveal benefits for the subirrigation sytem (Leskovar and Othman, 2016). Noteworthy is

Fig. 2.4. Subirrigation (ebb-and-flow) system for greenhouse vegetable transplant production in Speedling, Florida. Celery transplant trays are suspended on metal wire frames 20 cm above the concrete ground level.

that subirrigation may promote root growth beyond the bottom of the trays, potentially requiring root pruning, a practice that is not practical (Biernbaum and Versluys, 1998) or if in excess, can be detrimental during the transplant recovery after field transplanting, as earlier reported in tomato by Leskovar and Cantliffe (1992). In spite of so many advantages, the subirrigation system failed to gain extensive popularity commercially (Cantliffe and Soundy, 2000) possibly due to the complexity in the design, operation of the system and higher cost as compared to the simplicity, including complete automation of the overhead irrigation system (Leskovar, 1998; Liu *et al.*, 2012).

Control of Transplant Size

In containerized transplants, root confinement is the major factor regulating the transplant size, through interactive and complex processes. Techniques aimed at modifying transplant root and shoot morphology and physiology have been developed in the nursery to control or suppress plant height or stem elongation, enhance plant compactness or robustness, and condition or "harden" transplants to better adapt to the post-transplanting stress. Practical methods of keeping plants short and stocky before transplanting have been the subject of numerous research papers published between the mid-60s to mid-90s and nicely reviewed in the first edition of this book (Wien, 1997). These include mechanical stress, water stress, nutrient conditioning, transplant pruning, and manipulating day–night temperature. Another stage to control or maintain growth is when transplants have reached the ideal final size. This is important for nurseries to increase the marketing ability over a longer period and to minimize the damage during shipping and field transplanting in order to enable a successful establishment in the field (Agehara and Leskovar, 2015, 2017). However,

transplants can quickly outgrow the optimal size, producing overmature plants with spindly stems and excessive leaf growth, whereas their root growth is limited because of the small rooting volume of high-density plug trays (Marr and Jirak, 1990; Nishizawa and Saito, 1998). Such transplants are susceptible not only to damage during shipping and transplanting (Shaw, 1993; Garner and Björkman, 1996) but also to wind lodging in the field (Latimer and Mitchell, 1988; Garner and Björkman, 1999). The following section will discuss manipulation strategies to control transplant growth (size) by plant growth regulators, water, nutrition and light quality.

Plant growth regulators

In the last decade, plant growth regulators (PGRs) or biorational products have become potential tools to reduce or eliminate the risk of stressing young seedlings to the point of physiological injury as observed with some water or nutrient stress techniques. A main challenge when applying exogenous natural or synthetic compounds is the lasting response of the treatment, either short and rapidly reversible, or long and slowly reversible. Special attention should be given to synthetic growth retardants such as uniconazole (currently the only one registered in the United States for use in vegetable transplants) acting as gibberellin inhibitor with potent suppressive growth effects. Most synthetic PGRs such as daminozide, paclobutrazol, and uniconazole are commonly used in ornamental plug production to improve plant compactness, marketable value, and shelf life (Gibson and Whipker, 2001, 2003; Blanchard and Runkle, 2008; Currey *et al.*, 2012). Early work has demonstrated the beneficial effects of foliar applied abscisic acid (ABA) acting as a physiological antitranspirant, through the regulation of leaf transpiration by decreasing stomatal conductance in pepper (Berkowitz and Rabin, 1988; Leskovar and Cantliffe, 1992; Goreta *et al.*, 2007). In muskmelon transplants, exogenous ABA improved the maintenance of leaf water potential and relative water content, while reducing leaf electrolyte leakage (Agehara and Leskovar, 2012). The authors concluded that stress mitigation was also attributed to the ABA-induction of stomata closure. Since then, foliar applications of ABA have gained interest in the chemical industry as a method to control growth. However, the efficacy of ABA or other PGRs in general is not consistent since growth and physiological responses are dependent on multiple factors such as cell size, volume applied, time and frequency of applications, stage of development, environmental conditions of the nursery, crops and even cultivars. For example, the response to control pepper seedling growth during development was age-dependent, with a more effective growth suppression when ABA was applied at the cotyledon stage (Biai *et al.*, 2011; Agehara and Leskovar, 2014a, 2014b). In tomato, paclobutrazol reduced the internode length by 30% when applied to four-week-old seedlings as compared to 18% reduction when applied to six-week-old seedlings (Dikshit *et al.*, 2004). In recent studies ABA and uniconazole were evaluated as growth holding agents to prolong the marketability of mature bell pepper and tomato transplants. The study in bell pepper concluded that ABA applied at 3.8 mM (1000 mg L^{-1}) 3 days before maturity was most effective as holding agent with a reversible growth response 7 days after maturity, while that of uniconazole applied at 34 μM lasted until 16 days (Agehara and Leskovar, 2015). In tomato transplants the growth suppression by ABA was maximal when applied between 5 to 7 days, indicating the age-dependent sensitivity of tomato seedlings to exogenous ABA, while uniconazole produced a more compact plant with a 17% reduction in stem length (Agehara and Leskovar, 2017).

A more recent study with a pre-plant treatment of 1-MCP (1-methylcyclopropene), an inhibitor of ethylene action, has shown the effectiveness in suppressing ethylene-induced stress responses in tomato seedlings, accelerating the post-planting growth of tomato transplants and improving fruit yield by up to 25% (Agehara, Florida, 2017 personal communication). However, the response of tomato transplants to 1-MCP application appears to be cultivar and environment dependent, since 1-MCP improved shoot growth components across seven tomato cultivars at 30/20°C day/night; but only two cultivars exhibited positive responses at stress temperatures of 34/24°C (Leskovar and Othman, 2018).

Manipulating water and nutrition

Adequate irrigation and levels of N, P, and K are necessary for uniform growth and improved transplant quality in containers, thus both irrigation and fertilization, particularly N, are the most important strategies affecting the relative growth rates, and root/shoot dry matter partitioning in vegetable transplants. The frequency and level of irrigation and fertilization depends on the production season, with significant variances in the fall and winter versus spring and summer (Vavrina *et al.*, 1998). A review over a 58-year period on transplant nutrition, particularly N, has been nicely described by Dufault (1998). About 40% of the papers recommended N rates in the order of 300-400 mg L^{-1}, while 10% recommended N rates less than 100 mg L^{-1}. However, with improvements in root media components (e.g. peat, vermiculite), most nurseries currently use lower N (as well as P and K) levels applied at moderate to high frequencies as fertigation. In tomato transplants grown in the fall and spring season over a N range of 0 to 75 mg L^{-1}, plant size increased with 75 mg L^{-1} but total yield was best at 15–45 mg L^{-1} N in the fall and 45–75 mg L^{-1} in the spring (Vavrina *et al.*, 1998). A study conducted in iceberg lettuce transplants fertigated via a floatation system with 0 to 60 mg L^{-1} N, showed an increase in shoot:root ratio in response to N (Soundy and Cantliffe, 2001), resulting in bigger plants and larger head size at harvest when grown with 60 mg L^{-1} N. A following study in lettuce grown in a floatation irrigation system under Florida conditions suggested that best root growth necessary to achieve the highest pulling success rate from the trays, and subsequent yield and head quality, were obtained with a fertigation using N at 60 or 90 mg L^{-1} and applied two to three days apart (Soundy *et al.*, 2005). If transplants are over-irrigated or over-fertigated with increased levels of N they will produce long shoots which upon transplanting may tend to fall over the plastic mulch, resulting in scorching of leaves, broken shoots and losses in plant stands (Cantliffe and Soundy, 2000). Increasing the level of P and K in the transplant has led to less marked increases in plant growth as compared to N. Research in lettuce has demonstrated the need to apply P during production, but 15 mg was enough to obtain high quality and compact transplants, increasing shoot and root components (Table 2.1), pulling success, maturity, and field head weight (Soundy *et al.*, 2001a) (Table 2.1). Similarly, melon and tomato transplants showed little response to increasing K levels from 10 to 250 mg L^{-1} (Dufault, 1986; Melton and Dufault, 1991). This was also confirmed in lettuce grown with floatation irrigation, where K applied at 15 to 60 mg L^{-1} 2 or 4 days apart did not influence shoot and root growth components (Soundy *et al.*, 2001b). The authors concluded that pre-transplant K is not necessary if the peat+vermiculite media mix contains a minimum of 24 mg L^{-1} of extractable K.

To regulate the growth rate of transplants by nutrient management, especially N, opposite approaches have been advocated. The first is to keep transplants with a steady and slow growth during development using low nutrient levels, and then increasing just before transplanting. Under this scheme, it takes longer to produce a marketable transplant size with possible plant size regulation problems if field conditions are

Table 2.1. Shoot and root characteristics of lettuce transplants as affected by phosphorus (P) and nitrogen (N) nutrition 28 days after sowing (adapted from Soundy *et al.*, 2001a; by permission of ASHS).

Phosphorus applied ($mg \cdot L^{-1}$)	Leaf area (cm^2)		Dry shoot wt (mg)		Dry root wt (mg)	Root length (cm)	Root area (cm^2)	Root diameter (mm)
	N_1[z]	N_2	N_1[z]	N_2				
0	4.2	4.2	12.7	12.1	8.6	94	8.4	0.28
15	48.4	68.1	82.1	104.7	24.5	282	26.7	0.30
30	50.3	70.6	83.5	105.6	23.9	276	26.0	0.30
60	55.0	69.9	82.8	104.4	24.4	306	29.5	0.31
90	49.0	70.6	81.0	101.6	25.4	292	27.0	0.29
Response	Q**[y]	Q**	Q**	Q**	Q**	Q**	Q**	Q**

[z]N_1 = 60 $mg \cdot L^{-1}$; N_2 = 100 $mg \cdot L^{-1}$. [y]Q = quadratic; **Significant at 1% levels.

not optimum or ready for transplanting. The second and most common approach is to provide adequate nutrient levels during early growth, and then reduce the supply before transplanting. This practice promotes transplant "hardening," but caution should be noted since low levels of N can result in a small plant size, with significantly lower growth rates after field setting, delaying harvest and even reducing yields (Widders, 1989; Garton and Widders, 1990; Garton, 1992). A modification of the last approach was proposed by Dufault and Schultheis (1994) who developed the concept of pretransplant nutrient conditioning (PNC) in muskmelon and tomato. Here, N levels can be as high as 400 mg L^{-1} and then reduced to "harden off" the plants just before the transplants are ready for field planting. While the application of PNC in watermelon improved early but not total yield or fruit size (Graham *et al.*, 2000), these authors did not advocate this concept.

Light quality

Light is a nonchemical alternative that affects stem elongation through photoreceptors (protein pigments) such as phytochrome that sense red light (Pr) and far-red light (Pfr). During daytime, the phytochrome receptors are in the physiologically active Pfr form, resulting in the down regulation of elongation responsive genes; while at nighttime, the phytochrome receptor Pfr converts to the inactive form Pr, which increases the expression of elongation responsive genes (Nozue *et al.*, 2007; Soy *et al.*, 2012). Seedlings grown in plug trays at high density are exposed to mutual shading from neighboring plants causing a decrease in the ratio of red light (R) to far red light (FR), a shade avoidance response that results in the promotion of stem elongation, reduced leaf area, and branching. The morphological and physiological influences of supplemental artificial light intensity and photoperiod on seedling production have been well documented in winter production of containerized transplants, especially during mid-November to mid-February in northern regions of North America (Dorais and Gosselin, 2002). For example, supplemental light (100 µmol m^{-2} s^{-1} PPFD, 16-h photoperiod) increased shoot dry weight of celery, lettuce, broccoli, and tomato field transplants by 19 to 40%, and root dry weight by 21 to 97% as compared to control light (Masson *et al.*, 1991a, 1991b). Research using photoselective plastics that intercept FR wavelengths has demonstrated reductions in plant height on vegetable transplants. Li *et al.* (2000) evaluated R:FR ratios from 1.1 to 3.7 and obtained a 30% height reduction after four weeks of treatment with R:FR of 2.2. Under a structure with clear film and two FR-light absorbing films, stem elongation of cucumber, tomato and bell pepper transplants was reduced, promoting compactness (Cerny *et al.*, 2004). Similar effects were induced in tomato transplants using FR-light filtering as compared to clear plastic or no plastic (Evans and McMahon, 2004).

In the past, most supplemental artificial light has been provided by fluorescent light (FL) either single or in combination with incandescent light. Thereafter, high-intensity discharge (HID) lamps were introduced and became the standard for supplemental light. The most recent application of supplemental light with light-emitting diodes (LEDs) technology in the red (625–730 nm), green (500–560 nm), and blue (450–495 nm) spectrum is rapidly gaining attention in the nursery industry, especially for indoor closed production systems also known as plant factories. The high efficiency controlled closed system that uses multi-shelves and recycling watering technology was developed in Japan in the 1990s (Kozai, 2007) and expanded rapidly to other regions for growing compact transplants at high plant density in species like tomato, pepper, cucumber, lettuce, and herbs.

Because of the small size and narrow beam angles, LEDs can target light distribution which can be applied with combinations of spectral blends such as red:far-red or red:blue ratios (R:FR, R:B, respectively) to modulate selective photomorphogenic responses in seedlings. For example, pepper seedlings were shorter under a combination of red LED (660 nm) and blue fluorescent light or metal halide lamps (99:1 R:B photon flux ratio) at a PPFD of 300 µmol m^{-2} s^{-1} for 12 h day^{-1} as compared to longer seedlings grown under red or a combination of red and far-red (735 nm) LEDs (Brown *et al.*, 1995). Similarly, tomato seedlings grown under a red:blue or R:B:G (green, 520 nm) at a PPFD of

320 μmol m^{-2} s^{-1} for 12 h day^{-1} showed higher health index values and with shorter hypocotyls than those grown under red LEDs alone (Liu *et al.*, 2011). Leaf area and shoot fresh weight reduction responses were also confirmed in tomato seedlings when using a combination of red, green, and blue LEDs at a PPFD of 160 μmol m^{-2} s^{-1} for 18 h day^{-1} (Wollaeger and Runkle, 2014). A study with six tomato cultivars by Gómez and Mitchell (2015) using three R:B LED treatments (100:0; 95:5; and 80:20; with 627 and 450 nm for red and blue LED, respectively) as supplemental light (5.1 mol m^{-2} day^{-1}) across a daily light integral (DLI) of 0.4–19.1 mol m^{-2} day^{-1} found an increase of leaf area and stem diameter for all cultivars with the addition of blue light. However, plant responses to supplemental light are species- or genotype-specific as found by Liu *et al.* (2011) who reported an increase in dry mass of cherry tomato seedlings when exposed to blue LED as compared to either a combination of red, blue, and green LEDs, or monochromatic red LEDs. In cucumber seedlings, Hernández and Kubota (2016) reported decreases in seedling height and hypocotyl length, and increases in photosynthetic rate and stomatal conductance (Fig. 2.5) when the spectral ratio changed from 100R:0B% to 0R:100B% in phton flux (PF).

In rapeseed transplants, leaves grown under high blue photon flux (i.e. 50R-50B to 0R:100B%) had higher photosynthetic abilty (higher P_{max}) to utilize high photon flux (Chang *et al.*, 2016). Other studies comparing monochromatic LEDs and broad-spectrum white LEDs on lettuce and pepper

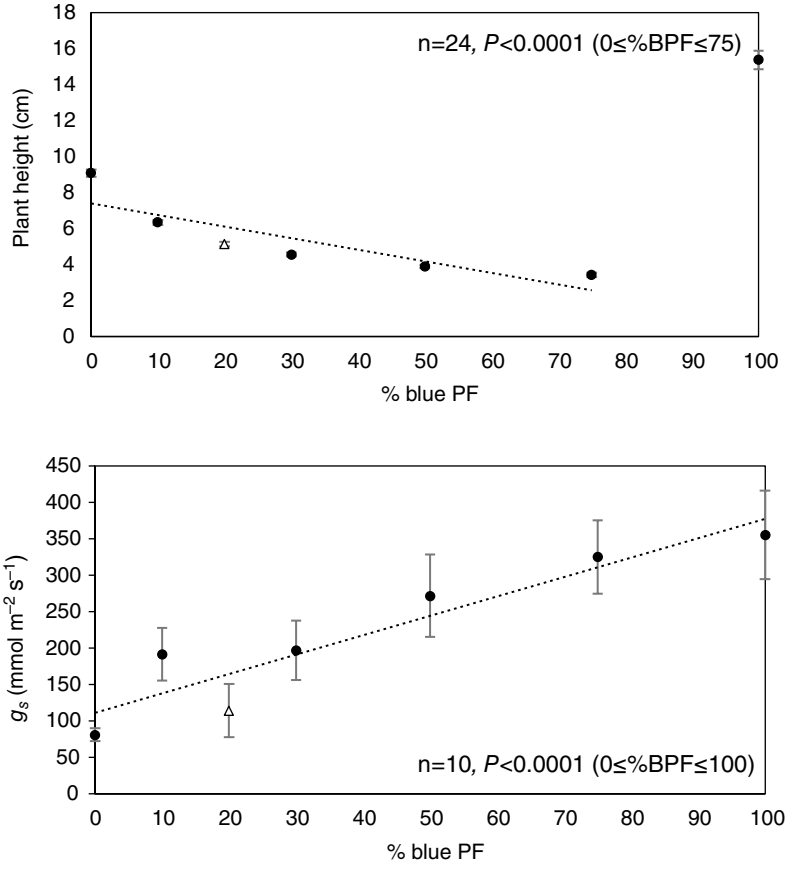

Fig. 2.5. Effect of light quality (increase of % blue photon flux) on plant height (top) and stomatal conductance (g_s) (bottom) responses of cucumber seedlings (Hernández and Kubota, 2016; with permission from Elsevier). Circles represent treatments containing B and R PF. Triangle represents the treatment containing green light (20B:28G:52R). Dotted line represents significant linear regression.

confirmed that blue light (lower photosynthetic quantum efficiency than red light) responses are species-specific and that the photobiological sensitivity changes with plant age (Cope *et al*., 2014). Another factor that can modulate hypocotyl elongation rate is the end-of-day (EOD) lighting with red (reduction) or far-red (promotion) LEDs (Chia and Kubota, 2010) or night break with red LED light. For example, tomato seedlings exposed to 10 min night break treatment with red light at an intensity of 20 μmol m^{-2} s^{-1} every 1 h, exhibited a decrease (32%) in plant height, a decrese in indole-3-acetic acid (IAA) and gibberellin 3 (GA_3) contents in the leaf and stem, an increase in stem diameter, and a delay in flowering, resulting in improvements in early yield (Cao *et al*., 2016). The state of knowledge of LED technology and physiological benefits of spectral combinations of LED lightning for vegetable seedlings and horticultural crop production are thoroughly discussed by Mitchell *et al*. (2015). With the gradual cost reduction of LEDs, it is highly possible that light quality management in vegetable nurseries will become a specialized activity to trigger and increase desirable photomorophogenic (e.g. compactness) and photochemical (e.g. antioxidants) responses to improve transplant quality and post-transplanting stress adaptation.

Grafted Transplants

The successful integration of vegetable grafting into current production practices has opened new opportunities for the vegetable industry worldwide to exploit vigorous rootstocks for effectively managing biotic and abiotic stresses. The science of vegetable grafting started in Japan and Korea in the 1920s. The first commercial use was reported for grafting watermelon onto gourd rootstocks in the 1930s in Japan. Since then the technique has been rapidly expanded into a commercial scale in eggplant in the 1950s (Oda, 1999), and tomato and cucumber in the 1960s (Lee and Oda, 2003), and ever since, its use has been consistently on the rise especially in the cultivation of vegetables in greenhouse and high tunnel production systems (Lee *et al*., 2010). Currently several nurseries in the United States and Canada are involved in vegetable grafting.

Grafting involves merging two independent genotypes to create a new composite plant or "*grafted transplant*" with desired traits from the scion (top) and rootstock (bottom). Because of the nature of a composite plant, the genotypic and phenotypic variability of grafted transplants depends on hydraulic and chemical signals through the xylem (root to shoot) or the phloem (shoot to root) and is due to the rootstock × scion × environment interaction (Venema *et al*., 2017). It is well known that signaling molecules involved in uptake, synthesis, transport, and metabolism can be synthesized in both root and shoot tissues. Superior rootstock traits are disease resistance, improved root vigor and architecture, and efficient nutrient and water uptake; while superior scion traits are taste, flavor, and yield. Due to the large genetic diversity within the Cucurbitaceae and Solanaceae families, and the existence of gene bank collections, breeders can now select potential best rootstocks for specific scions (Belén Pico *et al*., 2017). As a result, vegetable seed companies now offer specialized scion and F_1 hybrid rootstock combinations with desired traits of importance. A database of commercial vegetable rootstocks is available and updated annually in a vegetable grafting portal (USDA, 2016).

The production process and methods used for grafted vegetable transplants have been reviewed in a recent book edited by Colla *et al*. (2017). In short, the grafting method involves four steps: a) selection of compatible rootstock and scion cultivars; b) coordination of production of seedlings of rootstock and scion to graft transplants either manually or mechanically; c) healing of the graft union and recovery of the grafted plant at high humidity, moderate temperature and reduced light; and d) acclimation of grafted transplants (Lee *et al*., 2010) (Fig. 2.6). The most popular grafting methods are: splice, cleft, pin, hole insertion, and tongue. The scion can be cut above or below the cotyledon, making sure there is a match in the stem diameter with the rootstock. However, in tomato, the rootstock is typically cut below the cotyledon (Rivard and Louws, 2011) to avoid shoot regrowth or "suckering" from adventitious axial bud outgrowing the scion (Bausher, 2011). This practice is commonly used in the United States (Plug Connection, CA, personal communication). However, in other countries where

Fig. 2.6. Creating a grafted tomato transplant: (a) a grafting clip is positioned half-way on the rootstock stem cut below the cotyledon node; (b) rootstock and scion stems cut at 45° angle; (c) scion stem is aligned to rootstock with attention to match both stem diameters; (d) variation of grafting clipping and grafted above the cotyledon node; (e) acclimation at high humidity and low light; (f) grafted transplant below the cotyledon node; (g) grafted transplant above the cotyledon node with high root vigor for greenhouse production.

grafted transplants are utilized for greenhouse production (Korea, Spain) the graft is also performed above the cotyledons. A potential problem is the development of adventitious roots from the scion which in production fields could bypass the disease resistance of the rootstock (Meyer *et al.* 2017). Adventitious root formation is linked to the basipetal movement of auxin from the shoot meristem and young leaves to the graft union, which also promotes xylem tissue regeneration (da Costa *et al.*, 2013). A study in tomato suggested that 50% to 90% leaf removal during grafting was effective to reduce adventitious roots, and was suggested as a valuable technique to produce high-quality transplants for small-scale operations (Meyer *et al.*, 2017).

The main purposes for using grafted transplants are to overcome soil-borne diseases such as *Fusarium* wilt *(Fusarium oxysporum* f. sp. *lycopersici)*, bacterial wilt (*Ralstonia solanacearum), Verticillium* wilt *(Verticillium dahliae)* and root-knot nematode (*Meloidogyne* spp.) (Louws *et al.*, 2010), and to increase plant tolerance to abiotic stresses such as salinity (Colla *et al.*, 2010), heavy metals (Savvas *et al.*, 2010), cold (Venema *et al.*, 2008), lack or excess of water (Nilsen *et al.*, 2014), and organic pollutants (Schwarz *et al.*, 2010). Enhancement in water and nutrient use efficiency, as well as increase in yields in comparison with non-grafted plants have generally been reported across various environments (Djidonou *et al.*, 2013). Grafted transplants are five to ten-fold more expensive than ungrafted transplants, therefore a challenge for nurseries is to consistently produce uniform quality and safely transport to growers to use them successfully based on their local soil and environmental conditions. On-farm and high-tunnel economic research studies provided strong evidence of positive returns of tomato grafting under some conditions (Rivard and Louws, 2011).

Storage and Transport of Transplants

In the United States, most containerized large commercial vegetable nurseries are located in the southern states of California, Georgia, Florida, and Texas. Others that specialize in grafted tomato transplants are in Canada, California, and North Carolina. Local shipments near nurseries (200 km or less) transport transplants in the growing returnable trays (Cantliffe, 2009). This is in contrast to growers in northern, southern, and central states which often purchase transplants that are pulled and packed in boxes and shipped by truck from distant nurseries (200 to 2,000 km) from two to four days, risking deterioration in transplant quality such as depletion of starch reserves and stem elongation, and thus causing potential delays in stand establishment, and subsequent growth and yield. This is more pronounced when shipment occurs at a combination of ambient temperature and darkness. In an early study with young tomato transplants, the interaction of storage time (up to 8 days) and storage temperature (5 and 15°C) significantly affected transplant quality and yield (Leskovar *et al.*, 1991). Low temperatures and dim light (1–5 μmol m^{-2} s^{-1} PPF) have been reported to extend the storability of vegetable transplants (Kubota, 2003). During storage of six days at 10°C in darkness, less starch depletion and leaf water potential was reported for cabbage transplants that were preconditioned with water stress by withholding water for one day before storage (Sato *et al.*, 2004). A follow-up study simulated transportation air temperatures (6, 13, and conventional 19°C) combined with two light intensities (darkness or 12 μmol m^{-2} s^{-1} PPF) and evaluated tomato transplants with visible flower trusses over a four-day period (Kubota and Kroggel, 2006). The study concluded that 6 to 13°C temperatures and illumination significantly maintained the photosynthetic ability and overall transplant quality, while seedlings at 19°C were of poor quality, showing significant stem elongation in darkness (Fig. 2.7) and later on had flower abortion, delay fruiting of the first truss, and reduced yield. Under high transport temperature of 18°C, it has been suggested that ethylene accumulates in the containers, and is the main cause in delaying fruit development (Kubota and Kroggel, 2011). That study also showed that the ethylene inhibitor 1-MCP (1-methylcyclopropene) applied prior to 18°C transport temperature was as effective in reducing the percentage of the first tomato truss showing a delay in fruit development as lowering the transport temperature to 12°C.

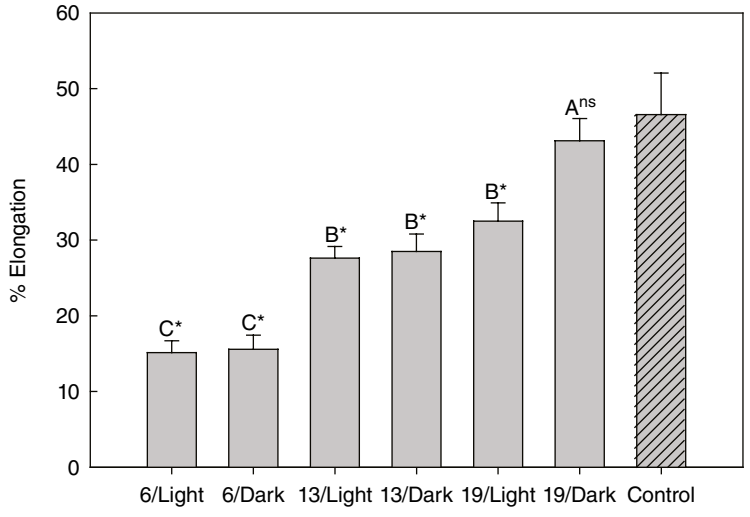

Fig. 2.7. Effects of air temperature (6, 13, and 19°C) and light intensities (darkness and 12 µmol m^{-2} s^{-1} PPFD) on stem elongation (% increase) during simulated transportation of tomato seedlings. Means with the same letter are not significantly different by Turkey HSD test at $P < 0.05$. Means with a single asterisk (*) are significantly different from the nontreated control by Dunnett's test at $P < 0.05$. (Kubota and Kroggel, 2006; with permission by ASHS).

Field Conditions

Transplant shock

After transplanting, plants are exposed to biotic and abiotic stresses that may reduce the root absorption area which is important for successful stand establishment. Until proper soil-root contact is established, transplants often experience transient drought/heat stress even under well-watered conditions. This sudden and severe water deficit results in "transplant shock" (Nitzsche *et al.*, 1991) mostly through the imbalance between transpiration demand and water uptake capacity. Transplant shock is very common in vegetable crops grown in semi-arid regions of the United States, where high air temperatures and drought stress typically delay root and shoot growth, with significant reductions in marketable yield. Production management strategies such as water and N deprivation during the final stages of growth, application of foliar antitranspirants and PGRs, or exposure to outdoor conditions for three to five days, have been used to pre-condition transplants or as "hardening" to increase field stress tolerance and reduce transplant shock. Exposing transplants to direct sunlight and wind conditions induces thickening of the leaf cuticle and thus can prevent excessive loss of moisture. Hardening also varies with the irrigation system used. For example, transplant hardening was obtained by growing them with the subirrigation system, which promoted a higher root:shoot ratio as reported for pepper (Leskovar and Heineman, 1994), tomato (Leskovar *et al.*, 1994), muskmelon (Franco and Leskovar, 2002) and lettuce (Nicola *et al.*, 2004). Most techniques that control growth, increase compactness, and reduce excessive stem elongation (described previously) will promote hardening and stress tolerance. Comprehensive studies on mechanical stress (brushing) have been published (Latimer, 1990, 1991; Latimer and Beverly, 1993) and reviewed (Wien, 1997; Cantliffe, 2009) and will not be repeated here. In the following section, conditioning methods with PGRs, irrigation, nitrogen, and cold temperatures to mitigate drought, heat, and chilling stress will be presented.

PGR conditioning for drought and heat stress

Unbalanced plant water status caused by heat, drought, and wind stresses may lead to sudden

and severe water deficits resulting in temporal wilting, leaf yellowing, burning, abscission, and/or apical de-topping (Nitzsche *et al.*, 1991; Leskovar, 1998). Transplants can perceive environmental stresses and use this information to modulate their regular patterns of growth and development. Altered responses to abiotic stresses are referred to as acclimation or hardening (Bruce *et al.*, 2007). To mitigate transplant shock and avoid wilting in the field, the application of PGRs and compounds named "anti-transpirants" has been investigated as a way to reduce stomatal conductance and prevent transpirational losses (Laurie *et al.*, 1994; Plaut *et al.*, 2004), and thus improve plant water status and turgidity. Early work in pepper by Leskovar and Cantliffe (1992), showed the efficacy of foliar ABA applied at 0.1 mM 28, 32 and 37day after seeding as an alternative for drought stress to control transplant growth in the nursery. Similar results were reported in tomato when using ABA analogs, especially as root dip applications (Sharma *et al.*, 2005a, 2005b). In those studies, tomato transplants improved their tolerance to transplant shock by reducing wilting through the maintenance of higher leaf water content. Leskovar *et al.* (2008) compared the effect of ABA, amioethoxyvinylglycine (AVG) and some commercial antitranspirants in mitigating transplanting shock on globe artichoke, tomato, and peppers. Artichoke is a species highly sensitive to heat and drought stress which cause mortality and lower stands. When ABA was foliarly applied at 1000 mg L^{-1} it provided drought tolerance, maintaining shoot water status via stomatal closure and improved leaf gas exchange during a dehydration period (Table 2.2) Conversely, film-forming antitranspirants were not effective to mitigate drought stress (Shinohara and Leskovar, 2014).

A dehydration-recovery study on pepper demonstrated the capacity of ABA applied at low concentration (0.1 mM) during a five-day exposure time to condition transplants through morpho-physiological responses (Goreta Ban *et al.*, 2017). ABA enabled the maintenance of water potential, reduced shoot growth, promoted lateral root branching, and developed adequate leaf anatomy for gas exchange. The authors suggested that ABA induced a "stress imprint" stimulus in plants facilitating a fast and protective response to recurrent stressful events.

Other molecules or signal hormone-like substances such as salicylic acid (SA) and jasmonic acid (JA) have been shown to have important roles in the regulation of seedling growth under water and drought stress. In bean and tomato plants exposed to stresses, SA modulated the membrane redox balance, mitigating the negative effect of reactive oxygen species (ROS) which are known to cause lipid peroxidation in membranes (Senaratna *et al.*, 2000; Yang *et al.*, 2004). Cucumber seedlings pre-treated with SA (0.50 mM) as seed soaking or as foliar prior to exposing them to a 14-day drought stress

Table 2.2. Relative water content, electrolyte leakage, water potential, photosynthetic rate, and leaf stomatal conductance of artichoke transplants in response to antitranspirant foliar application after four days of dehydration (Shinohara and Leskovar, 2014; with permission from Elsevier).

Antitranspirants[1]	Relative water content (%)	Electrolyte leakage (%)	Water potential (MPa)	Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mol \cdot m^{-2} \cdot s^{-1}$)
Control	41.3 b	31.0 ab	—3.00 b	0.7 b	0.01 b
ABA	83.9 a	11.1 b	—0.75 a	3.0 a	0.05 a
Antistress	41.3 b	44.1 a	—2.96 b	ND[2]	ND
Transfilm	41.5 b	31.1 ab	—2.64 b	0.79 b	0.02 b
Vapor Gard	36.8 b	42.8 ab	—3.38 b	ND	ND

Relative water content, electrolyte leakage, leaf water potential, photosynthetic rate and stomatal conductance at day of transplanting were 80.7, 8.6, 0.48, 13.0 and 2.30, respectively.

[1] Antistress (a.i. acrylic polymers) at 2.2%; Transfilm (a.i. polymeric terpenes and oxidized polyethylene) at 3.8%; Vapor Gard (a.i. di-1-p-menthene) at 2.2%.

[2] ND: data not detectable.

Means within columns followed by different letters are significantly different (LSD test, $p = 0.05$).

period, had improved physiological (chlorophyll content, chlorophyll fluorescence ratio) and growth parameters (shoot and root weights), increasing proline content in shoots and preventing increases in electrolyte leakage (Baninasab, 2010). Benzimidazole, an N-containing phenol (such as in the product Ambiol, Toronto, ON) is considered a plant growth regulator. When Ambiol was applied to tomato seeds at 10 mg L^{-1}, and seedling roots were then exposed to water stress, it significantly improved leaf area, shoot and root mass, and leaf photosynthesis (MacDonald *et al.*, 2010). Other authors hypothesized that Ambiol is involved in phytohormonal changes, since when applied to potato it decreased ABA concentrations by 50% while it increased IAA fivefold and zeatin threefold (Kirillova *et al.*, 2003).

Irrigation and N conditioning

In order to reduce the transplant shock period, nursery growers adjust irrigation strategies (frequency and time of irrigation) during development with typically less irrigations in the last week of the production cycle. Transient water stress has been used to control shoot growth and increased compactness in vegetable transplants in the greenhouse, a practice that alters dry-matter partitioning, seedling water status, and stomatal behavior (Liptay *et al.*, 1981; Berkowitz and Rabin, 1988; Leskovar and Cantliffe, 1992; Leskovar and Heineman, 1994). In muskmelon (*Cucumis melo* L.), it has been shown that preconditioning transplants with effective N supply using floatation irrigation in the nursery can modify root growth (Liptay and Nicholls, 1993), providing adaptive mechanisms to withstand microclimate shock in the field (Franco and Leskovar, 2002). Earlier work in jalapeño pepper transplants, supported the benefits of floatation fertigation in maintaining a uniform lateral root development and promoting transplant hardiness (Leskovar and Heineman, 1994). A study in globe artichoke addressed the impact of pre-transplant management of N and fertigation system on transplant quality and subsequent growth and physiology (Leskovar and Othman, 2016). Transplants fertilized with 75 mg·L^{-1} N (low N) had improved root components compared to those with 150 mg·L^{-1} N (high N). Low N promoted root surface area, root length, root branching and thinner roots, and less shoot area than high N. In addition, transplants in the low N regime were shorter and more compact, resulting in seedlings more tolerant to early field stresses.

Transplant conditioning for chilling stress

Chilling stress, particularly in chilling-sensitive species such as solanums or cucurbits, occurs when plants are exposed to suboptimal temperatures, typically night temperatures below 10°C and above 0°C for several hours. Chilled plants begin to lose turgor of the stem and leaves causing wilting, followed by death and drying out of the leaf tissue (Rikin *et al.*, 1976). Wilting and desiccation are exacerbated by wind causing severe stem damage on flaccid seedlings that may be twisted around repeatedly until the stem is severed. The underlying physiological processes occurring during chilling stress involves a reduction of water flow through roots, impairment of stomatal control and decrease in plant water status (Wien, 1997). Chilling injury can be further intensified under short exposures of direct sunlight causing photo-oxidation and the generation of superoxide anion radicals (Wise and Naylor, 1987; Peeler and Naylor, 1988). Exposing transplants to a period of non-freezing temperatures is known to cause cold acclimation, a process that involves morphological, physiological, biochemical, and gene expression changes. Some reported responses include: growth inhibition, increase in leaf thickness, increase in concentrations of cryoprotective osmolytes such as proline and carbohydrates; production of phytohormones ABA and brassinosteroids; putrescine, a free radical scavenger (Kagale *et al.*, 2007) and root 2,3,5-triphenyltetrazolium chloride (TTC)-reducing activity (Kagale *et al.*, 2007; Cuevas *et al.*, 2008; Jiang *et al.*, 2012). However, cold stress acclimation by exposing watermelon transplants to 2°C from 3 to 81 h was negative to vegetative and reproductive growth, reducing yield by 10% (Korkmaz and Dufault, 2001). In broccoli, when four- to eight-week-old transplants were kept at 2 °C for one or two weeks, there was a variable response in the antioxidant enzymes,

with an increase in peroxidase (POD) activity which was maintained throughout the growing period until harvest, while the increase of catalase (CAT) and phenolics were only observed at the transplant stage (Długosz-Grochowska *et al.*, 2012). That study showed no direct correlation on how CAT and POD functions in terms of their affinity and removal of hydrogen peroxide.

There is increased evidence that high levels of signal transducer molecules such as ABA, SA, JA, polyamines, and calcium can protect plants against chilling (Klessig and Malamy, 1994; Korkmaz, 2005). For example, in a chilling sensitive tomato genotype, two applications of exogenous ABA (1 mM), putrescine (0.1 mM), or 2,4-epibrassinolide (0.02 µM) when transplants reached three fully expanded leaves and five days later, provided cold tolerance by reducing the decline of photosynthesis and chlorophyll, and increasing proline, soluble sugar content, and root TTC-reducing activity (Jiang *et al.*, 2012). A three-year study in Poland (Kalisz *et al.*, 2015) evaluated chilling stress acclimation and the lasting "stress imprint" effect on young broccoli transplants subjected to 6, 10 and 14 °C for 7 and 14 days before planting. Broccoli seedlings recovered after chilling treatments, and two months after planting all treatments exhibited an increase in fresh weight as compared to the non-chilled 18°C control. Pokluda *et al.* (2016) evaluated coriander (*Coriandrum sativum*) transplants exposed to 6°C (chilling stress) or 18°C (control) following two foliar biostimulant treatments applied 44 to 51 days after seeding. Biostimulants positively increased stress biomarkers in chilled plants, such as transpiration, stomatal conductance and maximum quantum yield of PSII as compared to non-chilled transplants. In cabbage seedlings, freezing tolerance has been provided by simultaneous exposure of plants to water stress and low temperature (5°C) for seven days, which induced an increase in sugar accumulation (Sasaki *et al.*, 1998).

Concluding Remarks and Prospects

For the past 40 years, the use of containerized transplants has transformed the vegetable industry worldwide. Two main drivers for the fast increase in the volume of transplants produced presently are attributed to the switch from open pollinated to hybrid cultivars and to the development of innovative plug tray systems (Cantliffe, 2009). Growing high-quality transplants will continue to require a thorough knowledge of the seed quality factors affecting germination and emergence under diverse nursery conditions and a broad understanding of the physiological processes underlying transplant growth and morphology in a "root-confinement" environment and subsequent growth and development after field transplanting, especially in stressful environmental conditions. Seed enhancement technologies such as coating and priming with growth promoting agents have proven effective to increase the speed and synchrony of emergence and seedling vigor in numerous species used by the transplant industry. Similarly, nurseries are becoming highly experienced in the use of multi-cell trays with different cell volumes that are appropriate and cost effective for each crop species. However, despite the significant technological advances made by the seed and transplant industries during the last two decades, vegetable nurseries will continue experimenting and adapting major growing factors (irrigation and fertilization) affecting the rate of seedling development (root growth and transplant height) especially for the newly developed hybrids and novel crops. Dramatic genetic improvements have been achieved through conventional and modern breeding and now hybrid cultivars present "target" traits that may have the potential to modify the phenotype through improvements in resource use capture (water, nutrient, light), and indirectly modify their stress tolerance mechanism. These advances will create challenges and opportunities to nurseries to design specialized methods to enhance traits aimed at producing high quality transplants. The transient control of seedling growth will remain the main task for nurseries. Research on the application of single PGRs or bioactive compounds that have the capacity to modulate seedling growth has not been consistent and practical due to complex interactions with the crop species, developmental age and environmental conditions. Future research

combining the knowledge of signal transducer molecules and their synergy with nutrients such as N, and other non-chemical methods such as light control with species-specific spectral blends appear attractive for their effectiveness in the control of seedling elongation and stress acclimation to withstand transplant shock after field establishment.

A transformational practice that is gaining fast popularity in the United States is the use of grafted transplants, especially in solanaceous and watermelon crops. Despite the higher cost, up to ten-fold compared to non-grafted transplants, grafted seedlings provide growers alternative production systems to effectively manage soil-borne diseases and abiotic stresses such as temperature, salinity, and drought. Grafting has also been shown to enhance fruit yields and quality under protected culture and conventional and organic open field environments. Grafting combinations can be intraspecific (rootstock and scion belonging to the same botanical species) or interspecific (rootstock and scion belonging to different species of the same genus). Because there are multiple rootstock options to select, it is critical to expand research and nursery applications to better understand management factors affecting root and shoot growth (such as canopy vigor) of commercially available scion/rootstock combinations, or when using landraces and wild relatives known for their stress adaptive traits.

Finally, the current vegetable transplant production system in nurseries and field establishment in fields are very costly and high-labor intensive operations (Fig. 2.8), therefore automation is the key driver in the near future. Despite advances in mechanization for the plug technology in ornamental species and robotic systems used in the grafting technology as in Japan and Korea), automation of the transplant production system and mechanization of small-cell transplants continues to lag behind for vegetable nurseries and growers. In the future, the development of partial to full integrated robotic systems that control fertigation and light quality, and automatic field transplanters with vision system technology will be expected for the most demanded and expensive crops such as tomato, pepper, and watermelon. However, in addition to economic considerations and labor, the unique developmental pattern of each crop species and cultivar, and time to reach the target maturity will remain challenges. Very recently an automatic system (Fig. 2.9) has been developed in Spain and acquired by Tanimura & Antle in 2014 for commercialization in the United Staes (PlantTape®; http://www.planttape.com/). The system is completely integrated from sowing seeds into a tape, to germination, nursey growing and field transplanting, and has been used to harvest more than 1000 acres with broccoli, iceberg, and romaine lettuce in California in 2016. PlantTape® appears very attractive for large-scale growers using less growing materials, faster speed and more flexibility to plant at various stages of transplant development, which has important implications for planting schedules. However, this production system requires a complete change in components such as trays, greenhouse seeders, and transplanting, therefore small to medium growers may not afford to buy the entire system. In terms of transplant quality, this system ultimately will modify and potentially improve root and shoot growth balance (including taproot) as compared to growing in traditional trays imposing a more root-restricted environment. This new technology is an exciting opportunity for a variety of crops and transplant size and may constitute a new paradigm shift in transplanting.

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Fig. 2.8. Mechanical and manual transplanting operations: (a) tomato in California, and (b) lettuce in Spain.

Fig. 2.9. Integrated PlantTape® System: (a) lettuce transplants growing in a tape; (b) automatic discharge; (c) transplanting with multi-row capacity; and (d) field establishment.

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3 Regulation of Flowering in Crop Plants

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The transition from vegetative to reproductive growth is a key developmental decision in the plant life cycle. Plants have evolved sophisticated mechanisms and pathways to flower within a narrow seasonal window every year to maximize their reproductive success, and similarly the yields of grain crops depend upon optimizing flowering time to the climate. Plants are particularly reliant on day length (photoperiod) and temperature as seasonal indicators, and they integrate this information with endogenous signals such as hormones, carbohydrate status and age. In this way, plants are able to control flowering time to maximize their chances of reproductive success. Wheat, rice and maize account for about two thirds of the energy in human diets, and these crops are themselves the direct result of flowering. Flowering time has therefore been a key trait during domestication that has enabled crops to become adapted for growing in different climates. Furthermore, we shall see that in wheat and barley, for example, the architecture of the spikelet and number of fertile florets – traits that are strongly influenced by the underlying genetic network controlling flowering – play a major role in determining yield. In breeding programmes, the time between generations is a major rate-limiting step in the generation of new varieties, and we shall see how applying an understanding of the molecular pathways controlling flowering can enable the acceleration of breeding programmes.

In addition to its agronomic relevance, flowering time serves as a paradigm for understanding how developmental programmes are controlled in plants, and so the analysis of flowering time in crops contributes to a fundamental understanding of processes relevant to multiple areas of biology from evolution and comparative biology and population genetics through to understanding molecular mechanisms. While much has been learnt about fundamental processes in plant biology in the model system of *Arabidopsis thaliana*, this only represents a small proportion of the diversity of mechanisms and lifestyle strategies that plants have adopted. The elucidation of flowering pathways in more distant monocot crops has been particularly valuable in increasing our understanding of how plant development can evolve – for example, to exploit different cues to trigger flowering. Since humans have been cultivating crop plants for about 15,000 years, there is extensive scope to study which genes have been selected for in the process of domestication and how these have contributed to favourable traits.

While the major influences of temperature and photoperiod on different crops have been appreciated at a phenological level for hundreds of years, it is only recently that the underlying genes controlling the responses of crops to their environment have been identified and their mechanisms begun to be understood. As with many areas of plant science, the exploitation of

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the model plant *Arabidopsis thaliana* has been instrumental in establishing fundamental networks and mechanisms underlying the control of flowering. Recently, the expansion of genomic sequencing and genome editing are enabling progress directly in crops themselves. This is key since, while it is highly tractable, *Arabidopsis* is a model dicot, and so it will always be important to assess how relevant it is to crop plants. Nevertheless, because it is the most well understood plant for studying flowering time, we will begin with a survey of the major concepts that have been found in *Arabidopsis*. We will then examine what is known for key crops including tomato, wheat and barley. Finally, we shall discuss key agronomic challenges and areas of promise.

Arabidopsis

Arabidopsis thaliana is a highly tractable model system having a small genome, excellent genetics and a rapid life cycle, and being easy to transform (Somerville and Koornneef, 2002). This has enabled the discovery of many pathways and mechanisms controlling flowering, many components of which appear to be broadly conserved in crop plants. Pioneering genetic screens by Maarten Koornneef identified sets of mutants that could be attributed to different environmental sensing pathways, and their subsequent cloning revealed insights into the underlying mechanisms. Major pathways influencing the floral transition in *Arabidopsis* include the vernalization, photoperiod and ambient temperature pathways. These pathways converge on signals that activate flowering (Fig. 3.1).

Florigen

The presence of a signalling molecule that triggers flowering was proposed more than 80 years ago, and it was shown that leaves grown under inductive conditions are able to trigger flowering when grafted onto plants grown in non-inductive photoperiods (Kobayashi and Weigel, 2007). FT was identified as a gene necessary for accelerating flowering in *Arabidopsis* that is sufficient to induce very early flowering when overexpressed (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999). FT encodes a small globular protein that is expressed under inductive conditions in leaves, and is transported via the phloem to the shoot apex. At the apex, FT activates floral meristem identity genes that specify floral fate (Abe *et al.*,

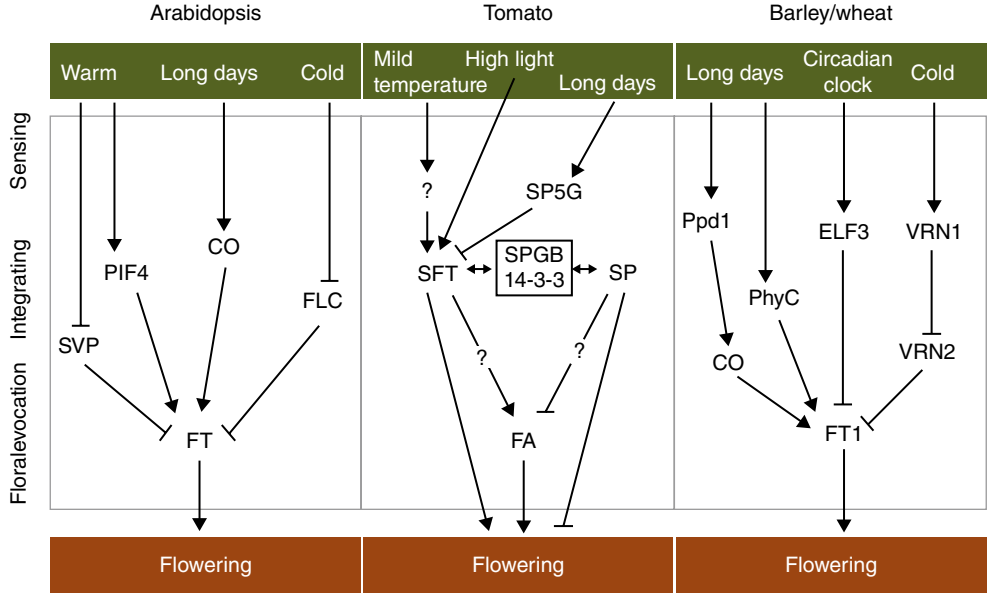

Fig. 3.1. Genetic pathways towards flowering in *Arabidopsis*, tomato and cereals.

2005; Wigge *et al.*, 2005). The identification of FT, which appears to be universally conserved as a floral activator in higher plants, provided a major unifying concept to understand how plants activate flowering. Despite considerable diversity in the cues and signals that activate flowering, with the same cue sometimes having opposite effects on flowering in different species, the regulation of FT-like genes as activators of flowering appears to be universal among the main crop plants studied.

Vernalization

Many *Arabidopsis* natural accessions show a requirement for a period of prolonged cold (typically around six weeks at 4°C) before they become responsive to inductive flowering signals. This enables plants to overwinter as vegetative rosettes, when they are more resistant to cold stress and do not have floral buds (Greenup *et al.*, 2009). This inhibition of flowering is controlled by a MADS-box transcription factor, FLOWERING LOCUS C (FLC), which binds to and represses the expression of key genes required for flowering. Prolonged cold results in the stable silencing of FLC expression via repressive chromatin marks. The sensing mechanism by which cold triggers FLC repression is still not clear. It has been proposed that the protein FRIGIDA (FRI), which is required for FLC expression, is destabilized by cold, and this leads to a reduction in FLC expression (Hu *et al.*, 2014). The FLC locus is also targeted by non-coding RNAs, and the expression of these in response to cold likely plays a role in shutting down FLC expression (Ietswaart *et al.*, 2012). Genetic screens have uncovered a set of genes that are required for efficient silencing of FLC by cold (Whittaker and Dean, 2017). VERNALIZATION INDEPENDENT3 (VIN3) is a plant homeodomain (PHD)-containing transcription factor that is expressed in response to cold and recruited to the FLC locus early on during vernalization. Subsequently, VERNALIZATION5 (VRN5), also a PHD protein, binds the FLC gene body and contributes to its silencing via the recruitment of a repressive polycomb complex. This repression of FLC by epigenetic marks is very stable, and persists until the next generation.

Photoperiod pathway

Arabidopsis is a facultative long-day plant, and long photoperiods (typically 16 hours) accelerate flowering compared to short days (eight to ten hours of light). A number of mutations eliminate the acceleration of flowering in long days, revealing the existence of a pathway to sense long photoperiods. Among the first of the photoperiod regulators identified and cloned was the gene CONSTANS (CO). CO loss of function alleles are insensitive to photoperiod, and flower as late as plants grown in short days. CO encodes a transcription factor with a zinc finger motif that directly binds the promoter of FT and activates its expression (Andrés and Coupland, 2012). The expression of CO peaks about 16 hours after dawn, and CO protein is stable in the light but degraded in the dark. In this way, CO protein accumulates at the end of each long day, enabling the activation of flowering (Searle and Coupland, 2004).

Ambient temperature

Warmer ambient temperatures accelerate flowering in *Arabidopsis*, and at 27°C under short days, plants flower almost as early as plants in long days at 22°C (Balasubramanian *et al.*, 2006). This acceleration of flowering is dependent on the up-regulation of FT gene expression. Multiple mechanisms have been proposed to account for this. Two transcriptional repressors, the MADS box proteins SHORT VEGETATIVE PHASE (SVP) and FLOWERING LOCUS M (FLM) both repress FT expression and it is proposed that warm temperature reduces their activity, enabling a commensurate increase in FT expression (Lee *et al.*, 2013; Posé *et al.*, 2013). A bHLH transcription factor, PHYTOCHROME INTERACTING FACTOR4 (PIF4), is also necessary for increasing FT expression in response to warm temperature (Kumar *et al.*, 2012), and PIF4 activity is increased both transcriptionally and post-translationally in response to warm temperature (Box *et al.*, 2015; Raschke *et al.*, 2015; Jung *et al.*, 2016).

Flowering control in other brassicaceae

A number of important crops are closely related to *Arabidopsis*, including *Brassica rapa*, *Brassica*

oleracea and *Brassica napus*. In these cases, strong conservation at the sequence and functional level can be directly observed for many key regulators such as *FLC*, enabling the relatively direct transfer of knowledge of the flowering pathways from *Arabidopsis* into these important crop species (Blümel *et al.*, 2015).

Tomato

Tomato is a day-neutral plant that was originally cultivated in Central and South America. Tomato has a perennial growth habit and tends to be grown as an annual. Unlike *Arabidopsis*, the vegetative and reproductive phases of tomato alternate regularly along the compound shoots. This is referred to as *sympodial growth*. The primary shoot terminates in an inflorescence after about 6–12 leaves and subsequent shoots form from axillary meristems immediately below the terminal inflorescence. While the flowering of tomato is largely unresponsive to photoperiod, flowering is accelerated when seedlings are grown at lower temperatures (10–15°C) compared to 27°C (Samach and Lotan, 2007). The other environmental variable having a strong effect on flowering time is light intensity, with high light accelerating flowering. Because of the sympodial growth, genetic mutations that perturb the floral induction pathway in tomato have particularly dramatic effects on plant architecture, and mutations in a floral repressor, SELF-PRUNING (SP) were identified early last century and exploited in commercial breeding since they cause premature and synchronized termination of shoots into flowers, facilitating the mechanical harvesting of the crop in one pass. Cloning of SP revealed it to be the homologue of TFL1, and it interacts with an FD-related bZIP transcription factor SPGB (SP associated G-box) and 14-3-3 adapter proteins, suggesting that, as in *Arabidopsis*, SP can antagonize flowering by repressing the targets that are normally activated by the FT-FD complex (Pnueli *et al.*, 2001). Consistent with this, the tomato homolog of FT, SINGLE FLOWER TRUSS (SFT) accelerates having the recessive form of the gene flowering when overexpressed, and *sft* mutants are late flowering. It is believed that the balance of activity between SP and SFT is particularly important in controlling the growth pattern of tomato, and by balancing the reproductive versus vegetative balance it is possible to adjust plant architecture. Little is known about the pathways by which environmental signals such as temperature and light intensity influence flowering in tomato. Likewise, the direct targets of SFT and SP are not known. A likely candidate is the LEAFY homolog FALSIFLORA (FA), and *fa* mutants are significantly late flowering, and when inflorescences do form they are devoid of flowers, but have leaf-like structures instead (Samach and Lotan, 2007).

Although cultivated tomato varieties are largely insensitive to photoperiod, some lines display a residual short-day behaviour. By looking at the closest wild relatives of domesticated tomato, *Solanum pimpinellifolium*, *Solanum cheesmaniae* and *Solanum galapagense*, it was observed that many accessions flowered considerably earlier in SD compared to LD. Subsequent QTL mapping identified two major QTL containing the genes SFT and SELF PRUNING 5G (SP5G, a SP paralog) (Soyk *et al.*, 2017). SP5G is a potent repressor of flowering, and mutations in the promoter are believed to have resulted in plants becoming largely photoperiod insensitive, which is thought to have facilitated the adaptation of these varieties to the longer summers in Europe when introduced in the 16th century. SP5G expression is controlled through the activity of phytochrome signalling, and it has been shown that night break experiments with red light cause a considerable increase in SP5G expression, suggesting that the phytochromes play a role in perceiving day length and transmitting this information to the flowering pathway via SP5G (Cao *et al.*, 2018). While SP and SP5G both control the vegetative to floral transition in different shoot systems, creating double mutants between these two genes results in plants that show dramatically early flowering and an improved harvest index of yield (Soyk *et al.*, 2017). The ability to directly target multiple genes of interest in a given crop background using CRISPR-Cas9 technology illustrates the potential of this approach to directly increase crop productivity.

Wheat and Barley

Wheat and barley are facultative long-day plants, and their ancestral wild progenitors have a vernalization requirement (Gol *et al.*, 2017).

Some varieties have lost the vernalization requirement, and these show spring growth types: flowering in the summer when planted in spring. The loss of the vernalization requirement as well as the selection of early maturity varieties that flower precociously are thought to have been important events in the adaptation of these crops to northern latitudes (Gol *et al.*, 2017). While the photoperiod and vernalization pathways have significant differences compared to what is known in *Arabidopsis*, both these pathways converge on FT-related genes to control flowering (Fig. 3.1).

While the floral transition is rapid and continuous once activated in *Arabidopsis*, the floral transition in wheat and barley is extended and occurs in two distinct phases that can be separated by several weeks (Gol *et al.*, 2017). Both these phases respond somewhat differently to environmental signals, and have different major genes regulating them. Furthermore, yield is a complex outcome of these two stages, depending on the total number of fertile florets that give rise to grain (Fig. 3.2). In the first transition, spikelet initiation occurs on the flanks of the shoot apical meristem and spikelet primordia are specified, marking the transition from vegetative to reproductive identity. During the second transition, floral morphogenesis and emergence (heading) of the spike occurs. This complex system therefore provides considerable plasticity and adaptability of flowering in wheat and barley. Vernalization primarily influences the vegetative to reproductive transition, while the photoperiod pathway has a large effect on the outgrowth of the spike and floret formation, a major determinant of yield. The short branch, or spikelet, has many florets, and if these are fertile they lead to seeds. The number and viability of fertile florets specified during the floral transition is therefore an important determinant of yield.

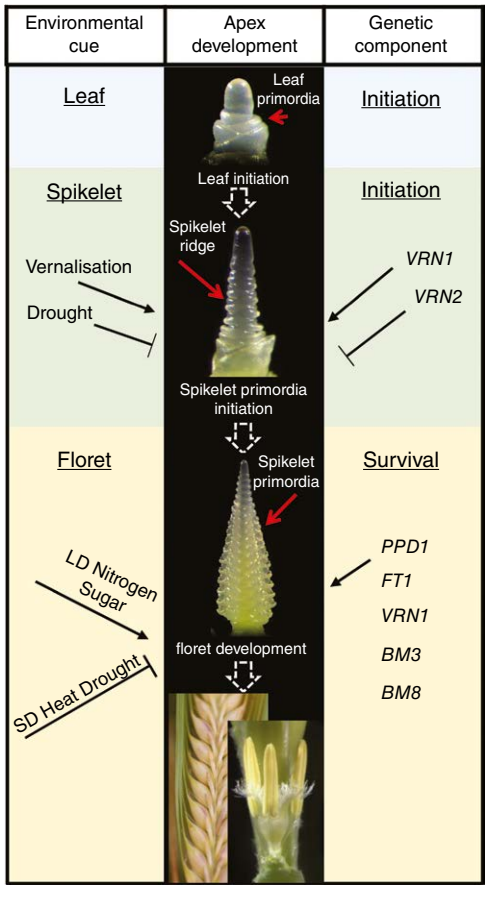

Fig. 3.2. Schematic representation of the development of the shoot apical meristem in response to different environmental cues in barley. The effects of environmental factors on spikelet primordia initiation and floret survival are given on the left-hand side. The effects of major genetic components on the timing of spikelet initiation and on floret survival are indicated on the right-hand side of the diagram (Gol *et al.*, 2017).

Vernalization

Winter wheat and barley have a vernalization requirement controlled by VERNALIZATION2 (VRN2). VRN2 is a strong floral repressor that likely represses gene expression directly as it has a duplicated zinc finger and CCT domain (Yan *et al.*, 2004). Following vernalization, the expression of VRN2 is repressed by VRN1, a gene encoding a MADS box transcription factor related to *AP1* and *FRUITFULL* in *Arabidopsis* (Shimada *et al.*, 2009). VRN1 probably plays an additional role in the floral transition, since as well as repressing VRN2 in the leaf, it is expressed in the shoot apex. VRN2 acts to repress the expression of wheat florigen, encoded by the FT-related gene VRN3. Once the vernalization requirement has been met, VRN3 gene expression becomes responsive to inductive cues such as photoperiod, in a similar way to FT in *Arabidopsis*.

Long days

Long days promote the expression of VRN3, a homologue of *Arabidopsis* FT, and flowering in wheat and barley. The precise molecular mechanism is not clear, but many key genes have been identified through genetic screens and homology approaches. Particularly important is the transcription factor Photoperiod1 (Ppd1), which encodes a pseudoresponse regulator (PRR) related to the clock genes PRR3 and 7 in *Arabidopsis*. The ancestral dominant forms of Ppd1 accelerate flowering, while recessive mutants in barley such as ppd-H1 have been selected in spring cultivars to delay flowering (Digel *et al.*, 2015). Similarly in wheat, a ppd1 mutant series show delayed flowering time (Shaw *et al.*, 2013), while insertions and deletions in Ppd-A1a and Ppd-D1a gene promoters show an acceleration of flowering (Wilhelm *et al.*, 2009; Seki *et al.*, 2013). Expression of Ppd1 is regulated by members of the evening complex: EARLY FLOWERING3 (ELF3) and LUX ARRYTHMO (LUX). Loss of activity of these genes in barley and wheat result in photoperiod-independent early flowering (Faure *et al.*, 2012; Campoli *et al.*, 2013). In both wheat and barley, mutant alleles in phytochromeC (phyC) result in a reduction in Ppd1 expression and delayed flowering (Chen *et al.*, 2014; Pankin *et al.*, 2014).

Maize

Maize, *Zea mays*, was domesticated about 9000 years ago from the wild grass *Teosinte* (Matsuoka *et al.*, 2002). Like many tropical plants, *Teosinte* is a short-day plant. Domestication and adaptation to more temperate climates was facilitated by the acquisition of a day-neutral flowering habit in *Zea mays*. The vegetative shoot meristem stops leaf initiation upon the induction of flowering and switches to a floral fate. The entire meristem is then consumed in formation of the tassel and inflorescence primordium. Maize has a wide range of flowering times from 35–120 days, indicative of the considerable genetic diversity in this trait (Colasanti and Muszynski, 2009). Compared to the other major crops, less is known of the underlying genes controlling flowering. Sequence analysis has revealed maize has a large number of florigen related genes (Danilevskaya *et al.*, 2008), with ZmZCN7 and 8 being the strongest candidates for having the main FT floral promoting role (Lazakis *et al.*, 2011). In short-day tropical maize, the expression of ZmZCN8 is promoted under inductive daylengths, and this FT-related protein is able to interact with the bZIP transcription factor ZmDLF1 that promotes flowering. This is likely under the control of the homolog of CO, ZmCONZ1, which is differentially expressed in response to photoperiods (Miller *et al.*, 2008). In day-neutral plants, it appears that nearly all the floral promoting activity is provided by the autonomous pathway. The key gene in the pathway is indeterminate1 (id1) (Coneva *et al.*, 2007); id1 expression in leaves through an unknown mechanism promotes the flowering pathway via ZmZCN8 and ZmDLF1. While much remains to be discovered, and there are likely more regulators, it is striking that maize shows significant conservation of many of the central components in the flowering pathway such as FT and *FD*.

Other vegetable crops

This review has focused on the major crops where flowering time is most well understood. A major theme emergent from these and other studies is that despite considerable diversity in the balance of environmental signals and their effects on flowering, key components appear to be universally conserved, most prominently FT and related genes. Many of the components have been found in major fruit crops including apple (*Malus* × *domestica*), grapevine (*Vitis vinifera*), lemon (*Citrus* sp.), onion (*Allium cepa*), pepper (*Piper nigrum*) and woodland strawberry (*Fragaria vesca*). Interestingly, FT and related florigen genes are conserved in all these plants (Blümel *et al.*, 2015). As well as flowering time regulation, the flowering pathways have large effects on crop yield. This has been directly demonstrated by gene editing the tomato inflorescence architecture gene *SELF-PRUNING (SP)*, a homolog of the *Arabidopsis* gene *TERMINAL FLOWER1*, which is related to FT. *SP* acts to counteract florigen signalling in tomato, and a mutation in *SP* was a key step in creating bushy tomato plants as loss of *SP* results in the conversion from indeterminate

to determinate growth (Pnueli *et al.*, 1998). By targeting the promoter of SP, it has been possible to create an allelic series for continuous variation in *SP* expression, and therefore architecture-related traits, demonstrating the value of combining genome engineering technologies with fundamental insights derived from model systems (Rodríguez-Leal *et al.*, 2017).

Outlook

As described above, flowering is a key trait enabling plants to adapt to different climates, and influences yield. Studying the floral transition in crops directly provides the opportunity to advance fundamental knowledge of how developmental programmes are regulated in plants as well as the chance to enhance crop resilience to the environment and accelerate plant breeding programmes. This is particularly relevant during a period of anthropogenic climate change, where it has been estimated that crop yields are likely to drop by about 10% for every 1°C increase in mean temperature (Battisti and Naylor, 2009).

Speed breeding

Field grown crops can typically only yield one or two generations per year. By extending the photoperiod to 22 h, with just 2 h of night, it has been possible to achieve up to six generations per year of barley and spring wheat (Watson *et al.*, 2018). This is a considerable acceleration, and offers the opportunity to increase the rate of plant breeding programmes for many crop plants.

Ectopic FT expression to accelerate flowering

The conserved role of FT as florigen makes it an ideal candidate to express ectopically to trigger early flowering. This is particularly relevant in trees, where the juvenile non-flowering phase can take many years, and was first demonstrated in poplar. For example, Trifoliate Orange (*Poncirus trifoliata*) flowers within 12 weeks of transfer to the greenhouse following transformation with FT. Cassava is a key crop where flowering is often the rate-limiting step for breeding as it is often either late and non-synchronous. Ectopic overexpression of the *Arabidopsis thaliana* FT was sufficient to greatly accelerate flowering in cassava. In a related approach in rice, Hd3a, a florigen, was placed under the control of a chemically inducible promoter. In this way, flowering in these rice lines can be triggered upon demand in the field by the application of an agrochemical (Okada *et al.*, 2017).

Effects of climate change on phenology

Plants are remarkably sensitive to temperature, and this is reflected in the observation that wild plants and crops have already shown an altered phenology in response to climate change. For example, the flowering time of wild plants in the UK has advanced in the last few decades (Fitter and Fitter, 2002). The existence of extensive genetic variation in the responsiveness of the flowering pathways to temperature suggests that within a certain range, farmers and breeders may be able to continue selecting lines best adapted to a new climate. For example, there are indications that particular wheat varieties are being grown increasingly further north, and these changes in range are resulting in a corresponding change in seasonal reaction to photoperiod, a major regulator of flowering behaviour. The issue of climate change is particularly complicated by the observation that increased variability will also occur, leading to the proposal that it will be necessary for farmers and breeders to develop a 'climate-smart' food system (Wheeler and Braun, 2013).

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4 Abiotic Stress Effects on Vegetable Crops

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Evidence that global climate is changing, and that this change is accelerating, has become a leading topic in science and international news in recent years. The United Nations' Intergovernmental Panel on Climate Change (IPCC, 2018) has summarized these findings: air and sea temperatures are at present 1°C higher than pre-industrial levels and predicted to rise by 1.5°C by 2030 (Fig. 4.1). The rise in average temperatures has been accompanied by an increasing frequency of heatwaves, and incidence of extreme events such as periods of drought, flooding, and violent storms (Wuebbles *et al.*, 2017). It is to be expected, therefore, that vegetable crops will be increasingly exposed to heat, drought, flooding, and salt stress, and an understanding of the effects of these stresses on these plants will be vitally important. Knowing how abiotic stress can be avoided or overcome by cultural practice and genetic means is the subject of this chapter.

General Stress Effects

The exposure of plants to abiotic stress, whether caused by drought, heat, cold, or high salinity, leads initially to quite similar reactions by the plant. At the cellular level, these stresses produce osmotic stress and cell dehydration (Wang *et al.*, 2003) and oxidative stress, leading to denaturation of functional and structural proteins (Fig. 4.2). The common stress response involves the perception of the stress factors, activation of transcription factors, and the mobilization of stress response mechanisms. Stress perception can occur at the level of the cell among organelles (Zhu, 2016), such as the endoplasmatic reticulum, the chloroplasts, mitochondria, or the cell walls.

The common response to the different stresses is complex in the number of genes that could be involved, but also presents opportunities to create plants that are capable of tolerance to multiple stresses.

Osmotic stress leads to rapid activation of multiple mitogen activated protein (MAP) kinases (Fig. 4.3), although how these produce the physiological actions in response to abiotic stress is not yet clear (Zhu, 2016). Similarly, salt, drought and osmotic stress treatments rapidly activate the SnRK2 family of protein kinases, that are linked to accumulation of abscisic acid (ABA) (Zhu, 2016).

The generation of reactive oxygen species (ROS) such as hydrogen peroxide due to abiotic stress (reviewed by Driedonks *et al.*, 2015) can result in membrane damage, but also lead to formation of transcription factors (Fig. 4.3), which in turn stimulate the formation of ROS scavengers.

Transcription factors such as heat stress transcription factors (HSFs) regulate the expression of stress-responsive genes such as heat shock proteins (HSPs). Transcription factors have been characterized in many plant species, and are activated by other abiotic stresses in

 Physiology of Vegetable Crops 2nd edition (eds H.C. Wien and H. Stützel)

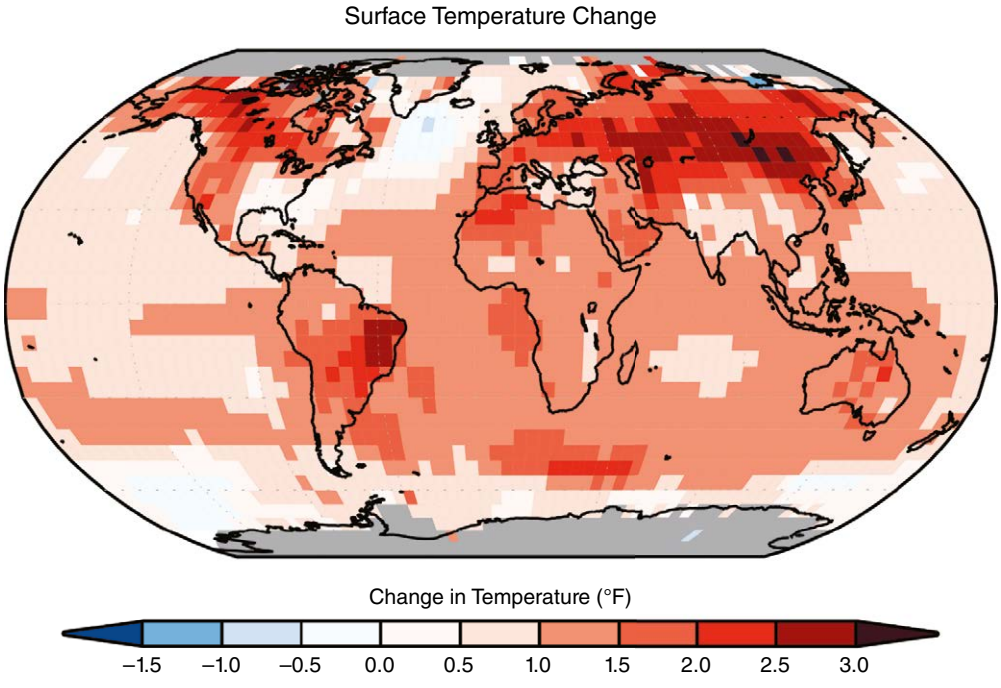

Fig. 4.1. Surface temperature change (in °F) for the period 1986–2015 relative to 1901–1960 from the NOAA National Centers for Environmental Information's (NCEI) surface temperature product. For visual clarity, statistical significance is not depicted on this map. Changes are generally significant (at the 90% level) over most land and ocean areas. Changes are not significant in parts of the North Atlantic Ocean, the South Pacific Ocean, and the southeastern United States. There is insufficient data in the Arctic Ocean and Antarctica for computing long-term changes (those sections are shown in gray because no trend can be derived). The relatively coarse resolution (5.0° × 5.0°) of these maps does not capture the finer details associated with mountains, coastlines, and other small-scale effects (source: Wuebbles *et al*., 2017).

addition to heat (Guo *et al*., 2016). These transcription factors are found in three major classes in crops like tomato, and have both stimulatory and inhibitory roles (Fragkostefanakis *et al*., 2015) in response to heat and other stresses. Overexpression of one HSF in tomato resulted in enhanced high temperature tolerance (Mishra *et al*., 2002), but their specificity to particular species will make common approaches for enhanced stress resistance difficult to achieve.

Heat shock proteins can be separated into six groups according to their molecular weight (Yu *et al*., 2016). The most abundant in vegetable crops like tomato is the HSP20 family, which has a protective function when plants are exposed to abiotic stresses such as heat, drought, or salt stress (Yu *et al*., 2016), preventing the misfolding of proteins exposed to stress. In potato, 48 HSP20 genes were identified, 14 of which were up-regulated by several abiotic stress factors (Zhao *et al*., 2018).

Sensitization to one stress, such as heat, could ready cell mechanisms in the plant so that the impact of another stress following the first one would be lessened in a process called cross-protection or priming (Hossain *et al*., 2018) (Fig. 4.4). For instance, exposure of tomato and pepper transplants to 20 hours at 38˚C hardened them to resist up to six days of 30˚ heat stress (Javanmardi *et al*., 2014). Similarly, exposure of tomato seedlings to mild drought stress or temperature near freezing, or low doses of the herbicide paraquat, hardened the plants to higher levels of the same stresses (Zhou *et al*., 2014). The sensitization treatments elicited production of hydrogen peroxide, and stimulated antioxidant enzymes (Fig. 4.4). When genes providing this cross-protection were silenced, the

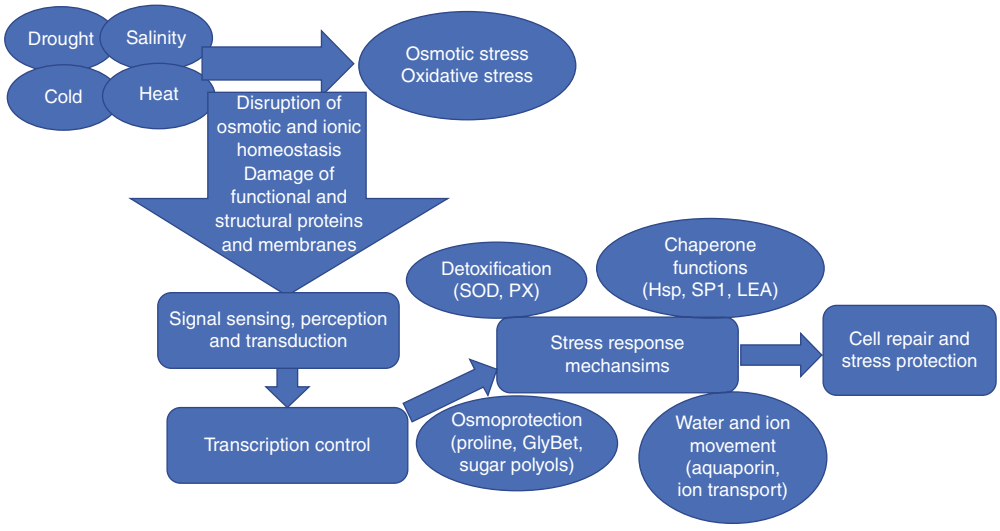

Fig. 4.2. Plant response to abiotic stress. Primary stresses such as drought, salinity, cold, and heat are often interconnected, and cause cellular damage and secondary stresses, such as osmotic and oxidative stress. The initial stress signals trigger the downstream signaling process and transcription controls which activate stress-responsive mechanisms to re-homeostasis and protect and repair damaged proteins and membranes. Inadequate response at one or several steps in signaling and gene activation may ultimately result in irreversible changes of cellular homeostasis and in destruction of functional and structural proteins and membranes, leading to cell death. *GlyBet:* glycinebetaine; *Hsp*: heat shock protein; *LEA*: late embryogenesis abundant protein; *PX*: peroxidase; *SOD*: superoxide dismutase; *SP1*: stable protein 1 (source: Wang *et al*. 2003).

tomato plants were damaged by the stress factors, indicating that the production of hydrogen peroxide and stimulation of antioxidant enzymes played key roles in cross-protection.

The role of exogenous agents and stimulants to generate tolerance to abiotic stresses in plants has become an active area of research and commercial development in recent years (reviewed by Beckers and Conrath, 2007; Calvo *et al.*, 2014). Early work focused on the role of salicylic acid in moderating effects of plant pathogen attack, but also revealed that this compound in low concentrations could lessen the effects of drought, salinity, and cold stress (Miura and Tada, 2014). In support of these findings, processing tomatoes treated with a synthetic source of salicylic acid showed lower levels of blossom end rot and higher yields under moderate drought stress than untreated plants (Giuliani *et al.*, 2015).

Plant biostimulants include microbial inoculants, humic acids, fulvic acids, protein hydrolysates, and seaweed extracts (Calvo *et al.*, 2014). The microbial inoculants may facilitate phosphorus solubilization in the soil, reduce ethylene levels in plant roots and improve drought resistance. Beckers and Conrath (2007) pointed out that priming can also occur between species growing next to each other, through the generation of volatile organic compounds when plants are damaged. A list of plant biostimulants available in Europe was recently published (Le Mire *et al.*, 2016).

Hydrogen sulfide is another compound that is generated at low levels in plant cells (Li *et al.*, 2016) in response to many abiotic stresses. When applied at low concentrations in the form of synthetic S donors, it produces some stress resistance. Melatonin plays a similar role in serving as an antioxidant that scavenges ROS and also reactive nitrogen species (RNS) (Wang *et al.*, 2018). Supporting this role, transgenic plants with higher levels of melatonin had higher total antioxidative capacity.

Plant hormones play a central role in the perception and mitigation of abiotic stress

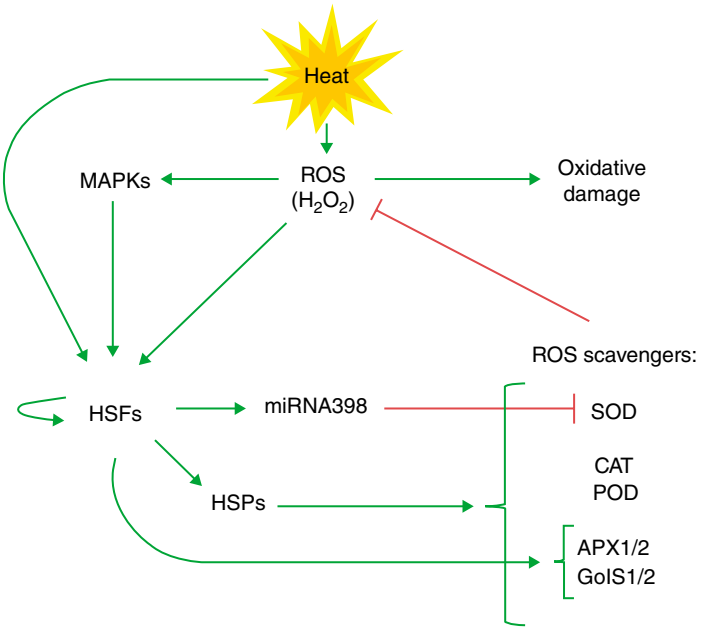

Fig. 4.3. Model describing the connections between heat shock transcription factors (HSFs), the production of heat shock proteins (HSP) and the generation of reactive oxygen species (ROS) in the heat stress response. In addition to directly activating transcription factors, high temperature leads to the accumulation of ROS. This, in turn, leads to a further activation of HSFs, either directly or indirectly via activation of a MAPK (mitogen-activated protein kinases). The HSFs bind to HSE in the promoter region of *HSF*, *HSP*, *miRNA398*, and ROS scavenging genes. The miRNA398-dependent down-regulation of a subset of SOD (superoxide dismutase) scavengers might play a role in the rapid ROS accumulation upon exposure to heat. This would support the activation of HSFs and thereby boost the induction of the heat-stress response in the short term. In the longer term, the induction and stabilization of other scavengers would start to suppress ROS levels to avoid excessive cellular damage (source: Driedonks *et al*. (2015). *APX*: ascorbate peroxidase; *CAT:* catalase; *miRNA398:* microRNA398; *POD*: peroxide dismutase.

(Kohli *et al*., 2013) (see also Chapter 6). When plants are exposed to growth-limiting conditions due to drought or salinity stress, abscisic acid (ABA) and ethylene build up and modulate GA, auxin, and cytokinin action through the action of the repressor protein DELLA (Kohli *et al*., 2013). Salicylic acid and jasmonates are also involved in these hormonal interactions in ways that are so far poorly understood (Verma *et al*., 2016).

Because of the vast array of environmental conditions and plant compounds, both plant-generated and exogenous, contributing to mitigation of the effects of abiotic stress, it is tempting to think that stress effects are easily dealt with. That is of course far from the truth for several reasons. First, stress may affect the plant at various times, often when the plant is particularly sensitive (see below). Secondly, stimulating the plant to develop cross-protection may not be feasible at specific plant stages. Fortunately, priming seedlings to harden them to field conditions before transplanting can be practical (Zhou *et al*., 2014) (see Chapter 2). Thirdly, the metabolic cost of producing defense-related compounds by the plant exposed to abiotic stresses must also be calculated. Few such accounting studies have been made to date, but Hulten *et al*. (2006) have shown that priming *Arabidopsis* against pathogen attack does not slow the plants' relative growth rate compared to untreated plants, but direct induction of plant defenses significantly reduced plant growth.

The other important method of increasing tolerance of plants to abiotic stresses is to alter the genetic steps involved in stress response

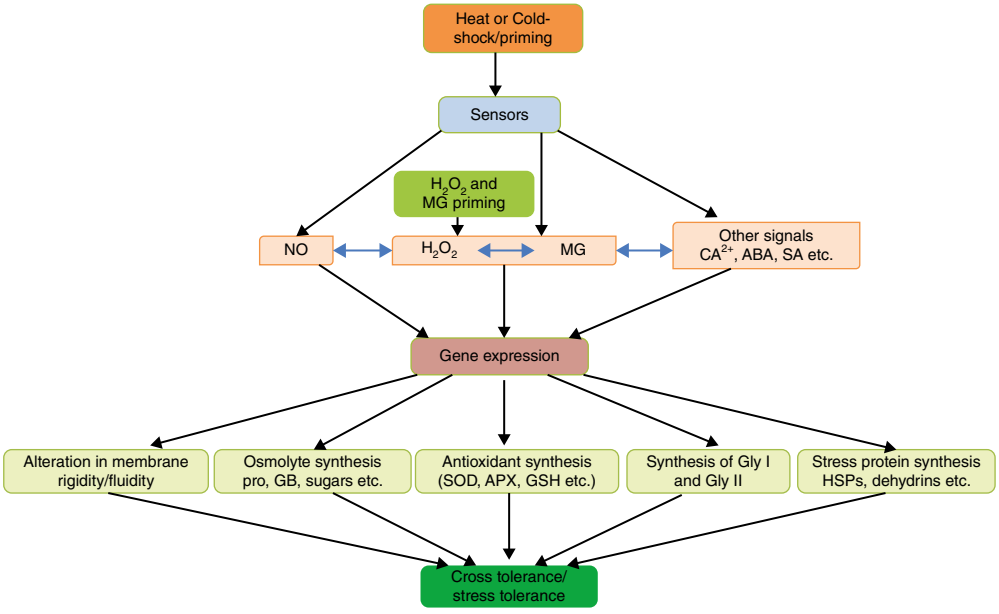

Fig. 4.4. Mechanisms underlying cross-stress tolerance in plants. Priming by heat- or cold-shock or chemical priming (H_2O_2 and MG) directly or indirectly triggers the production of endogenous signaling molecules like H_2O_2, MG, Ca^{2+} and NO. These compounds modulate gene expression and induce the acquisition of cross-tolerance/stress tolerance by modulating membrane fluidity and by stimulating the synthesis of osmolytes (Pro, GB, sugars, etc.), antioxidants (SOD, APX, GSH, etc.) and glyoxalases (Gly I and Gly II), as well as stress proteins (HSP, dehydrins) (modified from Li and Gong, 2011). *GB:* glycine betaine; *GSH*: reduced glutathione; *Gly I:* glyoxalase I; *Gly II:* glyoxalase II; *HSP:* heat-shock protein; *MG:* methylglyoxal; *SOD:* superoxide dismutase; *APX:* ascorbate peroxidase; *Pro:* proline; *NO:* nitric oxide; *ABA:* abscisic acid; *SA:* salicylic acid. Source: Hossain *et al*. (2018).

(Kotak *et al.*, 2007; Sanghera and Sharma, 2011; Nguyen *et al.*, 2018). Although the responses to several stresses involve similar mechanisms, there are many genes involved which are often specific to particular crops. The review by Nguyen *et al*. (2018) provides examples, frequently for *Arabidopsis* or major crop species. Attention has been focused on regulatory genes, and genes involved in regulating the levels of compounds such as antioxidants, protective proteins, and metabolites (Figs 4.3, 4.4). The new, more precise gene editing tools such as CRISPR/Cas9 appear to promise faster progress in developing more stress-tolerant crops (Rodriguez-Leal *et al.*, 2017; Haque *et al.*, 2018).

The severity of the impact of abiotic stresses on individual vegetable crops depends importantly on the life stages at which they are most susceptible to stress. In general, seedlings will be most sensitive to growth inhibition and death, with little capacity to resume normal growth after stress has been relieved. As plants get older, the time when reproductive tissues are being formed is a very sensitive period in vegetable crops that form fruits as the harvested product (see individual chapters on tomato, pepper, the Cucurbits, *Phaseolus,* peas, and sweet corn) (Zinta *et al.*, 2016). For head-forming vegetables, the period of head formation requires stress-free conditions if adequate yields are to be achieved (see chapters on lettuce, the Brassicas). The stress-sensitive period for root-forming and bulb-forming species is generally broader and less tied to a particular period of growth.

Temperature Effects

The many plant species that are eaten as vegetables originated in areas where temperatures are either temperate to tropical, and hence the temperatures at which they grow best also cover a wide range

Table 4.1. Approximate monthly temperatures for best growth and quality of vegetable crops. (source: Maynard and Hochmuth, 2007).

Temperatures,˚C			
Optimum	Minimum	Maximum	Vegetable
13–24	7	29	Chicory, chive, garlic, leek, onion, salsify, scorzonera, shallot
16–18	4	24	Beet, broad bean, broccoli, Brussels sprouts, cabbage, chard, collards, horseradish, kale, kohlrabi, parsnip, radish, rutabaga, sorrel, spinach, turnip
16–18	7	24	Artichoke, cardoon, carrot, cauliflower, celeriac, celery, Chinese cabbage, endive, Florence fennel, lettuce, mustard, parsley, pea, potato
16–21	10	27	Lima bean, snap bean
16–24	10	35	Sweet corn, cowpea, New Zealand spinach
18–24	10	32	Chayote, pumpkin, squash
18–24	16	32	Cucumber, cantaloupe
21–24	18	27	Sweet pepper, tomato
21–29	18	35	Eggplant, hot pepper, okra, roselle, sweet potato

(Table 4.1). Thus, optimum temperatures for radish would prevent okra from growing, and few *Brassica oleracea* vegetables would grow well in areas where sweet potato is well adapted. Nevertheless, heat stress and chilling stress effects will have similar expression in specific vegetables, even if the temperatures at which they occur differ among species.

High temperatures

The rise in global temperatures (Fig. 4.1) brings with it increases in heat stress, but also provides opportunities to expand production of vegetable crops on a national and regional level as warmer conditions lengthen frost-free periods (Bisbis *et al.*, 2018). In Europe, for instance, an earlier start of the growing season could permit production of longer-season crops, or more successive crops in a longer season. Similarly, Potopova *et al.* (2017) estimated that fruit-bearing vegetables grown in the Czech Republic have shown a 5 to 12% yield increase per ˚C temperature increase over the last 54 years, presumably because growing season temperatures had moved farther into the optimum range. Rising temperatures in current South American potato production areas may require development of better-adapted lines, and shift the cultivation to higher elevations (Schafleitner *et al.*, 2011). At the currently cooler sites, increased frost tolerance may be needed as cold spells would likely still occur. Predictive modeling of changes in temperature in Australia over the next 50 years has shown which current tomato production areas may be endangered, and where future cultivation of the crop might be feasible (Silva *et al.*, 2017).

The impact of heat on vegetable growth can be mitigated by focusing on a whole-plant perspective. For instance, it may be possible to escape the effect of high temperatures by fostering deep rooting to maintain leaf cooling (Fig. 4.5). Similarly, in leafy radish, heat tolerance was related to high stomatal density (Chen *et al.*, 2014). Under high temperature growing conditions, providing shade may lessen leaf temperatures (Diaz-Perez, 2013; Hernandez *et al.*, 2015). Heat stress protection mechanisms involving membranes and protecting against oxidative and osmotic stress were covered in the general section, above.

A major strategy to lessen the impact of heat stress is to identify stress-tolerant genotypes. For instance, plantings of leaf lettuce during seasons and locations warmer than normal identified lines better adapted to those conditions (Lafta *et al.*, 2017). Similarly, selecting tomato lines under hot field conditions in India revealed considerable genetic diversity in heat stress tolerance (Manish *et al.*, 2017; Rajiv *et al.*, 2017). The same strategy has been employed with the other major vegetable crops (see crop-specific chapters).

Selection for heat stress tolerance can be enhanced by use of a greenhouse, where interfering

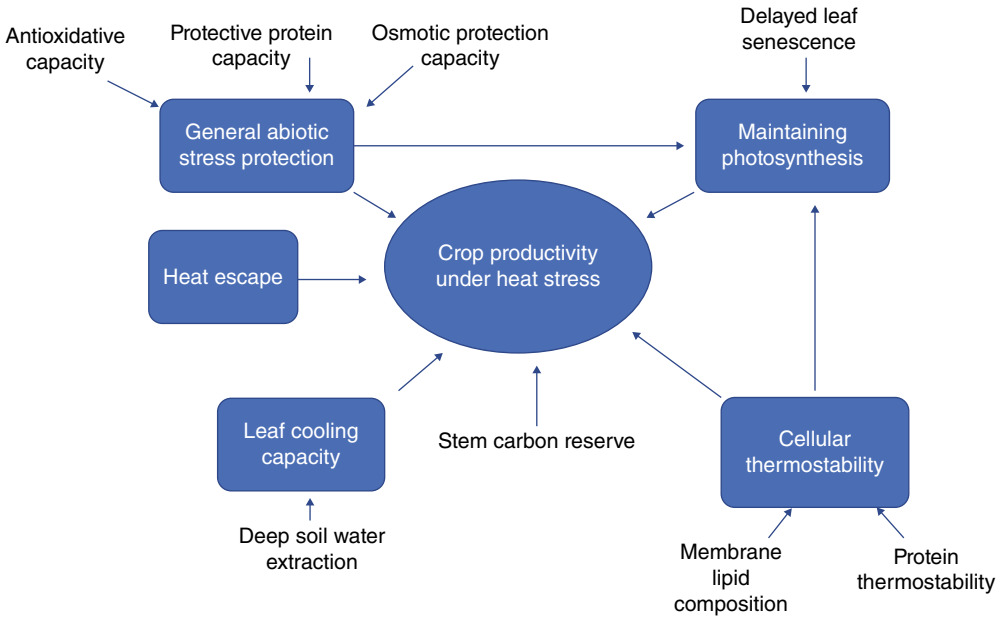

Fig. 4.5. Physiological and biochemical mechanisms relevant to crop productivity in heat-prone environments. (source: Xue and McIntyre 2011).

factors such as drought can be more easily controlled than in the field (Rainey and Griffiths, 2005). A comparison of heat stress response of snap beans selected in a greenhouse showed that the tolerant lines were also heat-tolerant in high temperature field conditions (Wasonga *et al.*, 2012). A further focus on processes most susceptible to disruption may speed progress: for peas, in which heat stress adversely affects pollen germination, timing of heat exposure to bud formation and flowering identified lines that produced pods in spite of the stress (Nikolova *et al.*, 2008). See also pollen heat stress research with tomato (Chapter 7).

Low temperatures

Anyone gardening in a temperate environment is familiar with chilling injury to tomato, beans, or cucumber following the first cool nights of fall: as the sun emerges, the leaves of these species wilt and collapse. Species grown as vegetables differ markedly in the low temperatures that they can endure without injury (Table 4.2). The classification must remain somewhat loose, however, since the process of hardening can lower the temperature at which plants are injured (see below).

As reviewed by Lukatkin *et al.,* (2012), the exposure of chilling-sensitive plants to temperatures below about 15˚C leads to a loss of leaf turgor, with a reduction in hydraulic conductivity and a failure of stomata to close. In *Phaseolus* beans, if plants survived the initial dehydration, leaf and root ABA levels built up, and led to stomatal closure and resumption of normal growth (Pardossi *et al.*, 1992). Application of ABA to the roots prior to chilling prevented chilling damage. The important role of ABA in chilling stress tolerance was also supported by Ntatsi *et al.,* (2012), who found that grafting of tomato seedlings unto rootstocks unable to produce ABA made the plants more susceptible to chilling temperatures.

Temperatures that subject vegetable crops to chilling injury range from just above freezing to 15°C, and are exacerbated by high light and air movement (Lukatkin *et al.*, 2012). Vegetable crops most sensitive to chilling include those of tropical origin (Tables 4.1, 4.2). Damage may occur in the seedling stage, to growing plants and to harvested products, and most often consists of loss of turgor and associated disorders. Postharvest damage due to chilling is outside the scope of this review, but

Table 4.2. Classification of cold hardiness of annual vegetable species. The process of hardening will shift temperature reaction of species among the frost-hardy species to the right.

Tender	Slightly hardy	Moderately hardy	Very hardy	Extremely hardy
+5 to 0˚C[z]	–5˚C	–5 to –10˚C	–10 to –20˚C	<–20˚C
Cucurbits Solanaceous Legumes[y]	Crucifers Celery, carrot Lettuce	Garlic, onions Spinach		Dried tissues, e.g. seeds

[z]Temperatures at which injury to tissue occurs.
[y]Sensitivity to injury may vary among plant parts: e.g. pea flowers more sensitive than leaves.

can be seen in Aghdam and Bodbodak (2013). A broad range of physiological processes are inhibited in affected plants, including water and mineral nutrient uptake, respiration, and photosynthesis. Fundamentally, chilling damage injures both the lipid and protein components of cell membranes (Lukatkin *et al.*, 2012).

Growth inhibition of seedling plants varies with species and the length of the seedling root (Rab and Saltveit, 1996a). Okra is sensitive to chilling with seedling roots of 1 to 7 mm, while tomato and cucumber are most susceptible when roots have extended to 7 mm. Chilling injury in cucumber roots is greatest in the root tip, thus inhibiting root extension in cold soils (Rab and Saltveit, 1996b). Smeets and Wehner (1997) confirmed these findings, showing that cucumber seedling susceptibility was higher at the first true leaf stage, compared to earlier. There is evidence both among cucumber and watermelon for cultivar differences in chilling temperature tolerance at the seedling stage (Kozik and Wehner, 2014). Part of the seedling growth inhibition of cucumber may be due to reduction of water and mineral uptake in cold soils (Dong *et al.*, 2017). If the growing medium was treated with salicylic acid, level of unsaturated fatty acids in the root cell membranes was increased to those found at normal temperatures, thus improving root membrane stability (Dong *et al.*, 2017).

Chilling-sensitive plants can be hardened to resist injury by exposure to mild stress by high or low temperatures (Lafuente *et al.*, 1991; Pardossi *et al.*, 1992), or to drought (Lukatkin *et al.*, 2012). Applications of ABA (Pardossi *et al.*, 1992), silicon (Liu *et al.*, 2009), cytokinins, GA inhibitors and antioxidants (Lukatkin *et al.*, 2012) also provide protection. The cross-protection mechanisms reviewed by Hossain *et al.* (2018), involve generation of ROS, which at low concentrations stimulate antioxidant production (see Fig. 4.4). For instance, genetically elevating the level of superoxide dismutase gene in winter squash (*Cucurbita moschata*) increased chilling tolerance (Chiang *et al.*, 2014).

The convenience of using young plants to detect genetic differences in susceptibility to chilling was used with tomato to screen seedlings at the five-leaf stage (Cao *et al.*, 2015). Accessions differed in leaf electrolyte leakage, chlorophyll content and chlorophyll fluorescence parameters after exposure to chilling for eight days and nights of 4˚C temperatures. Differential wilting indicated the importance of damage to cell membranes and to the photosynthetic apparatus by chilling injury.

When temperatures drop below freezing, the reaction of plant cells to freezing stress must be considered. Depending on the rate at which temperatures decline, freezing leads to the formation of ice crystals in the tissue. At cooling rates of more than 3˚C per hour, ice crystals may form inside the cells, leading to physical damage of membranes (reviewed by Guy, 1990). At slower cooling rates, the ice crystals form in the intercellular spaces, and liquid water from inside the cell moves to the extracellular solution and to the ice crystals (Guy, 1990). Temperature drop rates in nature are nearly always slow enough to prevent intracellular freezing, so the freezing process is one of cell dehydration and rehydration upon thawing. The cell acts as an osmotic system, with the state of the plasmalemma determining if the plant survives the cold shock or is damaged (Rodrigo, 2000).

The process of acclimation to cold can occur in exposure to low temperatures over several days. As a result, plants become hardened to below-freezing temperatures, and over a week, the lethal temperature declines significantly (Fig. 4.6)

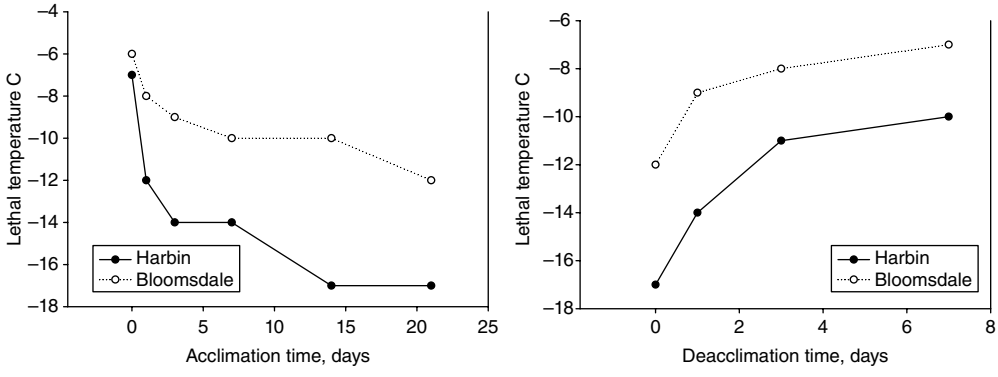

Fig. 4.6. Change in temperature at which 50% of leaves are killed as a result of freeze-hardening at exposure to 5/2˚C (day/night), and deacclimation at 20/17˚C (source: Fennel and Li 1985).

(Fennell and Li, 1985). The process of deacclimation can proceed even more rapidly if the plant is exposed to warm conditions. Hardening resulted in decreased permeability of thylakoid membranes in the spinach chloroplasts as well as increased capacity for vesicle expansion during the thaw cycle (Hincha *et al.*, 1987; Bailey-Serres *et al.*, 2012 *et al.,* Rao *et al.*, 2017).

The hardening process to increase freezing tolerance can also be achieved by exposure to drought stress in cabbage seedlings (Sasaki *et al.*, 1998). In addition to reducing electrolyte leakage after cold stress, cold acclimation combined with drought increases the levels of hexose sugars and sucrose in the plant cells. Presumably, the additional sugars contribute to protection of cell membranes during stress. The hardening agents discussed in the section on chilling, above, can also play a role in increasing freezing tolerance.

The avoidance of freezing injury in field-grown crops relies on a knowledge of the physics of air and plant temperature in a cooling environment (Perry, 1998). Especially on cool, calm nights with a clear sky, radiative cooling lowers air temperature. Since that cold air collects in the lowest areas, selecting field locations that avoid such "frost pockets" is an important first step. The fact that frost formation generates heat that can protect the tissue being covered by ice has fostered use of sprinkler irrigation during freeze events (Perry, 1998). Physical protection of vegetable crops with frost blankets or low tunnels is also often used.

The role of nucleating agents around which ice forms has provided other means by which freezing injury of plant tissues may be avoided (Skirvin *et al.*, 2000). Ice crystals form on plant surfaces around nuclei such as bacteria, especially if the leaf surface is wet (Wisniewski *et al.*, 2003). For these ice crystals to damage the plant, however, they must penetrate through the cuticle into the tissue. Coating the plant surface with hydrophobic materials prevents that penetration and can allow for significant super-cooling (Fuller *et al.*, 2003). Other approaches, such as flooding the plant environment with bacteria that are non-nucleating, have not succeeded and aroused alarm among environmentalists (Skirvin *et al.*, 2000). It may be possible, however, to breed supercooling plants with waxy cuticles and/or containing "antifreeze proteins" (Duman and Wisniewski, 2014). Progress to develop transgenic plants from transfer of genes from insects conferring supercooling properties has been slow, however, achieving only 1 to 3˚C protection (Duman and Wisniewski, 2014).

Moisture Effects

An adequate moisture supply during the growing period is a prerequisite for satisfactory production of vegetable crops, since the harvested product in most cases is high in water content. Both lack of water, and excess water in the root zone, can have detrimental effects on growth and yield, and are stresses common to vegetable production. It has been estimated that 70% of crop losses to abiotic stress were due to drought and flooding in 2000–2011, and that in 2011, flooding accounted for 70% of that loss (Bailey-Serres *et al.*, 2012).

Lack of moisture

Lack of moisture during part of the growing season is a common occurrence in the production of vegetables in the field. Drought stress can occur anytime, but it most often affects plants during the main part of the season, when plants are large enough to significantly deplete the soil water resources. Initial effects of a drought stress event are a reduction in expansion of leaf and stem, closure of stomata, reduced relative water content, and wilting of leaves (reviewed by Farooq *et al.*, 2009). In addition, leaf display to the sun may be reduced through leaf rolling (Blum, 1996).

As drought is prolonged, insufficient moisture adversely affects production of assimilates through reduced photosynthesis due to stomatal closure (Fig. 4.7), leading to reduced respiration rates and changes in assimilate allocation patterns (Farooq *et al.*, 2009). While translocation of carbohydrates declines during water stress, allocation to root systems is relatively less inhibited in some species such as maize and cassava (Robertson *et al.*, 1990; Leach *et al.*, 2011; Duque and Setter, 2013). An increase of ABA in the actively growing root tips coincides with this root growth, and with the stomatal closure during drought (Sharp *et al.*, 2004).

The effect of drought stress can be most damaging to plants at specific stages, adversely affecting yield formation. For instance, low water potential imposed on maize around pollination allowed embryos to form, but caused abortion and thus reduced kernel numbers (Zinselmeier *et al.*, 1999; Setter *et al.*, 2001). Although sucrose was translocated to the ovaries, it was not converted to starch due to inhibition of invertase activity.

Mitigating the adverse effects of drought stress involves several factors. Some mechanisms involve the escape from drought stress, such as early flowering, deep rooting, or increased efficiency of water use (Fig. 4.8) (Xue and McIntyre, 2011; Fang and Xiong, 2015). Others improve

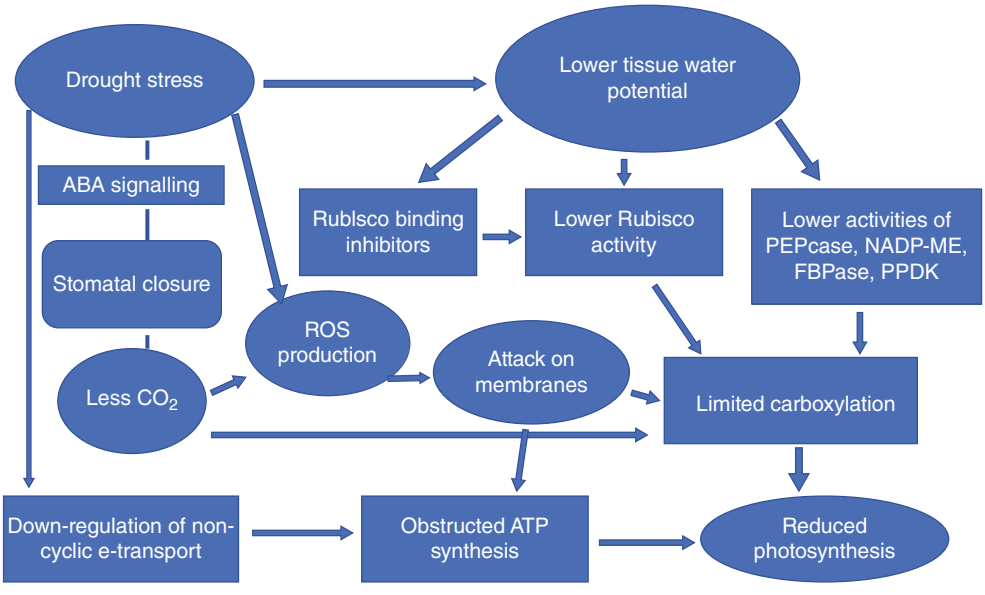

Fig. 4.7. The regulation of photosynthesis under drought stress. As available water is reduced, plants close stomata (plausibly through ABA signaling), which decreases CO_2 influx. Reduction in CO_2 not only reduces carboxylation directly but also directs more electrons to form reactive oxygen species (ROS). Severe drought conditions limit photosynthesis due to decrease in the activities of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), phosphoenolpyruvate carboxylase (PEPCase), NADP-malic enzyme (NADP-ME), fructose-1, 6-bisphosphate (FBPCase) and pyruvate orthophosphate dikinase (PPDK). Reduced tissue water content also increases the activity of Rubisco binding inhibitors (source: Farooq *et al*., 2009).

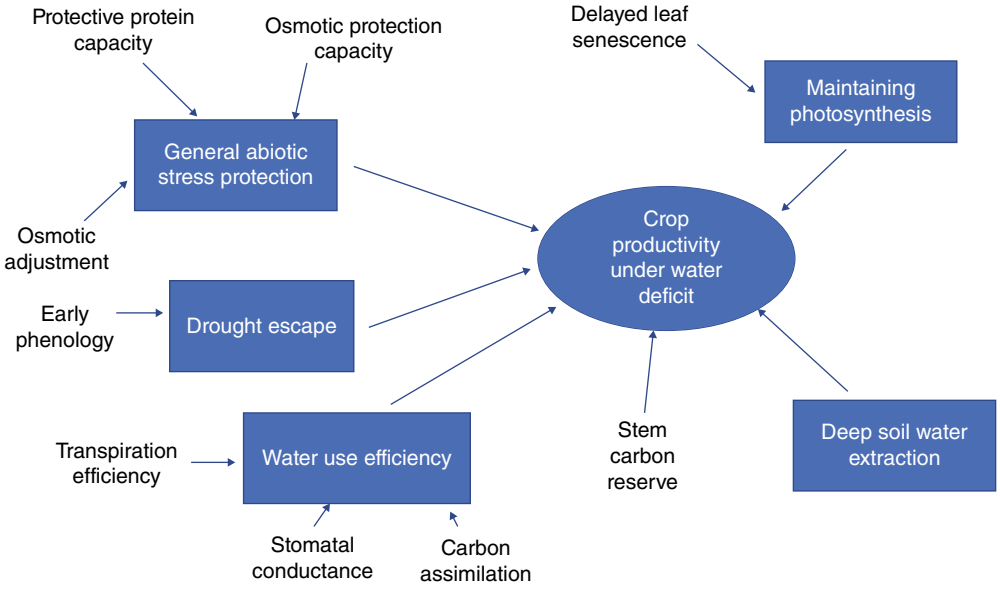

Fig. 4.8. Physiological and biochemical factors influencing crop productivity under drought stress (source: Xue and McIntyre 2011).

plant tolerance to the imposed stress through osmotic protection and production of antioxidants. In the area of stress protection, strategies to deal with drought are quite similar to those that mitigate other abiotic stresses such as heat and chilling temperatures (see Figs 4.2–4.5).

Avoidance of drought stress through grafting onto adapted rootstocks has shown promise recently for fruit-bearing vegetables (reviewed by Kumar *et al.* 2017). In tomato, selection of rootstocks for drought tolerance resulted in the most drought-adapted grafted plants, regardless of scion drought response (Altunlu and Gul, 2012). "Beaufort" and "Maxifort" were identified as most drought-tolerant of the rootstocks tested. Vigorous root growth and nutrient uptake was the basis of improved performance under drought stress for tomato (Rouphael *et al.*, 2008; Sanchez-Rodriguez *et al.*, 2014) and watermelon (Rouphael *et al.*, 2008). In pepper, rootstocks selected for improved growth under drought and also salt stress improved scion fruit yield under both these stresses (Penella *et al.*, 2017). Stress tolerance coincided with higher levels of leaf proline and a maintenance of photosynthetic rates. Other grafting studies with pepper resulted in variable top growth and yield under drought stress conditions, depending on the rootstock used, with some lines fostering maintenance of fruit yield despite reduced foliage, and others the opposite (Lopez-Marin *et al.*, 2017). These studies emphasize that selection of rootstocks and scions needs to be done under the stress conditions.

Exploiting genetic differences in response to drought stress is a strategy common to dealing with other abiotic stresses considered in this chapter. Where the stress factor can be routinely imposed, selecting among contrasting lines can be productive (e.g. Beebe *et al.* 2008 with common bean). In common bean, seed yield in dry environments was correlated with efficient partitioning of assimilates to the developing seeds, such as the pod harvest index (seed weight/pod plus seed weight *100) (Assefa *et al.*, 2013; Rao *et al.*, 2017). Although bean plants with strong, vigorous roots tended to have higher yields under drought stress, the practicalities of selecting for root type would be an obstacle (Polania *et al.*, 2017).

A comparison of drought tolerant and susceptible genotypes at the biochemical level has indicated the importance of an active antioxidant system to prevent oxidative stress damage (Kusvuran and Dasgan, 2017). In addition, accumulation of secondary metabolites and

osmolytes such as proline were also part of plant tolerance (reviewed by Kaur and Asthir, 2017). Nair *et al.* (2008) made similar findings with two cowpea cultivars differing in drought stress tolerance. The research shows that genetic changes in key processes may provide ways of developing lines with improved drought tolerance. Farman *et al.* (2017) identified two groups of genes involved in drought stress tolerance: those regulating the production of products that directly protect against stress, such as late embryogenesis active proteins (LEAs), water channel proteins and osmoprotectants, and those that regulate the expression of other genes such as transcription factors. They provide a table of successful manipulations of such drought-responsive genes in *Arabidopsis*, cereals and other field crops, and thus indicate the promise of such approaches for vegetable crops.

There has been some success in reducing drought stress damage by application of exogenous agents that counteract oxidative stress. For instance, salicylic acid and acetyl salicylic acid improved growth of *Ctenanthe setosa* (a tropical foliage plant) (Kadioglu *et al.*, 2011), reduced stress of muskmelon seedlings (Korkmaz *et al.*, 2007) and counteracted drought, chilling and heat stress in bean and tomato (Senaratna *et al.*, 2000). Similarly, application of salicylic acid or the osmolyte glycinebetaine lessened drought stress symptoms of sunflower at the flowering stage (Hussain *et al.*, 2008, 2009).

Flooding

Predictions of global climate change foresee rising sea levels, and increased incidences of severe storms with excess water (Wuebbles *et al.*, 2017), so scientists working on vegetable crops can anticipate more frequent encounters with flooding stress. In addition, the need to use land for more than one crop per season will require converting rice fields that are commonly flooded, to production of upland crops in the off-season (Lafta *et al.*, 2017), and thus will expose those crops to anaerobic conditions.

Research on the response of plants to flooding has focused primarily on crops such as rice that are grown in flooded soils (reviewed by Colmer and Voesenek, 2009; Jackson *et al.*, 2009, Bailey-Serres and Voesenek, 2010). The existence of *Oryza* species that can grow in flooded conditions has provided genetic mechanisms of flooding and submergence tolerance that are less relevant to vegetable crops that are grown in aerobic conditions. Flooding effects on field crops and vegetables were reviewed by Rao and Li (2003). In general, flooding resulted in stomatal closure, reduction in elongation growth and lower photosynthesis rates. Among vegetables, only a few studies have been made to find species differences in response to lack of oxygen in the root zone. For instance, in an experiment with potted plants, *Cucurbita pepo* was most flooding-tolerant, followed by tomato and cucumber (Walter *et al.*, 2004). Bean was most susceptible in the study. Jackson (1979) described the severe growth inhibition of pea plants in flooded soils after two to four days of flooding.

The degree to which adventitious roots form and the rate of their formation on flooded plants predicted recovery from flooding in tomato (Aloni and Rosenshtein, 1982). Adventitious roots form in tomato in response to lack of oxygen around the root system (Bradford and Dilley, 1978), and the production of the ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid). Ethylene formation increased the translocation of auxin to the flooded part of the plant and stimulated the growth of preformed root initials on stem and hypocotyl (Vidoz *et al.*, 2010). The translocation of ACC to the shoots of flooded plants increased ethylene formation in the tops and caused the epinastic curling of the tomato leaves (Bradford and Yang, 1980). The closure of stomata that was an early sign of flooding was accompanied by an increase in ABA, but was not affected by the ethylene build-up (Bradford, 1983). Flooding resulted in a decrease of cytokinin and gibberellin translocation from the root system (Jackson and Campbell, 1979; Bradford, 1983; Atkinson *et al.*, 2008), and is likely the cause of reduction of elongation growth. Foliar sprays of cytokinins partially reopened stomata in some experiments (Jackson and Campbell, 1979; Bradford, 1983), but not in others (Atkinson *et al.*, 2008), and will require additional research.

The formation of an anoxic root zone with flooding implies that mineral nutrient uptake will be directly affected (Rao and Li, 2003). For legumes, depending on nitrogen fixation, flooding deprives the plants of nitrogen gas, and oxygen for respiration. For plants dependent on inorganic N uptake, lower leaves develop nitrogen

deficiency (Reed and Gordon, 2008), which can be counteracted by N fertilization after stress relief (Nakano *et al*., 2018). In peas, plant nitrogen content was most decreased by flooding, whereas the other major nutrients were less affected (Jackson, 1979).

A major factor in the impact of flooding on crops is the incidence of soil-borne diseases as a result of anaerobic conditions in the root zone. Pathogens such as *Pythium*, *Rhizoctonia*, and *Phytophthora* occur frequently in flooded soils and can adversely affect crop survival (Rao and Li, 2003). For tomatoes grown in the tropics, grafting tomato scions onto rootstocks resistant to bacterial wilt disease (*Ralstonia solanacearum*) such as eggplant has been essential to overcome frequent plant death during the hot, wet growing season (Palada and Wu, 2007). Selection for flooding resistance among pepper species identified lines of *Capsicum baccatum*, *C. frutescens* and *C. chacoense* that improved bell pepper yields when used as rootstocks in the hot, wet season in the tropics (Palada and Wu, 2008). Shimamura *et al*. (2007) described the formation of aerenchyma in the hypocotyl and adventitious roots of sponge gourd (*Luffa cylindrica*) when flooded, which suggests that this species may be suitable as a rootstock for cucurbit vegetables in flood-prone environments.

For many years, avoidance of flood-prone soils has been a standard cultivation technique among vegetable growers. When wet, low-lying fields cannot be avoided, forming high beds that aerate at least part of the rootzone is advisable, although these plantings will be more drought-prone if the anticipated wet season does not occur.

Salt Stress

While irrigation is often used to avoid the effects of drought, the quality of soil and of the water can reduce the efficacy of irrigation. It is estimated that a third of irrigated lands are affected adversely by salinity (Marschner, 2005), and that 3% of the earth's surface has salt-affected soils (Panta *et al*., 2014). The high sodium content of many saline soils may also lead to destruction of soil structure and reduced drainage, and increases in waterlogging, which compounds the severity of the salinity effect (reviewed by Singh, 2017). In addition, rising sea levels have subjected more agricultural areas to flooding by seawater, especially in southern Asian countries such as Bangladesh and Pakistan.

The degree of soil salinity is measured by the electrical conductance of a soil paste, in deciSiemens per m ($dS.m^{-1}$) (Marschner, 2005; Scudiero *et al*., 2017). Soils can vary from non-saline (0–2 $dS.m^{-1}$), through slightly (2–4), moderately saline (4–8) to severely saline (>16 dS^{-1}) (Scudiero *et al*., 2017). For reference, it is useful to know that seawater typically varies from 44–55 $dS.m^{-1}$, and only a few species of plants, termed halophytes, can grow well at that salinity level (Flowers and Colmer, 2008). Most vegetables show growth inhibition at low soil salinity levels (Table 4.3), and attempts to improve salt tolerance among vegetable and other crop species has had limited success (Flowers, 2004). In the following paragraphs, plant reaction to salinity stress will be described, and progress achieved among vegetables in overcoming this stress factor is documented.

In addition to differences in salt sensitivity, plant species differ in the threshold level of salinity that begins the yield decline, and the slope of the yield/salinity curve (Fig. 16.21 in Marschner, 2005). Some halophyte species show growth and yield increases as salinity rises, while moderately salt-sensitive crops such as beets have a gradual downward slope, in contrast to *Phaseolus* beans, that show steep decline as the soil increases in salinity. Only a few crop species used as vegetables would be considered to be halophytes, and most are not grown or used widely, such as quinoa (*Chenopodium quinoa*), which can tolerate salinity levels up to 40 dSm^{-1}. Others include the leafy vegetables New Zealand

Table 4.3. Salinity tolerance of vegetable crops (Stepphun *et al*., 2005).

Crop	Part harvested	Soil salinity[z]
Asparagus	Spears	28.5
Bean, common	Seeds	3.34
Beet, red	Root	9.19
Cabbage	Head	6.62
Carrot	Root	4.26
Cucumber	Fruits	6.02
Lettuce	Leaves	4.83
Potato	Tubers	5.54
Tomato	Fruits	7.21

[z]Soil paste conductivity in $dS.m^{-1}$ that reduces yield of the harvested part by 50% below that of the same crop grown in a non-saline soil.

spinach (*Tetragonia tetragonoides*), seaside purslane (*Sesuvium portulacastrum*) (Panta *et al.*, 2014) and purslane (*Portulaca oleracea*) (Teixeira and Carvalho, 2009). But the majority of vegetable species would grow poorly in soils considered moderately saline (Table 4.3), and such areas are common in areas of low rainfall. For instance, in the dry western San Joaquin Valley of Southern California, soil salinity surveys have indicated that 45% of the 0.75 million ha of agricultural land has salinity values of more than 4 $dS.m^{-1}$ (Scudiero *et al.*, 2017).

Plants grown in saline medium are affected by salt in three major ways: i) water deficit induced by low water potential of the rooting medium, producing osmotic stress; ii) toxic effects of Na^+ and Cl^- ions, causing ionic stress; and iii) induced deficiency of major nutrients such as Ca^{2+}, K^+ or Mg^{2+} (Marschner, 2005). The dehydration effect is similar to effects of drought and heat stress described above (Fig. 4.2), eliciting a sequence of plant reactions to protect plant membranes and restore growth (Wang *et al.*, 2003). The most immediate effect is a reduction in leaf expansion, stomatal closure and lower photosynthetic rate (Greenway and Munns, 1980). In non-halophyte species that try to exclude salt from the root system (termed glycophiles), adaptation to counter the osmotic effect requires the accumulation of organic solutes such as sugars in the root system, and a reduction of above-ground surface area by increase in leaf succulence (Greenway and Munns, 1980). In halophytes, tissue tolerance of high levels of Na^+ and Cl^- allows these ions to accumulate to assist in turgor maintenance.

The toxic effects of Na^+ and Cl^- ions stems from the inability of susceptible plants from preventing uptake of these ions by the root system, and from translocating them to the leaves via transpiration (Munns, 2005). Lower leaves are first affected, turn yellow and abscise, followed by leaves higher on the plant. If the rate of leaf death exceeds rate of new leaf formation, the plant dies before the reproductive stage (Munns, 2005). Sodium ion translocation into above-ground parts is via the xylem, where, if isolated in leaf cell walls, it can accumulate to concentrations that again can cause dehydration of leaf cells (Tester and Davenport, 2003). If taken up into the cytosol, Na^+ exerts toxic effects by substituting for K^+ ions needed for the action of many enzymes. Few of the accumulating Na^+ ions are removed from leaves through transfer into the phloem, and translocation to growing points in the shoot and root would cause further toxic effects (Munns, 2005). It is thus apparent that a key attribute of salt tolerant plants is to exclude Na^+ from plant leaves. An inverse relation of leaf Na^+ concentration and salt tolerance has been found in tomato (Hajibagheri *et al.*, 1987) and durum wheat (Munns, 2005). Uptake of Na^+ and K^+ into the plant root system and transport within the plant is controlled by about 13 different gene systems, and thus would be difficult to alter (reviewed by Munns, 2005).

While much research on the tolerance of plants to salinity has focused on the influence of Na^+, the toxic effects of Cl^- ions are also important (Teakle and Tyerman, 2010). Sensitivity to Cl^- is particularly great in some legume species such as soyabean, but in *Phaseolus vulgaris*, shoot Na^+ concentrations correlated inversely with salt tolerance (Dasgan and Koc, 2009).

Differences in salt tolerances within species may include characteristics of both salt exclusion and inclusion. For instance, a study of six tomato cultivars showed one relatively salt tolerant line to have low Na^+ and Cl^- accumulation in the leaves at 7 dSm^{-1}, while another allowed higher levels of these ions in leaves without damage (Alfocea *et al.*, 1993). A salt-susceptible cultivar was stunted by the same high ion levels.

The toxic effects of sodium chloride in the soil on plant growth can be made worse by a lack of uptake of other essential ions needed for plant growth, especially Ca^{+2} and K^+ (Marschner, 2005). Thus addition of gypsum ($CaSO_4$) ameliorated growth of potato and beans growing on saline soils. On the other hand, irrigation with saline water increased Ca^{+2} disorders such as tipburn of lettuce and of Chinese cabbage (Mizrahi and Pasternak, 1985; Marschner, 2005), and blossom-end rot of tomato. Providing additional K^+ in the nutrient solution in order to reduce Na^+ uptake may also be counter-productive by inducing increased BER in tomato (Fan *et al.*, 2011).

Changes in root and shoot hormone levels when plants are exposed to salinity have increased understanding of salt-induced injury, and suggested adaptation mechanisms. In tomato, shoot ABA levels increased within 24 h of exposure to 10 dSm^{-1} salt stress (Albacete *et al.*, 2008), whereas cytokinin levels decreased markedly in the tops. In a similar study, senescence of lower leaves of tomato seedlings was correlated with a

decrease in zeatin and zeatin riboside content (Ghanem *et al.*, 2008). The important role of cytokinin production by roots in protecting against salinity was further supported by transgenic and grafting studies (Ghanem *et al.*, 2011) that increased root cytokinin levels and increased tomato shoot growth in saline conditions.

Grafting tomatoes onto salt-tolerant rootstocks has alleviated yield reductions from growth at moderate levels of salinity (Estan *et al.*, 2005, Martinez-Rodriguez *et al.*, 2008). Choosing rootstocks that excluded Na^+ and Cl^- from translocation to the tops boosted fruit yields by about 80% compared to non-grafted plants of the same cultivar when grown at 50 mM salt levels (7.3 dSm^{-1}). A broader study to identify rootstock characteristics associated with fruit yield under saline stress indicated however that quantitative trait loci that minimized changes in water status of the tops were most important (Asins *et al.*, 2010). Sodium ion, phosphorus and copper concentration of scion leaves, as well as the proportion of root weight were also positively associated with performance in salt stress conditions, indicating the multi-genic nature of salt tolerance.

Grafting studies with cucumbers also found that rootstocks that reduce transport of Na^+ to the shoot lessen the impact of moderate salt level (6.7 dSm^{-1}) on yield (Colla *et al.*, 2012). A commercial cultivar of cucumber was grafted on a *Cucurbita maxima* × *C. moschata* root, and grown in either Na_2SO_4 or NaCl solution (Table 4.4). Yields, plant dry weights and elemental compositions showed clear detrimental effects from the two salt types. The rootstock reduced leaf Na^+ concentration with both salt treatments, but failed to prevent a buildup of Cl^- ions in leaves of both grafted and ungrafted plants grown in NaCl solution. The results indicate that Na^+ -excluding roots can help cucumber performance at moderate salt stress levels. A similar study by Zhen *et al.* (2010) confirmed the efficacy of rootstocks that exclude Na^+ from cucumber tops, but also showed that oxidative damage played a role in salt stress. Tolerance of salt stress was linked to low hydrogen peroxide levels and high levels of superoxide dismutase, peroxidase and catalase enzyme activity in the roots.

Although the negative aspects of salinity on plant performance have been emphasized in this section so far, considerable recent research has highlighted the positive effects of mild salt stress on quality of the harvested vegetables (reviewed by Rouphael *et al.*, 2018). In general, enhanced dry matter content, improved sugar content, soluble solids (TSS) of fruits from salt stressed tomato plants have been widely reported (Leonardi *et al.*, 2004; Krauss *et al.*, 2006). In some cases, Vitamin C, lycopene and β-carotene were also increased (Krauss *et al.*, 2006), while in other studies, these were not affected (Leonardi *et al.*, 2004). Studies with melons (*Cucumis melo*) showed similar improvements in fruit TSS (Botía *et al.*, 2005) when irrigated with water with an EC of around 6 dSm^{-1}.

The timing of salt stress can in some cases influence the impact on yield and product quality. With melons, Botía *et al.* (2005) found that restricting salt stress to the fruit formation period allowed fruit yields to be maintained, while TSS and titratable acidity were enhanced. A similar experiment with cauliflower decreased curd yields no matter when salt stress was imposed, but increased the level of some curd glucosinolates (Giuffrida *et al.*, 2017) (see also Chapter 15).

To take advantage of such productivity improvements would require production systems in which salt water can be applied at will, such as

Table 4.4. Yield, plant dry weight and leaf elemental composition of cucumber grown on its own roots or grafted on a *Cucurbita maxima x C. moschata* rootstock (Colla *et al*. 2012).

Salt treatment	Graft treatment	Market yield	Dry weight, $g.plant^{-1}$		Element conc. in shoot, $mg.g^{-1}$		
		$Kg.plant^{-1}$	Shoot	Root	N	Na^+	Cl^-
Control	None	3.76a	91.4a	7.9	41.6	0.57e	20.6
	Grafted	3.82a	95.3a	8.3	40.6	0.52e	24.0
Na_2SO_4	None	2.20cd	57.1c	6.8	38.5	22.8a	28.2
	Grafted	2.66b	67.9b	7.7	38.9	8.4c	30.1
NaCl	None	2.09d	50.0d	5.9	33.1	14.4b	58.8
	Grafted	2.33bc	57.8c	7.3	35.5	4.3d	54.3

in greenhouse hydroponic operations. For field plantings, sources of saline water, as from wells, would need to be available (Botía *et al.*, 2005), but a build-up of salinity in fields where such methods are used would be a concern. In general, achieving the salinity level that minimizes yield depression but maximizes product quality in fluctuating climatic conditions is likely challenging.

Breeding and selection for improved salt tolerance has been a research objective for many years, but has had only limited success, given the multi-genic nature of stress (Flowers, 2004). As shown in the first section of this chapter, several stresses trigger similar responses in the plants (Wang *et al.*, 2003, and Fig. 4.3). For instance, tomato plants have been genetically engineered to increase the glycinebetaine content under salt and drought stress and thus confer greater tolerance to these stresses (Deepa *et al.*, 2011). Similarly, tomatoes expressing high levels of osmotin have shown greater tolerance to these stresses (Goel *et al.*, 2010). A third approach introduced a cell wall extension and reconstruction gene from hot pepper (xyloglucanendotransglucosyl-ase-/hydrolase) into tomato and improved both salt and drought stress tolerance (Choi *et al.*, 2011).

Concentrating on genes that regulate Na^+ compartmentalization in vacuoles, Zhang and Blumwald (2001) developed salt-tolerant tomato lines that accumulated Na^+ in leaf vacuoles but not fruits, and enabled plant growth in 200mM NaCl. The overexpression of a vacuolar Na^+/H^+ antiport gene from *Arabidopsis* markedly increased salt tolerance. Gouiaa and Khoudi (2015) achieved similar results with an overexpressed antiport gene from tobacco. Work with squash often used as rootstocks for cucumber identified a high affinity K transporter that is important in Na^+ transport in roots. When transgenically expressed in cucumber, the CmHKT1;1 gene facilitated the transport of Na^+ out of root xylem cells, reducing Na^+ transport to the tops (Sun *et al.*, 2018). When these transgenic plants were used as rootstocks for cucumber, salt tolerance was enhanced. This focus on transporter genes summarized by Zhang *et al.* (2004) appears promising, and will hopefully result in crops tolerant to salt stress.

At the cellular level, plants deal with salt stress by the expression of several SOS (salt-overly-sensitive) protein kinases in the roots, that regulate extrusion of Na^+ into the soil solution, and the loading into the xylem stream (Zhu, 2016).

The wider-ranging approach of comparing gene expression of plants either grown under non-saline or saline conditions has resulted in identification of many gene systems involved in salt stress response in radish (Sun *et al.*, 2015, 2016), broccoli (Tian *et al.*, 2014) and melon (Wang *et al.*, 2016). These studies emphasize the complexity of genes involved in salt stress response, and hint that lasting salt-tolerance may be a distant goal in vegetable crops.

Concluding Remarks

The stress factors impacting vegetable crops reviewed in this chapter can be grouped into those that can be avoided or moderated by physical structures, such as covers to mitigate cold, and irrigation to overcome drought. Heat, flooding, and salt are more difficult to avoid, and threaten to occur more frequently in this era of rapid climate change. It is fortunate that all these stressors have an effect in common: they subject the plant to dehydration and water loss, and thus elicit common reactions such as stomatal closure, osmotic adjustment, and the production of antioxidants. The similarity of responses thus produces the opportunity to cross-protect the plant from one stress by preceding that stress with a mild form of the same or another stress. This priming process has often been used, for instance in hardening transplants before exposing them to a harsher field environment. It is also possible to develop, through plant breeding, plants that are "pre-primed," or more resistant to abiotic stress factors, but the cost of having higher levels of osmotic substances in place on plant productivity needs to be considered. It is apparent that considerably more effort is needed to translate our understanding of fundamental reactions of plants to abiotic stress into improved plant productivity under field conditions.

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5 Models of Vegetable Growth and Development

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Modern science is characterized by a rapid increase of new knowledge, revealing more of the details of our natural systems. Inevitably, as the scope of our scientific understanding gets narrower, the deeper we get into detail. In the 17th century, René Descartes (1596–1650) proposed to study complex problems by splitting them up into smaller parts. This reductionism has led scientists to "know more and more about less and less until they know everything about nothing" (Konrad Lorenz). Thus, modern science faces the problem to integrate all the bits and pieces of knowledge in order to solve problems in complex systems. This is the background of the development of systems science and systems modeling during the course of the 20th century. In crop physiology, the challenge is to predict the physiological response to soil, climate, and management interventions on the crop level from our knowledge on the process level.

Vegetable Systems and Models

Vegetable crops are complex biological systems exchanging matter and information with their physical, biological, and human environment. Their complexity results from the multitude of structural units interacting with each other. The plants, the atmosphere, and the soil surrounding it, the other organisms around (pests, weeds, etc.), and the human interference are also a system of its own kind. This could be called a cropping system or an agro-ecosystem, of which the crop system–composed of the crop plants, which mutually affect each other, for example, by competition, protection, facilitation of disease transmission etc.–is a part. This illustrates that systems can be defined on various hierarchical levels, and each level has "emergent" properties, i.e. characteristics, which are unique for the particular hierarchical level (Trewavas, 2006). For example, leaf area index is a trait that is specific for the crop level, whereas photosynthetic capacity is typically expressed on the leaf level.

The vegetable crop system can not only be considered a component of a cropping system, it can also be subdivided into its components, which are the individual plants (Fig. 5.1). The plant system consists of different organs, i.e. leaves, stems, flowers etc. These are composed of various tissues, the latter of cells containing organelles, etc. In short, systems are composed of subsystems which are systems on their own. The hierarchical structure of systems means that a system can be decomposed to study its individual components (= subsystems), and the components can be reassembled to the system, provided that the relationships between them are known. In biological systems, these relationships are fluxes of matter and information, but also structural and geometric relationships like vascular connections or the position in space.

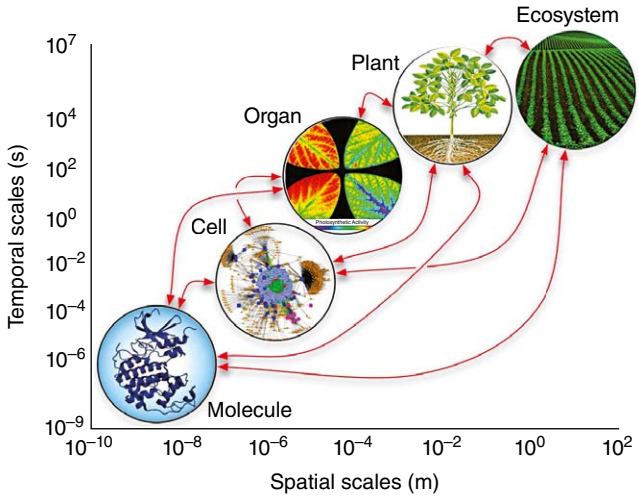


Fig. 5.1. Layers of organization of biological models across temporal and spatial scales. The y-axis represents real-time in which changes occur at each biological level; the x-axis represents the relative size or space which the biological level encompasses. The arrows indicate possible direct interactions among scales. Organ level image from Kim *et al*. 2001 (Marshall-Colon *et al*., 2017).

The complexity of (biological) systems is due to the potential of splitting them up into their components over and over—as Augustus de Morgan in *A Budget of Paradoxes* (second edition, ed. D.E. Smith, 1915) has put it:

> *Great fleas have little fleas upon their backs to bite 'em,*
> *And little fleas have lesser fleas, and so, ad infinitum.*

Models are simplified representations of reality. Simplification helps us to distinguish the more important from the lesser important features of a system. What is important and what not lies, however, to some degree in the judgment of the modeler. Models are therefore subjective. But the adequacy of the model structure and the importance of certain components also depends on the *purpose* of the model. Therefore, there is no "universal" model. In their review of crop modeling in horticulture, Gary *et al.* (1998) distinguish between models for plant production, research models, decision and policy analysis models, and models for teaching. While these categories appear somewhat arbitrary, it is obvious that there is a great diversity in concepts which will be highlighted in this chapter. As a matter of fact, almost all existing mathematical vegetable crop models can be categorized as research models–with little *direct* impact on practical vegetable production, probably with the exception of the "growing degree day" models used actively in predicting harvest scheduling. The purpose of research models is not only to better understand crop growth and development by integrating process descriptions, but also to identify gaps in our understanding which become evident when model predictions and (experimental) reality diverge. Of course, no model can be better than our understanding of its underlying physiology. In this chapter, we therefore focus on models that aim at improving systems understanding at the crop level. We will evaluate approaches for process description as well as model concepts.

Mathematical Models of Crop Development

Temperature and daylength are the driving variables for plant development. For many developmental processes, linear relationships between the daily developmental rate r_d (d^{-1}) and temperature have been observed:

$$r_d = a_d(T - T_b) \quad (1)$$

where T is the ambient temperature and T_b is the base temperature, i.e. the temperature below

which the developmental rate is 0, and a_d is a coefficient. For a_d, often the reciprocal of the thermal time required to reach a certain stage is used. For example, if a temperature sum of 200°C·d is required for the expansion of a leaf and $T_b = 0$, then a mean temperature of 20°C would result in $r_d = \frac{1}{200°Cd}(20-0)°C = 0.1\,d^{-1}$. Eqn.1 holds only for temperatures above T_b, and below the optimum temperature, T_o, the temperature at which r_d is maximal. Below T_b the development rate $r_d = 0$. For example, the rate of progress to ripening of tomato fruits (Adams *et al.*, 2001) and maturity of snap beans (Jenni *et al.*, 2000) has been modeled as a linear function of temperature, with base temperatures of 5.7°C for fruits of tomato cultivar "Liberto" and 0°C for the snap bean cultivars "Goldrush," "Teseo," "Labrador," and "Flevoro." A compilation of base temperatures for various vegetable species can be found in Maynard *et al.* (2007).

In vegetable crops, models of development can be used for the prediction of harvest time or for scheduling planting dates. In peas it has been standard procedure to estimate harvest time that is cultivar-specific with temperature sums. Using different base temperatures for the pre- and post-emergence phases was found to increase accuracy of prediction compared to utilizing one base temperature throughout (Friis *et al.*, 1987). Nevertheless, temperature sums for optimal harvest may vary with seasons, which makes tenderometer measurements indispensable (Fallon *et al.*, 2006). To estimate the probability of bolting in celeriac, Alt and Wiebe (2001) developed a transformed logistic function of temperature to calculate vernalization and de-vernalization (= negative vernalization) rates. Accumulated vernalization rates were related to bolting frequency in a saturation-type function.

In reality, the development rate r_d decreases above T_o until a ceiling temperature, T_c, above which the development rate $r_d = 0$. To also describe the decreasing development rates above T_o, Yin *et al.* (1995) developed a model based on the beta function:

$$r_d = a_d(T - T_b)^{\alpha}(T_c - T)^{\beta} \qquad (2)$$

where α and β are parameters. Figure 5.2 shows as an example the temperature dependence of the development rate of cassava from sowing to emergence. Yin *et al.* (1995) also present applications of the same function to leaf extension and vegetative development of maize. A similar approach based on the beta function has been proposed by Zhou and Wang (2018) and applied for predicting wheat and maize development:

$$r_d = r_{dmax}\begin{cases} 0 & T < T_b \\ \left(\frac{T-T_b}{T_o-T_b}\right)\left(\frac{T_c-T}{T_c-T_o}\right)^{\frac{T_c-T_o}{T_o-T_b}} & T_b \le T \le T_c \\ 0 & T > T_c \end{cases} \qquad (3)$$

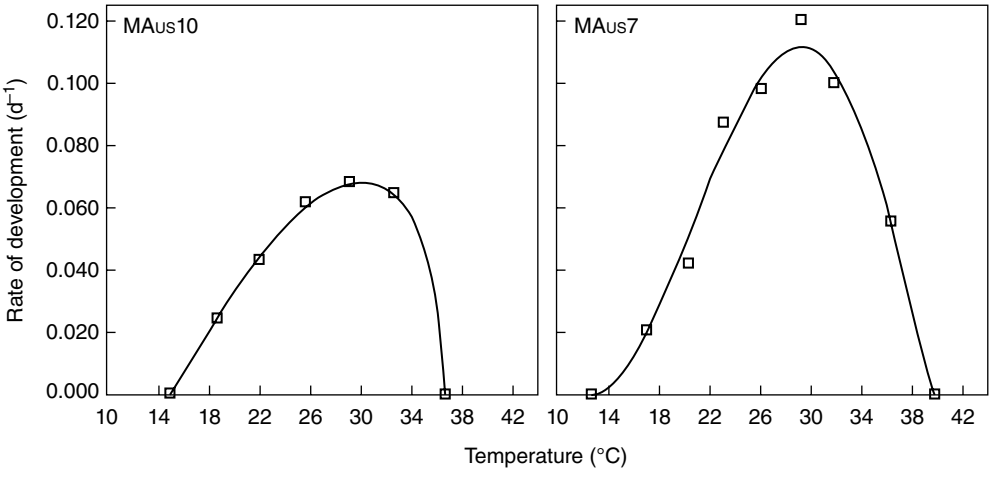


Fig. 5.2. Rate of development from sowing to emergence in two cassava cultivars as a function of temperature as modeled with a beta function (Yin *et al.*, 1995).

where r_{dmax} is the maximum rate of development. Combined exponential relationships yielding similarly shaped curves have been used to describe temperature response of germination and storage root growth of *Brassica* species (Andreucci *et al.*, 2016).

Often development is not only temperature driven, but also influenced by daylength. To combine photoperiodic and temperature effects, the concept of photothermal time as the accumulated product of temperature and relative daylength is used (Masle *et al.*, 1989; Brachi *et al.*, 2010). Extending eqn. (1) by the relative daylength l_d, i.e. the daylight hours as a fraction of the 24 hours yields the photothermal development rate r_{dp}:

$$r_{dp} = a_{dp} l_d (T - T_b) \quad (4)$$

When calculating photothermal development rates, Masle *et al.* (1989) propose to give the temperature of the daylight hours greater weight than nighttime temperatures. Some processes occur only above a certain minimum (base) photoperiod P_b. The relationship

$$r_{dl} = \max\left\{0, \left((P - P_b) + \left[\left(\frac{T - T_b}{T_o - T_b}\right)(P - P_b)\right]\right)\right\} \quad (5)$$

was shown to give a good description of bulbing in onions (Searle and Reid, 2016).

If development is limited by water availability, as may be the case in germination, development rate can be expressed as the product of effective temperature and effective soil water potential (Gummerson, 1986; Rowse and Finch-Savage, 2003). Similar to the thermal development rate r_d in eqns. 1–5, the hydrothermal development rate r_{dh} can be written as:

$$r_{dh} = a_{dh}(\Psi - \Psi_b)(T - T_b) \quad (6)$$

Rowse and Finch-Savage (2003) have used this concept to quantify the germination rates of onions and carrots (Fig. 5.3).

A critical point of using temperature sum for prediction purposes is that the recorded temperature, often the air temperature, is not always equal to the plant temperature. The difference between air and plant temperature strongly depends on the factors related to the light environment and the transpirational demand of the plants (Maes and Steppe, 2012), and should be taken into account when modeling crop growth (Parent *et al.*, 2018).

Artificial neural networks (ANN) are self-learning structures that may also be employed

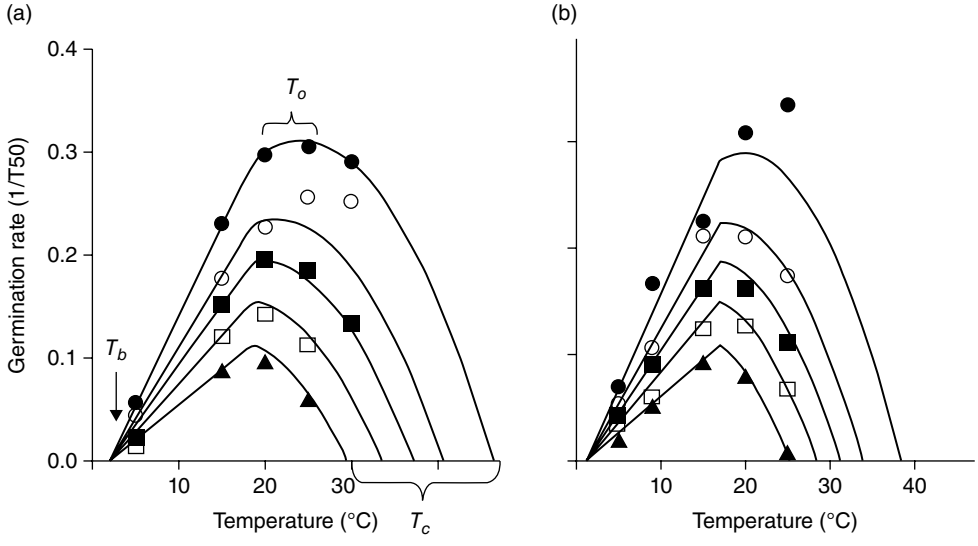


Fig. 5.3. The effect of temperature and water potential on the germination rate (1/T50) of carrot (a) and onion (b) seeds. T_b and Ψ_b are 1.9°C and –0.84 MPa, respectively, a_{dh} (eqn. 6) is 1/48.2 (MPa °C d)$^{-1}$. Closed circles Ψ_b=0; open circles Ψ_b=0.18; closed squares, Ψ_b=0.28; open squares Ψ_b=0.39; triangles, Ψ_b=0.51 MPa, respectively (Rowse and Finch-Savage, 2003).

to predict plant development. In peas, a maturity index for harvest planning was predicted with an ANN using radiation, rainfall, temperature (heat units), and an early/late plant indicator. With a lead time of seven days, the ANN produced an error lower than with the conventional manual method with a lead time of only two days, meaning that the ANN gave much more time for harvest planning (Higgins *et al.*, 2010).

Mathematical Models of Crop Growth

The description of plant growth with mathematical models has its roots in the 19th and early 20th century with attempts to quantify growth as dependent on the most limiting growth factor (e.g. by Liebig's law of the minimum or Blackman's optima and limiting factors–El-Sharkawy, 2011). While these early models could be considered statistical or empirical, improved physiological understanding and computing power has led to the development of more mechanistic models and the incorporation of genetic information.

Empirical crop models

A straightforward way to quantitatively describe the growth of vegetable crops or organs is the use of growth functions. These allow quantifying the dry or fresh weight W as a function of time t. The derivative of this relationship at any point in time yields the actual growth rate. Therefore, growth functions can be developed from equations for growth rate by integrating them over time (Hunt, 1982). For example, if the growth rate g of a plant or crop is constant, the integral yields a linear function of weight W depending on time t (Table 5.1). The assumption of a constant *relative* growth rate leads to the exponential growth function, and if the growth rate is proportional to the "growth still to be made'," i.e. the difference between the actual and the final weight, we obtain the monomolecular function.

Table 5.1. Some common growth functions for weight W as a function of time t

	Growth rate	Growth function	Parameters
Linear growth	$\frac{dW}{dt} = g = const.$	$W = W_0 + gt$	g: growth rate
Exponential growth	$\frac{dW}{dt} = rW$	$W = W_0 e^{rt}$	r: relative growth rate
Monomolecular growth	$\frac{dW}{dt} = a(W_f - W)$	$W = W_f(1 - be^{-at})$	W_f: final weight a: parameter describing the intensity of resource acquisition $b = 1 - W_0/W_f$
Logistic growth	$\frac{dW}{dt} = \frac{a(W_f - W)}{W_f}W$	$W = \frac{W_f}{1 + be^{-at}}$	W_f: final weight a: parameter $b = W_f/W_0 - 1$
Richards	$\frac{dW}{dt} = \frac{a}{c}\frac{W_f - W^c}{W_f}W$	$W = \frac{W_f}{(1 \pm be^{-at})^{\frac{1}{c}}}$	W_f: final weight a, b, c: parameters
Gompertz	$\frac{dW}{dt} = a(-lnW)W$	$W = W_f e^{-be^{-at}}$	W_f: final weight a, b: parameters
Weibull	$\frac{dW}{dt} = abt^{b-1}(W_f - W)$	$W = W_f(1 - e^{-at^b})$	W_f: final weight a: scale parameter b: shape parameter
Expo-linear	$\frac{dW}{dt} = r_m W$	$W = \frac{c_m}{r_m} ln\left(1 + e^{r_m(t - t_0)}\right)$	c_m: max. growth rate r_m: max. relative growth rate t_0: "lost time"

Sources: Hunt (1982), Goudriaan and Monteith (1990).

The logistic growth function combines both assumptions, the proportionality of the growth rate to the existing weight and to the growth still to be made.

In plants or plant organs, linear, exponential, or monomolecular growth of crops can be observed only for limited periods of time. Exponential growth occurs when the size of the growing organ or organism is more limiting than the resources necessary for growth, which is the case during early phases of growth. In contrast, monomolecular growth takes place towards the end of the growing period of an organ or organism, and between exponential and monomolecular phases growth is often nearly linear for some time. The sigmoidal growth functions (e.g. the logistic, Gompertz, Weibull, and Richards function) allow us to describe such situations.

Application examples include the use of the logistic function for describing fruit production of zucchini and sweet pepper (Lúcio *et al.*, 2015); the Gompertz function proved to be a good model to predict growth of lettuce (Tei *et al.*, 1996a) and to describe fruit growth of tomatoes at different temperatures (Fig. 5.4).

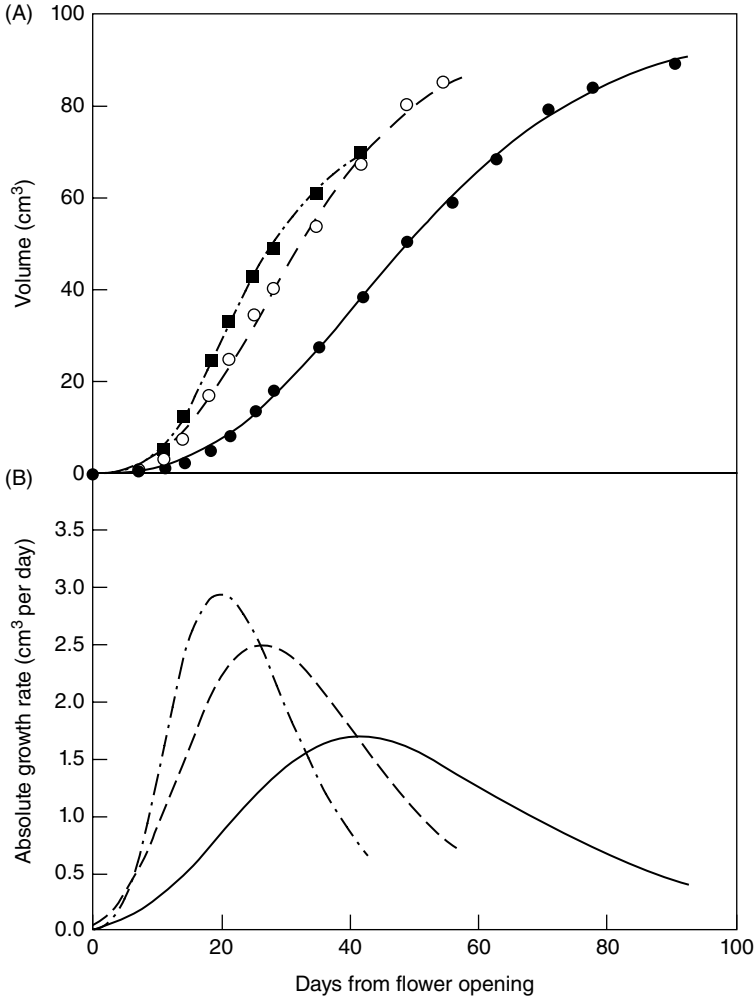


Fig. 5.4. The effect of heating or cooling individual trusses on growth (A) and absolute growth rates (B) of tomato fruit grown at 15(●, ——), 20(○,- - -), and 25°C (■,— - —). Points represent the mean volume of ten fruits calculated assuming fruits are spherical. Lines represent a Gompertz function (Adams *et al.*, 2001).

The Gompertz function was also applied to describe the cumulative yield of *Phaseolus* beans grown as a winter crop under different climatic conditions in Spain (López *et al.*, 2008). The function parameters could be related to the mean daily air temperature and the radiation integral over the harvest period. The Richards function was used for various processes of matter accumulation in vegetable systems, for example, to quantify fruit growth of tomato (Heuvelink and Marcelis, 1989) or nitrogen uptake by a number of vegetable crops (Feller and Fink, 1996). In addition to the above, Faridi *et al.* (2015) presented four novel sigmoid growth functions, namely Pareto, extreme value distribution, Lomolino, and cumulative β-P distribution, which so far have not been used in applications with (vegetable) crops.

Vegetables are often harvested during their linear growth phase, i.e. they go only through the exponential and linear growth phases and do not reach the phase of decreasing growth rates as, for example, cereals that are harvested at physiological maturity. This combination of exponential and linear and growth phases is represented by the expolinear growth function (Goudriaan and Monteith, 1990; Monteith, 2000), which has been used for growth prediction of peas and faba beans (Dennett and Ishag, 1998), pak-choi (Cho *et al.*, 2015), onions and red beet (Tei *et al.*, 1996a), lettuce and cauliflower (Aikman and Scaife, 1993), sweet pepper (del Amor *et al.*, 2008; Kim *et al.*, 2013), cucumber (Gómez-López *et al.*, 2004), and potato (Yuan and Bland, 2004).

The simplicity of empirical crop models is advantageous for rough prediction but often raises criticism of lacking physiological and mechanistic insights. Although the parameters of an empirical crop model may be physiologically interpretable (Table 5.1), they incorporate the effects of the environmental conditions, in which they are determined, on the physiological functions. This means that the model parameters can be only applied to a restricted range of environments and parameter differences between genotypes can be the result of complex interactions between genotype and environment.

Mechanistic crop models

Mechanistic crop models describe the processes of crop growth and development at various levels of detail. Process-based simulation modeling of crop growth became possible by the end of the 1960s when computer power allowed the synthesis of crop physiological processes (Bouman *et al.*, 1996). The early models described growth predominantly on the level of "potential," i.e. not limited by water and nutrients, nor reduced by pests, weeds, or diseases. Dry matter production as the result of photosynthesis and respiration, and dry matter partitioning as a function of light and temperature were the main processes (Fig. 5.5). From about the 1980s on, limiting factors such as water or nitrogen were given increasing attention by modelers. Evapotranspiration and nitrogen uptake and partitioning had to be coupled with soil processes such as water transport and nitrogen mineralization. More recent developments comprise effects related to climate change (CO_2, heat) and genetic effects.

Light interception and light use efficiency

Light energy is the driving force of plant growth. Models of light distribution in plant canopies provide the information necessary to calculate dry matter production or photosynthesis. In principle, light can either be absorbed, transmitted, or reflected by the green surfaces in a plant canopy. A major contribution to our understanding came in 1953 from the finding by Monsi and Saeki (2005) showing that light behaves in homogeneous canopies according to Beer's law (Beer, 1852), meaning that the fraction T of light being transmitted through a canopy depends on leaf area index penetrated, L, and the light extinction coefficient k:

$$T = \frac{I}{I_0} = e^{-kL} \tag{7}$$

where I and I_0 are the light intensities below and above leaf area index L.

The light extinction coefficient is an empirical dimensionless factor characterizing the uptake efficiency of a unit of leaf area, and typically ranges between 0.5 and 0.8 in vegetable crops, for example, 0.66 and 0.68 have been found for lettuce and red beets (Tei *et al.*, 1996b), 0.71 in a young tomato canopy and 0.45 in a closed tomato canopy (Chen *et al.*, 2014b), and 0.55 for cauliflower (Kage and Stützel, 1999). Ignoring light reflection from the canopy surface, the sum

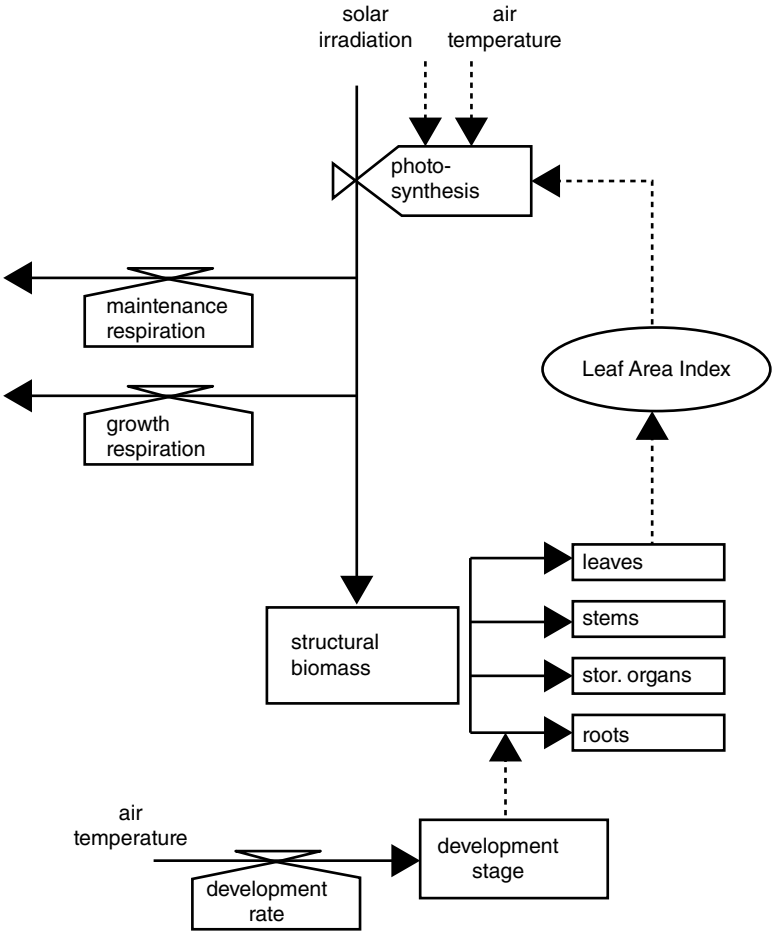


Fig. 5.5. Diagram of the relations in a typical "School of de Wit" crop growth model (SUCROS) for potential production. Boxes indicate state variables, valves indicate rate variables, circles auxiliary variables, solid lines (arrows) the flow of matter and dotted lines the flow of information (Bouman *et al*., 1996).

of relative light transmission, T, and relative light interception, Q, equals 1, thus T and Q may assume values between 0 and 1. This simple approach of calculating light interception holds for most field vegetable crops, as their canopies, at least in later stages of development, meet the assumption of a homogeneous medium. For distinct row crops or other heterogeneous canopies, however, complex radiation transfer models that are based on separation of the canopy into small elements are more appropriate (Chelle and Andrieu, 1999; Röhrig *et al*., 1999).

Multiplication of Q with the radiation integral over a certain period of time, S, results in the light interception integral which together with light use efficiency ε is in many models used to calculate dry matter production of a canopy over a given period (Monteith, 1977):

$$\frac{dW}{dt} = \varepsilon QS \tag{8}$$

Light use efficiency is an empirical value, ranging typically between 2 and 4 g dry matter per MJ photosynthetically active radiation intercepted (eg. for cauliflower–Kage and Stützel, 1999). This corresponds to the 5–8 g CO_2 fixed MJ^{-1} reported by Warren Wilson *et al*. (1992) for ambient CO_2 concentrations. Gallardo *et al*. (2011) report light use efficiencies as high as 5g MJ^{-1} PAR for the vegetative phase of muskmelon

in Spain, which decreased to 3.2 g MJ^{-1} in the generative phase. For greenhouse cucumbers and tomatoes grown under 1200 ppm CO_2, light use efficiencies of 5–8 and 7–10 g CO_2 MJ^{-1} are reported under ambient and enriched (1200 ppm) CO_2 concentrations, respectively (Warren Wilson *et al.*, 1992). Light use efficiencies also tend to increase with decreasing light intensity. For cauliflower crops growing under mean PAR intensities between 5 and 9 MJ m^{-2} d^{-1}, calculated light use efficiencies ranged between 3 and 4.2 g MJ^{-1} (Kage *et al.*, 2001). Differences in mean PAR between experimental sites can be the reason for large variations of the reported ε values in one and the same crop species (Chen *et al.*, 2019). Van Oijen *et al.* (2004) derived a simple relationship relating light use efficiency to light intensity I_0:

$$\varepsilon = \frac{\alpha\gamma}{1+\frac{\alpha k I_0}{P_{max}\delta}} \tag{9}$$

where α is the quantum efficiency of photosynthesis, γ is the conversion efficiency from CO_2 to dry matter, k is the light extinction coefficient, P_{max} the photosynthetic capacity and δ the photoperiod duration. Differences in mean PAR between experimental sites where ε is estimated can be the reason for the large variations of the reported ε values in a crop species.

A model for the prediction of harvest date of broccoli simulates the growth of individual plants as a function of light interception and light use efficiency (Lindemann-Zutz *et al.*, 2016a, 2016b). To estimate the start of inflorescence growth it utilizes an optimum function of temperature to predict vernalization. Aboveground dry matter produced is partitioned into stems, leaves and inflorescences. Harvest begin is defined to occur when the fresh weight of inflorescence and a 10 cm stem section reach a total weight of 500 g. Harvestability of a plant ends when the inflorescence fresh weight exceeds 500 g. The variation in head weight is generated in the model by assuming a normal distribution in vernalization rates.

Photosynthesis

Photosynthesis is usually measured as CO_2 uptake with gas exchange equipment such as the Li-Cor 6800 (Stinziano *et al.*, 2017) on the leaf level. Net photosynthesis rate P_n can be expressed as a function of the limiting factors, light intensity I incident on the leaf, and ambient CO_2 concentration C. A simple function easy to parameterize is the rectangular hyperbola (Acock *et al.*, 1978):

$$P_n = \frac{\alpha I \tau C_a}{\alpha I + \tau C_a} - R_d \tag{10}$$

where α is the light utilization efficiency, τ is leaf conductance to CO_2 transfer and R_d is day respiration rate. However, crop physiologists are often interested in the performance of the entire canopy and therefore single leaf photosynthesis needs to be integrated over the leaf area of the canopy to obtain canopy photosynthesis rate P_c:

$$P_c = \frac{\tau C_a}{k} ln \frac{\alpha k I_0 + (1-m)\tau C_a}{\alpha k e^{-kL} + (1-m)\tau C_a} - R_c \tag{11}$$

Io here describes the light intensity incident on the canopy surface, k is the canopy light extinction coefficient and m is the leaf transmission coefficient (Acock *et al.*, 1978). A major shortcoming of this approach is the assumption that all leaves in the canopy have the same properties, for example, the same photosynthetic capacity which is clearly not realistic. The fact, that (photosynthetic) protein concentration decreases from canopy top to bottom leaves, together with the assumption that photosynthetic protein concentration is proportional to photosynthetic capacity is represented in the canopy photosynthesis model of Johnson *et al.* (2010). Recently, a mechanistic model describing the protein turnover (synthesis and degradation) has been proposed to simulate the concentration of photosynthetic protein into the canopy depth (Pao *et al.*, 2019). In this model, protein synthesis rate of a leaf depends on the light energy received by the leaf and the nitrogen availability.

For simulating C_3 photosynthesis on the basis of biochemical processes, the model of Farquhar *et al.* (1980), often referred to as the FvCB model, has become a standard. It considers CO_2 assimilation rate A to be the result of the two processes catalyzed by Rubisco, carboxylation and oxygenation, and day respiration, taking place at rates V_c, V_o and R_d, respectively (Table 5.2). Photosynthesis rate is considered limited by either Rubisco (RuBP saturation), RuBP electron

Table 5.2. Additional equations of the FvCB model for C_3 photosynthesis (Caemmerer, 2013).

Assimilation rate	$A = V_c - 0.5V_o - R_d$
	$A = V_c(1 - 0.5\phi) - R_d$
Ratio of oxygenation to carboxylation φ:	$\phi = \frac{V_o}{V_c} = \left(\frac{1}{S_{c/o}}\right)\frac{O}{C_c} = \frac{V_{omax}}{K_o}\frac{K_c}{V_{cmax}}\frac{O}{C_c}$
Chloroplast CO_2 concentration for A = 0 (when R_d = 0), Γ^* (φ=2):	$\Gamma* = \frac{0.5O}{S_{\frac{c}{o}}} = \gamma * O$
	$\phi = \frac{2\Gamma *}{C_c}$
	$A = \left(1 - \frac{\Gamma *}{C_c}\right)V_c - R_d$
Photorespiration F (=0.5 oxygenation):	$F = \frac{\Gamma *}{C}V_c$
RuBP saturated rate of Rubisco	$W_c = \frac{C_c V_{cmax}}{C_c + K_c\left(1 + \frac{O}{K_o}\right)}$
RuBP saturated rate of CO_2 assimilation:	$A_c = \frac{C_c - \Gamma *}{C_c + K_c\left(1 + \frac{O}{K_o}\right)}V_c - R_d$

transport (RuBP limitation), or triose phosphate. Actual rate of photosynthesis A is the minimum of the three rates:

$$A = \min\{Ac, Aj, Ap\} \quad (12)$$

with
Rubisco limited A:

$$A_c = \frac{C_c - \Gamma *}{C_c + K_c\left(1 + \frac{O}{K_o}\right)}V_c - R_d \quad (13)$$

Electron transport limited A:

$$A_j = \frac{C_c - \Gamma *}{4C_c + 8\Gamma *}J - R_d \quad (14)$$

Triose phosphate limited A:

$$A_p = 3T_p - R_d \quad (15)$$

where C_c and Γ^* are the chloroplastic and compensatory (when A_c = 0 if R_d=0) CO_2 concentrations, O is the chloroplastic oxygen concentration, K_c and K_o are Michaelis-Menten constants for carboxylation and oxygenation, respectively, Tp is the rate of inorganic phosphate supply to the chloroplast, and J is the potential electron transport rate:

$$J = \frac{I_2 + J_{max} - \sqrt{(I_2 + J_{max})^2 - 4\theta I_2 J_{max}}}{2\theta} \quad (16)$$

where I_2 is the light absorbed by photosystem II (ca. 36% of the incident irradiance), J_{max} is the maximum electron transport rate and θ is a curvature factor. For details of the model see Caemmerer (2013). This model can be also extended for C_4 plants (Massad *et al.*, 2007). Gas-exchange in combination with chlorophyll fluorescence measurements have become a standard to estimate the parameters of the FvCB model. Despite the large number of methods proposed (e.g. Su *et al.*, 2009; Bellasio *et al.*, 2016; Moualeu-Ngangue *et al.*, 2017), it seems to exist neither a golden standard of measuring procedures nor for model parameterization.

Stomatal control

Since internal CO_2 concentration crucially depends on the opening of stomata, models predicting stomatal conductance are a prerequisite for biochemical photosynthesis models. Stomatal control is one of those fields where substantial

biophysical process knowledge is available, but the "control strategy" on the plant level is still speculative. A widely used approach is the semi-empirical Ball-Berry-Leuning model (Ball *et al.*, 1987; Leuning, 1995):

$$g_{sc} = g_0 + \frac{a_1 A}{\left(C_s - \Gamma^*\right)\left(1 + \frac{D_s}{D_0}\right)} \tag{17}$$

where g_{sc} is stomatal conductance for CO_2, A is the assimilation rate; D_s and C_s are the humidity deficit and the CO_2 concentration at the leaf surface, respectively; g_0 is the conductance as $A \rightarrow 0$, and D_0 and a_1 are empirical coefficients. Under drought stress, stomata close under the influence of root signals (Davies and Zhang, 1991). Of three model approaches tested to estimate the effects of drought stress on cauliflower crops, a simple empirical approach based on a linear-and-plateau relationship between stomatal resistance r_s and soil water potential ψ_S in the root zone resulted in the best model efficiency (Kochler et al., 2007):

$$r_s = \begin{cases} r_{s,min} & \psi_S \geq \psi_{Srs} \\ r_{s,min} + m_{Srs} \log(|\psi_S|) & \psi_{Srs} > \psi_S > \psi_{PWP} \\ r_{s,min} & \psi_S \leq \psi_{PWP} \end{cases} \tag{18}$$

where $r_{s,min}$ and $r_{s,max}$ denote minimum and maximum stomatal resistances, respectively, ψ_{Srs} denotes the soil water potential threshold at which stomata begin to close, and $m_{Srs} = r_{s,max}/\log(|\psi_{PWP}|)$. More than 35 empirical stomatal models have been proposed, as summarized in Damour *et al.* (2010).

To overcome the empirical part of present approaches, research tries to combine process-based and goal-directed "optimality" approaches (Buckley, 2017). An example of this is the model of Sperry *et al.* (2017) that calculates stomatal conductance from the maximum difference between photosynthetic "gain" and hydraulic "cost" functions.

Dry matter partitioning

In most models, dry matter partitioning is the process which distributes the dry matter from a dry matter or assimilate pool into the individual organs. Many models partition dry matter with empirical factors, often dependent on ontogeny (eg. Pearson *et al.*, 1997; Soltani and Sinclair, 2011) or based on allometric relationships (Stützel *et al.*, 1988; Kage and Stützel, 1999; Stützel and Aufhammer, 1991). A more functional approach is the concept of source- and sink-driven partitioning. Assuming that fruit growth is sink limited, dry matter partitioning to individual cucumber fruits was modeled by multiplying the total available assimilate flux with a partitioning factor for each fruit (Heuvelink and Marcelis, 1989; Marcelis, 1994). Partitioning factors f_i were calculated as the fractions of the individual fruit's sink strength to the total sink strength of all growing organs. The sink strength S_i of an organ i can be defined as its potential, i.e. not source limited, growth rate:

$$f_i(t) = \frac{S_i(t)}{\sum S_i(t)} \tag{19}$$

Experimentally, this source-unlimited growth rate has been determined by pruning competitive sinks, for example by leaving only one fruit per truss in tomato (Li *et al.*, 2015) to make sure that assimilate supply is not limiting. The potential growth rates for each organ can then be modeled over (thermal) time deriving a sigmoidal function like the Richards (Heuvelink and Marcelis, 1989; Marcelis, 1994) or the Gompertz (Li *et al.*, 2015) function. Wiechers *et al.* (2011) obtained more realistic results for dry matter partitioning in cucumber when they included fruit abortion and the dominance of older (bigger) fruits over younger (smaller) ones. Abortion was modeled to occur when the ratio A_T between the assimilates available for fruit growth and the sum of the sink strengths fell below a certain threshold. Likewise, dominance was assumed when the above ratio fell below a threshold D_T. Simulations obtained best results with thresholds D_T= 80% and A_T=30% (Wiechers *et al.*, 2011).

In asparagus, length growth of individual spears was modeled as a function of soil temperature around the spear tip, the spear length, and the soluble carbohydrate concentration of the storage root system (Graefe *et al.*, 2010). To include the effects of local growing conditions of individuals plant organs and their spatial relationships, the source-sink concept for partitioning has been incorporated into functional-structural plant models (FSPM, see below). This

has been shown for example in kiwi (Cieslak *et al.*, 2011) and peach (Allen *et al.*, 2005).

Nutrient dynamics

Estimating nitrogen fertilizer requirement to improve fertilizer application has been the objective of several models. The N_ABLE model (Greenwood, 2001) calculates a potential maximum nitrogen uptake rate based on the plant growth rate (modeled with a temperature sum function) and a critical nitrogen concentration, i.e. the plant N concentration necessary for optimal growth (Fig. 5.6). For these critical nitrogen concentrations N_{crit}, empirical power functions of plant dry weight W are common:

$$N_{crit} = a_c W^{-b} \quad (20)$$

where a_c and b are empirical coefficients (Table 5.3).

This potential actual nitrogen uptake rate is reduced as dependent on the available soil N concentration, which is the sum of the nitrogen released through mineralization of soil organic matter and fertilizer application. A significant part of the model deals with root growth and soil processes like N mineralization and transport. The N_ABLE model has been extended and revised with respect to routines for root development, N mineralization and water dynamics in the soil to model nitrogen dynamics in vegetable crop rotations (Rahn et al. 2010). This *EU-Rotate_N* model has been tested for various conditions (Guo *et al.*, 2010; Rahn *et al.*, 2010; Nendel *et al.*, 2013). While nitrogen uptake was usually predicted with sufficient accuracy, mineralization

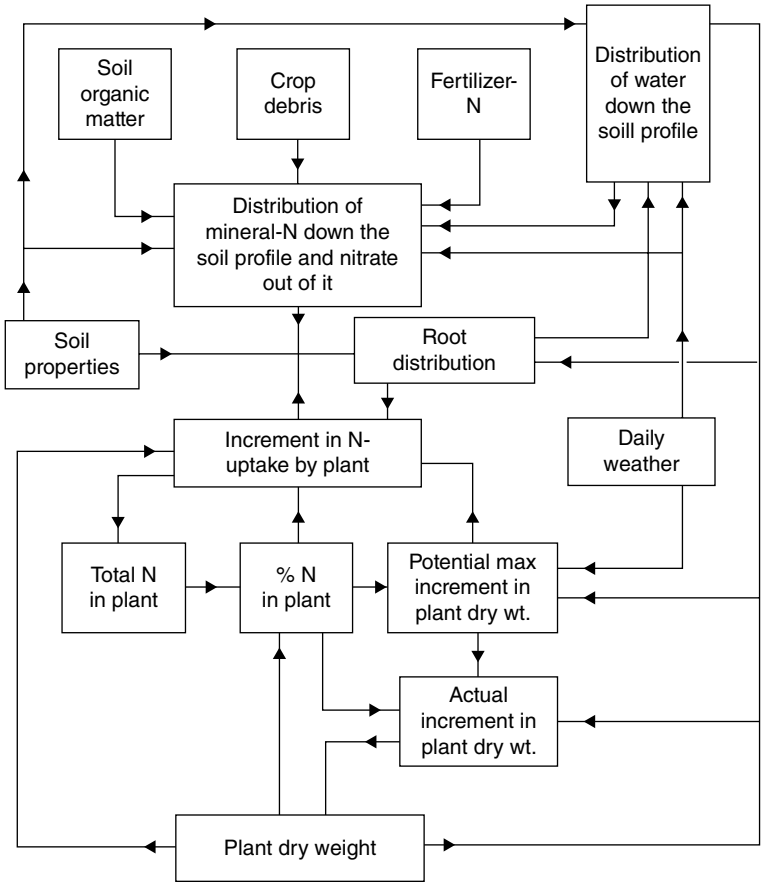


Fig. 5.6. Simplified flow diagram of the processes in N_ABLE. Boxes represent variable quantities or groups of quantities; lines and arrows represent the interdependence of the variables (Greenwood, 2001).

Table 5.3. Values for a_c and b in eqn. (19) to estimate critical nitrogen concentrations in some vegetable crops.

Species	a_c	b	Source
Pea	5.1	0.32	Ney *et al*. 1997 in Lemaire *et al*., 2008
Tomato	4.5	0.33	Tei *et al*., 2002
Muskmelon	7.55	0.126	Gallardo *et al*., 2011
Maize	3.4	0.37	Plénet and Lemaire, 2000

appears to be a challenge so that measurements of mineral soil N still are recommended (Nendel *et al*., 2013). The N_ABLE model is also the basis for the fertilizer recommendation model WELL_N (Rahn *et al*. 1996). The nitrogen fertilization model N-Expert (Fink and Scharpf, 1993; Feller and Fink, 1996) calculates fresh matter accumulation curves of a wide variety of vegetable species on the basis of empirical data and yield expectations provided by the user. Together with an empirical function of plant nitrogen concentrations, nitrogen requirements are calculated. Mineral N supply by the soil, either measured or simulated, is subtracted to give nitrogen fertilizer requirements. For simulating the nitrogen dynamics in greenhouse tomatoes and cucumbers, the uptake functions of N_ABLE were combined with the field soil-crop system model "Water Heat Carbon Nitrogen Simulator" (WHCNS) (Liang *et al*., 2018). Interestingly, sensitivity analyses indicated that the model had a higher sensitivity to soil hydraulic than to nitrogen turnover parameters.

Modeling spatial aspects of plant growth

Most plant growth models assume the whole canopy consisting of one leaf and do not explicitly consider plant morphology and the heterogeneity of environment and physiological function in the canopy (Fourcaud *et al*., 2007). The sunlit-shaded model improves this assumption by separating a plant canopy into two parts, one of which, shaded by the other, intercepting low light and having less photosynthetic proteins. This approach motivates the use of a multilayer model, where the canopy is divided into an arbitrary number of leaves with physiological properties similar to the gradient observed along the canopy depth. Structural plant models, however, describe the three-dimensional structure of a plant, thus allowing the representation of interactions between individual plant organs (or parts thereof), for example, with respect to carbon partitioning and apical dominance (Cieslak *et al*., 2011), and with their individual physical environment, such as single-leaf photosynthesis (Chen *et al*., 2014a, 2014b). These combinations of structural plant models with models for physiological processes of growth and development are referred to as functional-structural plant models, FSPM.

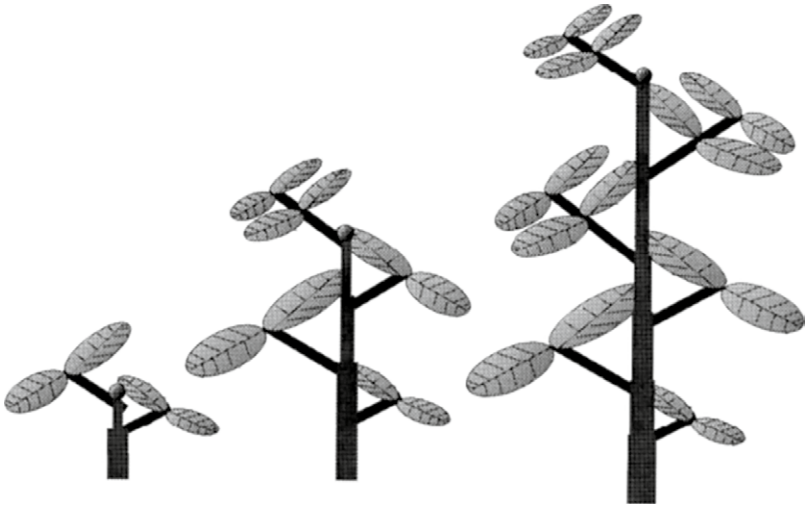
The basis for modeling plant structures is the definition of the morphological units, such as the organs, and their connections, the topological body plan of a plant (Vos *et al*., 2010). A powerful approach to model plant structures is the use of Lindenmayer systems (L-systems), a mathematical formalism introduced by Aristid Lindenmayer (1968). Briefly, L-systems are based on so-called production rules, which define what happens during each step of plant growth. The initial stage is called an axiom. For example (Diaz-Ambrona *et al*., 1998), if we start with an apical bud as an axiom and apply the production rules at each time step that each apical bud A forms an internode I, a leaf to the left +F and a leaf to the right −F, as well as a new apical bud, after 1, 2 and 3 time steps a plant would result as shown in Fig. 5.7.

I[+F]I[−F]A

I[+F]I[−F]I[+F]I[−F]A

I[+F]I[−F]I[+F]I[−F]I[+F]I[−F]A

In a parametric L-system, each individual component is assigned with a list of parameters or variables. This allows one to specify conditions for and intensities of morphological changes. For example, to model the phototropic movement of greenhouse cucumber leaves, differences in the red to far-red (R:FR) ratio between the two halves of a leaf were taken, as driving force and leaf movement was to occur until the difference in R:FR ratio between both halves was zero (Kahlen *et al*., 2008). That way, an important morphological adaptation influencing light interception could be simulated (Fig. 5.8).



I[+F]I[–F]A I[+F]I[–F]I[+F]I[–F] A I[+F]I[–F]I[+F]I[–F]I[+F]I[–F]A

Fig. 5.7. Visualization of a development sequence generated by an L-system. The images shown represent from left to right steps 1 to 3 of the main stem. The character below the pictures show the L-system description with I denoting an internode, F a leaf, and A an apical bud; square brackets denote that the element branches off the main axis, and + or – denote a turn to the left or right, respectively (Diaz-Ambrona *et al*., 1998).

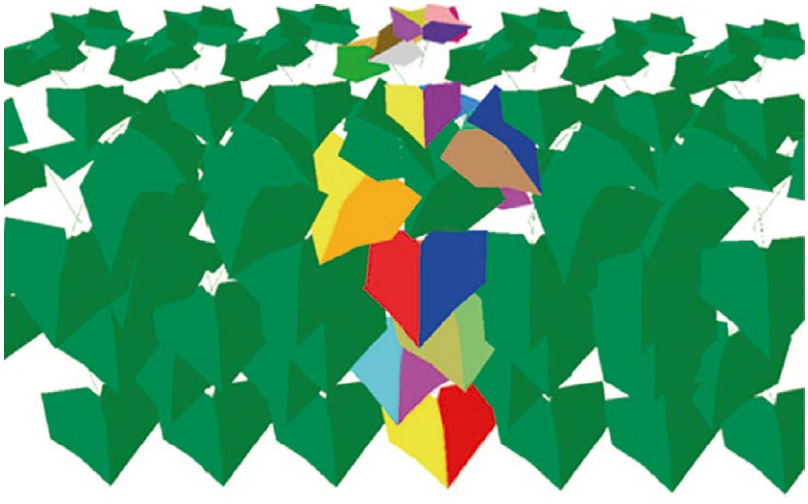


Fig. 5.8. Simulation of a cucumber canopy with the option for leaf tropism. The center plant is colored individually because it represents a typical canopy plant. Lower (older) leaves have aligned towards the inter-row space, whereas upper (younger) leaves are still in the process of aligning (Kahlen *et al*., 2008).

The construction of FSPM has several components (Fig. 5.9): to parameterize the architecture model, 3D data have to be obtained which may be through optical scanning or digitizing in an electromagnetic field (Kahlen and Stützel, 2007). The virtual canopy is then set up with graphical software like L-studio (Prusinkiewicz, 1998; Prusinkiewicz *et al*., 2000) or GroImp (Kniemeyer and Kurth, 2008). With raytracing, light interception is simulated, which is the basis for modeling matter production, for example, photosynthesis with the FvCB model. Comparison

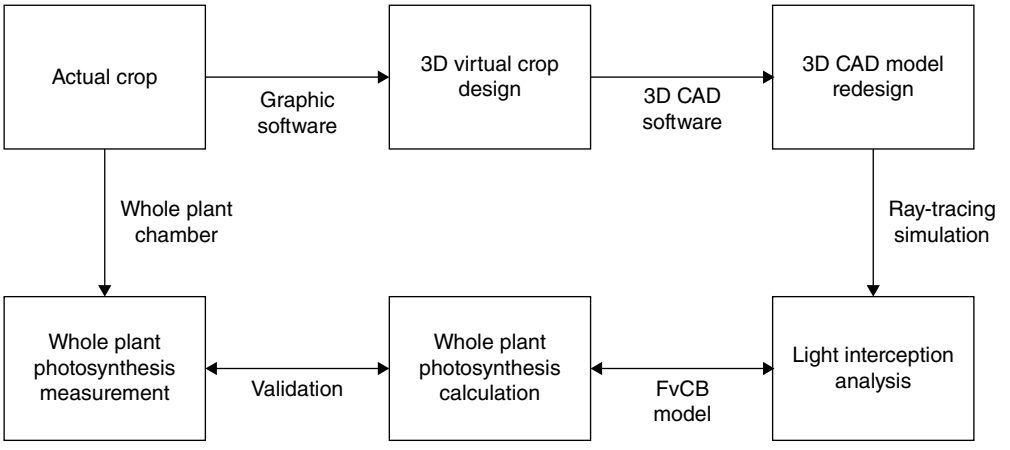


Fig. 5.9. A work flow for construction of a 3D plant model, calculation, and validation of whole plant photosynthesis rate for sweet pepper (Kim *et al*., 2016).

of simulated and measured photosynthesis shows whether the modeling efforts gave a good picture of reality.

Root growth and architecture

Modeling water and nutrient uptake under limited supply requires information on root distribution. A widely used empirical model (Gerwitz and Page, 1974) describes the percentage of roots P above a certain depth x:

$$P = 100(1 - e^{-fx}) \tag{21}$$

where f is a parameter. A two-dimensional model distributing roots with a diffusion equation showed good correspondence with measured data for tomato grown in a rockwool substrate (Heinen *et al*., 2003). However, the rooting depth investigated was only ca. 12–14 cm.

Similar to shoot morphogenesis, root architecture can also be described three-dimensionally with models like ROOTMAP (Diggle, 1988), SimRoot (Lynch *et al*., 1997), RootTyp (Pagès *et al*., 2004), SPACSYS (Wu *et al*., 2007), R-SWMS (Javaux *et al*., 2008) or RootBox (Leitner *et al*., 2010). Root elongation, direction and branching in the three-dimensional space are modeled as dependent on soil temperature, impedance, water, and nutrient content (Dunabin *et al*., 2013). It is not trivial to determine the time step of a root growth model. Whereas the concept of thermal time is widely adopted in shoot models, root growth rates of many existing root models are described as length per day, without considering temperature effects on root growth (e.g. SimRoot and RootBox). Due to the difficulties in root measurements, the responses of root growth rate to temperature remain largely unexplored and impede temperature-driven approaches to root growth modeling.

Genomic models

Parameters of crop models may be valid irrespective of crop species, or they may be species- or even genotype- (cultivar-) specific. Experimental determination of genotype-specific parameters over a range of cultivars is tedious and time consuming. Therefore, it appears logical to use the increasingly available genetic information for model parameterization. This is the background of what is generally referred to as "genomic modeling."

A good example to illustrate this is a model for leaf expansion rate dL/dt in maize as a function of meristem temperature T above a base temperature T_0, air vapor pressure deficit VPD and soil water potential Ψ (Reymond *et al*., 2003):

$$\frac{dL}{dt} = (T - T_0)(a + bVPD + c\Psi) \tag{22}$$

For model parameters a, b and c, 9, 6 and 7 quantitative trait loci, QTL (Miles and Wayne, 2008), respectively, were identified, and the values for the effects of these QTL on the parameter

values were estimated. Then, the model parameters for individual genotypes were calculated based on presence or absence of QTLs and their effect values, and leaf expansion rate was modeled with almost the same accuracy as with the original parameters. Similarly, flowering time of the lines of a *Brassica oleracea* population could be predicted based on nine QTL for three parameters of a QTL-based model (Uptmoor *et al.*, 2008).

In addition to providing more efficient ways for parameter estimation, genomic modeling is expected to improve the understanding of genotype × environment interactions (Bertin *et al.*, 2010). The basic idea is that QTL information describes the genotype including its reaction pattern to environmental factors, whereas environments are described by varying soil and climate inputs. It is important to keep in mind that genetic parameters in a QTL-based model are assumed to be intrinsically independent of the environment and the genotype-by-environment interactions are emergent properties of the process-based model. This assumption does not always hold true, especially for those parameters involved in complex physiological processes (Tardieu *et al.*, 2018). For example, the allelic effects of QTL of stomatal conductance have been considered a function of environmental variables (Alvarez-Prado *et al.*, 2018). The virtue of computer-based crop models is to allow the simulation of a wide range of genotypes and environments, for example, as a basis for adaptation studies.

Functional-structural plant models can also be used to estimate growth parameters on the organ and plant level. Determining these parameters for a (large) number of genotypes allows the estimation of QTL through a genome-wide association study (GWAS). Recent examples for such a combination of FSPM and GWAS (Fig. 5.10) are the *TraitCapture* platform (Brown *et al.*, 2014) and the *Phenomenal* pipeline (Chen *et al.*, 2019).

Modeling produce quality

In vegetables, important external quality traits are size, shape, sugar content, color, or surface damage of the marketed organs. Mechanistic descriptions of organ growth comprise biophysical processes like cell division, cell extension, water and dry matter transport, as in models of fruit growth (Fishman and Génard, 1998). Color of tomatoes after harvest was simulated with a first-order kinetic function of color and color

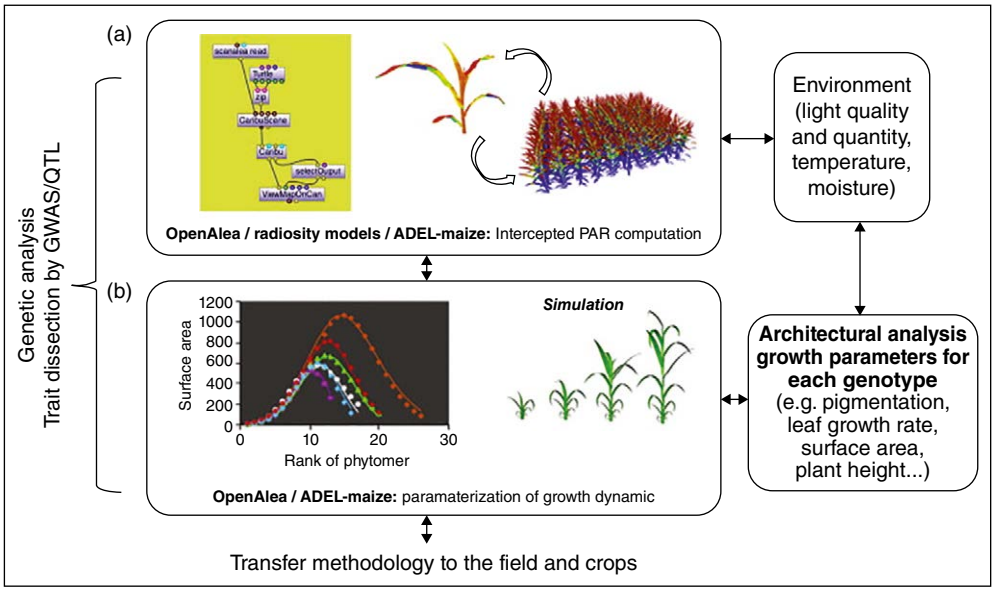


Fig. 5.10. (a) Functional plant models such as OpenAlea incorporate environmental factors as input along with (b) Structural models that include plant architecture to predict phenotype from environment and genetic variation. Figure adapted from Fournier and Andrieu (1999); (Brown *et al.*, 2014).

precursor content at harvest and storage time; likewise, firmness was considered a function of firmness and age at harvest and storage time (Schouten *et al.*, 2007).

Respiration and transpiration are important processes determining produce quality postharvest. For chemical processes like respiration and fermentation, a Michaelis-Menten approach as shown for tomato and chicory is obvious (Hertog *et al.*, 1998). Respiration can be described without inhibition by CO_2, with competitive, uncompetitive, or non-competitive (a 1:1 combination of competitive and uncompetitive) inhibition (Hertog *et al.*, 1998).

Glucosinolate synthesis can be modeled, subdivided into the biosynthesis of the core glucosinolate structure and methionine chain elongation using a Michaelis-Menten approach (Knoke *et al.*, 2009). Glucosinolate breakdown in broccoli could be predicted using exponential decay functions with rate constants for the formation of myrosinase and the decay of glucosinolates (Schouten *et al.*, 2009). The model was coupled with a gas exchange model (O_2 consumption and CO_2 production) and parameterized for varying CO_2/O_2 ratios in order to quantify the effects of modified atmosphere packaging.

Conclusion and Outlook

Over the past five decades, crop modeling has become an established field in crop science with activities on virtually all major crops and over all scales from molecule to ecosystem. A major deficit is incompatibility and incomparability of most models due to differences in model philosophies which translate into model structures, scale, and programming technology. A standardized framework of connectable tools and modules as is attempted in the "crops *in silico*" initiative (Marshall-Colon *et al.*, 2017) could therefore increase the power of modeling in answering biological questions on different systems levels. While it has been demonstrated that similar approaches on the cellular level in "systems biology" have facilitated the collaboration within the community, the challenge remains whether these approaches will leave the "biology ivory tower" and enter the sphere of agriculture and horticulture. Only by including processes of human interference and control models can the widening gap between scientific understanding and application in practical production be overcome.

From a practical point of view, larger production scales, increasing precision requirements for all operations in vegetable production and increasing complexity of the production chains call for better prediction instruments. The enormous progress in genetics and microelectronics, on the other side, generates more and more genotypic and phenotypic information which can be used in predictive modeling. The large quantities of sensor data gathered, for example by drones, need to be transformed into information. Making sense of "big data" requires models as tools to order and make practical use of complexity.

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6 Correlative Growth in Vegetable Plants

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Herbaceous annual vegetables grow by the allocation of carbon compounds to their organs through the processes of photosynthesis and respiration, primarily carried out in leaves, supported by uptake of water and mineral nutrients through the root system. The mechanisms by which the allocation of resources from these sources takes place, and how growth of the organs is regulated, can be termed "correlative growth."

Given the complexity of interactions that occur among leaves, roots, stems, and fruits, which act as sources in one instance, and sinks in another, it is difficult to study such an interacting system without introducing many artefacts. For instance, although partial leaf removal gives insight into the effect of leaves as sources, increases in the photosynthetic rate of the remaining leaf area may lead to erroneous assumptions of how many leaves are needed for optimum growth and yield (Wareing *et al.*, 1968). Feedback effects of sink removal may result in stomatal closure, accumulation of assimilates in leaves, and reduced productivity (Neales and Incoll, 1968; Plaut *et al.*, 1987). Fortunately, recent advances in top-root grafting (Venema *et al.*, 2017), and genetic manipulations of some vegetable plants and model plants like *Arabidopsis thaliana*, have greatly increased our understanding of how growth is controlled (Ruan, 2014; Ljung *et al.*, 2015). In this chapter, the interaction of plant parts above- and below-ground will be covered, followed by relations among vegetative and reproductive plant parts aboveground. Throughout, although examples will be taken from all over the plant kingdom, the emphasis will be on processes and mechanisms that may help improve productivity of vegetable crops.

Whole Plant Interactions

Our understanding of the regulation of plant growth, emphasizing the production, translocation, and use of carbohydrates, and root uptake of minerals and water, has grown significantly in recent years, aided by molecular tools (reviewed for tomato by Osorio *et al.*, 2014). In general, growth is directed by the interaction of sugars and plant hormones, influenced by environmental factors (Fig. 6.1) (Ljung *et al.*, 2015). Light, temperature, and other environmental factors are sensed by the aerial parts of the plant. This affects photosynthesis and the production of sugars, in turn regulating the levels of auxin (IAA) and the functioning of phytochrome interacting factors (PIFs). The latter are light-sensitive control factors also involved in sensing of circadian rhythm events (Ljung *et al.*, 2015). Cytokinins (CKs), gibberellins, and abscisic acid also affect growth, and these signaling pathways are linked with sugar and nutrient status. Cytokinins, IAA, and sugars function as long-distance signals, affecting for example, lateral root development and shoot branching.

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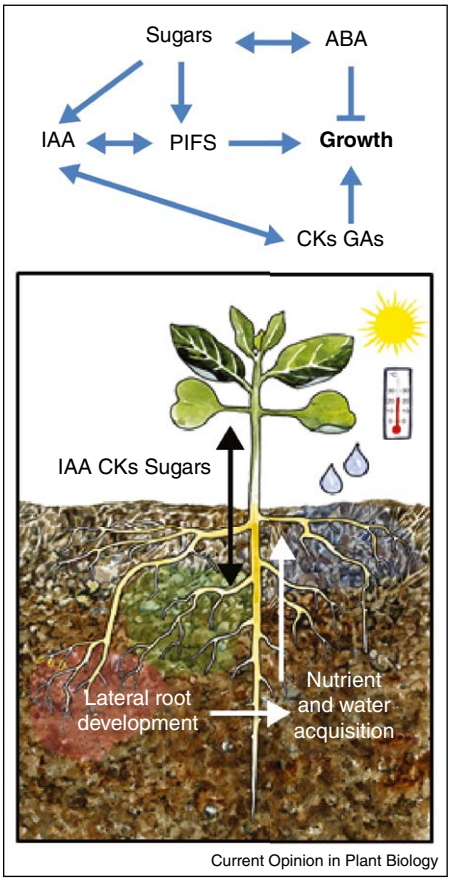

Fig. 6.1. Schematic diagram of the regulation of growth by sugars and growth hormones in plants. Abbreviations: ABA: abscisic acid; CKs: cytokinins; GAs: gibberellins; IAA: auxin, indole acetic acid; PIFS: phytochrome interacting factors (source: Ljung *et al*. 2015, with kind permission from Elsevier Science).

Auxins and sugars can be transported from shoot to root, inducing lateral root development to increase the uptake of water and nutrients from the soil, in turn increasing the growth capacity of the shoot. Signaling from root to shoot is also important for coordination of growth and development of the whole plant.

The central role of sucrose, the primary product of photosynthesis and chief compound translocated from source to sinks in the plant, has received significant attention (reviewed by Ruan, 2014). The activities of enzymes involved in sucrose metabolism play important roles in sugar partitioning and translocation, and their manipulation by molecular means has enhanced our understanding of ways in which crop productivity can be improved. For instance, by silencing a sugar partitioning protein in tomato leaves that reduces invertase activity, tomato fruit number and fruit weight was significantly increased, without altering plant biomass (Bermudez *et al*., 2014).

In the following paragraphs, examples of how plant organs interact, and our knowledge of how sugars and hormones are involved in growth regulation, will be given. In many cases, the examination of these interactions has led to realizations of the complexities involved.

Control of Branch Growth

In contrast to higher animals, plants begin postembryonic life in a very rudimentary state (Leyser, 2009). While animals develop before birth in a protected and relatively constant environment, higher plants are born with little of their final shape determined, and exposed to a changing environment that they cannot escape, lacking the capability to move. Adaptation in plants thus requires them to fit into that environment. The primary plant organs that bring about the plant's adaptation to above- and below-ground conditions are the shoot and root apical meristems. The shoot apical meristem has evolved to direct growth through stem extension and branching to optimize exposure to favorable light conditions, for instance. The root apex shifts growth toward water and nutrient sources, and to avoid physical obstacles.

Stem growth in the seedling stage of broadleaf plants is controlled by the vegetative apex, which forms a succession of phytomers, each consisting, in principle, of a node, an internode, a leaf, an axillary bud, and a root bud. It is not yet clear how the axillary meristems are formed, but recent research has indicated that they arise from quiescent zones between apex and leaf initials where cell division rates are low, and low levels of growth hormones exist (Wang *et al*., 2016).

The initiation of active growth of these dormant axillary buds has intrigued researchers for many years, for it allows the plant to more fully exploit the area in which it is growing, or to escape unfavorable growing conditions (Franklin, 2008;

Holalu and Finlayson, 2017). Bud growth is thus influenced by environmental factors such as the quantity and quality of light, and the amount of water and of the major mineral nutrient elements. The hormonal mechanisms of bud growth control will now be briefly described, and the way in which some of these environmental factors influence these mechanisms.

It has been known since the 1930s that control of the growth of dormant buds involves the

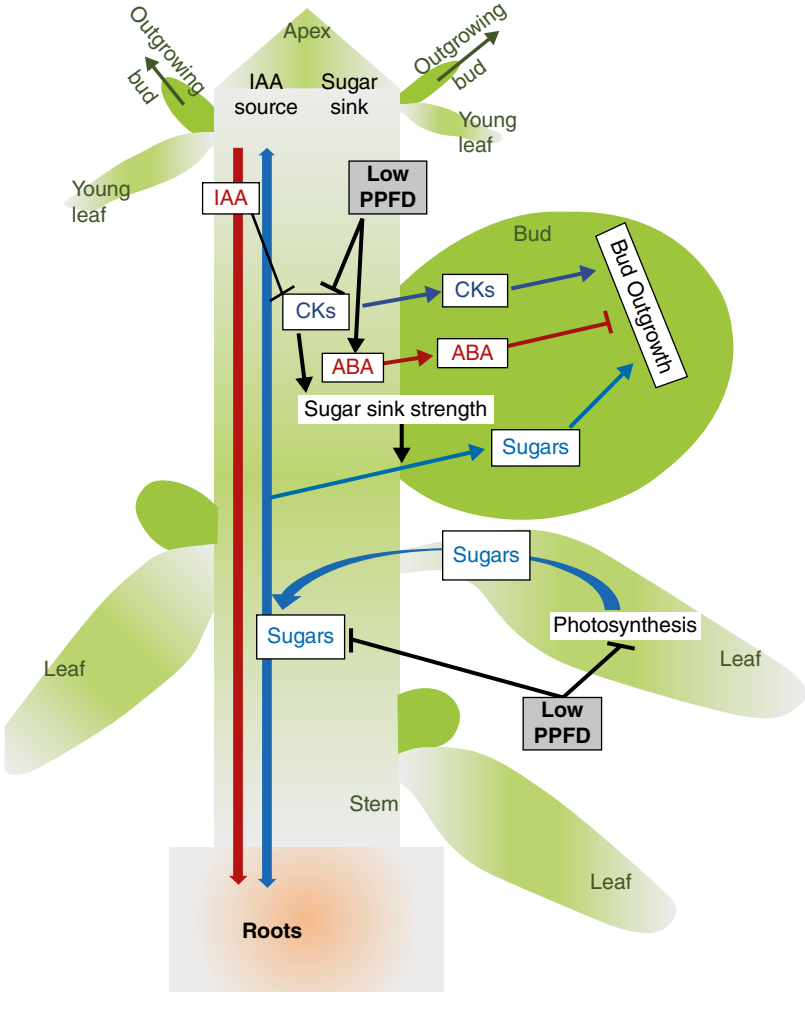

Fig. 6.2. Schematic representation of the relationships between CKs, ABA, IAA, and sugars in the regulation of bud outgrowth by PPFD (photosynthetic photon flux density or light) in an intact rose plant. Low PPFD reduces stem CK content, which appears to be a key limiting factor of bud outgrowth. The level of CKs transported from the stem to the bud, where they stimulate outgrowth, is likely to be dampened under low PPFD. The CK stimulation of bud sugar sink strength, shown in decapitated rose plants (Roman *et al*., 2016), may also be lowered. Low PPFD maintains a high stem ABA content, ABA presumably enters the bud where it inhibits outgrowth, antagonizing the effects of CKs. Low PPFD also reduces photosynthesis and stem sugar content. However, stem sugar content is not the main limiting factor for bud outgrowth since sugar supply is not able to override bud outgrowth inhibition under low PPFD. The reduced bud sugar sink strength under low PPFD may prevent the use of sugars by the bud for outgrowth. Although IAA is known to dampen stem CK content, low PPFD effect on CK is not mediated through changes in stem IAA content (source: Corot *et al*. 2017, by permission of *Frontiers in Plant Science*).

hormone auxin exuding down from the apical meristem (Thimann and Skoog, 1934) in a relationship termed "apical dominance." If the apex is removed, the buds start to grow, and can be inhibited again by applying auxin to the apical stump. Cytokinins can be applied directly to inhibited buds to stimulate their growth, but auxin application to growing buds does not stop their growth (Sachs and Thimann, 1967), implying that some other agent or condition is causing the inhibition. Bangerth (1994) showed that cytokinins produced by the roots are transported in the xylem stream to the rest of the plant, and provide the stimulus for bud growth when the polar auxin stream has been reduced after decapitation. When the apex is intact, cytokinin production is inhibited by the auxin (Muller *et al.*, 2015), and it is the absence of cytokinin at the bud that prevents bud growth.

Plants growing in the shade cast by others, or situated next to other plants at close spacing are exposed to light that is enriched in far-red wavelengths (725 nm) compared to red (650 nm) (low R:FR ratio). This change in light quality triggers a shade avoidance response characterized in part by an inhibition of branch growth (Tucker, 1977, 1981; Ballare *et al.*, 1991; Franklin, 2008; Holalu and Finlayson, 2017), and is another process regulated by the photoreversible compound phytochrome. In tomato, this bud growth inhibition was accompanied by an increased level of ABA in surrounding leaves and stem (Tucker, 1977). Tucker later found (1981) that a tomato variety with reduced branching tendency had higher stem ABA content than a normally-branching related line. ABA levels in buds of *Arabidopsis* transferred from low R:FR to high R:FR ratio decreased within three hours of the transfer (Holalu and Finlayson, 2017). In some species such as rose, the balance of ABA and cytokinins in the bud appear to control the initial growth of dormant buds (Corot *et al.*, 2017).

Another root-produced hormone has been found to inhibit bud growth. Strigolactone (SL) appears to act by inhibiting polar auxin transport in the plasma membrane of vascular-associated cells (Kohlen *et al.*, 2011; Shinohara *et al.*, 2013; Waldie *et al.*, 2014; Brewer *et al.*, 2015). By inhibiting auxin flow from inhibited buds, strigolactones also prevent the flow of cytokinin and growth compounds that would stimulate growth.

The complex interaction of stimulating and inhibiting factors in bud growth is illustrated by recent work with roses (Roman *et al.*, 2016; Corot *et al.*, 2017) (Fig. 6.2). Bud growth in roses is inhibited by darkness and stimulated by white light. The inhibition is accompanied by a rise in ABA and SL levels in the bud. Treatment with cytokinin overcomes the dark inhibition, but supplying sugars does not, indicating that sugar acts secondarily as an energy source. Other work with rose, and confirmed on peas (*Pisum sativum*) and *Arabidopsis*, indicated that sucrose acts as a signaling compound to stimulate bud growth in rose explants (Barbier *et al.*, 2015). Several non-metabolizable analogs of sucrose had similar effects. The finding that bud sucrose levels increased within two hours in pea plants that had just had their apex removed supports the important role of sugars in bud growth (Mason *et al.*, 2014).

Root-Shoot Relations in the Vegetative Stage

The growth of annual herbaceous plants after emergence and before flowering consists of the balanced development of above- and below-ground structures. The aerial parts consist of the leaves which form the main photosynthetic organs, borne by the stems and petioles. The plant is anchored by the roots, which also take up water and nutrients essential for growth. The roots depend on assimilates translocated from the leaves to supply the energy and carbon skeletons for growth and uptake of water and nutrient elements. Plant growth thus requires that both the above- and below-ground parts are developed in a balanced fashion, and that mechanisms exist to maintain that balance despite alterations in either or both environments.

Studies of the partitioning of assimilates to tops and roots early in the vegetative stage have commonly found that at a specific shoot/root temperature, allocation to shoots and roots is nearly constant (Brouwer, 1962a; Aung, 1974) (Fig. 6.3). If the balance is disturbed by leaf or root removal, growth of the affected part is accelerated, with the result that the balance is restored. Thus, defoliation leads to preferential growth of young leaves, rather than roots (Brouwer, 1962a;

Shishido *et al.*, 1993). Similarly, root pruning results in a cessation of top growth, and a stimulation of new root formation (Vuksani *et al.*, 2012). These temporary alterations in root:shoot ratio also occur when plants are subjected to shortages of essential inputs necessary for growth, as will be described below. The mechanisms governing the relative growth of the above-ground parts and the roots may differ depending on the nature of the disturbance, and are in most cases still poorly understood.

Brouwer (1962a) postulated a "rule of thumb" that helps to predict how the plant will react to perturbations of top or root environments. He stated that in situations of limited supplies of resources, the part of the plant nearest the source will be relatively stimulated, and growth of the part farthest from the source will be relatively inhibited. Thus, for instance, when a plant is transferred to low light conditions, growth of the tops (closest to the light source) is relatively less decreased than root growth, and root:shoot ratio decreases (Shishido *et al.*, 1993; Minchin *et al.*, 1994). In lower radiant flux densities, leaves become thinner (increased leaf area ratio), and thus maximize the capacity for light interception per unit plant biomass. Under conditions of limiting light, the relatively low amounts of assimilates produced may be used preferentially by the relatively stronger sinks, the apical meristem and developing leaves and stem tissue (Shishido *et al.*, 1993).

Growth of root systems is closely linked to sucrose supply, regulated by light intensity (Nagel *et al.*, 2006). Recent work by Spiegelman *et al.* (2015) with tomato showed that in addition to the translocation of sucrose from the shoot to the roots, a phloem-mobile protein cyclophilin (SlCyp1) stimulates root xylem development and secondary root growth. Transport of SlCyp1 is directly influenced by the light intensity under which the plants are growing, and thus increases root:shoot ratio with higher irradiance.

There is evidence from some plants that light quality can influence partitioning of assimilates. In soyabean, Kasperbauer (1987) demonstrated that end of the day exposure to far-red light resulted in enhanced stem and reduced root growth. It is possible that the stem elongation was mediated by gibberellins (Morris and Arthur, 1985). Similar examples of light quality effects on partitioning have been elucidated for storage root-bearing crops such as radishes and beets (Hole and Dearman, 1993). The example of light quality effects on root:shoot ratio fits less readily into the resource limitation scheme postulated by Brouwer.

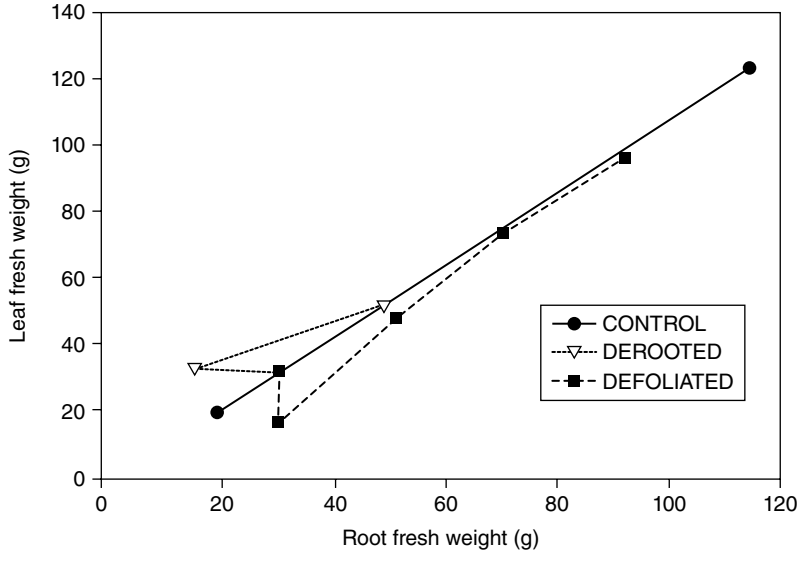

Fig. 6.3. The relationship between leaf and root fresh weight of bean seedlings grown in nutrient solution, as influenced by partial root or leaf removal (Brouwer, 1962a).

A common below-ground factor that alters root:shoot ratios is moisture supply. As soil water level is reduced from that optimum for plant growth, root elongation is less curtailed than shoot growth, and in some cases even slightly stimulated (Brouwer, 1962a; Creelman *et al.*, 1990). The relative growth of roots compared to shoots in dry conditions may be mediated by ABA in some plants. Although originally the source of the ABA was thought to be the roots (Hartung *et al.*, 2005), more recent work indicates that ABA is synthetized in leaves (Thompson *et al.*, 2007) as well as in roots and transported to the root system in peas and tomatoes (Manzi *et al.*, 2015; McAdam *et al.*, 2016). Other factors such as increases in pH of the xylem fluid during drought may also contribute to root-to-shoot signaling (Schachtman and Goodger, 2008).

Deficiencies of some mineral nutrients also produce an increase in the root:shoot ratio (Brouwer, 1962b). This phenomenon has been most closely studied with regard to nitrogen. Given the complex changes that occur as a plant becomes deficient in nitrogen, it has been difficult to determine which of these adjustments are primarily responsible for the root:shoot ratio change. In the early stages of nitrogen deficiency, leaves stopped expanding, and carbohydrates accumulated in leaves (Rufty *et al.*, 1988) and in the root system (Chapin *et al.*, 1988). The negative effects of nitrogen deficiency on photosynthetic rate were not apparent until later, so the plants temporarily had a surplus of carbohydrates, available perhaps to continue root growth. Induced nitrogen deficiency led to increases in leaf ABA levels within two days in tomato (Daie *et al.*, 1979; Chapin *et al.*, 1988), and may be responsible for the decrease in leaf expansion. Increased levels of ABA in the leaves may also be responsible for the reduced stomatal conductance prevalent in nitrogen deficient plants (Chapin *et al.*, 1988). Work with nitrate reductase-deficient tobacco plants has substantiated the role of root sugar levels in root growth (Scheible *et al.*, 1997). As these mutants accumulated nitrate in the leaves, leaf starch synthesis was inhibited and root sugar levels and root growth declined.

A suppression of stem growth may also be part of the alteration of root:shoot relations; de Jong *et al.* (2014) found that *Arabidopsis* subjected to low N conditions had enhanced auxin export from apical meristems and increased stem auxin content, leading to an inhibition of branch growth.

In phosphorus-deficient seedlings, shoot growth is more inhibited than root growth, resulting in an increased root:shoot ratio (Fredeen *et al.*, 1989; Cakmak *et al.*, 1994a; Yoneyama *et al.*, 2012) (Table 6.1). As with nitrogen deficiency (above), the reduced leaf growth may make assimilates available to the roots, allowing their continued development (Fredeen *et al.*, 1989). In agreement with these results, Cakmak *et al.* (1994b) found that sucrose export from primary leaves of P-deficient beans was not inhibited in comparison to seedlings grown on adequate P levels.

The recently-discovered plant hormone strigolactone has been shown to stimulate root elongation and root hair formation, especially under low phosphorus conditions in the soil (Yoneyama *et al.*, 2012; Kapulnik and Koltai, 2014). Strigolactone production is increased under low phosphorus levels in many species, and under nitrogen deficiency with crops such as lettuce (Yoneyama *et al.*, 2012) (Table 6.1), even though strigolactone exudation rates correlated only weakly with changes in root length.

The changes in root morphology stimulated by strigolactones can also be enhanced by plant selection. Strock *et al.* (2018) identified bean lines having roots with reduced secondary growth and enhanced root length, that more efficiently explore the soil for the immobile phosphorus under low P conditions. Beans with reduced secondary growth had roots that were a third longer, and top growth with nearly 68% more P. Without secondary thickening, the root systems facilitated the growth of mycorrhizae that help in P uptake.

The response of bean seedlings to potassium and magnesium deficiencies contrasts sharply with the reactions to −N and −P explained above. Potassium deficiency, and more drastically, magnesium deficiency, inhibited assimilate export out of leaves and curtailed growth of the root system (Cakmak *et al.*, 1994a, 1994b) (Fig. 6.4). More recently, research with tomato has found that inhibition of sink strength is one of the first changes that occurs on imposition of low potassium stress (Kanai *et al.*, 2012).

The use of top-root grafts combined with culture in hydroponic solution have increased understanding of the role of root and plant factors

Table 6.1. Plant growth and strigolactone exudation from the root system, ten days after treatment imposition, with plants growing in hydroponic conditions (source: Yoneyama *et al*., 2012).

Species	Treatment	Fresh weight, g/plant		Shoot/root ratio	Leaf number	Root length, cm	SL exudation[x]
		Shoot	Root				
Lettuce	Control	324	80	4.05	4.6	12.1	10
	-N	106	39	2.72	2.0	13.1	770
	-P	195	61	3.20	3.0	13.7	760
Tomato	Control	818	286	2.86	3.2	18.0	9
	-N	157	80	1.96	2.0	39.5	10
	-P	328	201	1.63	2.6	26.0	10,000

[x]Strigolactone exudation rate, pg.g^{-1}.24 h.

aside from root foraging ability in coping with low nutrient stress situations. For instance, some commercially-used rootstocks for greenhouse-grown tomatoes allow better plant growth in low K nutrient solution than the same cultivars on their own roots (Schwarz *et al*., 2013). A comparison of 16 rootstocks with various tolerances for growth under low K conditions indicated that poor performance was related to high levels of the ethylene precursor ACC in the transpiration stream (Martinez-Andujar *et al*., 2016). Similar work under low P conditions indicated that xylem exudate levels of the cytokinin trans-zeatin correlated positively with plant growth, while ACC again had a negative influence (Martínez-Andújar *et al*., 2017). Interestingly, the efficient P foraging rootstock lines also had higher levels of Ca in the transpiration stream. The research indicates the advantage of using suitable rootstocks for low nutrient situations, but also indicates that breeding new lines with these root traits could lead to plants adapted to low nutrient conditions.

The practice of grafting vegetable cultivars onto rootstocks that provide resistance to soil-borne diseases, or provide a means of reducing stresses from excess salts and adverse soil temperatures, has become commonplace is Asia and Europe, and is becoming popular in North America (reviewed by Lee *et al*., 2010 and Huang *et al*., 2015). The search for rootstocks with better stress adaptation has led to a greater understanding of the signals that pass from roots to shoots (reviewed by Aloni *et al*. 2010 and Venema *et al*. 2017). To the hormones that are at least partly synthesized in the root system such as abscisic acid (ABA), cytokinins, and the precursor of ethylene ACC, have been added such factors as strigolactones, mentioned above, and jasmonic acid, involved in formation of mycorrhizal associations. More recently, salicylic acid (SA) has been shown to build up in response to soil-borne pathogens, and to abiotic stress factors. At low levels, SA has been shown to enhance stress tolerance. This growing list of signaling agents, which in many cases interact, emphasize the complexity of root:shoot interactions.

Root-Shoot Relations in the Reproductive Stage

After flowering, the reproductive structures dominate demand for assimilates compared to vegetative structures and roots. Typically, in fruit-bearing crops such as tomatoes, cucumbers and eggplant, growth of shoots and roots are reduced in rate after flowering (Cooper, 1955; Van der Post, 1968; de Stigter, 1969). On the other hand, if fruits are removed, or when their growth is decreased at ripening, root growth rate increases again (Claussen, 1976) (Fig. 6.5). Fan *et al*. (2011), confirmed that root health of muskmelon is maintained during fruiting only if there is a balance in the number of vines and fruits per plant, and this number varies with the cultivar.

In crops in which the leaf canopy maintains photosynthetic activity over the reproductive period, root systems continue growing in proportion to the above-ground vegetative portion of the crop as successive flushes of fruits develop on the plants. For instance, Hurd *et al*. (1979) found in a glasshouse tomato crop that the root:shoot ratio of the vegetative parts of the plant was maintained at around 0.22 for most of the fruiting

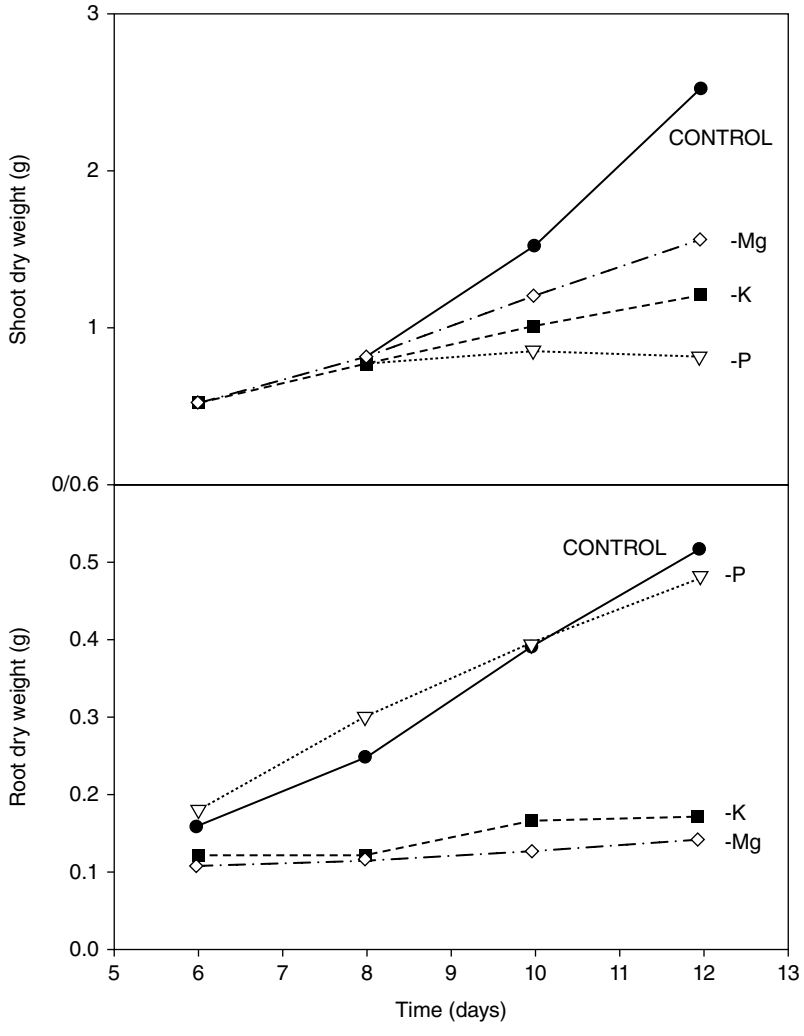

Fig. 6.4. Change in shoot and root weight of bean plants grown in full nutrients, or in solutions lacking phosphorus, potassium, or magnesium (Cakmak *et al*., 1994a, by permission of Oxford University Press).

period. The ratio was also not significantly altered if flower clusters were only allowed to set three fruits each. Similar results were obtained by Richards (1981), when tomato plant size was varied by growing the plants in containers of different root volumes. Pepper plants on which no, one or three fruits were allowed to develop also showed a constant relation of vegetative above- and below-ground growth (Nielsen and Veierskov, 1988). In muskmelon, continued root growth in the fruiting period was achieved by fruit and vine pruning (Fan *et al*., 2011). In all these cases, it is assumed that the demands of the developing fruits for assimilates and nitrogen compounds can be met by current photosynthesis, and does not require the dismantling of leaf proteins. Restricting fruit numbers by pruning, keeping fruit growth rates low by low temperatures, and by optimizing growth conditions with regard to nutrients, absence of disease and insect pests all serve to maintain photosynthesis and lessen the demand of the dominant reproductive sink.

In other instances, however, presence of fruits had a variable effect on root weight, and on the ratio of root to vegetative shoot weight. For instance, when fruit load was varied on a

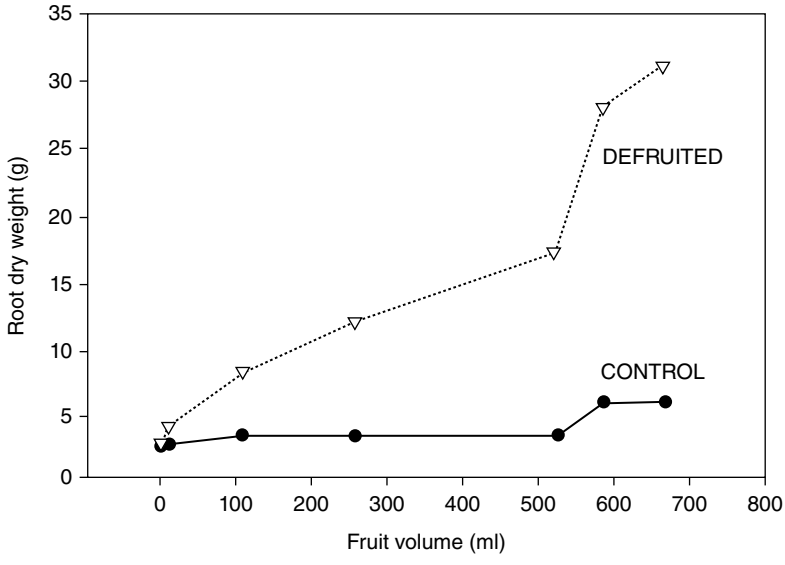

Fig. 6.5. Effect of presence of fruits on root growth in eggplant (Claussen, 1976).

glasshouse cucumber crop grown at 18°C, proportion of root growth decreased with increasing fruit numbers (Marcelis, 1994). The same variation in fruit load resulted in a constant but lower ratio of roots to vegetative shoot weight when the experiment was repeated at 25°C. In another experiment, glasshouse peppers responded to increased CO_2 in the atmosphere by increasing root growth only if the plants were defruited, but not if four or eight fruits were allowed to remain on the plants (Daunicht and Lenz, 1978). It is difficult to see how partitioning of assimilates to the roots in these cases could be determined by a priority system, in which roots were supplied only if there were surplus assimilates available.

Part of our difficulty in understanding the relation between shoots and roots in plants may be that dry weight changes in these organs do not correspond well with the changes in function of shoots and roots. Hurd *et al.* (1979) noted in glasshouse tomatoes, grown in nutrient film culture in which the roots could be viewed, that roots turned brown and stopped growing about 30 days after anthesis of the first flowers. This change was not apparent from the dry matter data. Similarly, Richards (1981) noted a close correlation between the leaf area growth of tomato plants and the number, rather than the weight of roots on the plants. He inferred that hormone production by the roots would be more closely related to the number of growing roots, with functioning root tips, where cytokinins, gibberellins, and ABA are manufactured (Itai and Birnbaum, 1991), than to the mass of the root system. Van Nordwijk and De Willigen (1987) similarly suggested that the functional equilibrium between roots and shoots should depend more on the relationship between root area and leaf area, than the ratios of their dry weights. More recently, techniques for describing root system architecture and functionality have been developed (reviewed by Zhu *et al.* 2011). For instance, use of clear tubes into which a camera can be lowered, termed minirhizotrons, showed that a bell pepper, grafted on a high-temperature tolerant pepper rootstock (S101), continued to grow and provide nutrients and water to the tops in a hot greenhouse (Aidoo *et al.*, 2018). Function of the root system was described by changes in root volume (Fig. 6.6), but was also reflected in the number of roots and root tips.

Transferring the tops of plants from their own root systems to those of others has been a common way of overcoming the adverse effects of soil pathogens or cold root zone temperatures (Lee, 1994; and Chapter 2). Research by Tachibana (1982, 1987, 1988) indicated that the root system of fig-leaf gourd (*Cucurbita ficifolia*) maintained water and phosphorus uptake in cold soils,

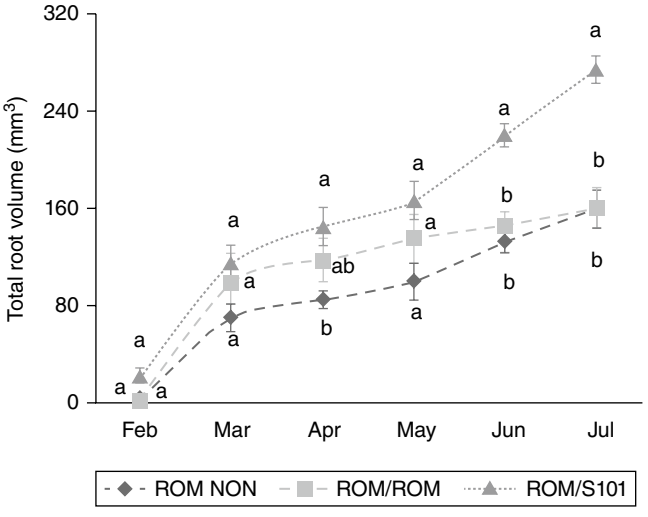

Fig. 6.6. Total root volume of "Romance" bell pepper seedlings grafted onto S101 rootstock (ROM/S101), self-grafted (ROM/ROM), or ungrafted (ROM NON), monitored monthly using minirhizotron cameras. February measurements were made at 81 d after transplanting. Plants were grown in a greenhouse in Israel in which maximum air temperatures averaged 40°C in April through June (source: Aidoo *et al*. 2018, with kind permission of Soil Science Society of America).

and showed continued cytokinin production, even when grafted to shoots of cucumber, that were not capable of such cold soil activity on their own roots. Detached roots of fig-leaf gourd maintained higher rates of respiration in low temperatures than cucumber, and may allow active mineral uptake in cool soils (Tachibana, 1989). Thus, the properties of the roots per se contributed to plant performance independent of the shoot. The improved vegetative growth was sustained into the reproductive period with higher fruit yields in cold soils (den Nijs, 1980). Choice of the rootstock species could confer tolerance to high temperatures in a similar manner. Li *et al.* (2014) found that *Luffa cylindrica* rootstock allowed growth of cucumber at 36/31°C, while grafting onto *Cucurbita ficifolia* roots aided growth at 18/13°C. In both cases, production of reactive oxygen species was reduced by the rootstocks under both extreme temperature conditions. The identity of the signal(s) transmitted from roots to tops in these experiments was not determined.

A similar improvement in cold weather growth was achieved by using the cold-adapted *Solanum habrocaites* as stock for tomato (Ntatsi *et al*., 2014). Again, the level of reactive oxygen species produced by the leaves was decreased compared to the plants growing on their own roots, or on roots from a cultivar not adapted to cold conditions. The interaction of tops and roots is however much more complex than the formation of root stress signals in cold soils. A detailed analysis showed many genes differentially expressed in tops of "Kommeet" tomato grafted either on the roots of a cold-sensitive cultivar or on *S. habrochaites* (Ntatsi *et al*., 2017) when grown at 15°C root temperatures. While genes related to reaction to the cold temperature stress were more strongly expressed in roots of the sensitive rootstock, cellulose synthesis genes were up-regulated in the cold-tolerant stock, confirming the continued growth of these roots in cold soils.

There is evidence of cultivar differences in tolerance of low soil temperature conditions. Aidoo *et al.* (2018) grew peppers in an aeroponic system, in which roots were subjected to either 7, 17 or 27°C, while tops were maintained at 25/18°C. At the lower root temperatures, one line had less reduction in photosynthesis and stomatal conductance than the other, and thus showed overall better growth.

In general, it has been found that as temperature of the whole plant increases, the root: shoot ratio declines until maximum top growth is achieved (Davidson, 1969). Working with pasture grasses, Davidson found that allocation to roots increases at both lower and higher temperatures than the optimum. The same trends appear to hold true with vegetables, but the range of temperatures explored are not sufficiently wide to show the full relationship. For instance, tomato plants kept in night temperatures of 24°C translocated a smaller proportion of assimilates to the lower stem and roots than if the plants were kept

at 9°C (Hori and Shishido, 1977). The trend was similar in the vegetative and the early reproductive stages, and respiration rates of the different plant parts reflected the translocation patterns (Shishido *et al.*, 1989). Cucumbers in the vegetative stage also showed increased assimilate distribution to tops in higher temperatures (Kanameha and Hori, 1980). In fruit-bearing cucumber plants, root:shoot ratio (including fruits) decreased from 0.07 to 0.03 as glasshouse temperature was increased from 18 to 25°C (Marcelis, 1994).

When shoot temperatures are altered separately from the root zone temperatures, the response depends on the temperature range to which each is subjected. For cucumbers, the optimum temperatures for shoot growth tend to be higher than for root growth, and temperature variation above-ground has more marked effects on productivity than below-ground variations (Kleinendorst and Veen, 1983). Considerable experimentation with glasshouse crops grown at relatively low air temperatures has indicated that increasing root medium temperatures can enhance plant growth and yield (Drews *et al.*, 1980; Gosselin and Trudel, 1983). A stimulation of root growth was also one of the first signs of growth enhancement when field-grown tomatoes were transplanted into soil covered with a clear plastic mulch (Wien *et al.*, 1993b). The mulch resulted in a 6°C increase in root zone temperatures during the first week after transplanting. Increased root growth was followed by enhanced branch growth and higher fruit yields.

The increased use of rootstocks to overcome biotic and abiotic constraints originating below-ground has led to the discovery that some stocks can have detrimental effects on fruit quality (reviewed by Kyriacou *et al.*, 2017 and Leonardi *et al.*, 2017). Among the scores of studies with grafted cucurbit vegetables, using *Cucurbita maxima* × *C. moschata* hybrids as stocks with watermelon has most frequently resulted in lower fruit sugar contents and increased titratable acidity (Leonardi *et al.*, 2017). Fredes *et al.* (2017) found a pumpkin-like off-flavor in watermelon fruits with the use of this rootstock, implying that the compound, (Z)-6-nonen-1-ol, was translocated from the root system into the fruit. Use of a citron melon rootstock (*Citrullus lanatus* var. *citroides*) avoided the production of off-flavors and had no detrimental effects on fruit sugar levels (Fredes *et al.*, 2017).

The reasons for variation in fruit quality when plants are grafted onto different stocks are many and varied. In grafted watermelon, Soteriou *et al.* (2014) noted that fruits set and matured eight days later than non-grafted controls, and delayed the peak of lycopene and soluble solids buildup. In muskmelon, grafting on hybrid pumpkin stocks led to increased fruit size, and a diminution of sugar concentrations in the larger fruits (Fu *et al.*, 2016). Similarly, if a change in the root system via grafting alters the balance of fruit numbers and vine leaf area, fruit quality could be adversely affected (Fan *et al.*, 2011).

Interactions Between Vegetative and Reproductive Parts

The interaction between vegetative and reproductive parts is often termed "source–sink relations," for the leaves, which make up a large part of the vegetative component of the plant, also serve as the principal source of assimilates for the reproductive tissues. Once rapid growth of the reproductive structures has begun, the developing fruits are generally the major sinks for the products of photosynthesis (Ho, 1992). Flower primordia, and the developing flower buds tend to have lower priority for assimilates than vegetative tissues in many vegetable crops, although there are exceptions. In the paragraphs below, examples of these interactions will be given, and the mechanisms that appear to be operating in each case.

The primordia of the first-formed flowers are being developed at a time when the plant is forming additional leaves, stem tissue and below-ground structures. If a vegetable crop such as tomato is faced with a limitation in assimilate supply at that time, vegetative primordia have precedence over the reproductive sinks (Ho, 1992). With indeterminate tomatoes growing under low light conditions, such lack of reproductive structure dominance is expressed in a failure of the first cluster to develop to anthesis, and with formation of additional leaves on the main stem (Kinet, 1977a, 1977b; and Chapter 7). Kinet (1977b) was able to overcome the detrimental effects of assimilate stress on cluster development by removing young leaves that were developing at the same time as the cluster. Other

research indicated that the competition between developing leaves and clusters was mediated by growth substances (Abdul and Harris, 1978; Kinet *et al.*, 1978). Application of GA_{4+7} (mixture of gibberellins 4 and 7) and benzyl adenine to the developing cluster when the cluster was first macroscopically visible allowed the flower buds to reach anthesis (Kinet *et al.*, 1978). Growth analysis revealed that growth of hormone-treated clusters was occurring at the expense of the plant's apical meristem.

A similar situation occurs in pepper (*Capsicum annuum*), in which assimilate shortages brought about by low light conditions or high temperatures result in flower bud abscission before anthesis (Wien *et al.*, 1993a; Marcelis *et al.*, 2004 and Chapter 8). Again, vegetative tissues, such as the developing leaves (Aloni *et al.*, 1991), and the expanded leaves (Turner and Wien, 1994) appear to have higher priority for the assimilates than the flower buds. The abscission is mediated by ethylene, and can be reduced by application of ethylene action inhibitors such as silver thiosulfate (Wien and Zhang, 1991). To date, stimulation of reproductive structure growth by direct application of growth-promoting compounds has not been successful in pepper.

In a detailed study of reproductive structure abscission of bell pepper, Marcelis *et al.* (2004) manipulated source strength (assimilate supply) by varying light levels, leaf area and plant spacing, and sink strength by application of heat stress or fruit removal. As source strength decreased, flower/fruit abortion increased, and as the growth rate of competing fruits increased, so did the loss of reproductive structures. Wubs *et al.* (2011) and Elings and deVisser (2011) confirmed the resource-based model of pepper reproductive structure abortion in simulations.

The balance between growth of vegetative and reproductive tissue can also be controlled by photoperiod. In some lines of peas, such as the photoperiod-sensitive line G-2 for example, long days bring about the cessation of apical vegetative growth, and foster pod and seed development, leading to eventual plant senescence. Under short days this same line supports both vegetative growth and production of flowers and pods. Davies and coworkers demonstrated that the rate at which flowers are developed on the plant lags behind leaf development in short days (Kelly and Davies, 1986, 1988; Sklensky and Davies, 1993). The continued leaf development may be mediated by high gibberellin levels in young leaves, and a relatively lower level of auxin. Under long days, lower gibberellin levels in developing leaves, and higher auxin levels in the floral parts may favor assimilate translocation to reproductive rather than vegetative tissues.

Once rapid growth of fruits has begun on the plant, assimilates tend to move preferentially to these reproductive structures (Wardlaw, 1990). This results in the cessation or reduction in the rate of new leaf and stem formation in such crops as cucumber, tomato and eggplant (Claussen, 1976; Hurd *et al.*, 1979; Marcelis, 1993a), and reduced root growth. Removal of fruits before they reach the active growth stage allows a continuation of leaf, stem and root formation on the plant, with little effect on the overall dry matter productivity. This appears to be the situation in some species, such as eggplant (Claussen, 1976) (Fig. 6.5). Similarly, in leafy green species such as African nightshade, making plants male-sterile by irradiation treatment increased leaf yields by 16% compared to the seed-bearing control plants (Ojiewo *et al.*, 2009).

In other species, however, removal of the strongest sink for assimilates may lead temporarily to reduced photosynthetic activity of leaves (Neales and Incoll, 1968; Bhatt and Rao, 1997). The inhibition of leaf photosynthesis rate after sink removal may have several causes. It has been linked in some species with stomatal closure within hours of translocation blockage, resulting from a build-up of ABA in the leaf blade (in pepper; Kriedemann *et al.* 1976; in soyabean; Setter *et al.*, 1980a, 1980b). Accumulation of starch in the plastids over several days may distort the membrane structure of the chloroplast of some species enough to lower gas exchange rates (Stitt *et al.*, 1995). More generally, blockage of carbohydrate export from leaves can lead to high acid invertase activity, hexose accumulation leading to a reduction in Calvin cycle activity (Goldschmidt and Huber, 1992). The formation of sugar phosphates after sink removal may in some cases result in a deficiency of inorganic phosphorus in the leaf (Plaut *et al.*, 1987). Assimilate accumulation in the leaf after sink removal has led in several species to reduced activity of ribulose bisphosphate carboxylase, the photosynthetic enzyme (in cucumber; Peet *et al.*, 1986; in tomato; Yelle *et al.*, 1989; see Stitt *et al.*, 1995).

It is important to note that sink removal will not invariably lead to adverse effects on photosynthesis. Most vegetable crops have alternate sinks such as branches, younger fruits, etc. that can become principal sinks after fruit removal. Heuvelink and Buiskool (1995) found, for instance, that dry matter production of glasshouse-grown tomato was not adversely affected until the plants had been reduced to one cluster. Less drastic pruning resulted in increased number and size of the remaining fruits. Similarly, Marcelis (1991) found that photosynthesis rate of glasshouse cucumber was not reduced by partial fruit removal treatments, but required that all the fruits be picked.

There is evidence that the correlative effects of rapidly enlarging fruits on vegetative tissues may be mediated in some cases by hormones. Tamas and co-workers showed that pod growth of determinate bean cultivars inhibited axillary branch growth during the reproductive period (Tamas *et al.*, 1979a). When pods were removed, branch growth resumed, and was accompanied by a decrease in branch ABA content. The branch growth inhibition could be re-imposed by substituting lanolin paste containing auxin in place of the growing seeds in the pods (Tamas *et al.*, 1981). The findings imply that the reproductive structures may function like the apical meristem of a vegetative plant, inhibiting the growth of branches through auxin diffusing from the pods. The inhibition may also involve the action of ABA on the axillary branches, although the origin of the ABA is not clear.

Interactions among reproductive parts

On vegetable crops that bear fruits, the developing reproductive structures are the dominant sinks for assimilates after flowering. The first-formed fruits take precedence over those developed later. This process of dominance takes similar form in different species, and has been described by many investigators.

In pod-bearing legumes, pods are located at the nodes of the earliest-opening flowers, generally the lower nodes of the main stem and the basal branches (De Moura and Foster, 1986a). Flowers further up the main stem and at later-formed nodes on the branches open successively later, and may set pods, but their development is adversely affected by the presence of the older pods at lower nodes. In cowpea (*Vigna unguiculata* L. Walp.), the younger pods typically abscise, or are inhibited from setting (Ojehomon, 1970). In soyabean, the oldest pods, that have reached mature length are most likely to survive an assimilate stress caused by heavy shade (Egli and Breuning, 2006). A similar priority order occurs in the individual cowpea racemes, on which the oldest flowers set fruit, and later-formed flowers and flower buds are shed (Ojehomon, 1968). If, due to insect damage or environmental stress factors the first flowers are not set, then the younger flowers at higher positions on main stem and branches form the yield on the plant. Typically, the first flowers to open after the stress is removed will then set pods (De Moura and Foster, 1986b; Ojehomon, 1970).

A similar pattern of intermittent fruit production on the plant occurs also in cucumber (de Lint and Heij, 1980) and in tomato (Hurd *et al.*, 1979). Analysis of node-by-node production of seedless cucumber fruits in glasshouse-grown plants indicated that about five fruits are formed in quick succession (De Lint and Heij, 1980). Fruits on the next nodes stay small and wither. The inhibition of later-formed fruits is also expressed in slower development rates of these fruits, and their delay in reaching marketable size. Marcelis (1993b) accomplished similar decreases in fruit growth rate by varying the ratio of fruit to leaves on the plant (Table 6.2). The results imply that the competing demands for assimilate by several simultaneously growing fruits is greater than the capacity of the plant to supply via photosynthesis.

In tomato, the cyclic production of fruits can be dampened somewhat by restricting the number of fruit on the earliest clusters (Hurd *et al.*, 1979). Fruit pruning results in the production of fewer, larger fruits. The degree to which the plants can compensate for reduced fruit numbers by increased fruit size is cultivar-dependent, and may be insufficient in drastic fruit pruning treatments (Favaro and Pilatti, 1987).

The position of individual fruits within a cluster, and the timing of their relative development play an important role in determining final fruit size. Tomato fruits formed from flowers at the proximal position on the cluster tended to have a higher cell number at anthesis of the flower, and to reach anthesis several days before

distal flowers (Bangerth and Ho, 1984). If natural pollination was prevented, and the fruits were set using synthetic auxin applied to the ovary, the size of distal and proximal fruits was equalized by triggering fruit set of both at the same time. In that situation, proximal fruits had fewer, and distal fruits more cells than the corresponding controls (Bohner and Bangerth, 1988).

The competition among bell pepper fruits on the plant also is manifested through a reduction in cell numbers in the later-formed fruits. Ali and Kelly (1992) found that removal of the first-formed fruit resulted in an increase in the size of later-formed fruit that was correlated with larger numbers of pericarp cells.

Dominance of the first-formed fruit may be exercised in several ways (Bangerth and Ho, 1984). The earlier fruit may constitute a stronger sink for assimilates, due to a higher pressure gradient between sink and source. This gradient may be in part mediated by the action of growth hormones such as auxins and cytokinins active in the growing fruit. However, levels of extractible auxins in the fruit have not correlated well with relative fruit growth (Ho *et al.*, 1982; Bohner and Bangerth, 1988). On the other hand, the rate at which auxin diffused out of the fruit was more closely related to fruit growth, with distal fruits having a lower rate than proximal fruits (Gruber and Bangerth, 1990). This lends support to the theory of "primigenic dominance," proposed by Bangerth (1989) as a mechanism that regulates the flow of assimilates among competing sinks. According to this theory, the first-formed fruit's greater auxin efflux would inhibit the auxin flow out of later-formed fruits at junction points. Assimilates would flow preferentially to the sink with the greater auxin flow.

Table 6.2. Effect of fruit number and temperature on fruit growth rate of "Corona" cucumber (Marcelis, 1993b), with kind permission from Elsevier Science.

Nodes with fruit (%)	Fresh weight increase rate, g day^{-1}	
	18°C	25°C
17	27.2	48.1
33	23.5	38.1
100	16.8	24.6

Much remains to be explained about the nature and location of these junctions, and the possible involvement of other growth hormones in primigenic dominance.

Another possible way in which dominance could be maintained by tomato fruits may be through the production of a growth inhibitor such as ABA. Abscisic acid content of competing tomato fruits has, however, not shown any relationship with fruit growth inhibition (Ho *et al.*, 1982; Bohner and Bangerth, 1988).

The importance of seeds in the dominance of competing reproductive structures has been apparent particularly from the work with beans (*Phaseolus vulgaris* L.). Tamas *et al.* (1986) found that seed removal from actively growing pods allowed growth of younger pods to continue, instead of being inhibited. If seeds were replaced by IAA or a synthetic auxin in lanolin, the growth-inhibiting effect of the older pods was restored. The larger fruits were found to export relatively more IAA than smaller ones, in parallel to their greater growth-inhibiting effect. In beans, this inhibition may be mediated by ABA, for Tamas *et al.* (1979b) found in earlier work that ABA content was higher in the inhibited pods. In this respect, the seed-mediated inhibition has elements in common with apical dominance of vegetative plants (see section on apical dominance above).

Seed-mediated dominance relationships have also been found in squash (*Cucurbita pepo* L.) by Stephenson *et al.* (1988) and in sweet pepper (Heuvelink *et al.*, 2004). Fruits containing more seeds inhibited the growth of later-formed fruits more than fruits with fewer seeds. The latter were also more likely to abort before reaching anthesis. It is not known if these effects are mediated by growth hormones. In tomato, the content of auxin and of ABA of seeds is several-fold higher than in the rest of the fruit (Bohner and Bangerth, 1988).

In some species, the functioning of a reproductive sink may be influenced by light, perhaps mediated by growth regulators. If individual fruits in glasshouse-grown cucumber were darkened, for instance, growth rate decreased, and the likelihood of fruit abortion increased (Schapendonk and Brouwer, 1984). More work is needed to elucidate the mechanism of this form of sink regulation.

Concluding Remarks

The correlations governing growth of the major organs of fruit-bearing vegetable crops have been characterized frequently, but the mechanisms that determine which part of the plant takes precedence when resources are limiting are still little understood. From the descriptions in the literature, we can predict for instance that developing leaves, stems, and roots will receive assimilates in preference to developing reproductive structures in the pre-flowering stage of growth. Once fruits have set, the latter become the dominant sinks, and the root system is lowest in priority in obtaining assimilates. A general sequence that determines sink activity might be initiated by an increase in growth hormone activity in a particular sink. This could lead to stimulation of enzymes catalyzing the accumulation or use of assimilates in that sink, resulting in increased translocation to the organ. Under conditions of limited assimilate supply, the activity of a subordinate sink would be inhibited, but the mechanism of that inhibition is even less well understood.

As has been stated repeatedly in this chapter, there are still many gaps in our knowledge on how growth correlations operate. The disruptive nature of experimental manipulation has limited the amount of information that can be obtained from conventional experiments which remove leaves, fruits, etc. Considerable progress has been made using genetic interventions that allow the experimenter to vary the levels or activities of growth hormones and of specific enzymes, and this will increase our understanding of how plants regulate the relative growth of their principal parts. Unfortunately, such studies have also highlighted the complexity of the gene systems involved in plant growth interactions, and the challenges we face in making improvements in plant productivity based on new findings.

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7 Tomato

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. . . a fruit that is almost universally treated as a vegetable and a perennial plant that is almost universally cultivated as an annual.

(Rick, 1978)

. . . serves as a model organism for the family Solanaceae and, specifically, for fleshy-fruited plants.

(Kimura and Sinha, 2008)

Introduction

History and botany

Tomato is the second most important vegetable crop after the potato, the most valuable fruit crop globally, and belongs to the family *Solanaceae*, genus *Solanum*, section *Lycopersicon*. In 1753, Linnaeus named tomato *Solanum lycopersicum*. Fifteen years later, Philip Miller moved it to its own genus, naming it *Lycopersicon esculentum*. Genetic evidence has now shown that Linnaeus was correct to put tomato in the genus *Solanum*, making *Solanum lycopersicum* L. the correct name.

In pre-Columbian times, the tomato was apparently not known to South American Indians since there is no name for it in their languages, no tradition and no archaeological remains in the Andean region (Rick, 1978; Harlan, 1992). Domestication took place in Mexico where truly wild tomatoes are unknown but weed tomatoes are common in the south of the country (Harlan, 1992). Tomato was first introduced in Europe in the middle of the 16th century (Rick, 1978; Kalloo, 1991), where they were planted as ornamental curiosities, and not eaten. An early introduction was probably yellow, since it was named "pomodoro" (golden apple) in Italy. Because it belongs to the nightshade family, tomato was sometimes considered poisonous, slowing down acceptance, and it is only recently that it became a major food crop.

Botanically, a tomato fruit is a berry consisting of seeds within a fleshy pericarp developed from an ovary. Based on the number of carpels, cultivars are grouped as: round tomatoes (two or three carpels); beefsteak tomatoes (more than five carpels); and the nowadays popular intermediate

Note: Parts of this chapter are taken from Chapter 3 (Heuvelink and Okello, 2018) and Chapter 4 (Heuvelink, Li and Dorais, 2018) of a recent book on the tomato crop (Heuvelink, 2018).

types (three to five carpels). Tomato fruits are composed of flesh (pericarp walls and skin) and pulp (placenta and locular issue including seeds). In general, the pulp accounts for less than one-third of the fruit fresh mass. Covering the epidermis is a thin cuticle. The cultivated tomato is diploid (12 chromosome pairs) and self-pollinating. Tomato is grown as an annual crop mainly in temperate climates, characterized by long summer periods and winter precipitation, but also in (sub)tropical climates. Two main tomato types are currently grown: i) the determinate or "bushy" tomato having a one time-limited flowering period followed by a period of fruit development and cultivated in open field for processed or fresh consumption tomatoes; and ii) the indeterminate tomato, used for protected production (high tunnels and greenhouses), producing inflorescences and flowers continuously throughout the plant's life.

Global tomato industry

A comprehensive and accessible overview of all aspects of the production of the tomato crop within the context of the global tomato industry has recently been published (Heuvelink, 2018). Global tomato production increased by more than sixfold in the last five decades, expanding from 27.6 million tons in 1961 to 171 million tons in 2014, with a cultivated area of 5 million ha (FAOSTAT, 2017). For example, annual tomato production per unit greenhouse area in The Netherlands increased by 113% between 1983 and 2010 (De Gelder *et al.*, 2012), which is mainly related to improved greenhouses and climate control, genetic improvement (e.g. higher light use efficiency; Higashide and Heuvelink, 2009), improved crop and pest management, and better control of the rooting environment. Fruit yield of processing tomatoes was genetically improved by 1.54% per year in California over a 20-year period (Panthee and Gardner, 2011).

Yield of field crops usually ranges between 40 and 100 t/ha, whereas yields from year-round cultivation in greenhouses in northwest Europe or North America easily exceed 500 t/ha, and yields as high as 700–900 t/ha are obtained in high-tech greenhouses without supplementary light (SL). With the use of SL, over 1000 t/ha have been produced (Verheul *et al.*, 2012).

The five leading producing countries are China, India, the United States, Turkey, and Egypt, representing 31, 11, 9, 7 and 5% of the total production, respectively, while the EU accounts for 11%. Processing tomato production represents 41% of the total harvested tomatoes and the largest producers are the United States, China, Italy, Spain, and Turkey (WPTC, 2016; Costa and Heuvelink, 2018). All processing tomatoes and most tomatoes for fresh consumption are grown in the open field, although greenhouse production for fresh consumption is expanding rapidly.

Model plant species

Carvalho *et al.* (2011) state: "In addition to its worldwide cultivation and economic importance, tomato has several characteristics that make it a convenient model plant species, such as a relatively compact genome (950 Mb) combined with a marker-saturated genetic linkage map, rich germplasm collections (Tomato Genetics Resource Center) and highly efficient transformation protocols." Tomato has a relatively short generation time, is easy to cultivate and is amenable to varied horticultural manipulations including grafting or cutting. Wild tomatoes are a valuable resource for desirable traits because they show great phenotypic variation (e.g. morphology and resistance against diseases and abiotic stresses) and can be crossed with cultivated tomato. These features make tomato ideal material for genetic research (Kimura and Sinha, 2008). Tomato is an established model to study fleshy fruit development and ripening (Osorio *et al.*, 2011) and tomato seed is considered a model system for germination research (Nonogaki, 2006). Its entire genome has been sequenced (The Tomato Genome Consortium, 2012), classical genetics research has created more than 1000 tomato mutants that include spontaneous and induced (via radiation and chemicals) mutations (Kimura and Sinha, 2008), and numerous large-scale functional genomics resources for tomato have been developed, resulting in a wealth of genomics resources (Fei *et al.*, 2011). This all makes tomato a prominent model system for research into plant genetics, pathology, and physiology. Guidelines for using tomato in

experiments with a controlled environment are provided by Schwarz *et al*. (2014).

Genetics, Plant Breeding and Biotechnology

The modern cultivated tomato, *S. lycopersicum* resulted from domestication of its wild progenitor, *S. pimpinnelifolium*, the only red-fruited of the wild tomato species which see commercial and home gardener use as so-called "currant tomatoes" for their intense flavor and attractiveness as garnishes. Key domestication loci notable in modern varieties include *fw.2.2*, *fas* and *lc* (see p. 150) which account for much of the dramatic alteration in fruit size from the pea or marble-sized wild species to currently used varieties. The Spanish explorer Hernán Cortés is credited with introducing tomatoes to Europe and drawings dating to not long after his return indicate striking similarities between those tomatoes and the varieties we recognize today (The Tomato Genome Consortium, 2012).

Genetics of the modern tomato

Much tomato breeding occurring since the time of Cortés through the mid 20th century was based on the limited germplasm available in Europe. While extraordinary genetic diversity is captured in tomatoes' cross-compatible red and orange (e.g. *S. pimpinellifolium*, *S. galapagense*, *S. cheesmaniae*) in addition to green or flavonoid accumulating wild relatives (e.g. *S. pennellii*, *S. habrochaites*, *S. neorickii*, *S. chmeilewskii*, *S. lycopersicoides*), their use in prior breeding was avoided due to negative effects on desirable fruit and other plant attributes. Notable improvement traits were selected from within this narrow germplasm base and spontaneous mutations. Examples include loci such as *ovate* (Liu *et al*., 2002) and *sun* (Xiao *et al*., 2008) which along with *fas* and *lc* broadened the repertoire of fruit sizes and shapes (Rodriguez *et al*., 2011). *Jointless1* and *2* removed the pedicel abscission zone resulting in fruit that separated at the calyx, a necessary feature to limit postharvest damage from the protruding remnant of pedicel abscission. *Jointless2* is preferred by breeders due to limited pleiotropic effects (Gomez Roldan *et al*., 2017). Wild-type tomatoes often develop a darker green shoulder on the calyx end of the fruit due to elevated plastid and chlorophyll accumulation in the fruit region with the greatest photosynthetic potential, an important aspect of tomato development as the fruit itself is estimated to contribute approximately 10 to 15% of its sink potential. During ripening, as the rest of the fruit achieves its characteristic red coloration, the green shoulder can become white, yellow, or orange. This aberrant coloration is considered less desirable to consumers yet is also a hallmark of heirloom varieties. Nearly 100 years ago, a spontaneous mutation, *uniform* (*u*) that eliminates the green shoulder emerged and is currently bred into most production varieties. It has recently been shown that *u* encodes a GOLDEN-LIKE2 (GLK2) transcription factor promoting higher plastid numbers, expressed at higher levels on the calyx end of the fruit and responsible for the green shoulder and ripening discoloration traits (Powell *et al*., 2012). In the 1960s, a spontaneous non-ripening mutation, *ripening-inhibitor* (*rin*) arose in a greenhouse of Cornell University vegetable breeder Henry Munger. While *rin*/*rin* fruit fail to ripen, *Rin*/*rin* hybrids produce slow ripening, long shelf-life and firm fruit readily deployed in the hybrid seed systems, yielding most modern production varieties with shelf life and textural characteristics essential to current high density production and long distance transport production chains. The *rin* mutation encodes a MADS-box transcription factor (see below), both essential to normal ripening and resulting from a gene fusion that eliminates ripening function and confers additional ripening repressor activity (Vrebalov *et al*., 2002; Ito *et al*., 2017). Both *rin* and *u*, while prevalent and essential to the needs of modern varieties, are also responsible for reduced quality noted by many consumers (Nguyen *et al*., 2014).

Increased exploitation of genetic diversity: roots to fruits

The tomato genome was sequenced in 2012 and this information is being deployed along with genome sequence data from over a thousand cultivars including modern, heirloom, and wild tomatoes (Tieman *et al*., 2017 and references

therein) to assist molecular breeding. Many modern breeding programs now utilize knowledge about the tomato genome, the chromosomal locations of important production and quality genes and available DNA sequence variation to develop and exploit DNA markers for targeted breeding and genome selection. While genetic modification has helped identify and functionally define important tomato genes, their practical utilization is currently through identification of natural genetic diversity and breeding for optimal alleles via genome-enabled selection methods.

Among tomato and its wild relatives are 12 members of the *Lycopersicon* clade which are intercrossable to varying degrees (Knapp and Peralta, 2016). While these wild species offer enormous genetic diversity, their use in breeding is limited due to their broad phenotypic effects when crossed with *S. lycopersicum*. Development of recombinant inbred and introgression populations from crosses between cultivated and wild parents has facilitated recent use of this vast genetic resource (Monforte and Tanksley, 2000 and references therein). Nevertheless, introduction of desirable traits including pathogen and abiotic stress tolerance loci from wild species can carry additional loci with negative impacts on fruit yield, size and quality.

Where tomato wild species have seen particular utility is in development of rootstocks. Tomato is highly amenable to grafting and rootstock selection can focus on soil-associated stress tolerances without concern for fruit traits. *S. habrochaites*, native to high elevations in the Andes, is exceptionally cold tolerant (Venema *et al.*, 2008), while *S. galapaganse*, which can grow adjacent to coastal waters, harbors salt tolerance with additional wild species useful in resistance to soil-borne diseases (Cuartero *et al.*, 2006). While tomato wild species have shown potential as rootstocks directly, most commercial rootstocks result from breeding selections derived from primary hybridizations between cultivated genotypes and wild species. Interestingly, recent research suggests promise for grafts between cultivated tomato scions and rootstocks from more distant relatives of tomato within the family Solanaceae such as eggplant (Petran and Hoover, 2014).

Tomato biotechnology

Ease of transformation along with a high quality genome sequence and short generation time contribute to use of tomato as a model for fleshy fruit development and additional aspects of plant biology (see below). Indeed, tomato is highly amenable to genetic transformation with modified or foreign DNA sequences and has the distinction of being the first commercialized genetically modified organism (GMO) crop. In an effort to develop higher quality fruit with reduced softening, researchers targeted a fruit-specific polygalacturonase (*PG2a*) gene for antisense repression (Sheehy *et al.*, 1988). *PG2a* is strongly induced during tomato ripening and catalyzes the hydrolysis of cell wall pectins. While more recent evidence suggests a more significant role for pectate lyase (PL) than *PG2a* in tomato softening, the resulting fruit, termed Flavr pathogens, presumably due to increased cell wall integrity, and could thus be harvested when more mature and of higher quality. Test marketing of FlavrSavr® was promising but production problems led to cancellation of the product. Similar GMO fruit were developed at the University of Nottingham (Smith *et al.*, 1988) and used by ICI Seed for sauce production where increased pectin integrity reduces energy/cost-intensive water removal needed to achieve the desired thickness. Sauce from GMO tomatoes with a reduced *PG2a* expression was produced in the United States and marketed in the UK for several years before the product was dropped. At the writing of this book, no GMO tomatoes are being produced for commercial use. This likely reflects a combination of: a) expense of required regulatory hurdles in a modest value crop where cost recovery is more difficult; b) resistance among many consumers to GMOs, especially in freshly consumed products; and c) the success of the seed industry in addressing many crop performance, production and fruit quality traits using molecular-assisted breeding and available natural genetic diversity.

Developmental Processes

Seed germination

Seed germination is a complex process, regulated by a large number of environmental and endogenous factors. Germination begins with

water uptake by the seed (imbibition or rehydration) and ends with emergence of the embryonic axis, usually the radicle. Radicle protrusion is sometimes referred to as "visible germination" (Bewley *et al*., 2013). In cultivated tomato, seeds are considered non-dormant, whereas some studies with wild types suggest that abscisic acid (ABA) plays a role in a slight dormancy. Seed germination of several gibberellin (GA) biosynthesis deficient mutants absolutely depends on the addition of GA to the medium during imbibition. Physiological, biochemical, and genetic evidence suggests a role for GA in weakening the structures covering the embryo during germination. GA biosynthesis in developing seeds is involved in embryo growth and the prevention of seed abortion (Kucera *et al*., 2005).

Tomato seeds characteristically germinate best in the dark, and in some cultivars light will inhibit germination. These responses are dependent on phytochrome action. Far red light has been reported to inhibit germination, whereas red light (at 37°C) promoted germination, suggesting that the presence of Pfr (the active form of phytochrome) in the seed is a prerequisite for germination. Germination in response to temperature has been described on the basis of thermal time, temperature sum, or heat units (i.e. degree-days). Constant heat units imply a linear relationship between germination rate and temperature. This seems unlikely, since optimum temperatures between 20 and 25°C have been reported. However, over the sub-optimal temperature range (13–25°C) this linearity has been observed (Bierhuizen and Wagenvoort, 1974). These authors reported a minimum temperature of 8.7°C for germination and a heat sum requirement of 88 degree-days to achieve 50% germination. In a further paper, however, they considered 13°C as the "practical" minimum temperature. Membrane lipid changes appear to be involved in cultivar differences in the ability to germinate at low temperatures, whereas failure to germinate at high temperatures may be due to thermodormancy, a condition thought to be related to an interaction between temperature and phytochrome action.

Stem and leaf development

At the tip of the main stem is the shoot apical meristem (SAM), a region of active cell division where new leaves and flower parts are initiated. It is dome-shaped and protected by newly-formed leaves. Leaves are arranged alternately with a 2/5 phyllotaxy. Shoot development in tomato can be separated into two different phases; during the initial vegetative phase the SAM forms metamers consisting of an elongated internode, a leaf and a bud (Fig. 7.1). After the formation of between 6 and 11 metamers, the primary shoot SAM is transformed into an inflorescence meristem (IM). While the IM develops into an inflorescence, a second phase of shoot development is initiated by the outgrowth of the bud in the axil of the youngest leaf primordium. This sympodial shoot grows vigorously, it displaces the developing inflorescence to a lateral position and transfers its subtending leaf to an elevated position above the inflorescence. After the formation of three leaves, the SAM of the sympodial shoot is also transformed into an IM and develops into an inflorescence. The main axis is again continued by the sympodial shoot in the axil of the youngest leaf primordium. In "indeterminate" cultivars the process is repeated indefinitely with inflorescences every three leaves. In "determinate" types each axis produces a limited number of inflorescences, and strong axillary buds develop at the base of the stem, producing a bushy habit, which is ideal for growing unsupported in the open. Side shoots are removed when "indeterminate" cultivars are grown. In tomato, several mutants are defective in axillary meristem initiation. The lateral suppressor (ls) mutant is characterized by the absence of sideshoots, except for the sympodial shoot and the lateral shoot immediately below (subfloral sideshoots; Schmitz and Theres, 1999). Comparative and functional analyses of tomato regulatory genes such as LATERAL SUPPRESSOR (LS), SELF PRUNING (SP), SINGLE FLOWER TRUSS (SFT) and FALSIFLORA (FA) have revealed mechanisms involved in shoot development and flowering time which are conserved among *Arabidopsis*, tomato, and other plant species (Lozano *et al*., 2009).

Leaf appearance or unfolding rate (LUR) is not necessarily equal to the rate of initiation of leaf primordia at the apex but, in the long term, equality is expected. LUR is predominantly influenced by temperature and shows an optimum response (De Koning, 1994). However, this relationship is close to linear between 17–23°C and

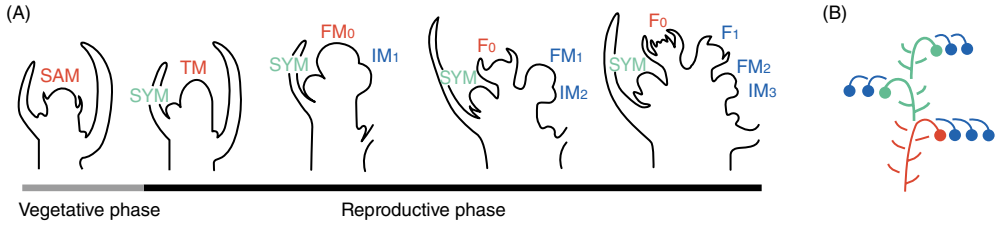

Fig. 7.1. Inflorescence development and architecture in tomato. (A) Successive steps of inflorescence development. The vegetative shoot apical meristem (SAM) initiates vegetative phytomers made up of an internode, a leaf, and an axillary meristem. When entering floral transition, the SAM takes an intermediate, transitional meristem (TM) fate whereas the last vegetative axillary meristem called the sympodial (SYM) takes over shoot growth. The TM initiates a new phytomer with a prominent inflorescence meristem (IM). TM and IM maturate toward floral meristem (FM) fate and become flowers (F). Each IM initiates another IM in the meantime of maturating to FM. (B) Schematic representation of a tomato plant. Colors represent different types of meristems (red: shoot apical meristem, SAM, called transitional meristem, TM, after floral transition; green: sympodial meristem, SYM; blue: inflorescence meristem, IM). (Périlleux *et al*., 2014).

as a rule of thumb, at 20°C, three leaves unfold each week. Meristem temperature determines leaf initiation rate and Savvides (2014) determined that meristem temperature can deviate substantially (–2.6 to 3.8°C for tomato) from air temperature under moderate environments.

De Koning (1994) observed no effects of fruit load, leaf removal, or planting density on LUR. Heuvelink and Marcelis (1996) also reported little influence of assimilate supply on LUR. However, Savvides (2014) reported a reduction in leaf initiation rate in young tomato plants when light level was reduced below 6.5 mol m^{-2} d^{-1}. This contrast with Heuvelink and Marcelis (1996) may be explained by assimilate supply limiting LUR in seedlings, because of the low leaf area and thus low light interception.

Like many other plant species, tomato shows strong internode elongation under a low red (R)/far-red (FR) ratio (a symptom of the so called shade avoidance response; Kurepin *et al*., 2010). The levels of endogenous growth-active gibberellin GA_1 and its immediate precursor GA_{20} were decreased by low R/FR ratio, whereas the levels of GA_1 catabolite, GA_8, increased (Kurepin *et al*., 2010). These authors also suggest that decreasing ethylene production under low R/FR ratio causes increases in stem elongation and GA levels. Reduction in length growth resulting from a so-called negative DIF (difference between day and night temperature) has been reported by many authors and for a large variety of crops including tomato (Fig. 7.2). Bours (2014) concluded that negative DIF affects growth by directly affecting the phase and amplitude of circadian clock genes, which in turn control downstream processes such as starch metabolism and hormone signaling pathways. Auxin and ethylene signaling pathways affected by negative DIF show significant crosstalk and interconnect with the circadian clock at several positions. Bours (2014) stressed the unique position of the photoreceptor phytochrome B in this regulation. Gibberellin metabolism is almost certainly involved in the regulation of stem extension by temperature (Langton and Cockshull, 1997).

Salt stress (high EC in the root environment) reduces stem elongation. Shaking both stresses and toughens plants, resulting in a reduced stem length, a phenomenon known as thigmomorphogenesis (Picken *et al*., 1986).

Flower differentiation and flowering

Samach and Lotan (2007) summarize progress in understanding the environmental cues that affect the initial transition to flowering in tomato and the genes that are involved in this transition and additional transitions occurring on the sympodial shoot. Environmental cues discussed are daylength, light intensity, and temperature.

In general, the tomato plant initiates 6 to 11 leaves preceding the first inflorescence (NLPI). The sensitive phase, where environmental treatments given to the plants, influence the position on the stem at which the first inflorescence will

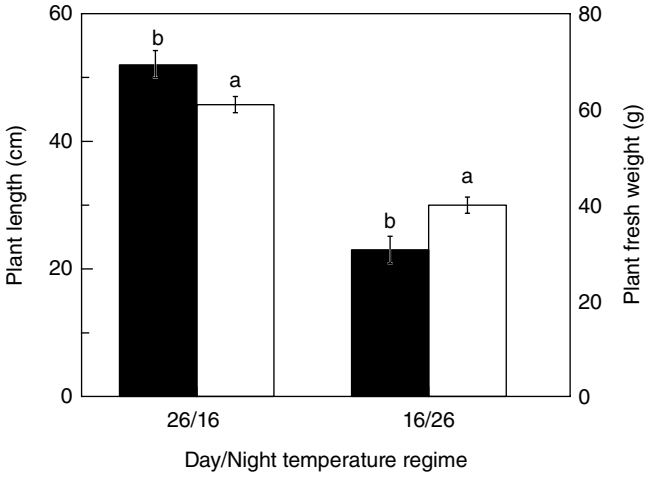

Fig. 7.2. Plant length (■) and plant fresh weight (□) of tomato plants (eight weeks after sowing, 40 days after start of temperature treatments) grown in climate cabinets at a light intensity of about 120 µmol m^{-2} s^{-1} at two different temperature regimes (daylength was 12 h; Heuvelink, 1989). Different letters indicate significant differences according to Tukey's HSD test at P=0.05.

develop, lasts approximately ten days, starting at cotyledon expansion, although this period can be longer depending on the cultivar. No single environmental factor can be regarded as critical for the control of flowering in tomato. Environmental factors like light, temperature, carbon dioxide, nutrition, moisture, and growth regulators directly or indirectly influence flower initiation.

The NLPI can be seen as the result of two processes: the rate of leaf initiation and the time to initiation of the first inflorescence which determines the end of the vegetative phase.

The influence of environmental factors on NLPI can be largely explained by the nutrient diversion hypothesis for flowering (Sachs and Hackett, 1969). According to this hypothesis the amount of assimilate available to the apex during the sensitive phase has to reach a certain minimum before flower initiation can take place (see review by Dieleman and Heuvelink, 1992).

The number of flowers initiated in an inflorescence depends on cultivar and environmental conditions. The inflorescence is a monochasial cyme in which the vegetative axis terminates in the king flower (Picken *et al*., 1986). Increased irradiance or decreased plant density and decreased temperatures positively influence the number of flowers formed in an inflorescence. Low air temperatures (<10°C) during inflorescence initiation promote inflorescence branching, usually resulting in more flowers per inflorescence.

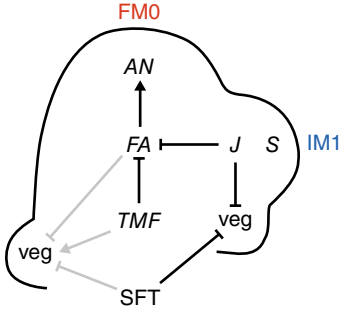

Fig. 7.3. Genetic control of meristem fate in tomato inflorescence. The left side of the diagram shows regulatory interactions (gray lines) at floral transition; the right side shows regulatory interactions (black lines) during the development of the inflorescence. Floral transition of the SAM is controlled by upregulation of *FALSIFLORA* (*FA*) in the meristem and by systemic SINGLE FLOWER TRUSS (SFT) signal, which both repress vegetative growth (veg). *TERMINATING FLOWER* (*TMF*) plays an antagonistic role and promotes veg, possibly by repressing *FA*. During inflorescence development, *FA* is required for maturation toward flower meristem (FM) fate, together with activation of the FM identity gene *ANANTHA (AN)*. By contrast, *SFT* is not required for FM identity but represses veg in the lateral inflorescence meristems (IM). This role is shared with *JOINTLESS* (*J*) that represses veg and prevents premature maturation of IM toward FM, possibly by repressing *FA*. By contrast, *COMPOUND INFLORESCENCE* (*S*) accelerates IM maturation. Repression lines and activation arrows do not mean direct interactions. (Périlleux *et al*., 2014).

Tomato flower induction (Fig. 7.3) occurs when the shoot apical meristem (SAM) is converted into an inflorescence meristem that produces floral meristems (FM; Périlleux *et al.*, 2014). Unlike SAM, the FM exhibits determinate growth through repression of *WUSCHEL (WUS)* by *AGAMOUS (AG)* gene encoded transcription factors (Sicard *et al.*, 2008). WUS promotes cell division and prevents premature differentiation. The FM produces four floral whorl primordia, sepals, petals, stamens and carpels. The fourth inner most whorl, the carpel primordia, fuse to form the ovary with locules that contain ovules. Carpel primordial size is regulated by INHIBITOR OF MERISTEM ACTIVITY (IMA). Carpel identity is controlled by *TOMATO AGAMOUS 1 (TAG1)* and *TOMATO AGAMOUS-LIKE 1 (TAGL1)* genes. Another group of carpel number regulators consists of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL/SPB) transcription factors (Karlova *et al.*, 2014).

Flower development after initiation is primarily influenced by temperature, higher temperatures resulting in faster flower development. Especially under conditions where photosynthesis is low, increased temperatures will stimulate flower bud abortion. High temperature effects on flower abortion may be a consequence of failure of fruit set rather than a direct effect of temperature (Sato *et al.*, 2006).

Before anthesis, cell division in the ovary temporarily ceases. Following successful pollination and fertilization, cell division resumes (fruit set) due to auxin and giberellic acid (GA) biosynthesis. Auxin transporters (PIN-FORMED; PIN) and receptors (TRANSPORT INHIBITOR RESPONSE 1; TIR1) are important during fruit set, development, and growth (Azzi *et al.*, 2015). Mounet *et al.* (2012) have shown that mutant tomato plants with silenced *SlPIN4* gene produce small parthenocarpic fruits. TIR1 controls degradation of AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressors in the presence of auxin leading to release of auxin response factors (ARFs). Parthenocarpic fruits are formed when *TIR1*, *Aux/IAA* and *ARF* are misexpressed (Ren *et al.*, 2011). Larger fruits form when transcriptional repressors of auxin transporters or receptors are silenced (Su *et al.*, 2014). Development of unpollinated ovaries is usually inhibited through reduced expression of genes encoding GA biosynthesis enzymes. Transcript levels of these genes (e.g. those encoding GA 20-oxidases) increase after successful pollination leading to synthesis of active GA_1 and GA_4. GA interacts with its receptor (GA INSENSITIVE DWARF1; GID1) to target DELLA proteins for proteolytic degradation hence release of GA-responsive gene repression. Silencing of *DELLA1* and repression of *GA20ox1* causes production of pollen with reduced viability, and small elongated facultative parthenocarpic fruits (Olimpieri *et al.*, 2011).

Following fruit set, cell division proceeds for about two weeks but can be longer (up to 25 days after anthesis) in some cultivars (Czerednik *et al.*, 2012). Cell division is a four phase process consisting of DNA synthesis (S) and mitosis (M) and two gap phases. During M phase, a cell divides into two. Newly formed cells expand during gap phase 1 (G1) in preparation for S phase when cellular DNA content doubles. S phase is followed by a second gap phase (G2) in which cell size doubles in preparation for M phase. Phase transition is regulated by catalytic cyclin dependent kinases (CDK) that are dependent on cyclins (CYC) for activation. Cyclins determine CYC-CDK complex stability, localization and substrate specificity. Seven CDKs and eight CYCs have been reported in tomato (Czerednik *et al.*, 2012). Activity of the CYC-CDK complex can be modified by i) proteolytic destruction of the CYC subunit (e.g. by the anaphase promoting complex/cyclosome; APC/C), and ii) inactivation by CDK inhibitors (e.g. SIM/SMR, WEE1 and Kip-related protein 1; KRP1). Cell cycle regulation can be upstream of CYCs and CDKs through transcription factors. E2F transcription factors are upstream cell cycle regulators that target genes (e.g. DP-E2F-LIKE1—*DEL1*) involved in DNA repair and chromatin dynamics at the transition between G1 and S. The RETINOBLASTOMA-RELATED (RBR) protein binds E2F transcription factors to prevent cell cycle progression. G1 to S phase transition occurs when CYC-CDK dimers phosphorylate RBR and release E2F transcription factors (Komaki and Sugimoto, 2012). Some proteins (e.g. FW2.2) inhibit cell division but their mode of action is still elusive. FW2.2 accounts for up to 30% of the variation in fruit fresh weight of domesticated and wild tomato (Frary *et al.*, 2000 refs). *FW2.2* gene expression increases in tomato fruits when grown at high temperature (Okello *et al.*, 2015b).

Fruit development and ripening

Tomato fruit development (reviewed by Klee and Giovannoni, 2011; Arrizumi *et al*., 2013; Karlova *et al*., 2014; Azzi *et al*., 2015; Heuvelink and Okello, 2018) is a four-phase process that begins with initiation of the floral meristem, carpel formation, and ovary growth. The second phase involves pollination, fertilization, fruit set, and resumption of cell division. During the third phase, cells expand and undergo endoreduplication. Initiation of ripening is the fourth phase and it marks the beginning of fruit senescence. A schematic representation of transcriptional regulation of the four phases of tomato fruit development is shown in Fig. 7.4.

The period between anthesis and fruit maturity decreases with increasing temperature from 14 to 26°C (De Koning, 1994, 2000; Adams *et al*., 2001). The fruit growth period can be well described by linearly relating its reciprocal (i.e. fruit development rate) to temperature. This means for harvest of ripe fruits a certain temperature sum has to be reached. For cv. Counter, accurate predictions of fruit growth period may be made assuming a temperature sum of 940 degree days and a base temperature of 4°C. However, the temperature sensitivity of the fruit development rate varies during fruit development, being high during the first weeks after anthesis when cell division and elongation take place, and then decreases when only cell elongation occurs. When the fruit is close to maturity, increasing temperature enhances fruit ripening (Monselise *et al*., 1978; De Koning, 1994; Adams *et al*., 2001). Other factors, like plant density, light intensity, carbon dioxide, air humidity, fruit load, plant age, or salinity in the root environment have no or only a small effect on fruit growth period. Severe drought stress shortens the duration of fruit growth period and fruit affected by blossom-end rot (BER) (see section on physiological disorders, p. 163) will ripen one to two weeks earlier.

The onset of fruit ripening coincides with the rapid slowdown of cell expansion, and onset of intensive metabolic transformations. Tomato is a climacteric fruit and ripening is associated with ethylene production and cell respiration peak in both attached and detached tomato fruit. As ripening progresses, fruit color changes from green to red as chloroplasts are transformed into chromoplasts, chlorophyll is degraded and carotenoids (mainly lycopene and to a lesser degree β-carotene) accumulate. This first becomes apparent at the blossom end of the fruit and progresses toward the stem end. Fruit softening and textural changes occur as the fruit cell wall is partially disassembled by enzymes and the ripe flavor develops as specific volatiles increase and the sugar–acid balance alters (see below). Ethylene biosynthesis in pre-climacteric immature and mature-green tomato fruit and ripening climacteric tomato fruit is by the conventional methionine to SAM to ACC to ethylene pathway (Abeles *et al*., 1992). Regulation of ripening via ethylene signaling occurs through ethylene biosynthesis and perception by receptors. Important regulators in the ethylene biosynthetic pathway include MADS-domain protein RIPENING INHIBITOR (RIN), COLORLESS NON-RIPENING (CNR), and NAC domain transcription factor encoded by the gene underlying the non-ripening (*nor*) mutation. Mutants (*rin, nor* and *Cnr*) in which the mutant alleles yield altered proteins (RIN and NOR) or reduced CNR expression, respectively, produce fruits that fail to fully ripen. Fruits of these mutants neither produce normal levels of ethylene nor ripen in response to exogenous ethylene application. Development of alternate alleles of these and other ripening genes via gene-editing confirms they encode necessary components of ripening control though suggest the spontaneous mutants may retain altered functions (Gao *et al*., 2019; Wang *et al*., 2019). Recent genome-scale analyses of DNA methylation and histone dynamics reveal a critical initial role of genome modification in releasing blocks to the activation of these and additional ripening transcription factors and their downstream targets (Zhong *et al*., 2013; Liu *et al*., 2015) and a central role of NAC-NOR in regulating ripening (Lu *et al*., 2018). These and additional aspects of the molecular genetic control of tomato fruit ripening are reviewed in Klee and Giovannoni (2011), Gallusci *et al*. (2016) and Giovannoni *et al*. (2017).

Once the fruit has attained sufficient maturity, typically around the mature green stage, exposure of these fruit to endogenous levels of ethylene hastens the onset of the climacteric and ripening. Once the ethylene concentration within the fruit surpasses a "threshold" level, it will promote its own biosynthesis (i.e., positive feedback) and autocatalytic ethylene production

will cause a rapid increase in production and accumulation within the tissues (Abeles *et al.*, 1992). The atmosphere within a tomato fruit is effectively isolated from the surrounding atmosphere by an impermeable skin and cuticle; about 95% of gas exchange occurs through the stem scar. Therefore, once ethylene has started its positive feedback climacteric rise, few external treatments can modulate its synthesis. Reduced temperatures and lowered oxygen atmospheres slow overall metabolism, but ripening will continue albeit at a slower pace. However, certain inhibitors of ethylene action (e.g., 1-MCP, ethanol vapors) appear to stop reversibly ethylene enhanced fruit ripening at almost any stage of ripeness (Saltveit and Sharaf, 1992).

During ripening the fruit can be partially green and red. Once ripe, however, high quality fruit have uniform red distributed over the entire surface of the fruit particularly if they harbor the *uniform* mutation mentioned above. Under proper conditions of temperature and humidity, tomato fruit progress through six well defined stages to the red-ripe stage. These stages are mature-green (1), breaker (2), turning (3), pink (4), light-red (5), and finally red-ripe (6) and they are based in practice almost entirely on the external color change of the fruit from green to red (i.e., destruction of chlorophyll and synthesis of lycopene). At the mature-green stage ("mature" or viable seed and no external red coloration), fruit have reached their final size and acquire the ability to continue to mature and ripen normally after harvest, though generally at a slower pace than fruit on the vine.

Ripening marks the beginning of senescence. Dark grown fruit are white in color due to lack of chloroplasts. They however, contain organelles (etioplasts and amyloplasts) that are capable of carotenoid accumulation and red color formation during ripening. Expression of carotenoid biosynthesis genes (e.g. *PHYTOENE SYNTHASE—PSY1*) change during ripening. PSY1 catalyzes the first step in the carotenoid biosynthetic pathway, is strongly induced at ripening initiation and mediates carotenoid flux of the ripening fruit toward accumulation of the characteristic red pigment of tomato, lycopene. LYCOPENE β-CYCLASE (LCYb) converts lycopene to the orange pigment β-carotene and is repressed by ripening induced ethylene resulting in lycopene accumulation. It is for this reason that early ripening tomatoes often appear orange as one is witnessing the activity of LYCb prior to its arrest. Flavonoids such as anthocyanin also accumulate in the fruit peel during ripening and significantly affect fruit color (Ballester *et al.*, 2010).

RIPENING INHIBITOR (RIN) regulates autocatalytic ethylene production genes and plays a role in aroma formation by regulating LIPOXYGENASE (LOX) genes (Qin *et al.*, 2012). *rin* mutants exhibit suppressed expression of ACC synthase (*ACS2* and *ACS4*; Barry *et al.*, 2000) and ACC oxidase (*ACO1*) genes that are involved in autocatalytic ethylene production. The RIN protein physically interacts with other ripening promoting transcription factors, for example TOMATO AGAMOUS-LIKE 1 (TAGL1) and FRUITFUL 1 and 2 (FUL1/TDR4 and FUL2/MBP7) to act as a regulatory complex (Wang *et al.*, 2019). TAGL1 is necessary for autocatalytic ethylene production and its repression completely inhibits ripening (Vrebalov *et al.*, 2009) while FUL1 and FUL2 appear to operate redundantly with maximal ripening effects when both are repressed (Bemer *et al.*, 2012). Interestingly, RIN appears to play a role in negative regulation of ethylene biosynthesis through promotion of the expression of two tomato transcription factors (APETALA2a (AP2a) and MADS-box Protein1 (MADS1)) that negatively influence fruit ripening (Dong *et al.*, 2013; Karlova *et al.*, 2014), possibly to damp the process. Plants in which *AP2a* is repressed produce fruits with earlier ripening initiation, orange ripe fruits, faster senescence and high levels of ethylene production. Expression of *MADS1* is high in mature green fruit but decreases as fruits ripen. Plants, with silenced *MADS1*, produce fruits that ripen earlier and produce more ethylene compared to the wild type. COLORLESS NON-RIPENING (CNR) on the other hand up-regulates the expression of ripening related genes like *PSY1*, *LOX* and *ACO1* (Qin *et al.*, 2012). Mutants (Cnr) are incapable of carotenoid biosynthesis because they lack phytoene and other carotenoid precursors. However, CNR also promotes expression of a negative regulator of ethylene biosynthesis, *AP2*. The final step in ethylene biosynthesis requires activity of ACO. Silencing of its transcription factor, HB-1 reduces expression of *ACO1* leading to inhibition of ripening. Additional recently discovered transcription factors, NAC domain protein (NAC4) and GRAS family (GRAS38) are positive regulators of ripening (Zhu *et al.*, 2014,

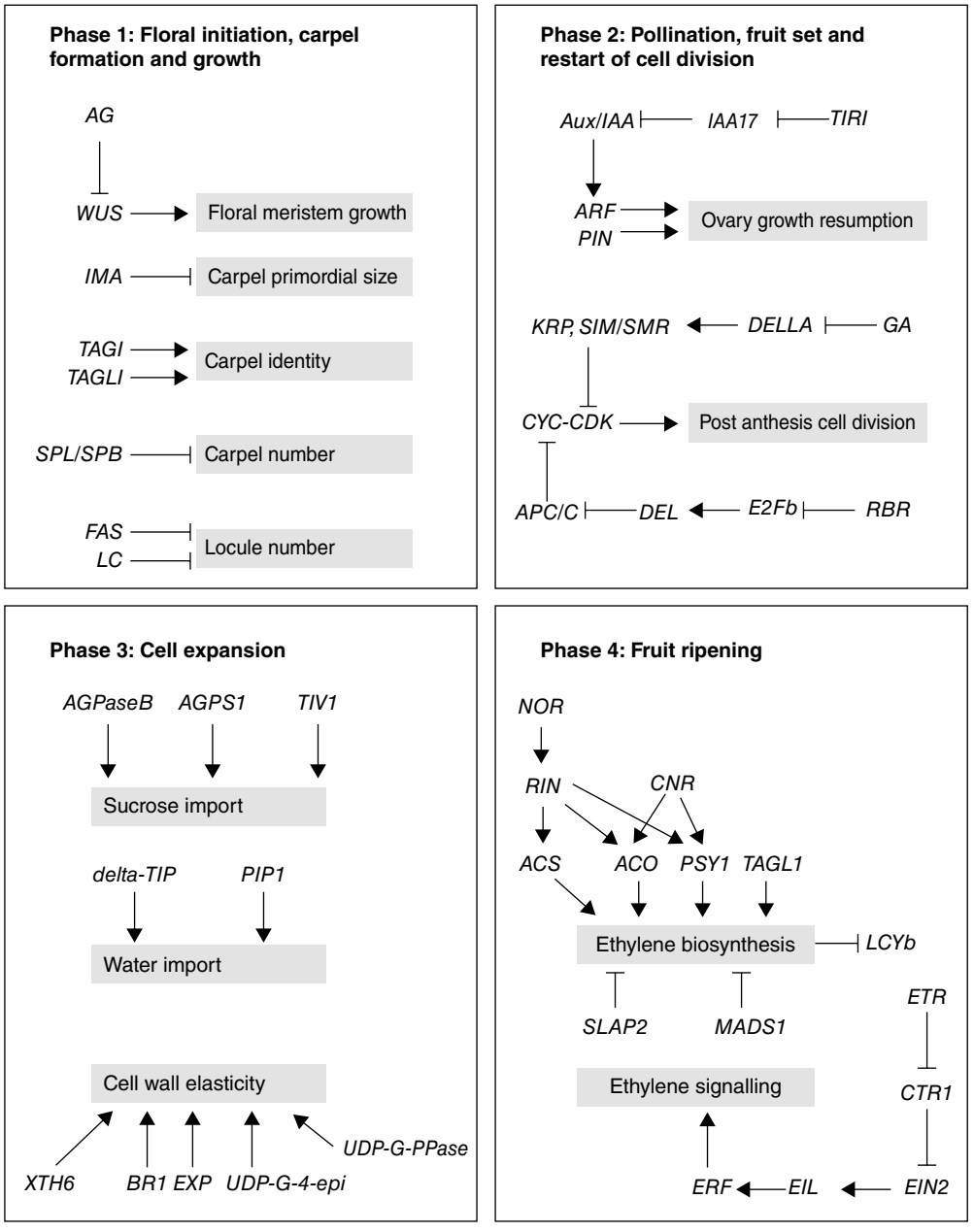

Fig. 7.4. Schematic representation of transcriptional regulation of the four phases of tomato fruit development (Heuvelink and Okello, 2018).
Abbreviations explained in the text.

instead of 2014, Shinozaki *et al*., 2018). Suppression of *NAC4* and *GRAS38* results in fruits with delayed ripening, decreased ethylene biosynthesis, increased firmness, suppressed chlorophyll degradation, and reduced carotenoid phenotype.

Ethylene receptors are encoded by *ETHYLENE RESPONSE* (*ETR*) genes, with ETR3/NEVERRIPE and ETR4 the most important in the fruit. ETR genes play a role in ethylene signaling by removing the block on ETHYLENE INSENSITIVE 2

(EIN2) exerted by CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1). The released EIN2 promotes EIN3/EIN3-like (EIL) transcription factor gene activity which activates transcription of *ETHYLENE RESPONSE FACTORS* (*ERF*). In general, ERFs positively mediate ethylene signaling. For example, ERF1 positively mediates ethylene signaling while ERF.B3 is active in feedback mechanisms that regulate ethylene production and responses. An additional tomato gene influencing ripening via ethylene signal transduction includes *GREEN-RIPE* which may be involved in facilitating the interaction of receptors with their Cu^{++} cofactor (reviewed in Gapper *et al.*, 2013).

Vegetative Growth

Tomato is usually described as a quantitative short-day plant, but vegetative growth is promoted by long days (Picken *et al.*, 1986). As mentioned above, the vegetative phase of a tomato plant is very short since the floral transition occurs, for most cultivars, when the third leaf is expanding. In tomatoes, vegetative growth and reproductive growth are thus proceeding concomitantly during the greatest part of the plant's life.

Leaf photosynthesis

Photosynthetic capacity of leaves varies widely according to light, water, and nutrient availability and these differences in capacity usually reflect Rubisco (Ribulose-1,5-*bis*-phosphate-carboxylase-oxygenase) content. Leaves in high light environments ("sun" leaves) have greater CO_2 assimilation capacities than those in shaded environments or lower in a canopy (Fig. 7.5) and this is reflected in the larger allocation of nitrogen-based resources to photosynthetic carbon reduction. Sun leaves have a high stomatal density, are thicker and have a higher ratio of Rubisco to chlorophyll in order to utilize the larger availability of photons (and hence ATP and NADPH). Shade leaves are larger and thinner, but have more chlorophyll per unit leaf dry weight than sun leaves. Shade leaves achieve a lower maximum rate of assimilation.

Whereas steady-state photosynthetic responses to environmental factors have been extensively studied, knowledge of dynamic modulation of photosynthesis remains scarce and scattered. However, incident irradiance on plant leaves often fluctuates. Dynamic photosynthesis is separated into sub-processes related to proton and electron transport, non-photochemical quenching, control of metabolite flux through the Calvin cycle (activation states of Rubisco and RuBP regeneration, and post-illumination metabolite turnover), and control of CO_2 supply to Rubisco (stomatal and mesophyll conductance changes; Kaiser, 2016). Increases in ambient CO_2 concentration and temperature (up to ~ 35°C) enhance rates of photosynthetic induction and decrease its loss, facilitating more efficient dynamic photosynthesis. Depending on the sensitivity of stomatal conductance, dynamic photosynthesis may additionally be modulated by air humidity. However, Rubisco activase and stomatal conductance are the main targets for improvement of photosynthesis in fluctuating irradiance.

Light use efficiency

As for many other crops, a linear relationship between cumulative intercepted photosynthetically active radiation (PAR) and dry mass production has been reported. The slope of this relationship is the light use efficiency (LUE), expressed in g dry mass per MJ of intercepted PAR. Biomass production is primarily determined by crop photosynthesis, while photosynthesis to a large extent depends on light interception, which furthermore increases with leaf area index (Lambert-Beer law; $(1 - e^{-k.LAI})$). The constant in this relationship is called the extinction coefficient k and for tomato its value is about 0.7 for diffuse radiation. In particular, internode length, leaf angle, and leaf shape affect the vertical distribution of light in a canopy. A more spacious canopy architecture due to long internodes and long and narrow leaves led to an increase in simulated crop photosynthesis of up to 10% (Sarlikioti, 2011).

Quantum yield or LUE, expressed as mol of CO_2 fixed per mol of photons absorbed, can be defined at leaf level but also at crop canopy level. Its value depends on light intensity, atmospheric CO_2 concentration and humidity, the composition of solar radiation (higher LUE at diffuse radiation; Li *et al.*, 2014), and root environment

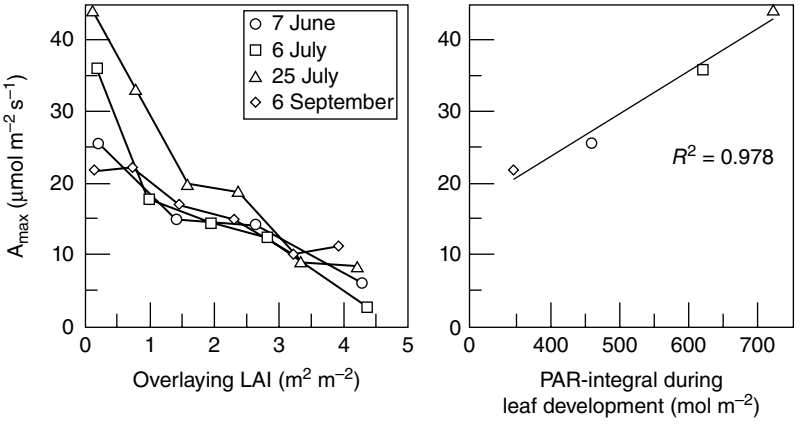

Fig. 7.5. The effect of overlaying leaf area index (LAI) on the A_{max} of leaves in vertically-grown tomato plants measured on four dates in summer (Panel A), and the effect of the PAR-integral received during 21 d of leaf development on the A_{max} of the youngest mature leaves (Trouwborst *et al*., 2011).

(soil moisture, salinity). At ambient CO_2 concentration, leaf LUE is about 0.05 mol mol^{-1}, with a maximum of 0.08 mol mol^{-1} without photorespiration, i.e. at very high CO_2 levels. A high temperature increases (photo)respiration, and consequently decreases LUE. Higher LUE of modern greenhouse cultivars (3.3 to 3.5 g per MJ PAR compared to 2.4 to 2.5 g per MJ PAR in open field tomatoes) results from a decrease in *k* and an increase in leaf photosynthetic rate (Higashide and Heuvelink, 2009). For field-grown fresh tomato, a LUE of 2.1 g MJ^{-1} (PAR) was observed (Scholberg *et al*., 2000).

Carbohydrate partitioning

Although the mechanism by which a plant partitions its resources between the different organs is of both theoretical and practical interest, it is still not fully understood. It is generally agreed that sinks play an important role in partitioning and in a tomato plant fruit are the most important sinks. Biomass allocation involves the transport of assimilates from sources to sinks. Hence, sources, the transport path, and sinks may influence allocation. Hormonal signals, such as cytokinins, abscisic acid, the ethylene precursor 1-aminocyclopropane-1-carboxylic acid and the auxin indole-3-acetic acid may coordinate assimilate production and biomass partitioning (Pérez-Alfocea *et al*., 2010). The order of priority in assimilate partitioning changes from the order of root > young leaf > flower in flowering plants to that of fruit > young leaf > flower and root in fruiting plants (Ho, 1996b).

Light can play an indirect or direct role in regulating source-sink relationships involved in allocation of photo-assimilate within the growing plant. The light intensity received by the plant affects the quantity of assimilates available to the plant organs and thus their degree of sink competition. Light also directly stimulates tomato fruit growth, due to mechanisms other than photosynthesis. This can be interpreted as a direct influence of light on tomato fruit sink strength. Fruit illumination stimulated cell division but had no detectable effect on fruit size, suggesting that effects of cell division are compensated by effects on cell expansion (Okello *et al*., 2015a).

Partitioning may be analyzed according to the concept of sink strengths. The term sink strength is used to describe the competitive ability of an organ to attract assimilates. Sink strength can be quantified by the potential growth rate of a sink, i.e. the growth rate under conditions of non-limiting assimilate supply, and depends on sink activity and size. Whereas sink activity is determined by processes such as phloem transport, metabolism, and compartmentation, sink size is determined by the cell number (Ho, 1996a). Sink strength of a truss is proportional to the number of fruit per truss. However, at high fruit numbers per truss, this is no longer so, as the

distal fruits show a reduced sink strength (De Koning, 1994).

The effect of removal of the middle leaf of each vegetative unit between two successive trusses has been investigated in a modeling study (Table 7.1). As expected, the reduced vegetative sink strength by leaf pruning increased partitioning to the fruits from 66 to 74%. However, yield was hardly affected as leaf pruning resulted in a lower LAI and hence lower light interception and total biomass production. In contrast, when leaf pruning was combined with delayed picking of older leaves to obtain the same LAI as in the control, a yield increase of 13% was predicted. Hence, a tomato cultivar with two instead of three leaves between trusses would improve yield, when combined with measures to keep LAI sufficiently high.

Increased temperature enhances early fruit growth at the expense of vegetative growth as the rate of plant development (new leaves and trusses) is higher (p. 144). The strong assimilate demand by the growing fruits at higher temperatures not only causes reduced leaf growth, but also delays growth of newly set fruits and stimulates flower abortion. As a consequence, after some time total sink strength of the fruits is low and the plant recovers vegetatively and good quality flowers develop. Subsequently, these flowers set fruit and form strong sinks resulting in the onset of a second cycle of strong fruit growth. This leads to a more or less pronounced alternation of fruit growth and vegetative growth (De Koning, 1989). On a possible direct effect of temperature on partitioning in tomato, literature data are not conclusive. For example, partitioning towards the fruits was only little improved when young producing tomato plants were exposed for three weeks at 24°C compared to 19°C (Heuvelink, 1995a). However, when a similar experiment was conducted for a period of two weeks during the anthesis of the sixth truss, the fraction of dry matter partitioned to the fruits was 0.68 and 0.80 at 19°C and 23°C, respectively (De Koning (1994).

Supply of assimilates from leaves to trusses in a multi-truss tomato plant is rather localized, the three subtended leaves of a truss being the principal suppliers. A truss together with the three leaves immediately below it has been regarded as a sink-source unit, although this relationship is not absolute. Removing a truss at anthesis resulted in yield increases on some of the remaining trusses closest to the one removed (Slack and Calvert, 1977). These observations seem to suggest that the transport path (phloem resistance) plays an important role in biomass allocation in tomato plants. However, a study in double-shoot tomato plants where on half of the plants no trusses were removed from one shoot and all trusses were removed at anthesis from the other shoot (100-0), whereas on the other plants, every other truss was removed from both shoots (50-50), showed that biomass allocation was the same for both treatments (Fig. 7.6). Hence, biomass allocation may be considered to originate from one common assimilate pool. This does not exclude that in intact plants, trusses are fed predominantly by the leaves nearby. It is also not conflicting with the observation by Slack and Calvert (1977) that excising a fruit truss at anthesis gave most gain in weight for trusses just below or above the excised one. This observation can be easily explained based on

Table 7.1. Average LAI, total and fruit dry weight, and fraction partitioned to the fruit in a simulation study for a greenhouse tomato crop (January till September). Dry matter partitioning is simulated based on sink strengths of plant organs. Leaf pruning (removal of one out of each three young leaves) was simulated by reducing the sink strength of each vegetative unit by one-third. Lowest, old leaves from a vegetative section were removed one week before fruits on the corresponding truss above the section were harvest-ripe. Seven fruits per truss were assumed. When delayed leaf removal was applied, removal of old leaves was delayed by two weeks compared to the control (Xiao *et al*., 2004).

Treatment	LAI ($m^2\ m^{-2}$)	Total dry weight ($g\ m^{-2}$)	Fruit dry weight ($g\ m^{-2}$)	Fruit fraction (%)
Control	2.4	3190	2093	66
Leaf pruning	1.7	2887	2125	74
Leaf pruning & delayed old leaf removal	2.3	3201	2362	74

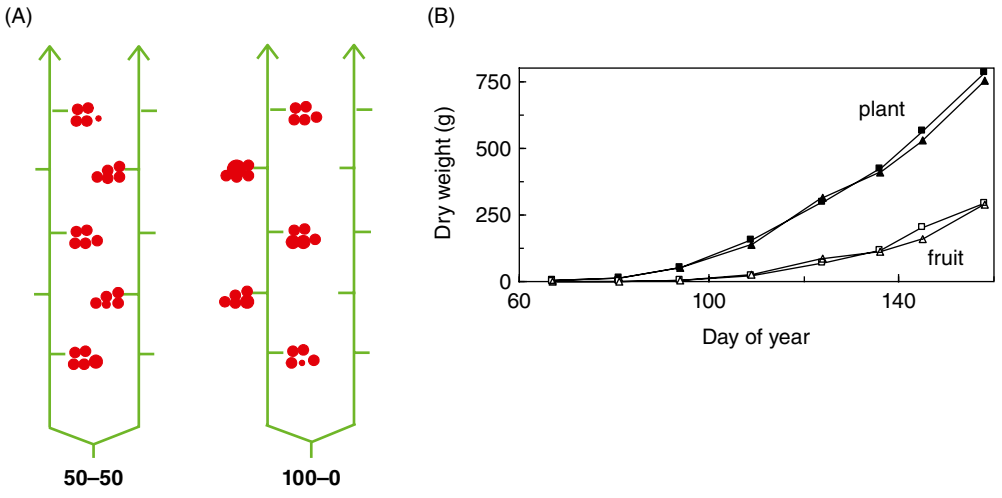

Fig. 7.6. (A) Schematic presentation of two pruning treatments on tomato plants with two equal stems: removal of every second truss at anthesis on each of the two stems (50–50) or removal of all trusses at anthesis from one stem and no truss removal from the other stem (100–0); (B) Total (closed symbols) and fruit (open symbols) dry weight increase for pruning treatment 50-50 (squares) and 100–0 (triangle). Experiment described in Heuvelink (1995b).

plant development without assuming a "distance effect" on assimilate partitioning (Heuvelink, 1996b). In conclusion, transport path plays only a minor role in biomass allocation in tomato.

Source:sink ratio

In a tomato plant, assimilate availability (source strength) is usually lower than assimilate demand (sink strength), which is shown by increased fruit size when some of the fruits are removed in an early stage. It was estimated that total assimilate demand is twice the assimilate availability, averaged over a whole growing season (De Koning, 1994), while for another tomato cultivar a source:sink ratio of 0.3 was reported by Bertin (1995). Increased light intensity and CO_2 concentration improve source strength, whereas sink strength primarily depends on temperature. Hence, competition occurs between vegetative and generative plant organs, among trusses and among fruits within a truss. For several cultivars, the altered source:sink ratio by leaf or fruit pruning did not influence the light saturated photosynthesis of young, fully expanded leaves measured under CO_2-limited conditions

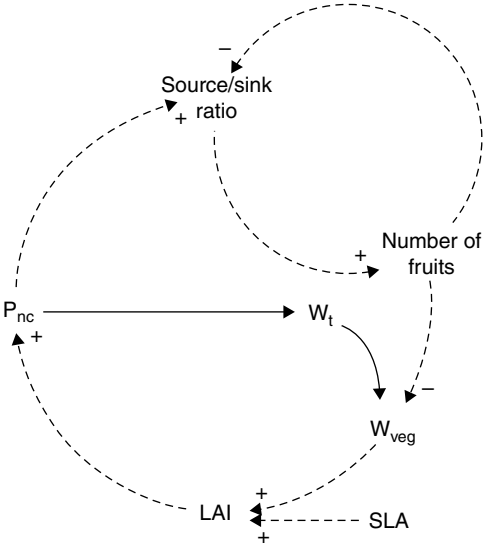

Fig. 7.7. A simplified representation of two important interactions (feedback mechanisms) between dry matter production and dry matter partitioning in an "indeterminate" tomato crop: (+) positive influence; (–) negative influence. Solid lines represent carbon flow, dashed lines represent information flow. LAI, leaf area index; P_{nc}, crop net assimilation rate; SLA, specific leaf area; W_t, total crop dry mass; W_{veg}, vegetative crop dry mass.

(Matsuda *et al*., 2011). Although soluble sugars accumulated in leaves of plants grown under high source:sink ratio, the amount of Rubisco was not affected by the altered source:sink balance. This study supports previous works suggesting that even a 50% decreased sink capacity did not lower leaf photosynthetic rate at ambient CO_2 conditions (Heuvelink and Buiskool, 1995). However, photosynthetic acclimation did occur under elevated CO_2, when plant sink strength was reduced by about 70% (Qian *et al*., 2012).

Two important interactions (feedback mechanisms) between dry matter production and dry matter distribution in tomato can be distinguished (Fig. 7.7): i) flower and/or fruit abortion at low source:sink ratio, resulting in fewer fruits on the plant and hence decreased sink strength and increased source:sink ratio; and ii) partitioning to the vegetative parts determining LAI and hence light interception and dry matter production. Fruit yield can be considered as the product of total biomass production and the fraction partitioned to the fruits. A larger number of fruits per truss will on the one hand increase partitioning to the fruits. On the other hand, as at the same time fewer assimilates are partitioned to the vegetative part, larger fruit numbers will lead to a lower LAI, the fraction of intercepted light and hence total biomass production. Conversely, individual fruit mass increases with decreased fruit number per plant, as competition for assimilates among fruits is reduced. This increase in individual fruit mass results from a higher average growth rate of individual fruits, as fruit growth period (time from anthesis until harvest ripe) is hardly affected by fruit load. At the commercial level, fruit pruning is generally applied to obtain a certain desired average fruit weight. Because of these two counteracting effects, fruit yield shows an optimum response to fruit number per truss (Heuvelink and Bakker, 2003).

Reproductive Growth

Fruit set

Fruit set, defined here as the proportion of open flowers which subsequently set fruit of a marketable size, may fail for many reasons. Flower/fruit abortion is defined as the proportion of flowers that fail to yield fruit of marketable size and hence fruit set is one minus abortion. In tomato, flower abortion occurs frequently, whereas fruit abortion is uncommon, although sometimes distal fruits stop growing at a small size and never ripen. Failure of pollen production (number and viability of pollen) or pollination, pollen germination, pollen tube growth, ovule production, fertilization, or fruit swelling may all result in poor fruit set. However, poor fruit set in low light conditions is most frequently caused by failure of pollen production or pollination. Abnormal flowers with rudimentary petals, stamens, and pistils produced under low light produce sterile pollen. The most critical stage for pollen development is meiosis, which occurs about nine days before anthesis in plants grown at 20°C. A day/night temperature of 32/26°C compared to 28/22°C did not cause a significant change in the number of pollen grains produced, however, it significantly decreased the number of fruit set, pollen viability, and the number of pollen grains released (Sato *et al*., 2006). Failure of tomato fruit set under a moderately increased temperature above optimal was due to the disruption of sugar metabolism and proline translocation during the narrow window of male reproductive development. Also failure in anther dehiscence at high temperatures has been mentioned (Arrizumi *et al*., 2013). At high humidities, pollen tends to remain inside the anthers, whereas at low humidities it may not adhere to the stigma. However, between 50 and 90%, the effects of relative humidity are small. Pollination can be adversely affected by abnormalities in flower structure. For self-pollination, the stigma must lie within the tip of the anther cone. Stigma exsertion beyond the cone enables the pollen to escape before reaching the stigma, and it may also lead to desiccation of the stigmatic surface. The length of the style is both genetically determined and affected by growing conditions. Low light, high nitrogen levels, and high temperature have been shown to increase stigma exsertion. Sato *et al*. (2000) conclude from their work that cultivar differences in pollen release and germination under heat stress are the most important factors determining their ability to set fruit. Humidity and light only marginally affect pollen tube growth.

Low light may reduce the size of flowers and ovaries and ovule development may cease under

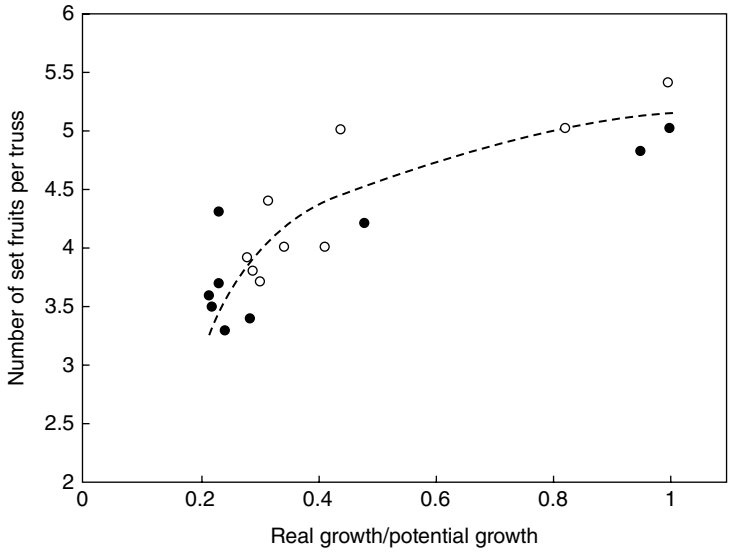

Fig. 7.8. Number of set fruits on the first nine inflorescences of beefsteak tomato "Capello," grown in CO_2-enriched (○) or non-enriched (●) polycarbonate greenhouses, as a function of the ratio between real and potential fruit growth, which is proportional to source:sink ratio, calculated at fruit set of each inflorescence, pruned to seven flowers (reprinted, with permission, from Bertin, 1995).

such conditions before or soon after embryo sac formation. Ovule viability may be adversely affected by high temperature (40°C), five to nine days before anthesis at about the time of meiosis in the ovule mother cells.

Fruit set is positively correlated with assimilate availability, which may be expressed as the source:sink ratio in the plant (Fig. 7.8). Tomato fruit set depends on successful pollination and fertilization, which trigger the fruit developmental program through activation of auxin and gibberellin signaling pathways. However, the role of each of these two hormones is still poorly understood. De Jong *et al*. (2009) reviewed the role of auxin and gibberellin in tomato fruit set and present a model integrating the role of both hormones (Fig. 7.9). The level of cytokinins is up-regulated five days after anthesis, suggesting a positive correlation between cytokinins and cell division. Application of synthetic cytokinin to pre-anthesis ovaries resulted in parthenocarpic fruit formation by activating cell division (Matsuo *et al.*, 2012). The precise role of seeds in the initiation of fruit growth in tomato has not been determined, although it has been suggested that they may be sources of auxin which stimulate fruit swelling. Gibberellins are also involved in fruit growth initiation, for high levels of endogenous gibberellins in the ovaries have been observed in cultivars which exhibit parthenocarpy.

Three sources of facultative parthenocarpy, pat-1, pat-2, pat-3/pat-4, are since long available for tomato breeding (Gorguet *et al.*, 2005), while recently three to four new loci have been identified, designated pat-6/pat-7 and pat-8/pat-9. Pat-6 and pat-8 are at the same locus and might be the same (Gorguet *et al.*, 2008). The parthenocarpic fruit development under control of these genes seems to be triggered by a deregulation of the hormonal balance in some specific tissues. Auxins and gibberellins are considered as the key elements in the parthenocarpic fruit development of those lines, mainly by an increased level of those hormones in the ovary, which may substitute for fertilization, to trigger fruit development. The presence of the pat-genes often results in pleiotropic effects like reduced taste, growth, and yield. This has hampered the release of commercial cultivars (Van Heusden and Lindhout, 2018).

Alternatively, obligate parthenocarpic tomatoes have been obtained by expressing the rolB gene from *Agrobacterium rhizogenes*, which enhances auxin sensitivity. This gene was expressed

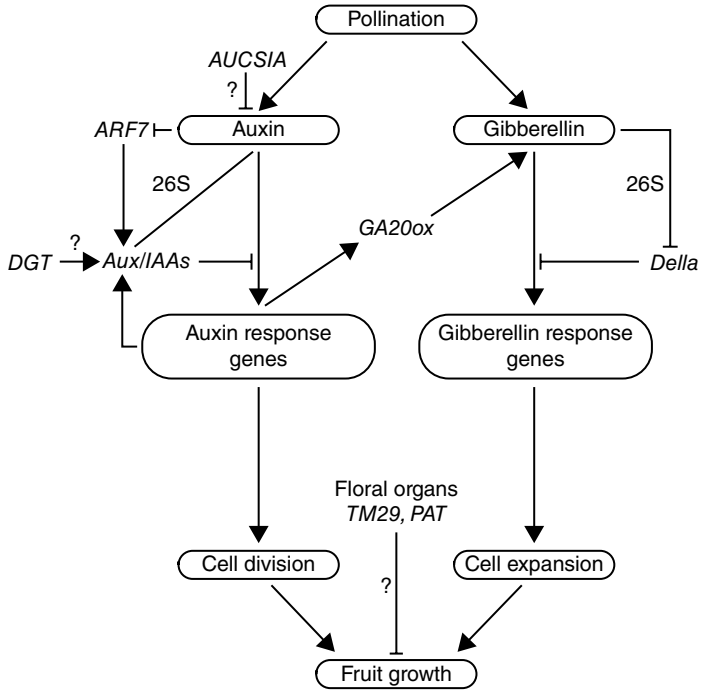

Fig. 7.9. A model integrating the role of auxin and gibberellin in tomato fruit set. The levels of both hormones increase after pollination, resulting in the activation of auxin and gibberellin response genes, which, in turn, will trigger fruit growth by regulating cell division and cell expansion. The auxin response is tightly regulated in a complex network, although the functions of some of the components in this network, such as AUCSIA and DIAGEOTROPICA (DGT) are not yet clear. Before pollination, the auxin response is inhibited by *ARF7* and *Aux/IAAs*, but upon pollination, these negative regulators are inhibited, or degraded by the 26S proteasome and the auxin response genes are transcribed. Some of these auxin response genes are *Aux/IAAs*, which create a negative feedback on the auxin signaling response. By contrast, the gibberellin signal pathway is subjected to positive feedback, as gibberellin stimulates the degradation of DELLA, a repressor of gibberellin signaling, through the 26S proteasome. However, gibberellin does not regulate fruit growth independently of auxin, since auxin seems to be able to stimulate gibberellin biosynthesis through the transcriptional activation of *GA 20-oxidases* (*GA20oxs*). Moreover, other factors such as TM29 and PAT, which might be derived from anthers, petals or sepals, also seem to have an important regulatory role in fruit set. The regulatory roles of other hormones, like ethylene, abscisic acid, and cytokinin are not included in this model, but these factors also contribute to the regulatory network that is required for the tight coordination, both temporarily and spatially, of fruit growth (De Jong *et al*., 2009).

using the TPRP-F1 promoter, which is highly specific for ovary, young fruits and developing embryos. Though these genetically modified tomatoes have given promising results in terms of quality and quantity of seedless fruit production, concerns from society still inhibit the release of transgenic cultivars (Van Heusden and Lindhout, 2018).

Cell expansion and endoreduplication

Cell expansion is a process that starts after fruit set and continues until the end of fruit growth. By the end of fruit growth, mesocarp cells can attain a size that is 30,000 times that of cells at anthesis. This increase in cell size is caused by import of water and sucrose and accumulation of macromolecules and organelles. Imported sucrose is broken down into glucose and fructose by vacuolar acid invertase. Glucose molecules are used in the formation of ADP glucose, the building blocks of starch. ADP glucose formation is catalyzed by ADP Glucose pyrophosphorylase (ADPGPP) while starch synthase catalyzes starch formation from ADP glucose. Towards the end of fruit growth, starch is broken down into fructose

and glucose in a reaction catalyzed by starch phosphorylase. The expression of genes encoding these enzymes markedly influences fruit growth. Silencing of the gene *TIV1* encoding vacuolar acid invertase biosynthesis for example causes reduced fruit size, high sucrose but low fructose and glucose content. Guan and Janes (1991) demonstrated remarkable differences in fruit size of *in vitro* grown fruits exposed to light or darkness. They attributed the large fruit phenotype observed in light grown fruit to higher rates of starch accumulation due to activation of ADPGPP by light.

Water uptake during cell expansion is driven by an osmotic potential gradient caused by carbohydrate accumulation. Water channels (aquaporins) facilitate water entry into cells. The expression of two aquaporin encoding genes (delta-tonoplast integral protein: *delta-TIP*; plasma membrane intrinsic protein: *PIP1*) increases between 28 and 42 days after anthesis (Prudent *et al*., 2010). Cell expansion following water and carbohydrate uptake depends on cell wall elasticity. Therefore factors influencing cell wall elasticity like new cell wall deposition, composition, bonding between wall components, and enzymatic modification of the wall significantly affect cell expansion. Expansin genes (*EXP*) encode proteins that act as a molecular grease between wall components thereby increasing cell wall elasticity. Prudent *et al*. (2010) studied the expression of two cell wall synthesis associated genes (UDP-glucose-4-epimerase: *UDP-G-4-epi*; UDP-glucose-pyrophosphorylase: *UDP-G-PPase*) and three cell wall degradation genes (polygalacturonase: *PG*; two xyloglucan endotransglycosylases: *XTH6* and *BR1*) in tomato. Their findings show that under non source limiting conditions, *UDP-G-4-epi* expression increases with fruit maturity but declines towards ripening. The expression of *PG* is similar to that of *UDP-G-4-epi* except that no decline in expression is observed towards ripening. Expression of *UDP-G-PPase* on the other hand does not change while that of *XTH6* and *BR1* drops initially and levels off during fruit development.

Cell expansion in tomato fruit is positively correlated with endoreduplication; a modified cell cycle in which cells undergo a repeated gap and DNA synthesis phase leading to ploidy levels ranging between 4C and 512C (where C is the haploid DNA content). Inhibitors of the CYC-CDK complex like WEE1, KRP, SIAMESE (SIM)/SIAMESE-RELATED (SMR) proteins promote endoreduplication. Promotion of endoreduplication also occurs through inhibitory competition between E2F transcription factors. For example, E2Fc transcription factors inhibit activity of the cell cycle promoting *DEL1* when they occupy the DNA binding site occupied by E2Fb during the normal cell cycle. Endoreduplication causes an increase in nuclear size. A positive effect of endoreduplication on transcription has also been shown in some studies. It is thought that the positive correlation between ploidy level and cell size stems from a causal relationship between nuclear and cytoplasmic growth as proposed in the karyoplasmic ratio theory (Chevalier *et al*., 2014).

Fruit growth

Fruit growth period varies widely among tomato varieties generally taking longest in large fruit cultivars. Cumulative growth of a fruit is expressed in the form of a sigmoidal curve (Fig. 7.10; Monselise *et al*., 1978) with an initial one-to two-week period during which absolute growth is slow (primarily reflecting cell division), followed by two to six weeks of rapid growth (mostly due to cell expansion) up to the mature green stage.

Sink strength of a fruit is primarily determined by fruit developmental stage. In developing tomato fruit, sucrose metabolizing enzymes may regulate sucrose unloading and sink strength and thus fruit dry matter accumulation. Acid invertase may not play an important role in the regulation of assimilate import by the tomato fruit (Dorais *et al*., 1999). However, overexpression of sucrose-phosphate synthase (SPS) in field tomato fruit increased sucrose synthase (Susy) activity by 27%, and 70% more sucrose was unloaded in transformant fruit (20 days after anthesis) compared to the untransformed control. Acid invertase and ADPGPP activities remained at similar levels or were slightly lower than in the untransformed control. Unexpectedly, the repression of Susy activity in the fruit (antisense cDNA of the tomato fruit specific Susy, TOMSSF) did not affect the unloading capacity when compared to the untransformed plants. Hence, four sucrose turnover cycles (I- sucrose degradation

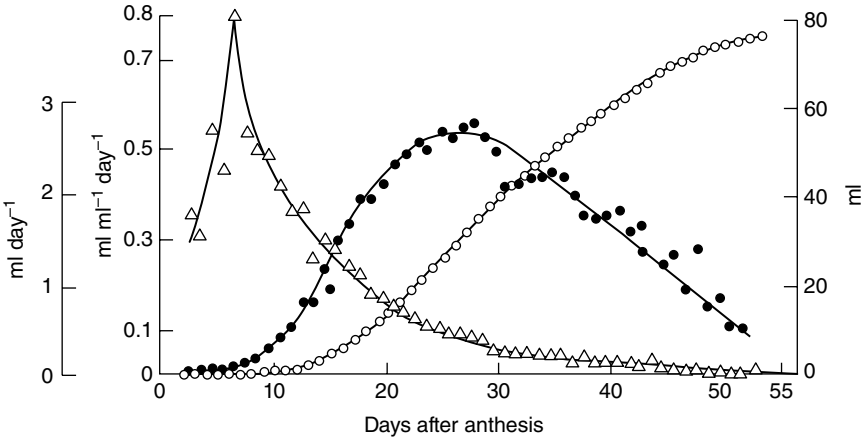

Fig. 7.10. Cumulative growth (○, ml), growth rate (●, ml day^{-1}), and relative growth rate, RGR (△, ml ml^{-1} day^{-1}) of tomato fruits (Monselise *et al*., 1978).

and resynthesis in the cytosol, II- sucrose degradation in the vacuole and resynthesis in the cytosol, III- sucrose degradation in the apoplast and resynthesis in the cytosol, and IV- starch degradation and synthesis in the amyloplast) may control sink activity of tomato fruit (Dorais *et al*., 1999). Thus, sink utilization (i.e. respiration, cellular structure, growth, and storage) determines the sucrose import rate. Sucrose metabolizing enzymes may affect the unloading rate but they are not the main regulatory factors.

Substrate cycles of sucrose and starch may provide metabolic flexibility and help to maintain the fruit as a carbon sink (Luengwilai and Beckles, 2009). Studies using transgenic lines whereby AGPase (the small subunit of ADPGPP) was overexpressed and suppressed, provided evidence that starch plays a more direct role in determining total soluble sugars and yield (Beckles *et al*., 2012). Indeed, partitioning of carbon to starch may increase sink strength in green fruit and then when it is degraded during ripening adds to the pool of sugars imported from the phloem. Approaches to manipulate fruit sink strength in order to improve harvest index and thereby crop yield and quality have been discussed (Herbers and Sonnewald, 1998), while biochemical factors contributing to tomato fruit sugar content have been reviewed by Beckles *et al*. (2012).

Different fruit size: genotype, temperature, and competition

Okello *et al*. (2015b) studied the physiological mechanisms for differences in fruit size of a small (cv. "Brioso") and intermediate-sized (cv. "Cappricia") cultivar and their response to two temperatures (21 and 27°C). These authors showed that differences in growth rate were more important than growth duration differences in determination of final fruit fresh weight differences between the two contrasting cultivars. The same was observed by Li *et al*. (2015) for three cultivars differing in fruit size. At cell level, it was observed that both cultivars differed in fruit size mainly because of differences in mesocarp cell number. Okello *et al*. (2015b) also noted that the reduction in fruit size at high temperature arose from reduction in cell volume and this occurred despite the 29% increase in cell number. Surprisingly, Bertin (2005) did not find differences in fruit size comparing 20 and 25°C; the higher cell number at 20°C was compensated by a reduced cell size. The higher cell number at 20°C resulted from an extended period of cell division, and the smaller cell size was due to a shorter period of expansion rather than a lower expansion rate. At the gene level, expression of three promoters (*CDKB1*, *CDKB2* and *CycA1*) and one inhibitor (*fw2.2*) of cell division was stimulated by fruit heating early during fruit development. Larger

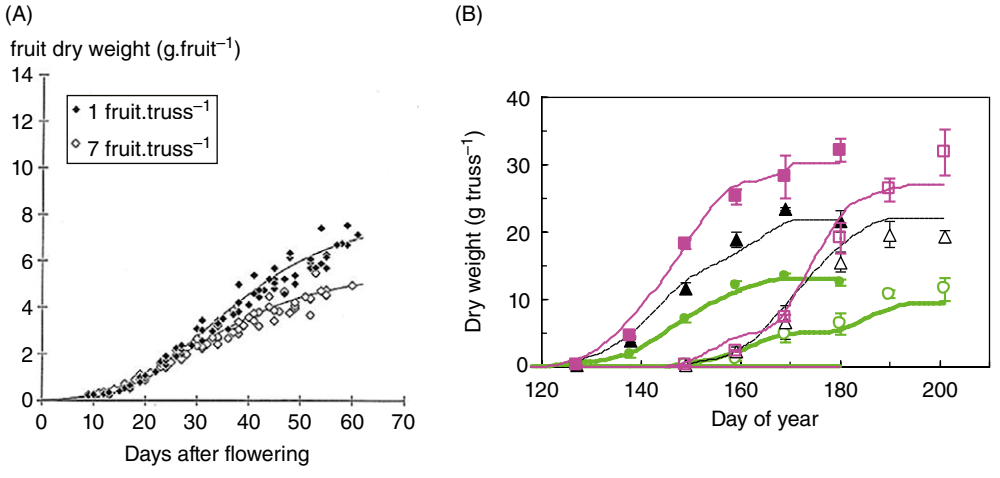

Fig. 7.11. (A) Measured (symbols) and fitted (Richards function, lines) tomato fruit dry weight as a function of days after anthesis for one (potential growth, ◆) or seven (◇) fruit per truss. (B) Measured (closed symbols, truss 3; open symbols truss 7) and simulated (lines, based on relative sink strength) tomato truss growth curves for plants with 3 (○, ●), 5 (△, ▲) and 7 (□, ■) fruit per truss. Vertical bars indicate standard error of mean, when larger than symbols (data from Heuvelink, 1996a).

cell number in "Cappricia" compared to "Brioso" tallied with higher expression of three cell cycle promoters (*CDKB2*, *CyCA1* and *E2Fe-like*) and lower expression of *fw2.2*. Other than the higher expression of *AGPaseB* in "Cappricia" at only one harvest stage, the expression of genes involved in promotion or inhibition of cell expansion did not tally with cell size observations in this study. The apparent mismatch between expression tendencies of some genes and cell and fruit level observations highlights the importance of downstream posttranscriptional regulatory mechanisms in fruit phenotype determination.

As tomato growth is usually source-limited, individual fruit growth is reduced when more fruits are growing on the plant (Fig. 7.11). The lower fruit growth rate and size of fruits when five fruits are retained per truss compared with only two fruits per truss results from the slow-down of cell expansion, whereas the number of cells is hardly affected in the proximal fruit (Bertin, 2005). However, within the inflorescence the decreasing gradient of fruit size from proximal to distal fruits is due to a decrease in cell number with similar cell size.

Fruit Quality Components

Beside the external fruit appearance (i.e. color, shape and size), the main quality traits of tomato are generally defined by its organoleptic attributes such as texture, sugars, organic acid content, and volatile compounds. The limited caloric supply, relatively high fiber content, and provision of minerals, vitamins, and phenols such as flavonoids make the tomato fruit an excellent "functional food" providing additional physiological benefits as well as meeting basic nutritional requirements (Dorais *et al*., 2008). In contrast to a substantial yield increase, tomato fruit taste and health value have declined over time. This is mainly related to the intensification of the production systems and the development and use of high-yielding, homogeneous firm, tasteless and mid- and long-life varieties developed for shipping and long-term storage. Recently QTL/genes for fruit flavor and health components were identified (e.g. Causse *et al*., 2011; Ruggieri *et al*., 2014; Sauvage *et al*., 2014; Tieman *et al*., 2017). By using new approaches such as metabolomics and consumer panels, substantial efforts have been made to better understand the chemical interaction between fruit components and consumer flavor preference (Tieman *et al*., 2012). In addition to genetics, quality traits also depend on the interaction between the environmental conditions and cultural practices during the course of fruit development, and are well documented by several reviews (Davies and Hobson 1981; Dorais *et al*., 2001; Ho, 2003; Dorais *et al*., 2008; Poiroux-Gonord *et al*., 2010; Etienne *et al*., 2013;

Ripoll *et al.*, 2014; Bertin and Génard, 2018). To explore the complex relationships between genetic, environmental conditions, and crop management, several process-based simulation models predicting processes underlying fruit quality were developed (Génard and Lescourret, 2004; Bertin *et al.*, 2010; Martre *et al.*, 2011; Kromdijk *et al.*, 2014). Examples of models are fruit growth models based on cell division and endoreduplication (Bertin *et al.*, 2007), cell division and expansion (Baldazzi *et al.*, 2012; Fanwoua *et al.*, 2013), carbon and water fluxes (Liu *et al.*, 2007), Genard and Gouble, 2005 respiration climacteric (Colombié *et al.*, 2017), sugar and acid metabolism (Lobit *et al.*, 2006; Prudent *et al.*, 2011; Etienne *et al.*, 2013), water and carbon fluxes (Baldazzi *et al.*, 2013), and by integrated models such as "virtual fruit" (Génard *et al.*, 2010) and multi-criteria optimization methods (Constantinescu *et al.*, 2016).

Fruit size and shape

While fruit shape is mainly determined by the variety (genetic), evidence showed that the final fruit size is highly correlated to the number of cells (Bertin *et al.*, 2003), which is strongly influenced by the interaction between genotype, environmental conditions and crop management (Bertin and Génard, 2018). Despite several explanations proposed to clarify the link between cell size and nuclear size or DNA content, the molecular basis of this correlation is still poorly understood (Chevalier *et al.*, 2011). In general, for modern cultivars, there is an opposite relationship between fruit size and fruit quality traits. Large tomato type varieties are often tasteless compared to cherry or cocktail varieties. Smaller fruits have generally higher vitamin C content while the greater skin to volume ratio of cherry tomatoes may enhance their flavonol content, which is mainly found in the skin (Dorais *et al.*, 2008).

Fruit texture

Fruit texture, defined by tomato firmness, meltiness, mealiness, chewiness, juiciness, crunchiness, particle size and shape characteristics, moisture and fat content (Bourne, 1980), interferes with flavor and aroma perception (Harker *et al.*, 2002; Causse *et al.*, 2003). Consequently, fruit texture, particularly fruit firmness, strongly influences consumer acceptability and purchasing (Causse *et al.*, 2010). Texture also impacts the shelf-life and shipping ability of tomato fruits (Seymour *et al.*, 2002). Fruit texture is complex because it involves various mechanisms as shown by the fruit softening during ripening (Dorais *et al.*, 2001; Toivonen and Brummell, 2008) and after harvest with the turgor loss, degrading enzymes and breakdown of the membrane and cell walls. During fruit growth and development, several studies reported that fruit texture is greatly determined by fruit internal structure such as the ratio between the pericarp and locular gel volume, thickness of different tissues, cell size, dry matter and soluble content, cell turgor, transport of solutes among cell compartments, chemical and mechanical properties of cell walls, cuticle structure and loss of water by transpiration (Dorais *et al.*, 2001; Bertin and Génard, 2018). Phenotypes and abiotic factors (e.g. light, temperature, water and nutrient availability, salinity) affecting these traits affect fruit texture (Dorais *et al.*, 2001; Dorais *et al.*, 2008).

Dry matter, sugar and organic acid content

In general, 5 to 10% of tomato fruit is dry matter, of which ~ 50% is represented by soluble sugars (mainly glucose and fructose in equimolar ratio; sucrose representing in general less than 5% of the soluble sugars), 15% by organic acids (mainly citric and malic acids; 7.7–10.4 meq 100 ml^{-1}; Davies and Hobson, 1981; Ho, 1996a) and amino acids (mainly glutamic acid, γ-aminobutyric acid and glutamine representing ~ 65% of the total amino acids), 8% by minerals (3–4% K, 0.6% N, 0.4% P), 1% by the cuticle and seeds, ~ 0.6 to 0.7% by fiber (lignin and polysaccharides from plant cell walls), and the remaining part by proteins, pectin, pigments, vitamins, and lipids (Davies and Hobson, 1981).

Fruit dry matter content is inversely proportional to fruit size, but positively related to total sugars content and to soluble solids and total solids ratio (Ho, 1996a). The transport,

accumulation and partitioning of sugars in the tomato fruit in relation to their metabolic pathways and enzyme activities are well defined (Dorais *et al*., 2001; Beckles *et al*., 2012; Bertin and Génard, 2018). Water import to the fruit is independent of assimilate concentration and is determined by plant water relations. Consequently, fruit size is inversely related to the salinity of the soil or growing medium, while the dry matter content increases with the salinity. Although fruit photosynthesis contributes to around 10 to 15% of the carbon required for fruit growth (Tanaka *et al*., 1974), fruit photosynthesis rarely exceeds its loss through respiration (~ 25% of imported carbon) and is not essential for fruit energy metabolism or development but plays a role for properly timed seed development (Lytovchenko *et al*., 2011).

Color and pigments

Color depends on fruit carotenoid concentration and distribution, and for some purple-fruited varieties on anthocyanin content (Jones *et al*., 2003). During tomato fruit ripening, carotenoids enhance by around 10 to 50 times, which is mainly related to the important accumulation of lycopene and β-carotene, and the loss of chlorophyll. Carotenoids are also a major class of compounds providing precursors to essential vitamins (e.g. β-carotene for vitamin A) and antioxidants, which constitute an important health value. Carotenogenic regulation has recently been reviewed by McQuinn *et al*. (2015). Proposed regulatory mechanisms of carotenoid biosynthesis include light, availability of substrates and metabolic sequestration and ethylene (Fanciullino *et al*., 2014).

Aroma volatiles

In addition to sugars (glucose and fructose) and acids (citrate, malate, and glutamate), tomato flavors are related to volatiles. For example, when the flavor-associated chemical composition of 48 modern cultivars were compared to 236 older *S. lycopersicum* accessions, a total of 13 flavor-associated volatiles were significantly reduced in modern varieties relative to heirloom varieties (Tieman *et al*., 2017). Thus, poor flavor of modern varieties is related to the dilution of many flavor volatiles that positively influence consumer liking. Although over 400 volatiles were identified in tomato (Buttery, 1993), only 16 of them contributed to the perception of tomato flavor (Baldwin *et al*., 2000). Volatiles include acyclic, cyclic, and heterocyclic hydrocarbons, alcohols, phenols, ethers, aldehydes, ketones, carboxylic acids, esters, and lactones (Lewinsohn *et al*., 2001). The most abundant volatiles reported among 152 heirloom tomato varieties were hexanal, cis-3-hexenal, cis-3-hexen-1-ol, 3-methyl-1-butanol, hexyl alcohol, 1-nitro-3- methylbutane, and 2-methyl-1-butanol (Tieman *et al*., 2012). However, some of the most abundant volatiles such as C6 volatiles did not contribute to consumer liking, whereas other less abundant ones did (Tieman *et al*., 2012, 2017). Apocarotenoid geranial was positively correlated with the perception of sweetness, independently of sugar concentration (Tieman *et al*., 2012). This finding proposes a novel way to increase perception of sweetness without increasing sugars as higher sugar content is generally only achievable with a penalty on fruit size (Tieman *et al*., 2017).

Vitamin C and polyphenols

Beside its important role in plants, ascorbic acid (vitamin C) is an important micronutrient for human diet because of its role as an antioxidant. Among varieties, its concentration varies from 80 to 400 μg g^{-1}. Ntagkas *et al*. (2018) concluded from their literature review that respiration and photosynthesis interact in light regulation of ascorbic acid biosynthesis via carbohydrate availability. Despite the fact that ascorbic acid is synthesized from D-glucose, its synthesis in tomato fruit is not limited by sugar content (Massot *et al*., 2010). Tomatoes are an important source of phenolic compounds, mostly restricted to their skin (98%) and placental tissue (Slimestad and Verheul, 2009). Chlorogenic acids and flavonoids are the main polyphenols in tomato. Polyphenols have diverse biological functions in plants and are known to play an important protecting role in response to biotic and abiotic stresses. In terms of human health, epidemiological studies showed strong correlation

between polyphenol intake and a decrease incidence of chronic diseases (Cherniack, 2012; Mursu et al., 2013). In tomato, the most important compounds for fruit quality are polyphenols, phenolic acids, phenylpropanoids, coumarins and flavonoids (Slimestad and Verheul, 2009).

Plant and Fruit Quality Disorders

Disorders of tomato leaves, stems and fruit, often referred to as physiological disorders, have both genetic and environmental components; in many cases the exact cause of the disorder is not well understood or involves a complex of factors. Although symptoms are characteristic and cultivars differ in susceptibility, the origin cannot be attributed to a single biological, environmental, or cultural factor. Several disorders (genetic disorders, stem disorders, hollow fruit, rough fruit, gold fleck, fruit pox) that are still economically important and described by Grimbly (1986) and in Kinet and Peet (1997) are not common in modern cultivars and production practices, and will not be discussed here. Flower and fruit abscission is discussed in the section "Reproductive growth" (p. 155). Blotchy ripening (non-uniform ripe fruit color) results from problems in lycopene development. The ripening process is discussed in the sections "Developmental processes" (p. 143), ("Fruit development and ripening", p. 148), and "Fruit quality components," (p. 160).

Blossom-end rot (BER)

Internally, BER first appears in green tomatoes as areas of white or brown locular tissue. Externally, a small, water-soaked spot appears, then enlarges, near the blossom scar. Affected tissue dries out, developing into a well-defined, dark, sunken, leathery spot. BER occurs when calcium and/or water levels in the root zone are low, but a complex interplay of anatomical, genetic and environmental factors determines when or if fruit develop the disorder. Interactions between daily irradiance, air temperature, water availability, salinity, nutrient ratios in the rhizosphere, root temperature, air humidity, and xylem tissue development in the fruit can all affect BER incidence (Dorais *et al.*, 2001). Fruit are most susceptible to BER when there is a rapid visible size increase. For control, selection of resistant cultivars, maintaining optimal salinity (< 4–5 dS m^{-1} in hot weather), avoiding drought, flooding, excessive daytime temperatures, high light and low humidity are recommended as well as adequate root zone calcium without excessive concentrations of competing cations (Adams, 1999).

BER symptoms first appear when a localized calcium deficiency in the distal end of the fruit results in leaky membranes, cell plasmolysis, membrane breakdown, and/or cell wall failure (Ho and White, 2005). The critical level for water soluble Ca^{2+} concentration in the distal part of young fruit is 0.18 μmol·g^{-1} FW and lower Ca^{2+} levels in the distal area of the fruit may irreversibly destabilize the cell structure of the young fruit, leading to BER (Vinh *et al.*, 2018). If water-soluble Ca (including both apoplastic and cytoplasmic Ca^{2+}) in the distal part of the young fruit exceeded 0.30 μmol·g^{-1} FW, BER symptoms were not observed (Vinh *et al.*, 2018). Based on a literature review.

Calcium availability in the distal part of the growing fruit requires balance between calcium transport and calcium demand. The use of a BER-resistant introgression line by Ikeda *et al.* (2017) provides evidence for the importance of Ca^{2+} transport but also illustrates the difficulty of separating the role of growth rates and tissue calcium levels in BER development. Fruit and leaf (but not root and stem) Ca^{2+} concentrations were higher in the resistant introgression line than the parent, suggesting that enhanced Ca^{2+} transport reduces BER. Expression of genes encoding for cation exchangers, Ca^{2+} -ATPases, a Ca^{2+} channel, and Na^{+}/Ca^{2+} exchangers were all higher in the introgression line, giving this line increased ability to accumulate Ca^{2+} in fruit and leaves. However, fruit growth rates were also lower in the introgression line.

Relationships between fruit growth rate, final fruit size, and calcium supply requirements are difficult to separate although, as discussed by Ho and White (2005), large-fruited cultivars are generally more susceptible to BER and BER increases with high temperature and high light during the period of rapid fruit growth (Ho *et al.* 1993). Comparing a large fruited (rapid fruit growth rate, susceptible to BER) and a medium fruited (slower fruit growth rate, less susceptible) tomato cultivar at a range of Ca:K ratios, Vinh *et al.* (2018) found that both had a similar

threshold level of calcium availability for the disorder to appear. Together with water-soluble Ca supply, rapid fruit growth rates explained just over half of the variation in BER incidence, suggesting fruit growth rate is important, but not the sole determinant of BER development.

Water availability to the fruit is inseparable from calcium availability and influences BER incidence because calcium is immobile in the phloem and enters the fruit mainly through the water-conducting tissue (xylem) as the fruit transpires. The supply of water and nutrients to fruit changes during fruit development (Hocking *et al*., 2016). Fruit often has low rates of transpiration and low xylem transport rates compared to leaves. These relatively low rates of fruit transpiration and competition with leaf transpiration mean that humidity can also affect BER incidence. Low daytime humidity, high temperature and high light, disproportionately increase leaf transpiration, decreasing the calcium supply to the fruit (Adams and Ho, 1993). High humidity, especially at night, can increase the fruit calcium content because of high root pressure (Gutteridge and Bradfield, 1983). Dorais *et al*. (2001) suggest deleafing to avoid excessive canopy transpiration, shading, roof sprinkling, greenhouse fogging, and keeping a proper fruit:leaf ratio. Olle and Williams (2017) suggest reducing BER in greenhouse tomatoes by using a negative DIF (higher night temperature than day temperature) to reduce plant height and Ca deficiency symptoms. Under negative DIF regimes, transpiration rates increase at night and decrease during the day, potentially bringing more Ca^{2+} into the fruit. They also reported higher Ca^{2+} in greenhouse plants grown under a far-red absorbing film which was also associated with reduced stem and shoot elongation.

De Freitas *et al*. (2014) tested whether BER susceptibility under water stress was related to the Ca^{2+} allocation between leaves and fruit by applying abscisic acid (ABA) to both the whole plant and only the fruit of water-stressed plants. Whole plant ABA sprays increased xylem sap flow and Ca^{2+} movement into the fruit, increasing water-soluble apoplastic Ca^{2+} concentrations, and reducing both leaf transpiration and BER compared to water sprayed and fruit sprayed treatments. Whole plant sprays also increased stem water potential and total fruit water uptake compared with water-sprayed controls. For the whole-plant ABA treatments, more functional xylem vessels were maintained during early fruit growth and development so there was less resistance to Ca^{2+} movement in the fruit and more Ca^{2+} could be moved to the distal end. Effects were greater with whole plant treatment than the fruit only ABA treatment, possibly because of the combination of increased xylem sap flow rate into the fruit and higher Ca^{2+} concentration in the xylem sap taken up by the fruit. Similarly, Barickman *et al*. (2014a, 2014b) found spray application of ABA to tomato plants decreased leaf Ca concentrations while increasing those in the fruit.

Saure (2014) proposed that Ca^{2+} deficiency is not the cause but a result of BER. Saure (2014) concludes that the actual cause of BER is abiotic stress, resulting in an increase of reactive oxygen species (ROS) and finally cell death.

Fruit cracking

Cracks can occur in circles around the stem scar (concentric cracking) or radiate from the stem scar (radial cracking). Shoulder check is a similar disorder occurring on the fruit shoulder (Huang and Snapp, 2004), while cuticle cracking, also called russeting, are very fine hair-like cracks limited to the cuticle and first layers of cells of the epidermis, located on the sides and bottom of the fruits (reviewed by Dorais et al., 2004). Cracking in all forms reduces marketability because fruit are unattractive, have decreased shelf life and are more vulnerable to water loss, disease and insects. Breeders have increased cracking resistance, but the episodic nature of damage makes it difficult to recommend effective control practices (Khadivi-Khub, 2015).

Cracking occurs when there is a rapid net influx of solutes and water into the fruit at the same time ripening reduces the strength and elasticity of the tomato skin, so maintaining uniform fruit growth rates (Peet, 1992) by, for example, increasing greenhouse temperatures gradually from night-time to day-time levels is helpful. High light intensity increases cracking because it raises fruit temperatures, fruit soluble solids and fruit growth rates. Avoiding rapid increases in soil water levels or decreases in nutrient solution electrical conductivity (EC) is critical. Li *et al*. (2002) found fruits were most susceptible to cracking when the

difference between the fruit growth rate in the new (low) and old (high) EC was maximal, rather than when the absolute growth rate was maximal. During the period of rapid fruit expansion in field grown tomatoes, Huang and Snapp (2004) found a consistent association between shoulder check and rainfall following hot, dry weather. Plastic rain covers reduced shoulder check.

Crack resistance is positively correlated with the thickness of the cuticular membrane, its stiffness, strength and resistance to breakage, flavonoids, lower fruit growth rates, and higher covalently bound pectins and susceptibility with higher activities of polygalacturonase, β-galactosidase and cellulase and higher levels of water-soluble pectin. Knoche and Lang (2017) suggest that fruit growth strains the fruit skin and cuticle. When the rate of cuticle deposition cannot keep up with the rate of increase in fruit surface area, cuticle failure resulting in cracking is likely. Dominguez *et al.* (2012) found that fruit growth rate during ripening, probably sustained by internal turgor pressure, is a key parameter in fruit cracking, because fruit ripening off the vine did not crack. Fruit cracking can occur at all stages of fruit growth, but mature fruit of large fruited cultivars are most susceptible (Olle and Williams, 2017). Fruit pruning to increase fruit size can increase fruit cracking (Abdel-Razzak *et al.*, 2016).

Inheritance of cracking resistance was thought to be complex, involving many genes, with low heritability and high environmental influence making selection difficult. Recently, however, Capel *et al.* (2017) have identified quantitative trait loci and codominant genetic markers for fruit cracking in the tomato genetic map constructed from a recombinant inbred line population, providing molecular tools for marker assisted breeding of cracking resistant tomato lines.

Oedema (intumescence injury)

Oedema appears as undifferentiated and callus-like swellings (blisters) on the leaf from abnormal division and enlargement of the epidermal and parenchyma cells. The turgid parenchyma cells erupt, then dry out, leaving necrotic areas and twisted, distorted leaves.

Oedema can appear when water provided to the leaves exceeds transpiration for a period of several days. However Kubota *et al.* (2017) also observed oedema in susceptible tomato cultivars grown in ultraviolet (UV) radiation deficient light environments such as light emitting diodes (LED) or UV-blocking polycarbonate greenhouse cladding. Decreasing watering and promoting transpiration by increased ventilation, higher temperatures and higher light is usually effective when oedema is caused by low transpiration. Under LED lighting, oedema can be prevented by a UV-B dose around 12.3–14.0 mmol m^{-2} d^{-1} (4.7–5.3 kJ m^{-2} d^{-1}) (Kubota *et al.*, 2017).

Environmental and Cultural Factors Affecting Growth and Productivity

Non-optimal light, CO_2 and temperature conditions and/or nutrition and water supply may limit growth and yield of tomato crops. The relative importance of these factors depends on the cropping situation. In heated winter greenhouse crops (in northern countries with long winter nights) light is most likely to be limiting, while CO_2 is the most limiting factor for heated crops under high solar radiation (e.g., Mexico). In unheated winter greenhouse crops (e.g., southern Europe and the Middle East), light, temperature, and CO_2 may be limiting. In the field, temperatures (too high or too low), light (short or cloudy days), water, nutrition, or lack of protection from weeds and pests and diseases may all be limiting. The scarcity and poor quality of water occurring in several areas constitute the most important deterrent to high yields of high-quality tomatoes.

Light

A light requirement equal to higher than 30 mol m^{-2} d^{-1} is reported for a tomato crop, while a light integral of 4.8 to 6.0 mol m^{-2} d^{-1} is suitable for tomato seedling production. In general, 1% reduction in light reduces tomato production by 0.7–1% (Marcelis *et al.*, 2006). To overcome light limitation in the winter, supplemental lighting (SL) is successfully used in northern countries (Dorais *et al.*, 2017). High pressure sodium (HPS) lamps are most commonly used, but LEDs receive more and more attention due to their long life,

high efficiency in transferring electricity into light and adjustable spectrum that allows optimization for photosynthesis, plant morphology and physiological function (Lu and Mitchell, 2016; Dorais *et al*., 2017). Additional research is still needed on optimal spectra for tomato growth and yield. The "coldness" of LED light as compared with HPS light makes it possible to bring the lamps into the crop canopy for interlighting. Top HPS SL combined with LED interlighting resulted in a 20% higher yield of tomatoes compared with top HPS lighting only (Moerkens *et al*., 2016).

Tomato plants need a minimum dark period of about 6-h (Dorais and Gosselin, 2002). When exposed to continuous light (CL), interveinal mottled chlorosis starting at the leaf/leaflet base appears as the most distinctive symptom (Dorais *et al*., 1995; Vélez-Ramírez, 2014). The CL-induced injury was related to photo-oxidative damage, early senescence and/or photosynthetic down-regulation (Dorais *et al*., 1995). CL-induced injury in tomato may arise from retrograde signals that counteract signals derived from the cellular developmental program that promote chloroplast development, such that chloroplast development cannot be completed, resulting in the chlorotic phenotype (Vélez-Ramírez, 2014). A dominant locus on chromosome 7 of wild tomato species (known to be tolerant to CL) that confers CL tolerance and the type III light harvesting chlorophyll a/b binding protein 13 (CAB-13) gene was identified as a major factor responsible for CL tolerance (Vélez-Ramírez, 2014). Introgressing the tolerance into modern tomato hybrid lines resulted in up to a 20% yield increase when plants were grown under CL compared to a 16 h photoperiod.

CO_2

CO_2 is the substrate for photosynthesis and as with all C_3 crops, current atmospheric concentrations of CO_2 are limiting to photosynthesis in tomatoes. CO_2 enrichment of greenhouse tomato crops increases the rate of diffusion of CO_2 into the leaf and therefore gross leaf and crop photosynthesis. It also increases photosynthesis by suppressing photorespiration and increases water use efficiency. A saturation-type of response to CO_2 is observed for photosynthetic rate, growth and yield. In (semi-)closed greenhouses where the venting is minimal, increased rates of photosynthesis due to the higher CO_2 concentrations and better climate control result in yield increase of around 10–20% compared with open (vented) greenhouses (De Gelder *et al*., 2012).

The appearance rate of leaves and trusses is not affected by CO_2 concentration. Moreover, CO_2 concentration has no direct effect on dry matter allocation to plant organs because sink strength rather than source strength determines assimilate partitioning. However, CO_2 enrichment in tomato plants increases fruit set and thus indirectly increases dry matter allocation to the fruit.

Acclimation of photosynthesis (Fig. 7.12) was noted in the early 1990s (e.g. Besford, 1993). Acclimation of plant photosynthetic capacity to high CO_2 is influenced by the plant sink strength, which is affected by environmental, genetic and management practices (Foyer *et al*., 2012). Therefore, elevated CO_2 concentrations do not cause feedback inhibition in a high-producing tomato crop when the plants have sufficient sink organs (fruits) to utilize the extra assimilates (Qian *et al*., 2012). This is clearly shown by comparing studies on CO_2 acclimation of young and mature tomato plants having different source/sink balance. The long-term CO_2 acclimation of C_3 plants to high CO_2 concentration is related to a substantial reprogramming of gene expression (Fukayama *et al*., 2009) in response to metabolic changes such as the down regulation of transcripts encoding Rubisco and other proteins associated with CO_2 fixation, and an up-regulation of those encoding proteins involved in RuBP regeneration and starch synthesis (Leakey *et al*., 2009; Foyer *et al*., 2012).

Temperature

Temperature has a direct influence on plant metabolic processes such as photosynthesis, respiration, and sink strength, which affect plant development and crop growth. The temperature influence on yield is mainly related to its effect on plant development through processes like leaf initiation rate, leaf area development, pollen quality, and hence fruit set.

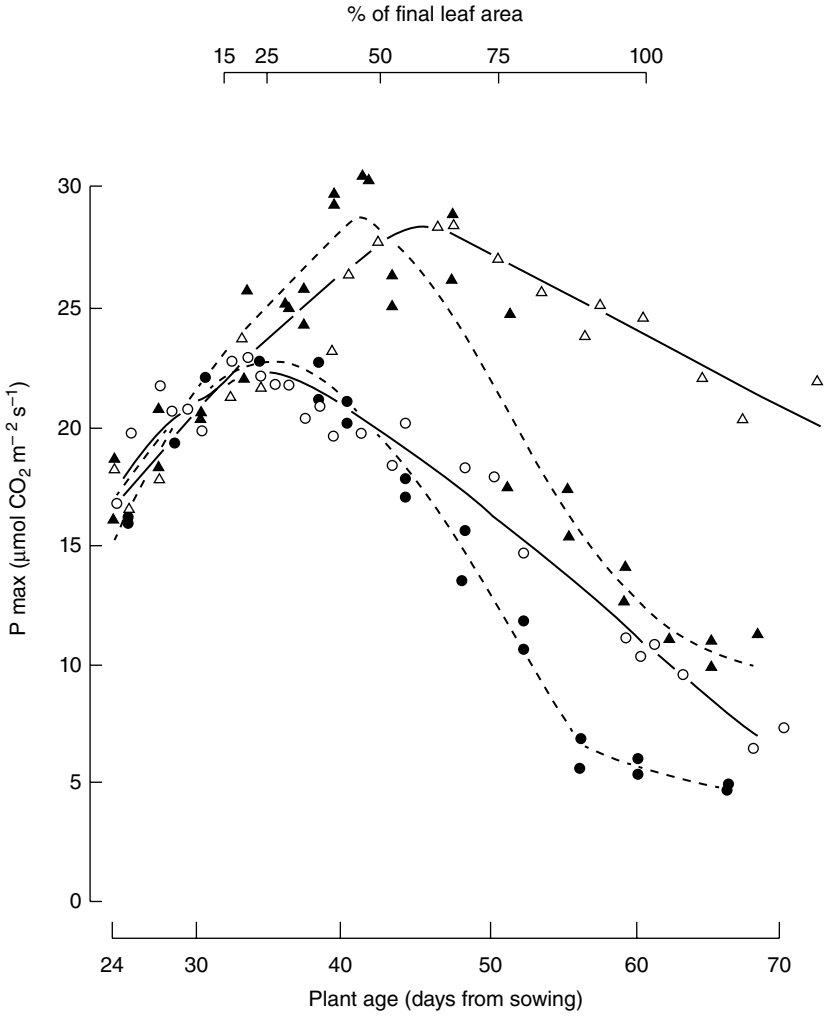

Fig. 7.12. Light-saturated rate of photosynthesis, P_{max} (at 1500 µmol m^{-2} s^{-1}) of the unshaded fifth leaf of tomato plants at various stages of development. Plants grown in 340 µmol mol^{-1} CO_2 (○, △) or in 1000 µmol mol^{-1} CO_2 (●, ▲) and measured in 300 µmol mol^{-1} CO_2 (○, ●) or in 1000 µmol mol^{-1} CO_2 (△, ▲) (reprinted, with permission from Kluwer Academic Publishers; Besford 1993, p. 444).

Optimal temperature

Temperature has a minor effect on gross photosynthesis of tomato, within the range of 15–25°C. The optimal temperature for gross crop photosynthesis is higher under high CO_2 concentration compared to ambient CO_2 level, although crop photosynthesis had a smaller response to temperature than leaf photosynthesis (Körner *et al.*, 2009). The optimum temperature for vegetative growth is between 18 and 25°C, and between 18 and 20°C for anthesis. The effect of temperature on the growth of closed canopies is mainly through maintenance respiration, which doubles with 10°C rise in temperature. For semi-determinate field-grown tomatoes, leaf area index increased linearly with degree days after planting (initial lag phase of 225°Cd; base temperature of 10°C) (Scholberg *et al.*, 2000). Increasing the temperature of the root zone from 14°C to 26°C increased the quantity of water absorbed during a day by 30% and the rate of Ca absorption by 45% (Dorais *et al.* 2001).

Non-optimal temperatures

Under suboptimal average temperatures and night temperatures, the relative growth rate for tomato plants is reduced because of thicker leaves (reduced specific leaf area, SLA). Photosynthesis is not affected by suboptimal night temperature (Fig. 7.13). Suppression of the shoot growth rates and leaf area expansion under suboptimal temperatures (e.g. 17/14°C compared with 22/18°C) was related to hormonal signaling (ABA, auxin), rather than a restriction in the rates of net CO_2 assimilation (Ntatsi *et al*., 2013). Within the cultivated tomato, there is only little genetic variation in response to temperature, which hampers breeding for equal production and quality at lower temperatures. However, in wild tomato species (e.g. *S. pennellii*, *S. hirsutum*) low-temperature tolerance is present (Fig. 7.13). It was shown that the decrease in SLA at lower temperature in cv. "Moneymaker" was related to the accumulation of non-structural carbohydrates (soluble sugars + starch), which was much less pronounced in more cold tolerant related wild tomato species (Venema *et al*., 1999).

Photosynthesis is reduced under supra-optimal temperature. Excessive temperatures (e.g. 40/30°C day/night temperature for 8 d or 35/15°C for 40 d) cause severe damage to the photosynthetic apparatus via the loss of grana stacking, the down-regulation of PSII activity and the reduced Rubisco activity (Ogweno *et al*., 2008; Zhang *et al*., 2012). In addition to the effect of heat on the PSII functioning and Calvin cycle, lower fruit sink strength resulting from a fruit set decline under supra-optimal temperature reduces the export of assimilates from leaf to fruit organs resulting in photosynthesis feedback inhibition and starch accumulation in the leaf (Zhang *et al*., 2012).

Day and night temperature

Relative growth rates of young plants strongly respond to the difference between day and night temperature (DIF; Fig. 7.2). For a mature crop, the long-term average temperature rather than the day-night temperature regime determines crop growth and yield. Temperature integration up to 24 days (amplitude 3 or 6°C) did not influence yield (De Koning, 1990). This provides possibilities for energy saving in greenhouse production.

Chilling injury

As tomato is a tropical species, no growth occurs below 12°C, and long-term exposure to these low temperatures causes chilling injury. Under conditions of high irradiance and low temperature as found in early spring, inhibition of photosynthesis may occur following the production of highly reactive oxygen radicals (Apel and Hirt, 2004); a reduced demand for photo-assimilates due to reduction or cessation of growth and development; a decrease of Calvin cycle turnover rates (Venema *et al*., 2005); a decrease of the photochemical quenching (Havaux, 1987); a decline of the specific activity of Rubisco (Brüggemann *et al*., 1994); and the inactivation of Rubisco (Venema *et al*., 2005). The circadian

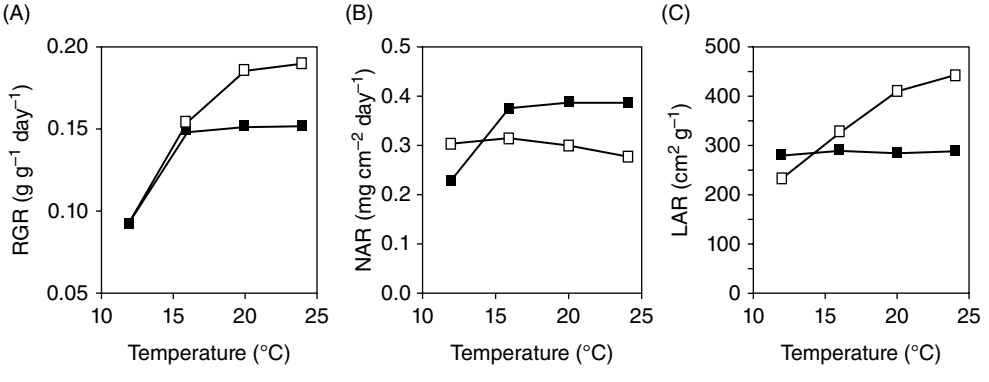

Fig. 7.13. Effect of temperature on: (A) relative growth rate (RGR); (B) net assimilation rate (NAR); and (C) leaf area ratio (LAR) of seedlings of *S. lycopersicum* "Moneymaker" (-□-) and *S. pennellii* LA716 (-■-). (Venema *et al*., unpublished).

rhythm controlling the activity of sucrose phosphate synthase (SPS) and nitrate reductase (NR), key control points of carbon and nitrogen metabolism in plant cells, is also delayed by chilling treatments (Jones *et al.*, 1998). In addition, low temperatures (e.g. 6°C for five days) alter the membrane lipid composition of tomato chloroplasts and decrease the number of granal thylakoids (Novitskaya and Trunova, 2000). Under chilling conditions, the incapability of tomato to adequately regulate stomatal conductance results in a high transpiration rate and plant wilting. Exposing young tomato plants during several days at high day and low night temperatures (e.g. nine days at 25/9°C compared to 25/15°C) reduces their capacity for CO_2 assimilation, which decreases the utilization of NADPH (Liu *et al.*, 2012). This reduction is accompanied by stomatal limitation of CO_2 supply and decline in Rubisco activity at the transcriptional level. On the other hand, low night temperatures increase soluble sugars and starch content as well as sucrose synthase activity (Qi *et al.*, 2011).

Humidity

Humidity, expressed as vapor pressure deficit (VPD), between 0.2 and 1.0 kPa, has little effect on carbon assimilation per se and plant growth, except when leaf or plant transpiration exceeds the water supply resulting in water stress (Grange and Hand, 1987). However, high VPD (1.5–2.2 kPa) reduces plant growth and yield because stomatal closure and a reduced leaf area reduce photosynthesis, and high VPD results in poor fruit set (pollen does not adhere on dry stigma). On the other hand, very low VPD (0.1 kPa), in addition to increasing the risk of diseases such as *Botrytis*, may reduce leaf area due to calcium deficiency (Holder and Cockshull, 1988), and therefore reduce fruit growth rate (Bakker, 1990) and yield. The ovule fertilization rate may also be affected due to a more difficult release of pollen. Long-term low VPD is also known to induce abnormal stomatal functioning, larger stomata, and changed stomatal density in several plant species including tomato (Fanourakis *et al.*, 2013; Arve and Torre, 2015).

Salinity

Salinity stress is largely used by producers to favor generative development in tomato (e.g. stimulate fruit growth on the first truss and improve fruit set; Lee, 2011) and to enhance fruit quality in terms of taste and nutritive value (Dorais *et al.*, 2008). In general, moderate salinity (e.g. EC of 6 dS m^{-1}) does not affect biomass and dry matter partitioning among fruit, vegetative parts, and roots, while high salinity (e.g. EC of 10 dS m^{-1}) reduces plant dry weight, and very high salinity (e.g. 17 dS m^{-1}) reduces dry matter distribution to the fruits (Dorais *et al.*, 2001).

Salinity may also reduce stem internode length, plant height, and leaf area of tomato (Najla *et al.*, 2009). Under Mediterranean field growing conditions, LAI, radiation use efficiency, shoot dry weight accumulation, and yield decreased with increasing water salinity from 0.5 up to 15.7 dS m^{-1} (De Pascale *et al.*, 2015). Shoot growth inhibition under salt stress may be regulated by hormones (e.g. cytokinin, abscisic acid, auxin/cytokinin ratio) or their precursors (Pérez-Alfocea *et al.*, 2010; Ghanem *et al.*, 2011; Lovelli *et al.*, 2012). Functional evidence about the role of metabolic and hormonal inter-regulation of local sink processes in controlling tomato fruit sink activity, growth, and yield under salinity were shown (Albacete *et al.*, 2014).

Other factors

Other factors such as the irrigation and nutrient management (Santos and Toress-Quezada, 2018) together with the type of growing system (Kubota *et al.*, 2018; Santos and Salamé-Donoso, 2018) impact tomato plant growth and fruit development. Water stress due to suboptimal irrigation management increases leaf abscisic acid concentration, which results in stomata closure, ethylene synthesis, buildup of leaf carbohydrates, and a reduced fruit size. On the other hand, excess irrigation results in epinasty, reduction of stem elongation, premature senescence of leaves, high abscisic acid content, and a poor root system (Dorais *et al.*, 2001).

Too high nitrogen levels promote vegetative growth at the expense of reproductive growth and result in poor fruit quality and high plant susceptibility to insects and diseases. On the other hand, suboptimal nitrogen supply reduces crop foliar development and consequently the quantity of assimilates available for the fruit (Dorais *et al.*, 2001). Nitrogen supply should be in balance with other nutrients such as potassium to avoid any physiological disorders and poor fruit flavor. An inadequate potassium supply reduces plant growth, fruit set and allocation of dry matter to leaves and roots (Dorais *et al.*, 2001). On the other hand, too high potassium supply may result in magnesium and calcium deficiency and reduced plant growth. Calcium is essential for cellular membrane stability and cell wall rigidity and plasticity. During periods of rapid plant growth, additional calcium supply is needed to respond to accelerated cellular enlargement and fruit development (Dorais *et al.*, 2001). Phosphorus limitation affects the functioning of PSII and low concentrations adversely affect reproductive growth as phosphorus is essential to the development of flowers, plays a role in cytokinin transport and stimulates the absorption and distribution of calcium in the fruit.

Summary

Tomato (*Solanum lycopersicum*) is a crop of great economic and scientific importance. Annual yields above 100 kg m^{-2} in greenhouse production have been reported. Tomato is a prominent model system for research into plant genetics, pathology, and physiology (e.g. it is an established model to study fleshy fruit development and ripening). In the last four years only, more than 6000 scientific papers have been published with "tomato" or "tomatoes" in their title. The tomato genome was sequenced in 2012 and this information is being deployed along with genome sequence data from over a thousand cultivars including modern, heirloom and wild tomatoes to assist molecular breeding. Tomato fruit growth is a four-phase process that begins with initiation of the floral meristem, carpel formation, and ovary growth. The second phase involves pollination, fertilization, fruit set, and resumption of cell division. During the third phase, cells expand and undergo endoreduplication. Initiation of ripening is the fourth phase and it marks the beginning of fruit senescence.

Besides the external fruit appearance (i.e. color, shape and size), the main quality traits of tomato are generally defined by its organoleptic attributes such as texture, sugars, organic acid content, and volatile compounds. The limited caloric supply, relatively high fiber content, and provision of minerals, vitamins, and phenols such as flavonoids make the tomato fruit an excellent "functional food."

Disorders of tomato leaves, stems and fruit (e.g. BER and cracking), often referred to as physiological disorders, have both genetic and environmental components, and in many cases the exact cause of the disorder is not well understood or involves a complex of factors.

Genetics, environmental factors and crop management all affect tomato crop growth and yield. Non-optimal light, CO_2 and temperature conditions and/or nutrition and water supply may limit growth and yield. The relative importance of these factors depends on the cropping situation. In general, 1% reduction in light reduces tomato production by 0.7–1%. Tomato plants need a minimum dark period of about 6-h. Continuous light results in leaf interveinal mottled chlorosis. Within the cultivated tomato, there is only little genetic variation in response to temperature, which hampers breeding for equal production and quality at lower temperatures. However, in wild tomato species (e.g. *S. pennellii, S. hirsutum*) low-temperature tolerance is present. Acclimation of plant photosynthetic capacity to high CO_2 is influenced by the plant sink strength, which is affected by environmental, genetic and management practices. Therefore, elevated CO_2 concentration does not cause feedback inhibition in a high-producing tomato crop when the plants have sufficient sink organs (fruits) to utilize the extra assimilates.

Studies on tomato have increased our understanding of many aspects of plant physiology and there is no doubt that tomato will also serve in the future as a "model and reference plant."

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8 Peppers

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The cultivated *Capsicum* peppers are herbaceous, frost-sensitive plants that in temperate areas are annual in growth duration, but in tropical areas may continue to grow and produce yield over several years. They are the source of capsaicin, the most commonly used spice in the world (Andrews, 1984). Pepper fruits provide a high nutritional value and are a rich source of vitamins A, B and C, iron, potassium, magnesium, β-carotene, folic acid, and fiber (Hulse-Kemp *et al.*, 2016). Pepper cultivars are cultivated for production of a green or a mature ripe fruit, used in salads and as a cooked or as a raw vegetable. Still others are the source of red food coloring, made from the dried, ground powdered fruit.

The *Capsicum* genus consists of 25–30 species; five of these *Capsicum* species were domesticated: *C. annuum*; *C. baccatum*; *C. chinense*; *C. frutescens*; and *C. pubescens*. The largest group of varieties is found among the *C. annuum* spp. which are grown in temperate and tropical areas worldwide (Paran and van der Knaap 2007), but thought to originate in Mexico and Central America (Andrews, 1984; Perry *et al.*, 2007). Fruit remnants dating back to 7000 BC have been found in caves in this area, and evidence for cultivation of this species has been traced to the period between 5200 and 3400 BC (Govindarajan, 1985; Perry *et al.*, 2007). The explorer Columbus imported peppers into southern Europe, from where they spread to the Middle East, Africa and Asia, and were introduced back into more northerly parts of North America (Andrews, 1984). The wild progenitor of *C. annuum* is thought to be the bird pepper, whose domestication occurred in Mexico. The fruit of wild bird pepper is small (about 1 cm in length), erect, red-coloured, pungent (hot), deciduous (falls off the plant when ripe), and soft-fleshed (Paran and van der Knaap 2007).

The other four species are also considered part of the cultivated *Capsicums*, but play a much smaller role in agriculture and commerce (Smith *et al.*, 1987). They include *C. frutescens*, comprising small, pungent-fruited peppers that serve as a source of tabasco sauce. It, and *C. chinense*, are thought to originate in the Amazon Basin of South America. *Capsicum baccatum* also traces its origin to central South America, and has been selected in Brazil for a range of fruit sizes and shapes (Nagai, 1989). *Capsicum pubescens* is the only cultivated pepper species that originated in cooler zones, and is thought to have come from the highlands of Bolivia (Andrews, 1984).

Most pepper species are diploid and have 12 pairs of chromosomes (2n = 2X = 24) which make the *Capsicum* species amenable to traditional breeding methods as they are inter-fertile to varying degrees owing to their similar genome structure (Hulse-Kemp *et al.*, 2016). However, crossability among the species is limited, so breeders have only been able to make limited use of differences in disease resistance

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among species (Greenleaf, 1986). Within *C. annuum*, a tremendous range in size, shape, pungency, and mature color of fruits has been selected that now forms the basis for the types used in commerce throughout the world (Andrews, 1984; Somos, 1984; Greenleaf, 1986; Naegele *et al.*, 2016).

World production of nearly 32 million metric tons of fresh peppers on 1.9 million ha ranks peppers in the middle range of vegetables in terms of popularity (FAO, 2016). Some 46% of production is centered in Asia, with China the principal producing country. The countries of southern Europe are the second-most important producing region, with 24% of world production. Although Africa and North and Central America are less important in fresh pepper production, total area devoted to peppers is probably underestimated, since cultivation of the crop for dry chili powder is important in these regions. The world production of chili and dry peppers is nearly 3.8 million tons on 1.7 million ha (FAO, 2016). In Mexico, for instance, about 31% of pepper production area is devoted to the dry product (Laborde and Pozo, 1982). Mexico, as probable country of origin of the crop, also has the most diverse array of cultivars and wild types that are harvested.

Despite the rich genetic diversity that exists within the crop, our knowledge of the physiology is limited, and restricted to a few cultivars, primarily of the large-fruited bell peppers within *C. annuum*. This trend has been reinforced by the growing importance of large-fruited peppers in European glasshouse production and under net houses in the Mediterranean basin and worldwide, and the need to understand the crop's physiology to improve cultivation practices. Nevertheless, production in the field of smaller-fruited types, loosely classified under the common name "paprika," has also generated important physiological information (Somos, 1984).

Seed Germination

The germination and emergence of pepper is slow at room temperature, and further delayed by cooler conditions. At 25°C, pepper required 3.5 days for radicle emergence, while at 15°C, 9 days were required (Watkins and Cantliffe, 1983). The optimum temperature for emergence and seedling growth of pepper was 26°C, emergence and seedlings shoot and root growth was inhibited at 19°C. Germination temperature had no significant effect on root length (Samarah *et al.*, 2016). Emergence from 1.2 cm soil depth took eight to nine days at temperatures from 25–35°C (Lorenz and Maynard, 1980), and was prevented altogether at temperatures lower than 15°C. The endosperm constitutes the principal barrier to radicle emergence. The growth promoter gibberellic acid (GA) may be involved in endosperm penetration during germination. Watkins and Cantliffe (1983) found that soaking the germinating seeds in GA_{4+7} (a mixture of gibberellins 4 and 7) increased the speed of radicle penetration. The activity of endomannanase, a cell wall degrading enzyme, also increased with GA treatment and during radicle penetration (Watkins *et al.*, 1985). It is possible that GA stimulated enzyme activity that enhanced endosperm breakdown in the area near the radicle tip.

Germination of pepper seeds may be preceded by a period of dormancy. In a survey of several cultivars of two species of *Capsicum*, Randle and Honma (1981) found that emergence took from 20 to 50 days from sowing at room temperature. Emergence times of the slow lines could be shortened to 20 days by storing the freshly harvested seeds at 24°C for two to three weeks. The slow emergence could also be eliminated by delaying the extraction of seeds from the fruit for 10 days after the fruit was fully ripe. While different *C. annuum* lines varied in emergence from 20–50 days, *C. frutescens* and *C. chacoense* lines tested required 51–61 days to emerge, respectively. These findings were confirmed by Edwards and Sundstrom (1987), who showed that three weeks seed storage of tabasco pepper (*C. frutescens*) increased seed germination from 40–70%. Although details of the dormancy mechanism have not been worked out, it is possible that a lack of GA may prevent prompt germination and emergence.

Bell pepper (*Capsicum annuum* L.) multiplication is performed by seeds, which are characterized by slow germination and low uniformity of seedling development (Bosland and Votava, 2012). As a consequence, direct sowing in the field is not considered as a viable alternative to the more costly plug transplants that are required for uniform field stands. A promising strategy that may permit direct field sowing is

the use of primed seeds because such treatment provides for enhancement of the physiological performance of seeds, which leads to a faster and synchronized development of the seedlings (da Silva *et al.*, 2015). Seed size affects the uniformity of pepper plants. Large seeds 3.5–4.2 mm in diameter and 0.36–0.4 mg seed weight emerged two days earlier with better standing and performance as compared to smaller seeds (< 3 mm and less than 0.3 mg) (Bosland and Votava, 2012). Furthermore, the small seeds failed to produce transplants meeting the minimum size requirements for transplanting.

In general, seed priming improves germination percentage and rate, emergence, seedlings growth and uniformity, and yields of peppers (Bosland and Votava, 2012). Priming of pepper seed by a variety of techniques (see Chapter 1) has generally demonstrated a considerable reduction in time of germination, but only a small gain in rate of emergence from the soil (Rivas *et al.*, 1984; Bradford *et al.*, 1990; Ilyas, 1993; Bosland and Votava, 2012). Sundstrom and Edwards (1989) showed that although the radicle emerged from the pepper seedcoat much earlier after priming, there was little stimulation of hypocotyl elongation rate in tabasco pepper. Addition of GA (GA_3 and GA_{4+7}) to primed seed had little added effect on emergence rate, however, indicating that the factor limiting hypocotyl elongation was not a lack of that growth promoting substance (Ilyas, 1993). On the other hand, it was recently shown that addition of brassinosteroid to the primed seeds reduced pepper germination time and increased seedling growth (da Silva *et al.*, 2015, Fig. 8.1). Further work is needed to translate the benefits from priming in hastened germination to similar stimulation of emergence rate and on the role of other plant hormones in seed performance enhancement. In addition to hormone priming, other treatments (potassium nitrate and PEG) have been found to improve germination and seedlings establishment in pepper (Bosland and Votava, 2012) in diverse germination temperatures. The main function of osmo-conditioning is to maintain an osmotic potential sufficient to prevent seed germination while permitting enough moisture to enter the seed to allow completion of the early metabolic steps required for germination (Bosland and Votava, 2012).

Vegetative Growth

In addition to a rather slow seed germination and seedling emergence rate, pepper also has a relatively slower seedling growth rate than some other vegetable crops. Comparative growth analysis of tomato, cucumber, and pepper indicated that pepper had a 25% lower relative growth rate than the other two species. The slower growth rate of pepper was not due to a lower productivity per unit leaf area (net assimilation rate), but to a reduced production of leaf area (Bruggink and Heuvelink, 1987). Pepper seedlings had significantly thicker leaves (higher specific leaf weight) than the other two species.

It is possible to reduce leaf thickness and increase the proportion of leaf area to total plant mass (leaf area ratio) by reducing incident light (Nilwik, 1981). These changes occur at the expense of the plant growth rate, however, and may thus be counterproductive. Nevertheless, use of light shade (25–50%) during seedling growth has been advocated to increase yield of

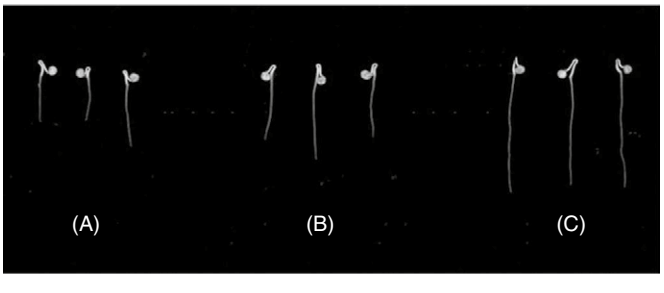

Fig. 8.1. The influence of seed priming treatments on germination and seedling growth. Seedlings from (A) unprimed seeds; (B) drum-primed seeds using only water (traditional); (C) drum-primed seeds including 24-epibrassinolide (source: da Silva *et al.*, 2015).

pepper in a tropical environment by maximizing leaf area production (Schoch, 1972). Furthermore, shade nets are used during seedling establishment after transplanting in the hot season and to minimize sun scald damage when fruit are exposed to direct sunlight. In general, under Mediterranean growing conditions, use of shade nets during flower bud development results in poor fruit set.

Two types of plant architecture can be found in pepper plants, an indeterminate type that grows like vines and a semi-determinate type in which the plant grows and, as fruit sets, the plant slows its growth. Unlike tomato, a true determinate type does not exist in pepper (Bosland and Votava, 2012). The rate of plant growth is also strongly influenced by the air temperature, which affects both the rate of dry matter production, and the partitioning of that dry matter into leaf tissue. Pepper growth in the vegetative stage has been found to be greatest at 25–27°C day and 18–20°C night temperature (Dorland and Went, 1947; Bakker and van Uffelen, 1988). Total plant mass and leaf area were optimal at 20–22°C mean temperature, and declined outside this range (Fig. 8.2). Day temperatures lower than night temperature, and a low night temperature of 12°C were also detrimental to vegetative growth. Lower growth temperature also reduces future productivity by increasing specific leaf weight, and decreasing the ratio of leaf area to total plant dry weight (leaf area ratio) (Nilwik, 1980a, 1980b).

Root growth by pepper seedlings has been studied in artificial and several field conditions. If allowed to grow undisturbed, pepper plants will produce a prominent tap root during early seedling growth (Stoffella *et al.*, 1988). In the field, direct-seeded peppers grown in a deep soil develop several prominent roots that may reach a depth of 3 m (Weaver and Bruner, 1927). If the plants are transplanted, root growth is shallower and more branched, with 80% of the active root system found in the upper 75 cm of soil (Hammes and Bartz, 1963; Bosland and Votava, 2012). In general, root weight is approximately 10% of the total plant weight. Root growth is proportional to shoot growth in the vegetative period in pepper (Nielsen and Veierskov, 1988), as has been found for many herbaceous plants (Brouwer, 1962).

Root growth and distribution are significantly influenced by soil management, including cultivation, and the extent and distribution of irrigation water. It is likely that root distribution will also be significantly affected by soil structure and density, as well as using polyethylene mulch, a common practice in pepper production. Experiments with tomato have shown that lateral root growth is greatly stimulated by use of plastic mulch (Wien *et al.*, 1993a). No similar studies have been made on this aspect in pepper.

Induction of Flowering

The production of flower primordia in *C. annuum* appears to be little influenced by daylength, occurring in the same time on plants grown under photoperiods 7–15 h long (Auchter and Hartley, 1924; Cochran, 1942; Bosland and Votava, 2012).

Most *C. annuum* cultivars developed a single stem with 8–15 leaves before the appearance of the first flower. The number of leaves that develop before the first flower seems to be controlled by temperature and cultivar (Bosland and Votava, 2012). Typically, two or three branches arise at the apical meristem, which again terminate in a flower after producing one node. Each shoot bears one or two leaves, terminates in a flower and then divides into two second-order branches. In the field, the same pattern is repeated for about five nodes, depending on the length of growing season (Somos, 1984). Under glasshouse conditions, where plants are typically restricted to two stems by pruning, many more nodes may form. At the end of an 11-month growing season, the stem may exceed 3 m in length (M.H. Esmeijer, Naaldwijk, the Netherlands, 1995, personal communication). There is also considerable genetic variation in branching patterns of *C. annum* (Somos, 1984).

The most important factor determining flower differentiation is air temperature, especially night temperature (Bosland and Votava, 2012). Subjecting pepper seedlings to 10°C night temperatures before flower initiation increased main stem leaf number by one or two leaves (Rylski, 1972a). This contrasts with tomato, in which exposure to 10°C at leaf stage 2 reduces the node to first flower significantly (Wittwer and Teubner, 1956; Calvert, 1957).

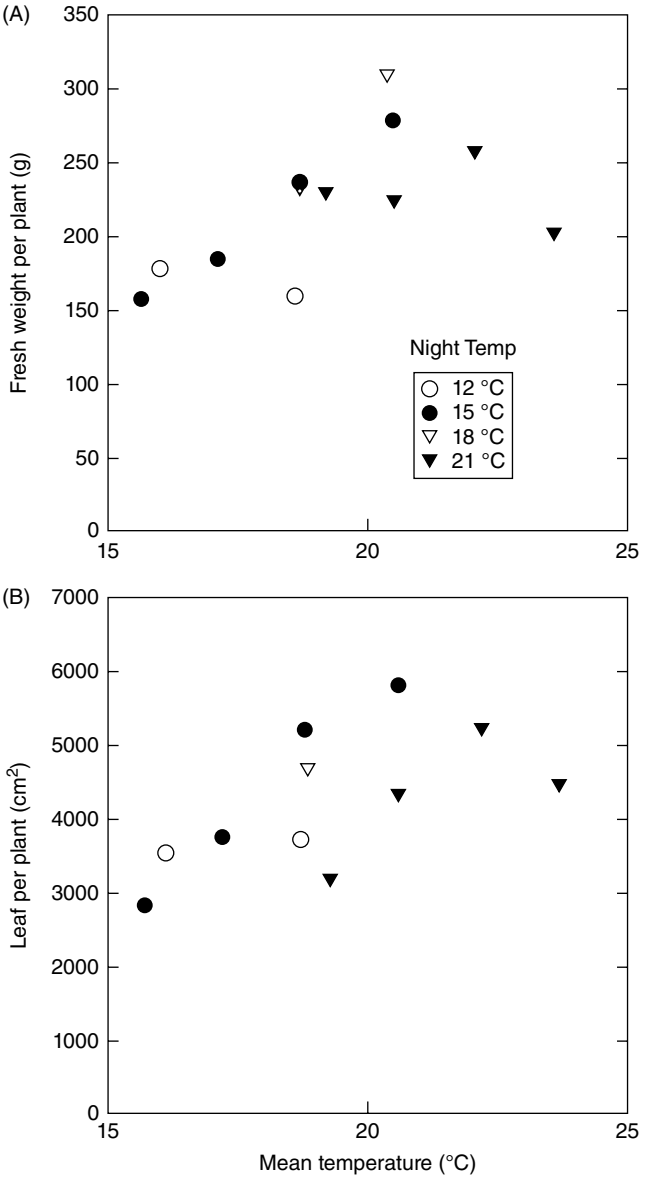

Fig. 8.2. The influence of mean and dark period air temperature on (A) vegetative plant fresh weight and (B) leaf area per plant for pepper plants grown in ambient light conditions in a glasshouse in winter (source: Bakker and van Uffelen, 1988).

Flower bud initiation and appearance is not so susceptible to environmental or nutritional changes (Eguchi *et al.*, 1958; Rylski, 1972b).

The results of *C. annuum* flower induction experiments indicate that this species is day-neutral in the photoperiods and temperatures which would normally be encountered in the field. More work is needed to determine if this is also true of other cultivated *Capsicum* species, especially those hitherto largely confined to tropical environments.

Flower Development and Fruit Set

The typical *Capsicum* flower is pentamerous, hermaphroditic, and hypogynous (Bosland and Votava, 2012). Detailed morphological landmarks (organogenesis and gametogenesis) during flower development from flower initiation until anthesis are presented in Fig. 8.3 and Table 8.1 (Sandoval-Oliveros *et al.*, 2017). Flowers of pepper are generally considered to be self-pollinated,

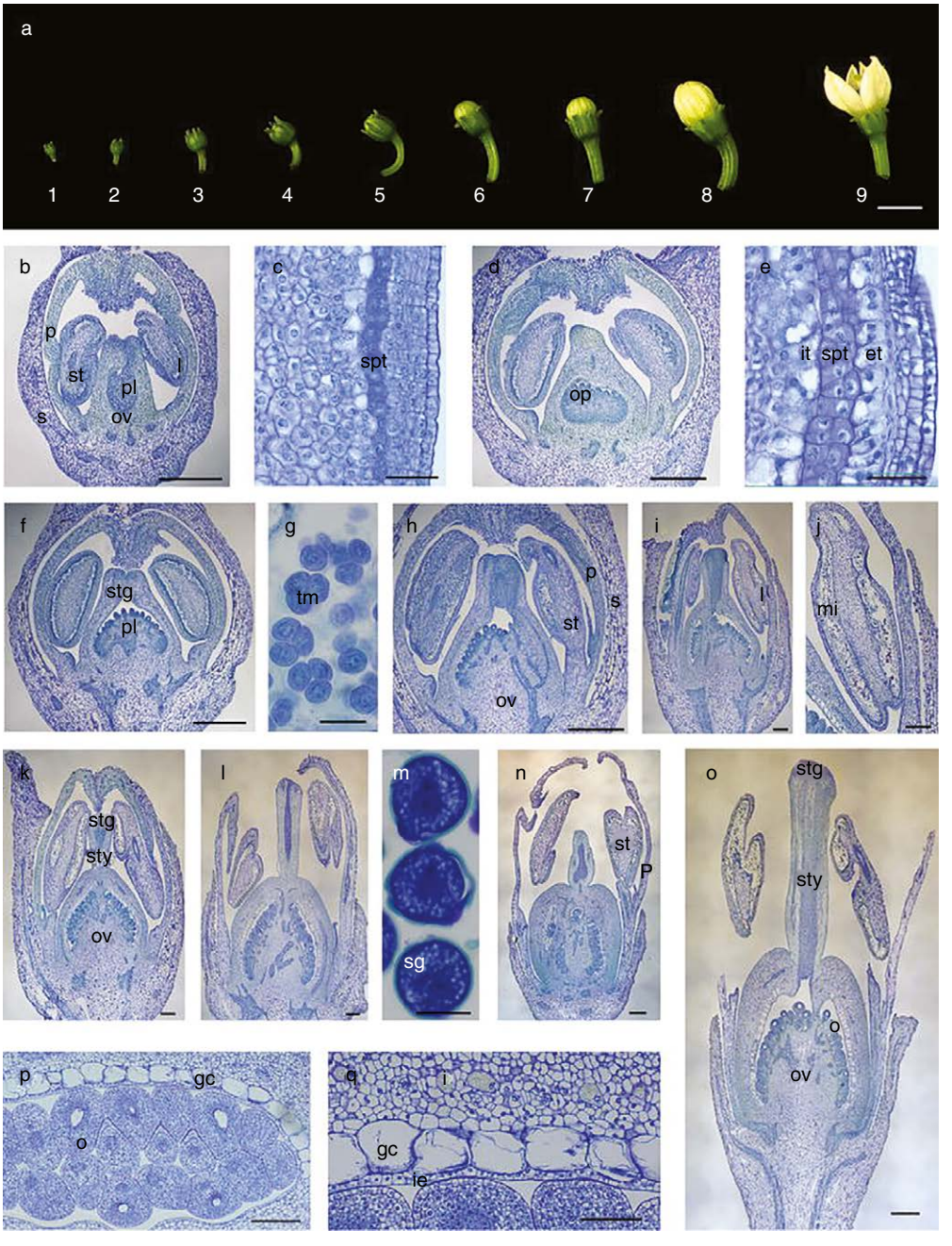

Fig. 8.3. Floral development in chili pepper (*Capsicum annuum* L. cv. Huichol). Pepper floral buds from 1 mm (stage 1) up to pre-anthesis (stage 9); b–q histological sections of flower buds of the nine floral stages shown in an external tapetum, gc giant cell, i idioblast, ie internal epidermis, it internal tapetum, l locules, mi microspores, o ovules, op ovule primordia, ov ovary, p petals, pl placenta, s sepals, sg starch granules, spt sporogenous tissue, st stamens, stg stigma, sty style, tm tetrad of microspores (Sandoval-Oliveros *et al*., 2017).

Table 8.1. Flower development stages of *Capsicum* associated with morphological changes and reproductive organ development landmarks (adapted from Sandoval-Oliveros *et al*., 2017).

Bud stage	Length buds (mm)	Morphological changes	Landmark
1	1	Green buds, calyx lobes closed, sepals and petals visible	Sporogenous layer became visible (microsporangia). Placental tissue became visible
2	2	Green buds, petals star curling over the stamens	Differentiation of pollen mother cells. Vacuolated internal tapetum and bicellular external tapetum Ovule primordial became visible
3	3	Green buds, the stigma star to form	Male meiosis. Tetrad of microspores. Beginning of megagametogenesis
4	4	Green buds, elongation of the style	Young, free microspores. Functional megaspore
5	5	Petals grow to the top of sepals	Unicellular pollen integument growth
6	10	Green petals emerge from the sepals	Polarized pollen; development of embryo sac
7	13	Protruding white petals	Bicellular pollen grain
8	15	Protruding white petals twice as long as calyx	Maturing microspores, exine becoming thick Mitotic divisions of the functional megaspore
9	17	Open flower before anthesis	Mature pollen grains with fusiform generative nucleus Mature embryo sac

although, unlike tomato, the anthers and stigma often do not touch each other (McGregor, 1976). On many cultivars, flowers are held horizontally or pendent, so that pollen can fall onto the stigmatic surface. Presumably, in the field, insects help to transfer pollen and increase fruit set (Erwin, 1932; Odland and Porter, 1941). Tanksley (1984) found an average of 41% out-crossing among tester lines interplanted in chili pepper fields. Plant movement by wind probably also contributes to pollination. Under the wind still conditions of glasshouses or insect-proof net houses, the introduction of bees during flowering increased the seed set and fruit size of the fruits produced (de Ruijter *et al*., 1991; Kristjansson and Rasmussen, 1991; Shipp *et al*., 1994; Serrano and Guerra-Sanz, 2006).

On the day of anthesis, the pepper flower begins to open by dawn, and most new flowers open within the first 3 h after sunrise and they open for less than 1 day. Anther dehiscence commonly lags the flower opening by 1 to 10 h (Kato, 1989; Bosland and Votava, 2012). The significance of such a delay may be questioned, since the stigmata of pepper flowers remained receptive for three days at 28/18°C day/night temperatures, and the pollen retained viability for three days after anthesis in these studies (Kato, 1989). A similarly long stigma receptivity period was found by Cochran and Dempsey (1966) for pimento pepper.

Pollen tubes started their germination on the stigmatic surface 2 h post pollination (HPP). Pollen tubes reaches the ovary within 10 h after germination started and fertilization occurred around 24 HPP (Tiwari *et al*., 2013; Yasuor 2017, unpublished data). However, these processes can be delay according to cultivar and environmental conditions (Kato, 1989; Reddy and Kakani, 2007; Fig. 8.4).

Parthenocarpy is a desired trait in sweet pepper, as it is expected to minimize yield fluctuations and enhance the total fruit production while providing the inclusion of a quality trait (Tiwari *et al*., 2011). This trait is even more important when fruit set and fruit development occur under stress conditions. Peppers have the capacity to set fruit parthenocarpically, especially under low temperature conditions (12–15°C night temperatures) (Rylski

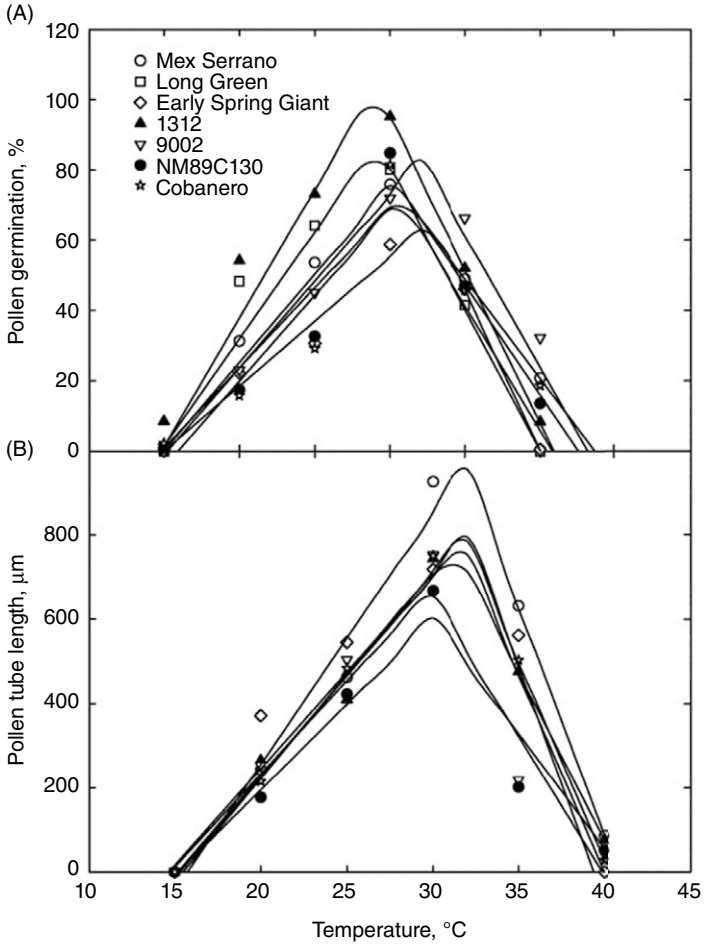

Fig. 8.4. Influence of temperature on (A) pollen germination percentage and (B) pollen tube length of various *Capsicum* species. (Source: Reddy and Kakani, 2007).

and Spigelman, 1982; Polowick and Sawhney, 1985; Tiwari *et al.*, 2011). Failure of seed set is at least partly due to the formation of abnormal and non-viable pollen and in reduced ability to germinate, but the mechanism by which the plant retains the fruit despite a lack of seed set is not known. In addition, early growth of the ovary, which leads to physical distance between the male and female flower organ, might result in seedless parthenocarpic fruit. The temperature optima for fruit set, and the topic of flower abscission, an important physiological disorder in pepper, is covered in the section on physiological disorders below. Parthenocarpy in pepper is receiving more attention currently with the introduction of small fruited seedless cultivars (sweet bite) to the fresh market.

Male sterility is controlled by both nucleic (Shifriss and Rylski, 1972) and cytoplasmic genes (Shifriss and Frankel, 1971). In both types of male sterility, anthers stayed small and shrunken, and were blue–violet in color, with little or no viable pollen. Cytoplasmic male sterility (CMS) is a maternally inherited trait that leads to the failure to produce functional pollen grains. In CMS line anthers, microspore mother cells (MMCs) showed initial abnormality of cytomixis at the early prophase, which might be caused by the genetic defect of MMCs involved meiotic

genes, which were responsible for the DNA fragmentation. The tetrad microspores in CMS were aberrant and pollen grains were crushed at tetrad stage. The tapetal cells in CMS anthers followed the premature programed cell death (PCD), which formed a large vacuole at the early prophase, and kept low metabolic activity during meiosis and completely degenerated at tetrad stage (Qiu *et al*., 2017).

Fruit Growth and Maturation

The process of fruit growth begins with the formation of the ovary during the early stages of flower differentiation (Fig. 8.3) (Cochran, 1938; Munting, 1974; Sandoval-Oliveros *et al*., 2017). In the period before anthesis of the flower, the basic structure of the ovary is determined, including the number of carpels to be found in the mature fruit. Cell division predominates during this stage, followed by cell enlargement after flowering (Kano *et al*., 1957; Munting, 1974; Sandoval-Oliveros *et al*., 2017). Some cell division activity is, however, maintained into later stages of fruit growth in long-fruited pepper types, especially at the basal part of the fruit (Kano *et al*., 1957; Munting, 1974). Pepper fruit formation differs from that of tomato and squash in that the shape of the ovary at anthesis gives less indication of final fruit shape (Sinnott and Kaiser, 1934; Houghtaling, 1935). From a globular ovary at flowering may form an ovoid or an elongated pepper fruit. Changes in cell shape and the plane and amount of cell division thus influence final fruit shape.

The temperature at which the plant is growing during this pre-anthesis period can also influence fruit shape. Subjecting pepper seedlings to 35°C from the time the third leaf was longer than 1 cm resulted in a significant increase in fruit locule number (Ali and Kelly, 1993), without increasing fruit size. If pepper plants are grown at low night temperatures (8–10°C) before flowering, the ovary tends to be larger and broader than that of plants grown at higher (18–20°C) temperatures (Rylski, 1973; Polowick and Sawhney, 1985). Fruits developed from these ovaries also have a decreased length: width ratio, and the style persists and forms a point on the blossom end of the fruit (Rylski, 1973). The increased ovary size of such cool temperature grown plants does not result in bigger fruits at maturity, however, even if normal pollen is used to ensure seed set. In fact, since normal pollen development is impaired at low temperatures, small, seedless fruit are often the result of growing pepper plants in cool conditions (Polowick and Sawhney, 1985). Selection of genotypes from a wide range of genetic variation under low night temperatures led to the development of varieties that can set normally-shaped fruits even under night temperatures of 8–10°C. This in turn extended the period of fruit setting, leading to a longer production season and higher yields (Elkind *et al*., 2008).

Conditions after anthesis also play a significant role in pepper fruit development. An important factor is the degree to which seed set has been successful. A direct linear relation between the number of seeds/seed weight per fruit and final fruit size was found in peppers grown under protective structure (Rylski, 1973; Yasuor *et al*., 2015, unpublished data, Fig. 8.5). Conditions which negatively influence overall plant growth can also reduce final fruit size (see next section). As fruit number per plant increases, the size of individual fruits tends to be smaller. Conversely, restricting fruit set allows the plant to develop the retained fruit to a larger size (Rylski and Spigelman, 1986). Unfortunately, the selection of pepper genotypes with large fruits has probably resulted in cultivars that are very susceptible to flower and flower bud abscission (Wien *et al*., 1993b).

The changes in carbohydrate levels in pepper fruit during development have been studied by several workers. During the initial growth phase after anthesis, rapid fruit growth coincided with accumulation of glucose and fructose, and lower levels of sucrose and starch (Nielsen *et al*., 1991; Hubbard and Pharr, 1992; Osorio *et al*., 2012) (Fig. 8.6). As growth rate of the fruit slowed, sucrose and starch accumulated. During fruit maturation, there was a further steep rise in reducing sugar content, and reduction in starch and sucrose levels. Fruit growth rate, and the level of hexose sugars was closely related to the activity of acid invertase in the fruit (Nielsen *et al*., 1991). This indicates that fruit growth may be regulated by the rate at which the imported sucrose is converted to hexose sugars in the fruit. At later stages of fruit development, cleavage of sucrose by the sucrose synthase

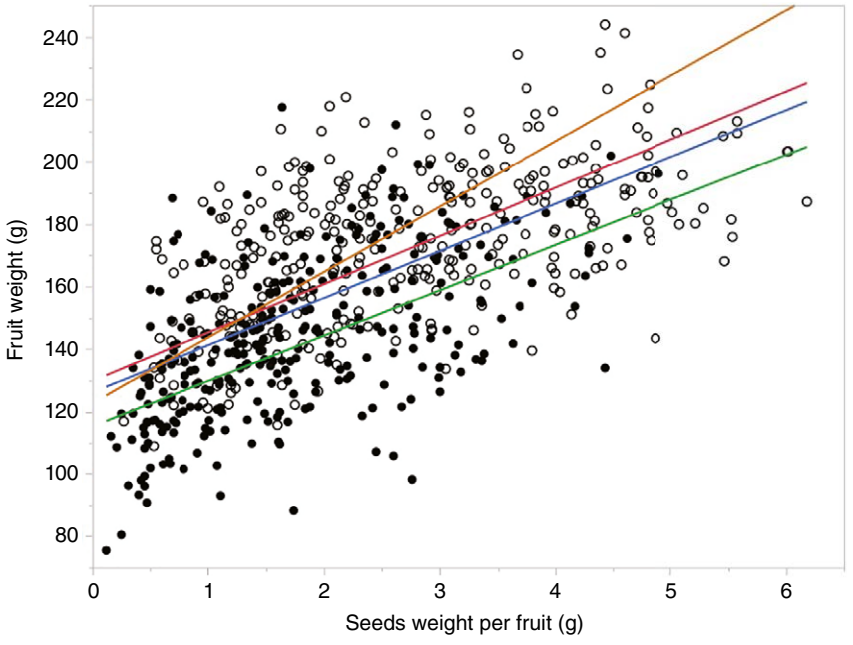

Fig. 8.5. The seed weight per fruit and fruit weight for different bell pepper cultivars grown under plastic (●) or insect-proof net (○) covered structures in the Negev desert during the summer (Yasuor *et al*., 2015, unpublished data). Each line is a linear regression of a different pepper cultivar.

enzyme plays an important role (Nielsen *et al*., 1991; Hubbard and Pharr, 1992). As in the case of sugars, pepper fruit development and ripening characterized with significant changes in other primary metabolites alterations (Osorio *et al*., 2012; Fig 8.6). Majority of the amino acids accumulated during fruit growth and maturation, with a peak around 50–60 days post anthesis. Similarly, citrate and ascorbate (dehydroascorbate) significantly accumulated during fruit development.

Peppers are important for the production of spice and of red food coloring as well as the fresh vegetable, directly consumed. The pungent compounds in hot peppers are primarily the flavorless, fat-soluble capsaicinoids, composed chiefly of capsaicin and dihydrocapsaicin (Govindarajan, 1985; Guzman *et al*., 2011). Capsaicinoids start to accumulate 20 days post anthesis and synthesis usually persists through fruit development. These compounds are found in the cross wall and placental region of the fruit, and not in the seed or pericarp tissues (Huffman *et al*., 1978). In pungent pepper types, the epidermal cells of these tissues synthesize the capsaicinoids in the endoplasmatic reticulum, and secrete the materials into a subcuticular cavity (Zamski *et al*., 1987; Guzman *et al*., 2011). At maturity, pungent fruit may be distinguished from the sweet types by the presence of blistered cells on the placenta, whereas the placenta of non-pungent fruits appears smooth.

In addition to the qualitative and quantitative genetic control on the expression of pungency, there is very strong environmental influence. Any environmental stress will increase the pungency level. For example, elevated night temperatures and water-deficit conditions induce an increased accumulation of capsaicinoids (Guzmann *et al*., 2011).

The red pigment in mature pepper fruit that is the source of food coloring is composed of a group of related carotenoids, principally capsanthin, capsorubin and cryptoxanthin (Govindarajan, 1985). These compounds mask the presence of the yellow pigments β-carotene and violaxanthin, which become prominent in yellow-fruited bell peppers at maturity. The color compounds are found in the outer pericarp layer of the fruit, and their formation is favored by moderate

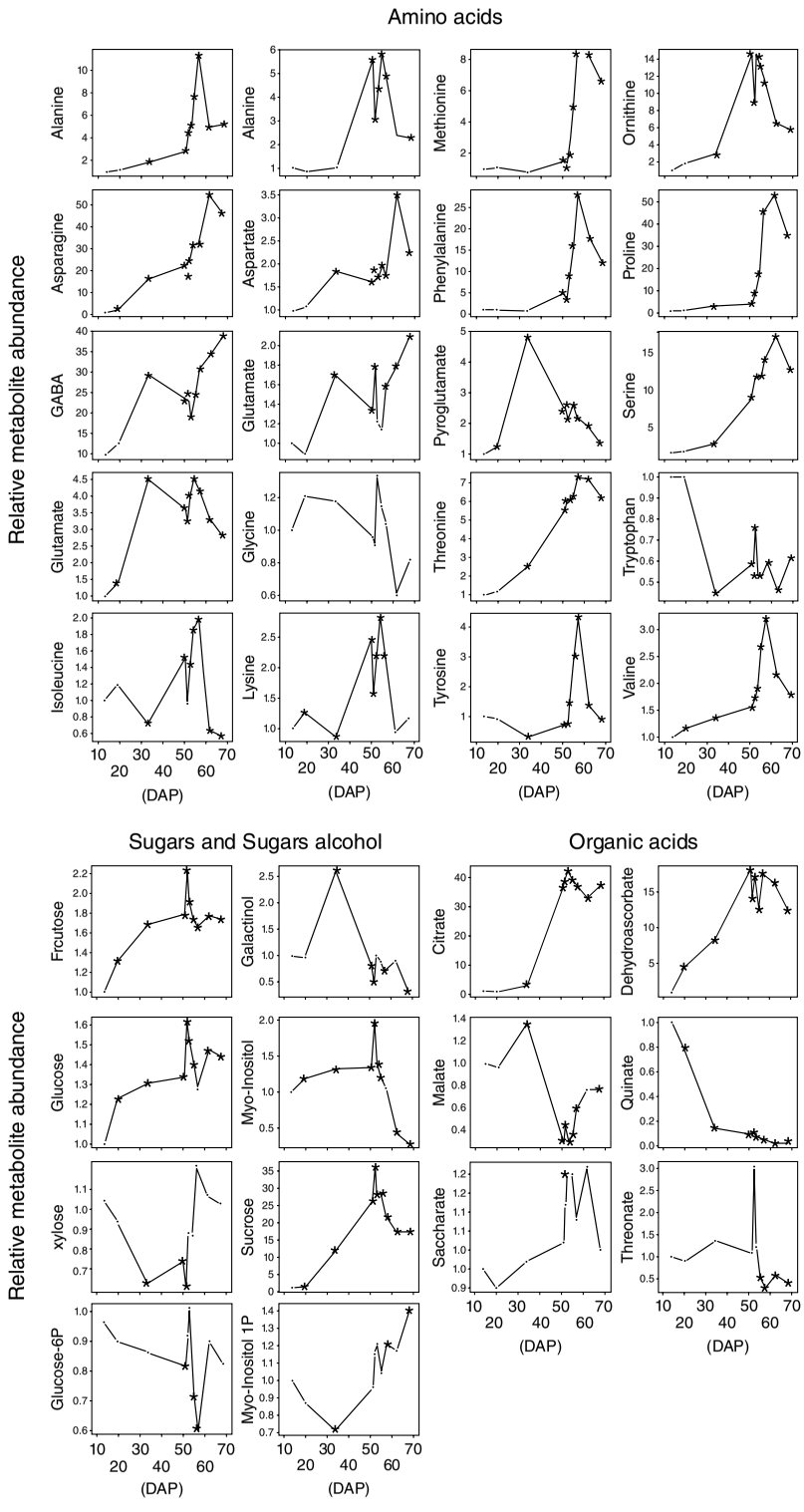

Fig. 8.6. Primary metabolite levels during pepper fruit development and ripening (Osorio *et al.*, 2012).

growing temperatures (Cotter, 1980; Guzman *et al.*, 2011).

The formation of red color is one of several changes of the maturing fruit of pepper. Pepper is generally not considered to be a climacteric fruit, because it is thought to lack the typical increase in carbon dioxide and ethylene production as it ripens (Saltveit, 1977). Research with hot peppers indicates, however, that at least some pepper cultivars do produce low levels of ethylene as the fruit are turning color (Gross *et al.*, 1986). Many types of peppers can be induced to turn color more rapidly by treatment of the plants with ethephon once color development has started (Cantliffe and Goodwin, 1975; Worku *et al.*, 1975), indicating that the ripening processes are not insensitive to ethylene.

Factors Determining Productivity

Reproductive growth and partitioning

As with many plants producing reproductive structures, the most actively growing organ on the pepper plant after flowering is the fruit (Hall, 1977; Beese *et al.* 1982) (Fig. 8.7). The characteristic that sets pepper apart from many other fruiting vegetables is that leaf photosynthetic activity is maintained even into late phases of fruit growth (Hall and Brady, 1977). In the field, if environmental conditions permit, additional flushes of vegetative and reproductive growth can occur as the first fruits are harvested.

The most obvious sign of assimilate competition among different organs on the pepper plant is the abscission of flowers and small fruits during the most active fruit growth period, resulting in a cycling of flowering and fruit set (Hall, 1977; Clapham and Marsh, 1987; Marcelis *et al.*, 2004; Wubs *et al.*, 2009). Flower and fruit were the most susceptible to abortion during the first week after anthesis (Marcelis *et al.*, 2004). Different evidence points to a negative linear correlation between source strength and flower/fruit abortion rate in pepper plants. More light, higher CO_2 concentrations, and lower planting density, increases the availability of assimilates per plant, and decreases fruit abortion. The cyclical pattern in fruit set is caused by changes in demand for assimilates and by sink strength of the competing organs (Marcelis *et al.*, 2004; Wubs *et al.*, 2009). High flower abortion occurs when fast growing fruit (at approx. three

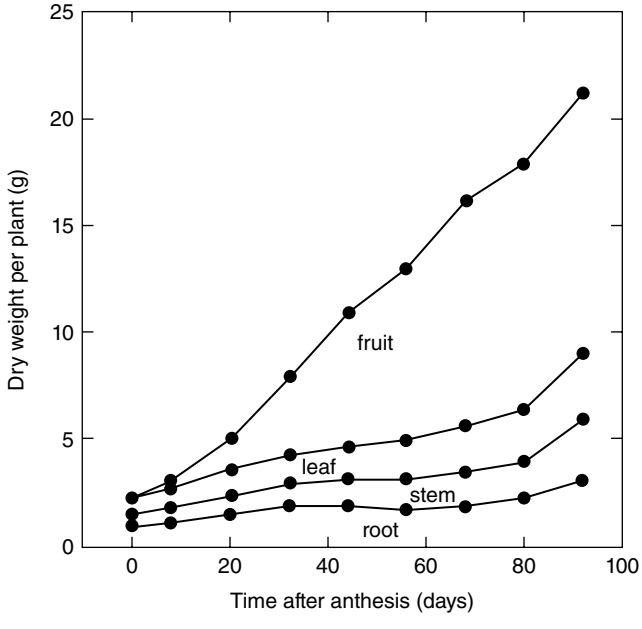

Fig. 8.7. Dry matter partitioning of bell pepper (cv. Market Giant) after flowering for pot-grown plants restricted to one fruit (Hall, 1977).

weeks after anthesis) are present, due to competition for assimilates. Fruit set increases when fast growing fruit are almost mature and have a low assimilate demand (Wubs *et al.*, 2009). Preventing the growth of reproductive structures by continuous flower removal eliminates the cycling, and leads to a faster growth rate of vegetative plant parts (Hall, 1977; Clapham and Marsh, 1987; Heuvelink *et al.*, 2002). In some cultivars, continuous flower removal resulted in accelerated senescence of leaves, a decrease in leaf photosynthetic rate, and a buildup of carbohydrates in stems and leaves (Hall, 1977; Hall and Milthorpe, 1978). The lowered gas exchange rates were correlated with increases in both stomatal and intracellular resistances to CO_2 diffusion as a result of the deflowering treatment (Hall and Brady, 1977). It is possible that the stomatal closure may be due to an increase in leaf abscisic acid (ABA) levels upon flower removal, but more work is needed to confirm this mechanism (Kriedemann *et al.*, 1976).

Environmental conditions for yield production

Temperature

The productivity of bell pepper is constrained by the adverse effects of high temperatures on fruit set, and the detrimental influence of low temperatures on fruit shape (Rylski and Spigelman, 1982; Aloni *et al.*, 1999). For high yields of good quality fruit, Bakker and van Uffelen (1988) found that mean air temperatures of 21–23°C were optimal during vegetative growth, followed by 21°C during the fruit growth period (Fig. 8.2). They also recommended that the amplitude between day and night temperatures be maintained at 7–9°C, for the low light conditions of the North European winter under which the experiment was conducted. As mean temperatures varied from the optimum, they found that the day/night temperature range needed to be increased to maintain high yields. Blondon (1978), working under artificial lights, and Dorland and Went (1947), experimenting in presumably higher light conditions of southern California, reached very similar conclusions. This would imply that these optimum temperatures were valid for a range of light conditions, a conclusion that needs to be checked experimentally.

In glasshouse production systems it is also possible to modulate root zone temperatures (RZT) if that is advantageous for growth and yield. Studies have shown that for plants grown at an air temperature of 23/19°C (day/night), fruit weight was maximal at 24 and 30°C root zone temperature (Gosselin and Trudel, 1986). At 30°C root zone temperature, fruit set and early yield were decreased, but the plants made up for the delay with higher later yields. High RZT might become a limiting factor for pepper growth and development when plastic mulches are used (Díaz-Pérez, 2010). Temperature increases of 1.5–2°C due to usage of different reflecting plastic mulches result in a significant inhibition of different vegetative growth parameters (above ground organs dry weight and size) and in reduction of fruit yield (Fig. 8.8). These high RZTs apparently primarily affect the water status and mineral uptake of the plant (Díaz-Pérez, 2010). There may also have been direct effects of the reflecting light on plant growth. More research is needed to better understand the effect of high RZT on pepper cropping, mainly because a high proportion of pepper and other vegetable crops are grown under passive non-environmental controlled insect proof net houses throughout the world (Elkind *et al.*, 2008).

The pepper plant's root zone (RZ) is exposed to varied temperatures (high and low) during the growing season. Pepper plants grown during winter in unheated net houses are exposed to low RZT (Dodd *et al.*, 2000; Aidoo *et al.*, 2017). These low RZT's affect many physiological process such as gas exchange, shoot and root phenology, and plant primary and secondary metabolic processes (Aidoo *et al.*, 2017). The impact of RZT temperature fluctuations were greater if the temperature decreased to 7°C than to 17°C. Also, different cultivars responded differently to RZT reduction (Aidoo *et al.*, 2017) or were growing at constant or diurnally fluctuating RZT (Dodd *et al.*, 2000). These results emphasize the important role of root temperature in pepper development and crop production.

Irradiance

The influence of light on the productivity of pepper differs depending on whether one is considering a

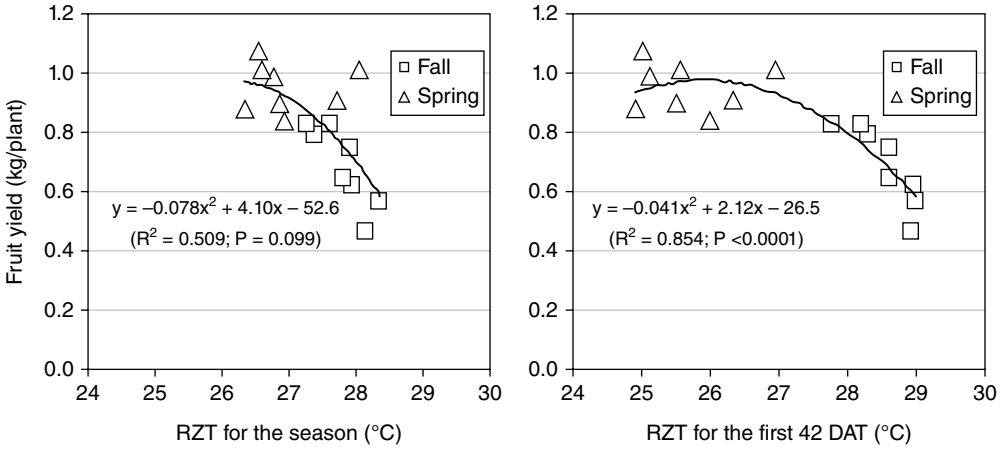

Fig. 8.8 Fruit yield of bell pepper plants as a function of mean root temperature (Díaz-Pérez, 2010). Bell pepper plants were grown in soil cover with various colored plastic film mulch in Tifton, GA, during fall of 2002 and spring of 2003.

glasshouse-grown crop during the low light conditions of the temperate winter, or a field of peppers growing in full sun outdoors. Demers *et al.* (1991) demonstrated that supplementing the reduced irradiance of Quebec's winter can significantly increase yield and fruit size (Table 8.2). The high cost of energy makes supplemental lighting prohibitively expensive in many countries, so growers avoid the adverse effects of low light conditions on fruit set and productivity by using that dark time of the year for vegetative growth (Bakker, 1989a).

In the field, we can be faced with the other extreme. Rylski and Spigelman (1986) reported that irradiance levels averaged 28 MJ m^{-2} day^{-1} in the Negev Desert of Israel during the summer growing season, compared to 0.7–1.54 MJ m^{-2} day^{-1} typical of glasshouses growing peppers in Holland in winter (Bakker and van Uffelen, 1988). Under those high light conditions, total fruit yields were reduced 19% compared to plants lightly shaded from transplanting, while marketable yields were decreased by 50% (Table 8.3). The shading treatments sharply reduced sunscald injury of the fruit (see below), and increased fruit size, perhaps because of an increase in seed number per fruit. The decrease in overall productivity of the plants grown in full sun may be due to lower leaf area measured (Díaz-Pérez 2014). Also, shaded plants showed improved mineral nutrient accumulation in leaves. Similar to the findings of Rylski and Spigelman (1986), slightly decreasing irradiance levels by using 17 mesh instead of 50 mesh nets produced bigger fruits. Smaller fruits were characterized with reduced average seed weight per fruit (Yasuor *et al.*, 2015, unpublished data; Fig. 8.5). The effect on seed development can be explained by the negative effect of high irradiance on pollen viability. Further research is needed to clarify the effect of excess irradiance on pepper flower organ development and functionality. The foregoing results imply that yield production of pepper will increase with increasing irradiance, only if temperatures remain in the optimal range, and plants have access to sufficient water. Under summer desert conditions, both temperature and high irradiance could have been limiting.

Carbon dioxide

A less expensive means of increasing the assimilation rate of glasshouse-grown peppers is to augment the atmospheric CO_2 content. As with many crops, peppers respond to this practice by increasing the proportion of fruit set (Nederhoff and van Uffelen, 1988) and increasing early production (Daunicht and Lenz, 1973). With regard to fruit yield, raising CO_2 levels by as little as 200 ppm was sufficient to increase the number of fruit harvested by 60% (Nederhoff and van Uffelen, 1988). Carbon dioxide enrichment has become a standard part of glasshouse pepper production in Holland (van Berkel, 1986).

Table 8.2. Effect of supplementary light treatments on the early and total marketable yield of glasshouse peppers grown in Quebec during winter (Demers *et al*., 1991).

	Fruit yield (kg m^{-2})			
Lighting treatment	Early	Total marketable	Fruits m^{-2}	Fruit size (g)
Control	0.18 a	0.39 a	4.0 a	117 a
0.9 mJ m^{-2} day^{-1}	0.75 b	1.52 b	14.5 b	127 b
1.4 mJ m^{-2} day^{-1}	1.24 c	2.45 c	22.6 c	128 c
1.8 mJ m^{-2} day^{-1}	1.55 d	2.99 d	27.6 d	128 c

Means in a column followed by different letters are statistically different at the 5% level.

Table 8.3. Effect of plant shading level on yield and fruit quality of bell peppers grown in a high light desert climate in Israel (reprinted from Rylski and Spigelman 1986, by kind permission from Elsevier Science).

Shading (%)	Fruit no. $plant^{-1}$	Fruit size (g)	Market yield (kg m^{-2})	Sunscald fruit (%)
0	8.1 a	86 c	2.0 c	36 a
12	8.5 a	97 b	3.1 b	20 b
26	7.7 a	108 a	3.7 a	8 c
47	5.4 b	111 a	2.9 b	2 d

Means in a column followed by different letters are statistically different at the 5% level.

Similarly, elevated CO_2 levels caused an increase in the pods number, yield, fruit size, seed number, and pungency of Habanero peppers (*C. chinense* Jacq.) (Garruña-Hernández *et al*., 2013).

Water

Under field conditions, insufficient moisture supply can adversely affect growth and yield of pepper. Pepper is a shallow-rooted crop so the amount and frequency of irrigations depend on soil type, bed type, plant size, and environmental conditions in the field or under protective structures (greenhouses and net-houses) (Bosland and Votava, 2012). Even small deficits, maintained for the entire season, can have large effects (Beese *et al*., 1982). Drought stress can restrict vegetative growth and development of leaf area, which then reduces yield (Biwalkar *et al*., 2015). In an irrigation experiment with sweet peppers grown under natural ventilated greenhouse conditions, for instance, reduced irrigation level with 80% and 60% replenishment of the evapotranspiration resulted in significant reduction of plant height and leaf area index (LAI). Measurements of the moisture status of the plants during the season showed little difference in leaf water potential and stomatal resistance between moisture treatments, indicating that monitoring these characteristics would help little in predicting when more irrigation was needed (Horton *et al*., 1982). Biwalkar *et al*. (2015) showed that reduced irrigation water availability resulted in fruits with reduced fruit length, width, girth, and weight, and with lower dry matter and ascorbic acid content. The pepper response to water deficit was also affected by cultivar and by fertigation regimes (Biwalkar *et al*., 2015).

Shorter, more severe water stress had more drastic effects on plant growth and assimilation rate (Alvino *et al*., 1990; Katerji *et al*., 1993). If there was sufficient time for recovery, the plants could resume the production of leaf area and yield, without detrimental effects on the latter (Alvino *et al*., 1990). Moisture stress imposed at some critical growth stages may have long-term effects from which recovery of full yield may not be possible. Pellitero *et al*. (1993) found in a field study that imposing stress during fruit set had detrimental effects similar to stressing the plants all season in a field experiment. Katerji *et al*. (1993) confirmed that pepper plants are particularly sensitive to drought during the fruit set stage, by imposing water stress of equal severity (−1.6 MPa predawn leaf water potential) on pepper plants at several stages of plant development.

In areas with regular and ample rain, irrigation is usually not needed. Irrigation is essential in arid and semi-arid regions to provide adequate moisture for pepper production

(Bosland and Votava 2012). This becomes even more important when low water quality is used (Yasuor *et al.*, 2017).

Variation of relative humidity in the glasshouse production environment can influence pepper fruit size, but appears to have no effect on yield (Bakker, 1989a). High relative humidity, especially at night, led to a 13% increase in fruit size (Bakker, 1989a), probably due to changes in water uptake by the developing fruit. Baer and Smeets (1978) showed that the large fruits developed under 95% relative humidity contained significantly more seeds than fruits on plants grown at lower humidities.

Ontogeny of yield

In trying to understand how yield is developed in pepper plants, it is necessary to consider the field, glasshouse and insect proof net house production systems separately, for they are quite different in crop establishment, early manipulation of the plants, and in the duration of growth.

Field-grown plants

Peppers growing in the field generally are not deflorated or pruned and thus tend to set the first flowers, if environmental conditions permit. Yield on field-grown plants of bell pepper is therefore comprised primarily of fruits on the main stem branches arising beyond the first inflorescence, and the first flowering nodes of basal branches (Gaye *et al.*, 1992). When fruits on those nodes are growing actively, fruit set at later-formed nodes is inhibited, so that a group of fruits of similar age develop and make up the main yield of the plant. If the season is long enough, the next flush of flowers may set when the first fruits are maturing, forming additional yield. If fruit set during the first flush of bloom is reduced due to adverse climatic factors, the cyclic pattern of yield production is not as pronounced.

Little information is known about the partitioning of fruit yield between main stem nodes and basal branches. Cooksey *et al.* (1994) found that transplanted chili pepper plants developed more branches than direct-seeded plants. Stoffela and Bryan (1988) varied in-row spacing from 13 to 50 cm, and left one to three plants per stand with direct-seeded peppers, but these treatments did not alter branch number per plant. Neither of these groups determined the yield of main stem and basal branch nodes separately. Cooksey *et al.* (1994) did note, however, that the harvestable fruits on transplants were spread over a larger vertical plane than on direct-seeded plants, and judged them to be more difficult to harvest mechanically.

Glasshouse and nethouse pepper

In commercial glasshouse production, pepper plants are typically transplanted into the production area, and pruned to two stems, with all basal and all additional upper branches being removed as they arise (Bakker and van Uffelen, 1988). In addition, fruit set is prevented on the first ten flower nodes to allow an adequate vegetative frame to be built up. The fruiting stems are supported vertically. Similarly, in insect-proof net houses pepper plants are also transplanted into the soil or soilless culture media. Unlike in greenhouse production no stem pruning is needed and pepper plants are left with two to three main stems. If not naturally aborted, fruits are usually removed from the bottom two to three nodes. In this production system, plant are supported horizontally ("Spanish system"). In comparison to the field-grown plants, glasshouse and net house peppers start producing fruits later, but the production season can extend over a much longer period. Whereas bell peppers in field plantings would typically be harvested over a two- to three-month period, the glasshouse production season can extend for eight months. Total marketable yields can therefore be considerably higher in the latter case. Even in a shorter harvest period of four months, Bakker and van Uffelen (1988) reported marketable yields of 10 kg m^{-2}, compared to typical yields from field experiments of 2–6 kg m^{-2} (Locascio and Fiskell, 1976; Hochmuth *et al.*, 1987; Stoffella and Bryan, 1988) and to typical yields from insect proof net house or greenhouses in the Mediterranean basin of 6–12 kg m^{-2} and 7–12 kg m^{-2}, respectively, depending on planting dates and crop duration (Yasuor *et al.*, 2013 and Yonatan Elkind, personal communication).

Chili or paprika peppers grown for chili powder used as food coloring were estimated to be grown on 15% of the total pepper hectarage

in the USA in 1976 (Andrews, 1984). This crop is generally direct-seeded and harvested when most of the fruits are dry, late in the season. Yields of dry fruits range from 0.1 to 0.7 kg m^{-2} (Beese *et al.*, 1982; Cooksey *et al.*, 1994). This crop often receives a lower intensity of management with regard to inputs of irrigation and fertilizer than the bell pepper crop.

Seed yield

When open-pollinated plants are harvested for seed, seed yields tend to be proportional to fruit yield (Osman and George, 1984). In a nitrogen rate experiment with "Anaheim Chile," Payero *et al.* (1990) found, however, that seed yields were maximal at the lowest N rate used (170 kg ha^{-1}), while fruit yields were not changed as nitrogen rates increased to 310 kg ha^{-1}. On the other hand, a greenhouse winter pepper crop produced larger fruits at low N rates, but increased N had no significant effect on seed yield (Yasuor *et al.*, 2013). The occurrence of cool or high temperatures during fruit set could also reduce the number of seeds per fruit, and thus lower seed yield (Rylski, 1973; Yasuor 2015, unpublished data; Figure 8.5).

Physiological Disorders

Flower bud and flower abscission

Abortion of reproductive organs is common in sweet pepper, even when grown in greenhouses under carefully controlled environments (Wubs *et al.*, 2009). Loss of flower buds and flowers is a problem occurring primarily in the production of large-fruited bell peppers, which are grown in temperate and subtropical environments. Stages susceptible to abortion are very young flower buds (< 2.5 mm), buds close to anthesis, and flower and fruits up to 14 days after anthesis (Erickson and Markhart 2002; Wubs *et al.*, 2009). The principal causal factors are high temperature, low light levels, drought stress, failure of pollination/fertilization, the presence of rapidly growing fruit on the plant and biotic agents like certain virus diseases, and insect pests (Wien *et al.*, 1989a; Marcelis *et al.*, 2004; Wubs *et al.*, 2009). Depending on when the stress occurs, the disorder can result in the delay of anthesis, the prolongation of the flowering period without fruit set, or the early termination of fruit setting. In regions with short growing periods, such as upstate New York, the disorder can result in total yield loss by delaying fruit development into the cool period when fruit grow too slowly to reach market maturity (H.C. Wien, personal observation, Elba, N.Y., August, 1988).

Under conditions of severe stress, the plant abscises open flowers as well as buds of a range of sizes. The reproductive structure loss can be so total that growers describe the plants as having "gone vegetative." In that situation, several weeks may be needed for development of new flowers. This phenomenon is also prominent when pepper plants are grown under passive structure such as insect proof net houses. Knowing which factors play a role in the abortion of reproductive organs, and what processes take place during abortion, could help to reduce the extent of abortion in pepper. More detail on the abiotic causes, the mechanism of the abscission process, and control methods are given below.

Causal factors

The most common cause of flower and flower bud abscission in pepper is high air temperature. Cochran (1936) found that fruit set was reduced at 27/21°C, and that no flowers set if plants were grown at 38/32°C in a glasshouse (Fig. 8.9). High night temperatures are more detrimental to fruit set than high day temperatures (Rylski and Spigelman, 1982; Aloni *et al.*, 1991). In the field, however, even 32/15°C (day/night) sufficed for complete flower and bud abscission of many cultivars (Wien, 1990). When high temperature is combined with moisture stress, abscission is further increased, although moisture stress at moderate temperatures is generally not sufficient for complete reproductive structure loss (Bereny, 1970; Cochran, 1936) (Fig. 8.9). The production of bell pepper under low light conditions frequently found in glasshouses in the winter often results in poor fruit set (Bakker, 1989b, 1989c).

Excess nitrogen fertilizer is frequently blamed by growers for poor fruit set under field conditions, yet evidence in the literature for this is difficult to find. In field experiments, increasing nitrogen rates result in increased yields up to a plateau, and perhaps a slight decline at highest rates (Locascio and Fiskell, 1976; Hochmuth

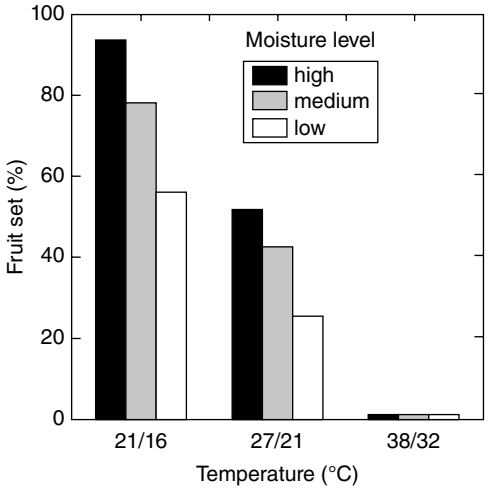

Fig. 8.9. Influence of air temperature and soil moisture on percentage of fruit set of "World Beater" pepper grown in pots in glasshouse compartments. Fruits were removed after setting. Data are averages of two years of experiments, 1932–1934 (Cochran, 1936).

et al., 1987; Crespo-Ruiz *et al.*, 1988; Hartz *et al.*, 1993; Yasuor *et al.*, 2013) (Fig. 8.10). It is possible that the lush, leafy growth that is associated with the effects of excess nitrogen fertilization is the result rather than the cause of poor fruit set. Furthermore, it was recently shown that under the high temperature present in passive structures (net houses and greenhouses) during flower development and fruit set, reduced N level and even complete N omission improved fruit set and yields (Yasuor *et al.*, 2013, 2015–2017, unpublished data).

Several biotic causal factors for pepper reproductive structure abscission have also been identified, including virus diseases, fungal pathogens and insect pests such as leafhoppers (Wien *et al.*, 1989a).

Presence of rapidly growing fruit can reduce the fruit set of later-formed flowers and also lead to flower bud abscission. Flowering and fruit set frequently resumes once the fruits reach mature size, resulting in cycles of fruit setting and abscission (Wien *et al.* 1989a; Marcelis *et al.*, 2004).

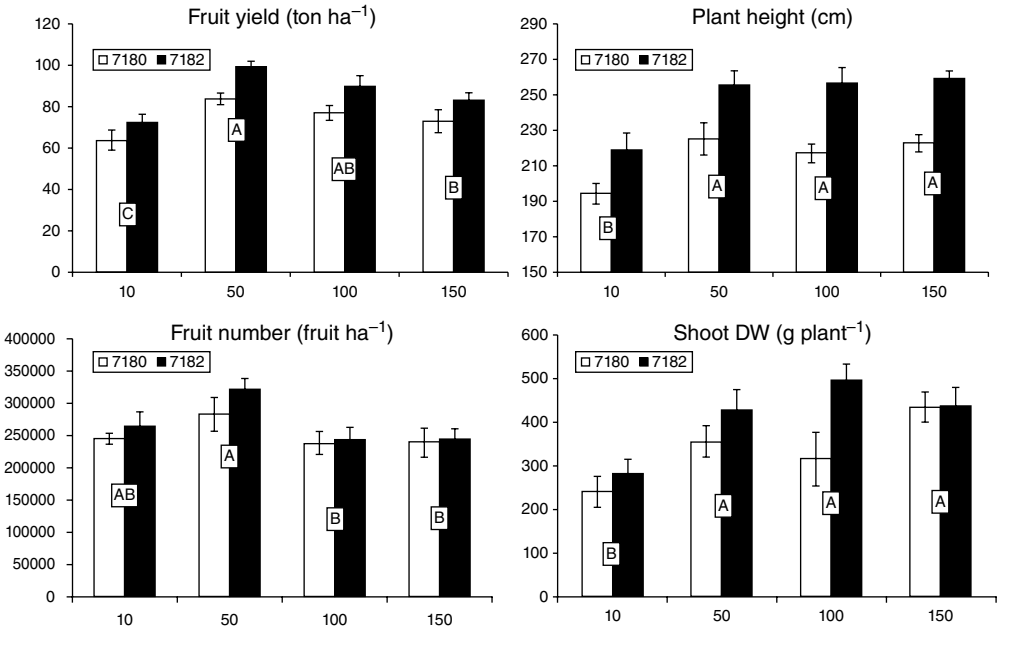

Fig. 8.10. Influence of nitrogen concentration (ppm) in irrigation water on fruit yield, fruit number, and plant height and shoot biomass of two greenhouse-grown pepper cultivars in Israel (modification of data present in Yasuor *et al.*, 2013). Pepper plants grown from summer until spring.

Mechanism of abscission

The physiological mechanism of pepper reproductive structure abscission resembles the way in which leaves are thought to abscise (Beyer and Morgan, 1971; Beyer, 1975). The hormonal control of abscission of vegetative and reproductive organs of many species, including the reproductive organs of pepper, can primarily be ascribed to the combined action of auxin and ethylene (Marcelis *et al.*, 2004). While the leaf, or in this case, the flower or flower bud is actively growing, auxin is translocated down the petiole or pedicel, and prevents the formation of an abscission layer at its base. Under stress conditions, ethylene is generated, which both reduces the polar auxin transport down the pedicel, and causes the formation of the abscission layer. There is good evidence that this model is operating in the case of heat stress abscission of pepper flowers (Wien *et al.*, 1993b). High temperatures reduced indole-3-acetic acid levels and particularly auxin transport capacity in pepper reproductive organs (Huberman *et al.*, 1997). Ethylene and its precursor, aminocyclopropane carboxylic acid (ACC), have been shown to increase in pepper reproductive tissue prior to abscission, when stressed by high temperature or low light (Wien *et al.*, 1989b, 1993b; Aloni *et al.*, 1994b; Huberman *et al.*, 1997). The resulting reduction in the endogenous IAA level in the abscission zone may lead to increased sensitivity of the zone to ethylene in the induction of abscission (Huberman *et al.*, 1997; Fig. 8.11).

The way in which high temperature and low light conditions initiate the hormonal changes described above is not entirely clear, but most likely involves a reduction of assimilate levels in the reproductive structures (Wien *et al.*, 1993b; Aloni *et al.*, 1996; Marcelis *et al.*, 2004). Aloni *et al.* (1991) found that heat stress reduced the translocation of assimilates to the reproductive structures, and the conversion of the translocated sucrose to reducing sugars in the flower buds. Acid invertase activity in the buds, but not the young leaves, was inhibited by heat stress. In buds of low light-stressed plants there was a similar lack of reducing sugars, but there was no evidence that invertase activity had been adversely affected (Wien *et al.*, 1989b; Turner and Wien, 1994b; Aloni *et al.*, 1996). In that case it appeared that mature leaves retained assimilates instead of translocating them to the reproductive structures (Turner and Wien, 1994a, 1994b). In addition to the negative effect of high

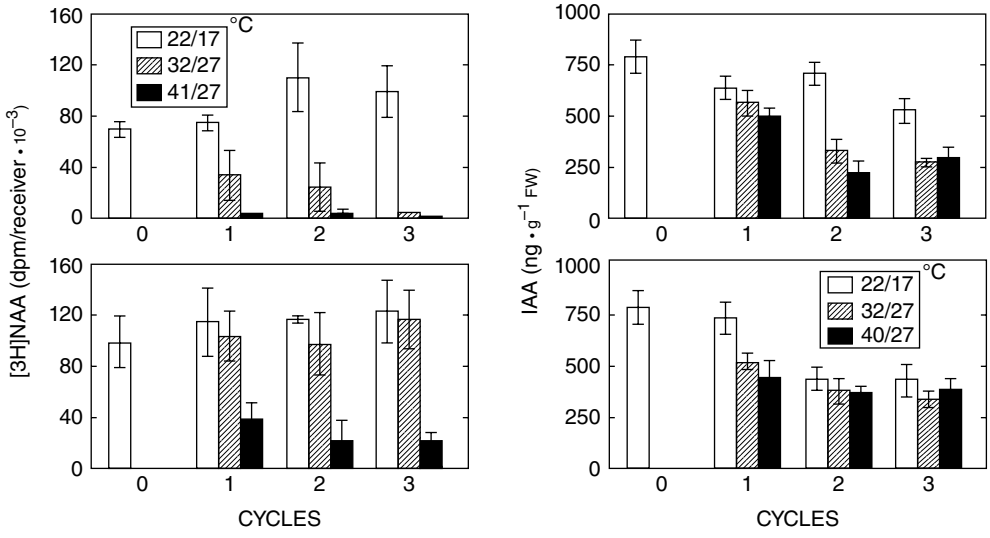

Fig. 8.11. Effect of high temperature exposure cycles on IAA content and [3H]NAA transport in pepper flower and fruitlets. (Huberman *et al.*, 1997).

temperatures on assimilate content, it was found that high temperature inhibited auxin biosynthesis in developing anthers, leading to sterility (Sakata *et al.*, 2010). More research is needed to clarify how auxin, ethylene and assimilates levels are affected by high temperature and their role in pepper flower development.

Control methods

The principal method by which reproductive structure abscission in pepper can be alleviated is using less stress-susceptible cultivars (Wien *et al.*, 1989a; Elkind *et al.*, 2008). There is considerable genetic diversity in reproductive structure abscission even among the bell peppers (Tripp and Wien, 1989; Wien *et al.*, 1993b). Unfortunately, the cultivars most susceptible to abscission within the bell pepper type also tend to be those with desirable large fruit type. It should be possible, however, to select lines which resist abscission of reproductive structures past the fruit set stage but then develop only a limited number of fruits to maturity. There are indications that some cultivars developed for glasshouse culture may have these attributes (Aloni *et al.*, 1994b).

Selection for stress resistance is most directly done by exposing plants to the stress conditions, be they high temperature or low light. If such environments are difficult to create on a large enough scale to permit the screening of breeding materials, it may be possible to use techniques that substitute essential elements of the stress environment, or that elicit the same response as exposure to the stress. For instance, cultivar screening for differences in sensitivity to ethylene use ethephon applications to explants or whole plants, and have shown good correlation with cultivar differences in stress sensitivity (Tripp and Wien, 1989; Aloni *et al.*, 1994b). It may also be possible to select lines less inhibited in polar auxin transport by flower pedicels when subjected to stress (Wien *et al.*, 1993b). Cultivar differences in assimilate partitioning to reproductive structures rather than mature leaves have also been demonstrated (Turner and Wien, 1994a).

Other ways in which abscission can be reduced include the moderation of temperatures in the field through frequent sprinkler irrigation, and the mitigation of low light stress in glasshouses by elevation of the CO_2 content (Nederhoff and van Uffelen, 1988). Application of synthetic auxins has not been effective in reducing low light-induced abscission, but application of the ethylene action inhibitor silver thiosulfate has reduced abscission (Wien and Zhang, 1991). The presence of the heavy metal silver limits use of this compound to non-food purposes.

Sunscald

When pepper fruit are exposed to high intensity sunlight after they have reached the mature green stage of growth, they are susceptible to tissue damage and bleaching called sunscald. The injury is caused by a combination of heat and light (Rabinowitch *et al.*, 1986). If tissue temperature rises to 50°C, only a 10-min exposure to intense sunlight is sufficient to cause damage (Barber and Sharpe, 1971). The threshold temperature in pepper is 38–40°C (Rabinowitch *et al.*, 1986), but injury at those temperatures requires at least 12 h exposure. The disorder is prevalent in high light environments, and can be a serious detriment to production of bell peppers. For instance, Rylski and Spigelman (1986) reported that unshaded pepper plants grown during the summer in the Negev Desert showed a 36% incidence of sunscald (Table 8.3).

Damage is caused by a combination of direct injury of the tissue due to the heating effect, and the generation of superoxide anion radicals through the action of light on chlorophyll at high temperatures (Rabinowitch and Sklan, 1981). Heating of the fruit in the dark causes the pericarp of the fruit to turn flaccid and brown, but not become bleached.

Pepper fruits are most susceptible to this disorder at the mature green stage, and when turning color from green to red. The immature green fruit are less subject to the disorder, and the fully ripe red fruit are not susceptible (Rabinowitch and Sklan, 1981). Chlorophyll must be present in the pericarp for sunscald to occur. Presence of the enzyme superoxide dismutase in the chloroplasts of the fruit can lessen or prevent injury by catalyzing the formation of hydrogen peroxide and oxygen from the superoxide radicals. The increased susceptibility of the mature green fruit to sunscald was correlated with a lower superoxide dismutase activity at this stage (Rabinowitch *et al.*, 1982).

Scientists have also found that pepper fruit could be conditioned to tolerate sun exposure without injury by giving the fruit a heat treatment in the dark. Rabinowitch *et al.* (1986) found that heating peppers for 6 h at 40°C allowed them to tolerate sun exposure with much reduced injury. The potentiating effect of the heat treatment lasts from about 15–36 h after the fruit have been removed from the high temperatures. The mechanism of the protective effect is not entirely clear, but in tomato, Rabinowitch *et al.* (1982) showed that heat treatment significantly increased superoxide dismutase activity.

Pepper breeders have long recognized the need to select plants that are less subject to sunscald, and select for the production of adequate leaf area, especially for large-fruited types. These cultivars unfortunately tend to have greater susceptibility to flower and flower bud abscission under stress (Turner and Wien, 1994a). To date no tolerant cultivar exists, leaving the ability to cope with this disorder to cultural practices.

Cultural practice management measures that can be used to reduce sunscald damage are to erect shade canopies over the fields that reduce light by 26–36% (Rylski and Spigelman, 1986; Díaz-Pérez, 2014). Recently it was suggested that suncald damage can be reduced by using grafted plants with improved canopy coverage (López-Marín *et al.*, 2013).

Blossom-end rot

Blossom-end rot (BER) of pepper appears as sunken dark circular lesions on mature green and riper fruit. The tissue breakdown may occur not only on the stylar end of the fruit, as the name implies, but also on the side. It occurs most frequently on large-fruited cultivars, but has been noted on the smaller-fruited pimentos as well (Hamilton and Ogle, 1962). The disorder appears to be quite similar in cause, occurrence and control methods to BER in tomato. Since it has been studied in much more detail in that crop, the reader is referred to Chapter 7 for more information on the physiology of the disorder.

Nutritional studies with pepper have confirmed that BER is caused by a localized deficiency of calcium in the fruit, brought about most directly by a lack of calcium in the root zone (Miller, 1961). The deficiency is worsened by limited water availability due to insufficient irrigation or use of saline water. Given the immobile nature of calcium in the plant, and the difficulty of translocating this element from one part of the plant to the other, BER can frequently occur even when there are adequate supplies of calcium in the soil (Bangerth, 1979). Since calcium moves in the xylem, it is translocated preferentially to organs with highest transpiration rates. Thus, even in normal situations, fruit calcium levels in pepper are very low, typically in concentrations of 0.2–0.3% (Marti and Mills, 1991b). There is also differential distribution of calcium in the fruit, with values of 0.2% at the stem end, and a range of 0.04–0.07% being found on the flanks and at the blossom end in one study (Marti and Mills, 1991b). This coincided with the symptom location described above. Of the total amount of calcium accumulated by the plant at maturity, only about 6% is found in the fruit (Miller *et al.*, 1979). Calcium deficiency becomes even more problematic when desalinated water is used for irrigation of BER-susceptible crops (Yermiyahu *et al.*, 2007).

Several approaches have been tried to remedy the BER problem in pepper. The most important step is to ensure that calcium levels in the soil are adequate, and the levels of other nutrients that might compete with this element for uptake are not excessively high. For instance, it was demonstrated with sand culture that high magnesium levels in the nutrient solution could induce BER (Hamilton and Ogle, 1962). Similarly, high nitrogen levels, particularly if supplied by a large proportion of ammonium nitrogen, also increased incidence of the disorder (Miller, 1961; O'Sullivan, 1979; Marti and Mills, 1991a; Bar-Tal *et al.*, 2001).

Some investigators have attempted to increase fruit calcium content by increasing transpirational flow to the tops or to the fruit, or by reducing transpiration of the foliage by application of antitranspirants. As with tomato, providing the plants with even water supply and avoiding moisture stress can greatly minimize the incidence of BER (Pill and Lambeth, 1980). Furthermore, additional night irrigation when calcium is supplied via fertigation might reduce BER in pepper.

The detailed physiology of this disorder in peppers is largely unexplored, but given the studies by Ho and co-workers on BER in tomato (e.g. Ho *et al.*, 1993), it should be possible to make rapid progress in our understanding of this malady. Further work is needed to elucidate the factors that affect calcium distribution in the pepper plant, and its availability to the developing fruits. A better understanding of the physiological mechanism of BER could be helpful to devise selection criteria that would allow breeders to develop cultivars with reduced susceptibility to the disorder. For example, Aloni *et al.*, (2008) demonstrated that the occurrence of BER in bell pepper is accompanied with alteration of apoplastic ascorbic acid levels, ascorbate oxidase activity and pH.

Other Fruit Disorders

Pepper fruits may show several other disorders, the most common of which are abnormalities in fruit shape or fruit color, or the formation of cracks in the fruit surface.

Abnormal fruit shape

Fruit shape can be influenced by temperatures during ovary formation (see section on fruit growth and maturation), or by the absence of seeds. The latter condition most commonly occurs if seed set has been inhibited by low temperatures (12–15°C night) (Rylski and Spigelman, 1982; Polowick and Sawhney, 1985). Such seedless fruits are frequently smaller, with thinner than normal pericarps, and of flat, irregular shape. They are often retained on the plant until fruit maturity, and although higher in assimilates, are seldom of marketable shape.

Color spotting

Several fruit discolorations have been described on bell pepper fruits, of largely unknown cause. Dark, circular spots of 2–7 mm diameter occurring on mature green fruits have been documented on peppers grown in Texas (Villalon, 1975), Queensland, Australia (Hibberd, 1981) and Florida (Ozaki and Subramanya, 1985). Villalon failed to isolate any pathogens from the lesions, which extended into the pericarp tissue. The disorder varied considerably among cultivars, with "Yolo Wonder" being found susceptible in both the Queensland and Florida studies, and "Early Calwonder" and "Sheba," "Early Bountiful" and "Superset" showing few symptoms in the Florida and Queensland studies, respectively (Hibberd, 1981; Ozaki and Subramanya, 1985). Incidence of black spot was more prevalent on soils high in calcium in Florida, and the calcium content of the affected areas of the fruit was reportedly higher in the Australian study.

Heat pale spots

Heat pale spots disorder is the appearance of yellow spots beneath the fruit cuticle (Aloni *et al.*, 1994a; Silber *et al.*, 2009; Yasuor *et al.*, 2015). Unlike sunscald, heat pale spots are not directly related to high light intensities, but to the air temperature around the fruits. Even more, this disorder is common even under shade net (Yasuor, unpublished data). Higher damage levels were observed when pepper fruit were exposed to 24°C night temperatures as compared to 17°C (Silva and Yasuor, unpublished data). Pepper cultivars differ in their susceptibility to the phenomenum (Aloni *et al.*, 1994a; Yasuor *et al.*, 2015; Silva and Yasuor, unpublished data). The susceptibility diversity is related to fruit antioxidant activity during fruit maturation and ripening. Examination of the affected tissue revealed high concentrations of calcium oxalate crystals (Aloni *et al.*, 1994a; Silva and Yasuor, unpublished data). Unlike the programmed cell death process, the heat pale spot appearance is a slow process and is usually observed during fruit color break (Silva and Yasuor, unpublished data). Recently it was found that manganese can reduce the severity (Silber *et al.*, 2009; Yasuor *et al.*, 2015). Much remains to be learned to develop cultivars or agronomical tools for better coping with the phenomenon which has become a major factor in reducing fruit quality in protective production during the hot season (Yasuor *et al.*, 2015).

Fruit cracking

The appearance of cracks on the surface of bell pepper fruits is associated with production of peppers at high night relative humidity and low night temperature (Rylski *et al.*, 1994). Cultivar differences in susceptibility were partly related to pericarp thickness. Somos (1984) found that the disorder was aggravated by uneven watering. In the Jalapeno pepper, the shallow surface cracks are a characteristic of the normal mature fruit (Andrews, 1984).

Concluding Remarks

This brief look at the physiology of the pepper plant reveals the pepper to be a rather demanding crop. During the long germination and emergence period, a well-watered soil at moderate temperatures is needed to continue plant development. The slow leaf area development rate makes the plant susceptible to competition by other plants. Although the induction of flowers seems to be relatively unaffected by environmental factors, many cultivars among particularly the bell peppers are very sensitive to the loss of reproductive structures once these have been initiated. On the positive side, once the fruits are growing actively, the plants very efficiently partition assimilates to both the fruits and to maintenance of the vegetative organs. The plants typically retain much of the leaf area in the late reproductive period, and if weather conditions permit, will continue to produce additional flushes of reproductive organs. Pepper fruit yield and yield fluctuation are highly affected by environmental conditions and other factors that determine plant ability to maintain source strength and therefore reducing flower and fruit abortion.

In comparison to many other vegetable crops, our depth of knowledge of the physiological processes in pepper is limited, particularly for the non-bell types. The increasing popularity of the crop, and the fact that it can be easily grown in pots in glasshouses and artificial environments should contribute to a rapid increase in research activity with this fascinating plant.

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9 Potato[1]

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History

More than 6000 years before the Spanish came to South America, the so-called "Irish" potato (*Solanum tuberosum* L.) was under cultivation in the highlands of the Andes (Hawkes, 1992). The potato was a central part of the culture of the Incas and of the civilizations that preceded them. Potatoes could be grown successfully at altitudes up to 3700 m above sea level.

A secondary center of origin for the potato was farther south, in Chile. Here the potato had been selected for ability to tuberize under longer photoperiods than those found in its primary home near the equator. The Chilean form, best represented by potatoes grown in the island of Chiloe (latitude 43°S) is usually designated subspecies *tuberosum* to distinguish it from *andigena*, the subspecies grown in Peru.

Although the *tuberosum* subspecies is far better adapted to the long days of European summers, evidence indicates that the *andigena* potato was introduced to continental Spain in about 1570 (Hawkes and Francisco-Ortega, 1992) and actually arrived in the Canary Isles some ten years earlier (Hawkes and Francisco-Ortega, 1993). From Spain, the potato gradually spread northward throughout Europe. It is likely that the first potatoes grown were poorly adapted to the long summer days of northern Europe and that they would have matured only after daylength had shortened, late in the autumn. Variants were gradually selected that could tuberize under longer days and were therefore earlier in maturity. Once such adapted clones had been selected, the climate in northern Europe was found to be almost ideal for potato production. The crop gained popularity at an extraordinary rate in many countries, due to its high yield potential and nutritional value.

By the end of the 18th century, the economy of the peasants in Ireland was as fully dependent upon the potato as had been true for the pre-Spanish cultures of the Andes. The Irish ate enormous quantities of potatoes, often more than 3 kg per person per day. So total was the dependence of poor farmers upon the potato that when the late blight disease (*Phytphthora infestans*) suddenly appeared in 1845, with devastating crop losses, the effects on the population were calamitous. During the next several years, perhaps a million people died of starvation or of diseases associated with extreme malnutrition. Another million Irish emmigrated to the United States, Canada, and various other parts of the world.

After the late blight epidemics, attempts were made to introduce late blight resistance through new germplasm collected in South America. It is probable that the germplasm for the second

[1]This chapter is an updated version of a chapter originally written by E. E. Ewing in 1997.

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introduction of potato came from the isle of Chiloe, because the European varieties that had been gradually selected from *andigena* were replaced with subspecies *tuberosum*. Analyses of chloroplast DNA show that almost all cultivars in Europe and North America now belong to the latter subspecies (Hosaka and Hanneman, 1988). However, Hardigan *et al.* (2017) provide evidence that many important traits selected for commercial production in cultivated potato today such as carbohydrate metabolism and glycoalkaloid biosynthesis contain gene signatures from numerous wild *Solanum* species representing more than 100 tuber-bearing relatives (*Solanum* section *Petota*).

Nutritional Value and Importance

Compared to other types of plant protein, the quality of potato protein is relatively high. The vitamin C content is also high; three medium potatoes (90 g each) will supply the adult requirement for vitamin C under average conditions. Potatoes also contain fiber and significant amounts of B vitamins as well as various minerals, most notably potassium. A medium-sized potato contains about the same number of calories as an apple or an orange—it is not "fattening" except for the gravy, butter, or cooking oil that may be added to it.

In world tonnage, the potato ranks after rice and wheat as the third most important crop for human consumption (https://cipotato.org/potato/facts/). Approximately three-quarters of the plant is comprised of the edible portion, tubers, while only about one third of cereals is comprised of the edible portion. Therefore, few if any crops surpass the potato in terms of potential yields of calories or of protein per hectare if calculated per day of growing season, and it ranks among the top half dozen or so food crops in total world production of both protein and energy. In addition, the potato is adapted to a wide range of growing conditions providing food security to countries in Asia and South America. Because of these attributes, the potato is considered the most important vegetable in the world, consumed by over 1 billion people.

Total worldwide production of potatoes is over 370 million tonnes per year. China leads the world in potato production with nearly 96.0 million tonnes produced annually followed by India, the Russian Federation and the Ukraine at 45.3, 30.2, and 22.3 million tonnes, respectively. The USA ranks 5th in potato production with 19.8 million tonnes per year, representing about 5.4% of the potatoes produced in the world. Almost all European countries have substantial potato production along with Africa and South America (https://www.potatopro.com/world/potato-statistics).

Botany

The potato is a member of the Solanaceae family. Thus, it is not surprising that its fruit resembles a small green tomato. Cultivated potatoes are tetraploids with 48 chromosomes and highly heterozygous. For this reason, once a selection is made it is propagated clonally. During potato breeding, true seeds are extracted from the fruits and handled much like tomato seeds. There is usually a period of seed dormancy for several months or more. The seedling will send out stolons from axillary buds just above the soil level (starting from the cotyledonary nodes), the stolon tips will grow under the soil, and tubers will form on the buried stolons. The tubers can then be used for clonal propagation, as described below. In most developed countries, the main value of the seeds is to plant breeders who use them to develop new cultivars followed by clonal propagation. It is also possible to grow the potato from true seeds and interest in producing potatoes from true seed has increased in recent years. For example, most wild type potatoes are in fact diploids and with proper selection, breeding of diploids to produce uniform hybrid true seed may represent the future of potato production (Jansky *et al.*, 2016).

A potato tuber is a shortened, swollen, starchy stem (Fig. 9.1). The tuber bears minute scale leaves, each with a bud in its axil. These buds form the "eyes" of the tuber, and the scale leaves form the "eyebrows." The sprouts that develop from the eyes come from buds that are in the axils of the leaves. The eyes are arranged in a spiral around the tuber just as leaves on the normal potato plant are arranged in a spiral. Like an ordinary stem, the potato tuber shows apical dominance. The first sprout to develop is from an

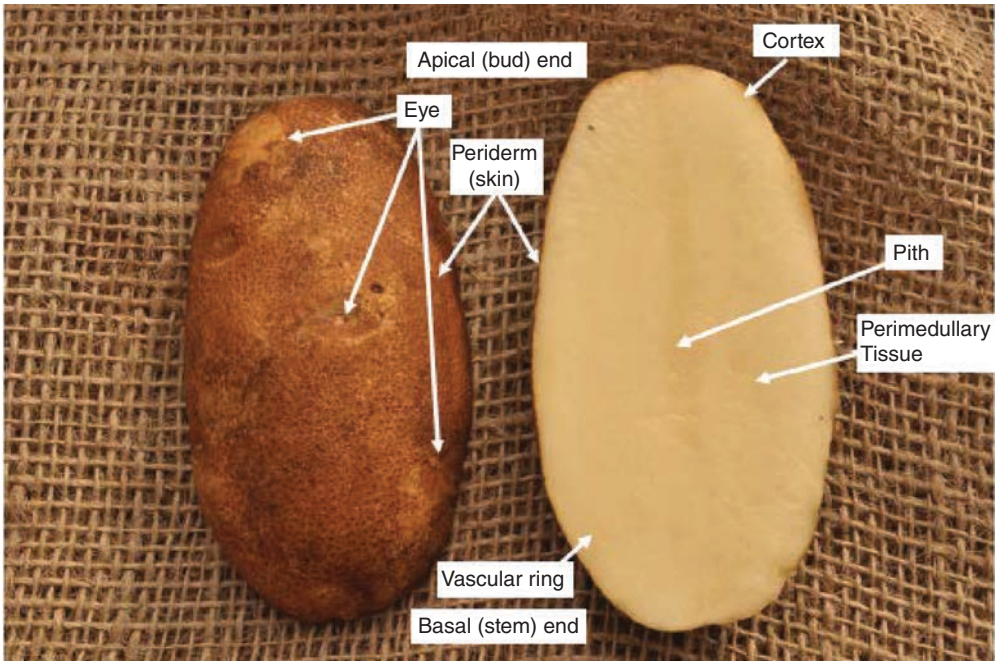


Fig. 9.1. External and internal potato tuber anatomy (photo: S. Gupta).

eye in the apical end of the tuber. If the apical sprout grows, sprouts from the other eyes are suppressed; if it is damaged (such as by seed cutting), the apical dominance is broken and most of the other eyes on the tuber will sprout. The potato tuber develops chlorophyll in the light and has the internal anatomy of an ordinary stem. The epidermis sloughs off early in the development of the tuber and is replaced by a corky periderm. This is the "skin" of the potato. Under the periderm is the cortex, the vascular ring, and the pith.

All plants obtained from the offspring of a single tuber are genetically identical, unless chance mutations have occurred. This means that all tubers of a given cultivar should be highly uniform unless they have become infected with a virus.

Potato Growth, Development, and Culture

The potato is considered a cool season perennial vegetable, but used in agriculture as an annual crop. Best yields are typically obtained in climates where the average growing season temperature is about 15–20°C although day/night temperature differential is also important (Benoit *et al.*, 1986). The root system on the potato plant is fibrous and not extensive. Ample soil water, therefore, is necessary whether from rain or supplemental irrigation to produce a viable crop.

The life cycle of the potato can be generally divided into five stages (Fig. 9.2) (Struik, 2007). The length of each stage will depend on cultivar and climatic conditions. Stage I is sprout development and occurs during the first 30 days after planting. The seed tuber is the primary source of nutrients during this stage. Growth stage II is vegetative and occurs between 30 and 55 days after planting. During this growth stage, roots begin to provide nutrients for haulms and leaf photosynthesis occurs to provide energy for biomass production. Growth stage III, corresponding to tuber initiation and tuberization, occurs between about 50 and 70 days after planting. During this stage, haulm (vines in the United States) growth and nutrient uptake increase exponentially. Tuber bulking occurs during growth stage IV, generally between 60 and 90 days after planting; although depending on the growing

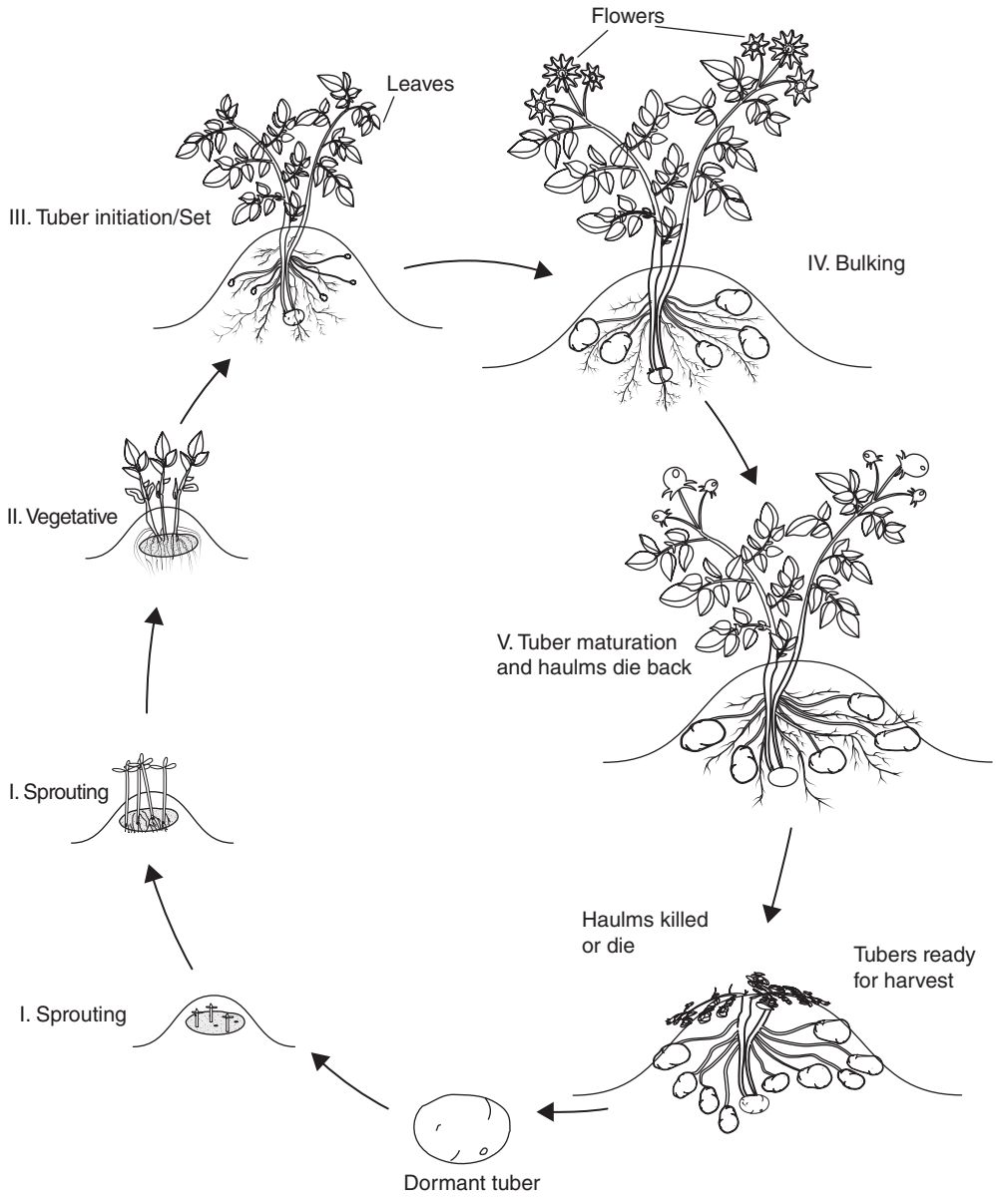


Fig. 9.2. Life cycle of the potato crop.

conditions, this stage could be longer and occur later for late maturing cultivars. During this stage, haulm growth and nutrient uptake slow down, flowering occurs, and mobilization of carbohydrates and nutrients from the haulms to the tuber takes place. Growth stage V is tuber maturity when the haulms begin to senesce and nutrients in leaves and roots are further transported to the tubers. After growth stage V, tubers remain in a state of dormancy until dormancy is broken and conditions allow sprouting to begin, starting the cycle again. All these stages will be discussed in more detail in subsequent sections.

Potatoes can be planted as whole or cut seed. Use of whole seed generally results in more stems and smaller tubers and is preferred by the European market. An advantage of whole seed is a lower transmission of seed tuber borne diseases such as bacterial ringspot. Cut seed is commonly used in the United States because larger tubers are preferred by the processing market and consumers for the fresh market.

In general, seed pieces should average 40–55 g. A propagule of this size is large enough and contains sufficient food reserves to get the new plant off to a rapid, vigorous start. Unfortunately, the seed tuber is also an ideal vehicle for the transmission of plant diseases from one generation to the next, including diseases caused by viruses, bacteria, fungi, and nematodes. Therefore, it is important to start with disease-tested tubers. The starting materials for production of disease-tested tubers are tissue culture plantlets produced by meristem-tip culture and maintained in vitro (Fig. 9.3). Such plantlets will form small tubers, called "microtubers," in vitro, and these can be planted directly in the field for propagation. Microtubers typically range in size from less than 250 mg to 1 g. An alternative is to transplant the plantlets from the test tube into greenhouse pots. Tubers produced in this way are called "minitubers," which might range in weight from 5 to 25 g or more.

Theoretically, microtubers are completely free from disease organisms when harvested from tissue culture. However, once microtubers or minitubers are planted in the field, even under ideal circumstances each successive crop of seed tubers will pick up more and more infection with virus and other disease organisms. Adding to the problem is the fact that multiplication rates are low: 1 ha of seed tubers will produce only enough seed tubers to plant about 10 ha in the next generation. A reliable supply of high quality seed tubers requires not only access to tissue culture plantlets, but also cool climatic conditions that are unfavorable for aphids that serve as vectors for many virus diseases. A final necessary ingredient is a governmental program for seed certification, including rigorous programs for inspection and quality control. Relatively few countries can meet this combination of requirements.

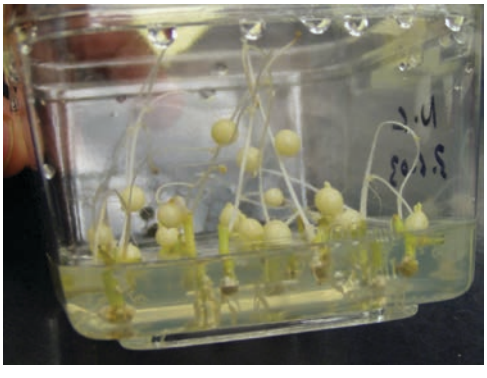


Fig. 9.3. Microtuber development in tissue culture (photo: S. Gupta).

There is also interest in finding alternative methods for propagation. These include transplanting locally grown tissue culture plantlets or cuttings thereof, planting microtubers or minitubers, and planting seeds (commonly referred to as "true potato seeds" or TPS to distinguish them from seed potato tubers). TPS can be seeded directly in the field, sown in beds for transplanting to the field, or sown in beds to produce tubers that can be planted in the field. Except for some of the diploid populations a disadvantage of TPS is lack of uniformity.

The quantity of tuber seed planted per ha will have a considerable effect on average tuber size. Cultivars that develop large tubers should therefore be spaced more closely. Cut seed tends to give somewhat fewer sprouted eyes per ha than whole seed. Therefore, cut seed should be spaced closer, increasing the quantity of seed per hectare by perhaps 10%. Although the quantity of seed planted per ha has a considerable effect on yield and tuber size, within reasonable limits the planting arrangement does not seem to have much influence (Sieczka *et al.*, 1986).

Sprouting and Shoot Growth

After harvest, tubers go through a dormant stage (or rest period), during which they will not sprout even if placed at warm temperatures. The length of dormancy depends on cultivar and environmental and physiological factors (Sergeeva *et al.*, 2012). Tubers in dormancy have very low levels of physiological activity (Suttle, 2004). Dormancy is characterized as a stage of temporary suspension of visible growth, but tubers are metabolically active as evident by the biochemical changes

that occur over time. In dormant tubers, cell division in the buds is absent and most cells in the meristem are arrested (Campbell *et al.*, 1996). Internal factors that accompany and possibly regulate dormancy progression and early sprout growth are discussed in detail by Suttle (2007).

The transition from a resting phase (dormancy) to an active phase is characterized as sprouting initiation, which is a metabolically high active state as reflected in tuber protein profile changes (Delaplace *et al.*, 2009; Sergeeva *et al.*, 2012). Temperature is one of the major environmental factors that most affects dormancy length. Low storage temperature is used to prolong dormancy worldwide (Eshel and Teper-Bamnolker, 2012). The combination of cultivar type, growing season and storage temperature greatly impacts the length of dormancy.

From dormancy to sprouting there is a shift in tuber physiology from starch synthesis to reserve mobilization. Physiological and biochemical changes like phloem unloading to phloem loading result in an increase in the starch and sucrose pools and accumulation of organic acids in growing buds etc., in the tuber (Viola *et al.*, 2007). Potato tuber sprouting is regulated by the signaling molecule sucrose, which activates trehalose-6-phosphate and the SnRK1 signaling network (Sonnewald and Sonnewald, 2014). Whether in storage or in the field, sprouting leads to higher rates of respiration as well as remobilization of storage compounds in the potato tubers, mainly starch and proteins (Suttle, 2004).

Sustained starch mobilization in the storage parenchyma appears to be induced at an advanced sprouting stage upon depletion of soluble carbohydrate reserves. Starch mobilization is accompanied by soluble sugar accumulation in the sprouts. Degradation of starch corresponds with enhanced activity of several enzymes such as α-amylase, β-amylase, starch phosphorylase (STP), maltase, and debranching enzymes. Starch cycling occurs during all stages of potato tuber development. Transcription and proteomics analysis by Li *et al.* (2017) revealed approximately 4000 differentially expressed transcripts and 700 differentially expressed proteins between dormancy and sprouting.

Dormancy and sprouting comprise a complex set of physiological processes that are regulated by endogenous hormones, such as abscisic acid (ABA). The impact of endogenous hormone levels from dormancy break to sprouting has been extensively reviewed (Suttle, 2004, 2007). Endogenous hormone levels provide strong evidence for a role of ABA in maintaining dormancy. Gibberellins and cytokinins are likely involved in bud dormancy release. An increase in cytokinin level leads to dormancy break and sprouting initiation (Lomin *et al.*, 2018). Exogenous application of gibberellin 3 (GA_3) is known to prematurely terminate tuber dormancy. The auxin, indoleacetic acid (IAA) appears to induce sprouting, as its concentration increases in tuber buds during dormancy release. Candidate genes associated with major phytohormones involved in tuber dormancy and sprouting have been identified (Bisognin *et al.*, 2018). Quantitative trait loci (QTLs) for dormancy and sprouting were mapped on chromosomes 2, 3, 5 and 7. Pathway analysis of the transcriptome indicated that phytohormones mainly gibberellic acid, brassinosteroids, ethylene synthesis, and signal transduction play important roles in tuber sprouting through various physiological processes such as cutin, suberine and wax biosynthesis as well as starch and sucrose metabolism (Li *et al.*, 2017).

Following dormancy, the number of sprouts that develop into stems and shoots depends on tuber size and physiological age of the seed tuber. Larger tubers generally have more eyes and therefore a higher potential to form more stems. The physiological age of a tuber is its stage of development starting at tuber initiation, as modified progressively by increasing chronological age, and is affected by environmental and genetic factors. Seed grown under conditions of stress such as high temperature tend to be physiologically older.

If transferred to warm temperatures by the end of dormancy, sprouting will begin at the apical end of the tuber and there will be a few sprouts per eye (Krijthe, 1962). Such tubers are physiologically young and exhibit a high degree of apical dominance. If tubers are stored at cold temperatures, below 4°C, for a long time after dormancy is broken, the sprouts that develop when they are finally moved to warm temperatures (10–15°C) will be noticeably different. Apical dominance will be less pronounced, so that many eyes will have sprouts, there will be more multiple sprouts at the eyes, and the sprouts will be more highly branched (Krijthe, 1962). Such tubers are physiologically older (Delaplace *et al.*, 2009).

Usually one or more shoots emerge from each eye, which may contain one or more buds (Struik, 2007). Well before plant emergence, the developing sprout grows adventitious roots, which constitute the root system. Also developing from the underground portion of the stem are stolons (or rhizomes), which may bear new tubers at their tips (Fig. 9.4). Initially all resources come from the seed tuber but as the shoots mature, they become independent and compete for resources such as light, water, and nutrients.

Each stem consists of various stem segments as illustrated in Fig. 9.5. The main stem of the potato plant terminates in a flower cluster. The cessation of growth of the main shoot axis may not be obvious because sympodial growth of one or more axillary branches just below the apex permits further shoot extension above the flower cluster. After developing up to six or more leaves, the new axillary branch(es) will terminate in a flower cluster in the same manner; but new sympodial growth may again occur (Almekinders and Struik, 1994). In this manner, the main axis may be extended by three or more levels of branching. Other axillary branches

Fig. 9.4. Roots, stolons and tubers of a "Russet Burbank" plant, grown in the sandy soils of central Minnesota, USA. Stolons are formed at the underground nodes of the main stem, and tubers are formed at stolon tips (photo: S. Gupta).

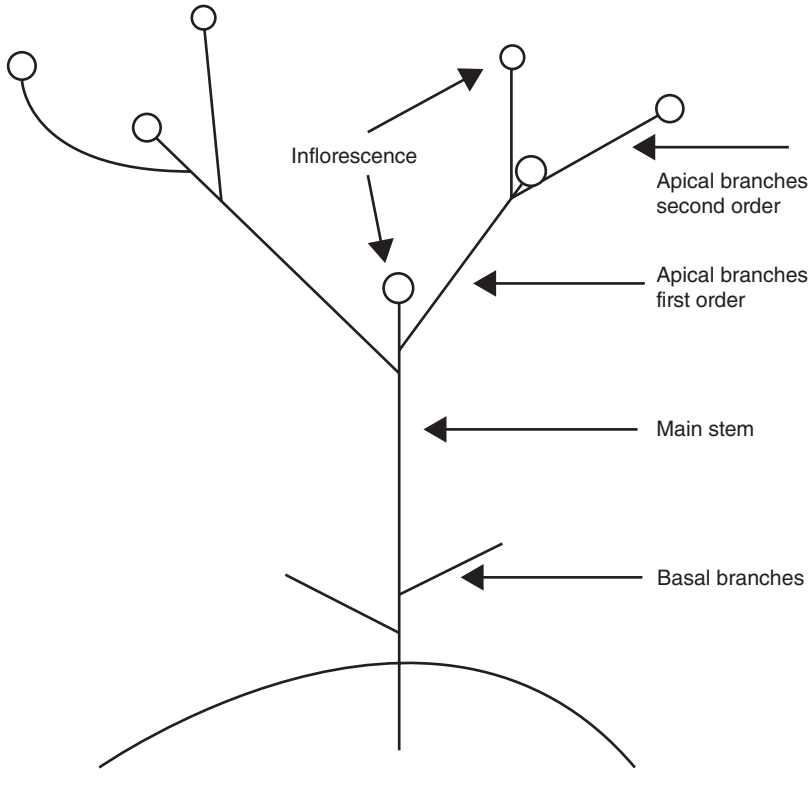


Fig. 9.5. Diagram of a potato shoot, showing positions on the main stem of apical and basal axillary branches. The main stem and all axillary branches eventually terminate in inflorescences, but new branches may form just below the inflorescence to continue the sympodial growth (Vos, 1995).

arise from nodes just above the soil level. Cessation of growth of the main axis associated with flower bud formation encourages basal axillary branching. The extent of axillary branching, both sympodial and basal, is of crucial importance in determining yield potential.

Two types of potato growth exist and depend on cultivar. One type is determinate and the other is indeterminate. Determinate cultivars tend to be smaller plants and have a shorter life cycle. Indeterminate cultivars are taller with numerous stem segments and a longer life cycle. They tend to have a higher yield potential than determinate cultivars, but require a longer growing season to mature.

Pattern of Stolon Formation

Stolons and then tubers form more readily in darkness than in light, although under certain conditions both can be made to develop in the light (Kumar and Wareing, 1972). Even aboveground axillary buds will form stolons and tubers if they receive the appropriate stimuli. Stimuli for stolons can be produced by the mother tuber in the absence of aerial parts, since stolon formation often begins before shoot emergence. Stolons also develop in the absence of a normal mother tuber, for example when true potato seed or microtubers are planted. Thus, it appears that stolon production can be affected by factors from both the mother tuber and leaves.

On potato plants grown from seed tubers, stolons develop first at the most basal node of the sprout and later at higher nodes (Cutter, 1992). In one study about half of the stolons formed at the most basal node, and roughly 10% of the remaining stolons formed at each of the next four higher nodes (Wurr, 1977). The main conditions that promote stolonization at basal nodes usually include an adequate moisture and darkness, but only in the presence of a dominant shoot apex (Brown, 2007). However, nutrients may also influence stolonization. According to Fontes (1997), the number and length of stolons are reduced when potato plants are cultivated at low-P levels. Gao *et al.* (2014) found that pot-cultivated potato plants supplied with N as nitrate (NO_3^--N) produced more stolons than those supplied with N as ammonium (NH_4^+-N).

Tuberization Process

The usual site of tuber formation is at the tip of an underground stolon, either the tip of the main stolon axis or the tip of a branch arising from an axillary bud (Fig. 9.4). Potato stolons are diagravitropic stems with long internodes and scale leaves. Stolons develop as branches from underground nodes and develop a hook at the tip (Peterson *et al.*, 1985). Plants that have reached the stage where they are capable of initiating tubers are said to be induced to tuberize. Induction is brought about by a "tuberization stimulus" that is produced in the leaves and is translocated to the site of tuber initiation (Gregory, 1956). The changes associated with tuber induction can be considered in the context of the competitive advantages they may have provided during evolution of the wild potato in the high Andes. As a storage organ, the tuber permits survival during periods of freezing temperatures. Dispersion of the wild plants takes place through seeds, but also through stolon growth, with longer stolons providing greater spread. Early in the growing season the best survival strategy is to have non-induced plants. These plants have the potential to produce more vigorous shoot growth to shade out competing species. Once vegetative growth is established, tuber induction begins.

Methods of studying tuberization

Because tubers form underground, and because exposure to light inhibits their formation, nondestructive observations of the process are difficult. One approach has been to devise special techniques that involve separation of the root zone from the stolon and tuber zone. A second approach is to take cuttings from stems, which under the right conditions are capable of tuberizing (Ewing, 1985). A third approach to the study of tuberization is to grow plants in vitro. While this technique is applicable for understanding tuberization in special cases and generating clean seed tubers for certification, extrapolation of results to full-sized plants should be done with caution. This is because in vitro plants are ordinarily grown on a sucrose medium, which has been shown to be involved in tuberization (Xu *et al.*, 1998).

In addition, shoots and roots in tissue culture normally are simultaneously exposed to either light or darkness, which may also affect tuberization responses. Recent studies on tuberization combine in vitro and soil tuberization analysis to elucidate tuber induction regulation (Teo *et al.*, 2017).

Tuberization stimulus and biochemical changes

After many years of research, biochemical aspects of tuberization have been elucidated, but the precise mechanism of the process is not completely understood (Hannapel *et al.*, 2017). In general, tuber induction is affected by various environmental cues such as photoperiod, temperature, and nutrition, which in turn control the synthesis and amounts of endogenous growth regulators (Mihovilovich *et al.*, 2014). Phytohormones as well as other metabolites also play a dominant role in tuber induction. However, as discussed by Hannapel *et al.* (2017) these are products of processes occurring downstream and are the result of an upstream switch.

As indicated above, the tuberization process begins with a signal originating in the leaves and moving down to stolon tips. Hannapel *et al.* (2017) identified three major mobile signals namely StCDF1, StBEL5 and StSP6A proteins originating in leaf. As the stolon tips begin to develop into tubers, endogenous activity of gibberellin (GA)-like compounds in the stolon tips decreases (Koda and Okazawa, 1983a). Another change associated with the earliest stages of tuberization is an increase in the concentration of "patatin," a glycoprotein that is one of the major storage proteins present in potato tubers (Paiva *et al.*, 1983). The tuberization stimulus is not necessarily a single compound; more likely, it consists of a particular balance between two or more compounds.

The tuberization stimulus has been shown to move across a graft union, proceeding from the leaves of induced scions to the underground nodes of non-induced stocks, where tubers were produced. Reciprocal grafts did not tuberize (Kumar and Wareing, 1973). Interstem grafts indicated that the stimulus is transported acropetally as well as basipetally (Kumar and Wareing, 1973). Other grafting experiments showed that characteristics of the leaf rather than the buried bud were responsible for the ability of clones that have long critical photoperiods to tuberize under long days (Ewing and Wareing, 1978).

Interspecies grafts have also provided interesting information. Transport of the tuberization stimulus was possible through a leafless segment of tomato or eggplant, but the leaves of these species diminished tuberization whether or not such leaves were exposed to short photoperiods (Okazawa and Chapman, 1962). "Mammoth" tobacco scions, which require short photoperiods for flowering, were grafted to leafless potato stocks from plants with a short critical photoperiod for tuberization (Martin *et al.*, 1982). Tuberization occurred on the potato stocks only if the scion was exposed to short days. When *Nicotiana sylvestris*, which requires long days for flowering, was substituted for the Mammoth tobacco in the scion, tubers formed on the stock only if the scion was exposed to long days (Martin *et al.*, 1982). The implication of these experiments is that the stimulus for flowering in tobacco was graft-transmissible to the potato, where it induced tuberization, and that this was true even if the stimulus was produced by a tobacco species that requires long photoperiods rather than short ones for flowering.

In temperate regions tuberization tends to coincide with flower production, but the relationship is not causal. Neither is there an absolute minimum age or size requirement for tuberization. For example, planting true seeds of very early maturing genotypes under short, cool days often leads to tuberization when only one or two leaves have developed beyond the cotyledons. However, other factors being equal, the larger the plant the more likely it is to tuberize. Under inducing conditions both young and old leaves are capable of producing the stimulus; so the greater the leaf area, the more stimulus is available for transport underground (Kahn *et al.*, 1983). If the plant was grown from a seed tuber, then factors from the tuber interact with factors from the leaves in determining the overall level of induction.

Assimilate level

An early hypothesis to explain induction to tuberize was that the level of non-structural carbohydrate in the leaf was the controlling factor. Short

photoperiods and cool temperatures would slow leaf growth, causing the accumulation of assimilate and a high C:N ratio, which in turn would bring about tuberization. Tuberization in modern cultivars is generally not sensitive to photoperiod and therefore factors other than daylength are involved.

With the discovery of plant hormones and evidence concerning their involvement in tuberization, the assimilate hypothesis was largely abandoned. However, it is still possible that high assimilate level is a contributing factor in induction, along with hormonal effects (Xu *et al.*, 1998). Increasing the sucrose concentration to at least 175 mmol l^{-1} greatly increases the frequency and size of tubers, and this is not simply an osmotic effect (Perl *et al.*, 1991). Another reason to suspect that high assimilate level is involved in induction to tuberize is that several genes which seem to be intimately associated with tuberization are "turned on" by high sucrose concentrations.

Hormones

Several plant hormones have been implicated in tuber initiation. Tuberization hormonal regulation during growth and development has been extensively reviewed (Navarre and Pavek, 2014). Here we present key hormonal changes impacting the tuberization process.

Gibberellins

High levels of GA_1 were recorded at initial stages of tuber initiation, which then declined sharply before any swelling of the stolen tip (Xu *et al.*, 1998). There is convincing evidence that GAs interfere with tuberization. Application of exogenous GA reduces tuberization, whether on whole plants, cuttings, in vitro plantlets, or excised sprouts cultured in vitro (Koda and Okazawa, 1983b). Long days, high temperatures, low irradiance, and high N fertilization all lower tuber induction; and all are associated with higher levels of GA activity (Menzel, 1983).

Cytokinins

Cytokinins are considered to be a primary tuber-inducing factor. Cytokinins were reported to accelerate and scale up tuber formation (Lomin *et al.*, 2018). The main biological effect of cytokinins is the induction of cell division (Romanov *et al.*, 2018). The addition of cytokinin frequently promotes tuberization in vitro (Palmer and Smith, 1970), and transfer of plants to cooler temperatures and shorter photoperiods has been associated with a temporary increase in cytokinin content of leaves (Langille and Forsline, 1974). It is often suggested that a high ratio of cytokinin to GA initiates the tuberization process (Melis and van Staden, 1984). Cytokinins and auxins are known to enhance tuber formation. A slight increase in the level of cytokinins can promote tuberization, while high levels of cytokinins can actually inhibit tuberization (Wang *et al.*, 2018).

Auxin

Although IAA has been studied less than the other known hormones with respect to its role in tuberization (Melis and van Staden, 1984), IAA is considered a key hormone that regulates potato tuberization (Wang *et al.*, 2018). The production and directional transport of auxin exists in stolons and acts synergistically with strigolactones, a new growth regulator, to control the outgrowth of the axillary stolon buds (Mason, 2013). IAA moves in the stolon tissue from the stolon tip to the basal part of stolon. Stolon tips are probably the site of IAA biosynthesis. Elevated levels of IAA have been reported in the stolon apex and stolon region during early tuber development stage. There is evidence from analysis of plantlets grown in vitro that IAA may interact with other hormones in regulating tuberization (Sergeeva *et al.*, 1994).

Abscisic acid

Treatment with chemicals that block the synthesis of endogenous GAs promoted tuberization in whole plants (Menzel, 1980), cuttings (Langille and Hepler, 1992), in vitro plantlets (Dodds, 1990), and sprouts cultured in vitro (Tizio, 1969). This led to the hypothesis that for tuberization to take place, a naturally occurring inhibitor or antagonist of GA synthesis is required. Abscisic acid (ABA) is a likely candidate, especially in view of the fact that there appears to be a close correspondence between the locations of several genes for the ability to tuberize under long days

and genes for ABA levels (Šimko *et al.*, 1996). ABA stimulates the tuberization process by counteracting GA, and sucrose regulates tuber formation by influencing GA levels (Xu *et al.*, 1998). ABA is normally regarded as a regulator that reduces GA-promoted processes in plant development.

Ethylene

Ethylene does not appear to play a direct role in potato tuberization (Suttle, 2007). The application of ethylene producing compounds to extremely old seed tubers caused a restoration of more normal sprout growth instead of sprout-tubers forming directly at the eye and GA activity was higher in the elongated sprouts than in the sprout-tubers. Apparently, ethylene stimulated GA levels, which had their usual effect of inhibiting tuberization. A different mode of action proposed for ethylene is that ethylene produced by friction between soil particles and the growing stolon tip might stop extension growth of the stolon, thereby facilitating tuberization (Vreugdenhil and Struik, 1989; Vreugdenhil and Van Dijk, 1989) .

Other hormones

Jasmonic acid is another hormone that tends to accelerate tuberization. Jasmonic acid and certain other closely related compounds enhance tuberization in vitro (Koda *et al.*, 1991; Cezano *et al.*, 2003). Jasmonic acid (JA) and its glucoside known as tuberonic acid glucoside (TAG) have been reported as a potato tuber forming stimulus (Yoshihara *et al.*, 1996). TAG is a metabolite of JA originating in leaves and transported to all parts of the plant. Accumulation of TAG in flower buds and stolons may induce flower and tuber formation.

The role of auxins in the tuberization process is well documented. A new class of hormones known as strigolactones (SLs) has recently been identified as a secondary signal that acts synergistically with auxins to regulate shoot branching (Mason, 2013). SLs have been detected in root exudates and proposed to act either directly on axillary bud outgrowth or indirectly by inhibiting auxin transport (Roumeliotis *et al.*, 2012). A system for the production and directional transport of auxin in stolons has been reported to act synergistically with SLs for control of axillary stolon bud outgrowth. An exogenously applied synthetic SL to stolons was found to reduce the number of tubers formed. Overall, these results suggest that auxins and SLs are antagonistic, with auxins promoting tuber formation and SLs repressing it (Abelenda *et al.*, 2011). However, further research on the role SLs in tuber formation and interaction with other hormones is warranted (Pasare, *et al.*, 2013; Sonnewald and Sonnewald, 2014).

Application of plant growth regulators

Application of various growth regulators such as auxins, chlormequat (CCC) and paclobutrazol to whole plants in season often produce increases in tuber set. Because the average tuber size is less, there is not usually a yield benefit but there could be a quality benefit (Herman *et al.*, 2016). Depending on market class there could be an economic benefit. However, there tends to be a good deal of variability from experiment to experiment in the degree to which tuber set is increased and the effect is also cultivar dependent. Here it should be borne in mind that a slight phytotoxicity to the leaves is often accompanied by a heavier set of smaller tubers, whether herbicides or growth regulators are applied (Ibrahim, 2018). A combination of GA and NAA seed treatment was used to alter the length-width ratio of processing potatoes (Dean *et al.*, 2018). Exogenous application of cytokinins had no effect on stolon elongation or tuber formation (Xu *et al.*, 1998).

Growth retardants like CCC and paclobutrazol that block GA biosynthesis can improve tuberization under environments unfavorable to tuber induction, especially for obtaining tuberization on in vitro plantlets (Hussy and Stacey, 1984). In addition to CCC and paclobutrazol, other examples of GA inhibitors include ancymidol and tetcyclasis (Vreugdenhil and Sergeeva, 1999). Many reports indicate that cytokinins and related compounds also improve in vitro tuberization (Vreugdenhil and Sergeeva, 1999). Growth regulators such as 2,4-D have been used to improve quality by enhancing the skin color of red potato tubers (Rosen *et al.*, 2009; Buhrig *et al.*, 2015).

Anatomical and morphological changes

Anatomical changes in the stolon during tuber initiation have been thoroughly reviewed by Cutter (1992) with more recent contributions by Xu *et al*. (1998) and Viola (2001). It is well known that increases in cell division, cell enlargement and starch deposition all occur before visible swelling of the stolon tip (Plaisted, 1957). It appears that the increase in cell enlargement precedes cell division (Cutter, 1992).

Tuber induction leads to tuber initiation, defined to have occurred if the swollen portion of the stolon tip is at least twice the diameter of the stolon (Ewing and Struik, 1992). Initiation typically occurs 20–30 days after emergence depending on cultivar and growing conditions. This phase will usually last about 10–14 days; although tubers may continue to form in some varieties throughout the growing season (Meredith, 1988). The tiny swellings are called "tuber initials." Many tuber initials fail to develop into tubers, but are resorbed; and their contents are reallocated to other parts of the plant. Such resorption may occur even to tubers that have attained diameters greater than 2 cm. Cho and Iritani (1983) reported that in some treatments nearly half of the tubers initiated were resorbed, but resorption was limited to tubers that weighed less than 10 g.

Tubers that are not resorbed are said to have been "set." The number of tubers initiated can vary much more than the number of tubers set (Struik *et al*., 1988). The mechanism by which this phenomenon operates is unknown, as is what determines which tubers will develop, which will remain as small tubers, and which will be resorbed. Even actively growing tubers can lose carbon compounds to other parts of the plant. In the case of resorption, so much is lost that the tuber shrivels and disappears. It seems probable that the non-structural dry matter from the resorbed tuber is redistributed to other tubers, although this has not been proven. Even after tuber set, the fate of the tubers is not predictable. Some remain small, while others on the same plant continue to grow. Continued tuber development and maturation generally are favored by environmental factors that promote tuber induction. Conditions such as high soil temperature can cause even large tubers to revert to stolon growth. Drought is an important factor in determining the proportion of tuber initials that set, and thus the number that will be resorbed (Krug and Wiese, 1972). This helps explain why irrigation often increases tuber number.

The reversion of tuber growth to stolon growth exemplifies the plasticity displayed by the potato plant. Struik (2007) presented two situations of how tuber number can change through the growing season. In the first situation, tuber initiation is excessive, and many are resorbed. In the second, initiation is less with little or no resorption. In general, the higher the tuber set, the smaller the average tuber size. In Fig. 9.6, "Russet Burbank" showed a tendency to resorb tubers through the season while tuber number in two other cultivars "Dakota Russet" and "Easton" stayed relatively constant.

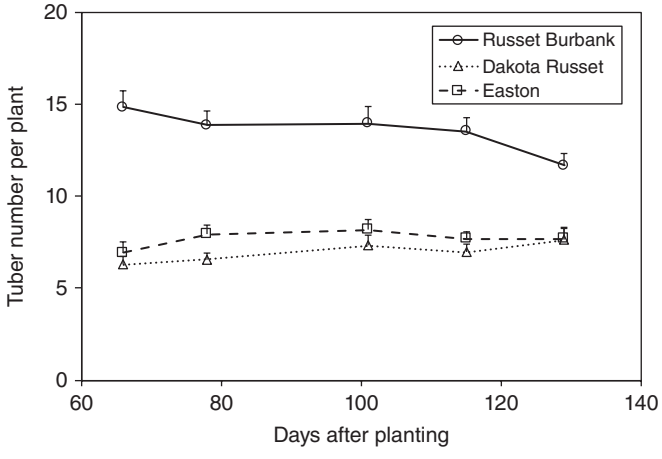


Fig. 9.6. Tubers per plant during the growing season in three cultivars, "Russet Burbank," "Easton" and "Dakota Russet." Tubers were resorbed in "Russet Burbank" and remained relatively constant in the other two cultivars (adapted from supplemental Fig. S6A in Sun *et al*. 2019).

There are also examples of tubers forming in various parts of the plant other than from stolons. Girdling of the stem at the soil level or other interference with translocation from leaves to stolons can cause tubers to form at aboveground buds. Interference with translocation from the leaves to the underground parts of the plant, as when stems are girdled by *Rhizoctonia* disease, may prevent tuberization of underground parts. In this event, axillary buds on aerial portions of the plant may tuberize, in spite of the inhibiting effects of light. Even flower buds have been shown to tuberize (Fig. 9.7); and new tubers can form directly from the buds of the mother tuber. Tuberization under varied conditions clearly shows the resiliency of potato as well as the ability to survive and reproduce under adverse conditions.

Physiological age of the mother tuber

Physiological age of seed tubers is an important consideration for assessing seed quality. Dominance of the growing apical bud over other lateral buds is one of the visible indicators of the tuber's physiological age (Eshel and Teper-Bamnolker, 2012). Apical dominance generally decreases as seed age increases. The physiological age of the seed tuber is a complex trait that affects not only seed quality, but also future crop performance, crop vigour and ultimately crop production (Struik, 2008). Physiological age is the result of several interacting factors such as genotype, sugar status of the tuber at the time of harvest, and the environmental conditions at harvest and during storage. This difference in physiological age is the result of management practices during crop growth and development and post-harvest storage conditions. Storage conditions that increase respiration rate can accelerate physiological aging. Physiologically older seed tubers are reported to have a higher rate of respiration. Warmer storage is associated with more rapid aging. Warm temperatures during the growing season of the crop used for seed may also cause a small increase in physiological age (Eshel and Teper-Bamnolker, 2012). However, some cultivars have been reported resistant to thermal aging. Exposure of seed tubers to diffuse light during sprouting ("green-sprouting") causes a drastic shortening of sprout growth (Scholte, 1989).

Fig. 9.7. Tubers formed from flower buds in "Russet Burbank" shortly before vine desiccation (photo: E. Souza).

A general view is that the greater the physiological age of the seed tuber, the greater is the potential contribution for tuber induction, and therefore an increase in tuber set. However, these differences are complicated by the fact that there are usually more stems due to a decrease in apical dominance, a smaller plant size, earlier tuberization, and earlier senescence when physiologically older tubers are compared to younger ones. The physiological age of potato seed tuber and sprouting initiation has recently been described in a more detailed review article by Wohleb *et al.* (2014).

Growth Rates of Individual Tubers – Tuber Bulking

A typical plant dug up in early to mid-season from a commercial potato field has a number of stolons, only some of which bear tubers (Fig. 9.4). Usually many more tubers are initiated than develop to a marketable size. Some are resorbed, some remain small until plant maturity, and others grow to variable sizes; but the first to be formed do not necessarily attain the largest size (Struik *et al.*, 1991).

The rate of initial growth may be important; tubers with restrictions in their mitotic processes during early development grew more slowly during later stages (Reeve *et al.*, 1973). One can only speculate as to which factors eventually become dominant in controlling the observed differences in growth rates among tubers as they enlarge further (see Struik *et al.*, 1991). Without this knowledge, it is difficult to manipulate tuber

size distribution or even to predict how a given set of environmental factors will affect it under field conditions.

Biomass production and partitioning to developing tubers (tuber bulking)

High biomass production depends upon having a leaf canopy that over a long part of the available growing season intercepts a high percentage of the incident radiance (Van Der Zaag, 1984). Under good growing conditions tuber dry matter continues in a nearly linear manner as long as the canopy is essentially closed (Fig 9.8). Not only is it important to achieve rapid closure of the canopy so that there is good interception of radiation early in the season, but maximum yields also require that the canopy continues to cover the soil late into the season. The presence of canopy late in the season depends upon leaves on axillary branches (both sympodial and basal); but axillary branching can be inhibited by strong induction to tuberize, an added reason why very strong induction can reduce yields.

The effects of early senescence in shortening the period of canopy closure are exacerbated by several pests. Senescent plants are more susceptible to leafhoppers aphids, early blight and *Verticillium* wilt; so if these pests are present, it is all the more important to avoid excessive induction. Otherwise the season may be severely shortened.

The effects of induction can be illustrated by the influence of cultivar (Fig. 9.8). Early maturing cultivars (e.g. "Red Norland") are typically able to tuberize even under the long, hot days of early summer. As tubers form on these cultivars, the growth of the rest of the plant is restricted. Sympodial growth stops soon after flower formation, and axillary branching at the base of the stem tends to shut down as well. Hence, although tuber yields are relatively good at early harvests, they may not measure up well at later harvests because the full growing season is not well utilized for biomass production. By contrast, late maturing cultivars (e.g. "Russet Burbank") do not tuberize as strongly until days are shorter or temperatures are cooler. Thus, late maturing cultivars have a longer season during which tops continue to grow. There are more sympodial branches and axillary branches at the base of the stem. The canopy intercepts essentially all of the incident radiation over a greater portion of the season, and larger reserves of photosynthate are manufactured for translocation to the tubers. This explains why late maturing cultivars have the potential for higher yields over a long season.

Genetic differences have the greatest effects on degree of induction, and hence on plant maturity; but cultural practices can modify the effects of cultivar. For example, planting date affects photoperiod and temperature, both of which affect induction to tuberize. The application of nitrogen fertilizer also has an effect discussed in more detail below, as does the physiological age of seed tubers. Seed tubers which are so young they are still dormant should never be planted, nor should they be so old that they will produce

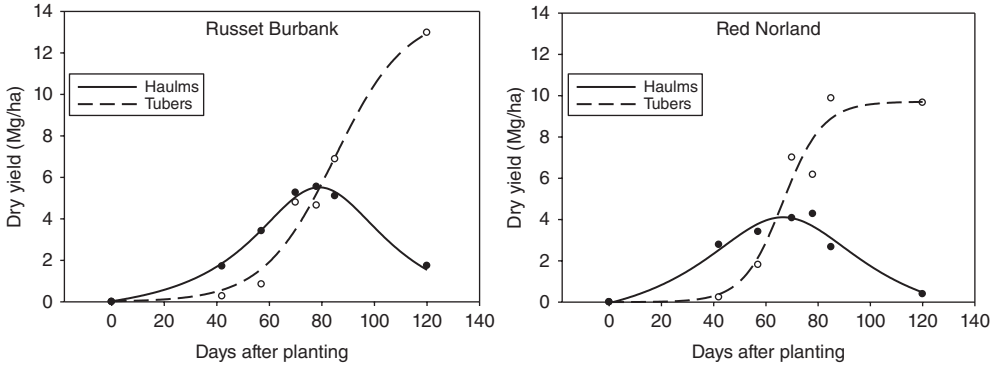


Fig. 9.8. Dry matter accumulation in tubers and haulms of cultivars "Russet Burbank" (left) and "Red Norland" (right), both grown in central Minnesota. The early cultivar, "Red Norland," started tuber bulking slightly earlier in the season and leveled off later in the season. "Russet Burbank" continued to bulk until vine desiccation (Rosen, unpublished data).

sprout tubers rather than normal sprouts. There is a range of options between these extremes. Better early yields will result from physiologically older seed, whereas younger seed potentially will produce higher yields if the growing season is long enough (Navarre and Pavek, 2014).

Growers who aim for early yields will sacrifice potential yield by promoting strong induction to tuberize early in the season. They will select an early maturing cultivar with a long critical photoperiod, choose physiologically old seed tubers, and use moderate to low rates of N fertilizer. Growers aiming for highest potential yields will select later maturing cultivars with shorter critical photoperiods, physiologically younger seed tubers, and high rates of N. In areas with very long growing seasons, such as the Columbia River basin, N fertilizer is applied later in the season through the irrigation water. This serves to limit induction, delay senescence, and prolong the period of maximum light interception.

In the tropics the speed of crop emergence and the size and persistence of the canopy will influence soil temperature through shading (Midmore and Mendonza, 1984), which in turn may have a major impact on earliness of tuberization and yield (Midmore, 1984). More shading and consequent cooling can also be obtained through closer spacing (Vander Zaag *et al.*, 1990), intercropping (Midmore, 1990), mulching (Midmore *et al.*, 1988), and irrigation. Irrigation affects cooling directly, but also indirectly through increased canopy development and consequent shading (van Loon, 1986). Soil cooling is especially important in tropical potato production, but may be beneficial under temperate conditions as well (Herman *et al.*, 2017).

Dry Matter Content of Tubers

Well over half of the potatoes consumed in North America are processed into frozen French fries, chips, dehydrated mashed potatoes, and other products. For most forms of potato processing, the higher the dry matter content of the raw product, the higher the yield of finished product. The percentage of dry matter in potato tubers commonly ranges from about 16 to 23%, depending upon cultivar and environment. The most widely used procedure to assess the dry matter content in potato tubers is by measuring the specific gravity (SG) of the tubers. Processors typically set a minimum dry matter content below which they will refuse to purchase the potatoes. SG is the reflection of dry matter content in the tubers (Wilson and Lindsay, 1969). Too low an SG results in high oil absorption and less product. Too high a SG results in brittle finished product and is not preferred by consumers. The ideal SG range for processing is 1.082 to 1.088. Dry matter content is important even for the fresh market. Potatoes low in dry matter (waxy potatoes) will have a watery texture; those high in dry matter will be more dry and mealy. Consumers vary widely in terms of which texture is preferred; but high dry matter tubers usually command a higher price, especially for baking and processing. A more recent consumer fresh market trend is for smaller potatoes with various colored flesh and skins. To meet this demand, selection of management practices and cultivars that result in high tuber set are required.

Potato cultivars vary widely in dry matter content. For example, cv. "Norland" tubers, which are mainly used for the fresh market, have a dry matter content of 16%, while cv. "Atlantic" tubers grown at the same location might be expected to have at least 22%. Environmental influence is also of major importance. High dry matter content is associated with high levels of irradiance and cool night temperatures (Zhou *et al.*, 2017b Zhou X or Zhou Z). Fertilization and other cultural practices can also have some effects.

Environmental Factors Affecting Tuber Bulking and Yield

Based upon best yields obtained in small experimental plots under ideal conditions, fresh weight yields of potato tubers can reach up to 100 t ha^{-1}. Of course, commercial yields fall far short of this, although recent yields in the state of Washington have been averaging about 86 t ha^{-1} (Mertz *et al.*, 2016). This compares to about 44 t ha^{-1} in The Netherlands and 10–15 t ha^{-1} in many developing countries.

Light

Commercial tetraploid potatoes are mostly photo insensitive and tuberization will become induced

even under very long photoperiods, especially if the other environmental factors (temperature, irradiance, and nitrogen level) are favorable for induction. This is particularly true of early maturing cultivars, which have even longer critical photoperiods than late maturing ones. Potato cultivars developed through breeding efforts are less sensitive to daylength and tend to be day neutral in their response to light (Teo *et al.*, 2017). In contrast, most wild *Solanum* species have a short critical photoperiod for tuberization; they will become induced only if the photoperiod is less than about 12 h. Short days/long nights favor induction to tuberize (Gregory, 1956) whereas long days tend to delay tuberization. The same is true of most accessions of *S. tuberosum* subsp. *andigena*, which are adapted to the short days and cool temperatures of their Andean home.

Irradiance level

Low levels of irradiance reduce induction of tuberization (Bodlaender, 1963), as does increasing the rate of nitrogen fertilization (Krauß and Marschner, 1976). While the effect of daylength on tuberization has been selected out of commercial potato cultivars, potato biomass production has been shown to be a function of photosynthetically active radiation (PAR) intercepted by the leaves as well as temperature. Jamieson *et al.* (2004) developed a simple model to describe potato tuber yield as a function of incident solar radiation received, the fraction intercepted by the canopy, the radiation use efficiency and the harvest index. Lowering irradiance decreases the partitioning of assimilate to the tubers (Menzel, 1985). This was true even when photosynthesis was not limiting. Tuber sink strength was found to be inversely associated with stolon length, which is genetically controlled (Kratzke and Palta, 1992). While intercepted radiation is still considered a determinant of yield potential, this parameter apparently does not adequately explain yield differences due to cultivar. In a more recent study, Oliveira *et al.* (2016) reported that radiation use efficiency (calculated from the slope of total dry biomass, haulms plus tubers, as a function of cumulative intercepted PAR) was more important than total accumulated intercepted radiation in explaining yield differences in three indeterminate cultivars. They also found that differences in tuber sink strength (ability of tubers to accumulate carbon compounds) were important in explaining yield differences under non-stressed (water, temperature, pathogens, nutrients) conditions.

The effects of lowering irradiance described up to this point resemble the effects of long photoperiods and high temperatures, but the effects on flowering are different. The abortion of flower buds is decreased by long days and high temperatures, whereas it is increased by shading the plants (Turner and Ewing, 1988). Apparently the reduction in available photosynthate from shading is overriding in its effects on retention of flower buds.

Temperature

Another important environmental factor is temperature; cool temperatures promote induction to tuberize (Gregory, 1956). It is commonly believed that night temperatures have more influence than day temperatures, but interpretation of experimental evidence is complicated by the fact that diurnal variation in temperature favors tuberization (Steward *et al.*, 1981). Both air and soil temperatures are important: cool air temperatures favor induction of leaves to tuberize, as reflected in cuttings (Gregory, 1956; Reynolds and Ewing, 1989a); and high soil temperatures block the expression of the tuberization stimulus at underground nodes (Reynolds and Ewing, 1989a). There is an interaction between photoperiod and temperature, such that the higher the temperature, the shorter the photoperiod required for tuberization of a given genotype (Snyder and Ewing, 1989). In general, short days and cool nights induce the plant to set tubers, while long days and warm nights inhibit or delay the tuberization process; however, exceptions do exist.

The relationship between intercepted radiation and dry matter is also influenced by temperature (Manrique *et al.*, 1991). Yuan and Bland (2005), for example, reported that potato yield was sensitive to temperature early in crop development, while light interception was important during the tuber bulking stage. Warmer temperatures have been shown to increase tuber number per stem and stolon stem length (Struik, 2007). Diurnal temperature fluctuations relative to a constant 20°C temperature increased tuber

yield in an indeterminate cultivar "Denali" and but had no effect on the determinate cultivar "Norland"(Bennett *et al.*, 1991). Relative to cool temperatures during the entire growth period, tuber yield increased when plants were exposed to warmer temperatures during the first weeks of growth followed by cool temperatures (Bennett *et al.*, 1991). Intuitively, this makes sense because cool temperatures early in the cropping season delay crop growth and canopy development. Potatoes are sensitive to frost, so low temperatures later in the growing season will also reduce the potential for biomass production. Yields of potatoes are particularly limited when grown in climates with shorter growing seasons. The same indeterminate cultivar grown in an ideal climate with a long growing season (140–160 days) such as the Pacific Northwest of the United States can have double the yield potential compared with a shorter growing season (110–140 days) such as the Upper Midwest and Eastern United States.

Whether senescence is accelerated or delayed by increasing the temperature depends upon the photoperiod and other conditions. If days are long, high temperatures may shift the partitioning away from tubers toward shoot growth to the point where plant senescence is delayed (Ben Khedher and Ewing, 1985). Conversely, if photoperiods are short enough to permit reasonable tuberization even at the high temperatures, then the more rapid growth and development at high temperatures may shorten the growing season (Vander Zaag *et al.*, 1990).

Some cultivars are more sensitive to high temperatures than are others (Ben Khedher and Ewing, 1985; Reynolds and Ewing, 1989b). Nevertheless, it seems safe to say that for all genotypes, high temperatures, and long photoperiods, decrease the partitioning of assimilate to tubers and increase partitioning to other parts of the plant.

Mineral nutrition

Potato is grown as an annual crop, which results in a plant that is highly dependent on readily available nutrients in the root zone. Commercial production in developed countries generally requires high inputs to optimize yields, especially nutrients. Under these conditions, potato plants may have a relatively low nutrient use efficiency, which can negatively impact the environment due to fertilizer loss, especially nitrogen (Davenport *et al.*, 2005; Bucher and Kossmann, 2007). For these reasons, a better understanding of potato plant nutrition and the influence of nutrients on potato plant growth and physiology will also be discussed in this chapter. While lack of any essential nutrient can affect plant growth and development, the focus of this section is on nitrogen (N), phosphorus (P), potassium (K), sulfur (S), and calcium (Ca), which can have a strong influence on potato yield and quality.

Nitrogen

Of all the essential elements, nitrogen is the most important in potato growth and production. Nitrogen is a constituent of amino acids, proteins, chlorophyll, nucleotides and nucleic ac ids as well as numerous secondary compounds (Bucher and Kossmann, 2007). In general, for most plants, a combination of NH_4^+ and NO_3^- is preferred over application of a single source of either form (Marschner, 1995). Nitrate assimilation occurs through the reduction of nitrate to nitrite by the enzyme nitrate reductase (NR). Then, the nitrite is reduced to an NH_4^+ by nitrite reductase in the chloroplast (Meyer and Stitt, 2001). Ammonium absorbed or produced by nitrate assimilation, or arising from photorespiration, is incorporated to amino acids (glutamine and glutamate), by the action of two enzymes, glutamine synthetase and glutamate synthase (GS) (Taiz and Zeiger, 2014). According to Mack and Schjoerring, (2002), NO_3^- reduction via NR and NH_4^+ assimilation via GS occurs predominantly in the potato shoots, and tubers are N autotroph organs with the capability of de novo synthesis of amino acids. They also reported that NO_3^- supply stimulated potato plant growth but reduced tuber number. However, under field conditions, the effect of N on tubers per plant is often not significant or inconsistently affected (Bélanger *et al.*, 2002; Souza *et al.*, 2019; Sun *et al.*, 2019).

There are two forms of GS, a cytosolic form (GS1) and chloroplastic form (GS2) (Bucher and Kossmann, 2007). Teixeira *et al.* (2005) observed that the GS2 isoform was present in leaves only, while the GS1 form was detected in all organs of potato, which indicates that GS1 can function in non-photosynthetic tissue such as tubers. More specifically, these researchers found potato GS is

encoded by three genes, one gene encoding GS2 (Stgs2) and two encoding GS1 (Stgs1a and Stgs1b). They also observed a similar pattern of expression between GS2 gene Stgs2 and GS1 gene Stgs1a, where their content decreased as leaves senesced. However, they observed that the GS1 gene Stgs1b progressively accumulated with leaf age, suggesting that the particular GS1 gene (Stgs1b) is mainly responsible for the production of glutamine for export from the senescing leaf to another plant organ such as tubers.

The amount of amino acids in different plant parts is a measure of N assimilation and partitioning. Muttucumaru *et al.* (2014) found that asparagine was the predominant free amino acid in potato tubers, and concluded that only a small amount was translocated from the potato leaf to the tuber. In contrast, glutamine, glutamate, and serine are the major amino acids transported from leaf to tubers.

Nitrogen is also a component of chlorophyll (Taiz and Zeiger, 2014). Thus, either deprivation or excess of N can be detrimental to biomass production and partitioning due to the negative effect on radiation use efficiency (Bangemann *et al.*, 2014). There is a parallelism in the effects of N on tuber induction and the effects on overall morphology. Induction to tuberize tends to decline with an increase in soil N level. The N form supplied can also influence stolonization. Gao *et al.* (2014) found that pot-cultivated potato plants supplied with N as nitrate (NO_3^--N) produced more stolons than those supplied with N as ammonium (NH_4^+-N).

High applications of N are associated with increased partitioning of dry matter to shoots rather than to tubers. Haulm growth is more responsive to soil N availability than by competition for light (Oliveira, 2000). According to Duchene *et al.* (1997) increasing N supply over the optimum level can delay or inhibit tuber formation, since aboveground biomass continues to accumulate, and stems continue to grow at the expense of the tubers, leading to a decrease in the harvest index of potato crop, which is defined as the tuber dry weight expressed as a percentage of the total plant dry weight. However, when the increased canopy for light interception has a sufficiently long season, adequate temperature and levels of irradiance at the end of season, tuber production is usually much higher in plants supplied with high N than those that receive inadequate amount of N. Sun *et al.* (2017), reported increased total marketable yield up to 231 kg ha^{-1} in five potato cultivars in one year and up to 319 kg ha^{-1} in the second year (Fig. 9.9), suggesting that environmental factors such as temperature, length of growing season and amount of rainfall influence N response.

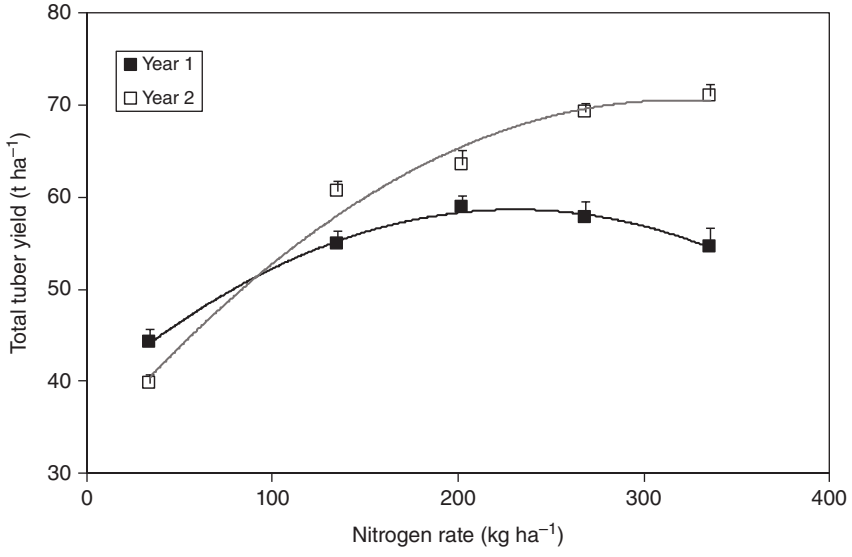


Fig. 9.9. Tuber yield as affected by N rate under a longer (open quadrats) and shorter (closed quadrats) growing season (adapted from Fig. 1, Sun *et al.*, 2017).

Phosphorus

Potato plants have a relatively high P requirement, as it is an important component of phospholipids, nucleic acids, coenzymes, and phosphoproteins (Bundy *et al.*, 2005). Thus, P influences plant metabolism by affecting cellular energy transfer, respiration, and photosynthesis (Marschner, 2012). In severely deficient soils, increased P availability increased tuber dry matter content, starch and protein and lowered sugar content (Leonel *et al.*, 2017). These compositional changes in turn affected the nutritional quality and potential uses for processing.

Orthophosphate is the preferred form for P uptake and assimilation. A large variability of orthophosphate acquisition and utilization efficiency has been observed between plant species and within cultivars of the same species indicating that P efficiency is under genetic control (Thornton, 2014). Czarnecki *et al.* (2013) reported that strigolactone levels significantly increased under phosphate-limiting conditions, and that increase was often associated with a rise in activity of arbuscular mycorrhizal fungi, leading to enhanced P uptake. The average removal of P with the potato crop is 25 to 35 kg P ha^{-1} (Davenport *et al.*, 2005). The majority of P (about 80%) is taken up during the tuber bulking phase (Fernandes *et al.*, 2011) and is an important nutrient involved in enhancing potato tuber yield and quality.

The influence of P on cell division strongly links plant growth with P supply. When cultivated under deprivation of P, the plant typically exhibits reduced tuber set, limited root, stem, and leaf growth, number and length of stolons, and reduced yield and quality of the tubers, as well as, late maturity compared with plants adequately supplied with P (Rosen *et al.*, 2014; Rosen and Bierman, 2008; Soratto and Fernandes, 2016).

In a greenhouse experiment, Fernandes *et al.*, (2014) reported about a 60% smaller root surface area and a greater capacity for P uptake in potato plants grown under P deficient conditions than those cultivated under P sufficient conditions. When potatoes are under P deficiency stress, the transcription factor gene (StMYB44) is overexpressed in potato tissues and organs and results in lower accumulation of phosphate in shoots (Zhou *et al.*, 2017a). They also found that the abundance of PHOSPHATE1 (StPHO1), a phosphate transport-related gene, was reduced in StMYB44 overexpression lines, which demonstrates that StMYB44 results in a reduction of phosphate transport in potato by suppressing StPHO1 expression.

Potassium, sulfur, and calcium

Potassium is responsible for regulating the opening and closing of the stomata which regulates the exchange of water vapor, oxygen, and carbon dioxide (Shabala, 2003). Previous research has shown that the guard cell-specific K^+ channels KST1 and SKT1 (Zimmermann *et al.*, 2001) mediate K^+ inward movement required for stomatal opening in potato. However, the expression of SKT1 is almost exclusively restricted to guard cells in the abaxial leaf epidermis, which is evidence of differential regulation of stomatal movements in the two leaf surfaces. The role of K for osmoregulation, not only controls the movement of water into the plant but also influences the redistribution of nutrients and carbohydrates within the plant (Westermann, 2005). Potassium is required by plants for sugar translocation and starch synthesis, and since potato tubers are rich in starch, the requirement for K is relatively high (Kumar *et al.*, 2007). The availability of K can also strongly affect tuber yields (Silva and Fontes, 2016). Kang *et al.*, (2014) observed that K uptake by potato continued to increase with increasing K, which demonstrated a luxury absorption of K. Based on numerous studies, it is clear that excessive K soil levels and/or excessive K applications can reduce tuber specific gravity. Furthermore, K sources with a higher salt index, such as potassium chloride can result in lower tuber solids than with lower salt index sources such as potassium sulfate.

Sulfur is an important component of amino acids and has roles in the activation of certain enzymes and chlorophyll synthesis as well as in the formation of many secondary compounds in plants (Marschner, 1995). Positive effects of S application on tuber yield have been reported (Pavlista, 2005; Barczak *et al.*, 2013). In addition to yield, tuber quality can also be affected. When potato plants are grown under S deprivation, the alteration of free amino acids and sugar levels in tubers affect formation of acrylamide and aroma compounds in tuber flour heated at high temperatures (Elmore *et al.*, 2007, 2010).

Survival of plant cells depends on sufficient calcium nutrition for stabilization of cell

membrane by bridging polar head groups of phospholipids at the membrane surface (Palta, 2010). Most Ca absorbed by potato roots is translocated to aboveground organs (Cabalceta *et al.*, 2005). Potato leaves accumulate 68 to 74% of total Ca taken up by the plant, whereas the proportion in tubers ranges from 5 to 12% (Paula *et al.*, 1986; Fernandes *et al.*, 2011). Kratzke and Palta (1985) provide evidence that specific tuber root hairs extract Ca to supply most Ca in the tubers. Within the plant, Ca is associated as a signaling molecule mediating plant response to environmental stresses and hormones (Hirschi, 2004). Since Ca is a component of cell membranes, Ca plays an important role in tuber quality and plant growth when plants are subjected to abiotic stresses. Previous studies suggest that Ca influences tuberization possibly by increasing GA, which results in suppression of the tuberization signal (Ozgen and Palta, 2005).

Carbohydrate Metabolism in Potato Tubers

Plants mobilize and store energy in the form of carbohydrate. Potato tubers are a rich source of carbohydrates, mainly starch. Bulking tubers store energy mainly in the form of starch to meet the energy demand during long-term storage and development of new shoots at the end of the dormancy. About 80% of the total tuber dry weight is starch. Starch is a complex carbohydrate consisting of long chains of glucose molecules and is synthesized in plastids by the action of several enzymes as show in Fig. 9.10.

Potato starch is comprised of two kinds of polysaccharides. One is a small linear molecule known as amylose and the other is a large, highly branched molecule known as amylopectin. Both are polymers of α-D-glucose units. Amylose is a linear polymer of up to several thousand glucose units, whereas amylopectin is a larger polymer regularly branched with α-1,6-branch points. These two molecules are assembled together to form a semi-crystalline starch granule. Potato starch granules are oval in shape and range in size from 0.1 to 50 micrometers in diameter. The ratio of amylase and amylopectin directly relates with starch quality. Different potato cultivars may contain different proportions of amylase and amylose pectin. Potato cultivars with high amylose contents are characterized as mealy cultivars in texture. Most of the russet cultivars are mealy in texture. Red, white, and yellow skin potato cultivars usually have high levels of amylopectin and are termed as waxy varieties.

The photosynthetic assimilation of atmospheric CO_2 by leaves yields sucrose and starch as end products. Disaccharide sucrose is continuously exported from the leaf cytosol to the parts of the plants, while polysaccharide starch concurrently accumulates as granules in chloroplasts. Sugars produced by photosynthesis move first from the mesophyll cells to the vascular tissues (the phloem). Sucrose is the main compound translocated from source leaves to sink tissue that is tuber. Starch is the main reserve carbohydrate in most plants.

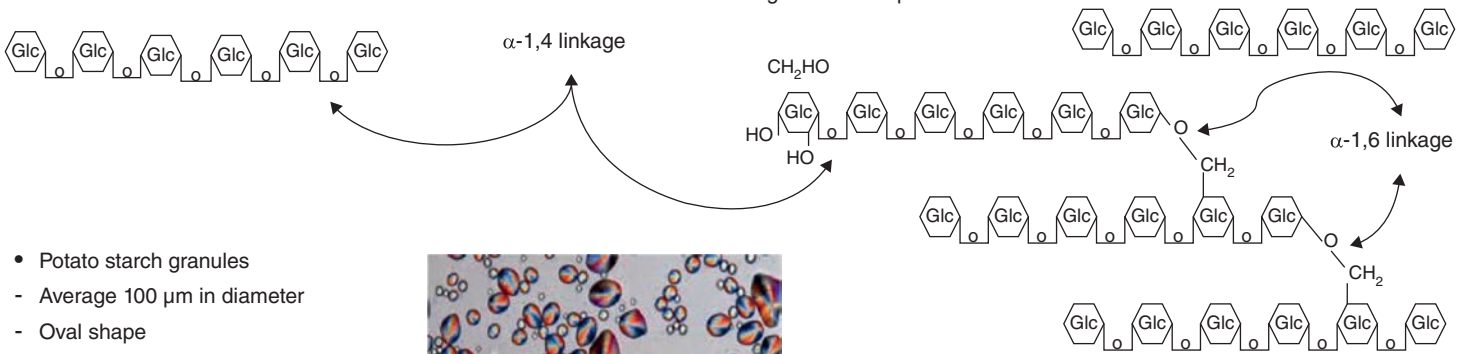
Starch metabolism in developing potatoes starts with sucrose translocated from leaves. Sucrose translocated from leaves to developing tubers can be used for energy production through the respiratory glycolytic pathway, structural polysaccharide synthesis and for starch synthesis for long term storage (Fig. 9.10). The majority of sucrose translocated to developing potato tubers is converted to starch by the action of several enzymes.

Starch synthesis in developing tubers

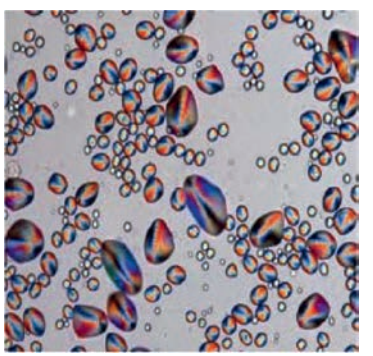
Developing potato tubers obtain most of their carbon from sucrose synthesized in leaves and delivered via a sucrose-proton co-transport system (Kuhn, 2003). Sucrose unloaded from phloem into a developing tuber is partitioned between starch, structural polysaccharides, storage as sucrose or hexose and entry into the respiratory pathways to provide ATP, reducing power and C-skeletons to developing tubers. About 50 to 70% of the sucrose translocated to developing tubers is incorporated into starch formation. Only 5 to 10% sucrose is utilized for structural polysaccharides (Rees and Morrell, 1990). The sucrose production and partitioning to developing potato tubers has been described in previous reviews (Hofius and Börnke, 2007). Potato tubers usually have a high sugar content early in their development because the rate of sucrose transport from the leaves exceeds the rate of conversion to starch. However, when the tubers reach maturity the sugar content reaches its lowest point.

- **Amylose**
- Linear molecule with α-1,4 glucan links
- 1000 glucose units

- **Amylopectin**
- Highly branched molecule with α-1,4 and α-1,6 glucan links
- 10,000 to 100,000 glucose units
- 20–25 glucose units per branch



- Potato starch granules
- Average 100 µm in diameter
- Oval shape
- Water insoluble



Mean length range from 30 to 44 µm

Fig. 9.10. Potato starch composition (S. Gupta).

The process of starch synthesis can be divided into three stages: i) initiation; ii) polysaccharide elongation and branching; and iii) termination of synthesis. There are several reports published on polysaccharide elongation and branching (Nazarian-Firouzabadi and Visser, 2017). However, information on starch synthesis initiation and termination is limited. Extensive research has been conducted on starch metabolism in potato tubers (Nazarian-Firouzabadi and Visser, 2017; Van Harsselaar *et al.*, 2017). The biochemical pathway of starch synthesis and degradation is shown in Fig. 9.11. Starch synthesis in potato tubers is a complex chain of reactions operating simultaneously. Several pathways are interconnected. Each pathway is mediated by several enzymes. The product of one enzyme could be the substrate for the other enzyme. Furthermore, enzymes have several levels of control. These include small molecules generated from other enzyme activities, changes in enzyme activity due to post-translation modifications, and/or development of new proteins known as isoforms.

The key enzymes involved in starch synthesis are ADP-glucose pyrophosphorylase or AGPase, starch synthase (SS) and branching enzymes (BE). The enzyme pyrophosphatase cleaves the pyrophosphate (PPi) generated by AGPase, driving ADP-Glc synthesis. ADP-glucose is the precursor that provides the glycosyl unit. AGPase consists of four sub-units and is highly regulated by the 3-phosphoglyceric acid (3PGA) and inhibited by inorganic phosphate (Pi).

The second enzyme in starch synthesis is SS. This enzyme may be granule bound or may be distributed between the stroma and starch granule. Once a glycosyl donor ADP-glucose is synthesized, then SS will continue to link the glucose units to form amylose. A third starch branching enzyme will transfer a segment of an α-D-1, 4-glucan to the carbon 6 of glycosyl units in the same glucan. Starch branching enzyme isoforms differ in the length of the glycosyl chain synthesis.

Starch degradation during sprouting and storage

At the time of sprouting, stored starch provides the energy and the polysaccharides for growth and development. Stored starch breaks down with the help of three main enzymes, amylase, disproportionating-enzyme or D-enzyme and various debranching enzymes (Critchley *et al.*, 2001). Part of the starch breaks down to form energy through glycolysis (Fig. 9.12). The end product of glycolysis, pyruvate, enters the TCA cycle to release CO_2 and H_2O, or starch breaks down to form reducing sugars, glucose, and fructose that can be used for polysaccharide synthesis.

In general, during cold storage the rate of starch degradation exceeds glucose consumption through glycolysis. Cold storage temperature below 7°C further increases reducing sugar formation. Reducing sugar formation due to cold

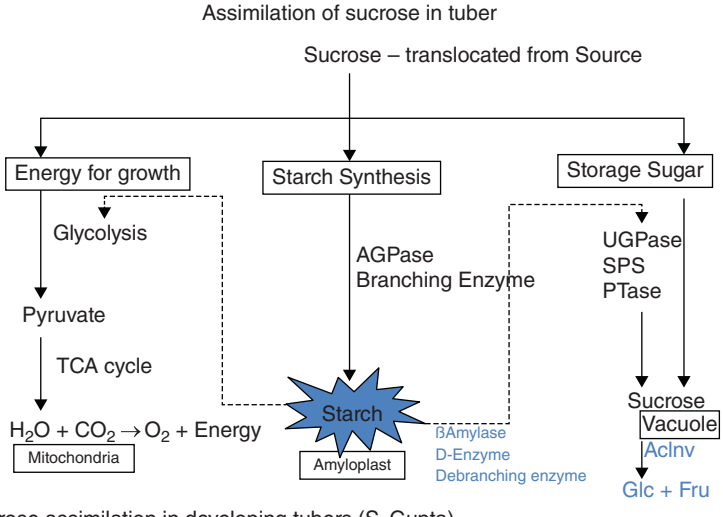


Fig. 9.11. Sucrose assimilation in developing tubers (S. Gupta).

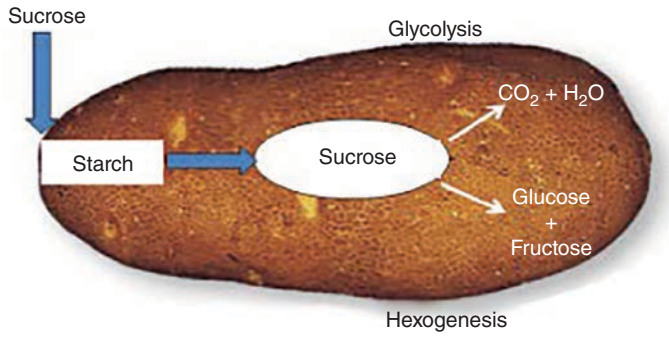


Fig. 9.12. Conversion of starch to sugar in a potato tuber (S. Gupta).

storage temperature is known as cold-induced sweetening (CIS). Excess glucose-1-P released from starch degradation enters the hexogenesis pathway and is converted to reducing sugars (glucose and fructose). The enzymes involved in hexogenesis are uridine diphosphate glucose pyrophosphorylase (UGPase), sucrose phosphate synthase (SPS) and phosphatase (Ptase). The first enzyme is UGPase, which takes glucose-1-P and combines with the nucleotide uridine triphosphate (UTP) to form UGP-Glc. The enzyme SPS combines UDP-Glc and fructose-6-P to form sucrose-6-P which then converts to sucrose by the action of the enzyme phosphatase. Sucrose mobilizes into the vacuole as a storage sugar (see review by Sowokinos *et al.*, 2000). Sucrose stored in the vacuole is hydrolyzed by action of the enzyme acid invertase to form reducing sugars. Recent research has been focused on reduction of reducing sugar formation due to the discovery of a potential cancer causing agent in processed starchy food products, known as acrylamide. Acrylamide is formed in a non-enzymatic reaction known as the Maillard reaction at processing temperatures above 120°C where reducing sugars combine with the free amino acid asparagine (Amrein *et al.*, 2003). Genomic strategies have been developed to alter the expression of key enzymes to lower tuber reducing sugars and asparagine (Halterman *et al.*, 2016).

Physiological Tuber Disorders

Raw potato tuber quality is of paramount importance to the potato industry. That includes size, shape, and freedom from disease. Some defects are also caused by unusual environmental conditions during growth and in storage and not by pathogens, insects or nematodes. These defects caused by unfavorable environmental conditions or improper handling of tubers are known as physiological defects or disorders. These defects can be external or internal. The losses due to these defects have not been systematically quantified but can be serious for growers. Several of these physiological disorders have been described previously in detail (Sowokinos, 2007; Mikitzel, 2014). Here, we briefly describe the major physiological disorders: sugar end defects (SED), internal brown spot (IBS), brown center (BC), hollow heart (HH), and secondary growth.

Sugar end defect

Sugar end defect is also known as dark end, or jelly end, translucent end, or glossy end. The names arise from the appearance of affected tissue at the stem end of the tubers, which becomes glassy or translucent. Because such tissues are deficient in starch and high in sugars, dark brown colors develop during production of French fries or chips (Fig. 9.11). Cultivars with long tubers seem to be most susceptible to the disorder. High soil temperatures are an important factor, especially if accompanied by drought. Signs of such defects are not visible in raw tubers. During the frying process, the stem end of the tuber develops a dark color. The symptoms, causes and physiological mechanism of such defect development have been described in a review article by (Thompson *et al.*, 2008).

Internal brown spot

Non-pathogenic necrosis or discoloration of tubers is known by several names such as internal brown fleck, internal browning, internal rust spots, internal necrosis, and internal brown spot (IBS). The usual symptoms of these defects are irregularly-shaped spots that could appear anywhere in the tuber. IBS is quite different from another physiological disorder known as brown center (BC). IBS was evident in tubers where calcium was reduced after tubers reached 5 cm in diameter (Davies and Monk-Talbot, 1990).

Brown center and hollow heart

When the discoloration occurs in the center of the tuber, it is known as brown center (BC). It is postulated that brown center is a precursor for hollow heart. These internal physiological disorders are usually associated with calcium deficiency and exacerbated by temperature and water stress. Environmental conditions like high temperature causes a disruption in the calcium supply which is required for the cell's metabolic processes resulting in tissue necrosis.

Calcium withheld during tuber initiation causes symptoms of BC (Davies, 1998). Loss of membrane integrity and stimulation of oxidative reactions have been reported in BC affected tissues (Sowokinos, 2007). Both IBS and roughness of smooth-skinned cultivars may be associated with Ca flux in potatoes (Yencho *et al.*, 2008).

The hollow center of the tuber is known as hollow heart (Raimo *et al.*, 2018). Hollow heart is characterized by an irregular shaped hollow cavity in the center of the tuber (Fig. 9.13, A). This is one of the common defects in potato production especially when high temperatures occur during tuber bulking stage. HH in large tubers has been associated with excessive rapid tuber enlargement due to changing environmental conditions and excess nitrogen supply (Hiller *et al.*, 1985).

Secondary growth

Under some conditions, growth of an individual tuber stops and there is a partial or complete reversion to stolon growth from one or more eyes. Because this condition is often associated with warm soils, the phenomenon is known as "heat sprouting." Heat sprouting is different from the normal breaking of tuber dormancy that occurs after tubers have been harvested and stored. It is more like an interruption in the dormant condition than a termination of it; subsequent to heat sprouting the tuber buds again become dormant.

Much more common than heat sprouts and chain tubers are "knobs"—formed when the initial reversion to stolon growth is incomplete, and secondary lateral growth leads to knobby protrusions at one or more eyes instead of heat sprouts or chain tubers. Other types of deformity include "bottlenecks," "dumbbells," and pointed-end tubers, the names of which are self-descriptive (Fig. 9.13, B). Collectively, these defects are known as "second growth." They can be considered as products of fluctuations in the

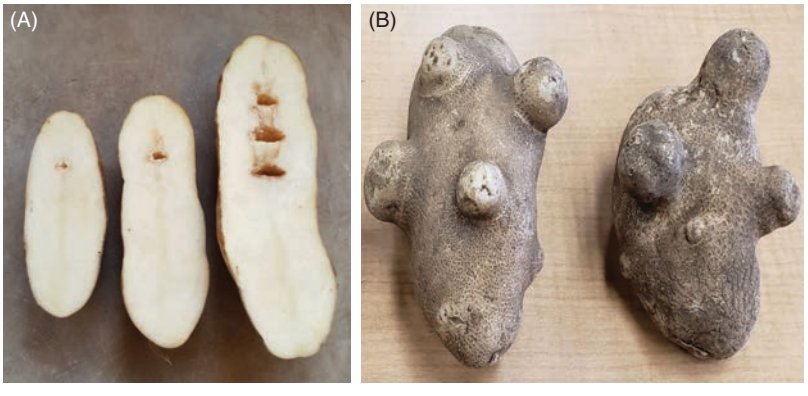


Fig. 9.13. (A) Hollow heart defect and (B) secondary growth (knobs) in potato tubers (S. Gupta).

intensity of the induction to tuberize, usually brought about by changes in temperature.

It seems likely that the knobs and secondary tubers also involve resorption from the primary tuber—that the dry matter is removed from the attached primary tuber rather than merely passing through the vascular bundles of the primary tuber from other parts of the plant (Scholte, 1977). Cultivar susceptibility is one of the major factors for secondary growth. Other environmental conditions like irregular or inadequate application of water causing water stress are also involved (van Loon, 1986).

Flowering and Seed Production

Seasonal fluctuations in daylength regulate important aspects of plant development such as the flowering transition or, in potato, the formation of tubers in andigena types. Daylength is sensed by the leaves, which produce a mobile signal transported to the shoot apex or underground stems to induce a flowering transition or, respectively, a tuberization transition. Factors other than daylength appear to be more important for flowering in cultivated (tuberosum) potatoes. Potato floral and tuberization transitions are controlled by two different florigen-like paralogues (StSP3D and StSP6A) that respond to independent environmental cues (Hannapel *et al.*, 2017).

Because conventional propagation depends upon seed tubers, flowers and fruits are usually ignored or have been viewed as a minor nuisance by traditional growers. In some areas, the seeds from potato berries (Fig. 9.14, B) fall to the ground and germinate the following season, presenting a "weed" problem. Not only do the seedling volunteers compete with the regular plants, but they may even set tubers that compromise the purity of potatoes grown as certified seed. Another concern with fruit set is that it diverts biomass production away from tubers. A few experiments have demonstrated that such an effect is measurable (Jansky and Thompson, 1990), but under most circumstances it is economically unimportant.

In contrast to production, flowering and fruiting are of major concern to plant breeders. Except for selection of chance mutations (usually for more desirable skin color), cultivar development depends on fruit set. Understanding flowering and fruit is even more important with the current interest in propagation from true seed (Jansky *et al.*, 2016).

The growth of the main stem is terminated by an inflorescence, but stem growth may continue from an axillary bud (Fig. 9.5). A new branch will again terminate in an inflorescence, and this process may continue for several stages, depending upon the vigour of the plant. The central axis of the potato inflorescence itself terminates in a flower, which is the first flower in the inflorescence to open (Fig. 9.14). This proximal flower tends to give the largest berry, the most seeds, and the heaviest seeds (Almekinders *et al.*, 1995).

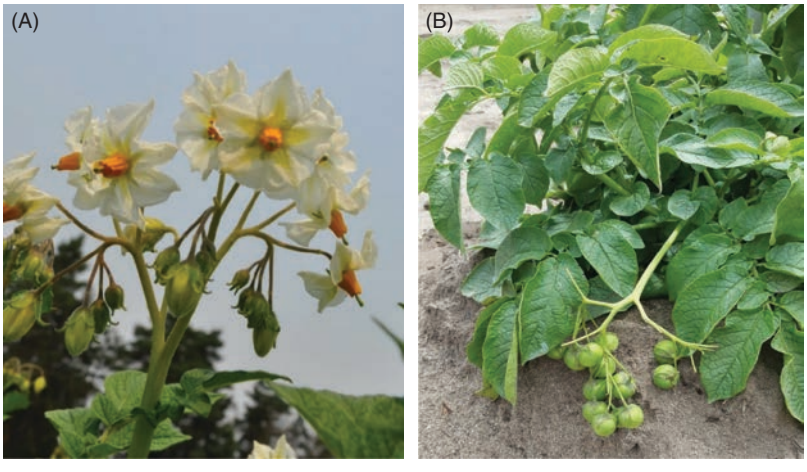


Fig. 9.14. Potato inflorescence (A) and fruiting (B) (S. Gupta and E. Souza).

The number of flowers that form is more a question of how many flower primordia survive than how many primordia are initiated. Many flower buds abort before they develop, which makes it difficult to study the effects of environment on primordia initiation. For this reason there is confusion in the literature as to whether flower initiation is favored by short or long days. For andigena types the flowering stimulus is initiated by long days. For the cultivated potato, any effect of daylength on flower initiation appears to be slight; but long days and moderately warm temperatures reduce flower bud abortion (Turner and Ewing, 1988; Almekinders and Struik, 1994). In studies with andigena types, the stimulus for flower initiation has been shown to be a translocatable type of flowering locus-like protein family that is sensitive to environmental signals (Rodríguez-Falcón *et al*., 2006; Navarro *et al*., 2011). A similar protein family is also responsible for tuberization (González-Schain *et al*., 2012).

The diversion of assimilate away from flower buds to competing tuber sinks may have some influence on flower bud abortion. Further improvement in flower production and fruit set can be expected as plant breeders interested in TPS production select for these traits.

Molecular Biology and Biotechnology

The potato lends itself to genetic engineering not only because it is among the crops most amenable to transformation and regeneration, but also because it is clonally propagated (Vayda and Belknap, 1992). Added to this, its value as a world food crop and the large number of pests that attack it have made the potato a prime subject for genetic manipulations. Transgenic plants are already available that carry resistance to various insects or diseases, and interest is high in inserting still other genes (Belknap *et al*., 1993). Various techniques for foreign gene transfer like particle bombardment, protoplast transformation, microinjection and embryo electroporation, agrobacterium mediated transformation have been used to produce genetically modified organisms (GMO).

The first biotech potato was commercialized as NewLeaf™ by Monsanto in 1995 for resistance to Colorado potato beetles. Resistance was achieved by inserting a bacterial gene using bacterial DNA for gene transfer. The variety was grown and processed until 2002 but was discontinued due to anti-GMO public pressure. Although there are various advantages offered by transgenic potato plants, consumer support is one of the most important factors in their adoption. Therefore, a regulatory clearance from various agencies like Food and Drug Administration (FDA), US Department of Agriculture (USDA), and Environmental Protection Agency (EPA) in the US is required before wide release and production for sale. These regulations are expensive and time consuming given lack of consumer acceptance. Consumer acceptance is essential for adoption of any new technology.

While transgenic potato plants are not being used commercially, they do offer exciting prospects for advancing our understanding of potato physiology. These include transgenic plants with varying levels of different hormones, so that effects of hormones on tuberization, tuber dormancy, flowering and other traits can be examined. Various molecular techniques have been used to advance our knowledge on the role of various key enzymes related to potato post-harvest physiology and increase productivity. Sweetlove *et al*. (1996) used this technique to transfer *glgC*-16 gene for one of the key starch synthesizing enzyme AGPase from *Escherichia coli* to study the enzyme mechanism and increase total starch content in the tubers.

Another approach is to use only genes from the same species for trait improvement and the resulting plants are known as cisgenic. Cisgenic is defined as the transfer of gene(s) within species and not from bacteria or any other organism. However, bacterial DNA is still used as vehicle to deliver the gene and remnants of the bacterial DNA may pass on to genetically modified plant. Therefore, to address public concern, a new technology was developed by the J.R. Simplot Company called Innate technology. The company used only plant DNA for potato gene transfer and introduced Innate™ 1.0 potatoes to the fresh and chip market in 2015 (Halterman *et al*., 2016). The technology was essentially similar to agrobacterium mediated transformation but the right and left border of transfer DNA were of plant origin and the gene transfer was within species.

An even newer technique was developed to transfer foreign genes into potato using RNA. The technique was known as RNA interference

(RNAi). The technique was used to reduce one of the key enzymes involved in cold-induced sweetening called acid invertase (Bhaskar *et al.*, 2010). Though the technique has several advantages over the previously used techniques, RNAi often results in incomplete gene silencing. This approach still requires regulatory agency clearance by USDA, FDA, and EPA in the United States and the European Food Safety Authority (EFSA) in Europe.

More recently, other methods have been developed for genetic improvement without the use of any bacterial DNA or the introduction of any foreign DNA. These methods enable precise addition, deletion, or substitution in plant DNA (Shukla *et al.*, 2009; Townsend *et al.*, 2009). Precise genome modification, also known as genome editing, is enabled by sequence-specific nucleases and cells' natural DNA repair mechanism at the DNA break point. Currently available methods include the use of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) (Puchta and Fauser, 2014; Clasen *et al.*, 2016), and clustered regularly interspaced palindromic repeats, commonly known as CRISPR/Cas9 (Doudna and Charpentier, 2014). The common term for these methods is known as "gene editing" and fits outside the traditional regulatory processes. While these new technologies offer exciting possibilities for potato improvement though a basic understanding of physiological processes, adoption is currently limited by consumer concerns, industry acceptance, and policies implemented by regulatory agencies. Sustained growth in potato cultivar development will therefore depend on traditional breeding techniques as well as use of new technologies as a tool for parent selection.

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10 The Cucurbits

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General Introduction, Evolution, Taxonomy

The family *Cucurbitaceae* is evolutionarily highly diversified with more than 800 species, including the most important vegetable crops worldwide. Most of the major clades in *Cucurbitaceae* have been cultivated, as fresh fruit, leafy vegetable, medicine, or ornamental plants (Fig. 10.1). For example, watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and melon (muskmelon or cantaloupe, *Cucumis melo* L.) are consumed as desserts, cucumber (*Cucumis sativus* L.) and bitter gourd (or bitter melon, *Momordica charantia* L.) can be used as ingredients for fresh salads or be cooked, and the leaves of bitter gourd and jiaogulan (*Gynostemma pentaphyllum* (Thunb.) Makino) are widely used as herbal medicine (Lin *et al.*, 2009). Tender shoots of many cucurbits are fast-growing and are consumed as high-yielding leafy vegetables, for example, ivy gourd (*Coccinia grandis* (L.) Voigt) and chayote (*Sechium edule* (Jacq.) Sw.).

The fruit flesh of luffa (*Luffa aegyptiaca* Mill. and *L. acutangula* (L.) Roxb.), the squashes and pumpkins are generally consumed after boiling or baking. The immature fruit of summer squash or marrows (*Cucurbita pepo* L.), and the mature fruit of all the principal squash and pumpkin species (*C. argyrosperma* C.Huber, *C. moschata* Duchesne, *C. maxima* Duchesne, and *C. pepo* L.) are used as food. Seeds of watermelon and some squash and pumpkin species are roasted and eaten as snacks, or ground as an ingredient of sauces. Several cultivars of *C. pepo* with hull-less seeds have been developed that facilitate the food uses of the seeds. With an oil and protein content of 46 and 34%, respectively, these could be exploited as alternative oil-bearing crops (Whitaker and Davis, 1962). Pumpkins and gourds (*Cucurbita pepo*) are frequently grown solely for the ornamental value of the fruits, in harvest displays and as part of the Halloween celebration, as well as the eye-catching fruit of squirting cucumber (*Ecballium elaterium* (L.) A.Rich.).

The family *Cucurbitaceae* has been used by humans for centuries. Today, the worldwide production of cucurbit vegetables reaches 241 million tons per year (FAO, 2017), with Asian countries producing 60–80% of the cucurbit vegetables worldwide (Fig. 10.2).

The major cultivated cucurbits can be classified into New World and Old World species with regard to their origin. Cucumbers are thought to have been first cultivated in India, where their use has been recorded as long as 3000 years ago (Whitaker and Bemis, 1976) and diffused westward to Europe through two independent routes between the 9th and 14th centuries (Paris *et al.*, 2012). *Cucumis melo* was thought to have arisen

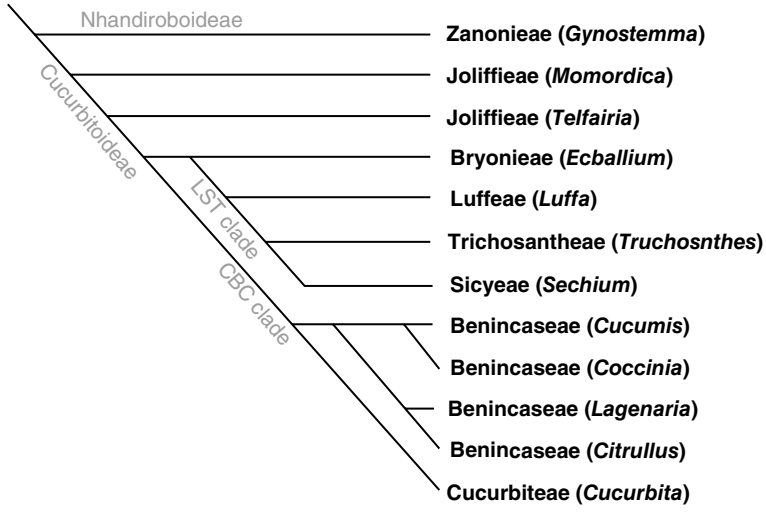

Fig. 10.1. Phylogenetic relationships of agricultural crops in *Cucurbitaceae*. The subfamily Nhandiroboideae includes medicinal plant jiaogulan (*Gynostemma pentaphyllum*). Most of the utilized plants belong to the subfamily Cucurbitoideae (adapted from Kocyan *et al*., 2007).

in the central part of Africa, and spread rapidly into Asia, where many cultivars have since been selected, but recent DNA sequences from plastid and nuclear markers suggest that the common ancestor of cucumber and melon originated in Asia, and their closest wild relatives are *Cucumis hystrix* from southeast Asia and *Cucumis picrocarpus* from Australia, respectively (Sebastian *et al*., 2010). The watermelon was cultivated in Egypt at least 4000 years ago (Paris, 2015) and is thought to have originated in northeastern Africa. However, a more recent study using well-resolved phylogeny of *Citrullus* suggests its origin in West Africa (Chomicki and Renner, 2015). The New World species include all the cultivated *Cucurbita*, which were domesticated following several independent occasions, dating back as far as 10,000 years ago (Sanjur *et al*., 2002; Kistler *et al*., 2015). From archaeological records, the *Cucurbita* species are among the most ancient of the cultivated crops in the Americas. The earliest documentation of *Cucurbita* in Europe dates from the early 16th century (Paris *et al*., 2006). In the last decades, the growth and productivity of cucumber has been intensively studied to optimize its cultivation in greenhouse production systems. Genomes of cucumber (Huang *et al*., 2009), melon (Garcia-Mas *et al*., 2012), bottle gourd (Wu *et al*., 2017), bitter gourd (Urasaki *et al*., 2017), and zucchini (Montero-Pau *et al*., 2018) have now been sequenced and documented in the cucurbit genomic database (http://cucurbitgenomics.org). This facilitates studies of the genetic controls of physiological processes among cucurbit vegetables.

Germination and Seedling Growth

The germination of cucurbit vegetable seeds requires relatively warm temperatures (Maynard and Hochmuth, 2007), and takes place within three or four days at 25–30°C. For cucumber and muskmelon, the lower limit of germination is 12°C (Table 1.5, this volume). Summer squash (*Cucurbita pepo*) germination showed a lower threshold between 5 and 10°C, and optimum germination between 30 and 35°C (NeSmith and Bridges, 1992).

When cucumbers are exposed to low soil temperatures during germination and emergence, they suffer chilling injury that increases the more the radicle has emerged from the seed (Rab and Saltveit, 1996; Mangrich *et al*., 2006). The injury appears as an inhibition of further root and hypocotyl elongation. To overcome such adverse effects, researchers have soaked germin-

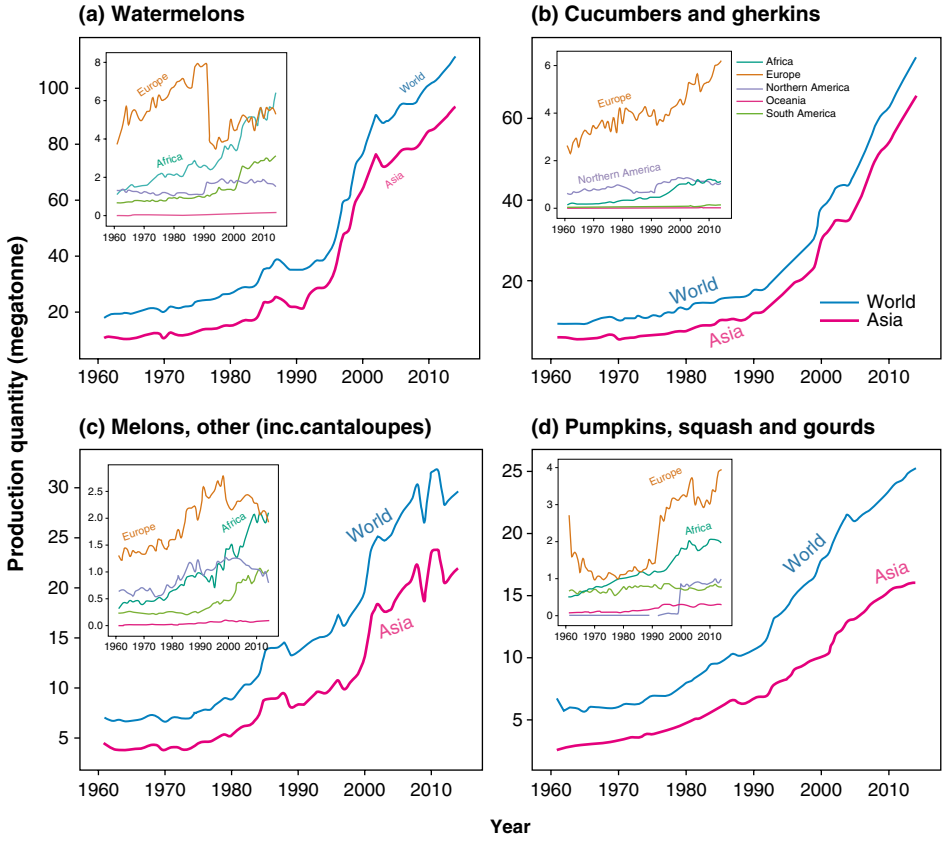

Fig. 10.2. Worldwide production of the major cucurbit vegetables from 1960 to 2014. The outer graphs show the trends in yield worldwide and in Asia. The inner graphs show the trends in yield in Africa, Europe, Northern and South America, and Oceania (FAO, 2017).

ating seeds in solutions of plant growth regulators including GA (Nelson and Sharples, 1980) or paclobutrazol (Ramin, 2009), with varying results. More promising appears to be the selection of cold-tolerant cucumber lines. For instance, after four cycles of selection for germination at 15°C, Nienhuis *et al.* (1983) improved low temperature germination from 32 to 94%. Gu *et al.* (2017) identified 22 cold-tolerant cucumber lines by growing them at 13°C. In muskmelon, Nerson *et al.* (1982) showed "bird's nest" cultivars developed in Iran to have significantly better cold temperature germination than standard viny and dwarf cultivars developed in the United States.

The low germination rate of cucumber at 15°C may be partly due to seed dormancy (Nienhuis *et al.*, 1983). Freshly harvested seed failed to germinate at this temperature and remained dormant until it had been stored for 84 days. Watts (1938) had also encountered this phenomenon in "Black Diamond" cucumber, and was able to overcome the inhibition by removing the seed-coat, or germinating the seed at 30°C. The presence of a germination inhibitor in the testa was further supported by the finding that soaking cucumber seed in acetone significantly improved cold temperature germination (Nelson and Sharples, 1980). These workers also showed that watermelon seed germination at 16°C could be improved by washing the seed for 2 h in water.

The improvement of watermelon seed germination and seedling emergence at low (15°C)

temperatures has continued to be a focus of research. Priming in solutions of potassium nitrate was most effective (Kang and Cho, 1996; Demir and van de Venter, 1999; Armin *et al.*, 2010; Barbosa *et al.*, 2016). The hard seed-coat of triploid watermelon poses an additional germination challenge (Zheng *et al.*, 2005) that can be overcome by hydropriming (see Chapter 1). Combinations of hydropriming and GA_3 (Phanna *et al.*, 2015), or KNO_3 and methyl jasmonate (Korkmaz *et al.*, 2004; Susila *et al.*, 2013) brought further improvements in cold soil germination and emergence speed.

The cucurbit seedling is among the most rapidly growing of any vegetable plant. It owes this characteristic to several properties. Seed size of the cucurbit vegetables is relatively large, varying from about 20 mg seed^{-1} for muskmelon to 150 mg for squash and pumpkin (Maynard and Hochmuth, 2007). The decorticated seeds contain on average about 49% oil and 35% protein (Jacks *et al.*, 1972), suggesting that a large store of reserve materials is available for seedling growth before the cotyledons and true leaves start to photosynthesize. Large seed size also implies large initial seedling size, giving the plant an early start in light interception and assimilation. Seedlings thus reach a size suitable for transplanting in three weeks, compared to tomato and pepper, which generally require six to eight weeks (Maynard and Hochmuth, 2007).

Seedling productivity of cucumber is comparable with that of tomato and pepper seedlings in terms of net assimilation rate (dry matter production per unit leaf area per unit time) (Bruggink and Heuvelink, 1987). Cucumber thus does not appear to have an inherently greater photosynthetic rate but apportions a large amount of its dry matter to leaf growth in the early stages (high leaf area ratio, leaf area to total dry weight).

Cucurbit seedlings are often planted in field conditions that could subject the plants to chilling temperatures (Korkmaz and Dufault, 2001). At temperatures between 4 and 15°C, cucumber, melon and watermelon roots produce damaging levels of reactive oxygen species that damage lipid membranes (Zhang *et al.*, 2012; Hou *et al.*, 2015). These effects are at least partially mitigated by endogenous levels of salicylic acid in the roots of cucumbers (Dong *et al.*, 2014), and by application of methyl salicylate (Seydpour and Sayyari, 2016) or acetyl salicylic acid (Korkmaz *et al.*, 2007). Stimulation of antioxidant systems in cucurbits has similarly been accomplished by seed or seedling treatments of paclobutrazol (Baninasab, 2009; Baninasab and Ghobadi, 2011), sucrose (Cao *et al.*, 2014) by brassinosteroids and abscisic acid (Yu *et al.*, 2002). In several of these cases, the treatments protected against other seedling stresses, such as high temperatures and salt stress, suggesting that genetically increasing the activity of antioxidant genes could boost stress resistance. Xi *et al.* (2010) successfully used this approach with *Arabidopsis*, but it remains to be seen if the protective effect could be extended past the seedling stage.

Under greenhouse conditions, where temperatures can be regulated, it has been found in the vegetative period that stem extension rate and the development of leaf area is linearly dependent on the mean air temperature within the range of 19–26°C (Krug and Liebig, 1980; Kahlen and Chen, 2015). The amplitude around these means made no difference as long as day temperature did not exceed 28°C. If night temperature was higher than day temperature, stem extension rate was slowed by the production of shorter internodes. The most economical temperature regime for the rapid production of greenhouse cucumber plants during the vegetative stage would be to set day temperatures at about 4–6°C higher than the night temperature. Nitrate uptake by cucumbers in cold soil was inhibited due to the decreased gibberellic acid metabolism by the roots, which could be restored by exogenous GA application (Bai *et al.*, 2016). Another approach to overcoming the growth-inhibiting effects of cold soils is to graft cucumber onto rootstocks of species less susceptible to cold root temperatures (den Nijs, 1980 and see Chapter 6). This approach, widely practiced in China, Taiwan, Japan, and Korea, allows cucumber and other cucurbits to be grown without soil heating (Huang *et al.*, 2015). More particularly with regard to root growth in cold soils, Tachibana (1982, 1987) found that *Cucurbita ficifolia*, one of the most common rootstocks used for cucumber, maintains active growth, water and nutrient uptake at 12 and 14°C. Differences in susceptibility to low soil temperatures between two cucumber cultivars were particularly well related to differences in phosphorus uptake at those temperatures (Tachibana, 1982,

1987). More recently, Zhang *et al.*, (2012) found that *C. ficifolia* produced lower levels of reactive oxygen species at chilling temperatures than cucumber. Progress has also been made to select *Cucurbita* sp. lines with increased tolerance to chilling temperatures for use as rootstocks for cucumber (Xu *et al.*, 2017).

Flower Differentiation

Although about 50% of the species in *Cucurbitaceae* are monoecious (Renner and Schaefer, 2017), sexual systems of cucurbits are highly diverse. For example, mating systems of *Cucumis* can be monoecy, dioecy, or hermaphroditism (Roddriguez-Granados *et al.*, 2017). Some dioecious species such as ivy gourd (*Coccinia* in Fig.10.1) have heteromorphic sex chromosomes (Devani *et al.*, 2017). The monoecious sexual type is most prevalent in *Cucurbita* spp., watermelon, bitter gourd, and bottle gourd. Many gynoecious cultivars are derived from crosses between gynoecious by monoecious parents, and produce significant numbers of male flowers, especially under long daylengths and high temperatures (Connor and Martin, 1971; Lower and Edwards, 1986). The percentage of female flowers can be increased significantly in crosses of gynoecious by hermaphrodite lines, and lines homozygous for the gynoecious trait have also been developed by use of ethylene-inhibiting chemicals such as silver nitrate (Lower and Edwards, 1986).

The regulation of sexual development is under genetic, environmental, and hormonal control. Genes related to sex determination have been investigated most in cucumber and melon and have been proposed to be conserved in cucurbits (Zhang *et al.*, 2014). After the initiation of floral meristems in the leaf axil, cucumber and melon develop anatomically similar "pre-sexual primordia." Further differentiation of these primordia into stamen and carpel can be arrested depending on genotype, epigenetics, and environmental triggers on hormone production (Boualem *et al.*, 2015; Roddriguez-Granados *et al.*, 2017). Composed of three genes, *CmWIP1*, *CmACS-7*, and *CmACS11* (Fig. 10.3), the genetic regulatory network of sex determination in cucumber and melon has been elucidated relatively recently (Boualem *et al.*, 2008, 2015; Martin *et al.*, 2009; Roddriguez-Granados

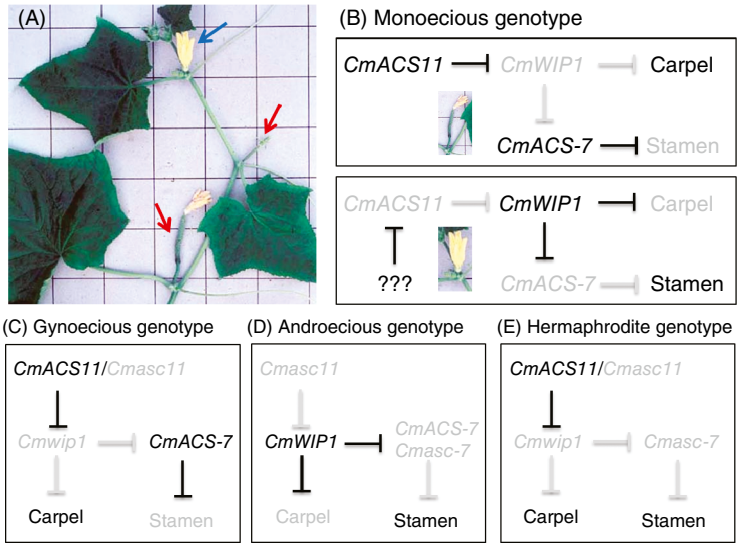

Fig. 10.3. Genetic network determining sex expression in *Cucumis melo*. (A) Monoecious cucumber vine with male (blue arrow) and female (red arrows) flowers. (B) Monoecious genotype. (C) Gynoecious genotype. (D) Androecious genotype (E) Hermaphrodite genotype (Boualem *et al.*, 2015).

et al., 2017). *CmWIP1* is an ethylene zinc finger transcription factor which induces carpel abortion and represses the expression of *CmACS-7* (Martin *et al.*, 2009), which encodes an ethylene biosynthesis enzyme (Boualem *et al.*, 2008). Since ethylene suppresses the development of stamens, expression of *CmACS-7* results in stamen abortion.

In monoecious genotypes, all three genes are dominant and the expression of *CmACS11* determines the fate of the flower primordia (Fig. 10.3B). According to this model, the mutating *CmACS11* gene of monoecious genotype should create the androecious genotype (Fig. 10.3D) and the double mutant of *Cmwip1* and *Cmacs-7* should be hermaphroditic (Fig. 10.3E).

This model might facilitate the breeding of cucurbits since the gynoecious flowering genotype is preferred for achieving high fruit number and yield. Therefore, most commercial cultivars of greenhouse cucumber are gynoecious. According to this model, gynoecious genotypes have recessive alleles of *Cmwip1* (Fig. 10.3C), and can be developed from monoecious genotypes by mutating the *CmWIP1* gene. This model also explains the previous observation that the gynoecious genotype can be homozygous or heterozygous (Frankel and Galun, 1977; Lower and Edwards 1986) and that homozygous gynoecious cultivars of cucumber produce male flowers at very low frequency (in many situations they will only produce female flowers—Lower *et al.*, 1983). Heterozygous gynoecious cultivars, especially those developed from crosses between gynoecious and monoecious lines, produce male flowers on a significant proportion of their nodes (Cantliffe, 1981; Lower *et al.*, 1983; Lower and Edwards, 1986). These cultivars are termed "predominantly female" by the seed trade and should be monoecious per se with frequent expression of *CmACS11*. The model in Fig. 10.3 implies that the expression of *CmACS11* is the key to regulating the female-to-male flower ratio in monoecious cucurbits. Although the signals triggering the expression of *CmACS11* are still unknown (Boualem *et al.*, 2015), previous studies on monoecious cucurbits indicate that it is dependent on environment, genotype, and hormones.

Environmental effects on sex expression

The sex type of flowers in the species of cucurbits having monoecious flowering habit shows a distinct ontogenetic pattern (Nitsch *et al.*, 1952; Shifriss and Galun, 1956). The basal main stem nodes are generally male, and these become interspersed with an increasing number of nodes bearing female flowers. At the upper part of the main stem, the plant may eventually form a zone of nodes bearing only female flowers. Cultivars differ in ratio of male to female flowers, and in the lowest node bear female flowers, which can appear between the tenth and thirtieth node. At least three environmental factors have an important influence on sex expression in cucurbits: temperature, light energy, and photoperiod. In general, the environmental conditions which encourage the buildup of carbohydrates and which reduce the amount of vegetative growth tend to favor female flower expression (Table 10.1).

High temperatures suppress the formation and delay the initiation of the female flower (Nitsch *et al.*, 1952). Mean temperatures are most important, but night temperatures also play a significant role, with warm nights leading to increased male flower production at a given mean temperature, compared to warm days (Nitsch *et al.*, 1952). The temperature influence may occur during flower primordia differentiation, as in cucumber, or during the development of the flower to anthesis. In *Cucurbita pepo*, low temperatures may inhibit male flower development after differentiation, leading to precocious female flowering (Rylski and Aloni, 1990; H.C. Wien, Ithaca, NY, 1990, unpublished data).

The effect of photoperiod on sex expression appears to be less striking than the influence of temperature and light conditions in most cultivars (Matsuo, 1968; Cantliffe, 1981). Short photoperiods tend to favor the production of female flowers in *Luffa cylindrica*, but not *Lagenaria siceraria* (Takahashi and Saito, 1986). Under long-day conditions, all flower primordia of *Luffa cylindrica* abort and the male flower buds first develop when daylength is shorter than 13 hours (Chen *et al.*, unpublished). It is difficult under field conditions to separate photoperiod from light quantity effects, since short photoperiods often coincide with periods of reduced light quantity. In that situation, it appears that light energy plays the more important

Table 10.1. Environmental effects on sex expression in *Cucurbitaceae*.

Treatment	Effects of treatment	Reference
Cucurbita pepo		
High temperature	Reduce female flower	Wien, 2006
High temperature	Delay the initiation of the first female flower	Nitsch *et al*., 1952
Warm night temperature	Increase male flower	Nitsch *et al*., 1952
Low temperature	Inhibit male flower differentiation	Rylski and Aloni, 1990
Low light + high temperature	Predominant male flower	Cantliffe, 1981
Low light, shading	Delay the initiation of female flower	Kooistra, 1967; Cantliffe, 1981
Luffa cylindrica		
Short photoperiod	Increase female flower	Takahashi and Saito, 1986
Lagenaria siceraria		
Short photoperiod	No effect	Takahashi and Saito, 1986
Cucumis sativus		
Long photoperiod	No effect or delay the initiation of female flower, depending on genotype	Matsuo, 1968
High nitrogen	Delay initiation of female flower	Ito and Saito, 1960
High plant density close spacing within rows	Increase male flower	Lower *et al*., 1983; Nienhuis *et al*., 1984

role, as in the example cited by Cantliffe (1981). If photoperiod is controlled by keeping the amount of light energy constant and extending day length with low intensity light, some cucumber cultivars are day neutral with regard to sex expression, and others show delayed female flowering in long days (Matsuo, 1968).

Effects of growth hormones on sex expression

It is well-known that ethylene is a feminizing hormone in cucurbits, implying that ethylene may locally remove the repression of *CmACS11* in monoecious plant (Fig. 10.3B). Evidence for the role of ethylene comes both from studies in which endogenous levels of the growth hormones are related to sex expression, and from the effect of applying the compounds exogenously to the plant. The capacity of ethylene to stimulate female flower development in the cucurbits first became known when researchers applied ethephon (2-chloroethylphosphonic acid) to cucumbers (Robinson *et al*., 1969). When applied to monoecious cucumber seedlings, the chemical eliminated male flowers on the lower nodes, and increased the number of female flowers. Ethephon stimulated the formation of female flowers on monoecious muskmelon, without much change in male flower numbers, while on andromonoecious plants there was an increase in perfect and suppression of male flowers (Karchi, 1970), in accordance with the gene regulatory network in Fig. 10.3. Ethephon application has thus become useful to ensure that the inbred line used as the female parent in hybrid combinations does not develop male flowers, and is used for this purpose in both cucumber and squash breeding (Shannon and Robinson, 1979; Lower and Edwards, 1986).

Treatment of *Cucurbita pepo* with ethephon increased female flower number, but decreased endogenous auxin activity (Chrominski and Kopcewicz, 1972). Under 20/15°C day/night temperature, treating pumpkin seedlings at two-leaf stage with the ethylene-producing chemical ethephon significantly increased the number of female flowers (Wien, 2006). Watermelon appeared to be more sensitive to ethephon applications than the other cucurbits, and the crop's reaction was opposite to that found for cucumber (Christopher and Loy, 1982). Exposure to concentrations of 30 µl l^{-1} retarded female flower formation, but had less effect on male flowers. In bitter gourd, low concentration of ethephon (25 mg l^{-1}) increased the total and the female flower number but the chemical decreased female flower

number under high concentrations (Banerjee and Basu, 1992).

The central role of ethylene in cucurbit sex expression has been further strengthened by the finding that inhibitors of ethylene formation or action have effects on flower formation that are opposite to those of ethephon. For instance, treatment of homozygous gynoecious cucumbers with the ethylene action inhibitor silver nitrate or the ethylene synthesis inhibitor aminoethoxyvinylglycine (AVG) resulted in the formation of male and perfect flowers (Atsmon and Tabbak, 1979). In contrast, both silver nitrate and AVG suppress female and perfect flowers of watermelon, with only a partial reduction of male flowers (Christopher and Loy, 1982). Silver nitrate and silver thiosulfate are used by cucumber breeders to produce inbred, all-female lines (Lower and Edwards, 1986). Treated gynoecious plants produce some male flowers and permit selfing, without the detrimental effects of elongated, brittle stems brought about by gibberellin application. According to the genetic model, inhibiting ethylene action of homozygous gynoecious cucumber should only induce perfect but not male flowers. This indicates that an unknown mechanism leading to carpel abortion might exist, or the genetic materials used in the previous study were not true homozygous gynoecious lines.

Determinations of endogenous ethylene levels in cucurbit seedlings have supported the regulatory role of this chemical in sex expression. In cucumber, seedling apices of gynoecious lines produced more ethylene than those of monoecious or androecious lines (Rudich *et al.*, 1972b, 1976). Ethylene evolution was higher for female than for male flower buds, and monoecious lines increased their ethylene evolution at the time that female flower primordia were developing, due to the expression of the ethylene biosynthesis enzyme, *CmASC-7*. Short daylengths stimulated ethylene evolution in comparison to long daylengths, in concert with their influence on female flower formation (Rudich *et al.*, 1972b).

In maize, it is known that jasmonic acid and brassinosteroids inhibit the carpel development and gibberellins are involved in stamen abortion (Zhang *et al.*, 2014). The effects of these hormones and auxin on sex expression in cucurbits are probably due to their crosstalk with ethylene since no direct evidence shows that gibberellin and auxin affect sex expression in cucumber and melon (Zhang *et al.*, 2014). For example, increasing female flower of cucumber, melon, and zucchini by treating their seedlings with a brassinosteriod also increased their ethylene production (Papadopoulou and Grumet, 2005). In other studies, brassinosteriods showed no effects on female flower induction (Manzano *et al.*, 2011).

The production of male flowers on nodes of cucumber that would normally produce female flowers can be brought about by the application of gibberellic acid (GA_3), and even more effectively, by GA_{4+7} (Mitchell and Wittwer, 1962; Atsmon and Tabbak, 1979). Similar results have been obtained with cucumber, *Cucurbita*, and muskmelon (Splittstoesser, 1970; Rudich *et al.*, 1972a; Atsmon and Tabbak, 1979). In bitter gourd, GA_3 increased the total and the female flower number at low concentration (40 mg l^{-1}) but decreased it at high concentrations (Banerjee and Basu, 1992). Higher levels of endogenous gibberellins have been found in monoecious and andromonoecious cucumbers than gynoecious cultivars, in parallel to their tendency to produce male flowers (Hemphill *et al.*, 1972). Environmental conditions that favored male flower development, such as high temperature and long daylength also increased the amount of gibberellins detected in the apical regions (Saito and Ito, 1963).

Flowering and Fruit Set

Flowering time

The time of flowering of the cucurbit vegetables is primarily determined by temperature, and its influence on plant growth rate. Temperature is also the principal factor determining the time of anthesis and duration of opening of individual flowers. Seaton and Kremer (1938) found that the flowers of *Cucurbita* had a minimum temperature for anthesis and anther dehiscence of around 10°C. Above this level, flowers would open at dawn, and remain open until about noon. Under cooler conditions, anthesis and anther dehiscence was delayed until the following day. As temperatures increased beyond 30°C, anthesis occurred earlier, and flowers closed by mid or late morning. This is similar to our observation in bitter gourd. In contrast, flower buds of bottle gourd open in the evening and wilt at

dawn (T.-W. Chen, unpublished data). The minimum temperature for opening of cucumber and watermelon flowers was found to be about 15°C, whereas muskmelon anthesis temperatures were between 18 and 21°C (Seaton and Kremer, 1938). The duration of flower opening for cucumber, watermelon, and muskmelon is generally for the entire daylight period of one day. The receptivity of the female flower, or of the female portion of perfect flowers of cucumber has been found to extend from two days before to two days after anthesis under growth chamber conditions (Le Deunff *et al.*, 1993). In the greenhouse, Munger (1988) reported that manual crosses were successful on the day of anthesis and the following morning, but under temperate field conditions, manual pollination success often decreased to low levels on the afternoon of the anthesis day.

Pollination

The cucurbit vegetables are among the vegetable crop species that require insects for pollination. This is most obvious for the crops that have separate male and female flowers, such as cucumber, watermelon, squash, and pumpkin. But even in muskmelon (*Cucumis melo*) which bears perfect flowers, pollinators are necessary.

The physiology of the plant may influence cucurbits' attractiveness to pollinating insects. For instance, the domestic bee *Apis mellifera* uses the flowers of cucurbits as both a source of pollen and of nectar (Free, 1970). The principal attraction in muskmelon flowers appears to be nectar. Plantings of a nectarless mutant had only sporadic visits by bees, and as a consequence, poor fruit set and low yields (Bohn and Mann, 1960; Bohn and Davis, 1964), even though pollen production by the mutant was normal. Similarly, accessibility of the staminal nectaries in *Cucurbita pepo* may influence the frequency of visits by honey bees (S.W. Cady, R. Glatz and H.C. Wien, 1994, Ithaca, NY, unpublished observations). Nectar production involves sucrose synthesis in the nectary parenchyma cell and secretion into extracellular space via a sugar transporter SWEET9 (Lin *et al.*, 2014). Several *SWEET* genes have been identified in cucurbits but their functions are not yet confirmed (Hu *et al.*, 2017).

Pollen germination and pollen growth

When pollen from the same plant or another plant of the same species is deposited on the stigmatic surface, pollen germination follows in less than 30 min. under normal conditions (Suzuki, 1969; Sedgley and Buttrose, 1978). Self-incompatibility is unknown in cucurbits, probably due to the switch between monoecy and dioecy (Renner and Schaefer, 2017). Optimal pH in vitro for pollen germination is between pH 8.0 and 9.0 in most of the cucurbit species except wax gourd, whose pollen shows the highest germination rate under pH 7.0. Cucumber pollen germinates over a wide range of temperatures, but pollen tube growth rates may be inhibited at the extremes, preventing fruitset (Matlob and Kelly, 1973). As temperature increased from 10 to 32°C, pollen tube elongation rate increased in snake melon (*Cucumis melo* var. *flexuosus*), but cucumber pollen tubes were only stimulated up to 21°C (Matlob and Kelly, 1973). There are also considerable genetic differences in pollen tube growth rates, ranging from 0.95–6 mm h^{-1}.

In some cucumber cultivars, the rate of pollen tube growth may not be rapid enough to allow fertilization of ovules over the entire length of the ovary (den Nijs and Miotay, 1991). At the same time as the pollen tubes are growing, the ovary is also elongating, and on some long-fruited cucumber cultivars, the middle and far end of the fruit may never be reached by the pollen tubes. As a result, the fruit enlarges at the blossom end, and relatively few seeds are formed (Varga and Bruinsma, 1990). The path of the pollen tubes to the ovary and ovules is primarily along the conducting tissue connecting the style and ovary, but once the ovary is reached, pollen tubes will also travel in the cavities between fruit lobes (Poole and Porter, 1933).

Fruit set and parthenocarpy

After fertilization, the developing gametophyte produces phytohormonal signals involved in fruit set and cell division. In many cucurbit vegetables, particularly in cucumber (Rudich *et al.*, 1977) and in *Cucurbita pepo* (Rylski, 1974; Robinson, 1993), fruit set and fruit growth are often achieved without pollination or the fertilization of ovules,

leading to so-called parthenocarpic fruit development (Li *et al.*, 2014). Many genes underlying the pathways of auxin, gibberellic acid, and cytokinin are related to parthenocarpic fruit set in different species (Joldersma and Liu, 2018), but in cucurbits, auxin is the most direct and evident phytohormone for fruit set. In general, the potential of fruit set is related to the ability of cell division and endoreduplication in the exocarp, pericarp, and placenta after anthesis. If cell division is interrupted, the fruit aborts. In cucumber, genes involved in cell division are highly expressed during the first two to four days after pollination (Fu *et al.*, 2010). Applying naphthaleneacetic acid (NAA), *N*-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU) and 24-epibrassinolide (EBR) on unpollinated cucumber ovaries at anthesis induces the expression of genes regulating cell division, therefore promotes parthenocarpic fruit growth. In contrast, GA_3 has restricted effects on these genes (Fu *et al.*, 2008, 2010). Transcriptome comparisons between parthenocarpic and non-parthenocarpic fruits also suggest the importance of maintaining cell division for fruit development (Li *et al.*, 2014). Since cell division is an energy-consuming physiological process, genes related to the breakdown of carbohydrates are also up-regulated during fruit set (Li *et al.*, 2014).

Coupling parthenocarpy with gynoecy is a main goal in cucurbit breeding. The tendency to set and develop seedless fruit is enhanced by cool weather conditions, and to a lesser extent, by short photoperiods (Rylski, 1974; Rudich *et al.*, 1977; Dean and Baker, 1983). In cucumber, parthenocarpic tendency is more strongly expressed in lines that have a higher proportion of female flowers. Even on monoecious lines, the tendency for parthenocarpic fruit production increases with plant age, as does the femaleness of the plant (Rudich *et al.*, 1977; Kim *et al.*, 1992).

There is considerable genetic variation in parthenocarpy in cucumber, and the characteristic has been viewed as a means of overcoming adverse environmental effects on fruit set or pollinating insects. In cucumber, it has been suggested that an incomplete dominant gene *P* regulates parthenocarpy, and parthenocarpic fruits were produced later in the heterozygous *Pp* genotype than in the homozygous *PP* genotype (Pike and Peterson, 1969). The potential of a node to produce parthenocarpic fruits has been linked to the endogenous level of indole-2-acetic acid (IAA), (Kim *et al.*, 1992). A recent study comparing endogenous auxins, cytokinins and giberellins levels and proteomics between a gynoecious parthenocarpic and a monoecious non-parthenocarpic cucumber line suggests a hormone-independent mechanism toward parthenocarpy (Li *et al.*, 2017). From a search in the cucumber genome database (Huang *et al.*, 2009) for genes expressed in the ovary, Li *et al.* (2014) suggested 14 putative genes related to parthenocarpy in cucumber.

The production of seedless fruits in watermelon does not occur without special measures, since naturally occurring parthenocarpy has not been reported in this species. Fruit set without seed set can be brought about by pollinating a self-sterile triploid watermelon with a diploid pollen parent (Kihara, 1951), by pollinating diploid cultivars with soft X-ray irradiated pollen (Sugiyama and Morishita, 2000) or by using growth regulators. Commercial production of seedless watermelon has been hampered by several factors. The abnormally thick seed-coat of the triploid seeds impedes germination and makes the use of transplants advisable. In addition, yields are decreased because one quarter of the field must be planted to a diploid pollinator cultivar. Consumer reluctance to accept that the small white empty seed-coats found in these parthenocarpic fruit are not seeds has also hampered acceptance of seedless watermelon cultivars. Pollinating pistillate flowers with intergeneric pollen has been used to produce seedless fruit. Pollinating watermelon with bottle gourd pollen induces 57% fruit set, close to the fruit set rate of self-pollination (65%, Sugiyama *et al.*, 2014). Sugiyama *et al.* show that pollen of luffa and bitter melon are able to reach the ovaries of watermelon, but unable to produce parthenocapic fruit. In contrast, pollen of bottle gourd is not able to induce fruit set of bitter gourd, luffa, and winter squash. Apparently, the phylogenetic relationships between watermelon and bottle gourd are close (Fig. 10.1). It would be interesting to test if parthenocarpy can be only induced by the intergeneric pollination of the closely related species.

Fruit development

The development of fruit in the cucurbit vegetables has been an object of fascination for many

years. Attention has focused on the large-fruited members of *Cucurbita pepo* and *C. maxima*, which can reach extraordinary size. Contests organized by pumpkin-growing clubs broke the record of 200 and 500 kg in 1976 and 1999, respectively (Fig. 10.4). Fifteen years later, a Swiss farmer broke the 1000 kg barrier, and fruit weights of 1190 kg have been achieved in 2016 in Belgium (http://www.giantpumpkin.com).

Such fruits are said to gain more than 20 kg, or about 800 g of carbon per day, while reaching the maximum fruit growth, but the physiological attributes needed to bring about such prodigious size increases have been largely unexplored (Savage *et al.* 2015). A recent study comparing two giant pumpkin varieties, "Atlantic Giant" and "Mammoth," with their small fruit progenitor Hubbard squash suggests that factors limiting fruit size are not photosynthetic capacity and leaf area but the ovary morphology to increase sink size and higher phloem area in the petioles to increase carbon transport (Savage *et al.* 2015). The ovaries of these giant pumpkins have more locules and larger cell diameter than Hubbard squash. Unfortunately, data related to other factors involved in fruit size (e.g. cell number or duration of cell proliferation) are not available from this study.

In *Cucumis melo*, differences in fruit size between genotypes and growing seasons are associated with the cell number in the pericarp, which is determined by the length of the cell proliferation stage (Higashi *et al.*, 1999). Later studies suggest that the expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene (*CmHMGR*) is involved in cell proliferation in the melon pericarp (Kato-Emori *et al.*, 2001; Kobayashi *et al.*, 2002). After cell division, cell enlargement contributes to the increase in fruit size. In watermelon (*Citrullus lanatus*), cell enlargement of the innermost fruit tissues continued until the cells had attained an astonishing 350,000-fold increase from their size at the end of the cell division stage. A gene involved in cell-size regulating pathways, *LITTLELEAF*, has been confirmed to affect the sizes of fruit, flower, and leaf (Yang *et al.*, 2018). Biophysical mechanisms related to fruit enlargement after cell division have been elucidated in grape (Zhu *et al.*, 2018), tomato (Cieslak *et al.*, 2016; Constantinescu *et al.*, 2016), and peach (Cieslak *et al.*, 2016), but not yet in cucurbits except a simple model proposed by Savage *et al.* (2015).

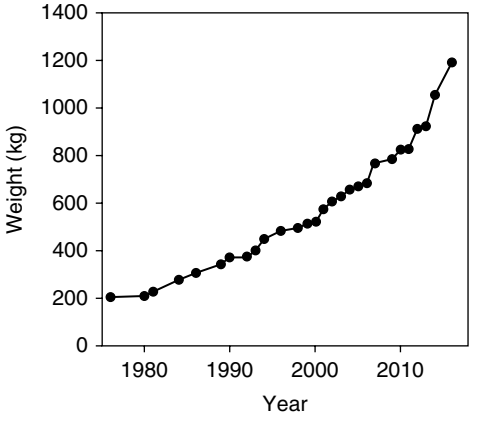

Fig. 10.4. World record weight of the pumpkin fruit (data from www.giantpumpkin.com).

Fruit growth in *Cucurbita pepo* and cucumber is characterized by an initial log-linear (exponential) phase, followed by a gradual growth rate decrease (Marcelis, 1992b), resulting in a quadratic relationship between growth rate and fruit size (Savage *et al.*, 2015). A comparison of cultivars ranging in fruit size from 40 to 7000 cm^3 indicated that fruit growth rates varied little, but the larger-fruited types had longer growth durations. Increase in fresh weight was closely related to volume growth, which in turn could be accurately predicted from the allometric relationships between volume, length, and circumference (Kahlen and Stützel, 2007). Fruit growth rates can be profoundly affected by the influence of the rest of the plant, and by environmental factors. Increasing assimilate supply, for example with higher irradiance, enhanced fruit growth rates (Marcelis, 1993). The higher assimilate levels resulted in increased number and size of fruit cells, if higher light was given early in fruit development. Later applications of high light increased cell size only.

To maintain the supply of carbohydrates during fruit growth, transport velocity in phloem, sieve tube area, and mechanisms related to phloem unloading in fruit are the key traits. A specific feature of the vascular system in cucurbits is the existence of two different phloem systems: a fascicular phloem located bi-collaterally within the main vascular bundle, and an extrafascicular phloem scattered in the stem. Differences in metabolome and proteome levels between these two phloem systems suggest that the

extrafascicular phloem functions in signaling transduction between organs and fascicular phloem is responsible for sugar loading into fruit (Zhang *et al.*, 2010). The principal translocated carbohydrate of the cucurbit vegetables has been suggested to be raffinose and polysaccharide stachyose, but sucrose can also compose 50% of the transport sugar in watermelon (Rennie and Turgeon, 2009). Upon reaching the fruit peduncle, this transport sugar is thought to be transformed into sucrose and hexose sugars in muskmelon and cucumber (Handley *et al.*, 1983; Hubbard *et al.*, 1989). In the phloem of cucumber fruits, sugars are unloaded into the surrounding apoplasmic pathway by sugar-specific transporters, for example sucrose transporter *CsSUT4*, located on the plasma membrane of the companion cell (Hu *et al.*, 2011). Gross and Pharr (1982) found that cucumber fruit peduncles contain the necessary enzymes to convert stachyose to sucrose. Peduncle extracts of *Cucurbita moschata*, watermelon, and muskmelon also had similar capabilities, but the responsible enzymes are still unknown (Hu *et al.*, 2011). After reaching the cell walls between companion cell and phloem parenchyma, sucrose is broken into hexose by cell wall acid invertase, or is transported directly into the phloem parenchyma through the sucrose transporter. In the early stages of growth, muskmelon fruit sucrose levels tend to be low, with soluble sugars made up almost exclusively of glucose and fructose (Hubbard *et al.*, 1989). During later stages of fruit growth, acid invertase activity drops, and sucrose phosphate synthase (SPS) enzyme activity increases (Hubbard *et al.*, 1989; Hu *et al.*, 2011). At the same time, sucrose levels also rise, until they make up nearly 50% of the fruit's soluble sugars. Hubbard *et al.* (1989) found that SPS activity correlated well with sucrose concentration at fruit harvest, when they compared melon cultivars with contrasting fruit sucrose content. Although reducing sugars make up between 2 and 3% of fruit fresh weight of cucumber and melon during development, starch levels of these fruits are less than 1% in both species (Schaffer *et al.*, 1987). It is therefore not possible to increase fruit sugar content of melons once the fruit has been detached from the plant (Bianco and Pratt, 1977). For optimum fruit quality, harvest should take place as close as possible to the time of maturity of the fruit.

The changes in carbohydrate content of the fruits of *Cucurbita* and of watermelon have not been intensively investigated. As in muskmelon, watermelon fruits show earlier increases in reducing sugars than in sucrose during development (Porter *et al.*, 1940). At maturity, the fruit typically has 10% total sugars, of which about 35% is sucrose. If the fruit is allowed to become overmature on the vine, or stored at room temperature, the proportion of sucrose increases to around 65% (Porter *et al.*, 1940). Interestingly, there is climacteric (e.g. melon) and non-climacteric (cucumber and watermelon) fruit ripening in cucurbits. A recent study shows that the molecular mechanisms controlling fruit ripening in melon is similar to that in peach and papaya, possessing a transcriptional feedback circuit triggered by ethylene (Lü *et al.*, 2018). In this circuit, ethylene activates the expression of the ethylene transcription factor EIN3 up-regulating the *NAC* gene. *NAC* further upregulates the fruit ripening gene and ethylene biosynthesis genes *ACC* and *ACO*.

Fruit dominance, competition and abortion

In indeterminate plant species, the influence of growing fruits on the development of younger fruit and further vegetative growth has been thought to limit yields. Within a single plant, the first fruit requires less time to reach a harvestable size than the younger fruit in gynoecious cucumbers (Wiechers *et al.*, 2011). In cucumber, McCollum (1934) showed the inhibiting influence was strongest in seeded fruits until the time the seedcoats hardened. Parthenocarpic fruits had a much weaker inhibitory effect. Denna (1973) extended these observations with comparisons of parthenocarpic glasshouse cultivars that were pollinated or allowed to set seedless fruits. Among 12 cultivars, the seedless plants produced 17% more fruit than their seeded counterparts. Denna also pointed out that seeds comprised a significant amount of the total plant dry weight, namely 10% for the glasshouse cultivars, and 20% for two pickling cucumber lines. From that, one would expect the yield depression from the first-formed fruits to be even stronger in seeded cultivars.

The causes for the inhibition have not been clarified due to the experimental difficulties, but

two major factors are thought to be involved. The first is competition for limited assimilates, and the priority of reproductive structures for these (Pharr *et al.*, 1985; Marcelis, 1992a). Pharr *et al.* (1985) calculated, for instance, that one actively growing cucumber fruit required the photosynthetic output of 40% of the plant canopy. During the growth of parthenocarpic cucumbers, the inhibition of vegetative growth is most marked during periods of rapid fruit growth (Marcelis, 1992a). The greater inhibitory effect of seeded fruits may be due to the fact that seeds contain 32% fat and comprise 20% of fruit dry weight (Denna, 1973) which require more assimilates to synthesize than carbohydrates. It has been conceptualized that the distribution of canopy assimilates among fruits in a plant would be proportional to the potential growth rate of the growing fruits (Marcelis, 1994), but a model in which this assumption was implemented did not match the observed abortion rate and growth duration of the individual fruits (Wiechers *et al.*, 2011).

The second is a dominance effect, implying that the hormonal factors from the older fruit inhibit the development of the younger fruit even before the canopy assimilate sources become limiting (Bertin, 1995). Schapendonk and Brouwer (1984) found, for instance, that just starving a cucumber plant of carbohydrates by defoliation will reduce fruit growth rate, but is not sufficient to cause the reproductive tissue to become necrotic. They used this as indirect evidence that hormonal influences are involved in the fruit-induced growth inhibition. It is also likely that hormonal activity in the developing fruit allows that organ to become a stronger sink for assimilates than other tissues. Although the responsible signal is still unclear, a recent study shows that the dominant fruit affects the metabolic and transcriptional programs 24 h after the pollination of the inhibited fruit (Shnaider *et al.*, 2018). Among the transcriptional changes of the inhibited fruit are those involved in gibberellin degradation, hormonal signaling, and, surprisingly, genes related to pollen development. This implies a potential effect of dominance on the fertilization success of the inhibited fruit.

Factors Affecting Productivity

Yield production in the annual herbaceous vegetable crops of the *Cucurbitaceae* is affected both by factors that influence overall biomass production, and those that determine the partitioning of assimilates to reproductive organs. As with other vegetable crops such as tomato and pepper, crop responses have been worked out in detail for glasshouse production systems, in which temperatures, light and CO_2 levels can be regulated. Accordingly, information is most complete for the climatic controls needed for optimum growth and yield of the gynoecious parthenocarpic cucumber grown in the glasshouses of northern Europe and North America. Productivity also involves issues of the timing and concentration of harvests. In pickling cucumber, where the fruits are harvested at a young stage, much effort has been expended to devise production systems and develop genotypes that give high yields in a short harvest period. Fruit quality is an important criterion in the production of muskmelon, watermelon, and winter squash. Production systems must provide conditions which allow fruit to develop acceptable sweetness and taste, and the size characteristic of the cultivar.

In the European production system, cucumbers typically are sown between January and April, and bear fruit during spring and summer. In winter production, supplemental lighting period of 20 h per day is typical to achieve year-round production, especially in the Scandinavian countries (Pettersen *et al.*, 2010). Besides the energy costs for supplemental lighting, the balance between the heating costs and optimal day/night temperature for plant growth is also an issue for off-season production. Considerable experimentation has determined that mean air temperatures of 18–24°C are optimum for greatest yield accumulation (Drews *et al.*, 1980; Liebig, 1980). As temperature increased, leaf appearance rate accelerated, and time to first harvest declined (Krug and Liebig, 1980). Plants started bearing earliest under high temperatures, but had a shorter harvest duration, and a reduced total yield (Liebig, 1980). Recent studies show that meristem temperature, instead of the air temperature, determines the leaf appearance rate (Savvides *et al.*, 2013; Savvides *et al.*, 2016). The deviation of meristem temperature from air temperature results from the cooling effects of the transpiration and is dependent on the morphological characteristics and of the young sprouts.

Variation of day and night temperature about the mean had no effect on earliness and

early yield of the cucumber cultivar "Farbiola" and "Corona" but higher day temperature accelerated the early fruit production in cultivar "Aramon" (Slack and Hand, 1980; Grimstad and Frimanslund, 1993; Papadopoulos and Hao, 2000). Usually, greenhouse cucumber plants are trained as single-stem system to facilitate fruit harvest. Short internode and stem length is therefore a characteristic of interest to produce more fruit under the limit of canopy height. Internode elongation is under the controls of light quantity (daily mean of photosynthetic active radiation, PAR), light quality (red:far-red ratio), and their interplays with air temperature (Kahlen and Chen, 2015). In cultivar "Aramon," internode length reduces by 1 cm per 70 μmol $m^{-2}s^{-1}$ increase in mean PAR 6-10 days before the internode has reached its maximum growth rate, probably close to the phase of cell division (Kahlen and Stützel, 2011). This study also suggests that low red:far-red ratio perceived by the whole stem around leaf initiation increases the internode length by up to 2.6 cm.

Earliness of yield production and marketable yield at a given air temperature can be boosted by increases in light levels (Liebig, 1980). This shows that the yield of greenhouse cucumber is often limited by the capacity of canopy photosynthesis, which acclimates to different light and temperature regimes. Canopy photosynthesis is determined by the size and the capacity of the photosynthetic apparatus, namely leaves. Although the fruits of *Cucumis* and *Cucurbita* have a significant chlorophyll content (252 and 850μg g FW^{-1}, respectively), their photosynthesis contributes only 1–5% of the total fruit carbon requirement (Aschan and Pfanz, 2003). The optimal leaf temperature for leaf photosynthesis rate under ambient CO_2 concentration is between 30–36°C in cucumber. Cucumber leaves developed under 15°C ambient temperature have their optimal temperature 3–6°C lower than those developed under 30°C, showing significant photosynthetic acclimation to temperature (Yamori *et al.*, 2010). Typically, leaf photosynthesis in a healthy and fully-expanded cucumber leaf under saturating light (about 1300–1500 μmol photon $m^{-2}s^{-1}$) and ambient CO_2 condition is between 20–27 μmol CO_2 $m^{-2}s^{-1}$. This corresponds to the biochemical capacity of the maximal electron transport and Rubisco carboxylation rates of about 170 e^- $m^{-2}s^{-1}$ and 140 μmol CO_2 $m^{-2}s^{-1}$, respectively (Chen *et al.*, 2014). These values show a significant plasticity to acclimate to light quantity (Trouwborst *et al.*, 2010, 2011; Moualeu-Ngangue *et al.*, 2017), light quality (Hogewoning *et al.*, 2010; Savvides *et al.*, 2012), and nutrient supply (Pao *et al.*, 2019). Since cucurbits are C_3 plants (except *Xerosicyos danguyi*, a Crassulacean acid metabolism species belonging to the Zanonieae clade) (Fig. 10.1) (Bastide *et al.*, 1993), CO_2 diffusion through stomata and mesophyll can restrict the instantaneous leaf photosynthesis rate of cucumber by more than 40%, depending on the environmental scenarios (Chen *et al.*, 2015; Moualeu-Ngangue *et al.*, 2016). Our data in cucumber, bitter melon, bottle gourd, pumpkin, zucchini and *Bryonia dioica* suggests that maximal stomatal conductance of healthy cucurbit leaves ranges from 0.5–1.2 mol $m^{-2}s^{-1}$ (Chen *et al.* unpublished), higher than many crop species (McAusland *et al.*, 2016). Many recent studies focused on the genetic variations in stomatal speed in reaction to fluctuating light conditions (Vialet-Chabrand *et al.*, 2017; Lawson and Vialet-Chabrand, 2019). Following the protocol of McAusland *et al.* (2016), we observed an overshoot of stomatal opening in reaction to a light signal of 1000 μmol photon $m^{-2}s^{-1}$ in all of the above mentioned cucurbits with a maximum rate of stomatal opening ranging from 0.12 mmol $m^{-2}s^{-2}$ in bitter melon to 0.49 in cucumber cultivar "Aramon" (Chen *et al.* unpublished).

Increasing ambient CO_2 level for glasshouse cucumber has become the standard practice particularly when glasshouse vents are closed. Concentrations of 700–1000 μl l^{-1} CO_2 are commonly used, and have been found to increase yields from 20–43% (Hand, 1984; Kimball, 1986). In situations where temperature control requires ventilation, CO_2 supplementation becomes more difficult. Under warm spring and fall conditions of North Carolina, Peet and Willits (1987) found that enrichment periods as short as 4.5 h still increased cucumber yields by 27%. A larger benefit could be obtained, however, by use of a passive rock storage cooling system that allowed the vents to be kept closed for more than 8 h per day.

Hydroponic systems are often used in greenhouse production of cucumber and melon. The presence of salt in the irrigation water, often in form of NaCl, and its accumulation in the hydroponic systems, can reduce the productivity of cucumber and melon, both classified as salt

sensitive species (Drew *et al.* 1990; Savvas *et al.*, 2005; Stępień and Kłobus, 2006) (and see Chapter 4). Grown under 50 mM NaCl, leaf area and shoot dry mass of cucumber cultivar "Aramon" was reduced by 56% and 53%, respectively, which resulted in a reduction of canopy photosynthesis by 52% (Chen *et al.*, 2018). The salinity effects on plant physiology can be classified in two categories: osmotic effects and ionic effects (Munns and Tester, 2008). In cucumber, the osmotic effects are essentially drought effects, which reduce organ expansion rate and stomatal and mesophyll conductance, resulting in a decreased photosynthetic apparatus and an increased stomatal and mesophyll limitation of leaf photosynthesis (Chen *et al.*, 2018). Under saline conditions, Na^+ and Cl^- enter the plant and their concentrations in the xylem sap increase. These ions are transported into the leaves with the transpirational stream and accumulate in the leaves. Excessive Na^+ accumulation in the leaf interferes with physiological functions and accelerates chlorophyll degradation (Shu *et al.*, 2012). Once the cells in the leaves become incapable of compartmentalizing Na^+ and Cl^- in the vacuole, chloroses or even necroses appear (Stępień and Kłobus, 2006; Munns and Tester, 2008).

Knowledge about the specific effects of Na^+ and Cl^- ions on physiological traits can only be obtained by using mixed-salt experiments, where the physiological parameters can be compared between NaCl, Na^+ dominant and Cl^- dominant treatments (Tavakkoli *et al.*, 2010, 2011). Treatments in a mixed-salt experiment are only comparable if the osmolarity and ion concentrations are equal for all treatments since differences in osmolarity affect ion fluxes into the shoots. For example, salt accumulation rates in leaves can be faster in cucumber plants grown under low than high salinity because a higher transpiration rate under low salinity increases the total ion flux into the leaves due to the smaller osmotic effects on stomatal conductance (Drew *et al.*, 1990). As a consequence, photosynthesis could be more restricted under low than high salinity due to the stronger ion toxicity (Drew *et al.*, 1990). Furthermore, salt concentration in the nutrient solution also affects the salt accumulation rate in leaves due to the non-linear relationships between ion concentrations in the nutrient solution and in the xylem sap (Munns, 1985). In many mixed-salt experiments either equal osmolarity (Colla *et al.*, 2012, 2013) or equimolar ion concentrations (Hajrasuliha, 1980; Martin and Koebner, 1995; Tavakkoli *et al.*, 2010) were used. Therefore, conclusions drawn from these experiments could be biased due to the differences in ion accumulation rates between treatments.

To improve cucumber performance under salinity, genotypic variation in physiological responses to salinity has been studied (Tiwari *et al.*, 2010). Beside the search for genetic material for breeding salt-tolerant cultivars, some horticultural techniques, for example, grafting on salt-tolerant rootstocks (Davis *et al.*, 2008; Edelstein *et al.*, 2011; Colla *et al.*, 2012; Huang *et al.*, 2013), CO_2 enrichment (Garcia-Sanchez and Syvertsen, 2006), additional nutrient supply (Dabuxilatu and Ikeda, 2005; Abdolzadeh *et al.*, 2008) or artificial alteration of light conditions in the greenhouse (Garcia-Sanchez and Syvertsen, 2006; Fini *et al.*, 2014), have been proposed to improve salinity tolerance of horticultural crops. Among these horticultural approaches, grafting cucumber onto pumpkin was demonstrated to be the most successful method to improve salinity tolerance (Mavrogianopoulos *et al.*, 1999; Colla *et al.*, 2012, 2013; Huang *et al.*, 2013). The physiological mechanisms of salinity tolerance acquired by grafting are still not well understood, but of the several mechanisms that have been identified so far, the restriction of Na^+ uptake by the roots of pumpkin is most evident (Edelstein *et al.* 2011; Huang *et al.*, 2013). Our experiment of grafting cucumber "Aramon" onto pumpkin cultivar "Becada" suggests that grafting enhances K^+ transport toward the young leaves and Cl^- transport toward the old leaves (Chen *et al.* unpublished). Furthermore, the pumpkin cultivar "Becada" excludes Na^+ perfectly under 60 mM NaCl so that the Na^+ concentrations in the leaf of "Becada" and of the grafted "Aramon" were not different from those grown under non-saline condition (Chen *et al.* unpublished). Since Na^+ inactivates enzymes in the leaf cell and interferes with physiological functions controlled by K^+, for example stomatal regulation (Chen *et al.*, 2015), grafting onto pumpkin improves K^+ homeostasis and thus leaf functions in cucumber under salinity. Nevertheless, pumpkin roots do not exclude Cl^- so that cucumber plants grafted onto pumpkin rootstocks have Cl^- contents similar to non-grafted plants (Colla *et al.*, 2012, 2013). In our previous experiments with cucumber grafted onto pumpkin rootstocks we did not find

evidence supporting that Cl^- would be accumulated to a toxic level in leaves because cucumber seems to have mechanisms avoiding over-accumulation of Cl^-. In two cultivars, "Aramon" and "Line-759," Cl^- accumulation rates were close to zero between 8 and 15 days after being grown under 60 mM salinity while their Na^+ accumulation rates were maintained similar to those between one and eight days after treatment (Chen *et al*. unpublished).

Physiological disorders

Flower gender imbalance

Under the cool conditions prevalent in temperate regions during planting, the development rate of male flowers of *Cucurbita pepo* is inhibited more than that of female flowers (Rylski and Aloni, 1990). This can result in the precocious development to anthesis of female flowers, and a lack of fruit set because of a dearth of open male flowers. The problem is especially pronounced in some hybrid cultivars of summer squash that flower early in the season. Applications of GA_{4+7} as flower buds become visible can hasten male flower development to anthesis, indicating that flower development may be under similar hormonal control as flower differentiation in cucurbits.

At high growing temperatures (e.g. 32/27°C), female flower expression and development is inhibited in *C. pepo* (Wien, 2006), a tendency mirrored by pumpkin plants grown in temperate and sub-tropical conditions (Wien *et al*., 2004). Some cultivars were less susceptible to delayed fruiting at high temperatures. The increase in male expression in warm conditions can also lead to formation of stamens in female blossoms and retention of petals on developing fruits of squash (Penaranda *et al*., 2007).

Sudden wilt of melons

The disorder is also called vine collapse, crown blight and late collapse by researchers in different parts of the world, and refers to the rapid wilting of plants just as the fruits are beginning to develop netting, and the vines have covered the ground (Martyn, 2007). Within days, the entire field may be affected, and vines may not recover. A range of pathogenic organisms have been associated with the disorder, and may be the primary causes of the observed symptoms. The causal organisms associated with the disease differ in different melon-growing regions. For instance, in New York, cucumber mosaic virus (CMV) and *Fusarium* wilt have been implicated (Zitter, 1995). In Texas and the American southwest, *Monosporascus cannonballensis* has emerged as the major causal agent of vine decline of both muskmelons and watermelons in the last 20 years (Martyn and Miller, 1996; Martyn, 2007). In Israel, Nitzany (1966) caused vine collapse under cool conditions by inoculating with CMV and *Pythium.*

The presence of rapidly growing fruits on the plants appeared to be key to development of the vine collapse symptoms on the plants in a number of these cases. Periods of cool, cloudy weather, followed by hot, sunny conditions increased incidence of the disorder (Zitter, 1995). In areas of persistent warm weather, melon plants with small root systems restricted by transplanting from small cells were more susceptible to the disorder than direct-seeded crops (Martyn, 2007). Although direct experimental evidence is lacking, it is thought that during the rapid fruit growth phase, demand for assimilates of the growing fruit is so high that root growth is reduced. If at the same time, pathogens attack the root system, or reduce the plant's capacity to produce assimilates, root death may result. A third factor that could further exacerbate the situation is adverse weather conditions that would reduce photosynthetic rates and reduce root function through waterlogging of the soil. Grafting melon transplants onto roots resistant to the pathogens has provided a way to avoid this serious disorder (Su and Lin 2008; Gisbert *et al*., 2017).

Hollow heart of watermelon

This disorder is characterized by the separation of the inner parts of the fruit into distinct segments, leaving hollow areas at harvest maturity. Hollow heart occurs more often in the first-formed fruit on the plant, as a result of excess nitrogen fertilization, and delayed harvests (Kano, 1993). The disorder is more prevalent under conditions of rapid fruit growth rate, when the rind is expanding more rapidly than the inner regions of the fruit (Sinnott, 1939; Kano, 1993). Ways of avoiding the condition include selection of less susceptible cultivars, and using cultural practices that moderate fruit growth rate and final fruit

size. These include adequate plant populations, moderate levels of nitrogen, and prompt harvests. In fields of seedless watermelons, having pollinizer plant frequency of 20 to 33%, and choice of pollinizer cultivar reduced incidence of hollow heart (Fiacchino and Walters, 2003).

Bitter fruit in summer squash

The sporadic occurrence of bitter fruit in plantings of zucchini and other summer squash types has caused serious medical problems in a few cases. The ingestion of as little as 3 g of such fruit can cause nausea, stomach cramps and diarrhea (Herrington, 1983). Consumption of bitter squash was responsible for 22 cases of food poisoning in Australia, and occasional similar incidents in the United States (Herrington, 1983; Rymal *et al.*, 1984) and one case of death in Germany has been reported in 2015. The bitterness is caused by cucurbitacins, tetracyclic triterpenes that occur naturally in the family *Cucurbitaceae* (Rymal *et al.*, 1984). These compounds can occur in all parts of the plant, although concentrations tend to be highest in the roots (Rehm *et al.*, 1957, Rehm and Wessels, 1957). Plants may have intensely bitter fruits, but non-bitter leaves or cotyledons (Rymal *et al.*, 1984). Concentrations of cucurbitacins may be several times higher in the placental region of the fruit, compared to the pericarp or the rind (Jaworski *et al.*, 1985). Thus the bitter fruit of summer squash would be potentially more dangerous than those of mature squash or pumpkin, in which the placenta is not eaten.

The origin of these occasional plants producing bitter fruit is not exactly known, but they are thought to have arisen from chance outcrosses to bitter-fruited wild or ornamental gourds during seed production, or through mutations. In some cases where bitter fruit could be traced to individual plants, the plant and mature fruit characteristics did not match that of the cultivar, indicating that genetic change had occurred (Rymal *et al.*, 1984).

Concluding Remarks

Some aspects of cucurbits physiology, e.g. pathogen resistance, grafting and quality, are not included in the current chapter due to the limited space, but they are covered by the excellent reviews published recently (Olczak-Woltman *et al.*, 2011; Colla *et al.*, 2017; Gur *et al.*, 2017;). The cucurbit vegetables are a unique group of species that have fascinated plant researchers for many years. Not only the fruit size, the fruit shape, striping patterns and morphological diversity on the fruit surface are also fascinating in cucurbits. By virtue of their large seeds, they begin growth rapidly, and achieve an efficient light intercepting plant canopy earlier than most herbaceous plants. They are aided in this by large, planophile leaves borne on rapidly growing stems, even though assimilation rates are not higher than in most other herbaceous crops with C_3 assimilatory pathway (Bruggink and Heuvelink, 1987). Reproductive growth has received much attention in these crops, particularly the factors determining cucurbits follow the gender of the flowers. In spite of the revealed genetic networks in cucumber and melon, there is still no direct evidence showing that other cucurbits follows the same genetic mechanisms of sex determination. Dioecious species in the genus *Coccinia*, close relatives of *Cucumis* (Fig. 10.1), possess heteromorphic or hormomorphic sex chromosomes (Sousa *et al.*, 2017) and might have differences in sex determination from cucumber and melon.

A greater understanding of the mechanisms of sex expression in watermelon, bottle gourd, and bitter melons may need to be preceded by the discovery of a wider range of flower genotypes similar to those that aided the investigations of cucumber sex expression physiology, or by searching for orthologues in the cucurbit genome database. A missing piece of the puzzle remains the epigenetic controls of female flower induction in the monoecious cucurbits (Fig. 10.3B). Rapid growth also characterizes the development of the fruit of many cucurbit vegetables. While the giant pumpkin cultivars of *Cucurbita maxima* present the most striking example of this, with growth rates of more than 20 kg (fresh weight) per day, glasshouse cucumber fruits have been shown to gain more than 200 g per day for short periods (Marcelis, 1993). Such a massive transfer of assimilates to one or more reproductive structures on the plant make it likely that growth of other plant parts will be curtailed during this period, for example, abortion of the fruits growing at the closest node (Wiechers *et al.*, 2011). This is probably the reason why cucurbit vegetable crops are known for the strong inhibition of vegetative,

and particularly root, growth after flowering, leading to a cyclic development of fruits (e.g. in cucumber, bottle gourd and bitter gourd, Chen *et al*., unpublished data). The factors that control the movement of assimilates to fruits, rather than to other parts of the plants are at present poorly understood. Hormonal signals presumably make the developing fruit a strong sink for assimilates, but the nature and control of these hormonal signals needs more investigation. Current advances in genomics allow cost-efficient genotyping. The next challenge is to phenotype different genetic materials (Tardieu *et al*., 2017). This will be a difficult task in the next ten years, especially in horticultural crops such as cucurbits which are large in plant size. Further work is also needed to compare the capacity of the cucurbit plant to produce assimilates with the demands of assimilatory products by the rapidly growing fruits. Such studies in pumpkin (Savage *et al*., 2013, 2015) need also to be done with other cucurbit vegetables, to understand the limits of fruit productivity, both in terms of fruit yields and of fruit quality factors such as sweetness. It may be that higher yields of fruits with acceptable soluble solids can only be achieved by improvements in the rates of photosynthesis, or more efficient respiration and translocation mechanisms. Our unpublished data suggested that nitrogen partitioning between photosynthetic and non-photosynthetic proteins in the leaf determine the genotypic differences of potential of photosynthetic capacity in cucumber, but we have much to learn before such statements, especially that about the relationship between photosynthesis and fruit quality, can be made with certainty.

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11 *Phaseolus* Beans[1]

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Phaseolus beans are grown as green vegetables and/or pulses. The most important species is *P. vulgaris* L. (2n = 2x = 22), the common or green bean. It is a major food legume for human consumption worldwide for its edible seeds and pods. It is also an important source of protein (about 22%), vitamins (folate), and minerals (Ca, Cu, Fe, Mg, Mn, Zn) in seed for human diets especially in developing countries in the tropics (Beebe, 2012). It is used for canning and freezing as well as the fresh market. Pods of green beans are prepared whole, sliced or cut, and the seeds may be harvested green as a vegetable (e.g. flageolet), or dry as a pulse. Green pods are a superior source of Ca, Fe, thiamin and niacin.

Pods are slender, green, yellow, black or purple in color, sometimes striped. They can be cylindrical or flat, straight, or curved, 1.0 to 1.5 cm wide and up to 20 cm in length and contain 4 to 12 seeds. The seeds are 0.5 to 2.0 cm long, kidney shaped, and highly variable in color (white, red, green, tan, purple, gray, or black) depending on the variety. For whole beans, there is a trend particularly in Europe to develop varieties which produce a large proportion of very fine (6.6–8.0 mm diameter) or extra-fine (< 6.5 mm) pods. Runner beans (*P. coccineus*) are grown in northern Europe mainly for the fresh market, and used as cut green beans.

Phaseolus beans have a wide diversity of seed and pod types, plant habits from bush to climbing bean, range of maturities, photoperiod sensitivity and neutrality, adaptation zones, wide range of disease and stress resistances and different nutritional quality components. Breeders use this genetic variability for crop improvement. Beans are a staple in developing countries in East Africa and Latin America (and also in some regions of Asia), where it is the main source of protein since it can account for up to 20% of the total daily protein intake per person. In Europe, there has been a notable increase in bean consumption in past years, due to a greater demand for healthy and functional food, and the current trend of vegetarian diets in Central Europe and the United Kingdom, in which beans and other pulses are included as meat substitutes. As part of the promotion on pulses during the International Year of Pulses in 2016, the Food and Agriculture Organization (FAO) recommended that beans and other food legumes be eaten daily as part of a healthy diet to prevent and manage chronic disease, and to address growing global obesity issues (fao.org/pulses-2016).

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 Physiology of Vegetable Crops 2nd edition (eds H.C. Wien and H. Stützel)

The genus *Phaseolus* includes five cultivated species (Debouk, 1999) but the common bean (*P. vulgaris*) is the most widely grown and the most economically important worldwide. *P. vulgaris* includes all dry edible bean seed types—pinto, kidney, navy beans, and different names for garden beans such as runner beans, string beans, half-runners, snap beans, French and haricot beans. The other four types include: the scarlet runner bean (*P. coccineus*, known for its red/scarlet flower color); year-long bean (*P. dumosus,* aka *P. polyanthus*, name changed in 1995); tepary bean (*P. acutifolius*); and lima bean (*P. lunatus*).

There are more than 30,000 domesticated and over 1000 wild accessions of common bean housed in the germplasm collection at Centro International de Agricultura Tropical (CIAT), Cali, Colombia. Domestication occurred in at least two major centers, the Andean and the Middle American, resulting in two major gene pools (Singh, 1992). The Andean gene pool is substantially less variable than the Middle American gene pool (Blair *et al.*, 2007). Within these gene pools are a total of seven races, including four Middle American (Mesoamerica, Durango, Jalisco, Guatemala) and three Andean (Peru, Nueva Granada, Chile). The complementarity of races and their contribution as sources of traits has emerged as an underlying theme of genetic improvement.

Common bean was introduced into Europe after 1492. Upon its introduction onto the Iberian Peninsula from the Americas, hybridization of the Andean and Middle American gene pools created novel genetic variation and Europe is considered as a secondary center of diversity for common bean (Angioi *et al.*, 2010). Europe's landraces and historical varieties of this species constitute a cultural good and a promising resource for genetic improvement.

The genus *Phaseolus* is characterized by adaptation to an extremely wide range of ecological niches, from humid rainforests to arid deserts (Freytag and Debouck, 2002). *Phaseolus* beans are cultivated over a wide range of climates from northern Europe and America to the tropics of America, Asia, and Africa. *P. vulgaris* is the most widely adapted species, *P. coccineus* being adapted to cooler climates and *P. lunatus*, and *P. acutifolius* to warmer climates. Related species are an attractive option for traits to broaden the genetic base of common bean, especially for adaptation to more extreme environments (Souter *et al.*, 2017). Species that can be crossed to *P. vulgaris* cover most of this ecological range, and the secondary gene pool (*P. dumosus* and *P. coccineus*, and wilds in *sensu strictu*, *P. costaricensis*, and *P. albescens*) and tertiary gene pool (*P. acutifolius*, *P. parvifolius*) and these two gene pools represent an additional and important genetic resource for the crop improvement. *P. lunatus* is the fifth domesticated species within the genus. It is classified into a quaternary gene pool in relation to *P. vulgaris*, and cannot be crossed with common bean.

In 2014, total world production of green bean was 21.7 million tons, harvested from 1.5 million ha with an estimated gross production value of US$8,815 million compared with the production of dry beans of 26.5 million tons, harvested from 30.6 million ha with an estimated gross production value of US$13,687 million (FAOSTAT, 2017). Its production spans from 60°N to 32°S latitude and from near sea level to elevations more than 3000 meters above sea level. Its symbiotic nitrogen fixation (SNF) ability is both of agricultural and ecological significance.

Beans have been used extensively as a convenient plant for physiological and biochemical research. At the whole-plant level, there has tended to be more work done on dry beans than green beans, because of their importance worldwide as a food crop and protein source. As a green vegetable, however, beans are also of major importance worldwide, probably second only to peas.

During the last two decades, researchers have focused on elucidating the various physiological and molecular components underlying abiotic stress responses of common bean (Rao, 2014; Araujo *et al.*, 2015). Although physiological studies provided a general overview of plant responses, there is need to further dissect, and eventually profit from, the mechanisms underlying plant adaptation to abiotic stresses (Mir *et al.*, 2012; Diaz *et al.*, 2018; Assefa *et al.*, 2019). This will lead to: (a) identifying stress tolerance-related traits; (b) elucidating the genetic basis of key traits (as major responsible genes or associated quantitative trait loci, QTLs); (c) integrating molecular biology and genomics approaches; and (d) generating better performing cultivars through breeding. Equally important will be to advance the understanding of the plant growth and yield responses to the combination of multiple abiotic stresses (Mittler and Blumwald, 2010; Yang *et al.*, 2013; Lynch, 2019).

Germination and Seedling Emergence

The seed size of *Phaseolus* varies considerably, from less than 130 mg in wild or non-cultivated types, up to 1000 mg or more, but most commercial cultivars of *P. vulgaris* have a seed size in the range of 200–350 mg. Cultivars of *P. coccineus* tend to have larger seeds.

Germination of *P. vulgaris* is epigeal, whereas *P. coccineus* is hypogeal. Both species germinate in about six to eight days under optimum conditions. Mature seeds do not normally show any period of dormancy. Water is imbibed through the micropyle, the raphe and the hilum, but uptake through the seed coat is negligible (Korban *et al.*, 1981). Seed germination begins when the seed takes in water rapidly, causing the inner layers to swell and split the seed coat and other coverings. The radicle then emerges and starts its downward growth into the soil. The hypocotyl elongates and straightens, raising the cotyledons above the ground. As the epicotyl begins to lengthen and straighten, the first leaves, called plumules, emerge.

Salinity adversely affects germination and seedling growth in beans. Cachorro *et al.* (1994) found that this effect could be reduced by adding calcium to the soil. The main effect of salinity seemed to be on K concentration and its transport from root to shoot. Increase in salinity from 0 to 180 mM of NaCl decreased germination of *Phaseolus* species by 50% (Bayuelos *et al.*, 2002). Salt stress is first perceived by the root system and it impairs plant growth by inducing an osmotic stress due to reduced water availability and ion toxicity due to solute imbalance in the cytosol. Greater salt tolerance in green bean cultivar "Corallo" was due to its capacity for Na retention in the roots and maintaining appropriate K/Na and Ca/Na ratios, limiting the accumulation of toxic ions into actively growing shoots (Assimakopoulou *et al.*, 2015).

Vegetative Growth

Growth of leaves and biomass

The development of bean (determinate and indeterminate plant types) passes through two main stages of vegetative (V), (7 to 40 days) and reproductive (R), (40 to 94 days) growth depending on the genotype and its growing conditions. Vegetative stages are determined by counting the number of fully expanded trifoliate leaves on the main stem while the reproductive stages are described by pod and seed characters. The first pod developing on the plant is described and followed to full size. At the time of first bloom (R stage), secondary branching begins in the axis of lower nodes which will produce secondary groups of blooms or pods. To determine the growth stage, the main stem is followed, which is readily discernible on both determinate and indeterminate plants. A trifoliate is counted when it is fully unfolded (Kandel, 2010). Briefly, V1 is emergence, V3 is first trifoliate leaf, V4 is third trifoliate leaf, R5 is preflowering, R6 is flowering, R7 is pod formation, R8 is pod filling, and R9 is physiological maturity. Under optimal conditions, the bean crop grows nearly exponentially until pod growth begins. Leaf area index (LAI) in *P. vulgaris* increases to about 40 days after emergence (DAE), depending on the cultivar (Fig. 11.1), and then declines with seed filling (50–65 DAE), as photosynthates and N are translocated to the developing seeds. Leaves at the lower nodes die first, followed by leaves higher up the main stem and later on the branches.

Virtually all (> 95%) of the incoming radiation is intercepted by the bush bean canopy when the LAI reaches 4 or more (Aguilar *et al.*, 1977). The net assimilation rate (NAR, which is equal to crop growth rate, CGR, divided by LAI) declines as the LAI increases to its maximum value at about the time when pod growth begins.

The specific leaf area (SLA = leaf area/leaf dry weight) increases to a maximum of about 600 $cm^2\ g^{-1}$, shortly after flowering, declining back to where it started at about 400 $cm^2\ g^{-1}$ (White, 1981).

Bush beans develop a rather shallow root system, the bulk of the roots growing in the top 20–30 cm and in a radius of 45–70 cm. This makes the plants generally susceptible to nutrient or moisture deficiency even over relatively short periods of time. Modern green bean cultivars ideally have highly concentrated flowering and pod set, so that they have relatively less ability to recover from any setback in vegetative growth which may occur due to stress.

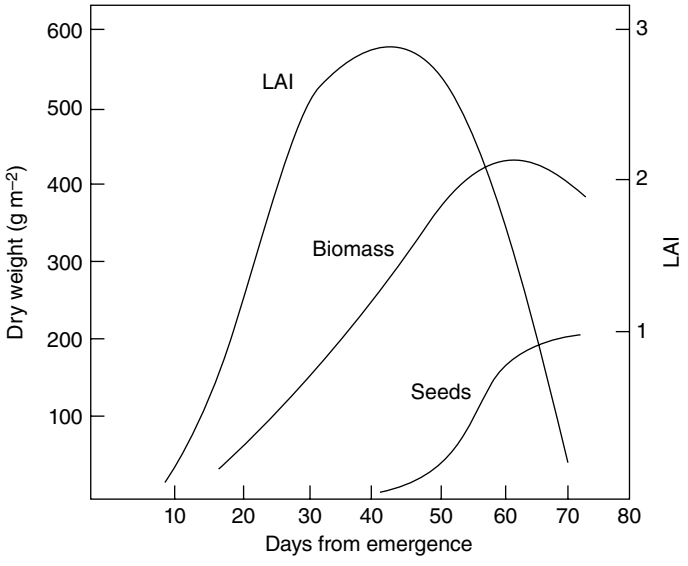

Fig. 11.1. Growth of LAI, biomass, and seeds in *P. vulgaris* cv. "Porrillo sintetico" (Laing *et al*., 1984).

Growth habit

A key driver of bean crop growth is interception of light by its canopy and its use in carbon assimilation to accumulate dry matter. Leaf area development is critical for light interception and entails several related physiological processes such as leaf addition, expansion, and senescence. These processes could vary by genotype and are affected by management factors such as sowing density. In common bean, higher sowing densities tend to limit individual plant branch formation, and consequently individual plant leaf area and vegetative mass (Ricaurte *et al*., 2016).

Beans are commonly classified into bush, half-runner and pole types. More detailed growth habit descriptions have been provided by Debouck and Hidalgo (1984), and their classification is illustrated in Fig. 11.2. Virtually all green bean production for processing is done with type I bush beans, because they provide the concentrated pod set required for machine harvesting. It has been possible to develop suitable type II (erect indeterminate bush) varieties for commercial dry bean production, and these tend to be higher yielding than type I beans in some environmental conditions. Type III (sprawling, indeterminate) cultivars are also used for dry bean cultivation, particularly in areas where drought stress frequently affects the crop, for example in Mexico.

Climbing beans (type IV) have the highest yield potential, and are grown on poles or trellis, or in some tropical countries they are intercropped with maize. Climbing is achieved by twining, or circumnutation, of the climbing shoot up a support. Aluminium ions applied to excised shoots at concentrations of 4–8 mmol aluminum chloride have been found to significantly slow the rate of twining without affecting the growth rate (Badot *et al*., 1993). Kretchmer *et al*. (1977) found that the climbing response is controlled in some cultivars by phytochrome which is unrelated to the photoperiod flowering response.

Reproductive Growth

Induction of flowering

Reproductive growth in beans is initiated by flowering and a period of pod and seed set. The number of pods or seeds that a bean crop community produces is determined during this period, and this number determines, in large part, the final pod or seed yield. At CIAT, Colombia, over 4000 accessions from the world germplasm collection of *P. vulgaris* have been screened for photoperiod response, and of these 39% were day-length neutral but the distribution of photoperiod response varied among growth habits and seed size

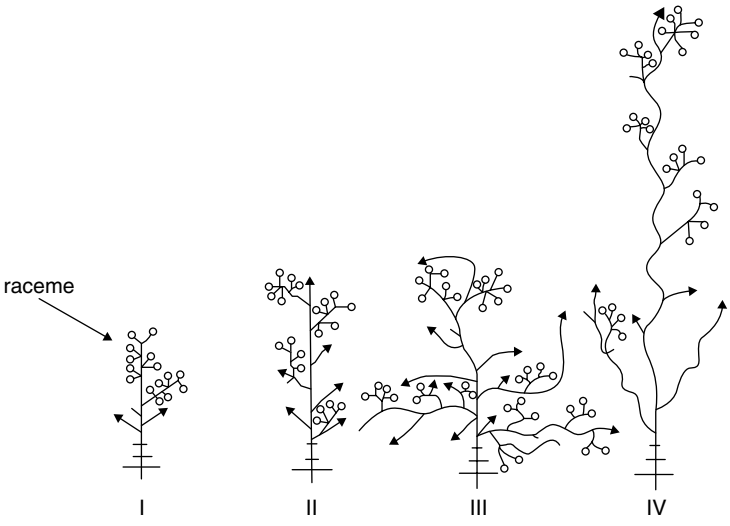

Fig. 11.2. Growth forms in *Phaseolus*: (I) determinate bush, (II) indeterminate bush, (III) indeterminate, straggling, and (IV) indeterminate, climbing (adapted from Debouck and Hidalgo, 1984).

classes (Masaya and White, 1991). Little screening of other *Phaseolus* species has been done, but it is likely that the primitive condition in the genus is for short-day adaptation, with day-neutral genotypes being selected as part of the domestication process and further enhanced by modern breeding.

Short-day plants have a quantitative response to photoperiod which is affected by temperature (Gniffke, 1982). There is a tendency for a higher proportion of climbing cultivars to be photoperiod sensitive, and Coyne (1967) found a genetic linkage between indeterminate growth habit and photoperiod response. It appears there are two major dominant genes for photoperiod sensitivity, with modifier genes controlling a quantitative response (Wallace, 1985). There is no juvenile phase in beans, the plants being equally sensitive to photoperiod at all stages of vegetative growth (Zehni *et al.*, 1970).

The effect of increasing temperature in day-neutral cultivars is to reduce the number of days to flowering. By contrast, the effect of increasing temperature is to enhance the photoperiod response in short-day plants, further delaying flowering (Enriquez, 1975). Temperatures of the rooting zone and subsoil may be key factors in determining days to flowering, seed germination, and tap or lateral root formation. Recently, Bhakta *et al.* (2017) found that temperature, solar radiation, and photoperiod play major roles in controlling flowering time in common bean.

Pollination and pod growth

Bees are essential for achieving pod-set in *P. coccineus* but not in *P. vulgaris* which is self-pollinating. The flowers of *P. coccineus* are larger and contain more nectar than those of *P. vulgaris*: 5.7–8.2 mg nectar containing 28–37% sugar for *P. coccineus* compared with 2.0 mg nectar containing 43% sugar for *P. vulgaris* (Wroblewska, 1993).

The anthers dehisce in the bud just before it opens, usually at night. Once the pollen reaches the stigma, the pollen tubes grow down the hollow style and fertilize the ovules within 12 h, the ovules nearest the style being fertilized first.

Commercial varieties of green beans (*P. vulgaris*) take about 25 days from pollination to the stage at which the green pods are ready for harvesting, when they are approaching their maximum length and fresh weight (Fig. 11.3). Thereafter the seeds continue to develop for another 20–30 days by which time the pod is ripe and the seeds are dry.

The distribution of pods on the plant depends on the cultivar and its growth habit. In determinate cultivars (type I), flower primordia are formed on the raceme in the axil of the uppermost

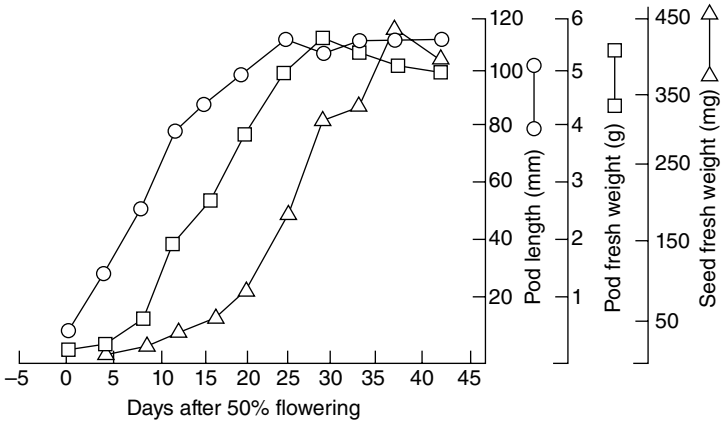

Fig. 11.3. Pod and seed growth of *P. vulgaris* cv. "Black Turtle Soup" (adapted from Izquierdo, 1981).

leaf of the main stem first, and flowering then proceeds downwards to the lower nodes and along the branches (Ojehomon, 1966). By contrast, in type II cultivars such as "Porrillo sintetico" the first flowers to open normally arise from nodes 6 to 7, and flowering then proceeds upwards and downwards on the main stem and along the branches (Laing *et al.*, 1984).

The typical commercially grown crop (type I or II) is of relatively short duration and does not have time to develop a large LAI before pod set begins. Evidence suggests that the first formed reproductive structures have a strong competitive advantage for the supply of assimilates. There is a tendency, therefore, for almost complete pod set from the first-opened flowers, followed by a high rate of abscission of later-formed flowers. Even in optimum conditions about 60–70% of the flowers and young pods are shed. Bean cultivar is conservative in the allocation of its resources, giving precedence to the survival of a limited number of pods over a short space of time. This tendency ensures a more uniform pod set and has been selected for in modern cultivars.

Factors Limiting Growth and Yield

After photosynthesis and respiration, the extent of senescence and abscission are major determinants of final yield. Failure of pod set, which might be caused by high temperature at pollination, can cause flowers to abort. Only one ovule needs to be fertilized to prevent pod abscission (Halterlein *et al.*, 1980). Older pods may drop if there is an inadequate supply of photosynthate (Tanaka and Fujita, 1979). The amount of carbohydrate stored in the stem at flowering varies considerably, even in the same cultivar, and Adams *et al.* (1978) found differences between genotypes in both the amount of carbohydrate stored and its mobilization after flowering. This could be related to the incidence of abscission and the ability of the plant to tolerate stress.

The occurrence of senescence and abscission appears to depend mainly on the source–sink balance in the plant, such that tissues which are at a competitive disadvantage are eliminated. There are likely to be other mechanisms also at work, such as endogenous growth regulators (White and Izquierdo, 1991). Key factors influencing the supply side of the source–sink balance are light, temperature, and water and N supply.

Light

In a high radiation environment, the maximum net photosynthesis of the canopy has been found to be 35–40 mg CO_2 dm^{-2} h^{-1} at saturating global solar radiation values of 600–650 W m^{-2} and LAI values of 4.5 (Sale, 1975). However, the maximum photosynthetic rate at leaf level can be reached at lower levels of solar radiation of 300 W m^{-2} (White and Izquierdo, 1991). Penetration of light

through the canopy is critical and it depends to a large extent on the orientation and distribution of the leaves. The pulvinus of the bean leaflets and the trifoliate leaf structure allows the bean plant to orient its leaves in relation to the sun and maximize canopy light interception (Wien and Wallace, 1973). At higher plant densities, types I, II and III growth habits reach close to 100% light interception at flowering time (Ricaurte *et al.*, 2016).

Temperature

Changes in temperature affect development during the reproductive growth stage and high temperatures during the flowering stage lead to abscission of flowers and a low pod set, resulting in yield loss (Porch and Hall, 2013). Day temperatures higher than 30°C or night temperatures higher than 20°C could result in yield reduction. Day temperatures below 20°C will delay maturity and cause empty mature pods to develop. Reproductive responses are all related to phenological development, plant–water relations, photosynthetic parameters, and shoot growth. High temperature affects many physiological processes, including photosynthesis and allocation of photosynthates to reproductive structures.

Various authors reported on the effects of high temperature on common bean during and after anthesis, and during day and night hours (Omae *et al.*, 2012; Porch and Hall, 2013). Beans are very sensitive to high temperatures above 30°C at flowering time, showing increased abscission of flower buds, flowers, and young pods, and poor fertilization and seed development in the pods. Greater tolerance of high temperatures would be very desirable and there is some evidence for genetic variation (Ofir *et al.*, 1993; Weaver *et al.*, 1985; Monterroso and Wien, 1988; Porch and Hall, 2013).

In the snap bean cultivar "Tenderette," all reproductive parameters (pods and seeds per plant; pod length and weight) declined when night temperatures reached 27°C (Konsens *et al.*, 1991). These effects were aggravated by day temperatures above 27°C. Genotypes selected for high temperature tolerance may show less reduction of pollen viability at high temperatures than susceptible ones. Pollen viability can be used as a selection method to evaluate heat tolerance. High temperature-induced water deficit at the time of anthesis could limit reproductive development. Snap bean cultivars with a smaller midday drop in leaf water content yielded better than the cultivars with a larger midday drop in leaf water content (Omae *et al.*, 2005). Heat-tolerant cultivars have higher biomass allocation to pods and higher pod set in branches leading to higher harvest index in the heat-tolerant compared to the heat-sensitive cultivars (Omae *et al.*, 2012).

Beans are also sensitive to low temperatures, which can limit their production in the early part of the season. At a temperature of 14°C, Farlow (1981) found that seed set was reduced and that this was mainly due to a reduction in ovule fertility rather than pollen germination. Differences among genotypes for tolerance to suboptimal temperatures were reported by Dickson and Boettger (1984). Breeding of green beans for cold tolerance, using a tolerant line NY 5–161 from Cornell and selecting in the growth chamber at 16°C for 30 days, was reported by Holubowicz and Legutko (1995). The unifoliate and the first trifoliolate leaf stages were the most sensitive to freezing temperatures in common bean (Meyer and Badaruddin, 2001). Their estimated temperature to cause 50% mortality was −3.25°C, although regrowth after survival was limited, meaning few plants made it to maturity. Interspecific introgression of portions of the tepary bean genome into common bean is a promising method for increasing tolerance to extreme temperatures in common bean (Souter *et al.*, 2017).

Water

For maximum production of 60- to 120-day beans, water requirements vary between 300 and 500 mm depending on climate (Allen *et al.*, 1998). The water requirement during the seed filling period depends very much on whether the pod is harvested green or dry. For green beans, the total growing period of the crop is relatively short and during the seed filling, which is about ten days long, the crop evapotranspiration (ET) is relatively small because of the drying of the leaves.

For dry beans, the seed filling period is longer and the decrease in crop evapotranspiration is

relatively greater. The growing period depends on the number of pickings, and when three or four pickings are taken the harvest period is 20 to 30 days.

Beans are very sensitive to both water deficit (drought stress) and excess rainfall. Approximately 60% of bean production regions worldwide are affected by drought (Rao, 2014). In most commercial green bean production areas, supplementary or regular irrigation is required. But very lush growth should be avoided as this can cause problems with lodging and may lead to increased disease levels.

Pan evaporation and daily crop factors (water use/pan evaporation) can be used for scheduling the irrigation of green beans (Smittle and Dickens, 1992). Remote (satellite, airborne and unmanned aerial vehicle imageries) and proximal sensors (mounted on tractors, poles or towers, and portable specto-radiometers) are able to detect soil and crop water status (Alvino and Marino, 2017; Araus and Kefauver, 2018). Recent advances in use of soil moisture sensors, wireless communications, ET measurements, remote sensing, computer technology, and cloud computing offer many potential opportunities to develop robust irrigation advisory tools (Cahn and Johnson, 2017) that can help bean growers to accurately determine and meet crop water needs.

There is good evidence that deep roots are associated with drought resistance in beans (White and Singh, 1991; Polania *et al*., 2017). Ensuring adequate soil preparation and fertilization, to encourage deep rooting, will reduce the risk of drought stress. A close relationship between LAI and ET has been observed in green beans by Bonnano and Mack (1983). The onset of drought interrupts photosynthesis and tissue expansion, because the stomata close, normally at low leaf water potentials, and this restricts gas exchange (O'Toole *et al*., 1977; Walton *et al*., 1977). With continued drought stress, the plant water potential declines, resulting in wilting and the loss of the ability of the plant to orient its leaves.

P. acutifolius has been found to be more drought resistant than *P. vulgaris* (Cory and Webster, 1984; Rao *et al*., 2013), and there is some evidence that beans can be bred for improved drought resistance (White and Singh, 1991; Beebe *et al*., 2013; Rao *et al*., 2017). There are various possible mechanisms to reduce water loss including: (i) increased leaf thickness and/or reduced cell size; (ii) differences in stomatal sensitivity to humidity; (iii) differences in the amount of cuticular wax; and (iv) differences in leaf pubescence, orientation, color, or size. Recent work indicated that stomatal control and low stomatal conductance were clearly associated with drought tolerance, conserving water during stress, and increasing water use efficiencies (Traub *et al*., 2017).

Remobilization of photosynthates and N may increase in some genotypes under drought stress (Polania *et al*., 2016a, 2016b; Rao *et al*., 2017), and this would permit greater root growth and improve osmotic adjustment. Traub *et al*. (2017) found no drought effect on free proline, but malate, glucose, fructose, inositol, and raffinose all increased, sometimes enough to osmotically adjust leaf tissues. Abscisic acid (ABA) was found to be especially drought responsive and grafting studies indicated that shoot identity controlled ABA levels in stressed roots and that root identity had little or no effect on stomatal behavior (Traub *et al*., 2017).

Beans are also very sensitive to waterlogging, when the soil water is above field capacity. Leaf and root growth have been found to stop after two days of flooding, associated with a severe reduction in transpiration and a rise in the leaf ABA concentration (Wadman van Schravendijk and van Andel, 1985). Prolonged hypoxia/anoxia caused by flooding or waterlogging affects root hydraulic conductance and mineral uptake which causes stomatal closure, wilting and chlorosis in leaves. Soltani *et al*. (2017) analyzed the genetic architecture of flooding tolerance in the dry bean Middle-American diversity panel using a greenhouse phenotyping protocol and found that race Durango/Jalisco was the most flooding tolerant at the seedling stage, whereas pigmented small seeded genotypes were the most tolerant at germination stage. Brief periods of waterlogging can also enhance the root and hypocotyl disease caused by *Pythium*. Li *et al*. (2016) screened 194 common bean varieties from 37 countries for their resistance to root and hypocotyl disease and tolerance to waterlogging and found ample genetic variation that can be exploited to combine disease resistance with waterlogging tolerance to develop new cultivars for disease-prone regions with irregular but intensive rainfall patterns.

Soil nutrients and nitrogen fixation

Nutrient requirements

Fertilizer requirements for high production are 20 to 40 kg ha^{-1} N, 40 to 60 kg ha^{-1} P and 50 to 120 kg ha^{-1} K. Bean is capable of fixing N which can meet its requirements for high yields. However, a starter dose of N is beneficial for good early growth. Beans, like all legumes, have a relatively high N demand during pod fill, and senescence and abscission may result from competition for N (Sinclair and de Wit, 1976). The relatively high protein content of bean seeds (20–24%) implies N content of about 4%, which means that 40 kg of N is required for every 1 t of seed yield. Where a top dressing of N is required, this is normally applied at the 2–3 trifoliate leaf stage. Further top dressing may be required when rainfall is high and when *Rhizobium* inoculation is not used or nodulation is ineffective. Beans respond well to high fertility levels. Being a short season crop, most of the fertilizer is applied at planting time in order to establish optimal vegetative growth before flowering. Beans are very sensitive to Mn, Zn and Fe deficiencies, which can adversely affect photosynthesis.

Adaptation to low phosphorus availability in soil

Phosphorus (P) availability in soil is a major limiting factor to bean productivity on more than 50% of bean producing area, especially in tropical soils and root architecture has a great influence on the ability of bean plants to acquire P from the soil (Rao *et al.*, 2016). Beans can establish symbiotic interactions with arbuscular mycorrhizal fungi, leading to the formation of phosphate acquiring mycorrhizae. Long-term research using common bean contributed to defining root traits and their role in enhanced soil exploration and P acquisition (Lynch, 2011). One of the key mechanisms to increase access to P is greater *topsoil foraging* resulting from root architectural, morphological and anatomical traits. The ideotype of *topsoil foraging* incorporates: (i) early root vigor and preferential production of roots in topsoil; (ii) greater root branching and the production of long root hairs; (iii) high root length density in the topsoil and the proliferation of lateral roots in P-rich patches; (iv) greater root length/mass quotient, either through the development of thinner roots or the formation of root aerenchyma; and (v) the partitioning of a greater proportion of biomass to the root system. Shallower root growth angle of axial or seminal roots increases the topsoil foraging and thereby contributes to greater acquisition efficiency of P from low P soils. Greater inhibition of secondary root growth under P stress reduces root costs, increases P capture and improves growth in low P soil (Lynch, 2019).

Adaptation to acid soils

The soil pH for commercial production of beans should not be below 6.5 ideally, but beans are produced down to pH 5.5 in some areas. Al toxicity in acid soils affects as much as 40% of the global bean production area, especially in the tropics (Rao, 2014). Mechanisms of Al resistance in common bean were defined using the Al resistant genotype "ICA Quimbaya" and the Al sensitive "VAX-1" (see review by Yang *et al.*, 2013). It was shown that the induced and sustained Al resistance of "Quimbaya" is mediated by reducing the stable-bound Al in the apoplast thus allowing cell elongation and division to resume. Resistance to Al in common bean is attributed to the release of citrate by the root apex which is mediated by the multidrug and toxin extrusion (MATE) citrate transporter gene. Al resistance was mainly dependent on the capacity to sustain citrate synthesis, thereby maintaining the cytosolic citrate pool that enables exudation. The initial Al-induced inhibition of root elongation in both Al-resistant and Al-sensitive genotypes was correlated with the expression of the 1-aminocyclopropane-1-carboxylic acid oxidase gene (Yang *et al.*, 2013).

Symbiotic nitrogen fixation

Beans are able to establish intimate symbiosis with rhizobia to form nodules, where atmospheric N_2 is reduced to ammonium via SNF. This reduced N is exported from the nodule to the plant, so that the plant obtains a source of N and the bacteria is provided with photoassimilates in exchange (Peoples *et al.*, 2009). As a promiscuous host legume in terms of nodulation, green and dry beans are able to associate with a broad and diverse range of rhizobia, although the

competitiveness for nodulation and SNF capacity of most of these strains is generally low (Muñoz-Azcarate *et al.*, 2017). Beans can supply at least part of their N requirement through SNF and compared to other grain legumes, beans have lower SNF capacity. The estimated mean value of N derived from the atmosphere (Ndfa) for common bean across different geographical regions of the world was 39% compared with the values of other widely-grown legume crops between 54% to 65% whereas the values for soybean and faba bean were 68% and 75%, respectively (Peoples *et al.*, 2009).

Selection based on bean varieties with the best performance in highly mechanized and heavily fertilized monoculture systems in the 20th century is often reported to be the cause of the current relatively low SNF ability. But climbing and indeterminate varieties consistently have higher nodulation and SNF abilities, compared with most bush-type cultivars due to the relatively longer period of fixation during the growth cycle in climbing type cultivars (Barbosa *et al.*, 2018). In addition to genetic background, several abiotic factors can greatly influence the SNF ability of common bean.

Green beans are mostly grown without inoculating with *Rhizobium* and do not nodulate effectively in most commercial production areas. As a consequence, and by contrast with peas, green beans are normally fertilized with relatively heavy doses of N, sufficient to inhibit any nodulation that might otherwise occur.

Given the desirability of reducing N applications, for environmental reasons as well as cost, some work has been done on SNF in green beans, but much more has been done with dry beans. With green beans, Neuvel and Floot (1992) in the Netherlands found that N applications could be reduced by 25–50 kg ha^{-1} without significantly reducing yields, by mixing granular *Rhizobium* inoculant with the seeds.

Environmental factors including biotic and abiotic stresses such as P deficiency, drought, and pest and diseases affect SNF performance (Ramaekers *et al.*, 2013; Polania *et al.*, 2016b; Diaz *et al.*, 2017; Barbosa *et al.*, 2018). Among these limitations, SNF is highly sensitive to drought (Devi *et al.*, 2013; Polania *et al.*, 2016b), with possible interactions among stresses. Identification of parental genotypes to use in breeding that combine superior SNF ability under stress with other desirable traits could be a useful strategy to confront the new challenges of climate variability and problem soils.

Harvest Prediction

The combination of weather, soil, and genotype determines the time to harvest, and being able to use this information to predict the time to harvest is important, particularly for processing companies who need to plan the daily intake of product into the factory.

Time to harvest

Bush green beans produce pods ready for fresh harvest in about 45–65 days and 55–75 days for the pole types. Models based on heat units work on the basis that the crop must accumulate a certain number of day-degrees above a base temperature before flowering begins, and this type of model has been used to predict the harvest date of green beans (Gould, 1950) (see also Chapter 5). Scarisbrick *et al.* (1976) found that the heat unit system could explain phenological variation and predict harvest maturity also in dry beans. Heat unit methods of predicting harvest maturity are not widely used, however. This is perhaps because most of the cultivars used for commercial green bean production are determinate and have a relatively constant time to flowering in a particular location. Local knowledge of the performance of each cultivar is used to program plantings.

An example of the heat unit approach is the work on season extension in Germany by Scheunemann (1991), who recorded the number of days to reach specific growth stages together with the summed temperatures, in sequential plantings from mid-May to July. Plant development was affected most by the occurrence of stress days (minimum temperature < 9.5°C). Adequate rainfall/irrigation and effective long-range weather forecasting were essential for the accurate prediction of harvest date.

Fereira *et al.* (2006) used yield and quality variables (alcohol-insoluble solids, dry matter content, seed:pod ratio, fiber content, length of 10 seeds, Kramer shear press, color, lipid content,

and mineral composition) of four green bean varieties to determine the time to maturity. They found that the optimum harvest date, which corresponds to 10% dry matter content and 6.6% of alcohol-insoluble solids, could be set at thermal times after first flowering of 356, 384, 429, and 417 day-degrees for the varieties of "Alcade," "Carlo," "Cleo," and "Mutin," respectively. There is need to establish the relationships between pod size and harvest time to predict time to harvest.

Crop phenology and yield

To predict not only harvest date but also the crop yield, models can be developed to simulate growth. A model known as "Beangro" has been developed for dry beans by Hoogenboom *et al.* (1994), and an update of that has been developed called "Cropgro-dry bean" (Hoogenboom *et al.*, 1995). The model could presumably be modified to work for green beans. Recently Zhang *et al.* (2017) developed a model to predict node addition rate in common bean which could be incorporated into existing crop simulation models (Hoogenboom *et al.*, 2012) in an effort to convert them into gene-based simulation models that can provide a more comprehensive account of plant processes from planting to harvest using genotype and environmental data. Others have developed similar models which can cope with different growth habits (Gutierrez *et al.*, 1994; Ricaurte *et al.*, 2016).

Potential applications for a model would be: (i) optimizing agricultural management practices to obtain maximum yield at minimum cost; (ii) risk analysis by looking at the effects of weather changes over a number of seasons; and (iii) predicting harvest maturity and yield for individual fields in any one year. Clearly the model would need to be carefully validated with measured observations in any particular region.

Seed development and storage compounds

Seeds develop from fertilized ovules in the pod, and bean seed storage compounds are present in the embryo. Seed development in beans is highly related to nutrient metabolism and transport as an intense sink activity. The main seed storage compounds are carbohydrates, proteins, lipids, and phytic acid (phytin). The purpose of accumulation of these compounds in the seeds is to feed the embryo during development and guarantee seed germination and plantlet emergence. The protein is stored in protein storage vacuoles in the cell and on germination is rapidly hydrolysed to provide a source of reduced nitrogen for the early stages of seedling growth.

In *P. vulgaris* and *P. lunatus* vicilin is the major storage protein, whereas *P. coccineus* tends to have more legumin although it is highly variable for the ratio of vicilin to legumin (Durante *et al.*, 1989). In *P. vulgaris* the vicilin protein is known as phaseolin, and is about 50% of total protein in the bean seed, the other major protein being phytohemaglutenin, which is a lectin (Bollini and Chrispeels, 1978). Although phytohemaglutenin is the lectin commonly found in bean seeds (up to 10% of total protein), in some wild accessions of *P. vulgaris* a lectin-related protein, arcelin, can reach levels of up to 40% of total protein, and this protein has been associated with resistance to seed storage insects (van Schoonhoven *et al.*, 1983; Osborn *et al.*, 1988).

Phytohemaglutenin lacks sulphur amino acids and is highly toxic to monogastric animals, lowering the nutritional value of beans (Pusztai *et al.*, 1979). The level of α-amylase inhibitors is up to 5% of total protein, and these are also toxic to animals because these negatively affect starch utilization by the animal (Lajolo *et al.*, 1984). Like arcelin, α-amylase inhibitors are active against some storage insect pests (Huesing *et al.*, 1991).

Understanding how seeds of common bean develop and accumulate storage compounds and the major genes involved in this process may potentially lead to molecular breeding tools, either transgenic or not. The exact characterization of temporal and spatial expression of seed-specific genes may reveal interesting promoters to be used with genes of interest that will not be expressed in other parts of the plant, leading to energy economy of the entire physiology, reflecting, thus, in higher yields.

Biofortified Beans

Biofortification is the process of breeding for improved nutrient content in seeds of dry beans

and pods of green beans and is considered a sustainable and cost-effective strategy to address malnutrition in developing countries where beans are consumed daily. In beans, improvement of mineral content is advantageous precisely because the baseline seed iron content is high (55 mg kg^{-1}) and variability for the trait is great, with values ranging up to 110 mg kg^{-1}, allowing initial breeding attempts to be much more successful than in the cereals in overall iron and zinc content increases in seeds (Beebe, 2012; Blair, 2013; Andersson *et al*., 2017). The HarvestPlus initiative of the CGIAR on biofortification estimated that an addition of approximately 40 mg kg^{-1} to baseline iron levels in beans can meet a large proportion of the recommended daily intake of iron. The target areas for achieving impact with biofortified beans are in iron deficiency anemia prone areas of Central America, northeastern Brazil, and the Great Lakes region of Africa where the crop is important and consumption rates are high. In recent years, several bean biofortified cultivars were released in developing countries (one in Bolivia; three in Brazil; four in Colombia; nine in DR Congo; two each in El Salvador, Guatemala, Honduras, Nicaragua, and Panama; ten in Rwanda; and six in Uganda) (Andersson *et al*., 2017) and these cultivars play a key role in improving food and nutritional security in developing countries of the world.

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12 Peas

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Peas (*Pisum sativum* L.) are commonly grown, and are a popular food, in the temperate and to a lesser extent in the subtropical, regions of the world. Many different types of peas are produced and prepared in a variety of ways. In developed countries, succulent types are a favorite for freezing or canning, while in developing countries, dry peas are a common, practical, and reliable food because they are easily stored. Dry peas have been used for thousands of years whereas the consumption of green peas, eaten immature as a fresh vegetable, is a development dating from the early 17th century. Edible flat-podded peas are used in oriental cooking, and edible-podded peas ("snap peas") are used as a fresh or frozen vegetable. Dry peas are exceptionally versatile and are used in soups, mixed with other vegetables, made into noodles, and also used as a garnish to add color to a variety of dishes; rehydrated and dyed green they are particularly popular in English pub food as "mushy peas." The difference between garden (fresh) and field (dry) pea varieties lies in the carbohydrate content: garden peas contain higher sugar and lower starch content than field peas. By far the largest use of peas in developed countries is as a protein source for animal feed that is rich in many of the essential amino acids, especially lysine; however, they are deficient in methionine and cystine. Peas are rich in calcium, phosphorus, iron, sodium, and potassium.

Peas were domesticated in the fertile crescent of southwest Asia and they make a frequent appearance in archaeological remains as far back as 7000–6000 BC (Zohary and Hopf, 1973; Smartt, 1990). After domestication, cultivation spread north into Russia, west into Europe, east into India and China, and eventually to the western hemisphere soon after the discovery of the New World. Production of both green and dry peas (Table 12.1), particularly in Canada, has increased over the last 30 years as the pea crop has found increasing usage as an animal feed. In 2017 the top US green pea-producing states were Minnesota, Washington, Wisconsin, Oregon, and New York, while Montana and North Dakota led in the production of dry peas. Green pea production in Minnesota in 2017 totaled 88 million kg from 18,600 ha. Green peas, regular

Table 12.1. Pea production in 2017.

Country	Country rank	Quantity (metric tons)	Area (ha)
Production quantity of green peas			
World		20.70 million	2.67 million
China	1	12.59 million	1.57 million
India	2	5.34 million	530 thousand
USA	3	243 thousand	48 thousand
Production quantity of dry peas			
World		16.20 million	8.14 million
Canada	1	4.63 million	1.77 million
Russia	2	3.29 million	1.30 million
China	3	1.52 million	1.05 million

http://www.factfish.com/statistic/peas%2C%20green%2C%20area%20harvested

 Physiology of Vegetable Crops 2nd edition (eds H.C. Wien and H. Stützel)

Gregor Mendel 1822–1884. Painting by Jane Burrell, University of Tasmania.

Fig. 12.1. Mendel's tall *LE* (left) and dwarf *le* (right) peas.

and edible podded, are also widely grown in home gardens, but the acreage is difficult to quantify.

Pea Physiological Genetics

Much of our current understanding of pea physiology comes from studies of the genetic diversity of peas and pea mutants, especially by Ian Murfet and Jim Reid at the University of Tasmania in Australia (Murfet and Reid, 1993). Pea genotypes were the basis of the pioneering work of the Austrian monk Gregor Mendel in Brno (now in the Czech Republic) in the mid-19th century that established the fundamental laws of inheritance (Fig. 12.1). Germplasm collections are maintained at numerous centers throughout the world including the United States.[1]

To date, four of Mendel's seven genes have been sequenced (Table 12.2). Two of the other three genes, GP (pod color) and FA (fasciation), are amenable to candidate gene approaches on the basis of their function, linkage relationships, and synteny between the pea and *Medicago* genomes. However, even the gene (locus) identity is not known for certain for the seventh character, the pod form. The pea genome has recently been sequenced. Combined with a detailed analysis, this provides insight into pea evolution, both in the wild and during domestication, and accelerates understanding of the molecular basis of agronomically important traits (Kreplak *et al.*, 2019). Nonetheless the homolog to almost any Arabidopsis gene can now be isolated and characterized in pea, and the use of related genomes such as Medicago has overcome many of the limitations.

Seed Anatomy and Germination

The mature pea seed consists of an embryo and two fleshy cotyledons surrounded by a testa. The point

[1]The US collection of peas containing landraces, cultivars, and wild relatives is maintained at Pullman, Washington, by the National Plant Germplasm System operated by the US Department of Agriculture, Agricultural Research Service. That collection numbers 6135 accessions and is readily available to geneticists, breeders, plant physiologists and other interested individuals on request.

Table 12.2. Genetic characteristics examined by Mendel and a summary of the genes, phenotypes, and presumed mutations involved (From Reid and Ross, 2011).

Trait	Dominant phenotype	Recessive phenotype	Symbol group	Gene function	Molecular nature of mutation
Seed shape	Round	Wrinkled	*R*	Starch branching enzyme	0.8-kb insertion
Stem length	Tall	Dwarf	*LE*	GA 3-oxidase	G-to-A substitution
Cotyledon color	Yellow	Green	*I*	Stay-green gene	6-bp insertion
Seed coat/flower color	Purple	White	*A*	bHLH transcription factor	G-to-A at splice site
Pod color	Green	Yellow	*GP*	Chloroplast structure in pod wall	Unknown
Pod form	Inflated	Constricted	*V*?	Sclerenchyma formation in pods	Unknown
Position of flowers	Axial	Terminal	*FA*	Meristem function	Unknown

of attachment of the seed to the internal wall of the pea pod forms a scar known as the hilum. Near the hilum is the micropyle, a small opening in the testa, where the pollen tube enters the ovule and deposits the sperm cells at fertilization. The hilum, micropyle, and raphe (a bulge created by the embryonic root or radicle) are positioned along the line of separation between the two cotyledons (Fig. 12.2). The embryonic axis is composed of the radicle, hypocotyl, epicotyl, and young shoot or plumule. The seed coat is composed of two different cell layers, the testa and the integuments. The inner parenchymatous cells senesce before maturity and are then crushed by the developing cotyledons (Murray and Collier, 1977). The food, mainly carbohydrates, is stored in the cotyledons. Round pea seeds have 65% amylopectin (branched starch) whereas wrinkled peas have only 2%, with the rest being unbranched amylose. Round (smooth) peas have a more efficient conversion to starch. The starch branching gene in wrinkled peas contains an extra piece of DNA (a transposon) that inhibits gene activity, so amylopectin formation is inhibited (Bhattacharyya *et al.*, 1993). Wrinkled peas have some sucrose at maturity, so the seed shrinks as it dries.

Conditions for germination are optimum at 17°C and emergence takes three to five days (Fig. 12.2). The rate of germination and emergence decreases with progressively lower temperatures and ceases at 4°C (Wagenvoort and Bierhuizen, 1977). Pea embryos can leach large amounts of solutes in the first few minutes or hours of imbibition due to the porous nature of

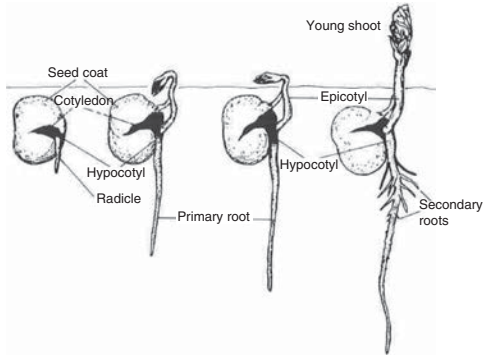

Fig. 12.2. The pea seed and germination.

the cell membrane in the dehydrated state. These components attract soil fungi which feed on the young seedling causing various seedling diseases, such as damping-off caused by *Pythium* spp. or *Rhizoctonia solani*.

Imbibition begins immediately after the seed is placed in the soil and comes in contact with water.[2] This phase lasts approximately 20 h and is characterized by a rapid uptake of water by the cotyledons and embryo, with a nearly doubling of seed size. In the early part of imbibition (approx. 6 h), nucleic acids and proteins become rehydrated. Once cellular organization has taken place osmotic water accumulation into the vacuole takes place. Near the end of the germination process water uptake slows and numerous metabolic changes take place. The seed can tolerate dehydration in the initial stages of imbibition, but once the

[2]There are also hard-seeded types which do not do this reliably. They carry the *A* gene and are usually small and round.

seed begins metabolic activity dehydration causes damage to the embryo and cotyledons.

The second phase of germination continues for several days and is characterized by degradation of materials stored in the cotyledons. Starch, protein, and phytic acid concentrations decrease, while the concentrations of soluble carbohydrates, amides and inorganic phosphorus increase. The initial increase in sucrose after imbibition is due partly to the hydrolysis of sucrose oligosaccharides. Starch mobilization in the wrinkled pea seeds begins slightly earlier than in round (smooth) pea seeds because the amylose in the wrinkled seeds is more susceptible to breakdown by starch-degrading enzymes than is the more complicated amylopectin of round seeds (Monerri *et al.*, 1986). The third phase of germination starts approximately five days after the start of imbibition and is characterized by decreased oxygen uptake and the start of the senescence of the hypogeal cotyledons. Many of the cellular components become disorganized causing the macromolecules (DNA, RNA and protein) in the cell to break down into their component parts, followed by transport to the developing embryonic axis.

The embryo also passes through three phases during germination (Sutcliffe and Bryant, 1977). Phase I corresponds to the imbibition phase and is primarily characterized by water uptake and the initiation of metabolism. The rate of metabolism increases during phase II and many dramatic ultrastructural changes take place in the cells. Golgi bodies and mitochondria show increased development of their membrane systems and some division of the mitochondria may occur (Yoo, 1970). The endoplasmic reticulum also proliferates during this phase. However, there is little change in the fresh weight of the axis or nucleic acid content. The cells in the radicle elongate causing it to emerge from the testa and allow oxygen to enter the seed. The increased oxygen concentration causes an increase in respiration, triggering the third phase of germination for the embryo. During this phase, there is an increase in the fresh weight of the axis and a net synthesis of DNA and RNA.

Respiration in the germinating seed increases slowly during the initial stages of imbibition and shows distinct stages. During the first stage, a rapid increase in respiration occurs due to activation of the enzymes involved in glycolysis and the hexose phosphate pathway. The second stage corresponds to a moisture content of 40–50% and is marked by a transition to a higher level of respiration due to completion of mitochondriogenesis. The third stage corresponds to the increase in water uptake as the axial cells vacuolate and elongate, and reflects the respiration of cells involved in active metabolism.

Once the radicle has emerged from the seed it begins to elongate downward (positive gravitropism) followed by the emergence and upward growth of the epicotyl. Secondary roots and root hairs begin to grow on the root as the taproot and shoot elongate. Root growth occurs through cell division and elongation in a relatively small part of the root tip.

The initial growth rate of pea seedlings is dependent on seed size; this effect is carried long into development and cannot be overcome by photosynthesis (Veierskov, 1985). The cotyledons are also very important to growth of the plant, as they appear to provide important factors that the plant is unable to synthesize photosynthetically.

Germination percentages and seedling vigor are affected by the genotype, physiological age of the seed at harvest, subsequent handling, and length and conditions of storage. Handling affects the condition of the testa and seed coat, and is a critical determinant of seedling vigor. Under optimal conditions seed remains viable for about 10 years.

Vegetative Growth

Early growth

Vegetative growth begins after germination. The plumule remains curved as the epicotyl emerges from the cotyledons and grows upward (Fig. 12.2). The curvature of the plumule is induced by the natural production of ethylene, and protects the shoot apex from mechanical damage during passage through the soil to the surface. When the plumule hook emerges from the soil and is exposed to light, stem elongation slows, the tip straightens, and the first leaf becomes visible. In the dark, tall (*LE*) seedlings elongate at the rate of about 1.5 mm/h with dwarf peas

somewhat less depending on the degree of dwarfness (Behringer *et al.*, 1990); *le* dwarf pea internodes reach 70% of the height of *LE* tall pea internodes in the dark (about 16cm for *LE* internode 2–3), and 50% of the height for internodes in the light (about 5 cm for *LE* internode 4–5) (Reid, 1983). The stem elongation rate drops rapidly, within a minute or so, in response to light, dropping to 20% of the original rate within eight minutes and to about 5% in about two to three hours (Fig. 12.3) (Behringer *et al.*, 1992; Behringer and Davies, 1993), but slowly recovers over the next six hours in tall peas. Even eight seconds of light is sufficient to give a transient response. The immediate response is to blue light received via the blue-responsive pigment cryptochrome. Red light acting through phytochrome takes longer, about 90 minutes, and the response to white light or daylight is the combination of these responses. Changes in growth rate are hormonally regulated, especially by the natural plant hormone gibberellin (GA; see below). On exposure to light the sensitivity to gibberellin of pea seedlings rapidly falls, so that the rate of stem elongation of plants in the light is much lower than in the dark, even though their GA_1 content is higher (O'Neill *et al.*, 2000). In addition, the level of growth-active GA_1 falls and then slowly recovers (Reid *et al.*, 2002).

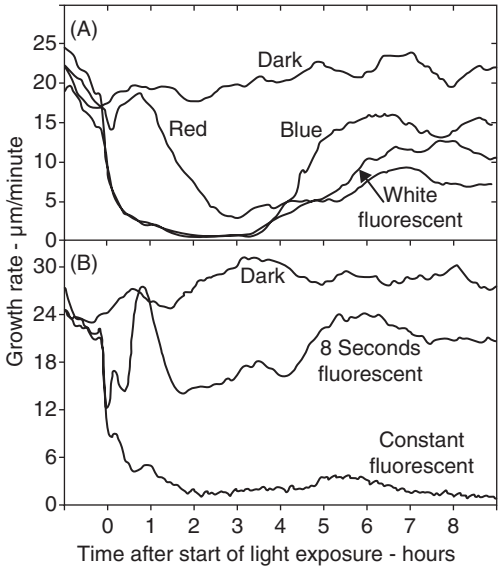

Fig. 12.3. Rapid changes in pea seedling growth on exposure to the light. Pea seedlings respond to blue and red light contained in white light by a reduction in the growth rate. The response to blue occurs in seconds whereas the response to red is slower, taking about one to two hours. In addition, the response to blue is more transient with a rapid increase in elongation if the light is removed. After an initial large decrease in the growth rate the growth rate partially recovers, associated with an increase in GA_1 biosynthesis. (A) Changes in the growth rate of dwarf pea (cv. Sparkle) seedlings in red, blue and white fluorescent light. (B) Changes in the growth rate of dwarf pea (L203) in response to a brief 8-second exposure to white fluorescent light: there is an immediate response to the blue component, followed by recovery and then a later decline attributable to the red component of the fluorescent light followed by a slower recovery of the growth rate. Data derived from Behringer *et al.*, 1992 and Behringer and Davies, 1993.

Photosynthesis: chloroplast biogenesis

The plumule hook changes from a white or pale yellow to a dark green as a result of chlorophyll synthesis after emergence. In the hypogeal seedlings of pea (Fig. 12.4), where the cotyledons do not emerge from the soil, the greening process only occurs in the true leaves. Some components of the chloroplasts are already present in etiolated seedlings, including pro-lamellar bodies and a core protein of the photosystem I reaction center (Fig. 12.5). The first stacked membranes in the chloroplast are observed after eight hours of light; fully developed grana are apparent on the third day from the start of illumination (Fig. 12.5C). The core proteins of photosystem II appear over the first eight hours of light, accompanied by the appearance of antenna proteins of both photosystems (Rudowska *et al.*, 2012).

Stem growth

Internode length steadily increases in the light as development proceeds, up to about the eighth internode, when a tall pea typically has an internode of about 14 cm and a dwarf pea 3.5 cm (Fig. 12.6) (Mackenzie-Hose *et al.*, 1998). New internodes are produced approximately every three days, depending on the growing conditions. During vegetative growth, pea stems develop between 20 and 25 nodes. Beyond the third node, each node has a compound leaf

Fig. 12.4. Changes in wild-type (seven-day-old) seedlings after transfer from darkness to white light. The time (in hours) since transfer is indicated (source: Reid *et al*. 2010).

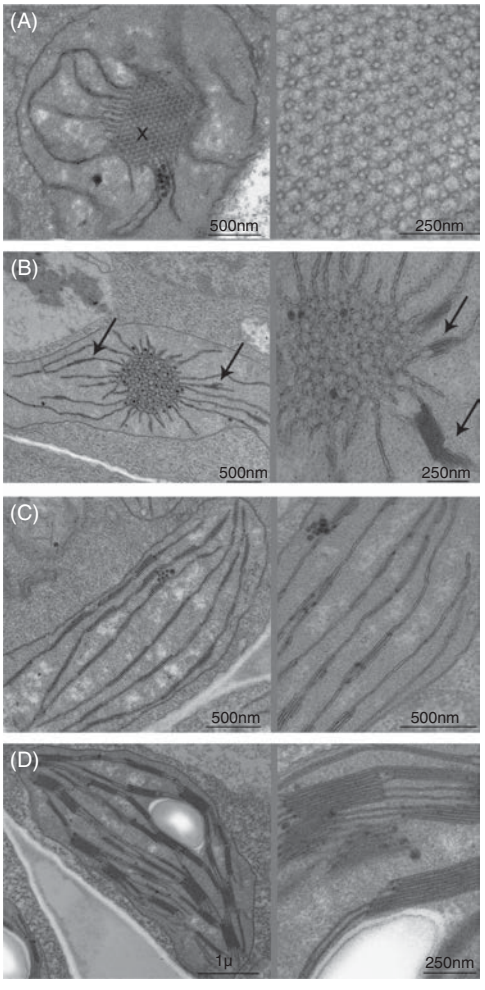

Fig. 12.5. Electron micrographs showing changes in mesophyll plastid development of pea seedlings following the start of illumination during the first day of light after eight days of germination in darkness; left side—whole plastid, right side: details of inner membrane structure. (A) Just before the light was switched on (x—paracrystalline Prolamellar body); (B) After 8 h of light (arrow—first stacked membranes formed); (C) After the third night from start of light; (D) After the third night and 3 h of light (modified from Rudowska *et al*. 2012.)

comprised of two leafy stipules at the base, a petiole with two or three pairs of leaflets, and terminating with three to five tendrils (Fig. 12.7). The first two nodes often remain below the soil surface with only two small rudimentary stipules at these nodes. Occasionally, basal branches or tillers are produced at these nodes. Stem growth is promoted by auxin (indole-3-acetic acid) moving downwards from the stem apex (Yang *et al*., 1993) and by gibberellin synthesized in the young tissues. The length of each node is controlled primarily by the level or responsiveness to gibberellin. The native active gibberellin is GA_1: if the GA_1 level is high the resulting stem is tall, but if it is reduced by deficiencies in GA biosynthesis the resulting plants are dwarf with varying degrees of severity, depending on the mutant and the biochemical step regulated (Table 12.2) (Fig. 12.7). The most common gene regulating height is Mendel's tallness gene, *LE*, (Fig. 12.1) for which the biochemical function, gene sequence, and the nature of the mutation were identified in 1997. *LE* regulates the final step in the biosynthesis of GA_1 from its precursor GA_{20} via the insertion of an −OH group at carbon number 3 on the gibberellin molecule. The recessive *le* form has a single base substitution (G to A) in the DNA leading to an alanine to threonine substitution in the protein, causing a decrease in the enzyme activity of the GA 3β-hydroxylase [oxidase], and a reduction in GA_1 by 95% (Lester *et al*., 1997). Height is also affected by several other modifying genes involving gibberellin biosynthesis and signal transduction, and phytochrome level and signaling (Table 12.2). Auxin also promotes the

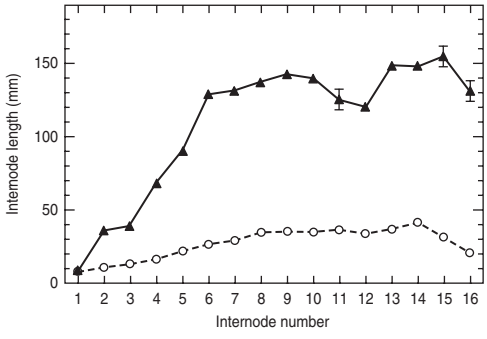

Fig. 12.6. Internode lengths of tall (*LE*) and dwarf (*le*) peas during growth (after Mackenzie Hose *et al*., 1998).

biosynthesis of GA_1 (Reid *et al*., 2010). Notable among the GA biosynthesis mutants is a tiny pea mutant named *nana* (gene *na*) with internodes only a few millimeters long; *nana* has a block in the three-step conversion of *ent*-kaurenoic acid to GA_{12} (Reid *et al*., 2010). In the plant the GA is detected by binding to a protein receptor, which activates a signal transduction chain finally resulting in the growth of the cells of the stem. A principal component of the signal transduction chain is a negative regulator, rather like a brake, called a DELLA[3] protein, that is negated by the GA, enabling growth to speed up in the presence of GA_1. However, if the DELLA protein is mutated to be non-functional, growth speeds up regardless of the level of GA_1. The mutant genes *la* and *cry-s*, combined, result in an inactivation of the DELLA negative-regulator and results in an ultratall, skinny, light-green pea plant nicknamed *slender*, regardless of the presence of any mutations in the GA biosynthesis pathway or the endogenous levels of GAs (Fig. 12.7). Another slender mutant *sln* results from a high level of GA_1 as the catabolism of GA_1 is inhibited because of a defective GA2oxidase. The root growth in *le* dwarf plants is identical to wild-type (WT) tall plants, but in ultradwarf *na* plants it is only about 40% of wild-type (WT) (Fig. 12.8), yet the root growth of the DELLA mutants is similar to WT regardless of the presence of *na* (Silva and

Fig. 12.7. Phenotypes, GA status and genotype of one-month old pea plants.

Davies, 2007); root growth in *na* plants can be restored by applications of GA.

The steep reduction in stem elongation as germination brings shoots into the light (Fig. 12.3) depends on the perception of the light by cryptochrome, the blue light-receiving pigment, and phytochrome, the red/far red-receiving pigment, so any alterations of these pigments impairs the

[3]Signal transduction proteins with the conserved N-terminal domain DELLA: aspartate-glutamate-leucine-leucine-alanine.

response to light. At least part of the light response is due to a drop in GA_1 levels caused by the down-regulation of the expression of the GA3ox1 gene that controls the conversion of GA_{20} to GA_1, and by up-regulating GA2ox2, which codes for a GA 2-oxidase that converts growth active GA_1 to inactive GA_8 (Reid *et al*., 2002). In addition to the photoreceptor mutations (Fig. 12.9 and 12.10), the *long1* mutation interrupts signaling from the phytochrome and cryptochrome photoreceptors. In the light *long1* shows no reduction in GA_1 because the GA_1 catabolism enzyme GA2ox2 is impaired resulting in a plant that remains ultratall (Fig. 12.9) (Weller *et al*., 2009b). LIP1 encodes an ubiquitin-ligase involved in the degradation of growth-inhibiting photomorphogenic-related factors. The *lip1* mutant displays a light-grown phenotype when grown in darkness with short stems along with open and expanded leaves because the growth-inhibiting factors are not broken down via ubiquitination; when grown in the light it has a dark-green, dwarf phenotype (Fig.12.10) (Weller *et al*., 2009b). The down-regulation of ethylene production also plays a role in the control of photomorphogenic development by phytochrome and cryptochrome, as *ein2*, a negative ethylene-receptor mutant, displays enhanced leaf expansion demonstrating that ethylene signaling constrains leaf expansion during deetiolation (Fig. 12.10) (Weller *et al*., 2015).

Fig. 12.8. Root growth in wild-type and *na* mutant (right) plants (source: Reid *et al*., 2010).

Shoot branching

Peas show strong apical dominance, so that the loss of the shoot tip results in the growth of the axillary buds into lateral branches. Growing shoot tips produce the hormone auxin (indole-3-acetic acid; IAA) that moves downwards within the stem and inhibits the outgrowth of axillary buds on the stem below (Fig. 12.11).

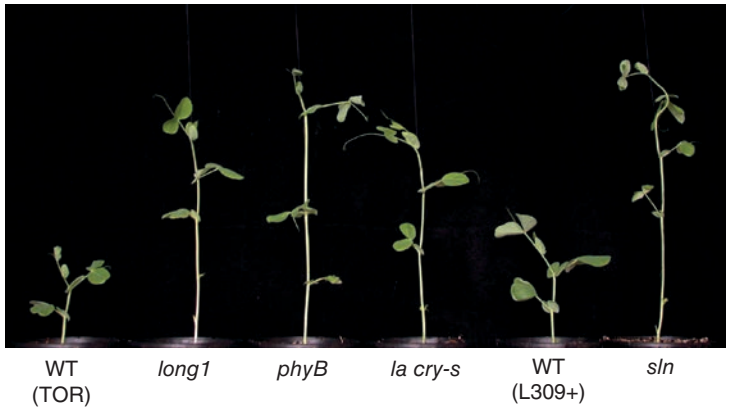

Fig. 12.9. Phenotypes of wild-type and mutant seedlings under greenhouse conditions (source: Weller *et al*. 2009b)

Fig. 12.10. Phenotypes of wild-type and mutant seedlings in red light (left) and darkness (right) (source: Weller *et al*., 2015.).

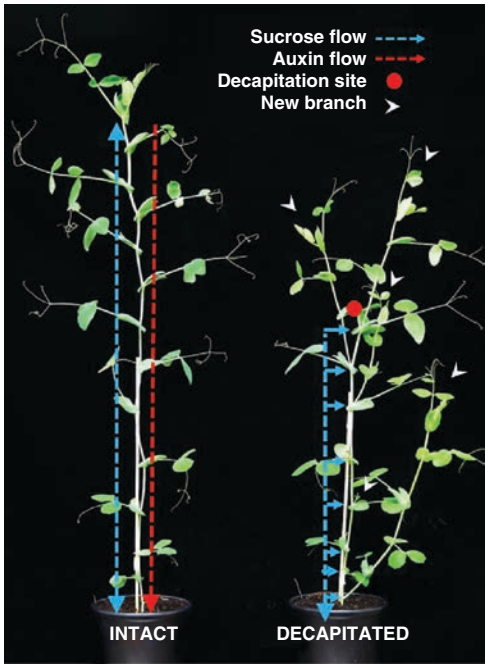

Fig. 12.11. Intact and decapitated branching pea plants with the factors that influence branching. Auxin acts to inhibit bud outgrowth via two upwardly mobile hormones, maintaining high strigolactone and low cytokinin content, which inhibit and promote bud outgrowth, respectively. After the loss of the growing shoot tip, auxin levels decrease and sucrose is rapidly redistributed to axillary buds, promoting their growth. (source: Barbier *et al*., 2012).

Auxin cannot enter the buds and therefore acts via secondary mechanisms. Auxin acts to inhibit bud outgrowth via two upwardly mobile hormones, maintaining high strigolactone and low cytokinin content, which inhibit and promote bud outgrowth, respectively (Ferguson and Beveridge, 2009; Barbier *et al*., 2017, Barbier *et al*., 2019). Strigolactone and cytokinin signaling is mediated by BRANCHED1/TEOSINTE BRANCHED1 (BRC1/TB1), a bud-localized transcription factor that inhibits bud outgrowth (Braun *et al*., 2012). The strong demand for sugars by the growing shoot tip also contributes to the suppression of bud growth. When the shoot tip is removed auxin levels progressively decrease down the stem; but this occurs too slowly to account for the early bud growth. In contrast, after the loss of the growing shoot tip, sucrose is rapidly redistributed to axillary buds where it represses the expression of *BRC1*, resulting in rapid bud release (Mason *et al*., 2014, Barbier et al., 2015); this effect is mediated by trehalose-6-phosphate (Fichtner *et al*., 2017).

Leaf development

Mature WT pea leaves have three types of lateral organs: stipules at the leaf base, with lateral leaflets and terminal tendrils in place of leaflets in the blade. In general, the lower nodes have single leaflet pairs while later nodes may have two or often three leaflet pairs. The number of nodes is primarily dependent on the cultivar. Pea leaves typically have up to three pairs of proximal leaflets, up to four pairs of distal tendrils and a terminal distal tendril. There are generally five leaf primordia at successive

stages of development in the shoot apex at any one time. Development of pea leaflet primordia is acropetal, while development of the distal tendrils follows a basipetal pattern. Pea leaves have cuticular wax on the upper surface and leaf color can range from yellow-green to a deep blue-green depending on the cultivar. Tendrils are contact-sensitive, filamentous organs that permit climbing plants to tether to their taller neighbors. The thigmonastic coiling of tendrils following a mechanical perturbation involves an initial rapid bending driven by a hydraulic-driven contraction on the ventral side of the tendril followed by a subsequent growth on the dorsal side (Jaffe *et al.*, 2002). The TL gene, which directs tendril development, encodes a transcription factor (Hofer *et al.*, 2009). The tendril-less (*tl*) loss of function mutation transforms the tendrils into leaflets. The conversion of tendrils into leaflets in *tl* mutants demonstrates that the pea tendril is a modified leaflet, inhibited from completing laminar development by *TL*. The stipule is an autonomous lateral organ, with the *COCH* gene as a master regulator for stipule development (Kumar *et al.*, 2009). *COCH* is essential for initiation, growth and development of the stipule, and together with *ST* mediates the developmental pathway for a peltate-shaped simple WT stipule; the recessive *st* allele greatly reduces stipule size.

Various genes modify leaf structure and form (Fig. 12.12). The most prominent of these genes are the *af, st* and *tl* genes, which in combination, produce eight distinct morphologies. The 'afila' leaf type, controlled by the *af* gene, is characterized by the conversion of leaflets to tendrils. This particular plant type is known as "semi-leafless". The semi-leafless morphology with stipules and extensive tendrils (*af ST TL*; Fig. 12.12D) enables the plants to grow more upright because of the entwining tendrils. The reduced leaf area of these plant types allows for greater light penetration into the canopy. The more open the canopy is, the more it reduces the amount of shading to the lower leaves and increases the photosynthetic activity of the developing pods. It also has the potential for reduced foliar disease as a result of increased air movement and reduced humidity within the crop canopy. The semi-leafless morphology produces greater yields than the conventional leaf types as the result of improved standing ability and the increased penetration of light into the canopy, and is the main type used in most commercial field-scale varieties (Kielpinski and Blixt, 1982). The completely leafless type (*af st TL*) has not proven useful in the development of new cultivars because of significantly reduced yields.

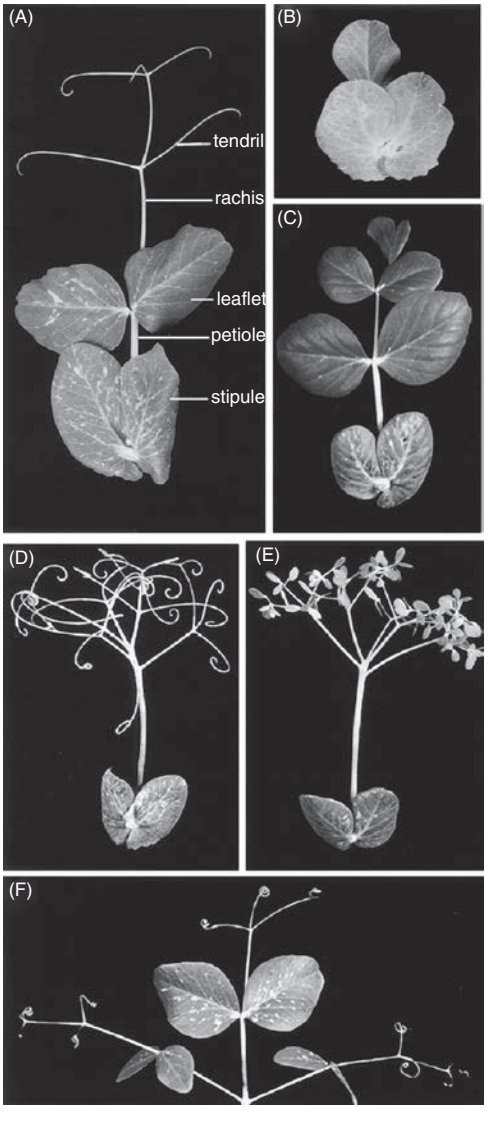

Fig. 12.12. Morphology of wild-type and mutant pea leaves. (A) A wild-type compound leaf. (B) A *uni* mutant (C) A *tl* mutant. (D) An *af* mutant leaf. (E) An *af tl* double mutant. (F) A *coch* mutant (source Gourlay *et al.*, 2000).

The compound leaf primordium of pea represents a marginal meristem (a "blastozone)" that initiates organ primordia, in an

acropetal manner, from its growing distal region (Figure 12.13). The *UNIFOLIATA* (*UNI*) gene (orthologous to the Arabidopsis floral meristem identity gene *LEAFY* (*LFY*)) promotes the transient indeterminate growth of the leaf. *UNI* is important in the maintenance of the leaf marginal meristem (Gourlay *et al*., 2000). *UNI* is expressed in the marginal meristem of the leaf during the period in which organ primordia are initiated, and promotes its maintenance. It is then downregulated at the time of leaf primordium determination. *UNI* expression is important in pattern formation in the compound leaf primordium, controlled by the genes *AF*, *TL*, and *COCH*. Mutant *uni* leaves have reduced complexity due to precocious differentiation. Successive leaves become progressively more dissected; leaves from lower nodes are less complex than leaves from higher nodes, possessing fewer lateral leaflets and/or lateral tendrils (Bar and Ori, 2015). Prolonged *UNI* expression is associated with increased marginal meristematic activity in the complex leaves of afila (*af*), cochleata (*coch*), and afila tendril-less (*af tl*) mutant plants. *UNI* expression is negatively regulated by *COCH* in stipule primordia, by *AF* in proximal leaflet primordia, and by *AF* and *TL* in distal and terminal tendril primordia. Lamina inhibition to produce tendrils requires *UNI*/*LEAFY*-mediated *TL* expression in organs emerging in the distal region of the leaf primordium (Hofer *et al*., 2009). Auxin location is known to regulate primordia activity and may be important in the regulation of leaf development as *UNI* is associated with high auxin levels, whereas *AF* suppresses auxin levels (DeMason *et al*., 2013).

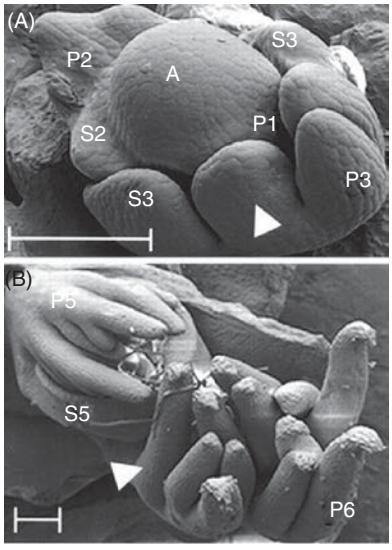

Fig. 12.13. Stem apical meristems showing the effects of the *af* mutation on early leaf development. (A) Wild-type and (B) *af* leafless mutant. A, vegetative shoot apex; P1 to P6, plastochron 1 to 6 of leaf development; S2 to S5, stipule primordia present on P2 to P5 primary marginal blastozones. White arrowhead indicates in (A) leaflet primordia; (B) developing tendrils. Bars = 100 μm (source: modified from Gourlay *et al*., 2000.)

Nitrogen fixation

Being a legume, pea nodulates in association with *Rhizobium* bacteria to enable nitrogen fixation, so that legumes as a group are a crucial lynchpin of many agricultural systems (Smýkal *et al*., 2012, 2016). Pea has the largest and best characterized range of plant hormone mutants of any legume species for the investigation of nodulation. Nodulation at the whole-root level consists of at least two spatially separate programs, infection at the root epidermis, and nodule organogenesis originating in the root inner cortex (Fig. 12.14). The process commences with the exchange of chemical signals between the epidermal root hair and the *Rhizobium* bacteria in the soil (Nelson and Sadowsky, 2015; Ibáñez *et al*., 2017). The perception of compatible Rhizobia-produced NOD-factors by the plant host induces physical changes that enable colonization. This includes root hair curling, and infection thread formation, with transmission of the bacteria from cell to cell in a membrane-bounded infection thread. The bacteria finally take up residence in membrane-bounded vesicles in the cortex, leading to the establishment of the nodule through cell division in the inner-cortical cell layers of the root. Nodule development and nitrogen fixation, like many plant processes, are influenced by plant hormones (Ferguson and Mathesius, 2014). Auxin and cytokinin are involved in nodule initiation, growth, differentiation, and positioning. Auxin accumulation at the site of nodule initiation, regulated by auxin transporters in the cell membranes, is crucial to nodule development

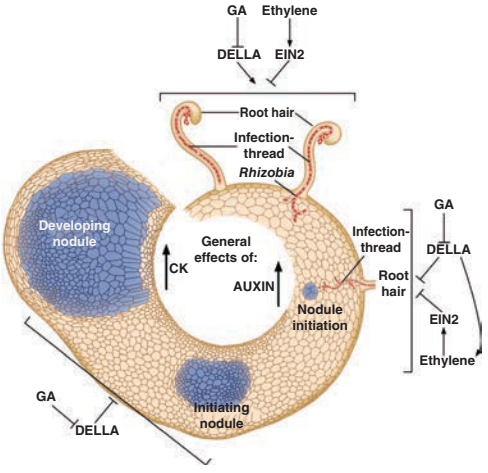

Fig. 12.14. Schematic diagram of nodule development in pea roots with the hormonal influences on nodule development. Top: infection of root hairs by *Rhizobia* bacteria; Clockwise from right: progressive nodule initiation and development. → = promotion; ⊥ = inhibition (source: Davies, 2018.)

Fig.12.15. A typical pea flower.

(Kohlen *et al.*, 2018). Rhizobium infection rapidly induces the upregulation of several cytokinin biosynthesis genes, leading to cytokinin accumulation and response in the region of the root where nodulation takes place (Gamas *et al.*, 2017). Gibberellins acting through DELLA proteins have an opposing role in different cell layers of the root, suppressing events leading to infection thread formation in the epidermis, but promoting nodule organogenesis in the inner cortex and the ultimate function of nodules as nitrogen-fixing organs (Davies, 2018; McAdam *et al.*, 2018).

Flower Formation

Flower buds initiate on the apical meristems approximately 20 days before they become visible. At the time of initiation, flower buds are enclosed by six leaf primordia, which expand and eventually expose the developing flowers. The pea flower (Fig. 12.15) is described as papilionaceous because of its superficial resemblance to a butterfly when the petals unfold. The calyx of the flower consists of five basally united sepals. The corolla consists of two wing petals, a large, showy standard petal, and a keel, which is formed by fusion of two petals early in floral bud development. The keel surrounds the anthers, stigma, style, and ovary. There are ten stamens in the complete pea flower, nine of which have joined filaments and one that remains free. The style is densely covered with trichomes, and terminates with the stigma which is surrounded by numerous papillae. Within the ovary, two rows of ovules alternate on the left and right halves of the fused margins of the carpel. The enclosing nature of the keel around the stamens and carpel ensures that the pollen is deposited on the stigma to effect self-pollination. After germination, the pollen tube grows through the style and micropyle and releases sperm into the embryo sac, and, by double fertilization, the embryo and endosperm are formed.

Phenology of flowering

Most pea cultivars are facultative long-day (LD) plants. However, some are day neutral, while others are obligate LD plants that fail to flower in photoperiods shorter than 12 hours if unvernalized (Weller *et al.*, 1997b). Flowers are borne in axillary racemes that develop sequentially as the stem continues growth, rather than on the terminal shoot meristem, leading to an indeterminate growth habit. All genotypes produce a number of vegetative nodes before flowering, ranging from four in the earliest varieties to

over 100 in the late-flowering varieties grown under non-inductive conditions. The extent of the reproductive phase also varies considerably with genotype and environment, with as few as one to over 50 flowering nodes expanded before arrest of the apical meristem. Once flowering has commenced it is usually stable, although under certain conditions some genotypes may undergo a period of vegetative reversion before again resuming flower production (Weller *et al.*, 1997b). Pea flowers are self-fertile and pollination often occurs before the flowers are fully open.

The genetic control of flowering

The mobile floral signal "Florigen" has been shown to be a protein encoded by the *FLOWERING LOCUS T* (*FT*) gene in Arabidopsis. In pea *GIGAS* is *FT* (Hecht *et al.*, 2011). However in pea, there are several *FT* genes, leading to a much more complex regulation of photoperiodic flowering in pea compared to *Arabidopsis* (Weller and Ortega, 2015). In the stem apex FT protein forms a complex with the transcription factor FD that then directly up-regulates the expression of floral genes, such as *AP1* (using Arabidopsis gene nomenclature).

Over 20 loci related to flowering time and inflorescence development have been identified in pea (Table 12.3) (Weller *et al.*, 2009a; Weller and Ortega, 2015). Two major loci are known that delay flowering under non-inductive short-days (SD): recessive alleles at the *HIGH RESPONSE* (*HR*) locus cause early flowering in SD and reduce, but do not eliminate, the photoperiod response, whereas recessive alleles at the *STERILE NODES* (*SN*) locus confer complete daylength insensitivity (Murfet, 1985). Much of the flowering time variation in peas is controlled by *HR*, with a single, widespread variant conferring altered circadian rhythms and the reduced photoperiod response associated with the spring-flowering habit (Weller *et al.*, 2012). This factor is likely to have permitted the successful prehistoric expansion of legume cultivation to northern Europe. *SN* controls developmental regulation of genes in the *FT* family and rhythmic regulation of genes related to circadian clock function. *SN* and two other circadian clock genes, *HR* and *DIE NEUTRALIS* (*DNE*), have a complex relationship in which *HR* regulates expression of *SN*, and the role of *DNE* and *HR* in the control of flowering is dependent on *SN* (Liew *et al.*, 2014).

Table 12.3. Flowering loci in pea The effect (or inferred effect) on flowering of the functional (wild-type) allele is indicated as either "+" (promoting flowering) or "–" (inhibiting flowering). The molecular identity refers to the Arabidopsis ortholog or the type of protein encoded (modified from Weller and Ortega, 2015, plus Rubenach *et al.*, 2017).

Developmental role	Pea locus	Promotes or inhibits Flowering	Molecular identity
Light	*FUN1*	+	*PHYA*
perception/	*LV*	–	*PHYB*
signaling	*LIP1*	–	*COP1*
Circadian clock	*SN*	–	*LUX*
	DNE	–	*ELF4*
	HR	–	*ELF3*
	PPD	–	*ELF3b*
Photoperiod response	*LATE1*	+	*GIGANTEA*
Floral signal	*GIGAS*	+	*FT (FTa1)*
Floral signal	*LF*	–	*TFL1 (TFL1c)*
integration	*VEG1*	+	MADS box
and	*VEG2*	+	*FD*
Inflorescence	*DET*		*TFL1 (TFL1a)*
development	*UNI*		*LFY*

A third locus, *LATE FLOWERING* (*LF*), inhibits flowering in both LD and SD. If the *LF* gene is inactivated it causes extremely early, photoperiod-insensitive initiation of flowering (Weller and Ortega, 2015), but remains responsive to photoperiod in several other respects, suggesting that *LF* is not involved directly in the photoperiod response mechanism. The fourth locus, *EARLY* (*E*), confers early initiation of flowering in some genetic backgrounds, but this effect is dependent on interactions with other loci. Allelic differences at the *HR*, *SN*, *LF*, and *E* loci interact to specify an extremely wide range of flowering times in plants in non-inductive conditions. This ranges from the genotype with alleles *lf sn*, which may flower as early as node 7 and is completely insensitive to photoperiod, to genotype with alleles *LF SN HR e*, which flowers relatively late under LD and may not flower at all under SD (Weller *et al.*, 2012). Another circadian-clock-related gene causing late flowering is

LATE1, an ortholog of Arabidopsis *GIGANTEA* (Hecht *et al.*, 2007). *LATE1* shows strongly rhythmic expression, and *late1* mutants affect the expression rhythms of key circadian clock genes. *DNE* has a role in the circadian regulation of several clock genes, including *LATE1*, and controls flowering through a *LATE1*-dependent mobile FT stimulus (Liew *et al.*, 2009).

Light perception is, of course, essential for photoperiod regulation of flowering. Mutants for the phytochrome-A photoreceptor are largely insensitive to LD and have a LD-specific late-flowering phenotype (Weller *et al.*, 1997a). Neither PHYB nor CRY1 are involved in promotion of flowering. Increased PHYA leads to photoperiod-insensitive early flowering (Weller *et al.*, 2004). A dominant mutant at the *LATE BLOOMER2* (*LATE2*) locus is late-flowering with a reduced response to photoperiod. *LATE2* acts downstream of light signaling and the circadian clock to control expression of the main photoperiod-regulated FT gene, indicating a role in photoperiod measurement (Ridge *et al.*, 2016).

The ancestral forms of pea are adapted to overwinter in the vegetative state, and show a strong vernalization requirement under SD. Vernalization in late varieties is represented by a decrease in the number of nodes prior to the appearance of flowers in response to cold temperatures in the region of 4°C. For example, plants with alleles *lf, e, SN, HR* respond quantitatively to the number of days of vernalization: 22 days of vernalization resulted in a decrease in the node of first flower from node 62 to node 38 (Reid and Murfet, 1977). Early expansion following domestication was accompanied by selection for the spring habit, typified by earlier flowering under SD and reduced response to both photoperiod and vernalization. This habit is primarily conferred by recessive alleles at the *HR* locus (Weller *et al.*, 2012). More recent selection of recessive alleles at the *SN* locus has resulted in even earlier flowering and effective insensitivity to both factors (Liew *et al.*, 2014).

Floral meristem identity

The stem apical meristem (SAM) undergoes a transition from a vegetative meristem to a primary inflorescence (I1) meristem, with indeterminate growth. This I1 meristem, produces secondary inflorescence meristems (I2), which in turn will generate floral meristems (F). The I2 usually produces one to two floral meristems before it ceases growing (Benlloch *et al.*, 2015) (Fig. 12.16). Mutants of the *DETERMINATE* (*DET*) gene have a determinate inflorescence that produces one to two normal lateral I2s and an apparent terminal flower on the main stem, but examination shows that the I1 meristem develops into an I2 meristem that produces a flower in a lateral position and terminates in a stub. The *VEGETATIVE1* (*VEG1*) locus regulates the production of I2 meristems. *veg1* mutant plants fail to produce flowers under any growing condition because of a blockage in I2 meristem identity acquisition, replacing secondary inflorescences with vegetative branches (Berbel *et al.*, 2012). (Fig. 12.16)

The number of flowers produced in each I2, as well as the number of I2s produced by the primary inflorescence, is characteristic of each cultivar. Pea usually produces between one and two flowers per secondary inflorescence. Recessive mutations of two genes, *fn* and *fna* cause an increase in the number of flowers per I2. Mutations in the flowering time genes *HIGH RESPONSE* (*HR*) and *STERILE NODES* (*SN*), involved in photoperiod response, also strongly influence this trait, with the number of flowers being decreased by recessive *sn* alleles and increased by dominant *HR* alleles. *SN* and *HR* not only control the activity of the I2 meristem in pea, but also affect the duration of I1 meristem activity, since the number of I2 nodes produced before I1 meristem arrest is decreased by recessive *sn* alleles and increased by dominant *HR* alleles.

Floral meristem identity is controlled by *PROLIFERATING INFLORESCENCE MERISTEM* (*PIM*) and *UNIFOLIATA* (*UNI*) genes, homologs to Arabidopsis *AP1* and *LFY*, respectively. In the loss-of-function *uni* mutants, floral meristems are not correctly specified and rather than flowers they produce proliferating structures, mainly formed by sepals and carpels. Two other genes considered to participate in the control of I2 meristem identity in pea are *GIGAS* and *VEGETATIVE2*. Plants with severe mutations in the *gigas* locus show an extreme non-flowering phenotype under LD conditions. Similar to *veg1*, *gigas* mutants show apparently normal vegetative

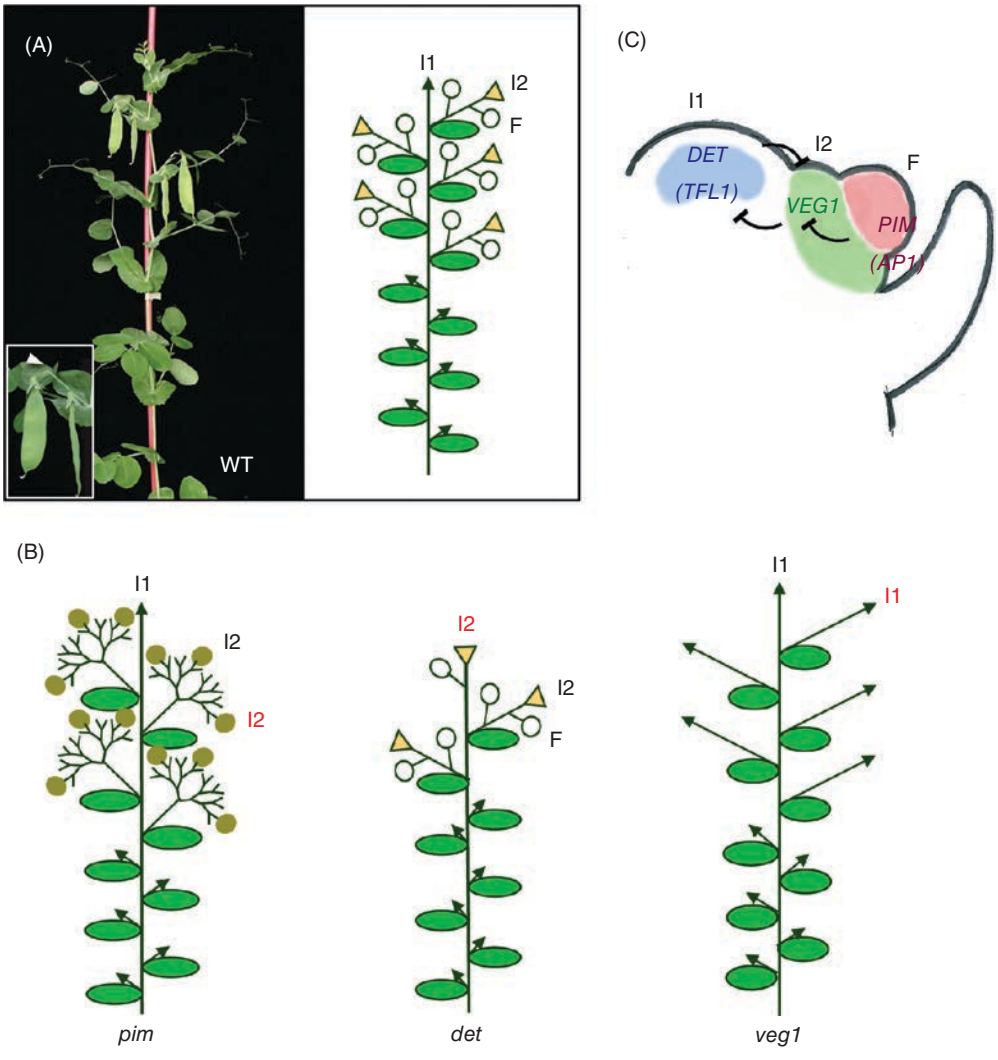

Fig. 12.16. Meristem identity genes in pea. (A) Picture and diagram of a pea wild-type plant. The main primary inflorescence (I1) shows indeterminate growth (arrowhead). Upper nodes of the plant contain secondary inflorescences (I2) which produce 1–2 flowers (F, open circles) and terminate into a stub (triangles). The inset shows a close up of a secondary inflorescence with two flowers (pods) and the stub (arrowhead). (B) Diagrams of meristem identity of the *pim*, *det*, and *veg1* mutants. In the *pim* mutant, flowers are replaced by proliferating I2s with abnormal flowers (closed circles). In the *det* mutant, the primary inflorescence is replaced by a terminal secondary inflorescence. In the *veg1* mutant, the I2s are replaced by vegetative branches with I1 identity. (C) Model for specification of meristem identity in the compound pea inflorescence. In the pea inflorescence apex, *DET* expression in the primary inflorescence meristem (I1), *VEG1* in the secondary inflorescence meristem (I2) and *PIM* in the floral meristem (F) are required for these meristems to acquire their identity. Expression of these genes in their correct domains is maintained by a network of mutual repressive interactions (source: Benlloch *et al*., 2015.)

development, and later in development the induction of inflorescence markers, such as upregulation of *DET* and bud outgrowth, indicating that the transition from vegetative to I1 meristem also takes place in *gigas* mutants. However, expression of *PIM* and *VEG1* is never induced under LD in the inflorescence of the *gigas* mutants, which indicates that I2 specification does

not take place (Hecht *et al.*, 2011). *GIGAS* corresponds to one of the pea homologs of the *FLOWERING LOCUS T* (*FT*) gene in *Arabidopsis*. Loss-of-function mutations in *VEG2* also cause a phenotype related to I2 meristem development. The *veg2-1* mutant displays a non-flowering phenotype similar to *veg1*, while *veg2-2*, a weaker allele, shows a delay in flowering and a conversion of I2 inflorescences into flower-bearing branch-like structures with indeterminate growth, which resemble the primary I1 inflorescence of WT plants. *VEG2* is a pea ortholog of *FD* (Sussmilch *et al.*, 2015). As in *gigas* mutants, expression of *PIM* and *VEG1* is never detected in the "inflorescence" apex of the *veg2* mutant. *STAMINA PISTILLOIDA* (*STP*) is another gene involved in specifying the floral meristem; *stp* primary inflorescences often terminate prematurely in an aberrant sepaloid flower (Taylor *et al.*, 2001). The most severe allele, *stp-4*, results in flowers consisting almost entirely of sepals and carpels. *stp* mutations also reduce the complexity of the compound pea leaf.

Pod and Seed Development

Initial growth of the fruit precedes the exponential growth of the enclosed seeds (Pate and Flinn, 1977). The early "flat pod" stage begins soon after anthesis and is characterized by elongation and increased width of the pods. The walls of the fruit thicken as the fruit inflates into a hollow envelope. This development is characterized by differential growth of the outer and inner layers of cells. The endocarp, the layer of cells lining the inner cavity of the pea pod, is composed of sclerified cells which form a fibrous layer. The lack of sclerified cells in pod tissue can be brought about by the action of the *p* and *v* genes. Each of these genes reduce the lignification of the pods while the presence of both *p* and *v* nearly completely eliminate pod parchment. Maximum pod wall dry weight coincides with the onset of seed filling at about 15 days after anthesis. Cultivars are in use that produce pods that lack parchment and are completely edible. The most widely known and used edible podded type cultivars are the flat podded "sugar" peas used in Oriental style cooking. "Snap pea" types combine the action of the *p* and *v* genes with the *n* gene for cylindrically shaped pods that are fiberless and completely edible.

The end of the "flat pod" stage corresponds to the final stage in seed abortion after which the seeds will grow to maturity. This corresponds to the end of cell division in the embryo and the beginning of seed fill, which occurs about 20 days after anthesis. The "round pod" stage represents the period of seed filling and is characterized by the pods taking on a rounded appearance as the seeds fill the inner cavity. A color change in the pods and seeds marks the end of the round pod stage and the beginning of seed maturation.

Seed growth (Fig. 12.17) is composed of three phases of rapid growth separated by two lag phases (Hedley and Ambrose, 1980; Le Deunff and Archidian, 1988). The initial growth phase lasts about 14–19 days after anthesis and is characterized by the formation of the embryo and surrounding structure. The growth of the testa and accumulation of endosperm is maximized during this phase. Growth rate of the embryo is very low especially in round-seeded types. The moisture content of the seed at this phase is nearly 85%. The first lag period is characterized by a decline in the growth rate of the testa and endosperm and lasts no longer than two days. The second growth phase is characterized by a reduced growth rate of the endosperm and a rapid increase in embryo fresh weight. As a result of increased embryo growth, the volume of the liquid endosperm declines.

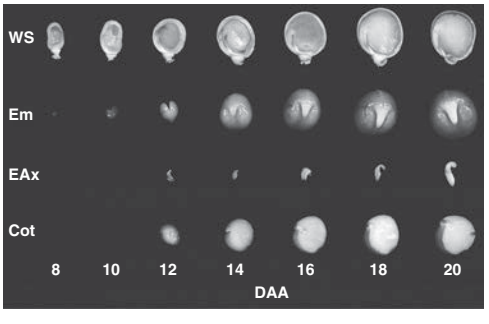

Fig. 12.17. The development of pea seed tissues over 20 days from anthesis. WS: Bisected whole seeds; Em: embryos; EAx: embryo axes; Cot: cotyledons (source: Nadeau *et al.*, 2011.)

The volume of the embryo and embryo sac increase at different rates. Initially the developing testa comprises the larger sink and grows more than the embryo. At the point of maximum endosperm content the embryo becomes the greater sink and grows relative to the testa. The cotyledons begin to accumulate storage materials and the moisture content drops from 85 to 55%. At the termination of this phase the liquid endosperm is exhausted as the embryo fills the embryo sac, cell division in the cotyledons is completed, RNA and protein synthesis increases, and starch accumulation is initiated. This phase is shorter for wrinkled than round seeded genotypes, and is followed by a second lag phase where there is a decline in the absolute growth rate of the testa and embryo with continued disappearance of the endosperm. The third growth phase is characterized by the maximum absolute seed growth rate which subsequently declines towards maturity, followed by the period of desiccation of the seed. As the seeds fill, the cotyledons act as a strong sink for sugars, amino acids, and phosphate. They soon consume the endosperm and enlarge to occupy the entire interstitial cavity. As the pod elongates, the peduncle curves downward from the weight of the pod. The final moisture content of the mature seed ranges from 14 to 18%.

Pollination and fertilization trigger strong sink activity in the ovaries, and normal fruit growth requires the presence of the seeds. GA_1 plays an important role early in pea seed development by regulating the development of the embryo and endosperm. Pollination and fertilization events increase GA_1 biosynthesis enzymes and decrease those of GA_1 catabolism (Ozga *et al.*, 2009). GA_1 is important in determining the rate of seed growth and sink strength (Nadeau *et al.*, 2011). Auxin is also required for normal seed size and starch accumulation (McAdam *et al.*, 2017). The coordination of growth between the seed and ovary tissues also involves plant hormones. The uncommon auxin, 4-Cl-IAA is produced in the seeds and is then transported to the pericarp, where it differentially regulates the expression of pericarp GA biosynthesis and catabolism genes to modulate the level of bioactive GA_1 required for initial fruit set (Ozga *et al.*, 2009).

Transcriptional control during pea seed development is a highly coordinated process. There are 459 and 801 genes differentially expressed at early and late seed maturation stages between vegetable pea and grain pea, respectively. Mitogen-activated protein kinases (MAPKs) and their cellular localization define apical and basal regions during formation of an apical-basal axis during embryo development (Winnicki *et al.*, 2017). Soluble sugar and starch metabolism-related genes are activated during the development of pea seeds leading to the accumulation of sugar and starch in the seeds. Differential expression of genes involved in sugar and starch biosynthesis at late development stages shows a negative correlation between soluble sugar and starch biosynthesis in vegetable pea (high seed soluble-sugar and low starch) and grain pea (high seed starch and low soluble-sugar) pea seeds (Liu *et al.*, 2015).

Photoassimilate partitioning to seeds

Pea fruit and seed growth are primarily supported by photoassimilate produced in the vegetative organs of the plant. Approximately two thirds of the assimilate accumulated by the seed is derived from the leaves and stipules directly subtending the pod (Harvey and Goodwin, 1978; Jeuffroy and Warembourg, 1991), and also from the pod itself (25%). Translocation of assimilates from other nodes occurs in smaller amounts. Assimilate partitioning depends on the relative developmental stages of the pods, the number of reproductive nodes and the number of pods on the plant. Sucrose uptake into the phloem of the vegetative tissue occurs via proton-cotransport. The pods are also capable of assimilating CO_2 from the atmosphere and they function to recycle CO_2 respired from the developing seeds. Pea pods also export small quantities of pod-produced sucrose and malic acid back into the rest of the plant via the xylem; the malic acid provides evidence that the C4 photosynthetic pathway may be operating in pod tissue (Hamilton and Davies, 1988). The phloem delivers assimilate to the seed coat or testa through the funiculus, and outward movement into the tissues of the testa is largely by diffusion (De Jong *et al.*, 1996). Sucrose is hydrolyzed to

glucose and fructose upon entry into the testa (Murray and Collier, 1977). Sugars and all other compounds diffuse from the testa into the peri-embryo space and are taken up by the embryo, as there is no cellular connection between the testa and the embryo. The source to sink partitioning of nitrogen is mediated by plasma membrane-localized proteins involved in the phloem loading of amino acids and their import into the seed cotyledons via epidermal transfer cells. Phloem loading of amino acids exerts a regulatory control over pea biomass production and seed yield, and the import of amino acids into the cotyledons is the limiting factor in seed protein levels (Zhang *et al.*, 2015).

More rapid fruit growth is associated with increased carbohydrate accumulation in the fruit as opposed to supporting continuing vegetative growth (Kelly and Davies, 1986, 1988). Transport from source to sink is activated by GA_1, which increases phloem sink unloading in the ovary, and a strong increase in sucrose exported from the source leaf occurs when the GAs reach the leaf adjacent to the ovary (Estruch *et al.*, 1989). An increase in the amount of radiolabeled carbon entering the unfertilized ovary can be seen with the application of GA to the ovary (Jahnke *et al.*, 1989), and the rate of fruit growth is correlated with the level of GA_1 in the developing fruits right from an early stage of growth (Zhu and Davies, 1997).

Net remobilization of mineral nutrients to the developing seeds takes place from both the vegetative tissues and pod wall during seed growth, but continued uptake and translocation of minerals to source tissues during seed fill is as, or more, important than remobilization of previously stored minerals. The proportion of the total shoot mineral nutrients (excluding N) partitioned to the seeds was P: 82%, S: 65%, K: 50%, Mg: 50% and Ca: 5%; micronutrients: Fe 70%, Cu 70%, Zn: 32% and Mn 21% (Sankaran and Grusak, 2014).

Desiccation and maturity

The final stage in seed growth is physiological maturity, followed by harvest maturity. Physiological maturity is attained when the vascular connection between the pod and mother plant is severed (Le Deunff and Archidian, 1988). It is characterized by a color change in the seeds and pods. This leads to a progressive loss of moisture down to approximately 18–14%, causing seeds to dry from the outside to the inside. There is a gradual build-up of desiccation tolerance during the second phase of seed growth. The slow dehydration during the seed filling stage from 85 to 55% prepares the seed for the rapid dehydration after physiological maturity.

Abscisic acid (ABA) accumulates at high concentrations in legume seeds (King, 1982) and regulates the production of storage proteins. ABA is essential not only for stimulation of filling but also for the inhibition of precocious germination (Finkelstein, 2010). This is aided by the presence of osmotic solutes (Barratt *et al.*, 1989).

Plant Senescence

Senescence of the plant follows, and requires the development of fruits. Impending senescence of the entire plant is first noticed as a slowing of apical growth, or apical arrest: the apical bud decreases in size and often assumes a more open appearance due to the presence of numerous flower buds, at the same time as elongation growth is reduced. Apical growth then ceases, and the apical tissues become chlorotic. Although the apical buds at this stage are clearly in the mid stages of the senescence process, they are not dead and can be rejuvenated. Such regrowth often occurs as the fruits and seeds mature, although in such cases the regrowth is a very brief weak flush of growth, which, upon the development of one or two more pods, soon ceases, and the progress of senescence continues. As time progresses, further degradative processes of senescence continue in the arrested apex, so that the tissues become necrotic and die. At this stage no further apical growth can occur. Starting at about the time of apical arrest, the leaves and the rest of the plant visibly start to senesce. The completion of this leaf senescence follows the death of the apical bud (Davies and Gan, 2012).

Unlike most peas, where senescence invariably follows fruiting, apical senescence in the G2 pea genotype, dictated by the presence of two dominant alleles *SN* and *HR*, is regulated by photoperiod (Fig. 12.18). Under LD, the G2 plants flower, fruit, and senesce, while under SD the plants flower and fruit but continue vigorous

growth. The presence of fruits is needed to induce senescence (Fig. 12.19). A physiological transition is initiated at flowering that normally results in a resource allocation to the developing fruits that is detrimental to the maintenance of vegetative tissues. G2 peas allocate less photosynthate to their vegetative buds in LD, when they senesce after flowering, than in SD, when the plants continue to flower without senescing, showing the importance of resource partitioning in the mediation of senescence phenomena (Kelly and Davies, 1988). Before visible senescence symptoms appear in the bud, the differences in the development of flowers and pods show a differential commitment to reproduction triggered by the different day lengths, under which the plants were growing. The flowers and pods of the pre-senescent LD plants develop far more rapidly (Fig. 12.20) (Kelly and Davies, 1986), correlating with the greater resource allocation to reproduction, well before senescence symptoms are visible, while SD plants allocate less photosynthate to the flowers, leading to slower development, and more to the vegetative tissue of the apical bud. The endogenous auxin and gibberellin content of floral and vegetative tissues within the apical buds of these peas correlates with this resource allocation (Zhu and Davies, 1997): a higher gibberellin content of the apical vegetative tissues within the apical bud was associated with vigorous vegetative growth, slower floral development, and continued growth, whereas the greater rate of floral bud growth, which precedes senescence, was associated with a higher indoleacetic acid content in the floral buds. Likewise plants bearing the alleles *ar* and *n*, which have smaller seeds and lower total seed yield, showed a resurgence of growth after apical growth had initially stopped, which is when normal plants undergo senescence. Recessive alleles *ar* and *n* impose a

Fig. 12.18. Peter Davies (left) with Zhu Yuxian (now Professor and member of the Chinese Academy of Sciences) and a short-day-grown G2 pea plant.

Fig. 12.19. G2 pea plants grown in: left: long days (16 h light); right: short days (9 h light). The long day plant has ceased growth and is undergoing senescence whereas the short day plant continues growth indefinitely (P. Davies).

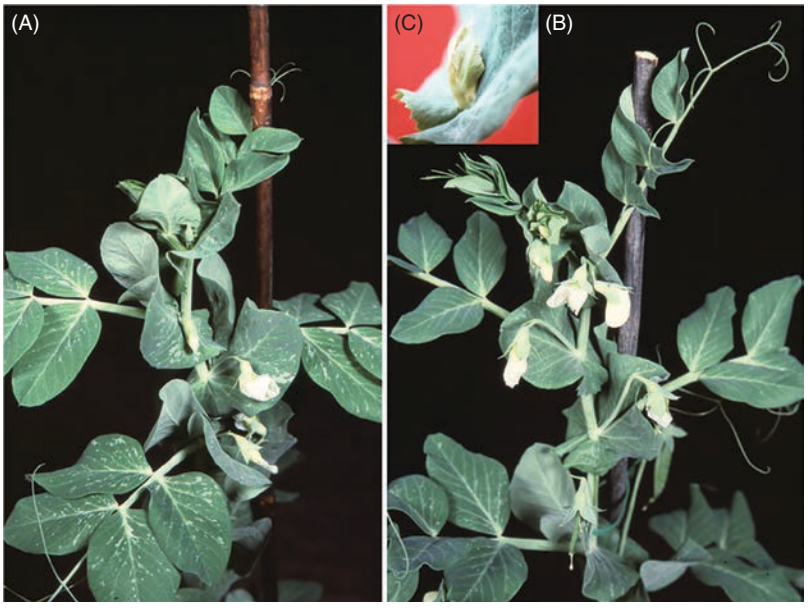

Fig. 12.20. Shoot tips of G2 pea plants just after the start of flowering, grown in (A) short days, in which indeterminate growth occurs, and (B) long days, in which senescence takes place after the production of a certain number of flowers and fruits. Insert (C) shows the apical bud of a LD-grown plant (x4 magnification) in a state of arrest and senescence after further growth has ceased. Note that under LD the development of flowers and fruits is more rapid than under SD, so that flower buds open closer to the stem apex, even right up amongst the developing leaves of the apical bud, causing the apical bud to display a more open appearance. This shows that the signal initiating the transfer of resources to reproductive growth and impending senescence starts very early in the reproductive phase (source: Kelly and Davies, 1987.)

lower metabolic drain as a consequence of their restrictive effects on hilum anatomy and pod morphology, respectively, leading to a reduction in sink capacity (Murfet, 1985). As a consequence, the developing seed crop fails to cause plant senescence and death at the usual developmental time.

As carbohydrates are abundant it is unlikely that carbohydrate partitioning in favor of the reproductive structures is the mechanism behind either apical arrest or the overall senescence of the plant. More likely it is the reallocation of other resources to reproduction that leads to the cessation of vegetative growth and the ultimate demise of the plant (Davies and Gan, 2012). Indeed, during leaf senescence mineral nutrients are mobilized out of senescing leaves to the following extent: N: 65%, P: 80%, K: 45%, S: 70%, although there was no mobilization of Fe, Mg, Ca or any other micronutrients (Maillard *et al.*, 2015).

Factors Affecting Productivity

Leaf morphologies

Optimum planting density is different for each of the leaf morphologies, being higher in leafless and semi-leaflet types. Ninety-five per cent light interception in conventionally-leafed plants is achieved with a leaf area index (LAI) of approximately 5 (Kruger, 1977). The semi-leafless type (Fig. 12.21) is the main type used in most commercial field-scale varieties (Kielpinski and Blixt, 1982). While tendrils have a greater photosynthesis per unit area in the totally leafless mutant, there is a 50% drop in yield compared to fully leafed varieties (Harvey and Goodwin, 1978). The completely leafless type (*af st TL*) has not proven useful in the development of new cultivars because of significantly reduced yields.

Fig. 12.21. Semi-leafless (*af*) peas in the field (courtesy of Rebecca McGee, USDA-ARS Pullman, WA.)

Climatic requirements

Peas are a cool season crop and are grown successfully in cool but not excessively cold climates. Seeds will germinate from 4 to 24°C. Germination at about 15°C , average growing season temperatures of 13–18°C and maximum temperatures of 20 to 21°C produce optimum yields (Fletcher *et al.*, 1966). Maximum temperatures exert a strong influence on both pod formation and the number of peas per pod. The combination of a warm spring (during the seedling tillering) and a cool summer (during the reproductive period) produces a high yield while the combination of cold spring and a hot summer produces a low yield. Lower temperatures early in development allow for greater total biomass accumulation by fostering sufficient root growth to support large vegetative growth (Van Dobben, 1962). Late plantings result in the first flowering node being lower on the plant, due to the photoperiod response to LDs. The earlier flowering under these situations results in decreased vegetative growth since reproductive growth triggers a switch in partitioning from vegetative sinks to reproductive sinks.

Although pea plants are tolerant of mild frost in the vegetative stage, frost at flowering causes heavy pod losses, and frost at pod set produces deformed and discolored seeds. High temperatures above 26°C during blooming and fruit filling negatively affect yield (Pumphrey *et al.*, 1979) by reducing flowering and fruits per plant (Davies *et al.*, 1985).

Water and soil

Evenly distributed rainfall (about 800–1000 mm per year) will maximize pea production (Kay, 1979), although the crop is grown successfully in areas of much lower rainfall (e.g. the crop in Australia where rainfall may be as low as 400 mm per year). In areas where irrigation is necessary, the soil must be deep and capable of storing moisture, in which case 800–1000 m^3 of water per hectare is required during the growth cycle for optimum yields. Under high temperature conditions, maximum economic yields are reported to be obtained when soil moisture content is kept at 60% of field capacity from emergence to just prior to flowering, and at least 90% of field capacity during flowering. Peas are sensitive to short exposures to anaerobic soil conditions, especially just before flowering and during fruit filling (Davies *et al.*, 1985; Ney *et al.*, 1994). Damage from waterlogging is generally more severe at warm temperatures.

Peas are cultivated in a wide range of well-drained soil types. The crop grows best on loams, clay loams or sandy loams overlying clay. Yields tend to be reduced when the crop is grown in sandy soils that do not retain moisture. Peas grow well on soils with moderate levels of calcium and a neutral or slightly acid pH.

Although peas are not considered a deep-rooted crop, they tend to be intolerant of shallow or poorly drained soils, possibly because of the increased incidence of root disease. Peas have a taproot system with numerous lateral roots that form a circle 50–75 cm in diameter and are known to penetrate depths of 100–120 cm (Torrey and Zobel, 1977; Kay, 1979). The crop is not well suited to the extremely leached soils characteristic of high rainfall areas of the tropics and subtropics because of acidity and high temperatures.

Mineral nutrition

Adequate amounts of each of the 14 essential plant nutrients are necessary for satisfactory pea yield (for additional information on mineral nutrient requirements see Muehlbauer and Summerfield, 1988). Certain nutrient deficiencies may result in poor seed quality, reduced pod formation and reduced nitrogen fixation by the root nodules, and an overall reduction in plant growth and yield. Nitrogen, phosphorus and potassium accumulate in large quantities in peas, and a crop of 1000 kg of dry seed may contain up to 43 kg N, 4.2 kg P, 9.2 kg K, 0.6 kg Ca, 1.2 kg Mg, and 0.8 kg S.

Nitrogen. Effectively nodulated pea crops seldom respond to applications of inorganic N fertilizer because such applications reduce nodulation and N_2 fixation, promote weed growth and delay crop maturity (Bezdicek *et al*., 1982). A "nitrogen hunger phase" is often experienced by pea crops planted in cool and wet soil, before the advent of significant symbiotic N_2 fixation. The lack of nitrogen at this early growth phase can be corrected by the application of a small starter dose of inorganic nitrogen fertilizer placed beneath, but not in contact with the seeds at the time of planting. Applications of an appropriate strain of *Rhizobium leguminosarum* is essential when peas are planted into a field for the first time or if peas have not been planted in a particular field for several years. *Rhizobium leguminosarum* is specific for a number of genera in the *Viceae* tribe of legumes that include *Lens, Lathyrus,* and *Vicia.* Longevity of rhizobia in soils planted to peas is long lived; however, it is recommended that inoculum be applied when planting pea crops. Special care is needed in the choice of fungicides for seed treatment and the timing and method of application to prevent potential toxicity to *Rhizobium* (Roughley, 1980).

Large concentrations of residual N in the soil, high salinity, and extremes of pH inhibit nodulation and N_2 fixation. Salinity can increase in the root zone due to the formation of a "plow pan" caused by compaction from heavy machinery. Impervious to water and root penetration, a plow pan layer causes the accumulation of toxic concentrations of salt in the root zone. Pea crops affected by salt accumulations are especially vulnerable to disease attack. The effects of the plow pan layer can be reduced by deep tillage that will also allow free percolation of water and leaching of toxic salts. Crop rotations with strongly tap-rooted crops can also reduce the effects of a plow pan layer.

Phosphorus. Peas will respond to phosphorus fertilization where soils are deficient, as is common in severely eroded soils. Rates of P fertilization can be readily determined by a calibrated soil test. In most instances, a broadcast application and incorporation of between 44 and 66 kg ha^{-1} P_2O_5 can be made in early spring to correct deficiencies. Where equipment is available, however, excellent responses to small amounts of P are obtained by placing the fertilizer in bands beneath the seeds, thereby obtaining good uptake by the pea plants, but not creating a high fertilizer salt concentration in the immediate vicinity of salt-sensitive pea seedlings (Muehlbauer and Dudley, 1974; Koehler *et al*., 1975). Fertilizer salt concentration, however, is not a problem where triple superphosphate is used.

Potassium. Where soil tests indicate potassium deficiencies, K_2O applications of about 22 kg ha^{-1} are beneficial. However, K applications should be made according to rates determined by trials for the area in question.

Sulfur. Sulfur should be applied where soil deficiencies are detected. Applications made to other crops grown in rotation may have sufficient carry-over benefits to the pea crop. Applications at the rate of approximately 17–22 kg ha^{-1} is generally sufficient to correct deficiencies.

Magnesium. Magnesium should be applied where soils contain less than 0.5 mmol available Mg per 100 g of soil.

Trace elements. Deficiencies of one or more of the trace elements, such as manganese, iron, copper, zinc, boron, and molybdenum may be corrected by applications of animal manures that usually contain small quantities of these elements. If animal manures are not available, applications of inorganic amendments to soil can benefit the pea crop.

Manganese. A deficiency of manganese can go undetected but is often characterized by retarded growth and necrotic spotting of the leaves. In severe cases, the seeds take on a symptom known as "marsh spot," characterized by a necrotic spot on the adaxial surface of the cotyledons.

Iron. Severe iron deficiency can be overcome by foliar applications of ferrous sulfate. Two applications each at the rate of 0.9 kg ha^{-1} are sufficient to correct deficiencies.

Molybdenum. Correction of any molybdenum deficiency is necessary (Cutcliffe, 1986), and usually accomplished by seed treatment at the

rate of 35 g ha^{-1} in the form of sodium molybdate with a "sticker" used to ensure uniform adherence to the seeds. Broadcast applications of ammonium molybdate ($(NH_4)_6MoO_7$) at the rate of 1.1 kg ha^{-1} to a soil with gypsum have also been used successfully to correct deficiencies. Fertilization with $NaMoO_4$ at the rate of 0.5 kg ha^{-1} can also be used to prevent deficiencies.

Boron. Extensively cropped soils commonly respond to small applications of boron. Applications of B between 2 and 4 kg ha^{-1} are beneficial to legume crops. Boron fertilizer, usually borated gypsum, is broadcast applied and incorporated by plowing or disking during seedbed preparation. Banded applications are often toxic. Since peas are extremely sensitive to excess of B, applications should not exceed 4 kg ha^{-1}.

pH. Peas are sensitive to both acid (below pH 5.5) and alkaline (above pH 9.5) soils. Optimum nutrient uptake occurs in slightly acidic soils (pH 6.5) because nutrient availability at that pH is most favorable (Halvorson, 1982). Correction of soil acidity by liming results in improved growth, reduced root disease, and improved yields. Excessive liming of acid soils, however, may result in manganese deficiency since manganese becomes less available in alkaline substrates (Kay, 1979).

Nutritional deficiencies and toxicities in peas can be diagnosed by soil testing, visual plant symptoms, or by plant tissue analysis. Unfortunately, the foliar symptoms of plant deficiencies are not distinct unless they are severe, and are often confused with other pathological or physiological disorders.

Physiological Disorders

Water congestion

Pea crops grown under conditions of excess soil moisture, relatively high temperatures and high humidity may develop a condition generally referred to as "water congestion." Typically, the terminal edges of the young leaflets discolor, become deformed and turn brown. Substantial areas of the pea foliage may be affected. The abnormality is generally considered to be of minor importance but may be serious under conditions of high moisture and high humidity (Kraft and Pfleger, 2001).

Hollow heart

Hollow heart is a disorder characterized by a sunken area in the adaxial surface of the cotyledons of pea seed and brought on by rapid drying during seed maturation. Seedlings from hollow heart affected seeds have delayed germination and lack vigor as the cotyledons are often attacked by soil borne damping-off pathogens during imbibition and germination (Harrison and Perry, 1973).

Blonde peas

"Blonde" is a disorder of peas characterized by variations of light green and yellow (blonde) peas in crops harvested for freezing or canning. The condition is thought to be brought about by shading of the pea pods by excessive vine and leaf growth. Also, the condition may be caused by lack of sunshine and cloudy conditions during pod filling. Genetic variation among pea cultivars in susceptibility exists and the increased use of semi-leafless cultivars are considered to have reduced susceptibility due to increased light penetration into the plant canopy.

Bleached peas

"Bleached" pea is a disorder caused by moisture, usually from light rains after the peas have reached dry seed maturity. Apparently the dry peas take up moisture leading to enzyme activation and a breakdown of chlorophyll. The condition causes color variations and is a market down-grading factor in dry peas. Some cultivars have a degree of resistance but when conditions are favorable for bleaching, all cultivars can be affected.

Pea Types

Pea cultivars vary greatly in size and shape of the plants, pods and seeds. Growth habit of all pea cultivars is indeterminate, but some recently released cultivars have a tendency toward determinacy. The move towards determinacy allows a greater majority of the peas to be in the proper stage for processing. The immature peas of the canning and freezing types are sugary when in the proper stage for processing, but become wrinkled upon dry-seed maturity. Canning-type cultivars have a light green color

when in the processing stage, while the freezer type cultivars have a dark green appearance at the same stage. Breeders have attempted to develop cultivars that can be used for both canning and freezing. Peas for home gardening are generally characterized as having large pods and ovules with a high number of peas per pod. Often home garden peas have an indeterminate plant type so as to extend the harvesting period. The various types of peas and their uses are described as follows.

Dry peas

An indeterminate growth habit and smooth and starchy seeds generally characterize dry peas. Seeds can be smooth green or yellow. Regular green or yellow dry peas are usually grown as a field crop in rotation with cereals. They are harvested by direct combining (Fig. 12.22) when seed moisture content is about 12% or less. Dry peas are used either as whole green or yellow peas or they are decorticated and split. Split peas are generally used in soup-making.

Marrowfat peas

Marrowfats are a large, flattened and somewhat dimpled green pea that most likely originated in England in the 16th or 17th century. They are favored in that country where peas generally are very popular. Marrowfat peas differ completely from other dry pea types in their seed type and quality traits. The plant habit of marrowfats is typically dwarf with very large leaves and heavy vines that are generally short and usually well branched. They are generally mid-season or late to flower and as a result are late maturing when compared to Alaska types. The seeds are about twice the size of a typical Alaska pea. In the UK, marrowfats are usually reconstituted and canned and are often referred to as "mushy peas" when served. Marrowfats are used extensively in Southeast Asian countries. In those countries, marrowfats are either fried or roasted with various flavors and packaged in small containers for use as a snack food. This particular use has been expanding in that area of the world and the market potential for marrowfats seems to be quite large. Good quality marrowfats

Fig. 12.22. Harvesting dry peas by combine showing the upright plant habit of a semi-leafless (*af*) pea variety (photo courtesy of the USA Dry Pea and Lentil Council, Moscow, Idaho).

are large seeded with good dark green color, although some countries have the option of using artificial coloring to improve appearance.

Edible-podded types

Edible-podded types have been in use for centuries. The original edible-podded pea has flattened pods and is generally used in the very immature stage in Asian cooking such as a stir-fry. The "snap pea" type has a cylindrical pod that is thick, fleshy and breaks easily when bent, similar to a snap bean. Snap peas are usually picked and consumed when the seed in the pod has developed to nearly its full size.

Freezer pea types

Freezer pea types generally have a dark green testa that imparts the typical dark green color to frozen peas. Similar to canner peas, they are harvested (vined) when tenderometer readings are between 95 and 105. The tenderometer is a device used to measure maturity and suitability for producing a high-quality product. Tenderometer readings above 105 indicate progressive increases in starch content in the peas and a concurrent reduction in edible quality. The time from planting to processing maturity is estimated by the processing industry by using accumulated heat units. Cultivars considered to be early, mid-season and late maturing require 1200, 1350 and 1500 accumulated heat units, respectively, calculated on a base temperature of 40°F (Chapter 4). The timing of planting of pea cultivars is critical to the continuous operation of pea processing factories. Soon after vining the raw product is quickly transported to the processing facility, usually within the hour, and quickly and efficiently processed. Crops that are quickly processed retain quality, while delays reduce the quality of the finished product.

Canned peas

Canned peas typically have a lighter green testa compared to freezer peas. The darker colored freezer peas are usually unsuited to canning because they tend to turn brown from the high temperature when processed and canned. Canner peas are also harvested at tenderometer readings of 95–105. Rapid processing is necessary to retain quality.

Austrian winter peas

Austrian winter peas have pigmented stems, flowers and seeds and are sometimes referred to as *Pisum arvense* or *P. sativum* spp. *arvense*. This particular type of pea is entirely cross-compatible with *P. sativum* and should not be considered a different species. They are usually grown as an autumn-sown crop in relatively cold regions. They have winter hardiness comparable to winter wheat and survive most winters. The crop is also used as green manure, usually at more southern locations. The crop, harvested as dry seed, has been used in the Far East as a filler in the manufacture of An-Paste, a sweet confection commonly used in Japan. The crop is often used as feed for pigeons and other animals but generally not used as human food. In times of shortages of smooth yellow peas, the Austrian winter pea can be decorticated and split to produce yellow split peas. Nearly all the production of Austrian winter peas is in the U.S. Pacific Northwest. Winter hardy germplasm, such as the "Austrian Winter," is being used in breeding with edible dry pea germplasm to develop suitable winter-hardy dry pea varieties that can be fall planted with the reasonable expectation they will survive harsh winters and produce higher yields when compared to conventional spring sown varieties.

Concluding Remarks

Peas played a prominent role in the discovery of the laws of genetics by Gregor Mendel in the mid-1800s and have regained their status as a model leguminous plant used in genetic, genomic and physiological research. Since domestication in the Near East region peas have been distributed around the world and can be successfully produced in a wide range of environments. They possess versatility as a fresh vegetable, dry pulse and as feed for livestock. The short duration of the pea growth

cycle fits well into rotations with cereals and other field crops, and such use is credited with improving soil structure and nutritional status while providing a break crop against diseases affecting other crops in the rotation.

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13 Sweet Corn

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Maize (*Zea mays mays*) was domesticated from *Zea mays parviglumis*, an annual teosinte, 6000 to 10,000 BP (before present) in southwest Mexico (Doebley, 2004). Sanchez Gonzalez *et al.* (2018) published a review of recent ecogeographic information of the teosintes. Crosses between domesticated maize and its wild *Zea* relatives can be made easily, but teosinte has not been widely used in modern maize breeding.

Maize is also one of the few grasses widely consumed as vegetable (green corn). Green corn is consumed at the immature stage, usually between 20 and 28 days after pollination (DAP), when the kernels are soft and succulent, but may or may not be sweet. Green corn is often the common grain maize harvested at an immature stage. When corn is consumed green in Mexico it is called *elote* and in parts of South America it is called *choclo*. An important class of green corn, widely consumed throughout East Asia, is waxy maize, based on the *waxy1* (*wx1*) allele that results in endosperm starch being 100% amylopectin rather than the typical mix of amylopectin (75%) and amylose (25%). Sweet corn norn is distinguished by the presence of one of more defective alleles in the endosperm starch synthesis pathway resulting in increased sugar. Until the 1960s, all sweet corn was homozygous for the *sugary1* (*su1*) (Tracy, 2001). Generally, sucrose makes up at least half of the increased sugar, with fructose and glucose contributing equally to the remainder (Creech, 1965).

Based on archeological records, people have cultivated *su1* corn for at least 3000 years, the oldest samples being from the Andean "Chullpi" complex. Maize races homozygous for *su1* existed in Mexico and what is now the southwestern USA precontact (Tracy, 2001). It is likely that *su1* corn existed in north central and eastern North America as well. Historical records and morphological and genetic analysis indicate that modern sweet corn originated in what is now the northeastern United States and was derived from "Northern Flint" germplasm (Tracy, 2001). The earliest English written record of what is most likely *su1* corn was in 1801 in Bordley's *Husbandry* (Stutevant, 1972). An often repeated, but apocryphal, tale of sweet corn origins is that in 1779 sweet corn was brought to New England by a Captain Bagnall, following the American army's mission to destroy the food supplies of the Haudenosaunee people. The only report of the event was an uncorroborated secondhand account written 40 years later by an individual with a pseudonym (Plymotheus, 1822).

Named cultivars derived largely from "Northern Flint," appeared in catalogs in the 1840s and 1850s (Tracy, 2001). An important exception was "Stowell's Evergreen" released around 1850. It was derived from a cross between "Menomony Soft Corn," likely a "Southern Dent," and northern sweet corn (Downing, 1851). The first widely accepted hybrid, "Golden Cross Bantam," released in 1933, is still sold. "Golden

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Cross Bantam" had excellent quality and worked well both for commercial canners and fresh market growers. The underlying origins of sweet corn are clearly based on "Northern Flint"/"Stowell's Evergreen" but, over the last 30 years, non-sweet germplasm has been incorporated into the breeding pool (Gerdees and Tracy, 1994; Tracy 2001).

Economics

In 2015, the United States grew more than 230,000 ha of sweet corn producing 1.3 Gg for fresh market and 8.6 Gg for processing, totaling a farm gate value of $1.4 billion (USDA, 2016). In 2015, 43% of commercial U.S. sweet corn was grown for the fresh market and 57% was processed (43% canning and 57% freezing). Minnesota, Washington, and Wisconsin are the leading states for processing. Florida and California lead for fresh market production (USDA, 2016).

Initially a crop of eastern North America, sweet corn is now grown throughout the world (Lertrat and Pulum, 2007). Sweet corn consumption and production in Japan and Taiwan grew rapidly in the 1970s and 1980s. European markets and production developed in the 1980s. South Africa, Australia, Argentina, Chile, and Brazil all produce significant amounts of sweet corn. The most explosive growth is in East Asia. Thailand exports canned corn around the world, and China's domestic production and consumption is growing rapidly. Statistics compiled by the FAO are not helpful in understanding world sweet corn production because they report green maize production, confounding all of the different green maize types.

A primary reason for the spread of sweet corn production and consumption was the development of supersweet corn (Marshall and Tracy, 2003). Supersweet corn, based on the *shrunken2* (*sh2*) allele, converts sugars to polysaccharides at a reduced rate than traditional *su1* sweet corn. Thus, supersweets maintain their quality under warmer growing and post-harvest conditions. Also, the high sugar content inherent in *sh2* endosperm increases the success in developing new hybrids when developing tropical sweet corn. The lower sugar content and more subtle quality factors of *su1* limited introgression of non-*su1* germplasm.

Nutritional Quality

Perhaps because of its name, the senior author has had more than one distraught parent contact him because their pediatrician or other authority dismissed sweet corn as a nutritious food, while the parent recognized it was the only vegetable their child would eat. In fact, sweet corn is an excellent source of a number of vitamins, phytonutrients, and minerals including folate, niacin, thiamine, vitamin C, lutein, zeaxanthin, and cryptoxanthin-ß (USDA, 2018). It is also a good source of dietary fiber, iron magnesium, potassium, and zinc. Recent work has focused on the genetics and improvement of carotenoids in sweet corn (Baseggio *et al.*, 2018). White kernelled corn lacks lutein, zeaxanthin, and cryptoxanthin-ß.

Factors Determining Sweet Corn Quality

When eating sweet corn, we bite into a developing kernel. Around 20 DAP, the kernel consists of the developing endosperm and embryo and succulent immature pericarp (ovary wall) (Fig. 13.1). The first sensation we get is tenderness determined by the pericarp thickness (Tracy and Galinat, 1987). We then perceive flavor, largely determined by endosperm sugar content (Reyes *et al.*, 1982). Finally, we perceive texture, ranging from creamy, to starchy. Texture is determined by the ratio of insoluble polysaccharides (starch) to water soluble polysaccharides (WSP) (Culpepper and Magoon, 1927; Dodson-Swenson and Tracy, 2015).

Endosperm development

Following double fertilization, endosperm develops in four stages: coenocytic, cellularization, growth and differentiation, and maturation (Bosnes *et al.*, 1992). The syncytial occurs during the first 72 hours after fertilization, forming a single-celled, multinucleate coenocyte. At the end of coenocytic stage, mitosis increases within the coenocyte and cellularization begins. Cell wall deposition proceeds from the outer endosperm toward the center until the endosperm is completely cellularized (Scanlon and Takacs, 2009).

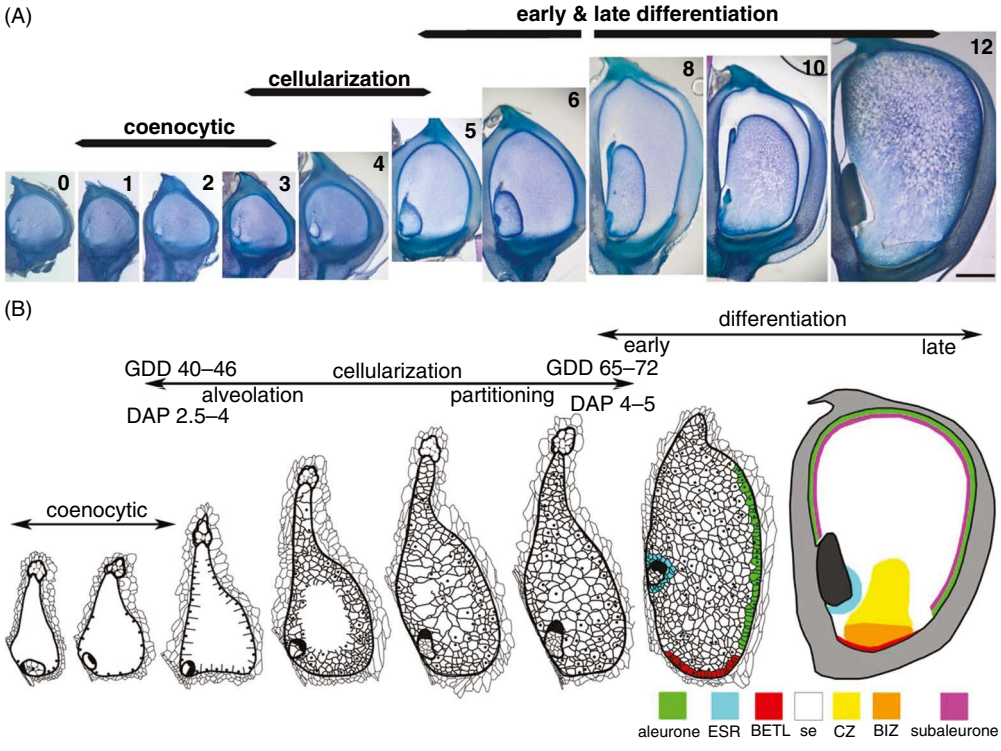

Fig. 13.1. Micrographs and model of B73 maize early endosperm development from a coenocyte to a differentiating endosperm before accumulation of storage metabolites. (A) Micrographs are of progressively older kernels (0–12 d after pollination [DAP]) longitudinally sectioned and stained with toluidine blue. (0) Kernel with unfertilized embryo sac. (1–3) Kernels similar in size with small coenocytic endosperm. (4) Enlargement of endosperm obvious with development of bulbous base. (5–6) Significantly larger and fully cellular endosperm by 5 DAP with identifiable embryo. (8–12) Later stages of cellular endosperm showing rapid growth and more than doubling of endosperm size in each successive image, or approximately every 2 d. All micrographs are presented with embryo to the left and endosperm base at same level. Bar = 1 mm. (B) A schematic highlighting major cytological transitions, timing of transitions, and location of differentiated cell types. Abbreviations: BETL, basal endosperm transfer layer; BIZ, basal intermediate zone; CZ, conducting zone; ESR, embryo surrounding region; GDD, growing degree days; SE, starchy endosperm (Leroux *et al*. 2014).

Following cellularization, four distinct endosperm tissues develop: basal endosperm transfer layer; embryo surrounding region; aleurone; and starchy endosperm. Differentiation is visible 6 DAP (Olsen, 2001). Starchy endosperm goes through a rapid phase of expansive cell division and elongation, first terminating in the central endosperm 12 DAP and ending at the endosperm periphery 20 to 25 DAP (Duvick, 1961). The peripheral layer differentiates into aleurone (Brown and Lemmon, 2007). In most mature maize kernels, aleurone consists of a single layer of cuboidal cytoplasmic cells surrounding endosperm. Aleurone cells remain intact and alive throughout kernel development and germination (Scanlon and Takacs, 2009).

As cell division and elongation terminates, endoreduplication initiates in starchy endosperm, greatly increasing the amount of nuclear DNA (Grafi and Larkins, 1995). Cell size and mass within starchy endosperm also increase during endoreduplication, coincidental with rapid accumulation of starch and storage proteins (Schweizer *et al*., 1995). Synthesis of starch and storage proteins peaks with onset of kernel maturation at 12 to 15 DAP (James and Myers, 2009). With the end of endoreduplication, approximately 16 DAP, starchy endosperm cells

undergo programmed cell death, starting in centrally located cells and spreading to the periphery 24 to 40 DAP (Young *et al.*, 1997a).

Endosperm starch accumulation and storage

Starch in nonmutant maize endosperm is 25% amylose and 75% amylopectin (Hannah, 2005). Starch is a glucose homopolymer, produced via a complex integrated pathway. Understanding the enzymatic steps in this pathway has been accomplished using maize as a model organism (Preiss, 1991; Nelson and Pan, 1995; Hannah, 2005) (Fig. 13.2). Within this pathway, four groups of enzymes play critical roles in determining the amounts and types of carbohydrates that accumulate in the kernel: adenosine diphosphate glucose pyrophosphorylase (AGPase); starch syntheses; starch branching enzymes; and starch debranching enzymes (Hannah, 2005).

Endosperm mutants in sweet corn

At least eight loci have been used in commercial sweet corn (Table 13.1). All but one of these code for enzymes in the pathway. To understand their epistatic relationships and effects on sweet corn endosperm quality and seed physiology, Boyer and Shannon (1984) divided them into two classes. The three mutants in class1 are expressed in the cytosol, and result in severe

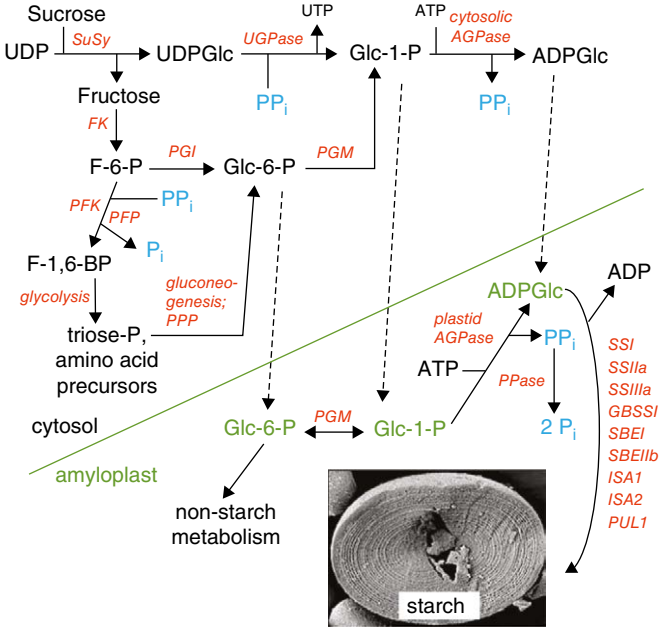

Fig. 13.2. Pathway of the central carbohydrate metabolism in developing maize kernels. The map is based on the recent literature on maize or higher plant biochemistry. Enzymes are red, cytosolic substrates are black. and substrates in the amyloplast are green. Personal communication Alan Myers. ADPGlc, adenosine diphosphate glucose; AGPase, ADP-glucose pyrophosphorylase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; DBE, starch debranching enzyme; FK, fructose kinase; F-6-P, fructose-6-phosphate; GBSS1, glucose bound starch synthase; Glc-1-P, glucose-1-phosphate; Glc-6-P, glucose-6-phosphate;ISA, isoamylase; PFK, phosphofructokinase-1; PFP, diphosphate—fructose-6-phosphate 1-phosphotransferase; PGM, phosphoglycerate mutase; PPP, pentose phosphate pathway; Pi, inorganic phosphate; PGI, phosphoglucose isomerase; PPase, plastidial soluble inorganic pyrophosphatase; PPi, pyrophosphate; PUL, pullulanase; SBE, starch branching enzyme; SS, starch synthase; SuSy, sucrose synthase; UDP, uridine diphosphate; UDPGlc, uridine-diphosphate glucose; UDPase, UDP-glucose pyrophosphorylase; UTP, Uridine triphosphate.

Table 13.1. Wild type genes encoding enzymes that are involved in the starch synthesis in maize endosperm (source: Hannah, 2005).

Gene	Enzyme	Class	Source
Amylose-extender1 (*Ae1*)	Starch branching enzyme IIa	2	Fisher *et al*., 1996
Brittle1 (*Bt1*)	Adenylate transporter	1	Sullivan *et al*., 1991
Brittle2 (*Bt2*)	AGPase small subunit	1	Hannah and Nelson, 1976
Dull1 (*Du1*)	Starch synthase III	2	Gao *et al*., 1998
Shrunken2 (*Sh2*)	AGPase large subunit	1	Hannah and Nelson, 1976
Sugary1 (*Su1*)	Isoamylase1	2	James *et al*., 1995
*Waxy*1 (*Wx1*)	Granule-bound starch synthase	2	Nelson and Rines, 1962
Sugary Enhancer1	unknown	2	Ferguson *et al*., 1978 1979

reductions in starch and total carbohydrates, and large increases in sugar. At 18 to 21 DAP, endosperm homozygous for a class1 mutant has four to eight times more sugar than starchy endosperm (Laughnan, 1953; Cameron and Teas, 1954; Creech, 1968; Tracy, 2001). Due to high sugar levels class 1 cultivars are often called "supersweet."

Class 1 mutants are generally epistatic over the class 2 mutants, which are expressed in the plastid. Class 2 mutants change proportions of polysaccharides and sugars in the kernel, with small reductions in total carbohydrate content. An eighth allele, *se1*, is widely used in commercial sweet corn, but its function is unknown. Combinations of any of these alleles result in synergistic effects which further reduce polysaccharide concentration and increase sugar content.

Sugary1 (su1)

The traditional allele used in sweet corn, *su1*, is also designated *su1-ref*, as the reference allele. *Su1* encodes an isoamylase-type debranching enzyme and *su1* is loss of function mutation, increasing sugars and WSP. Visual phenotype of *su1* endosperm is wrinkled and glassy (Garwood and Creech, 1972). Twenty DAP, dried *su1* kernels contained 15.6% total sugars, 22.8% water-soluble polysaccharide (WSP), and 28% starch, while starchy kernels contained 5.9% total sugars, 2.8% (WSP), and 66.2% starch (Creech, 1968).

In *su1* corn, sugar content reaches a maximum around 19–22 DAP. After this time, sugar is converted into starch and WSP, resulting in a loss of sweetness over later harvest dates. The mature kernel will have as little as 5% sugar and 1:1 ratio of starch to WSP. Conversion seems to be accelerated after harvest, with unrefrigerated *su1* ears losing 50% of their sugar in 24 hours. Because sugars are converted to WSP using a refractometer to measure or predict sweetness is not effective (Hale *et al*., 2005). Cultivars based on *su1* are no longer used in the fresh market and have been replaced by high sugar types. In seed catalogs *su1* varieties are often listed as "traditional" or "old fashioned."

Shrunken2 (sh2) and brittle2 (bt2)

John Laughnan (1953) of the University of Illinois suggested that *sh2* would be useful in the sweet corn industry. Supersweet hybrids have been instrumental in the increasing popularity of sweet corn around the world. Wild type *Sh2* codes for the large subunit of AGPase. AGPase is tetrameric with two large and two small subunits. Knocking out AGPase results in increased sugar and decreased polysaccharides. The visual phenotype of dry mature *sh2-r* kernel is shrunken and opaque (Garwood and Creech, 1972). Twenty DAP, *sh2-r* kernels contained 34.8% total sugars, 4.4% water-soluble polysaccharides, and 18.4% starch (Creech, 1965). The *sh2-r* mutant greatly reduces the weight of the kernel (Laughnan, 1953; Cameron and Teas, 1954).

Over the past 40 years *sh2-r* has come to dominate in sweet corn industry. In the United States, over 70% of all sweet corn that is currently used for processing is *sh2-r*. The transition to *sh2-r* from traditional *su1* sweet corn hybrids is due to greater sugar content, higher kernel moisture content, and longer postharvest shelf life (Carey *et al*., 1982). Sugar levels are high enough so there is no need to add refined sugar to the canned product. Shelf life of *su1* declines quickly after harvest with rapid moisture loss and con-

version of endosperm sugars to polysaccharides, resulting in a narrow window for processing and fresh market sales (Wong *et al.*, 1994). In *sh2-r* sugar conversion to polysaccharides is reduced, resulting in longer postharvest periods (Garwood *et al.*, 1976). However, *sh2-r* has reduced germination relative to *su1*. Improvements in seed production, seed treatments, and breeding have reduced, but not eliminated, the negative seed quality issues associated with *sh2-r.*

The *shrunken2-intermediate* (*sh2-i*) allele was produced by Dr. Gyula Ficsor using EMS treatment of the mature kernels (Neuffer, 1996). The *sh2-i* produces a small amount of AGPase and has an intermediate phenotype compared to *sh2-r* with more starch produced. Combining *sh2-i* with *su1* results in cultivars of exceptional quality (Dodson-Swenson and Tracy, 2015). The WSP content in the double mutant *sh2-i su1-r* was greater than in *sh2-r* and *sh2-r su1-r*. Sugar content in the double mutant *sh2-i su1-r* was greater than in *su1-r* or *sh2-i.*

The product of *Bt2* is the small subunit of the AGPase enzyme. Recessive *bt2* has the same effects on endosperm carbohydrates as *sh2.* Singly *bt2* is not widely used in sweet corn but is used in combinations with other mutations.

Sugary enhancer1 (se1)

The *sugary enhancer1* (*se1*) gene (Ferguson *et al.*, 1978, 1979) does not fit neatly into an endosperm class. For use in sweet corn, *se1* is always combined with homozygous *su1*. When in combination with *su1*, homozygous *se1* results in sugar levels near those of *sh2*, and WSP levels similar to those of *su1*; resulting in a high-quality, sweet and creamy endosperm (Gonzales *et al.*, 1976). In terms of both preharvest and postharvest, *su1 se1* loses sugars at a rate similar to *su1* (Ferguson *et al.*, 1979). For maximum quality, sugary enhancer types should be harvested at peak quality and consumed rapidly after harvest. Given its very high quality and the need for rapid consumption, sugary enhancer has been popular in local roadside markets, although it is losing market share to high quality supersweets.

Brittle1 (bt1)

Brittle1 codes for a membrane transporter required to move ADP-glucose from the cytosol into the amyloplast. Boyer and Shannon (1984) placed it into class1, but its effects aren't as severe as those of *sh2* and *bt2*. The kernels are heavier and somewhat less sweet than *sh2* kernels, in turn germination and seedling vigor is better. While not widely used in temperate sweet corn, Dr. James Brewbaker of the University of Hawaii has developed successful tropical cultivars based on *bt1* (Brewbaker, 1971). Many tropical sweet corn breeders have used the Hawaiian germplasm. Nevertheless, it appears that today *sh2* is predominant in the tropics (Letrat and Pulum, 2007).

Combining endosperm mutants

Given the number of genes affecting sugar content in maize endosperm, numerous gene combinations are possible. Some, such as *su1 se1*, rapidly attained commercial success, whereas others, such as *ae1 du1 wx1*, did not (Marshall and Tracy, 2003). New combinations are developed as breeders search for high-quality cultivars with excellent seed quality.

The most common combination is the double homozygous recessive, *su1 se1*. A new type that has very high quality but has yet to attain significant market share is homozygous for *su1* and heterozygous for two defective alleles at *Sh2*, *sh2-r* and *sh2-i*. Another common gene combination is partial modification, which is based on two or more recessive genes at least one of which is homozygous while the other is heterozygous in the seed that is planted (Courter *et al.*, 1988). In the grower's field, the heterozygous gene segregates on the ear produced for consumption. Thus, 25% of the kernels express both endosperm mutants. Kernels in which both mutations are expressed usually have higher sugar content. Seed of such hybrids is produced by crossing an inbred, homozygous-recessive at one locus (e.g. *sh2*) and homozygous-dominant at another (e.g. *Bt1*), with an inbred homozygous-recessive for both *sh2* and *bt1*. The seed from this cross would have the genotype, *sh2sh2 Bt1bt1*. In the ears produced by the grower, 75% of the kernels on the ear are *sh2sh2 Bt1-* and 25% are *sh2sh2 bt1bt1*. Thus, 25% of the kernels from such a hybrid will have elevated sweetness compared to the *sh2sh2 Bt1-* kernels supersweet.

Combinations, in which three recessive genes are used, leading to partial modification are available and often called trigenic or "triplesweets." In this case, the grower plants seed with two genes heterozygous and one homozygous. After meiosis and pollination in the grower's field, 44% (7/16) of the kernels have elevated sugar levels. An example of trigenic combination is *su1su1 Se1se1 Sh2sh2* (Courter *et al.*, 1988).

Other Quality Factors

Aroma and tenderness

In addition to sweetness, sweet corn flavor is affected by compounds that impart distinctive aromas, especially during cooking. It is possible to select for improved corn flavor in a breeding program by using taste tests. The biochemistry of aroma-causing compounds is complex, but sulfur-containing compounds, such as dimethyl sulfide and hydrogen sulfide play a role (Williams and Nelson, 1973; Flora and Wiley, 1974a, 1974b; Dignan and Wiley, 1976; Wiley, 1985; Azanza *et al.*, 1994).

Tenderness is very important in processed corn since, unlike sweetness, it is difficult to modify. Tenderness, the resistance of the pericarp to chewing (Huelsen, 1954), is negatively correlated with pericarp thickness (Bailey and Bailey, 1938). Pericarp is the outermost layer of the corn kernel and is derived from the ovary wall. Relatively few genes control the inheritance of pericarp thickness (Richardson, 1960). In some crosses, a major gene appears to condition thinness with several modifying genes for thickness (Tracy and Galinat, 1987). In hybrids, the inheritance of pericarp thickness tends to be additive, with a slight tendency toward the thin-parent value (Richardson, 1960). Pericarp thickness can be altered by selection. It is determined either by bite testing or measurement with various types of micrometers (Tracy and Schmidt, 1987).

Kernel Color

Kernel color, another important quality trait, is determined by three parts of the kernel. Sweet corn usually has the *P1-ww* allele resulting in a clear pericarp and white cob (Coe *et al.*, 1988). Beneath the pericarp is the aleurone, the outermost layer of the endosperm. Most sweet corn carry alleles that inhibit anthocyanin development in the aleurone (Coe *et al.*, 1988); however, there are some hybrids that have colored kernels due to anthocyanin production in either the pericarp or the aleurone. These pigments are water soluble so steaming or boiling results in loss of color.

Beneath the aleurone is starchy endosperm, which is either yellow or white controlled by the *Y1* gene with white being recessive to yellow (Coe *et al.*, 1988). Modifying genes influence the shades of yellow and white. Commercial cultivars are classified as yellow, white, or bicolor. Bicolor hybrids are produced by crossing a yellow inbred, *Y1Y1*, with a white inbred, *y1y1*. When the resulting F_1 (*Y1y1*) pollinates in the farmer's field a segregation of three yellow to one white kernel will appear on the ear. There are strong regional preferences for endosperm color, with bicolors being particularly important in the Northern United States and Japan. White endosperm hybrids are popular from the Mid-Atlantic region through the South. Yellow corn is the most important type for processing.

Endosperm Mutants and Germination in Sweet Corn

When compared to wild type maize, germination of class1 mutants is reduced (Rowe and Garwood, 1978). One proposed reason for reduced germination in these mutants is decreased starch concentrations in mature kernels, resulting in decreased energy stored for the emergence (Douglass *et al.*, 1993). Kernels of these mutants weigh less than wild type kernels (Wann, 1980; Schmidt and Tracy, 1988). Decreased starch concentration, along with high sucrose levels, appears to delay α-amylase transcription and reduces starch hydrolysis during early stages of germination (Young *et al.*, 1997b). There may also be a negative relationship between kernel sugar concentrations and germination and emergence (Douglass *et al.*, 1993; Zan and Brewbaker, 1999).

In addition to direct effects of increased sugar and decreased starch concentration due to endosperm mutants, there are other kernel properties

affecting germination and many are negatively affected by *sh2-r*. Since *sh2-r* reduces polysaccharides content, the endosperm can shrink away from the pericarp as the kernel dries, creating air pockets between the endosperm and pericarp (Styer and Cantliffe, 1983; Juvik *et al.*, 1992), leaving the pericarp vulnerable to mechanical damage. Damaged pericarp reduces germination in all endosperm types (Kohler, 1957). During imbibition, in kernels with intact pericarp water movement is primarily through the tip cap. When the pericarp is broken, water rushes into the kernel and may interfere with cell membrane reorganization (Parera *et al.*, 1996; Tracy, 2001). This results in leakage of soluble cellular contents, including sugars, salts, and other electrolytes from the seed (Wann, 1986). Electrolyte leakage is significantly greater in *sh2* kernels than in *su1*, and it is negatively correlated with percent germination (Styer and Cantliffe, 1983; Wann, 1986; Schmidt and Tracy, 1988). A damaged pericarp creates a number of problems, including membrane damage and leakage. Solute leakage during germination can also increase infection by fungal pathogens (Styer and Cantliffe, 1984; Wann, 1986; Tracy and Juvik, 1988; Headrick and Pataky, 1989; Headrick *et al.*, 1990; Parera and Cantliffe, 1991). Imbibing cold water (< 15°C) increases the damage. Other factors that influence germination in sweet corn include genetic background, health of the maternal plant, and seed maturation at harvest (Tracy, 1993; Marshall and Tracy, 2003).

Growing High-Sugar Sweet Corn Seed

Seed of high-sugar types is much smaller than *su1* seed. Seed of *su1* averages 5500 kernels per kg, whereas sugary enhanced (*su1su1 se1se1*) averages 7700 kernels per kg, supersweet (*Su-1Su1 sh2sh2*) averages 8400 kernels per kg and *su1su1 se1se1 sh2sh2* averages 14,300 kernels per kg (Marshall and Tracy, 2003). The much smaller seed of the high-sugar and *su1*-high-sugar combinations have made it necessary to use precision planters rather than the older plate-type planters used to produce *Su1* or *su1* hybrid seed.

Ample moisture is necessary for germination of high-sugar corn seed. It also is essential that the seed be planted no deeper than 5 cm, as compared to the maximum of 7 to 8 cm for *su1* seed. High-sugar seed generally needs warmer soil temperatures (> 16°C) to establish a good, uniform stand. Most *su1* sweet corn requires a minimum temperature of 10°C. After the high-sugar seedling has passed the 15-cm stage and the stand is established, agronomic practices are the same as for *su1* (Huelsen, 1954). Differences in producing the high-sugar seed and *su1* seed arise again at harvest time.

Harvesting and Conditioning High-Sugar Sweet Corn Seed

High-sugar seed is commonly harvested when the kernel moisture is between 50 and 55% moisture, higher than for *su1* seed, which is commonly harvested at a moisture of ~ 30%. The high-moisture content and the tenderer pericarp of the high-sugar and *su1*-high-sugar combinations has necessitated the specialized design and manufacture of modified pickers to harvest the ears. Harvested ears of high-sugar types are not husked in the field, as were ears of *su1* corn or field corn. Ears with husks are brought into the conditioning plant, where specialized husking beds built specifically to handle the high-sugar ears remove the husks. Husked ears are placed in drying bins.

High-sugar seed loses moisture more slowly than *su1* sweet corn seed, thus drying the ears takes longer and is more expensive. Temperature and drying rate are critical to good seed quality. In general, temperatures should be less than 37°C and the drying rate should be rapid, which requires large air volumes to be moved through driers as quickly as possible.

During the drying process, extreme shrinkage of high-sugar endosperm results in the embryonic axis protruding from the kernel, which makes the axis vulnerable to mechanical damage. To reduce physical damage to the pericarp and embryo, the shelling, milling, sizing, kernel sorting, transporting, treating, and packaging processes have been modified to handle high-sugar seed (Marshall and Tracy, 2003). Much of the equipment has been rubberized. The sheller has been modified by reducing the cylinder speed and reconstructing the cage in which the cylinder is housed to allow bypass of pre-shelled seed.

Milling and sizing machines have been modified to cope with seed half the weight and size of *su1* seed. Electric-eye instruments have been installed to remove discolored seed. Transporting the seed from process to process in the seed-conditioning plant has been modified to eliminate abrasion, falls, and pressure that can cause mechanical damage to the high-sugar seed.

After conditioning, high-sugar seed is treated with seed protectants. The pathogens of most concern at the seedling stage are *Pythium* spp., *Fusarium* spp. and *Rhizoctonia zeae* Voorhees (Headrick and Pataky, 1989; Pataky and Eastburn, 1992). Also, in the mid-1980s, *Penicillium oxalicum* was recognized as a problem of seedling-stand establishment in *sh2* hybrid seed-production fields and commercial-hybrid sweet corn fields. The *Penicillium* problem known as "five-leaf die back" led to the conditional use of systemic seed treatment fungicides for control (Pataky and Eastburn, 1992; Wilson *et al*., 1993). In the United States, all sweet corn seed is customarily treated with a combination of seed treatment fungicides. Usually the seed treatments are applied with a water-soluble polymer to help bind the protectants to the seed and keep dust to a minimum. Producing supersweets without seed treatments is problematic. Under cold conditions *Pythium* will be a problem and even under more favorable warm soil conditions *Penicillium* will cause stand loss. Some organic farmers who grow supersweet sweet corn are germinating seeds in sterilized soil in greenhouse and transplanting at the three or four leaf stage.

Anatomy and Development

The classification of the 300 races of maize in the 1940s and 50s was based on morphology, especially of the ear, and ecogeographical adaptation (Wellhausen *et al*., 1952). Ear and kernel shape differ greatly among the races as does length of growing season and plant stature. Maize is adapted from lowland to highland (3500 m) tropics and temperate regions from arid areas such as the southwestern US to high rainfall (> 5500 mm) areas. Despite wide adaptation, the basic anatomy and physiology of the maize plant is consistent across the races (Fig. 13.3). The main difference is the size of the plant, which is generally related to the length of growing season; longer-season varieties are larger and more robust. Numerous monographs, articles, have described in detail the anatomy and development of maize (Kiesselbach, 1950; Cheng and Pareddy, 1993).

Maize is an annual grass with a solid culm. Upon germination, the first five nodes stay below the soil surface and form a condensed crown. Adventitious roots emanating from the crown make up the secondary root system, which becomes the permanent root system. In some varieties, adventitious roots grow from above ground internodes and may contribute to the permanent root system. Early in development the meristem remains below or near the soil surface laying down internodes, nodes, buds, leaves, and reproductive tissues. Maize is a determinate plant and the apical meristem develops into the male inflorescence. The leaves consist of blades and sheaths, and the sheaths wrap tightly around the culm, offering structural support. Depending on the genetics and environment below ground nodes may initiate vegetative branches, called tillers or suckers. Female inflorescences (ears) are initiated at the nodes in the middle part of the culm. Depending on genetics and environment one to six ears may be initiated. At modern agricultural planting densities only one ear, (usually the uppermost) will produce kernels. The male inflorescence is formed when the meristem is within the whorl of leaves. An interesting exception to this is the extremely short season variety "Gaspé Flint," in which the tassel is already initiated in the embryo. After tassel initiation, culm internodes elongate rapidly and the tassel emerges from the whorl. As the tassel emerges the ear shoots emerge from leaf axils. The reproductive period begins at this time. Reproductive phase in sweet corn in no way differs from that of other types of maize, which has been well described (Bedinger and Russell, 1994).

Environmental Factors Affecting Growth, Development, and Quality

Due to the economic importance of maize, the effects of light, temperature, water, and nutrients are well studied (Tollenaar and Dwyer 1999;

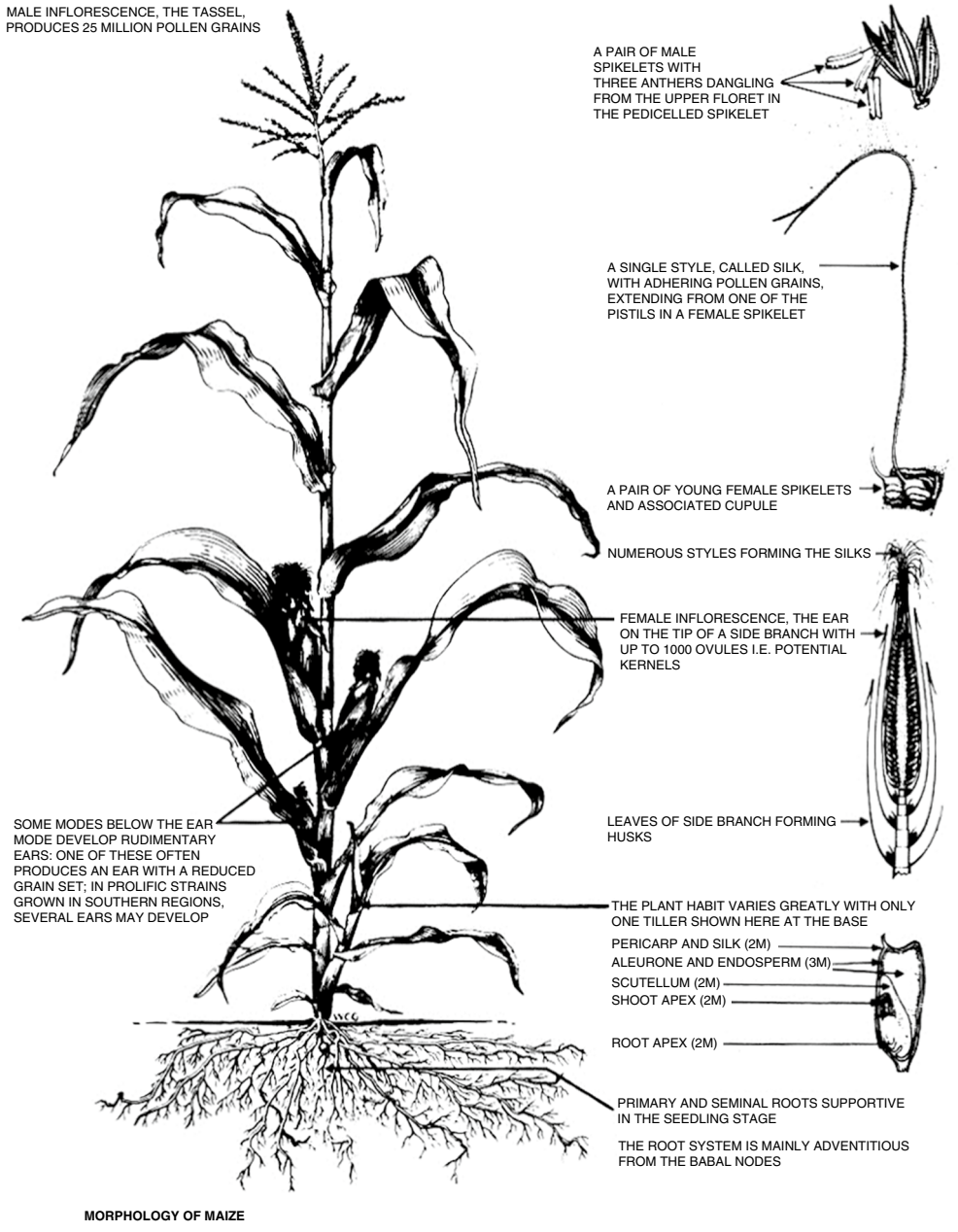

Fig. 13.3. The morphology of maize (Galinat, 1985).

Hoeft *et al.*, 2000; Chen *et al.*, 2015). Sweet corn performs similarly to grain corn in response to light, temperature, water, and nutrients. Differences are due to the shorter growing period of sweet corn, especially post-pollination, and a greater range of planting dates.

In grain maize production, maize crops are planted such that they make full use of the growing season. In north temperate areas, this would result in spring planting from March (southern Corn Belt) through mid-May (northern Corn Belt). This maximizes the amount of

light available (longest light period ~ 21 June) and warm temperatures, which maize, a C4 plant, tolerates well. Photosynthesis is maximized by high light intensity and long days, and warm temperatures accelerate growth rates. In temperate regions, development of reproductive structures is initiated during a period of increasing daylength and grain filling occurs under shortening daylength. Post pollination plants have a 50- to 60-day grain filling and maturation period. Grain maize cultivars have been bred to maximize the available growing season for specific maturity zones, which are based on accumulated growing degree days (GDD) or heat units. Growing degree days are calculated as the sum of daily average temperature minus 10°C (Cross and Zuber, 1972). If daily average is below 10°C, then 10°C is used: if the temperature is greater than 30°C, then 30°C is used. A base temperature of 10°C is usually assumed. Developmental response to temperature is linear between 10 and 30°C (Fischer and Palmer, 1984).

Sweet corn has a shorter growing season than grain maize as it is harvested 18 to 25 DAP depending on GDD. Sweet corn generally requires fewer days to pollination, depending on the cultivar, flowering between 45 to 80 days after planting.

In contrast to grain maize, sweet corn is planted every week of the year somewhere in the continental United States. In the northern states, it is planted as early as the local grain maize, experiencing lengthening days and warming temperatures, and as late as mid-July, experiencing shortening day length and cooling temperatures. In the south, Georgia, Florida, Texas, and Mexico, sweet corn is planted from late August through April. In these environments, flowering is induced under short days (> 11h) and a wide range of temperatures.

Light

Maize is a quantitative short-day plant (Kiesselbach, 1950), and photoperiod can influence leaf number and timing of flowering. Short days tend to reduce leaf number and accelerate tasseling and silk emergence (Huelson, 1954). Temperate sweet corn cultivars show a range of responses to day length. Sweet corn bred for summer production in the northern USA may be unadapted to the short hot days of winter plantings and plant height may be reduced in height by 1 m or more and flowering accelerated by as much as 21 days compared to growth under long day plantings. Cultivars developed under short day environments for winter production in the southern United States, usually perform similarly under long day conditions. Both sweet corn and grain maize cultivars bred for the tropics require day lengths shorter than 13 h for induction of flowering (Fischer and Palmer, 1984; Hung *et al.*, 2012).

Temperature

Given adequate light, water, and nutrients, temperature is the primary determinant of time to flowering and from flowering to ear harvest (Tollenaar *et al.*, 1979). Maize has a relatively high optimum temperature for photosynthesis, but hot temperatures can reduce seasonal productivity by accelerating developmental rate, shortening growth phases, reducing leaf area duration and increasing respiration (Duncan, 1975). Temperatures above 35°C are usually unfavorable for photosynthesis and reproductive development. Photosynthetic rate is affected by daytime temperatures, whereas developmental rate and respiration rate are determined by day and night temperatures. Therefore, the optimum growing environment for maximum productivity is one with warm days and relatively cool nights.

Cool nights are particularly important post pollination because cool nights slow the maturation rate of the kernels, extending the "harvest window" when kernels maintain high sugar content and tender pericarp. This is one reason why most sweet corn processing facilities are located in northern states. Maximum productivity occurs with daytime temperatures ranging from 24–30°C and average night temperatures about 13°C (Peirce, 1987).

Maize is chilling-sensitive suffering photosynthetic depression, photoinhibition, and chilling damage at temperatures below 5°C (Wolfe, 1991). Most maize cultivars don't grow at temperatures less than 12°C. There are genotypes that tolerate cooler temperatures (Hotchkiss *et al.*, 1997). Maize is intolerant to freezing temperatures and will die if exposed to temperatures < –2°C for even short periods of time. However, sweet corn kernels are not chilling sensitive. To

maintain quality, harvested ears should be either processed or chilled to near 0°C as soon after harvest as possible to slow respiration and maintain quality. Chilling is required for fresh sweet corn that will be shipped long distances.

Water and nutrients

Sweet corn has a relatively high water use efficiency (carbon fixed per water transpired). But it is not tolerant of drought conditions due to its relatively shallow rooting systems (e.g. 0.5–0.75 m), and sensitivity of the reproductive and kernel maturation phases to water stress. Short periods of drought during the reproductive phase negatively affect synchrony between pollen shed and silk receptivity for fertilization (Wolfe *et al*., 1988). This results in poor pollination and kernel development. Drought can also cause poor husk cover, leading to bird damage on exposed ear tips. Several studies have indicated that high temperatures and excessive rainfall are associated with lower sugar concentration (Culpepper and Magoon, 1927; Straughn and Church, 1909).

Mineral nutrition and pH requirements are similar to field corn (Heckman, 2007), except that sweet corn has a shorter growing season and produces less biomass. Maximum yield requires relatively high levels of nitrogen fertilizer. Recommendations can range from 100 to 165 kg nitrogen ha^{-1}, depending on soil type, cultivar, plant density, and rainfall or irrigation frequency. To minimize leaching of nitrates below the root zone, nitrogen applications are usually split between pre-plant and later side-dressings (before plants exceed about 30 cm in height). Phosphorus and potassium fertilizers are also applied as needed based on soil test results and local experience. Sweet corn does not have a high requirement for trace elements, but on light sandy soils and at certain pH levels, boron, zinc, magnesium, or manganese may be inadequate for normal growth.

Summary

Over the last 50 years, sweet corn has expanded from a crop grown in temperate North America to one grown on every continent with arable lands and in the tropics as well as temperate zones. Sweet corn production continues to expand rapidly in tropical areas of South America and east and southeast Asia. Among the reasons for this expansion are the increased use of high sugar mutants that maintain quality for longer periods of time, both on the plant and post-harvest, and breeding that has adapted the crop to more environments. Another reason for the expansion of sweet corn production is increasing incomes of people all over the world. To some degree sweet corn is a luxury and as people have more expendable income markets expand. It is likely that there are more sweet corn breeders today in tropical regions than in temperate regions. Further because of current research on endosperm genetics we can expect to see increases in eating quality (Dodson-Swenson and Tracy, 2015). There are efforts improving nutritional quality (Baseggio *et al*., 2019). Given these factors it is likely that sweet corn will continue to grow in worldwide importance.

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14 Lettuce

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Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables grown in North America, and forms an important part of temperate climate production systems in Europe and other regions (Ryder, 1999). World lettuce production has been increasing steadily, reaching 25 million metric tons (mmt) in 2014, with half grown in China (FAO, 2017) (Table 14.1). China is also primarily responsible for the production increase, which rose from 4 to 14 mmt between 1994 and 2014 (Table 14.1). In the United States, 3.8 million metric tons of lettuce was produced on about 107,000 ha, putting it first in importance among vegetables ahead of sweet corn and onions (USDA, 2017). California is the principal growing area, accounting for about 73% of national production.

Lettuce is an ancient vegetable crop that has been cultivated in the Mediterranean basin since around 4500 BC. Early forms of the crop were depicted on Egyptian tombs as having long stems, and pointed, short, upright leaves. They were probably cultivated for the edible oil in their seeds, rather than for the leaves (Ryder and Whitaker, 1976; Harlan, 1986). By the time of the ancient Greeks, forms whose leaves were consumed either fresh or cooked had been selected. Types which form heads did not appear until 1543 in Europe (Helm, 1954; Ryder and Whitaker, 1976).

Modern lettuce cultivars can be grouped into four or five types, according to plant form and predominant use (Ryder, 1979). The "crisphead "(or "iceberg") forms firm, closed heads resistant to mechanical damage and is tolerant of long-distance shipping. It is the most important type grown, and makes up the bulk of production in North America. The "butterhead" type forms loose, open heads, and has soft leaves easily damaged in handling. Leaf lettuce also shares this fragile nature, and both types must be well protected in shipping. "Cos" or "romaine" lettuce has erect, elongated leaves that form into a loose, loaf-shaped head. "Stem" lettuce is grown for its thickened, parenchymatous stem, harvested when the plant is still in the vegetative stage (Helm, 1954). In summarizing the physiology of the lettuce crop, the growth and function of the principal types, namely the crisphead, butterhead and leaf lettuce will be emphasized, because these have been most intensively studied.

Seed Germination

Lettuce is sown directly in the field in the major North American production areas but transplanted in Europe and Asia (Ryder, 1999). The production cycle may be as short as 60 days, and populations of 50–70,000 plants ha^{-1} would make transplanting uneconomical in many areas (Zink and Yamaguchi, 1962; Ryder, 1986). Since uniformity of growth is vital to achieve high yields

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of marketable product for a single harvest, much effort has gone toward ensuring that a maximum number of plants emerge at the same time. Unfortunately, two properties of lettuce seeds make this goal more difficult to achieve than with many other crops. First, lettuce seed is sensitive to high temperatures. If lettuce seed is subjected to temperatures of 30°C or above during the imbibition stage of germination, it becomes dormant and is delayed in germination (thermodormancy) (Ikuma and Thimann, 1964; Gray, 1977). The second phenomenon is the inhibition of some cultivars from germinating if the seed is subjected to dark conditions, termed skotodormancy (Borthwick *et al.*, 1954). These two forms of dormancy have been extensively studied, and practical ways have been found to overcome the constraints.

The lettuce reproductive structure is actually a fruit, consisting of an embryo surrounded by an endosperm and an outer pericarp (Fig. 14.1). The inhibition of the seed germination is brought about by the failure of the radicle to break through the endosperm that is acting as a physical barrier, and by the inhibition of growth of the seedling imposed by growing conditions (Takeba and Matsubara, 1979). For instance, if seeds are exposed to 30°C instead of 20°C from the start of imbibition, germination percentage is reduced unless the seedcoat is slitted or punctured (Prusinsky and Khan, 1990). At 35°, the embryonic axes could no longer generate enough force to break through the endosperm (Takeba and Matsubara, 1979).

Skotodormancy refers to the inhibition of lettuce germination in the absence of light. In classic work in the 1940s, Borthwick and his colleagues at the USDA in Beltsville, showed that light at 650 nm wavelength was most effective to stimulate germination, while 730 nm light inhibited germination, and that in a series of light

Table 14.1. Area harvested and production of lettuce and endive for China, the United States, and the world in three years, from 1994 to 2014. (FAO, 2017).

	China		United States		World	
Year	Area harvested, ha x 1000	Production, mmt	Area harvested, ha x 1000	Production, mmt	Area harvested, ha x 1000	Production, mmt
1994	170	4.21	119	3.98	655	14.26
2004	500	10.50	127	4.80	1,046	22.49
2014	584	13.66	107	3.79	1,158	24.98

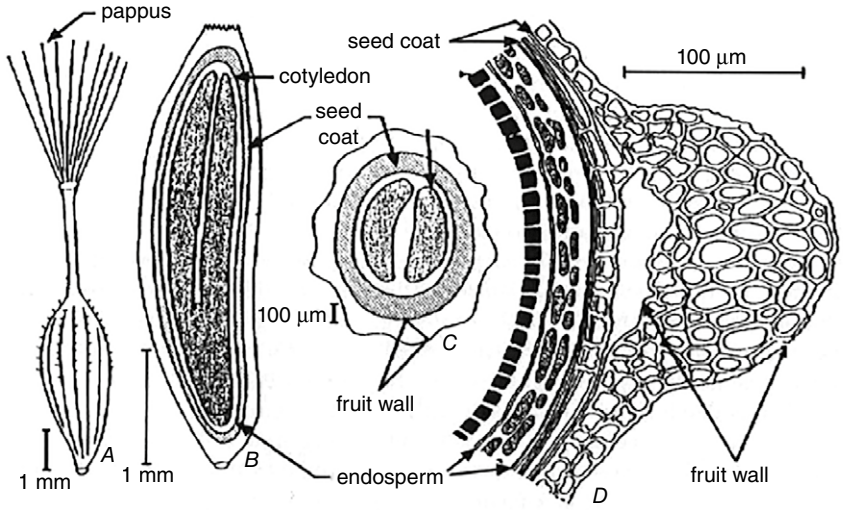

Fig. 14.1. Morphology of a lettuce achene. A. Entire achene with pappus. B. Longitudinal section. C. Cross-section. D. Portion of wall and endosperm. From Ryder (1999). With kind permission of CAB International.

exposures, the wavelength of the last treatment determined the effect (Borthwick *et al.*, 1954). The research established the foundation for work on the photoreversible pigment phytochrome, later found to play a role in many physiological processes in plants. In skotodormancy, the inhibition of germination is also mediated by the endosperm (Ikuma and Thimann, 1963; Tao and Khan, 1979). If the endosperm is removed, light-sensitive lettuce germinates fully in the dark. By treatment with light during imbibition, the force required to puncture the pericarp was greatly reduced, allowing the embryo to emerge. The light-sensitive stage commenced 1.5 h after the seed started to take up water (Ikuma and Thimann, 1964).

Under conditions allowing seed germination, the endosperm cells in the area where the radicle emerges deteriorate, the cells shrinking and loosening (Sung *et al.*, 2008). The separation of endosperm cells has been linked with an increase in the enzyme endo-beta-mannanase in the same area (Nascimento *et al.*, 2001). More recently, cellulase enzyme activity in the micropylar end of the endosperm has also been correlated with loosening of these cells (Chen *et al.*, 2016).

Sensitivity to high temperature was highest if heat was imposed from the start of moisture imbibition (Gray, 1977) for a minimum of 8 h duration. A shorter delay in germination occurred if seeds were subjected to high temperatures during the onset of cell division, which occurs at about 12 h after sowing (Gray, 1977). If exposure to heat was delayed until after the onset of cell elongation and cell division in the embryo, germination was irreversible (Cantliffe *et al.*, 1984). Thus any technique that allows the seed to reach the cell division stage at temperatures between 15 and 22°C would permit normal emergence at high temperatures. This is the basis for the use of various seed priming treatments to permit normal emergence of lettuce in high temperature environments.

Research has implicated the involvement of growth hormones in lettuce germination, and provided means of at least partially alleviating thermo- and skotodormancy. In cultivars that are able to germinate at high temperatures, ethylene was given off during the imbibition period, whereas inhibited seeds showed no ethylene production (Prusinsky and Khan, 1990; Huang and Khan, 1992). Seed treatment with the ethylene-generating chemical ethephon, or the ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid), or gassing with ethylene alleviated the thermoinhibition to a limited extent but could not restore germination at 35°C (Sharples, 1973; Abeles, 1986; Prusinsky and Khan, 1990). The combination of pericarp slitting and ethephon treatment resulted in additional increases in high temperature germination rate, leading Prusinsky and Khan (1990) to conclude that the formation of ethylene by the seed may be inhibited at high temperatures by anoxic conditions caused by an impervious endosperm. Recent findings (Yoong *et al.*, 2016) link ethylene with the stimulation of gibberellin formation that enhances elongation of the root and hypocotyl, facilitating radicle emergence.

Furthermore, treatment of light-requiring lettuce seeds with gibberellic acid allowed them to germinate in the dark (Tao and Khan, 1979), and seed treatment with the gibberellin synthesis inhibitor tetcyclasis prevented lettuce seed germination (Khan, 1994). A buildup of abscisic acid in the germinating seed, and upregulation of ABA synthesis genes were associated with heat-induced seed germination inhibition (Argyris *et al.*, 2008). The relative levels of ABA and GA may be key to regulation of lettuce germination (Yoong *et al.*, 2016).

The cytokinins may also play a role in allowing lettuce to germinate at high temperatures. Evidence for this has come primarily from improved germination after additions of cytokinins to the seeds (Sharples, 1973; Abeles, 1986). These chemicals may act by increasing seedling growth potential at high temperatures (Takeba and Matsubara, 1979), but the mechanism for this improvement is not clear. Sharples (1973) found that seed treatments combining ethephon and kinetin improved high temperature lettuce seed germination more than either chemical alone, and suggested that this be used as a seed treatment in hot environments.

Marked differences in the germination response of lettuce cultivars to high temperatures have been noted by Coons *et al.* (1990). Ten crisphead cultivars had high germination percentages at 30 but not 35°C when germinated in water in the light. When the germination tests were repeated in –0.3 or –0.6 MPa NaCl solution, germination rates declined, but some cultivars, notably "Coolguard" and "Empire," were less affected. A larger study in 2013 by Lafta and Mou showed that 20 genotypes among 150 tested had only a slight decrease in germination

when planted in petri dishes in light at 34°C (Lafta and Mou, 2013). This is encouraging the selection for improved high temperature germination capability in lettuce breeding programs.

Lettuce cultivars vary widely in their requirement for light with regard to germination (Borthwick *et al.*, 1954). At temperatures of 20–25°C, most field-grown cultivars of lettuce in the USA do not require light for germination. There is, however, a strong interaction between temperature and the light requirement. "Grand Rapids" will germinate readily in the dark at 15°C, but is strongly inhibited at 20°C and above. Cultivars which do not require light at 20°C may show improved germination in light compared to dark after being subjected to heat or osmotic stress during imbibition (Borthwick *et al.*, 1954; Guedes and Cantliffe, 1980). Given this interaction between temperature and the light effect, there is probably no cultivar that is truly non-responsive to light with regard to germination.

From the practical standpoint, a light requirement for germination could pose a serious impediment to seedling emergence. Wooley and Stoller (1978) showed that photosensitive lettuce seed planted 6 mm deep in soil was significantly delayed in emergence compared to seed placed 2 mm below the surface. Less than 1% of incident radiation penetrated fine-textured soil to a depth of more than 2.2 mm. The problem is made worse by seed coatings, unless these dissolve promptly on being moistened, or split open. As long as soil temperatures are low during the planting season, emergence should be satisfactory, but when planting into warm soils, special measures such as priming may be needed.

Seed priming

The inhibition of lettuce seed germination by high temperature and by darkness are both triggered during the early imbibitional stages (Ikuma and Thimann, 1964; Gray, 1977). If the seed can pass through these sensitive stages without being subjected to these inhibiting factors, germination and emergence are then less affected by environmental influences. Research has generally substantiated this theory. Seed priming in an osmotic solution at –1.5 MPa improved emergence of "Empire" lettuce in hot soils in California's Imperial Valley from 20% for untreated controls to 46–69% (Valdes *et al.*, 1985). The results were confirmed in controlled high temperature conditions in the laboratory (Valdes and Bradford, 1987). Similar positive results from priming were obtained by Kim *et al.*, (2000).

Lettuce seed can also be primed by exposure to a medium, either liquid or solid, of around –1.5 MPa for no longer than 12 hours, at a temperature of 15°C, which allows the seed to germinate but not to exert the radicle (Guedes and Cantliffe 1980; Khan 1992). Research also showed that the priming solution should be imbibed at 15°C and in light, with good aeration for no longer than 12 h (Guedes and Cantliffe, 1980). The seed can be dried at temperatures lower than 20°C and stored for up to a year without losing the benefits of priming (Khan, 1992).

Investigations into the physiological mechanisms of priming have confirmed the mode of action of stress inhibition of germination outlined above (Khan, 1992). Genes encoding for the enzymes in the gibberellin and ethylene synthesis pathway were enhanced during priming (Schwember and Bradford, 2010). In addition, lettuce seed showed an increase in endo-beta-mannanase activity during priming that continued during the subsequent drying period (Nascimento *et al.*, 2001), and presumably initiated the endosperm softening process.

Seed pelleting

Precise placement of lettuce seeds in the field is necessary if the costly practice of thinning the excess seedlings is to be avoided. The small, thin lettuce seed is difficult to sow in exact positions, so methods have been developed of coating the seeds with materials that change the shape to nearly spherical. The coating materials exclude light, however, and as a result may exacerbate the low emergence rate obtained under high temperature conditions (Zink, 1955). Development of splitting coat technology has overcome this constraint by allowing the coating to split open when hydrated (Hill, 1999).

It is now common to combine priming with seed coating to overcome the disadvantages of pelleted lettuce seed (Valdes *et al.*, 1985; Valdes and Bradford, 1987; Hill, 1999; Kim *et al.*, 2000). In addition, intensive research and development

by the seed industry, probably based on some of the physiological processes described above, has resulted in improved lettuce stands and yields (Kim *et al*., 2000).

Head Formation

The head of lettuce is an assemblage of leaves closely packed together over the growing point of the plant. The process of head formation in crisphead lettuce consists of a series of changes in leaf morphology and leaf orientation that transform the rosette structure of predominantly horizontal leaves to one where successive leaves become progressively more erect and in-arching (Johnson, 1983). This change in plant morphology is accompanied by an inward curvature of the leaf midrib and a broadening of leaf shape (Bensink, 1971; Sugiyama and Oozono, 1999). The arching-over of the developing leaves is aided by entrapment of the distal leaf margins by the base of the rosette of older leaves. Head formation results from the accumulation of young leaves under the layer of leaves covering the growing point (Johnson, 1983). Thus, for head formation to succeed, several morphological preconditions must be met (Dullforce, 1962): large individual leaves, a slow rate of stem elongation, short petioles and a high rate of leaf production.

The importance of the shape of the head-forming leaves was pointed out by Bensink (1958, 1971) in his detailed studies of lettuce head morphology. He showed that for a butterhead lettuce plant in the head-forming stage, leaf width increases more rapidly than leaf length as one goes from the outer part of the frame to the head (Fig. 14.2). This results in a pronounced decrease in the leaf length:width ratio, which is

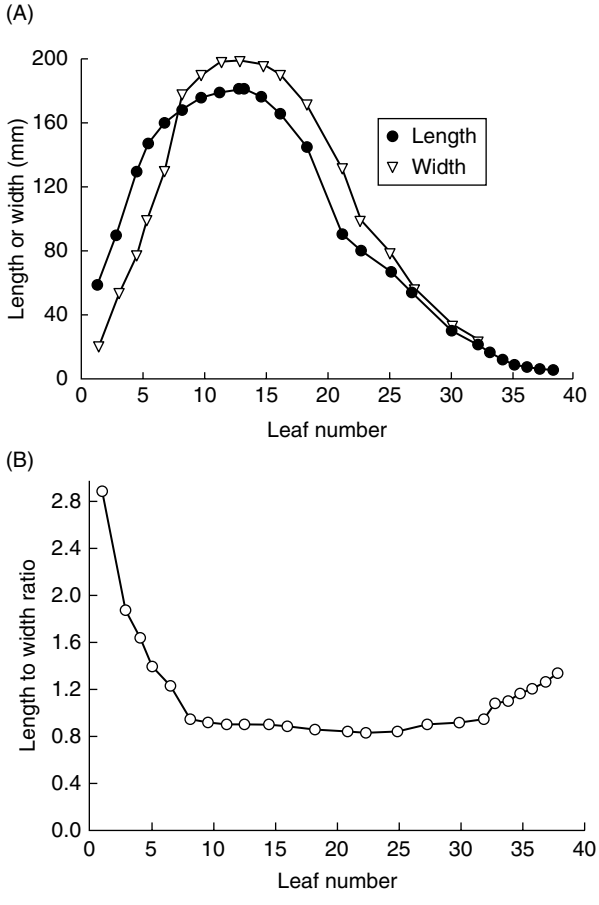

Fig. 14.2. (A) Length and width of successive leaves of a butterhead lettuce plant, numbered from the stem base upwards. (B) Length:width ratio of the same leaves (Bensink, 1971). With kind permission from Elsevier Science.

indicative of the head-forming tendency of the plant. Bensink further pointed out that leaf shape is strongly influenced by the environmental conditions under which the plant is growing. Under low light, leaves tend to be long and narrow. As light levels increase, their shape becomes progressively broader, with reduced length:width ratio (Fig. 14.3). Temperature interacts with light, enhancing leaf width at high temperatures and high light conditions, and resulting in narrower leaves if high temperatures occur under low light conditions. At 10°C, although growth is slow, leaf shape is favorable for head formation even under low light conditions. Applications of gibberellic acid to lettuce plants caused similar morphological changes in leaf shape as occurs when plants were grown under low light and high temperature conditions. It is therefore possible that this hormone is involved in the regulation of leaf shape and of head formation in lettuce.

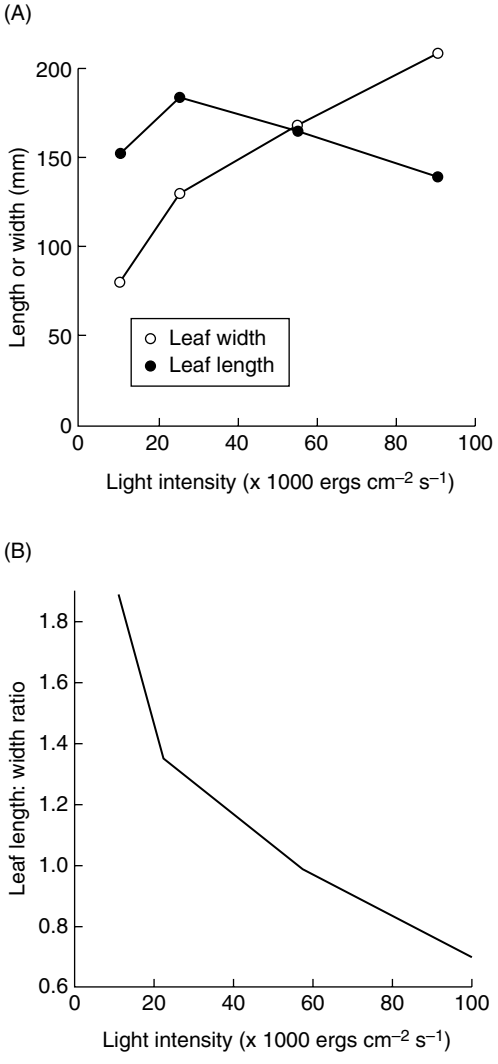

Fig. 14.3. (A) Maximum leaf length and width of butterhead lettuce when grown at 30°C, in relation to incident light level. (B) Length:width ratio of the same leaves (Bensink, 1971). With kind permission from Elsevier Science.

Bensink (1971) explored the cellular basis for these leaf shape responses, and found that leaf width was correlated directly to the number of cells in the leaf lamina, determined by the length of the cell division stage of the developing leaf primordia. Leaf length was determined by the length of individual cells in the leaf midrib.

Another morphological factor that influences head formation is the length of the stem. Environmental conditions that foster bolting, such as long photoperiods and high temperatures also prevent head formation (Dennis and Dullforce, 1974). The prevalence of such conditions in the northeastern United States and in Japan was a major deterrent to lettuce production in these areas during the summer until bolting-resistant cultivars were developed (Knott *et al.*, 1939; Shibutani and Kinoshita, 1966).

Head formation in cos lettuce proceeds in a slightly different manner than in crisphead lettuce (Nothmann, 1976). Instead of a marked change in leaf shape aiding in the curvature of the head-forming leaves over the apex, in cos lettuce the progressive curvature of the leaves is not accompanied by leaf shape changes. Under the high temperatures of the Israeli summer, head formation was prevented by bolting. In spring crops, abnormally twisted leaves in the developing head also inhibited heading (Nothmann, 1977). The cause for this disorder is not known, but has also been described in crisphead lettuce by Zink (1959).

Under field conditions, heading lettuce cultivars generally produce 13–20 frame leaves before head formation begins (Zink and Yamaguchi, 1962; Wurr *et al.*, 1987). The time when the head-forming leaves are first visible and start to cup is termed "hearting," and has been shown to be critically important in determining final head size (Wurr *et al.*, 1987; Wurr and Fellows, 1991). If the plants are growing under high

light conditions and average temperatures below 12°C during this period, large heads tend to form (Wurr *et al.*, 1992a). On the other hand, if plants are subjected to low light conditions during this period, head weights are reduced or maturity delayed. The morphological basis for this association may be the large size of the frame leaves formed under high light conditions, allowing larger heads to develop (Gray and Steckel, 1981; Wurr and Fellows, 1991). Under low light, leaves with increased length:width ratio, and smaller size would delay the head formation process.

Factors Determining Productivity

Aside from the effects on leaf morphology, light and temperature are major determinants of the plant growth rate, as expressed in the increase in leaf number (Bensink, 1971; Wurr *et al.*, 1981). As long as adequate levels of water and nutrients are available, increasing temperatures between 10 and 30°C, and increasing light levels between 1 and 26 MJ m^{-2} day^{-1} speed up the number of leaves formed per unit time and their size (Bensink, 1971; Wurr *et al.*, 1981; Wurr and Fellows, 1991). This also translates into larger plant biomass and greater harvested yield (Glenn, 1984).

Light

To obtain high yields of lettuce, it is necessary to have cultural practice and climatic conditions that permit the plants to make rapid initial growth. The sooner leaf cover of the soil surface has been achieved, the quicker the plants can use all the incident radiation in growth. In field-grown crops, close spacing and using transplants rather than direct-seeding advances the time of complete light interception. In glasshouses, raising temperatures to accelerate growth has also been found useful (Bierhuizen *et al.*, 1973; Frantz *et al.*, 2004), although the combination of low light and high temperatures may result in the formation of long, narrow leaves that delay head formation (Bensink, 1971). There are also genetic differences in the formation of a large light-absorbing surface. Brouwer and Huyskes (1968) found that the accelerated growth of a new lettuce line was due to its more rapid soil cover, and resultant greater light interception per unit fresh weight. The photosynthetic rates of the slow and rapidly growing lines were not different.

Light interception can also be maximized by growing plants in solution culture, which allows them to be repositioned without disrupting growth. Culture in moveable troughs, or on styrofoam blocks floating on tanks of nutrient solution (Glenn, 1984), are examples of systems currently in use (see also Chapter 2).

When other factors inhibiting growth are removed, lettuce productivity will be directly related to the incident light energy. In a series of solution culture trials conducted in spring and fall in a glasshouse in Arizona, Glenn (1984) demonstrated that the time required to produce a lettuce head weighing 120 g decreased as solar radiation increased (Fig. 14.4). Others working in controlled environment chambers have made similar findings (Verkerk and Spitters, 1973; Cracker and Seibert, 1983; Knight and Mitchell, 1983a, 1983b, 1988).

Under field conditions, response to increased light levels may be masked by adverse effects of increased temperature or reduced water status of the plants in the high light environment. For example, whereas Glenn (1984) measured continued growth increases up to 500 $\mu moles.m^{-2}.s^{-1}$ incident radiation in his glasshouse trials, Mattei *et al.* (1973) found that lettuce dry matter production was maximum at 150 $\mu mol.m^{-2}.s^{-1}$ in field plantings in Italy and the UK. These differences emphasize the need for high levels of water management for the lettuce crop, and the necessity to avoid temperatures in excess of 30°C.

Temperature

Temperatures considered optimum for growth of lettuce average 18°C, with a range from 7 to 24°C (Lorenz and Maynard, 1980). The lettuce crop is thus produced in areas and seasons where such temperatures reliably occur, such as the Salinas Valley in California in spring through fall, during the winter months in California's Imperial Valley, in the summer in central and northern Europe, and in the winter in Mediterranean countries (Simko *et al.*, 2014). In fact, Kimball *et al.* (1967) determined that lettuce production in the western USA was centered in areas that had

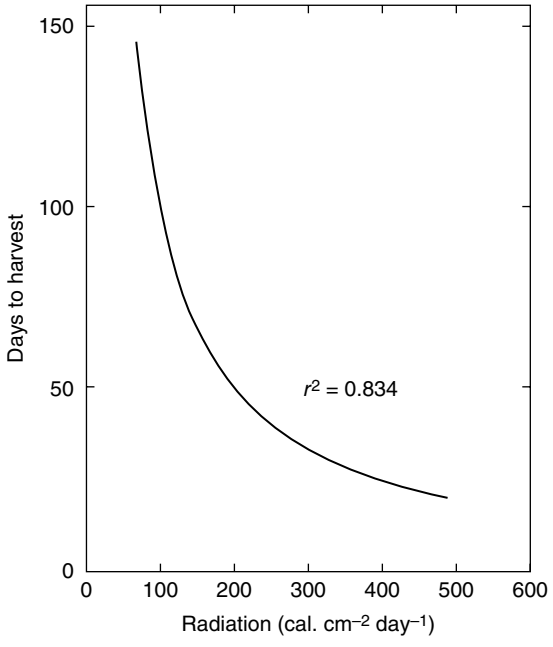

Fig. 14.4. The dependence of days to harvest of lettuce on solar radiation, combining data from England, and midwestern and western USA (Glenn, 1984). With kind permission from Elsevier Science.

at least two months of temperatures of 17–28°C (day) and 3–12°C (night). At temperatures higher than these ranges, the cultivars grown in these areas develop a high incidence of bolting and tipburn (see "Physiological disorders," p. 348).

Concerns with rising temperatures in lettuce production areas have given rise to several efforts to understand the mechanisms of heat tolerance in lettuce, and to select for genotypes with better adaptation. Lai and He (2016) subjected lettuce lines differing in heat stress susceptibility to chronic heat stress, and measured the physiological parameters most acutely affected. With increasing temperatures, photosynthesis rates and dry matter production were most quickly affected, whereas chlorophyll fluorescence was unchanged until the plants were exposed to 46°C. Choong *et al.* (2013) found that overall plant growth, and root extension rates, were greater in heat-tolerant lettuce lines.

The stress hormone abscisic acid (ABA) appears to play an important role in drought and heat tolerance in lettuce. Transgenic lettuce plants that overexpressed the ATHSP17.8 gene from *Arabidopsis* increased resistance to drought and salinity stress through a hypersensitivity to ABA action (Kim *et al.*, 2013). It remains to be seen if such genetic changes can lead to better heat tolerance in lettuce production fields.

The direct approach of selecting lettuce lines under high temperature conditions have also shown significant variation in heat tolerance (Gong, 1998; Souza *et al.*, 2008; Han *et al.*, 2012, 2016a, 2016b; Lafta *et al.*, 2017), indicating that considerable improvement in lettuce heat tolerance can be accomplished by conventional means. For instance, in field plantings in three locations in California under non-stress and heat stress conditions, 36 leaf lettuce accessions showed considerable variation in growth and the incidence of physiological disorders (Table 14.2) (Lafta *et al.*, 2017).

Temperature is the main factor determining the rate of growth of lettuce during seedling emergence and the early growth period (Bierhuizen *et al.*, 1973; Scaife, 1973). Growth rate depends on the temperature of the growing point, and this organ is located close to the soil surface in this rosette plant. It is thus not surprising that soil temperature is more closely correlated with plant growth rate than air temperature (Scaife, 1973; Wurr *et al.*, 1981). This information has been used to shorten the lettuce production season in glasshouses. Boxall (1971) found that heating the soil to 18°C decreased the length of the production cycle of butterhead lettuce by 14–17 days compared to plants grown in unheated soil under minimum air temperature of 7°C during

Table 14.2. Yield and physiological disorders of four leaf lettuce cultivars grown in the San Joaquin (S.J.), Imperial (E.C.), and Salinas (Sal.) Valleys of California. Plantings were timed to provide maximum temperatures of 32°C, 39°C and 21°C during the growing periods for the three locations, respectively (Lafta *et al*., 2017).

	Yield, g per plant			Bolting, percent			Tipburn count[z]		
Cultivar	S.J.	E.C.	Sal.	S.J.	E.C.	Sal.	S.J.	E.C.	Sal.
"Black Seeded Simpson"	454	145	198	42	100	0	1.5	0	0
"Grand Rapids"	392	115	137	92	92	0	0	2.3	0.5
"Salad Bowl"	350	175	179	8	0	0	0	0	0
"Slo Bolt"	350	90	214	0	0	0	0.8	1.6	0

[z]Tipburn count: number of leaves per plant with black or brown leaf edges.

winter in the UK. At the other extreme, reducing root zone temperature has allowed head formation in some lettuce cultivars grown under tropical conditions (Lee and Cheong, 1996).

Predicting maturity

The relatively short growth period from planting to maturity of lettuce allows more than one crop to be produced in a season in most locations. The objective with multiple plantings is to maintain a steady level of production over a period of time, to minimize marketing problems. The scheduling of plantings to achieve steady supply thus becomes important, and should be based on an understanding of the factors that determine when lettuce will reach marketable size.

In the production of leaf and butterhead lettuce, the acceptable size at harvest has not been closely defined, and can vary considerably (e.g. in Germany, plant fresh weight of 200 g is usual for spring-grown crops of leaf lettuce, and more than 350 g in summer) (Laber and Lattauschke 2014). One needs to know the time required to reach this marketable size to estimate the length of the production cycle. As shown above, growth rate of lettuce is accelerated by increased irradiance and higher temperatures.

In production systems in which control of irradiance and temperature are possible, such as in greenhouses, a constant crop growth duration can be achieved. Thus, on cloudy days, artificial light is given to reach a set daily irradiance total, and shade is applied during periods of high light (Both *et al*., 1997). With good glasshouse temperature control, it is therefore possible to produce a uniform lettuce plant weighing 150 g in 35 days, winter and summer, with photosynthetically active radiation totals of 17 $mol.m^{-2}d^{-1}$ (Both *et al*., 1997).

The prediction of maturity of crisphead lettuce is somewhat more complicated than for butterhead and leaf lettuce described above. Many researchers have found that maturity estimates based on a sum of heat units accumulated during the growing season are not reliable (Maderiaga and Knott, 1951; Zink and Yamaguchi, 1962; Kristensen *et al.*, 1987). Correcting the growing temperature with the irradiance received improved the accuracy of the prediction. The results were, however, still too variable to be practical (Wurr *et al.*, 1988). Another approach has been to predict maturity of the lettuce head by modeling the effect of temperature and irradiance on the fresh weight gain of the head after hearting (Wurr *et al.*, 1992b). Unfortunately, this approach was also not successful, for it assumes that head lettuce will be harvested at a constant head size or weight. The most important characteristic that determines harvest date is head density, and this varies not only with temperature and irradiance during heading, but also with these and other yet unknown factors even before heading begins (Wurr *et al.*, 1992b). Thus, in spite of considerable study, the most reliable predictor of harvest date in head lettuce is still the date estimated from the date of planting on the basis of the experience of previous years (Zink and Yamaguchi, 1962; Gray and Morris, 1978; Wurr *et al.*, 1988, 1992b) (Fig. 14.5).

Lettuce factories

The short growth cycle of lettuce, and its importance in commerce and the food chain, has given

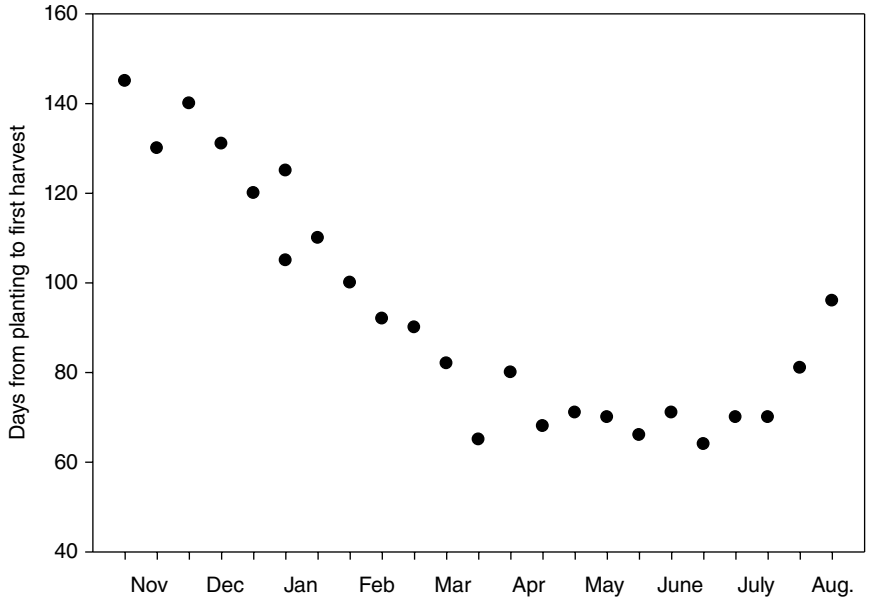

Fig. 14.5. The relation between days to first harvest and planting date for 312 crisphead lettuce crops grown in the Salinas Valley, California, 1953–58, with planting dates from November to August (Zink and Yamaguchi, 1962).

rise to many attempts to grow the crop in artificial environments (Duggan-Jones and Nichols, 2015) and thus to optimize growing conditions beyond what can be done outdoors (e.g. Higashi *et al.*, 2015); The interest in such "lettuce factories" has been further encouraged by the development of light-emitting diodes (LEDs), that produce light of narrow wavelengths without accompanying heat (Mitchell *et al.*, 2015; Stutte, 2015). These lights help to create artificial environments in which varying aspects of the light regime can be changed. Manipulation of light spectra using proportions of red, blue, green, and white LEDs have identified the ratios that maximize productivity and crop quality (Stutte *et al.*, 2009; Nicole *et al.*, 2016). For instance, addition of blue light (440nm) to an array of red (640nm) LEDs increased anthocyanin production in red lettuce grown at the same irradiance (Stutte *et al.*, 2009). Similar results were obtained by adding ultraviolet light (310 to 324nm) three days prior to harvest (Goto *et al.*, 2016). Such changes in light quality and quantity also brought about a 30% lowering of nitrate levels in the harvested lettuce (Bian *et al.*, 2016).

To save energy, LEDs can be operated either in a pulsed or steady mode. Pulsing the light 50 or 75% of the time produced lettuce growth of similar amount and quality as having the lights on constantly (Kanechi *et al.*, 2016; Son *et al.*, 2016). Alternate 12-hour periods of red and blue LED lights allowed lettuce plants to reach harvest weight four and ten days sooner than with simultaneous red and blue, or using fluorescent lighting, and used 40% less energy than with fluorescent lamps (Ohtake *et al.*, 2015). Adding light diffusers or changing light fixture design to distribute light more evenly over the canopy improved photosynthesis and growth of lettuce plants (Saito *et al.*, 2013; Kang *et al.*, 2016). Experiments with length of light and dark cycles have shown that lettuce grown in chambers illuminated by LED lights with a 16-hour light, two-hour dark cycle yielded 30% more fresh weight than under a 16/8 hour cycle (Kang *et al.*, 2013; Hiroki *et al.*, 2014).

Increased carbon dioxide concentration above ambient conditions has been a standard practice in greenhouse lettuce production for many years, but is not feasible in periods of sunny weather, when ventilation is needed for temperature control (Both *et al.*, 1997). In plant factories using LEDs, heat load from lamps is less of a concern, and carbon dioxide increases have enhanced lettuce growth until it reached a plateau at 1000 ppm (Duggan-Jones and Nichols, 2015; Jung *et al.*, 2016). Other atmospheric manipulations

are also possible: Korean researchers found that increasing the anion concentration in the air could boost shoot fresh weight by 64% after four weeks of treatment (Song *et al.*, 2014).

While such research encourages the development of factories to produce all our food (e.g. Frazier, 2017), calculations of the energy costs of such facilities compared to their outputs are sobering. Even using sunlight in a hydroponic greenhouse facility, lettuce production requires 82 times more energy than producing the crop in the field in Arizona (Barbosa *et al.*, 2015). Simulations of lettuce production in a greenhouse compared to a plant factory in four locations in the United States also show that the factory requires two to three times more energy, and emits three to seven times more CO_2 (Harbick and Albright, 2016). It is thus likely that plant production in factories will be restricted to high value food crops such as some lettuce types, and "medicinal" crops such as marijuana (Frazier, 2017).

Flower Induction

Lettuce is a quantitative long-day plant that can also be seed vernalized. A lettuce plant induced to flower undergoes a dramatic change in morphology, from a rosette with a short stem to a plant more than a meter tall, with elongated internodes and prominent terminal flowers on a many-branched stem. The transition from vegetative to reproductive growth may in head-forming cultivars involve the growth of the stem under the restraint of the previously formed head. Thus, climatic conditions that encourage head formation may delay time to flowering because stem elongation is retarded by the head.

Photoperiod and temperature effects

Although lettuce is considered a quantitative long-plant, studies on cultivar differences in photoperiod response indicate at least three broad categories of genotypes with regard to flowering in lettuce (Waycott, 1993). American crisphead cultivars have little response to photoperiods between 10 and 13 h, but are sensitive above this range; European butterhead types tend to show linear decreases in time to flowering with increasing daylength across the entire daylength range. A third group includes early-flowering genotypes that are nearly day-neutral (Ryder, 1988). Within each of these groups, there may also be cultivar differences in sensitivity to daylength. For example, within European butterhead cultivars, those cultivated in winter tend to be more susceptible to bolting than those adapted to summer conditions (Wiebe and Krug, 1985; Krug, 1986).

Temperatures under which the plants are growing can have a profound effect on when they will flower. Under conditions optimum for head formation (19/11°C), flowering is delayed both by heading and its resultant constraint on stem elongation, and by the retardation of plant growth rate (Thompson and Knott, 1933; Rappaport and Wittwer, 1956a; Ito *et al.*, 1963). In leaf lettuce, where head formation is not a factor, flowering is delayed by 12 days by 5°C cooler temperatures (Fig. 14.6). In "Grand Rapids", the delay was due to a slower growth rate, but the other two cultivars also had increased leaf numbers at the cooler temperatures.

Vernalization

The third major factor influencing flowering in lettuce is low temperature during seed germination and seedling emergence. Knott *et al.* (1937) demonstrated that if lettuce seed is exposed to 4°C for 5–20 days, 24 h after the start of germination, seedstalks appeared 3–5 days sooner than on the unvernalized controls. Subsequent work by Rappaport and Wittwer (1956a) and Prince (1980) indicated that at least 13 days of low temperature are needed, and that the low temperature treatment must be started within 3 days of germination.

As with the effect of photoperiod, cultivar differences have been found, with many apparently insensitive to seed vernalization (Prince, 1980; Thompson and Kosar, 1948). The effect is reversible in the wild *Lactuca serriola* by periods of high temperatures following the vernalization treatments as long as the seeds have not germinated (Marks and Prince, 1979).

There is limited evidence that lettuce seeds forming on the mother plant can be partially induced to flower by exposure to low temperatures during the seed development period (Wiebe, 1989).

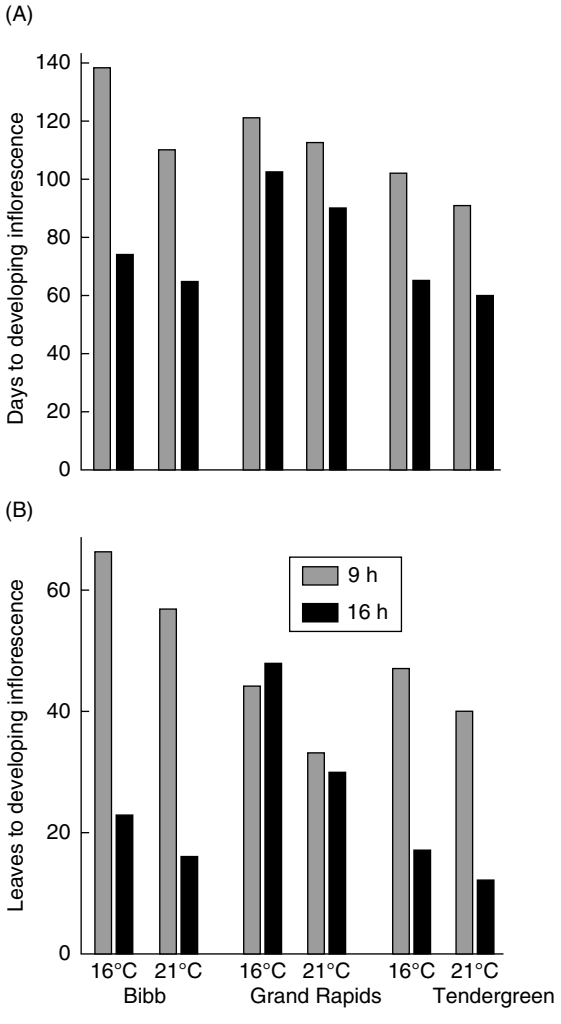

Fig. 14.6. Influence of photoperiod and night temperature on (A) days to developing inflorescence and (B) the number of leaves below the inflorescence for three leaf lettuce cultivars (Rappaport and Wittwer, 1956b).

The combined effects of daylength, vernalization and growing temperature tend to be additive in the induction of flowering in lettuce (Fig. 14.7). Thus, growing the plants under 21°C night temperatures at 16 h photoperiod, after seed vernalization, resulted in flowering after 135 days, while 188 days were needed for flowering under cool nights, short photoperiods and lack of seed vernalization. Crisphead lettuce cultivars selected for production in the northeastern United States are often sown in cool soils, and exposed to long photoperiods and warm nights during the season. They have therefore needed to have a higher level of resistance to bolting than cultivars selected for the cooler production areas of California, where lettuce is grown throughout the year (Ryder, 1986).

Genetic and hormonal factors in lettuce flowering

The time of lettuce flowering has been shown to be under the control of six genes, that cause variation of flowering time from very, very early (45 days) to very late (140 days) (Ryder and Milligan, 2005). While the early flowering trait is useful in shortening generation time in breeding, selection for late bolting and flowering is essential in the final product (Ryder and Milligan, 2005).

Research in recent years has provided more detail on the genetic and morphological details of lettuce flowering. The flowering locus T gene has been found in lettuce apical meristems induced to flower (Fukuda *et al.*, 2011). Lee *et al.* (2003)

noted that the first sign of flower initiation was the formation of a dome shape of the apical meristem. Genes responsible for the formation of GA_1 have also been associated with flower induction (Fukuda *et al.*, 2009). A sobering reminder of the complexity of flowering comes from a comparison of two lettuce lines differing in resistance to bolting. These flowering differences were associated with the differential expression of 12,204 genes (Han *et al.*, 2016a). Twelve MADS box genes were more closely related to flower induction than genes regulating GA metabolism. There is much more to be learned about the flowering process.

Seed Production

The floral axes of lettuce normally attain a height of 75–150 cm, and form a branched, bushy crown (Hayward, 1948). The inflorescence consists of a cymose cluster of heads, each of which may contain 15–25 flowers. The terminal flower capitulum usually flowers first, followed by lateral heads and those on branches. The weight of individual seeds reaches a maximum in 10–14 days after anthesis of the flower, but flowering on a single plant may extend over 40–50 days (Soffer and Smith, 1974; Globerson, 1981). Mature seeds are therefore found on the plant before flowering is completed, and the first-formed seeds may be lost to shattering unless they are harvested repeatedly by shaking the seed heads into bags (Foster and van Horn, 1957). The long plant maturation time also points out the need for seed production in areas where the post-flowering period is rain-free and where low relative humidity prevails (Hawthorn and Pollard, 1954). Accordingly, in the United States, lettuce seed production is practiced primarily in the dry interior valleys of California, Idaho, Oregon, and Arizona under irrigation.

The climatic conditions under which the seed develops on the mother plant can have a considerable influence on seed characteristics. High production temperatures (30/20°C day/night temperature) resulted in lower seed yields and smaller seed size than growth at 25/15°C (Gray *et al.*, 1988). Under cool conditions (20/10°C) yields were further reduced, but the seeds were 63% larger than at 25/15°C. Steiner and Opoku-Boateng (1991) also noted a reduction of seed weight, and of seeds per capitulum

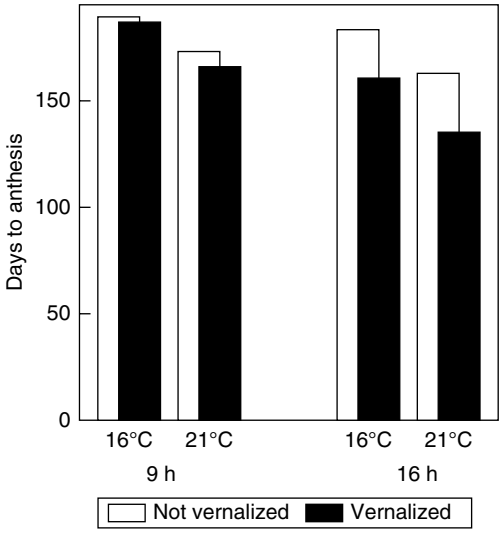

Fig. 14.7. Influence of photoperiod, seed vernalization and night temperature on days to anthesis of 'Great Lakes' crisphead lettuce (Rappaport and Wittwer, 1956a).

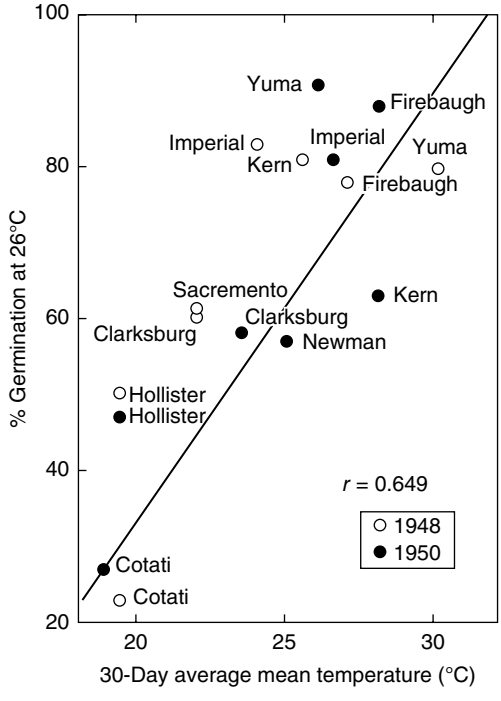

Fig. 14.8. The relation of germination percentage at 26°C and the average mean temperature for the 30-day period preceding seed harvest (Harrington and Thompson, 1952).

as temperatures increased in seed-production fields of lettuce in the San Joaquin Valley of California. High temperatures during seed development resulted in lowered seed vigor, but increased the capacity of the seed to germinate under warm conditions (Fig. 14.8) (Harrington and Thompson, 1952; Steiner and Opoku-Boateng, 1991; Contreras *et al.*, 2009). On the other hand, seed developed at 20/10°C failed to germinate at 30°C (Gray *et al.*, 1988). The day-length at which the mother plant is grown can also influence seed weight: seeds developing under long days tend to be heavier than those from short-day-grown plants, and keep for a longer time without deteriorating (Contreras *et al.*, 2008).

The physiological basis for the difference in seed yield with temperature has not been worked out. Since seed number produced per inflorescence was reduced both at high and low temperatures, pollination and fruitset may have been adversely affected at these temperatures. Lettuce is predominantly self-pollinated, with the pollen shedding from the inside of an anther cone (fused staminate tube) onto the style that grows through it at anthesis (Jones, 1927).

Water stress can also result in reduced seed yield, especially when it occurs during the reproductive period (Izzeldin *et al.*, 1980). As with high temperatures, the late stress treatments reduced the number of seeds per head. Highest seed yields were obtained if the plants were exposed to moderate water stress during the vegetative period, followed by adequate water.

Production techniques and environmental conditions that result in seed yields of uniform quality have been shown to also result in seed that produced uniform seedlings (Wurr *et al.*, 1986). Paradoxically, plants grown at low or high temperatures produced seed more uniform in size and vigor than if produced at intermediate temperatures (Gray *et al.*, 1988).

Treatment of the seed-producing plants with GA_{4+7} (a mixture of gibberellins 4 and 7) prevented head formation and advanced date of flowering by 9–31 days, increased the synchrony of flowering and hastened plant maturity compared to untreated plants (Gray *et al.*, 1986). Seed yields were substantially increased, and the size and uniformity of seedlings grown from those seeds was also improved (Wurr *et al.*, 1986). There was no evidence of carry-over effects of the GA treatment on seedling germination or susceptibility to bolting.

The positive relationship between seed size and vigor of the resulting seedling has been noted by a number of researchers (Smith *et al.*, 1973a, 1973b; Sharples, 1970; Wurr *et al.*, 1986). In an extensive series of studies, Smith and colleagues determined that both seed size and weight influenced seedling performance. For optimum uniformity, they found that seed had to be separated into size classes according to seed thickness or width, and into weight grades within each size (Smith *et al.*, 1973a, 1973b). Plants produced from thin and light seeds gave significantly smaller heads at maturity than the heaviest seeds in each of the seed width categories. It is important to note, however, that such relationships between size and vigor of seeds may only apply to seeds within a seedlot, and not between seed crops. Nevertheless, seed grading by size and weight can significantly increase the uniformity of the head lettuce crop.

Physiological Disorders

Tipburn

Tipburn is characterized by necrosis of the edges of young, rapidly expanding leaves of lettuce. When severe, the disorder may develop on more than half of the area of these leaves. The necrosis is often visible from the outside in cos and butterhead types, but in crisphead lettuce, the affected leaves are in the head, and are not found until the head is utilized. During periods of hot weather, the disorder can be so severe that it will require the abandonment of entire fields of crisphead lettuce (Misaghi *et al.*, 1992). The risk of tipburn also constrains the length of the production season in areas where temperatures become progressively hotter, such as Arizona and parts of California (Misaghi *et al.*, 1992; Simko *et al.*, 2014). Generally, the disorder develops on the growing plant in the field or glasshouse, but can also become a problem on harvested lettuce heads if they are stored at high temperatures, or not cooled quickly enough after harvest (Misaghi *et al.*, 1992). This increases the potential economic threat of this disorder, but also provides a quick means to check for tipburn susceptibility

of lettuce lines in a breeding program (Ryder, 1986). The disorder has been the focus of much research over the years, much of which was summarized in review articles by Collier and Tibbitts (1982), Saure, (1998), and Olle and Bender (2009).

Tipburn is caused by a calcium deficiency in the young, rapidly expanding leaves. Many researchers have shown that these tissues have lower calcium contents than expanded leaves (e.g. Collier and Huntington, 1983; Collier and Tibbitts, 1984; Cresswell, 1991). Barta and Tibbitts (1991) confirmed these findings with a sensitive detection technique. Leaf tissue about to develop tipburn had significantly lower calcium values than adjacent areas of the same leaf, and than the same areas on leaves of non-affected plants (Fig. 14.9). Foliar sprays of calcium of the young leaves of butterhead lettuce plants prevented the disorder (Thibodeau and Minotti, 1969), while the application of organic salts such as oxalates induced the disorder.

The marginal necrosis of young leaves that is a characteristic sign of the disorder may be partly due to the collapse of the affected tissue because of membrane and cell wall damage. However, the damage may also be caused by toxicity from latex released from ruptured lacticifers at the edge of the leaf (Tibbitts *et al.*, 1965). Calcium deficiency may be involved both in the weakening of leaf cell membranes and cell walls, as well as the loss of integrity of the lacticifer cell walls, allowing their rupture and the spilling of latex (Tibbitts *et al.*, 1985).

The relatively immobile nature of calcium in the plant has made tipburn a difficult problem to control. While foliar sprays of calcium salts have been effective in open-headed and leaf types, where sprays can be directed to the areas most susceptible, the technique is ineffective on the

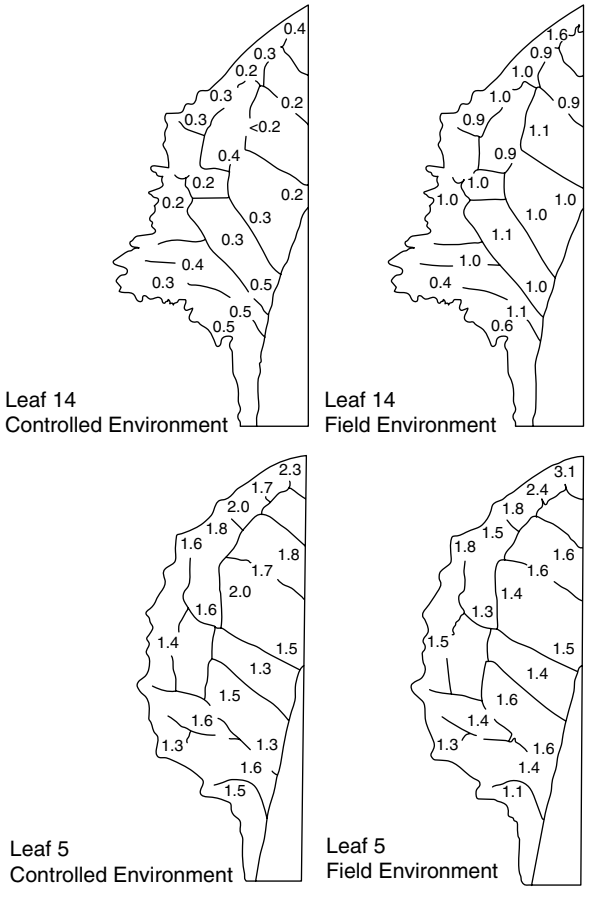

Fig. 14.9. Concentration of calcium in 20-mm long lettuce leaves, expressed as mg Ca g–1 dry weight, from plants grown in controlled environment or in the field. Leaves numbered from plant base (Barta and Tibbitts, 1991).

crisphead types. The disorder often develops on plants growing in soils with ample supplies of calcium, so increasing soil calcium levels also is not an effective control strategy. Understanding the factors which govern the movement of calcium in the lettuce plant may however help to devise strategies for tipburn control.

Calcium is transported in the plant primarily in the xylem, and therefore moves preferentially to actively transpiring tissues (Bangerth, 1979; Barta and Tibbitts, 1986). As a consequence, areas of the plant that have low transpiration rate by virtue of their location receive little calcium. By covering the young leaves of lettuce plants with a plastic cap that reduced transpiration, Barta and Tibbits (1986) induced tipburn on 53% of the covered leaves, compared to 1% in the uncovered controls. Calcium contents were 0.63 and 1.48 mg g^{-1} dry weight, for covered and uncovered leaves, respectively. In the opposite approach, Goto and Takakura (1992), Frantz *et al.* (2004) and Lee *et al.* (2013) eliminated tipburn in butterhead lettuce by blowing air onto the young leaves as heads started to form. Thus, maximizing plant transpiration rate by providing ample water and lowering relative humidity in glasshouse-grown lettuce crops during the light period could help in tipburn control, and there is evidence to this effect (Collier and Tibbitts, 1984).

Manipulation of transpiration rate is unlikely to be effective in alleviating tipburn in crisphead lettuce, where the sensitive tissue is enclosed and prevented from transpiring. For these plants, encouraging root pressure, which transports water and associated nutrient elements to all parts of the plant regardless of age, shows more promise for tipburn control. Root pressure occurs under conditions where transpiration is not functioning, and can be encouraged by increasing night-time relative humidity, and by reducing the resistance to water movement into the plant. Maintaining adequate soil water, and having a low night-time osmotic pressure of the soil solution are two ways in which water uptake can be maximized. This approach was moderately successful in reducing tipburn in butterhead lettuce grown in solution culture (Cresswell, 1991). Increasing relative humidity to above 95% during the dark period also decreased tipburn compared to lower humidities, and increased inner leaf calcium levels from 0.85 to 1.12 mg g^{-1} (Collier and Tibbitts, 1984).

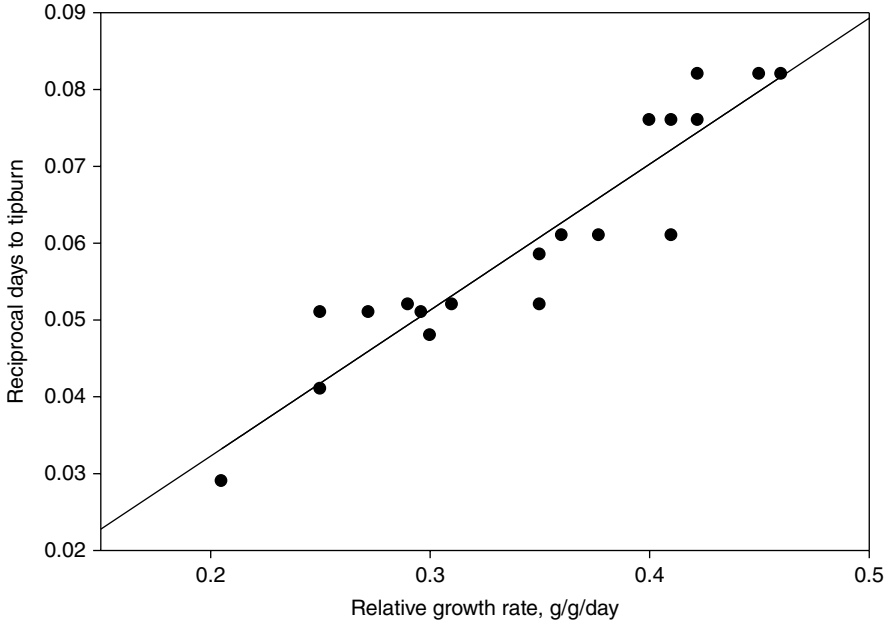

Fig. 14.10. The relationship between relative plant growth rate and the reciprocal of the number of days until tipburn first appears on the plants. Line equation y= –0.006 + 0.19x (r2 = 0.88) (Cox and Dearman, 1976).

The chances that a calcium deficiency will occur in the young leaves depends not only on the supply of the mineral to these organs as well as the demand of the leaves for calcium. As temperatures and light increase, so does growth rate, and with it the requirement for nutrients. Accordingly, Cox *et al.* (1976) found that the relative growth rate of lettuce, when varied by changing the temperature and light conditions, was closely tied to the time at which tipburn developed (Fig. 14.10). Similarly, tipburn incidence in serial plantings of crisphead lettuce through the year in Hawaii was most severe when growing temperatures were highest (Yanagi *et al.*, 1983). Other conditions which increase plant growth rate can also result in increased tipburn. For instance, Crisp *et al.* (1976) found that widely spaced lettuce was more affected by tipburn than the same cultivar grown at close spacing. The higher tipburn incidence in lettuce grown in controlled environments than in the field may also relate to the faster growth rates achieved in the growth chamber (Barta and Tibbitts, 1991; Sago, 2016). The disorder thus determines the maximum productivity that can be achieved by the lettuce plant. This was confirmed by Frantz *et al.* (2004), who quadrupled lettuce growth rate by combining high light levels, increased CO_2 and a growth temperature of 30°C by controlling tipburn with an air stream directed at the lettuce apex in growth chamber conditions.

Considerable genetic variation in tipburn incidence has been shown, related in some cases to plant morphology. For instance, Jenni and Hayes (2010) found that among Romaine lettuces, open head, partly closed and completely closed head types had 38, 56 and 74% tipburn incidence, respectively. Tipburn incidence varied among genotypes, environments and their interactions, but were not repeatable over years. There was a similar lack of consistency in tipburn incidence among 37 genotypes of leaf lettuce grown in five plantings in California (Lafta *et al.*, 2017). In a more hopeful note, Jenni *et al.* (2013) found one quantitative trait locus that described 38 to 70% of variation in tipburn incidence in multiple trials.

Brown rib

This disorder, variously termed brown spotting, brown rib and rib discoloration, has been linked to day temperatures of 35°C or more, occurring at two weeks after the start of heading in crisphead cultivars (Friedman, 1954; Jenkins, 1959; Jenni, 2005). Discoloration starts as a flecking and streaking brown area on the inner surface of the major veins near the outside of the head, and may spread to minor veins (Jenni, 2005). There are considerable cultivar differences in disorder severity, and sensitivity appears to be controlled by several genes (Jenkins, 1959; Jenni *et al.*, 2008). Three to five days of high temperature are sufficient to cause the disorder (Jenni, 2005). Under field conditions incidence of the disorder correlated with head temperature 5 mm below the head surface (Jenni *et al.*, 2012). Jenkins (1959) reported that the disorder could be lessened by sprinkler irrigation use when temperatures exceeded 27°C.

Concluding Remarks

Lettuce is a rather demanding and difficult crop to grow. At all stages of the life cycle, environmental conditions have a major influence on growth and development. Seed germination is controlled by light in some cultivars, and all are subject to inhibition by high temperatures. The higher incidence of bolting, tipburn, and puffy heads with prominent ribs at high temperatures, and other internal defects, also contribute to restricting production to areas where mean maximum air temperatures do not exceed 26°C during the growing period.

Soil conditions for lettuce production are also exacting. To get a good stand of uniform plants, the direct-seeded crop must be precision-planted at an exact depth into a well-prepared seedbed. Advances in priming and seed pelleting have made plant establishment more precise. Good control of soil water content is important to permit stress-free growth, and to avoid tipburn and other defects. The ability to provide optimum water conditions is a major factor contributing to the predominance of the western states in American lettuce production. Currently, 91% of American lettuce is grown in California, Arizona, and Colorado (USDA, 2017).

The ability of lettuce to grow under cool, and frequently low light, conditions has made it an important crop for winter glasshouse production,

particularly in Europe. Although yields are much lower than under higher light conditions, concentrating on high quality butterhead and leaf lettuce makes the crops economically feasible.

The current attempts to adapt the crop to grow in artificial, high input environments are contributing to our understanding of the growth and development processes of lettuce. Use of LEDs allows for light pulsing, novel daylength regimes, and light quality variations to enhance lettuce color and human nutrition quality (Mitchell *et al.*, 2015). Stacking several layers of plants in growth rooms operated year-round may overcome the variability and uncertainties of the field environment, in spite of high energy input needs.

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15A Cauliflower, Broccoli, Cabbage, and Brussels Sprouts

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The many different selected forms of *Brassica oleracea* present probably the most striking example of polymorphism in the group of plants considered vegetables. The progenitor of this diverse set of plants is described by Helm (1963) as a polymorphic perennial herb that generally had a height of 60–100 cm, and a moderately branched stem. It is thought to have originated on the coast and islands of the Mediterranean 3 million years ago, and spread from there throughout Europe, and secondarily to East Asia (Hammer *et al.*, 2013; Arias *et al.*, 2014). Earliest selections were probably made to reduce the content of bitter glucosinolates found in abundance in the wild types. The single-stemmed kales, with either smooth or ruffled leaf shape, were probably first consumed by people of the Mediterranean area as early as 600 BC (Thompson, 1976).

The ancient *Brassica* family plants were used in several ways. Study of classical literature and archeological evidence showed that 30 taxa were used either as medicines (83%), food (40%), or for magical or ornamental purposes (Toscano *et al.*, 2013). A similar diversity of species and their uses continues into the present day in parts of Italy, such as Sicily (Romano *et al.*, 2013).

The precise path of selection for the cabbage family vegetables that are popular today is not exactly known. Thompson (1976) speculated that as *B. oleracea* spread northward, types having a biennial flowering habit became predominant, requiring a cool period of two to three months to induce flowering. From among these, types were selected with a much-shortened stem and a large apical bud surrounded by many leaves. Cabbage occurred as early as in the 12th century in Germany (Helm, 1963). Selection of plants with short, swollen fleshy stems resulted in our present-day kohlrabi, first described in the 1500s in the same area. The selection for types with short, heavily branched fleshy inflorescences apparently occurred in the eastern Mediterranean area. Cauliflower was described in the 16th century, but broccoli apparently did not appear until 100 years later (Thompson, 1976). The latest arrival of the common cole crops is Brussels sprouts, which was selected, as the name implies, in Belgium in the 18th century.

Study of the *Brassica* family of vegetables has been aided by the detailed work on *Arabidopsis thaliana*, a weed in the *Brassicaceae*. Although *Arabidopsis* diverged from the common ancestor of the *Brassica* crops 17 million years ago (Golicz *et al.*, 2013), they share many gene systems and physiological mechanisms. For instance, Schiessl *et al.* (2017) could compare the 35 gene systems that govern flowering in *Arabidopsis* for similarities among the *Brassica* vegetables in processes such as vernalization, photoperiod pathways and temperature regulation of flowering. In Chinese cabbage, a QTL for extreme late bolting was identified by Ajisaka *et al.* (2001).

 Physiology of Vegetable Crops 2nd edition (eds H.C. Wien and H. Stützel)

Cabbage, cauliflower, and broccoli are vegetable crops of worldwide importance, occupying 6.7% of the 56.2 million ha of total vegetable area (FAOSTAT, 2017). Production of these *Brassica* crops has increased dramatically worldwide over the last 20 years, especially in the major production area, Asia (Table 15.1). The increase is especially large in cauliflower and broccoli, perhaps in concert with increases in standards of living. Of the three crops, cabbage currently occupies about twice as much land as cauliflower and broccoli combined, and produces about 2.8 times as much product.

Juvenile Period

The induction of flowering in *Brassica oleracea* is brought about by relatively low temperatures, in the process called vernalization (see Chapter 3), but the temperatures are effective only if the plants are past what is called the juvenile period.

Evidence for the existence of a juvenile period is obtained by exposing plants of various ages to low temperatures and observing how many leaves are formed on the main stem before the apex changes to a reproductive structure. If the plant is still juvenile when the cold treatment is imposed, the final leaf number will be similar to that of plants not cold-treated (Fig. 15.1). After the plants have reached the adult vegetative stage, cold treatment will reduce the leaf number formed, but also the rate at which plants grow. It is therefore physiologically more adequate to determine the effectiveness of vernalization not by chronological time to particular reproductive events, but by leaf number formed (Sadik, 1967; Wiebe, 1972a; Hand and Atherton, 1987).

The existence of the juvenile period has been clearly demonstrated by Stokes and Verkerk (1951), Wiebe (1972a), and Thomas (1980), who found that germinating seeds could not be vernalized. There are, however, instances where the existence of a juvenile phase has been questioned. In cabbage (Nakamura and Hattori, 1961) and in cauliflower (Fujime and Hirose, 1979) it has been shown that there was a slight reduction in final leaf number with vernalization of imbibed seeds, suggesting that there was no juvenile period. It seems likely that there are large differences in juvenility between cultivars and species and it may be that sensitivity to induction decreases and then increases as plants get older.

Changes in plant morphology that occur when cole crops reach the adult stage have been documented by a number of workers. From the standpoint of overall development, Stokes and Verkerk (1951) found that Brussels sprout plants showed a pronounced thickening of the stem, and enlargement of axillary buds when they became sensitive to induction by cold. This change occurs when the plants have formed about 30 leaves plus leaf initials and appears to be a feature generally applicable to Brussels sprout cultivars with a range of maturities (Thomas, 1980). In cabbage, transition from juvenile to adult stage is less pronounced, but appears to occur when stem diameter reaches about 6 mm (Boswell, 1929; Ito and Saito, 1961).

Table 15.1. Worldwide and Asia production of cabbage, Chinese cabbage, and cauliflower/broccoli for the three years 1994–1996, and 2014–2016 (source: FAOSTAT, 2017).

		1995	2015	Increase, %
Cabbage area, mha[z]	World	1.99	2.80	41
	Asia	1.20	1.74	45
Cauliflower/broccoli	World	0.74	1.31	77
area, mha	Asia	0.46	1.04	126
Cabbage prod'n, MMT[y]	World	45.2	70.9	57
	Asia	29.2	54.1	85
Cauliflower/broccoli prod'n., MMT	World	12.9	24.7	91
	Asia	8.8	19.5	122

[z]mha = million ha.
[y]MMT = million metric tonnes.

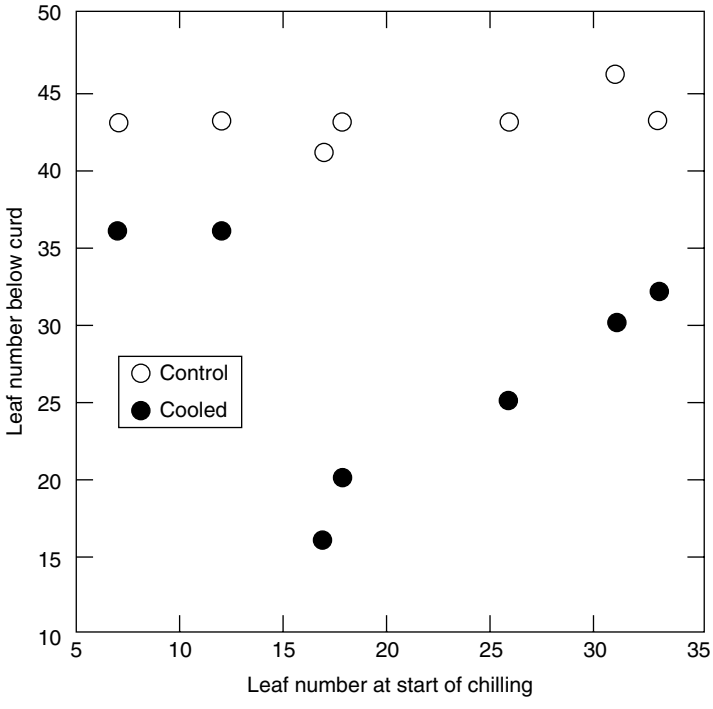

Fig. 15.1. Effect of a 28-day exposure to 5°C, begun at various plant ages (leaf numbers) on total leaves formed below the curd by "White Fox" cauliflower (Hand and Atherton, 1987). Plants became adult after formation of 17 leaves (by permission of Oxford University Press).

In cauliflower (Wiebe, 1972c), broccoli and Brussels sprouts (Stokes and Verkerk, 1951), adult vegetative plants have a distinctly domed apical meristem that is broader than that of the juvenile plants (Fig. 15.2). A pronounced enlargement of the apex occurs after plants are exposed to "relative cold" when they have reached the adult stage.

The length of the period during which exposure to cold will not induce flowering appears to be governed primarily by genotype, although it can also be influenced by environment. Wiebe (1972a) found that cauliflower plants grown under low light, and/or partially defoliated, formed more leaves before the curd than intact plants grown under full light. These findings support the views of Hand and Atherton (1987), and Williams and Atherton (1990), that during the juvenile period, assimilates are used preferentially for leaf growth rather than for apical meristem growth. Thus, under conditions of limited resources, leaf growth would be prolonged at the expense of meristem development.

Differences among cultivars in the leaf number marking the end of the juvenile period are variable. For instance, Wurr *et al.* (1982) found that in cauliflower, leaf number below the curd varied from 21 to 98 among a range of genotypes. This may reflect differences in the end of the juvenile period. On the other hand, seven Brussels sprout cultivars all reached the adult stage with about 30 leaves and initials, while some cauliflower cultivars became sensitive after only 10 leaves were formed (Wiebe, 1972a; Thomas, 1980).

From a practical standpoint, the existence of a juvenile period permits the plant to grow to an adequate size before it can be induced to flower. In climatic situations where a mild cold period would allow the crop to survive but not grow actively, a cole crop such as cabbage can be planted at the beginning of the cold period and survive until a later warm period without being induced to flower. Indeed, such overwintering of cabbage used to be a common practice in the eastern United States (Boswell, 1929).

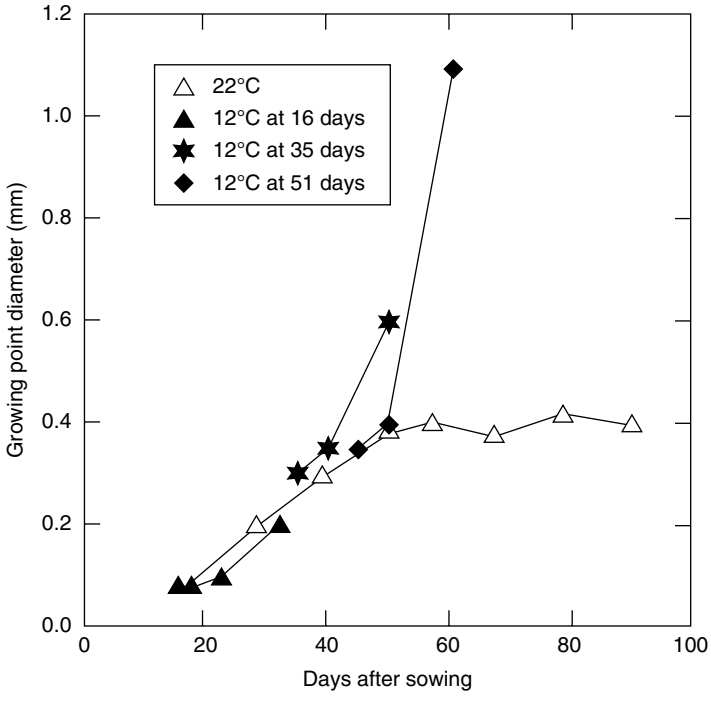

Fig. 15.2. Change in apex diameter when cauliflower cv. "Aristokrat" was exposed to 12°C at different times after sowing (Wiebe, 1972c). Plants reached the adult stage after about 42 days growth at 22°C.

Curd and Head Development in Cauliflower and Broccoli

Curd and head morphology

The curd of cauliflower has been described as a prefloral structure that shares some of the characteristics of both the vegetative and reproductive apices (Sadik, 1962; Margara and David, 1978). Like the vegetative shoot, it retains the 5:8 phyllotaxy of leaves, but leaf development has been reduced so that only bracts are formed in the curd. The lateral buds of the shoot meristem are elongated and much branched, and the apices of these branches form the surface of the curd. These apices are partly differentiated into prefloral structures, with masses of rudimentary flower buds visible microscopically (Sadik, 1962), but bud development is arrested at this early stage. The entire structure, much shortened and thicker than a normal flowering shoot, can give rise to the future inflorescence of the plant, if the right environmental conditions prevail. If not harvested, much of the curd becomes necrotic or decays, unless the distinct chilling requirement for flower bud initiation described by Fujime (1983) has stimulated the further development of some primordia into true reproductive structures (Sadik, 1962). The formation of flower buds is accompanied by bud elongation so that they project out of the curd, giving it a grainy appearance, termed "riceyness." As the reproductive structures develop, marked elongation of branches takes place, most often at the edge of the curd. By the time of anthesis, the flower stalks may project 20–50 cm above the curd (Sadik, 1962).

In broccoli, branched inflorescence development proceeds directly to the formation of flower buds without the formation of the intermediate pre-floral stage. While heads are marketable the flower buds are still small, but if the heads are not cut, the buds continue development to open flowers.

The morphology of cauliflower curds and broccoli spears is determined by the rate of production of branch primordia on the flanks of the apical meristems, the number of branch primordia produced before those formed first start

producing their own branch primordia, and the duration of the pre-inflorescence stage (Kieffer *et al.*, 1998). The latter is longer in cauliflower than in broccoli. If the first two parameters are relatively stable, semi-spherical curds with smooth surfaces are formed like in cauliflower and broccoli. However, when these parameters increase during curd development, the pyramidal structures of Romanesco curds are formed. In the regulation of curd structure, the gene *BoCAL* (syn. *BoCAL-a*), an ortholog of the *Arabidopsis* CAL (*CAULIFLOWER*) gene in conjunction with the *BoAP1* gene seem to be involved (Fig. 15.3).

Environmental factors in curd and head induction

The formation of curds in cauliflower, and heads in broccoli is primarily influenced by temperature. Estimates of the precise temperatures allowing vernalization to proceed vary but Wiebe (1972a),

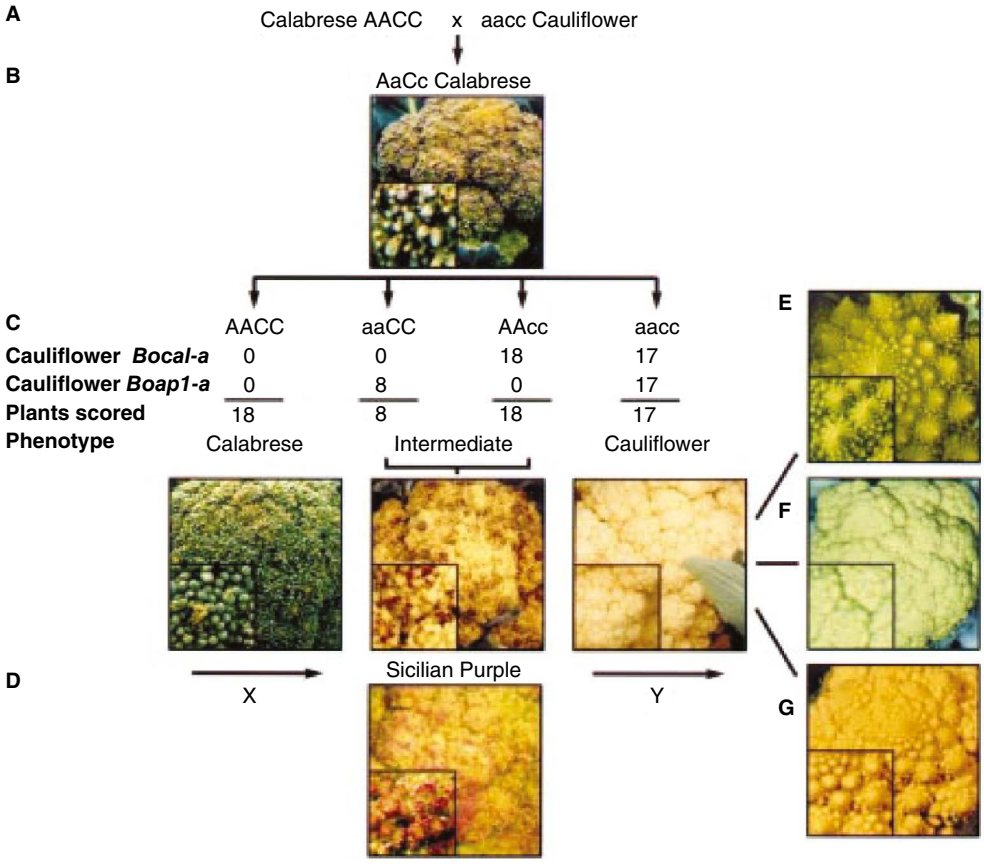

Fig. 15.3. A genetic model of the interactions between the *BoCAL-a* and *BoAP1-a* loci required to explain the curding phenotype of *Brassica oleracea* var. *botrytis*. AAD *BoAP1-a*, CCD *BoCAL-a*. Lower-case letters denote presumptive mutant alleles (e.g. cc D homozygous *Bocal-a*). A. DH parental genotypes and phenotypes. B. F1 of the cross; Calabrese broccoli phenotype suggests mutant alleles at both loci are recessive. C. DH offspring generated from the F1. Insets show magnified view. D. Theoretical "two-step" cauliflower domestication process. X denotes mutation of either the *BoCAL-a* or *BoAP1-a* loci in heading broccoli type (characterized by Calabrese broccoli (*B. o.* var. *italica*) in this instance) results in a "ricey" curding population phenotypically similar to both the Sicilian Purple types (shown here at the onset of anthocyanin expression) and the intermediates of this cross. Y denotes introduction of a further mutation into the second of the two loci results in stronger control over floral induction and development of the classic curd phenotype of cauliflower E–G. Examples of diverse regional forms of cauliflower curd from Italy (Smith and King, 2000).

Atherton *et al.* (1987), and Wurr *et al.* (1988) all showed that in cauliflower as the temperature increased, the relative rate of vernalization increased to a maximum and then declined. Wiebe (1972a) showed maximum response between 7 and 12°C while Wurr *et al.* (1993) developed a model for summer/autumn cauliflower with no vernalization below 9 or above 21°C and the maximum rate of vernalization between 9 and 9.5°C. For different cultivars in the same maturity period, Grevsen and Olesen (1994) proposed a symmetrical relationship with no vernalization below 0 and above 25.6°C and the maximum rate of vernalization at 12.8°C. Thus, vernalization in summer/autumn cauliflower proceeds most rapidly at moderate temperatures and slowly or not at all at low and high temperatures. At the latter many temperate cultivars fail to form curds but continue producing leaves at the apex. The response of vernalization rate, i.e. the reciprocal of the time from the end of the juvenility period to floral transition, to temperature can be described with a trapezoidal function (Fig. 15.4). Compared to typical summer cultivars adapted to temperate climates, winter cultivars have not only a narrower range of inductive temperatures, but also lower maximum vernalization rate. Cultivars adapted to tropical conditions have an even wider range of inductive temperatures and higher maximum vernalization rates than temperate summer cultivars. According to Wiebe (1990), the highest temperature capable of bringing about curd formation varies from about 16°C in some cultivars, to nearly 30°C in others. The knowledge of the genetic background of vernalization is still incomplete but is has been shown that the transcription of the *BoFLC2* gene, responsible for late flowering, is reduced by vernalization (Ridge *et al.*, 2015).

Broccoli has a similar temperature response to cauliflower, although the upper temperature limit for head formation may be higher. For instance, in Wiebe's study, the cultivar "Coastal" formed heads at a constant 27°C (Wiebe, 1975). Fontes and Ozbun (1972), on the other hand, prevented head formation in "Waltham 29" broccoli by raising it at 24/27°C day/night temperature.

Light conditions during vernalization were not important as long as optimum temperatures were used (Wiebe, 1972b). However, if the night temperature was raised from 12°C to 22°C at a reduced light intensity of 2.5 klux, curd formation was delayed and leaf number was increased. This may imply that adequate carbohydrate levels must be present in the plant to permit the differentiation of curds. Several investigators have related curd initiation to apex carbohydrate level and prevented it by reducing carbohydrates through growing plants in the dark or raising the plants in CO_2-free air or during periods of high temperature (Sadik and Ozbun, 1968; Wiebe, 1974). Similarly, Atherton *et al.* (1987) hastened curd initiation by applying sucrose to intact plant apices. It may be, as Atherton and co-workers suggest (Atherton *et al.*, 1987; Williams and Atherton, 1990), that low temperatures are required to reduce competition for assimilates between developing leaves and the apical meristem by suppressing leaf growth. The lack of correlation between apex sugar levels and differentiation to reproductive growth found by Wiebe (1974) for cauliflower, and Fontes and

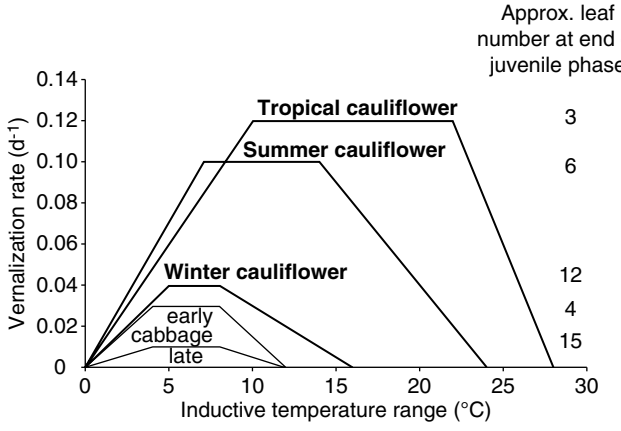

Fig. 15.4. Vernalization rates of different cauliflower and head cabbage cultivar types (source: Wiebe, 1972b, 1987).

Ozbun (1972) for broccoli, may imply, however, that other factors, such as hormones, are also involved (see below).

Cultivar Differences

Through breeding and selection, a range of cauliflower cultivars has been developed to produce satisfactory yields in specific environments ranging from the intermediate altitude tropics to the winter season of western England. The differences in adaptation have resulted in cultivars that contrast greatly in their response to temperature. Cauliflowers normally grown in the tropics can produce curds at mean temperatures of 25°C, after forming about 40 leaves (Wiebe, 1975). These cultivars typically have a short juvenile period and can be induced to flower after one to two weeks of low temperatures (Sadik and Ozbun, 1967). Thus, when grown in temperate areas, they typically form only small plants before initiating a curd, and often have ricey curds that quickly elongate to start flowering (Wiebe, 1975). Prolonged exposure of these cultivars to temperatures of 12–15°C in the early stages results in the formation of green broccoli-like heads instead of curds (Fig. 15.5)

The feature distinguishing over-wintered cultivars in mild temperate climates from those maturing in one growing season is the vernalization requirement (Sadik, 1967). In his experiments, a six-week period of 5°C was needed to induce curd production and flowering in the cultivar "February-Early-March." More extensive field studies with a range of these winter-heading cultivars by Wurr *et al.* (1981b), indicated that they may also have a lower temperature threshold for curd induction than summer cultivars, and a lower base temperature for curd growth. This should increase their growth adaptation during the cold season but some of them take more than 11 months to mature and suffer the risk of crop loss in colder than usual winters.

Cultivar differences in head size may be explained by recently discovered genes. A Curd Development Associated Gene 1 (CDAG1) was identified in cauliflower (Li *et al.*, 2017), which increases curd sized through increased cell number. This gene was shown to inhibit the transcriptional expression of the endogenous organ-size related (OSR) genes, ARGOS (Auxin-Regulated Gene involved in Organ Size) and ARL (ARGOS-Like).

Hormonal effects

It has long been conjectured that the induction of cauliflower curd formation could be mediated by growth hormones but attempts to substantiate this through direct measurement of endogenous compounds have only partly succeeded. The most consistent link between curd induction and hormone levels has been made for the gibberellins. Thomas *et al.* (1972) measured an increase in

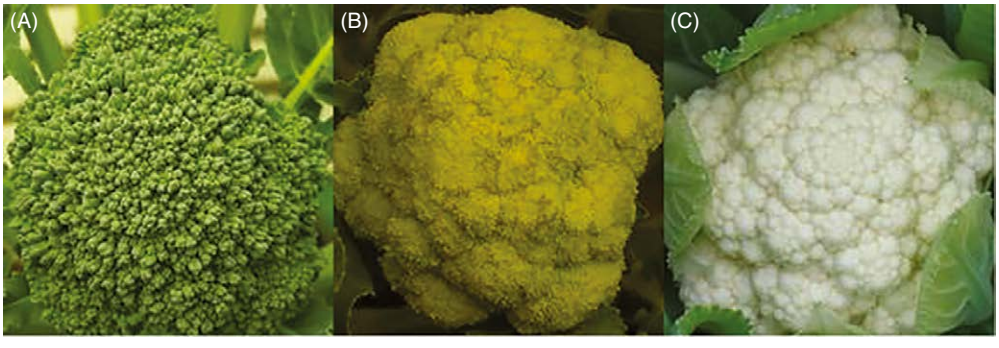

Fig. 15.5. Temperature effect on the stage of arrest of *Brassica oleracea* cv. "Green Harmony" F_1 grown under three different day/night temperature regimes during reproductive development. (A) At 16 °C/12 °C, curd arrested at floral bud stage (broccoli-like head). (B) At 22 °C/17 °C, curd arrested at floral primordium stage (intermediate curd). (C) At 28 °C/22 °C, curd arrested at inflorescence meristem stage (cauliflower-like curd). (Duclos and Björkman, 2008).

gibberellin-like substances about two weeks before curds were initiated in cauliflower. If the plants were exposed to cold, the hormone peak was increased, but curd initiation was delayed past the end of the sampling period. In support of these findings, Kato (1965) measured a slight increase in gibberellin activity with cold treatment of cauliflower, as did Fontes *et al.* (1970), after cooling broccoli plants. Further evidence supporting gibberellin involvement in curd induction came from Booij (1989, 1990c) who found in an extensive series of field plantings that GA_{4+7} (a mixture of gibberellins 4 and 7), but not GA_3, applied when the plants had just reached the adult vegetative stage, reduced the number of leaves to the curd. Recently, both GA_3 and GA_{4+7} accelerated the vegetative-generative transition and reduced node number of cauliflower and broccoli, with GA_{4+7} having stronger effects than GA_3 (Duclos and Björkman, 2015). However, GA_3 cannot substitute for cold in vernalization (Guo *et al.*, 2004).

The situation is less clear when considering growth inhibitor experiments. Application of the growth retardants ancymidol, chlormequat, or daminozide to vegetative plants had no effect on curd diameter (Booij, 1989). Daminozide, but not chlormequat, inhibited flowering and partially negated the effect of a cold treatment on leaf number in broccoli (Fontes and Ozbun, 1970). Paradoxically, the daminozide treatment also caused a large increase in endogenous gibberellins in the plants (Fontes *et al.*, 1970), whereas chlormequat did not change gibberellin levels. Much more work is needed to resolve these conflicting results. In all the experiments reporting endogenous gibberellin levels, gibberellins were detected by lettuce hypocotyl bioassay. It is vital that these findings be checked with modern direct measurements of the levels of endogenous gibberellins, and that the identity of the specific hormones be established.

Flower Induction in Cabbage and Brussels Sprouts

In contrast to cauliflower and broccoli, flower induction in cabbage and Brussels sprouts is only required for seed production. Indeed, in both cultivars flower development detracts from crop quality. To form flowers, cabbage and Brussels sprouts plants must be exposed to vernalizing temperatures once they have reached the end of the juvenile period. Optimum vernalizing temperatures have generally ranged from 4 to 10°C, although there are cultivar differences in the temperatures that are effective (Ito and Saito, 1961; Heide, 1970; Friend, 1985). Where temperatures fluctuate, both the mean temperature and the diurnal pattern influence how fast a plant comes to flower. Ito and Saito (1961) found that flowering was delayed, but not prevented, by exposure of cabbage to daily cycles of 16 h at 9°C, and 8 h at 27°C (Fig. 15.6). No differentiation of reproductive structures occurred if 8 h at 9°C and 16 h at 27°C were given, even after 120 days exposure. Similar findings were made by Heide (1970) for cabbage, and Verkerk (1954) for Brussels sprouts. If long durations of vernalizing temperatures were applied, high temperatures at the end of the cold period were ineffective in reversing the induction (Heide, 1970).

Plant age and size when inducing temperatures are applied also play important roles in determining the rate and effectiveness of the flower induction. Among plants of the same age, the largest were most strongly induced by a marginal cold treatment (Ito and Saito, 1961). The effect of plant age in cabbage may be caused by juvenility which prevents flower induction totally in young plants and may only gradually disappear in older plants. Ito *et al.* (1966) were able to increase the vernalization requirement in successive cuttings taken from cabbage plants, which may indicate a partial reversion to the juvenile state.

Under conditions marginal for induction of flowers in cabbage, factors which encourage stem elongation appear to aid flowering as well. For instance, exposure of cabbage to long day conditions after vernalization caused a marked increase in stem elongation, and marginally increased the number of flowering plants, compared to plants kept in short days (Heide, 1970). Frequent applications of gibberellic acid to adult cabbage plants grown at high altitude locations in Kenya also resulted in flowering of some cultivars that remained vegetative without the treatment (Kahangi and Waithaka, 1981). However, gibberellic acid applications are not effective in inducing flowering of all cultivars (Kahangi and Waithaka, 1981; Friend, 1985).

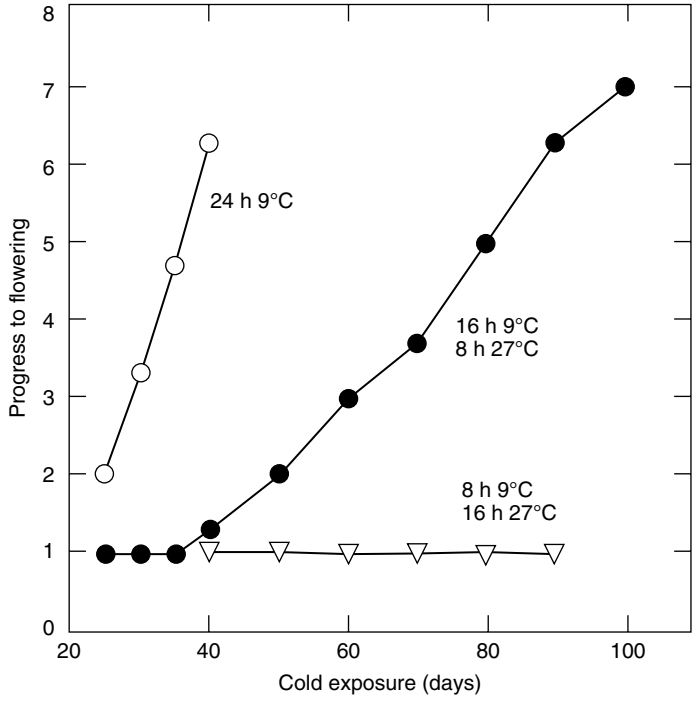

Fig. 15.6. Progress toward flowering of the cabbage apical meristem as influenced by plant exposure to increasing durations of 9°C, for 24, 16 or 8 h day^{-1}. Flower development stages: 1 = vegetative, 2 = dome-shaped, 3 = early reproductive, 4 = mid reproductive, 5 = sepals formed, 6 = petals formed, 7 = stamens formed (source: Ito and Saito, 1961).

In many plant species flower induction is an after-effect of the temperature treatment, with little change apparent in the apical meristem during the cold period. However, in *Brassica oleracea*, the apical meristem changes morphologically to the generative state while vernalizing temperatures are applied (Stokes and Verkerk, 1951; Ito and Saito, 1961; Wurr *et al*., 1993) and it is likely that vernalization occurs as the direct result of inducing temperatures.

The duration of low temperature treatment needed to induce flowering generally ranges from 10 to 50 days or longer in cabbage and 50–80 days in Brussels sprouts (Stokes and Verkerk, 1951; Ito and Saito, 1961; Heide, 1970; Thomas, 1980) though there are considerable differences between cultivars. In Brussels sprouts, an annual genotype has been identified that flowers without cold treatment (Wellensiek, 1960). Perhaps this is not surprising since cultivated cole crops are thought to have arisen from an annual ancestor grown in the Mediterranean region (Helm, 1963).

If cabbage or Brussels sprouts plants are given vernalization treatments insufficient for complete flower induction, partial reproductive development results (Stokes and Verkerk, 1951; Ito and Saito, 1961). Weak induction in cabbage resulted in the formation of an elongated stem and leaf bracts, no differentiation of flower structures, but the continuation of leaf and resumption of head formation. With successively stronger induction, reproductive growth was increasingly more complete, and the rate of development became more rapid. Unfortunately, we know virtually nothing about the chemical and hormonal changes that differentiate the development of partially and fully induced individuals.

Cabbage Head and Brussels Sprout Bud Formation

The process of head formation in cabbage appears to be similar to that described for lettuce in Chapter 14. The assemblage of layers of leaves

over the growing point requires the maintenance of a short stem during the heading period (North, 1957). As heading begins, leaves become broader and sessile, and more erect in their posture (North, 1958; Kato and Sooen, 1978). The inward curvature of the leaf edges, combined with their upright position leads finally to the formation of the head. Leaf production continues at a high rate in spite of the increasing confinement by previously formed foliage (North, 1957). As more leaves form, and these start to expand, the head gains in weight and firmness until it reaches a density acceptable for harvesting. Size of the head at harvestable density is determined by the cultivar, and cultural practices including such factors as the space available per plant, water and nutrient supplies during the growing season.

The factors that lead to erect frame leaf posture of cabbage and other head-forming *Brassica* species such as Chinese cabbage, have been under investigation. Kato (1981) pointed out the important role of the frame leaves in providing photosynthate for plant growth, and for creating environmental conditions that allowed younger leaves to grow more erect. Removal of inner frame leaves just as heading was beginning markedly delayed head formation, and resulted in the younger leaves assuming a horizontal attitude (Kato and Sooen, 1978; Kato, 1981; Hara *et al*., 1982), whereas removal of outer frame leaves had little effect on inner leaf attitude. Kato postulated that shading of the leaf bases of the inner frame leaves led to their inward curvature. In support of this theory, head formation was advanced by tying up the frame leaves of cabbage (Kato and Sooen, 1978). A comparison of 14 spreading or heading Chinese cabbage cultivars indicated that an acutely angled basal part of the leaf midrib was essential for erect leaf posture and ultimate heading (Nishijima and Fukino, 2005).

Search for hormonal factors in the formation of heads has shown that placement of the synthetic auxin naphthalene acetic acid (NAA) on the abaxial leaf surface near the leaf tip increased cupping, while placement on the opposite side caused them to become more horizontal (Ito and Kato, 1957). Gao *et al*. (2017) showed that auxin levels are high in upper and basal areas of cupping leaves of Chinese cabbage. Application of polar auxin transport inhibitors prevented leaf cupping. Increasing leaf auxin levels by introducing auxin synthesis genes from *Agrobacterium* led to earlier head formation (He *et al*., 2000), further emphasizing the importance of auxin in head formation. Information of the involvement of other hormones has been contradictory, particularly when relying on compounds applied to the plants (Kato and Sooen, 1980). For instance, application of gibberellins increased leaf cupping, but gibberellin inhibitors had no effect (Thomas, 1976; Kato and Sooen, 1980). Abscisic acid levels in leaves of cupping Chinese cabbage lines were lower than those in non-cupping lines (Gu *et al*., 2017). The key role of microsomal RNAs in regulating the timing and type of leaf curvature was highlighted by Wang *et al*. (2014) and Ren *et al*. (2018). There is need to broaden these studies into cabbage.

Studies of cultivars that form heads at different times have provided more insight into the process of heading. Earliness of head formation was related to an early start of leaf broadening (North, 1957; Tanaka and Niikura, 2003). The broad leaf shape may have reduced light intensities at the leaf bases. Kato (1981) further stated that late head-forming cultivars may be more sensitive to light with regard to leaf posture than early cultivars.

The head formation period ends with the attainment of the correct head density for harvesting. If the head is not harvested on time, further expansion of the inner leaves, and resumption of stem growth, result in the splitting of the head (North, 1957). Stem extension will occur even if the plant has not received sufficient low temperature exposure to bring about flower initiation, and seems to be part of the periodic growth cycle of the plant. Cabbage plants allowed to continue growth in an environment that does not induce flowering will show periodic stem extension followed by head formation (Fig. 15.7; Miller, 1929).

Cabbage breeders have selected against head splitting tendency, to permit the grower more latitude in harvest time. Work is needed to explain the physiology of cabbage stem growth and relate it to plant hormonal changes.

In Brussels sprouts, the axillary buds expand to form the harvested product. Bud formation begins at from 60 to 84 days after planting in a temperate environment (Everaarts and Sukkel, 1999), and is a cultivar characteristic that is also indicative of harvest date. Growth of the

Fig. 15.7. Cabbage not induced to flower by growing in a glasshouse in Ithaca, NY for 18 months at 20°C. Three periods of head formation alternated with stem extension (Wien, unpublished).

lateral buds is stimulated by removal of the plant apex at the time of bud formation (Thomas, 1972; Fisher and Milbourn, 1974), and this "topping" is a common practice in Brussels sprouts production. The increased bud growth appears to be caused by disruption of apical dominance, and stimulation of auxin production by the buds (Thomas, 1972). Application of auxin to the apical stump reduced bud growth rate and lowered bud auxin content. The involvement of root-produced cytokinins in lateral bud growth was indicated by bud growth stimulation with application of synthetic cytokinins (Thomas, 1976), and by enhanced bud growth at nodes below a point on the stem if it was girdled by steam (Thomas, 1983). The cytokinins would presumably be translocated up from the root system. Recent studies on branching of non-heading Chinese cabbage suggest the involvement of strigolactone as an inhibitor of bud growth (Cao *et al.*, 2017), but it is not known of a similar mechanism operated in Brussels sprouts.

Factors Determining Productivity

Cauliflower

Cauliflower has distinct responses to temperature for the phases of juvenility, vernalization, and curd growth (Wurr *et al.*, 1995) and this makes it a difficult crop to grow. Leaf production and expansion rate increase with temperature up to the end of juvenility but vernalization has a specific temperature range as previously described. If temperatures exceed this range, curd formation could be delayed or interrupted by further leaf formation. At temperatures lower than the optimum, leaf area development could be curtailed, leading to buttoning, or the production of ricey curds (see section on physiological disorders below). Cauliflower is also very demanding in terms of water and fertility requirements (Nonnecke, 1989). Only with a combination of temperature and other conditions permitting uninterrupted growth of the plant can sufficient leaf area form to allow the production of marketable curds. As a consequence, the major part of cauliflower production in temperate countries has been concentrated in areas where predictably moderate temperatures and adequate control of moisture conditions can be found. Under optimum temperature and moisture conditions, marketable yields of 12–15 t ha^{-1} can be achieved (Dufault and Waters, 1985; Maynard and Hochmuth, 2007). With increases in plant density, yields can be increased, but the resulting decrease in curd size (Salter and James, 1975; Thompson and Taylor, 1975) may not meet marketing criteria, except when sold as mini-cauliflowers (Salter, 1971). Both groups of workers also noted that some cultivars show virtually no change in marketable yield with increased plant density, while other cultivars are much more responsive. The latter also showed a slight increase in uniformity of maturity at close spacings (Salter and James, 1975).

In many countries, markets demand large curds with a smooth, white surface. To achieve this, maximum growth rates have to be achieved which requires adequate moisture and nutrient supply. For environmental reasons, nitrogen dosage should not exceed the demand. Linear relationships between photosynthetic capacity and leaf nitrogen content have been found which could serve as a physiological basis for fertilizer recommendations (Fig. 15.8)

Variation in curd maturity

Variability of plants in a crop frequently results in maturity covering a period of several weeks. Since harvesting can account for 20–40% of the total production cost, measures to reduce the

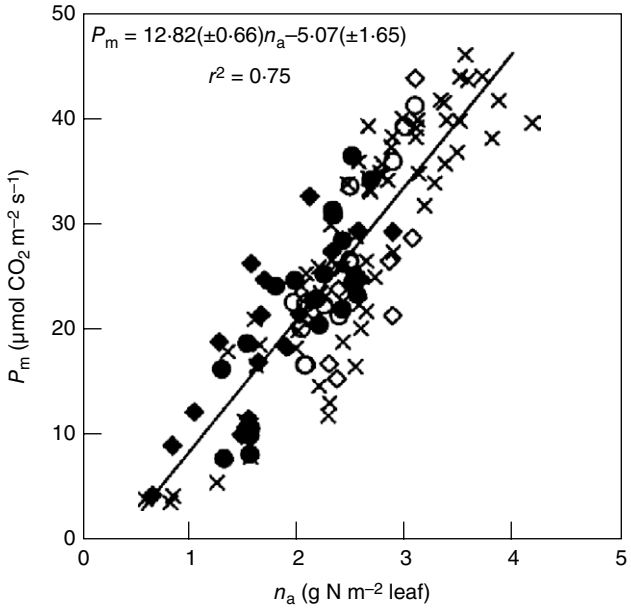

Fig. 15.8. Relationship between gross photosynthetic capacity, P_m, and protein-N content per unit leaf area, n_a,of cauliflower crops grown with two levels of nitrogen supply under different light conditions (unshaded, shaded with a net that absorbed 14% of PAR) in a glasshouse experiment (○, 145 g N 1^{-1}, light; ●, 35 g N 1^{-1}, light; ◊, 145 g N l^{-1}, net; ◆, 35 g N l^{-1}, net), and a field experiment (x) (Alt *et al.*, 2000).

number of times the crop is cut would make it cheaper to grow (Wheeler and Salter, 1974). Efforts to shorten harvest duration have focused both on the improvement of plant uniformity through modification of production practices, and through techniques that would synchronize curd initiation and growth.

Until the 1970s, cauliflower in Europe and North America was commonly sown in protected or outdoor seedbeds, and plants "pulled" for transplanting to the field with little or no soil adhering to the roots (Salter and Fradgley, 1969). By sowing the crop directly in the field, Salter and Fradgley halved the typical harvest duration of 20–30 days. Smaller improvements were achieved by more uniform spacing in the seedbed and by transplanting younger plants. In recent years, plant uniformity has been dramatically increased through the use of container-raised transplants. For instance, Wurr *et al.* (1990a) reported harvest durations of 14–22 days for "White Fox" cauliflower raised in cells, compared to 29–43 days for bare-rooted plants. Similar figures were quoted by Nonnecke (1989) from research on Long Island. In fact, the desire of a New York cauliflower grower to improve the uniformity of his cauliflower crop led to his developing the "Todd planter tray" in the 1960s which began the change to use of individual container trays for transplanting (Chapter 2).

Variation in the time of curd maturity is the result of accumulated variation among plants at earlier stages of growth. Booij (1990a) found that 55% of the variance in duration of harvest was due to the combined effects of variation in the duration of curd initiation and variation in temperature during curd growth. Some of the variation in curd initiation can be reduced by producing more uniform plants as explained above but the genetic uniformity of cauliflower has also been improved with the introduction of hybrid cultivars.

Another approach has been to reduce plant-to-plant variation in curd initiation by using treatments that encourage more synchronous curding. Salter and Ward (1972) discovered that subjecting cauliflower transplants to cold storage (two weeks at 2–5°C) caused a remarkable shortening of the harvest period compared to use of bare-rooted untreated transplants. Best results were obtained with plants that had produced 10–15 leaves and leaf initials at the start of the cold treatment, and so presumably were still in the juvenile stage. Cold storage of older plants or prolonging the duration of storage beyond three weeks reduced effectiveness of the treatment but when a wider range of genotypes was tried, the harvest duration was shortened with some cultivars and lengthened with others (Salter and James, 1974). Wurr (1981) suggested

that this was because the cold treatment worked only at a specific growth stage which varied according to genotype. However, the physiological basis for this response has not been identified. Salter and James (1974) postulated that the treatment helped to satisfy the cold requirement for curding, and thus synchronized the start of curd initiation, but subsequent work suggests that the effect is caused by some mechanism which as yet is not understood. Several workers have attempted to link the cold treatment to changes in apex hormone levels, particularly gibberellins. In one set of trials where treatment resulted in shortened harvest duration, there was a concomitant increase in gibberellin levels in the plant apex prior to curd initiation (Thomas *et al.*, 1972) but Wurr *et al.* (1981a) found that cold treatment increased gibberellin levels and reduced the harvest period in only one of four cultivars.

The variable and unpredictable results of the cold treatment of juvenile cauliflower plants illustrate that much remains to be explained about the vernalization process in *Brassica oleracea.* These results, and the contradictory findings with regard to the effect of seed vernalization (e.g. Wiebe, 1972a; Fujime and Hirose, 1979), indicate the need to look more intensively at the physiological processes governing the change from juvenile to adult vegetative state in cauliflower.

Continuity of production

To provide continuity of supply of produce throughout the growing season an accurate assessment of the duration of crop growth when planted at particular times of the season is needed, so that serial plantings can be made at appropriate times. Despite considerable effort by Salter and co-workers (Salter and Laflin, 1974), variation in weekly production of 50% was all that could be achieved. Principally, these workers simultaneously planted cultivars with different periods to maturity and staggered planting times. Wiebe (1980) conducted similar experiments and came to the same conclusions. Periods of high temperatures during the season caused much disruption in supply by hastening the harvest of crops close to maturity but delaying curd initiation on recently planted crops. The latter resulted in a reduction in supply six weeks later. Wiebe estimated that for conditions in Hannover, Germany, a series of ten plantings would be needed to achieve a continuous supply to market, but that weather fluctuations would cause unavoidable variations in amount of product. Martin (1985) and Wurr *et al.* (1990b) produced curvilinear relationships between the time taken for a crop to grow from transplanting to maturity and its time of transplanting, which can be used to aid crop scheduling.

Predicting maturity

Much of the variation in time to maturity is due to variation in the time from transplanting to curd initiation (Booij, 1987; Grevsen, 1990). Consequently, a technique has been developed to sample the crop once just after curd initiation to determine curd size and variability and then using a simple model of curd growth to predict when curds of any required size will be produced (Wurr *et al.*, 1990b). The technique has been successfully developed for commercial use. The model is based on the quadratic relationship between log curd diameter and the accumulated degree days above a base of 0°C (Wurr *et al.*, 1990b). Typically, it allows a grower to predict harvest maturity about four weeks in advance, compared to a week's advance notice by visual inspection.

Since the most expensive part of this technique is the sampling process, efforts to predict the time of curd formation in the crop have received emphasis. From a series of plantings of "White Fox" cauliflower, some of which were grown under polyethylene covers for part of the curd initiation period, Wurr *et al.* (1993) characterized the temperature response of curd formation as previously described. According to their measurements of apical meristem diameters, apical expansion towards the initiation of a curd was most rapid at 9°C, and ceased above 21°C. Lower limits of apex growth could not be precisely determined because of a lack of temperature points below 9°C. The model will allow the prediction of the time of curd initiation of this cultivar, once it has formed the necessary 17 leaves plus initials to become an adult vegetative plant and has been tested on field-grown crops of four cultivars (Wurr *et al.*, 1994). Grevsen and Olesen (1994) have also produced a model to predict the time of initiation. To be widely useful, such models will need to take into account the considerable

variation among cultivars in the temperatures needed for curd formation (Salter and Laflin, 1974; Wiebe, 1980; Booij, 1987). For accurate maturity predictions, it may be necessary to determine the temperature responses of each commercially important cultivar. Such efforts may be helped by recent modeling work with broccoli (Lindemann-Zutz *et al.*, 2016a).

Broccoli

The rapid increase in popularity of broccoli in North America during the 1960s through the 1990s has been followed by steady production, mostly carried out in California and Arizona, in locations and seasons that provide the cool temperate conditions that the crop demands (Ward *et al.*, 2015; USDA, 2017). The ability to supply the market year-round has been a key factor in Western U.S. dominance of production, coupled with a lack of cultivars adapted to the temperature fluctuations of the Eastern United States (Farnham and Bjorkman, 2011a, 2011b). Breeding and selecting broccoli lines that form acceptable heads when plants are exposed to air temperatures in the low 30s°C (Farnham and Bjorkman, 2011a) may thus broaden the area of broccoli production.

The most important factor influencing yield in broccoli is the population density at which the crop is grown. As with most crops, yield per unit area increases with number of plants, until a plateau is reached (Thompson and Taylor, 1976; Ward *et al.*, 2015). Although head size at maturity decreases as the plant population increases, consumers accept a range of head sizes. In the major production areas of the United States, broccoli is predominantly direct-seeded, allowing planting at higher plant densities than would be economically feasible with the transplanted crop (Le Strange *et al.*, 2010). In addition to high yields, close spacing has other advantages: plants have a lower incidence of hollow stem (see below) (Sanderson and Fillmore, 2010), they produce few side-shoots, and the main shoot has fewer leaves, reducing trimming waste at harvest (Thompson and Taylor, 1976). The reduction in side-shoot numbers concentrates the maturity so that single harvests of the entire crop are feasible (Cutcliffe, 1975a; Chung, 1982).

Broccoli has been shown to be quite responsive to nitrogen fertilizer, with yields plateauing at rates of between 200 and 350 kg/ha, depending on the method and timing of applications (Thompson *et al.*, 2002; Vagen *et al.*, 2007; Tremblay *et al.*, 2009). The broccoli head is a strong sink for nitrogen, with translocation taking place from the leaves to the head, at least at low N fertilization levels (Vagen *et al.*, 2007; Conversa *et al.*, 2013) (Fig. 15.9). When N rates were varied, head yields increased, but the fraction of plant N that was found in the head stayed at about 30% (Vagen *et al.*, 2007).

At higher N rates, mineral N residues can be elevated, potentially contaminating groundwater. Basing N rates on the nitrogen-supplying power of the soil, and adjusting by the nitrate content of leaf midribs at the time of supplemental N applications has helped to adjust fertilization to environmental and economic considerations (Belec *et al.*, 2001; Vagen *et al.*, 2007). Nitrate test strips and hand-held meters that measure chlorophyll and phenolic compounds are speeding up the estimate of nitrogen adequacy in soils and plant tissues (Tremblay *et al.*, 2009; Fortier *et al.*, 2010).

Predicting maturity

In areas where growing season temperatures are sufficiently invariate and predictable, crop scheduling of broccoli has been practiced by relying on the maturity characteristics of specific cultivars (Titley, 1987). Thus, most of the production season of southeastern Queensland in Australia permitted the use of cultivar-specific crop durations. In more typical environments, such as southern England, crop duration shows a curvilinear trend with time of planting (Wurr *et al.*, 1991). The longer crop growth periods from early or late season transplantings are primarily due to reduced growth rates at the prevailing low temperatures. Marshall and Thompson (1987a, 1987b) were able to predict maturity by calculating crop duration from sowing in day-degrees (thermal time), and improved it further by including solar radiation in the calculation.

Predictions of the time of maturity based on environmental conditions from transplanting incorrectly assume that the environment influences vernalization and head growth similarly. This problem can be avoided by beginning the

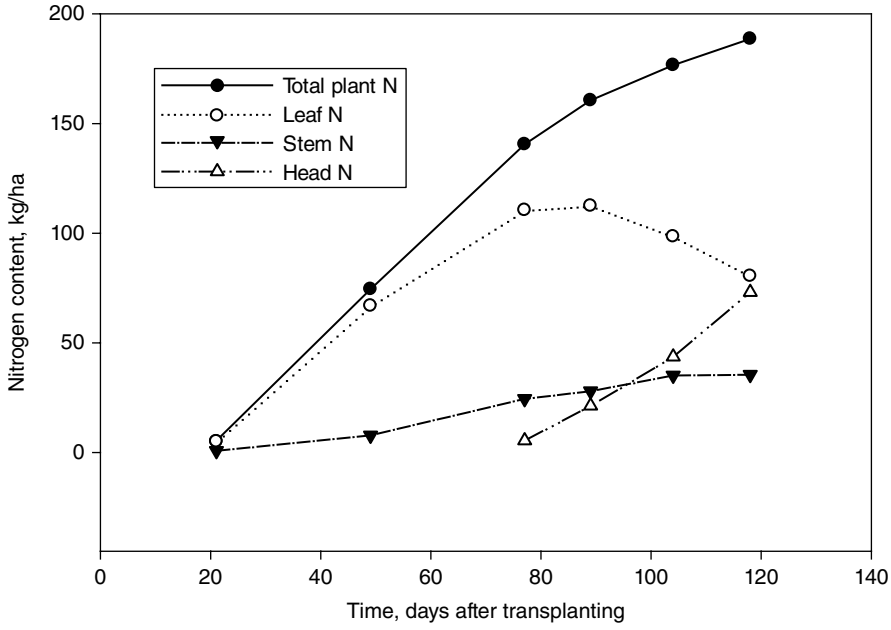

Fig. 15.9. Nitrogen content of a broccoli crop (average of two cultivars) grown in the winter season in southern Italy, fertilized with nitrogen at transplanting and 51 days later, totaling 130 kg/ha. (Conversa *et al*., 2013).

calculations after crop sampling has indicated that the head has been initiated. This approach, used by Wurr *et al*. (1991), resulted in accurate predictions of head maturity and has been developed into commercial prediction software which also takes account of differences in plant density. As in the experiments of Marshall and Thompson (1987a), the prediction was improved by including solar radiation in the model. A more recent model (Lindemann-Zutz *et al*., 2016a, 2016b) calculates floral transition including vernalization effects, head and stem growth as a function of light interception and light-use efficiency (see Chapter 5), and predicts a harvest window defined by the proportion of harvestable plants of 500g with 10 cm stem length as the beginning, and with the same weight consisting only of inflorescence (stem length 0 cm) as the end of a possible harvest period (Fig. 15.10).

Cabbage

The yield of cabbage is made up of two principal components: the number of plants per unit area and the size of the head. Productivity is altered by changes in the size of these components, and in the growth duration of the crop.

Head size is closely related to the amount of space available to each plant. As plant population increases the heads become smaller, decreasing to a minimum that may be smaller than acceptable in the market (Stoffella and Fleming, 1990; Kolota and Chohura, 2015). For instance, a head weight of from 1 to 2 kg is considered acceptable for fresh market cabbage (Maynard and Hochmuth, 2007). Highest marketable yield of that size range would come from plant populations of 55,000 to 74,000 plants/ha (Kolota and Chohura, 2015), and would be affected by the cultivar grown. At high populations, the shape or rectangularity of the space available per plant can also affect final head size, with decreases in in-row spacing lowering yield (Barrett *et al*., 2015). Head size is influenced directly by availability of major nutrients to the plant. Hara and Sonoda (1979a, 1979b) found that for satisfactory yields there must be adequate levels of N, particularly during the early head formation stage, and P and K during the earlier stage of outer leaf expansion. They identified the critical contents of N, P and K in the outer leaves which

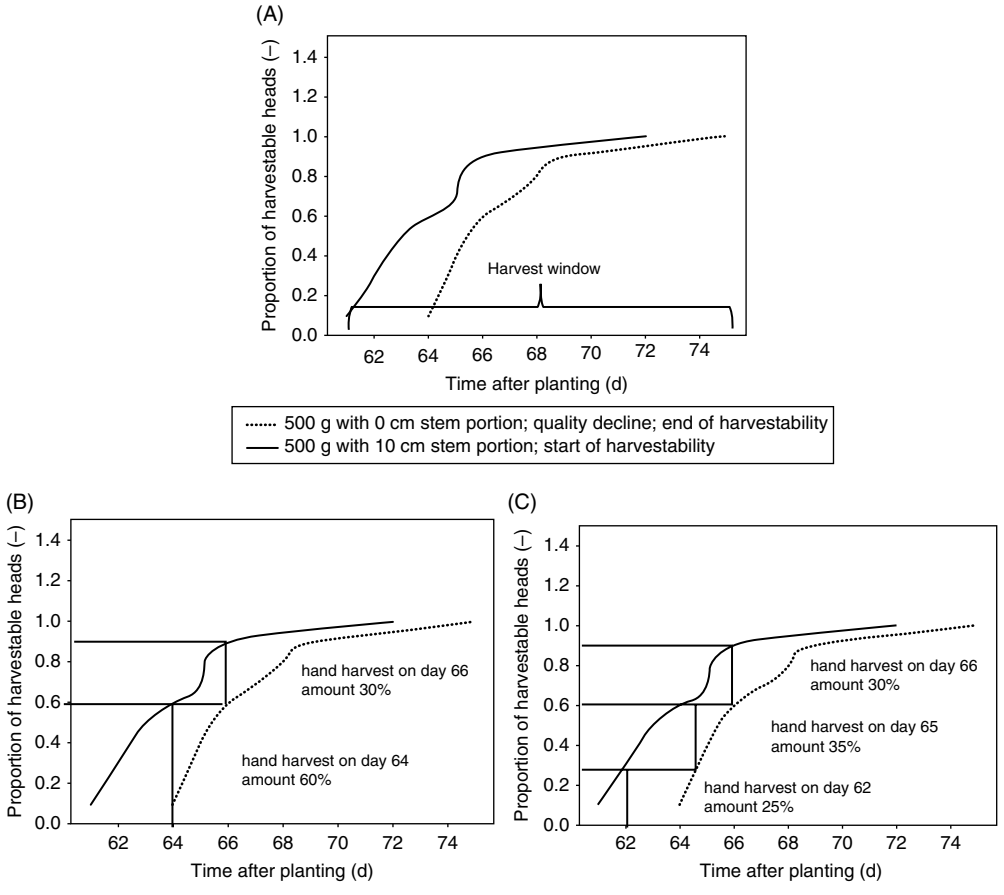

Fig. 15.10. Example of simulated proportion of harvestable heads against time after planting for weather data of Hannover, Germany, 2011 plating date (125 DOY). A: The harvest window is delimited by the first plants harvestable with 10 cm stem and the last plants harvestable without a stem portion; solid line describes the proportion of plants becoming harvestable with a 10 cm stem, dotted line describes the proportion of plants becoming unmarketable. B: Example for a scenario with two harvest dates, the vertical solid lines marking a date for one selective hand harvest corresponding to a certain amount of harvested heads (solid horizontal lines), in this example one selective hand harvest is carried out on day 64 after planting and another on day 66 after planting. C: Example for scenario analyses with three selective hand harvests on days 62, 65 and 66 after planting (Lindemann-Zutz *et al*., 2016b)

resulted in a 50% decrease in head yield as being 1.3, 0.1 and 0.3%, respectively (Hara and Sonoda, 1979b). Cabbage yields have frequently been shown to respond to increasing N rates up to broad optima of 300 to 500 kg/ha (Feleafel and Mirdad, 2013; Barrett *et al*., 2018b), but the detection of significant nitrate levels in the soil below the root zone at higher rates point to the environmental costs of those higher rates. The fate of N in the plant at maturity is also a factor in yield determination. Erley *et al*. (2010) found that high N retranslocation from leaves to heads increased yield of late cabbage cultivars, and reduced N residues in the soil at harvest.

Other factors which have been found to reduce cabbage plant growth and head weights are cultivation practices that damage the root system (White, 1977), and growth in compacted soils of a "no-till" soil management system (Knavel and Herron, 1981). Presumably, any stress factor which results in poor plant growth would also bring about reduced final head size. Factors

that might be included are drought, waterlogging, insect and disease incidence, and shading and nutrient stress by weeds.

The determinants of head size in cabbage are similar to those in lettuce, indicating that the beginning of head formation is the most important stage (Wurr and Fellows, 1991; see Chapter 14). Cervenski *et al.* (2012) found that the area of unfolded leaves when the head starts to form determined the quantity of assimilates available for translocation to the head.

Optimum temperatures for growth and yield of cabbage are listed by Maynard and Hochmuth (2007) as 16–18°C, but the crop is frequently grown in locations with average temperatures which exceed this range. For instance, cultivars have been successfully developed for growth in the lowland tropics, where cool season temperatures average 26°C (Kutty *et al.*, 2017). At these higher temperatures, the length of the growing season is reduced, and the yield is lowered (Knott and Hanna, 1947; Sundstrom and Story, 1984; McKeown *et al.*, 2010).

The estimate of yield potential of cabbage plantings can be done accurately close to harvest, when plant numbers per unit area, head diameter and an assumption of head density can be combined (Kleinhenz, 2003). A more rapid method employs a handheld instrument that measures reflectance from the canopy of red and near-infrared light to determine canopy completeness (Govaerts *et al.*, 2007). Ji *et al.* (2017) found that these values, termed the normalized difference vegetation index (NDVI) taken at 110 days after planting, was closely correlated to cabbage yield at 130 days. The correlation was further improved by adjustment for the cumulative temperature at the time of measurement (Ji *et al.*, 2017). Yield estimation on a larger scale using remote sensing would seem a logical next step.

Attempts to predict the maturity date of cabbage by calculation of heat sums have had limited success. Trials in the Florida winter season over 12 years showed a 4–16% variation in harvest time for individual cultivars, whether calculated on the basis of heat units (limits 0 and 25°C), or the time to maturity (Strandberg and White, 1979). To improve the accuracy of predictions it may be necessary to include some measure of solar radiation in the calculation, as in cabbage crops grown in Florida in winter (Barrett *et al.*, 2018a). Given the slow accumulation of heat units in fall-grown cabbage crops, a wide variation of time to harvest can be expected (Isenberg *et al.*, 1975). The fact that cabbage growth rate is proportional to the space available per plant further complicates maturity predictions (Stoffella and Fleming, 1990), but provides a practical indicator of impending harvest time. Growers know that the crop is ready for harvest when the heads at the end of the row start to split.

Brussels sprouts

Brussels sprouts is a crop best adapted to cool growing conditions (optimum temperatures of 16 to 18°, range 4 to 24°C), and a growth duration of from 100 to 120 days (Maynard and Hochmuth, 2007). A tolerance of frost allows the crop to be grown well into fall and early winter (Fisher and Milbourn, 1974).

The growth pattern of the Brussels sprout crop is sequential, with most of the leaf, stem and root weight forming before sprout growth starts (Abuzeid and Wilcockson, 1989; Booij *et al.*, 1997). In the principal production areas of Europe, rapid bud growth begins in mid-September, and can last through December (Abuzeid and Wilcockson, 1989 and Fig. 15.11). By the time of sprout harvest, the proportion of biomass going into sprouts varies from 25 to 40% (Fisher and Milbourn, 1974; Booij *et al.*, 1997). Sprout growth is from current photosynthesis, with little evidence that senescing leaves retranslocate dry matter to the active growing points (Wilcockson and Abuzeid, 1991). Studies with labeled carbon indicate that leaves nearest the developing buds provide photosynthate for bud growth.

It would seem logical that by increasing the number of plants per unit area, the number of sprouts formed and the yield of sprouts would also increase. In the range of densities of from 1 to 4 plants/m^2, several studies have shown little effect on yield (Fisher and Milbourn, 1974; Bortnes, 1990; Everaarts *et al.*, 1998). As plant population increases, average bud size at harvest declines (Everaarts and De Moel, 1998). Increasing the plant population increased yield only when the growing season was long enough so that the large number of buds could reach marketable size (Kirk, 1981). In general, maximizing the length

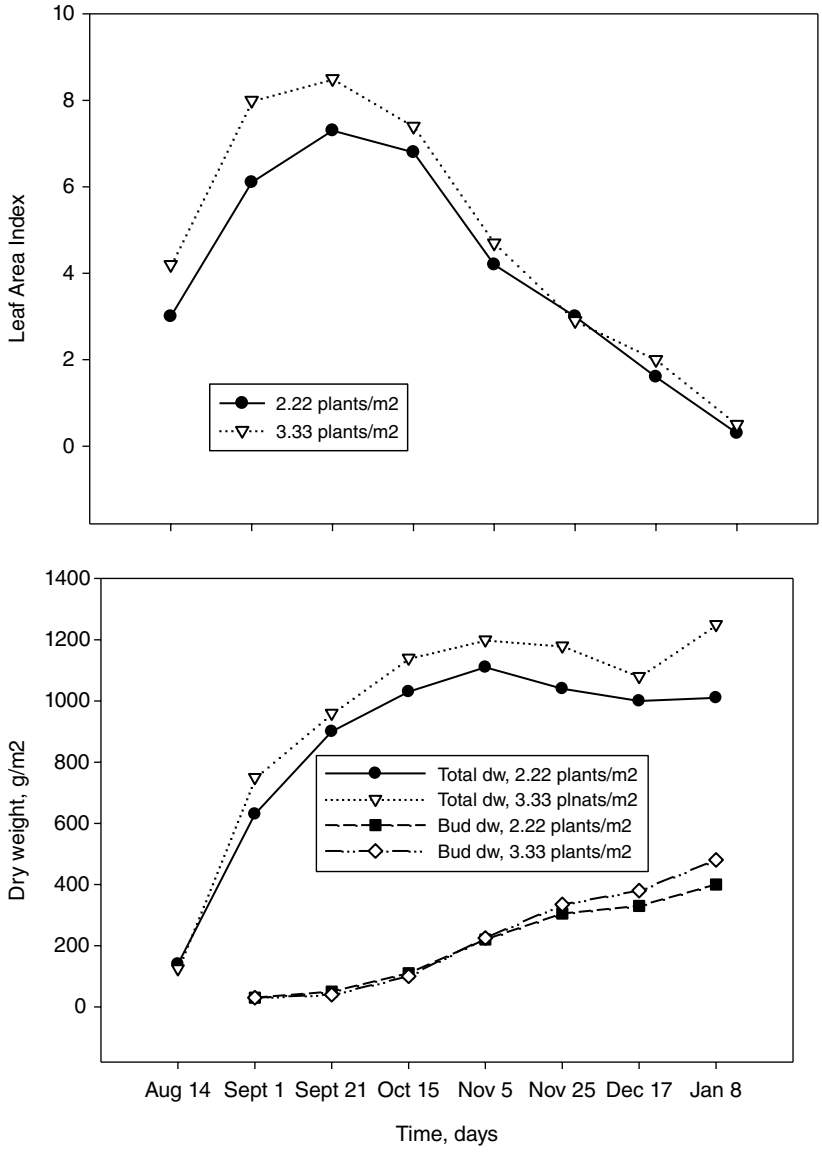

Fig. 15.11. Leaf area index and dry matter accumulation over time for Brussels sprouts grown at two densities in 1984 (Abuzeid and Wilcockson, 1989).

of the growing season has been a successful strategy to increase yields (Fisher, 1974; Abuzeid and Wilcockson, 1989; Everaarts and De Moel, 1998).

Another important yield-determining factor in Brussels sprouts is the size distribution of sprouts on the stem. At wide spacings, the lowest buttons grow first, and the upper buds develop later, leading to a marked increase in button size down the stem. At higher plant populations, the size uniformity is increased, permitting once-over harvesting of the crop (Fisher, 1974).

Increases in bud size uniformity have also been achieved by removal of the growing point of the plant, mentioned earlier. This labor-intensive procedure, termed "topping" or "stopping," is done either by hand, removing the apical bud, or using hammers or clubs to crush the plant apex. By destroying apical dominance, sprout growth

is accelerated, especially at the upper nodes of the plant, when done when the basal buds have 10 to 20 mm diameter. The time at which plants are stopped has a strong influence on how they react. If the apex is removed before sprouts have started to grow, upper axillary buds may grow into leafy shoots without a stimulation of bud enlargement. Stopping at progressively later dates allows more nodes to form on the plants, thus increasing yields (Cutcliffe, 1970). However, late stopping reduces the effect of the treatment on bud size stimulation (Metcalf, 1954; Jones, 1972; Fisher, 1974), and selection of cultivars that do not benefit from stopping has been a dominant selection goal. Physiological studies indicated that by removing the plant apex, assimilates that would otherwise have gone to stem and leaf growth were diverted into bud development, without negative effects on overall plant production (Jones, 1972).

The temperature requirements for optimum production of Brussels sprouts were outlined by Kronenberg (1975b). For late-maturing cultivars, he stipulated average monthly temperatures of 17–21°C during a three- to four-month period of early growth, followed by two months of 12°C during sprout development. To permit harvest of the crop during winter, he showed that climatic regions where early winter temperatures of 10 to 15°C rarely occurred, were favored for the crop (Kronenburg, 1975a). In Europe, these include the coastal areas of Holland, Belgium, and Spain, and parts of the UK, where the bulk of the crop is grown. Movement out of these areas of favorable temperature curtails the growing season and reduces yield.

Physiological Disorders

Blindness

Blindness is the loss of the growing point and has been variously reported to be associated with low temperatures (Salter, 1957), molybdenum deficiency, which can also cause misshapen leaves and leaves without blades (whiptail), insect damage, scorching by fertilizer and pesticide, daylength, light intensity, moisture stress, and seed quality, though the scientific evidence for some of these is patchy. The blindness disorder arose most often when winter crops of broccoli were started under temperatures near freezing especially under low light conditions (Wurr *et al.*, 1996). It could not be induced by cold alone, and there were marked differences among cultivars in the disorder (Forsyth *et al.*, 1999). More recently, Jonge *et al.* (2016), were able to induce blindness in germinating seeds of cabbage, kohlrabi and broccoli by letting the seeds imbibe water at 1 to 3°C for 14 days. They found that affected seedlings showed a marked down-regulation of genes involved in DNA replication and repair in the area where the apical meristem would form, thus halting its development (Fig. 15.12).

Hollow stem of broccoli and cauliflower

Broccoli and cauliflower show hollow areas in the stem, extending from below the head or curd to where the stem is normally cut. The disorder has been shown to be most severe where individual plants could grow rapidly such as: wide spacing, high nitrogen fertilizer levels, warm weather, and adequate moisture (Zink, 1968; Cutcliffe, 1972; Hipp, 1974; Scaife and Wurr, 1990) and to differ between cultivars (Cutcliffe, 1975b). The walls of these cavities are commonly not discolored, but there is potential for infection and spoilage after harvest. The disorder appears similar to the transverse cracking in celery caused by boron deficiency but boron concentration in cauliflower curds and young and older leaves was not correlated with hollow stem severity (Scaife and Wurr, 1990). Similarly, Everaarts and dePutter (2003) found no relation between the boron content of the stem at harvest, and the presence and extent of the disorder. Furthermore, micronutrient applications have not been successful in alleviating hollow stem (Hipp, 1974; Kojoi *et al.*, 2009).

Hipp (1974) found that the incidence of hollow stem showed an inverse curvilinear relationship with the duration of the broccoli growing period. Crops taking 110 days or more from sowing to maturity resulted in a low occurrence of the disorder while its incidence was much greater when plants grew more rapidly (Fig. 15.13).

Slowing the growth rate of individual plants by close spacing has most consistently reduced hollow stem incidence (Zink, 1968; Boersma *et al.*, 2009; Sanderson and Fillmore, 2010).

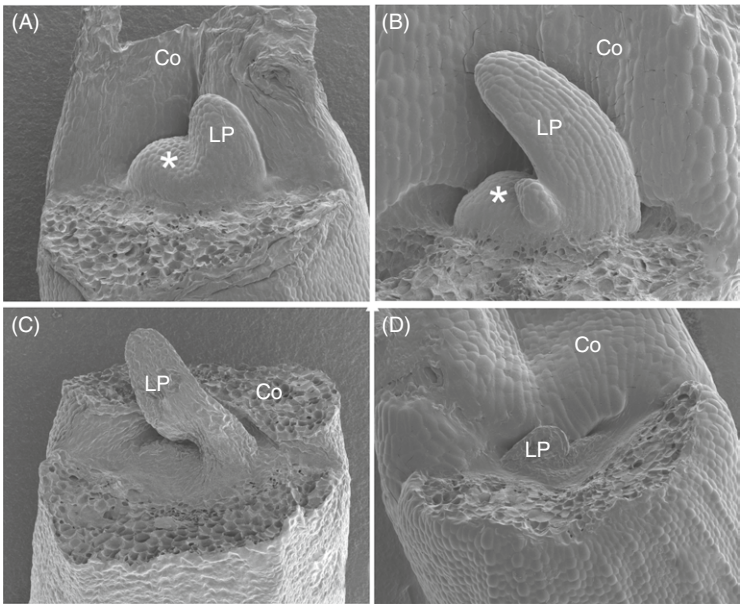

Fig. 15.12. Scanning electron microscopy picture of the phenotypic difference between a functional normal and arrested shoot apical meristem (SAM) in *B. oleracea* seedlings from cultivar "Stanton" F1. (A) Three-day old seedlings with a functional normal SAM and one leaf developing. (B) Four-day old seedling with one leaf emerging and functional normal SAM. (C) Three-day old seedling with an aberrant SAM area and one leaf-like structure. (D) Four-day old seedling with an aberrant SAM area. Asterisks indicate the SAM, Co, cotyledons; LP, leaf primordium (Jonge *et al*., 2016).

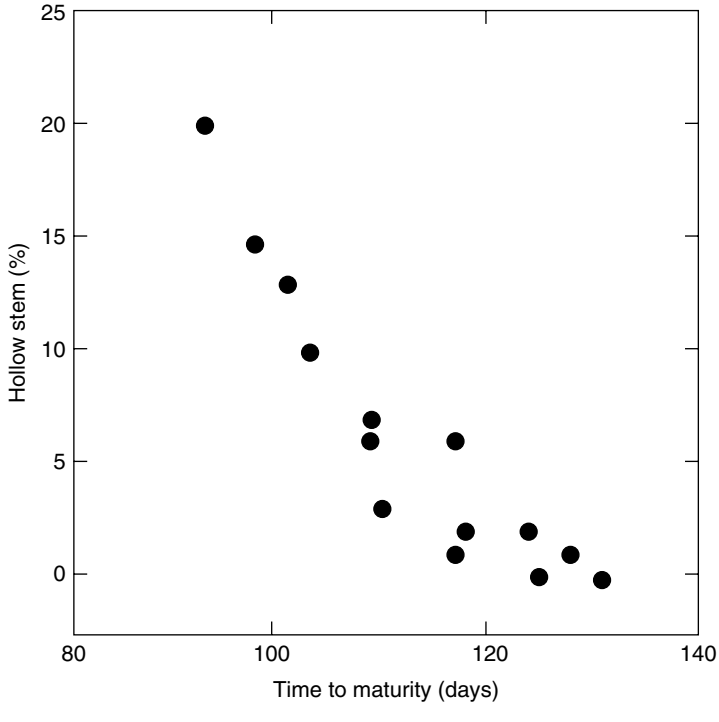

Fig. 15.13. Influence of maturity date (rate of growth) on incidence of hollow stem in broccoli (Hipp, 1974).

A close examination of broccoli stems showed that inner stem tissue started to fracture first in the pith just below the developing head, appearing as a mechanical fracture (Boersma *et al.*, 2013).

Tipburn and related calcium deficiency disorders

Physiological disorders related to a lack of calcium in the affected organ are common in the cole and other vegetable crops (reviewed by Olle and Bender, 2009). In the head- and bud-forming crops, the symptoms are similar to tipburn of lettuce, described in Chapter 14. In cabbage, tipburn appears as necrotic spots or areas in the margins of the rapidly expanding leaves in the middle part of the head (Walker *et al.*, 1961). In Brussels sprouts, similar symptoms occur in the sprouts, but the disorder has been termed internal browning (Millikan and Hanger, 1966). Severe calcium deficiency in this crop can also occur as a marginal necrosis of the rapidly expanding leaves near the shoot apex (Millikan and Hanger, 1966; Maynard and Barker, 1972). Tipburn of cauliflower also appears in the margins of immature leaves near the developing curd (Rosen, 1990) and the curd may be discolored if the dead leaf tissue touches it. Secondary pathogens may gain entry to the weakened areas and provide a source of curd infection. Culture of cauliflower in greenhouses and growth chambers can result in a more severe calcium deficiency disorder, the production of translucent or "glassy" curds (Krug *et al.*, 1972).The discoloration of broccoli heads termed "brown bead" may also be caused by Ca deficiency (Jenni *et al.*, 2001).

Work with cabbage has revealed one aspect not mentioned in Chapter 14: the importance of root growth. Since calcium is largely immobile in the phloem, its uptake is restricted to the young apical zone of the root (Marschner, 1995). Soil conditions which restrict root growth, such as anaerobic conditions, compaction and acid pH, can lead to lack of calcium uptake, and deficiency in the above-ground parts (Scaife and Clarkson, 1978). Accordingly, Walker *et al.* (1961) found increased cabbage tipburn in waterlogged areas of the field. Relative humidity is also important since a shortage of calcium is likely to occur when transpiration is limited. Wiebe *et al.* (1977) showed that in outer leaves, calcium content increased during the day in proportion to the transpiration, while in inner head leaves calcium was transported mainly at night when the head mass increased due to increasing plant water potential (Everaarts and Blom-Zandstra, 2001). High levels of competing cations in the soil, such as potassium, also can increase tipburn incidence (Cubeta *et al.*, 2000).

Foliar sprays of calcium-containing salts were generally ineffective in preventing the appearance of symptoms of these disorders in the cole crops (Walker *et al.*, 1961; Rosen, 1990). To have even slight ameliorative effects, applications had to be weekly or more frequent, and continued during the active growth period of the susceptible tissue (Millikan *et al.*, 1971). Realistically, a more practical means of avoiding the calcium deficiency disorders in cole crops is the use of resistant cultivars. Cultivar differences have been noted in cabbage tipburn (Walker *et al.*, 1961; Peck *et al.*, 1983), in cauliflower tipburn (Rosen, 1990) and with internal browning of Brussels sprouts (Millikan and Hanger, 1966). Some of these differences may be based on the rate of growth and earliness of particular cultivars, which expose them to the conditions causing the disorder (Rosen, 1990). By selecting cultivars under such conditions it may be possible to identify genotypes with a combination of characteristics giving lower susceptibility. The promise of selection for cultivars resistant to tipburn may be aided by the discovery of several QTLs associated with resistance in Chinese cabbage (Li *et al.*, 2010).

Buttoning of cauliflower and broccoli

The term buttoning refers to the production of small exposed curds of cauliflower or heads of broccoli. It commonly occurs in early cauliflower crops that are transplanted after being raised in greenhouses or cold frames (Skapski and Oyer, 1964; Birkenshaw *et al.*, 1982) and in broccoli where it has been described by several authors (Baggett and Mack, 1970; Miller *et al.*, 1985).

More commonly, buttoning occurs when relatively large transplants, which have been growing under favorable conditions in the greenhouse, are transplanted into cool field environments that lead to rapid curd induction (Skapski

and Oyer, 1964; Wiebe, 1981; Wurr and Fellows, 1984; Booij, 1990d). Although Skapski and Oyer produced the disorder on transplants that had already initiated curds prior to field planting, Booij and Wurr and Fellows demonstrated that plants which formed curds shortly after transplanting could also show buttoning. The key factor in buttoning appears to be the failure of the plants to produce a leaf area adequate to support a curd of marketable size. Several investigators have shown that there is a close linear relationship between the leaf area at harvest and the size of the curd (Fig. 15.14), (Skapski and Oyer, 1964; Wiebe, 1981; Wurr and Fellows, 1984; Booij, 1990d).

In general, buttoning is most likely to be caused by conditions restricting vegetative growth such as frost, bird damage, poor soil structure, shortage of nitrogen, high soil salinity, and low soil moisture (Wiebe, 1981). Transplanting shock on large plants has been shown to reduce the amount of leaf growth, in comparison to that made by direct-seeded plants. In addition, a plant which has initiated a curd will produce no more leaves, setting a finite limit on total leaf area. Direct competition between the growing curd and the young leaves may also limit leaf area development. Wiebe showed that removal of young curds resulted in a 37% increase in the area of expanding leaves.

To minimize the occurrence of buttoning, cauliflower and broccoli plants must make adequate vegetative growth in the field before curd or head initiation. In the case of the transplanted crop subjected to cool spring field conditions, use of younger, smaller transplants that are still juvenile when planted, is advisable. Use of cultivars with a longer juvenile period may also help (Baggett and Mack, 1970). Minimizing transplant shock and maximizing conditions for vegetative growth with optimum fertility, soil and water management, and the use of protective covers in the field can allow the plant to make adequate growth before curds begin to form. Direct-seeding also avoids the problem, but may be an unrealistic option for growers trying to produce an early crop.

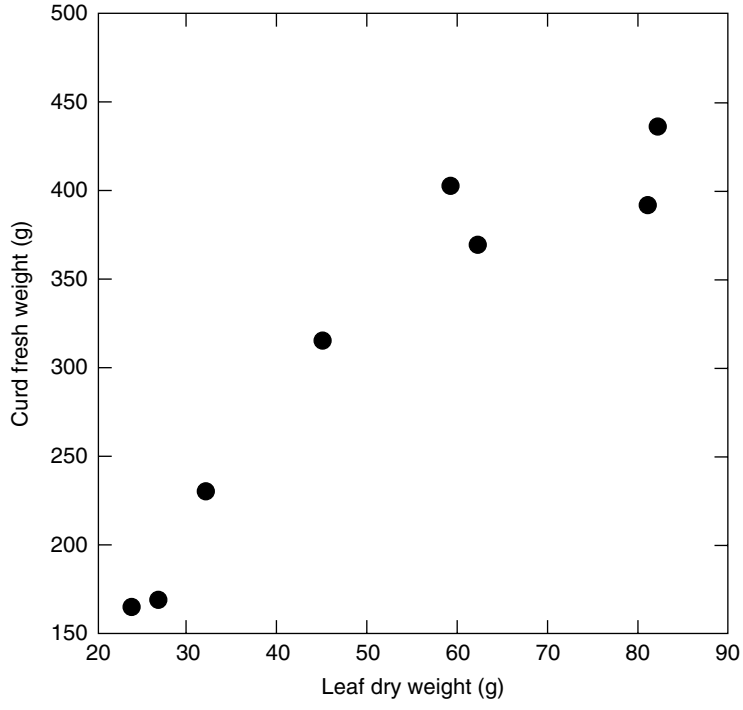

Fig. 15.14. The relationship of leaf dry weight at 92 days after sowing on curd weight at harvest for "All The Year Round—Lero" cauliflower grown in England. Plants of different sizes produced by transplanting from 36 to 64 days after sowing (Wurr and Fellows, 1984).

Since most crops that button are too physiologically advanced at transplanting, the problem could be minimized by developing simple models describing the growth and development of cauliflower and broccoli plants during plant raising. Such models could predict the level of apex development during plant raising and thus allow transplant growth to be manipulated to minimize the chance of buttoning occurring.

Cauliflower curd disorders

The temperatures allowing curd initiation to occur are cultivar specific and temperatures outside this range during early curd development may lead to the formation of structures that represent either a partial reversion to the vegetative state, or to more complete reproductive structure development.

If cauliflower is subjected to temperatures above the optimum for curd formation shortly after initiation, the curd develops bracts around individual florets, or in more extreme cases, the normally sessile white bracts on the peduncle of the curd elongate and become leaflike (Wiebe, 1972b, 1973a; Fujime and Okuda, 1996). For instance, Wiebe (1973a) induced bracting in "Aristokrat" cauliflower when subjecting the plant to three weeks of 25°C, beginning when the apical meristem had reached a diameter of 0.5 mm. The highest risk of induction of bracting was estimated to occur at a curd diameter of around 12 mm (Grevsen *et al.*, 2003). Bracting has also been induced in cauliflower by foliar sprays of the plant at the curd formation stage with ethephon (Booij, 1990b). Since ethephon is converted to ethylene, this implies that other stresses, which also cause the production of ethylene by cauliflower plants, may also induce bract formation. Gibberellins applied to the cauliflower apex at curd initiation can also increase bracting (Duclos and Bjorkman, 2015). The tendency to form bracts has been shown to be a simply-inherited genetic trait (Kop *et al.*, 2003).

When plants are exposed to relatively low temperatures after the beginning of curd formation, particularly after a period of high temperatures, development of individual flower buds in the curd is carried farther than in a normal curd, and the curd takes on a "ricey" appearance (Sadik, 1962; Wiebe, 1973b, 1975; Fujime and Okuda, 1996). The risk of induction of riciness was found to be highest at an apex diameter of around 0.35 mm (Grevsen *et al.*, 2003). Generally, the curd retains the normal white color, but the surface becomes grainy. In extreme cases, when cultivars adapted to the tropics are exposed to prolonged cool temperatures, the flower buds become green, and the curd resembles a broccoli head rather than the curd (Wiebe, 1975). However, curds are usually white until exposed to sunlight, when they turn cream and then yellow, becoming unmarketable. Some genotypes may even develop a pink or purple coloration in the curd (Crisp and Gray, 1979). Curds can also show blackish-brown discoloration caused by the direct effect of ultraviolet light (Nieuwhof, 1969).

The reaction of tropically adapted cauliflower cultivars to cool temperatures (Fig. 15.5) illustrates one extreme of the range of responses that is possible within cauliflower. The adaptation to high temperatures of these cultivars implies that they would rarely encounter heat sufficient to cause the development of leafy curds. At the other extreme, cauliflower cultivars that require a cold period for curd formation would form ricey curds only very infrequently. In general, one would expect to see a high frequency of curd disorders in cultivars being grown at or beyond the range of temperature conditions for which they were originally developed. Successful commercial cauliflower production is therefore

Fig. 15.15. Reaction of broccoli to heat stress. Left: "Marathon" uneven bead size and color when plants exposed to high temperature during head development; right: heat-tolerant selection. Plants grown during summer season in Geneva, NY, during which night temperatures exceeded 16°C during head development (Eastern Broccoli Project, https://blogs.cornell.edu/easternbroccoliproject/; with the kind cooperation of Dr. Thomas Bjőrkman).

frequently restricted to regions in which temperatures during the growing season are predictable and relatively consistent. This includes the maritime areas of Salinas Valley, California, Long Island, New York, and certain coastal areas of Holland, England, Wales, and Brittany in France.

Broccoli head disorders

Exposure of broccoli plants to high temperatures (35°C) during head initiation can lead to cessation of head development, or the formation of pale green beads of uneven size across the head (Fig. 15.15). Plants are sensitive to this disruptive effect at the beginning of head development, when the apical meristem is 1mm in diameter (Bjorkman and Pearson, 1998). There were considerable cultivar differences in susceptibility, with some lines producing only leaves when grown in South Carolina summer conditions, and others forming acceptable heads (Farnham and Bjorkman, 2011a). Breeding and selection appears to be producing results, and there are hopes that broccoli lines adapted to warm summer conditions will soon be available (https://blogs.cornell.edu/easternbroccoliproject/).

Concluding Remarks

The wide range of plant types within *Brassica oleracea* that have been developed for human consumption bring with them a bewildering range of optimum growing environments and hence requirements for cultural practice. Selection within each type has permitted the development of cultivars adapted to conditions ranging from the tropical to the cool temperate, and for seasons within these from summer to winter. New cultivars derived from crosses between cauliflower and broccoli, Brussels Sprouts, and kale, and broccoli and kale (e.g. Johnnyseeds.com) have erased these boundaries even more.

The variation in head/curd morphology in cauliflower and broccoli makes challenging the development of cultivars that have stable head and curd characteristics in spite of environmental variation. We have made little progress in selecting cultivars of cauliflower less sensitive to temperature variations, and that has restricted its successful cultivation to areas of predictably moderate temperatures. Perhaps by applying selection pressure for insensitivity to the physiological disorders that are induced by extremes of temperature, such as bracting or riceyness, a more widely adapted cultivar could be developed. The finding that bracting can be induced by ethephon foliar sprays (Booij, 1990b), and by application of gibberellin (Duclos and Bjorkman, 2015), may indicate ways in which such selection might be made. The search for broccoli lines that can be successfully grown in warmer climates has been more productive, and may soon allow this crop to be grown in areas with higher summer temperatures (https://blogs.cornell.edu/easternbroccoliproject/).

The recent focus on the production by the brassica vegetables of compounds which enhance their human nutrient content or increase pest repellent properties will likely increase consumption and use of these crops, and provide alternative uses (see Chapter 15B).

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15B Glucosinolates in *Brassica*

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The Function of Glucosinolates and Their Breakdown Products in Humans and Plants

The order Brassicales is characterized by a specific group of secondary plant metabolites, namely the glucosinolates, which are sulfur and nitrogen-containing compounds (Fahey *et al.*, 2001; Verkerk *et al.*, 2009; Clarke, 2010). These compounds are part of the plant's defense strategy against insects and plant pathogens (Hopkins *et al.*, 2009; Vig *et al.*, 2009; Ntalli and Caboni, 2017). For human nutrition, glucosinolates are of special interest due to their health-promoting properties in general and the cancer preventive properties of their breakdown products in particular (Traka and Mithen, 2009; Veeranki *et al.*, 2015). In case of injury to the plant cell, glucosinolates are enzymatically decomposed by the endogenous enzyme myrosinase and various degradation products, such as nitriles, epithionitriles, and/or isothiocyanates, are released (Hanschen *et al.*, 2017). Of note is that, while some glucosinolates, such as sinigrin and progoitrin, have bitter taste (Fenwick *et al.*, 1983), isothiocyanates are associated with the pungency of brassicaceous vegetables and have been shown to confer anti-cancerogenic (Lippmann *et al.*, 2014; Kumar *et al.*, 2015; Veeranki *et al.*, 2015; Palliyaguru *et al.*, 2018), anti-inflammatory (Bentley-Hewitt *et al.*, 2014; Herz *et al.*, 2016), and anti-diabetogenic effects (Waterman *et al.*, 2015; Guzmán-Pérez *et al.*, 2016). Isothiocyanates can interfere in all phases of cancer development. For example, they can protect the cell from tumor initiation as they increase detoxification of carcinogenic compounds. Moreover, isothiocyanates can inhibit the promotion and progression of mutated cells as they induce apoptosis, inhibit angiogenesis, and have anti-inflammatory and anti-proliferative effects (Hanschen *et al.*, 2014).

Recently, a review of human clinical trials targeting diverse health preventive effects of glucoraphanin/sulforaphane and gluconasturtiin/2-phenylethyl isothiocyanate was published (Palliyaguru *et al.*, 2018). For example, after a daily consumption of a broccoli sprout beverage containing 600 μmol of glucoraphanin and 40 μmol of sulforaphane for 12 weeks, excretion of the airborne pollutants benzene and acrolein, which are associated with lung cancer, was increased by 61% and 23%, respectively (Egner *et al.*, 2014). However, consumption of glucosinolate-rich plants may also have adverse effects, such as induction of goiter (Schöne *et al.*, 1997). Here, especially, the glucosinolate progoitrin and other 2-hydroxyalkenyl glucosinolates are linked to antinutritive effects because oxazolidine-2-thiones, potent goitrogenic compounds with a bitter taste, can be released from them (Astwood *et al.*, 1949; Tripathi and Mishra, 2007). By breeding rape varieties with low glucosinolate content and by processing the rape

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seed meal, the antinutritive properties of rape in animal nutrition were mainly overcome (Tripathi and Mishra, 2007). In a human trial with cooked Brussels sprouts, a vegetable rich in progoitrin, no goitrogenic effects were observed (McMillan *et al.*, 1986), which was attributed to cooking the vegetable. Nevertheless, the thiocyanate ion can interfere with iodine availability and thus is a goitrogenic compound. It can be formed from unstable isothiocyanates as well as by metabolism of nitriles or thiocyanates. However, by supplying enough iodine, goitrogenic effects from thiocyanate ions can be overcome (Schöne *et al.*, 1997; Tripathi and Mishra, 2007; Lee and Kwon, 2015). Thus, in a balanced human diet, goitrogenic effects from glucosinolates will be rare.

Exposure of *Brassica* plants to stress may shift growth from primary to secondary metabolism, and promote glucosinolate synthesis (Herms and Mattson, 1992). As glucosinolates play a key role in the plant's defense system they can be triggered by herbivore attack (Textor and Gershenzon, 2009; Rohr *et al.*, 2012). Of the different glucosinolate groups, the indole glucosinolates, in which a concentration increase by up to 20-fold can be induced, are favored against herbivores (Cipollini *et al.*, 2003; Textor and Gershenzon, 2009; Sotelo *et al.*, 2014;). For example, the specialist mealy cabbage aphid (*Brevicoryne brassicae*) was observed to increase in plants with low 3-indolylmethyl glucosinolate concentration, whereas the specialists cabbage white butterfly (*Pieris rapae*), cabbage whitefly (*Aleyrodes brassicae*), and crucifer flea beetle (*Phyllotreta cruciferae*) were not affected by the 3-indolylmethyl glucosinolate concentration in different plant genotypes (Santolamazza-Carbone *et al.*, 2014). Glucosinolate hydrolysis products, especially isothiocyanates, are toxic for many insects, bacteria, and fungi (Burow and Wittstock, 2009). For example, *Arabidopsis thaliana* accessions high in sinigrin and releasing high levels of allyl isothiocyanate had the highest antifungal effects on the fungal plant pathogen *Verticillium longisporum* (Witzel *et al.*, 2013). However, plants also contain specifier proteins which inhibit the formation of isothiocyanates in favor of the formation of other products. For example, after the specialist insect cabbage white butterfly fed on *Arabidopsis thaliana* plants, simpler nitriles were released, which could be linked to increased nitrile specifier protein activity (Burow *et al.*, 2009). As specialist insects are attracted by isothiocyanates released from the glucosinolates, it is believed that increased nitrile formation makes the plants less perceptible for ovipositing specialist insects or may attract enemies of the herbivores (Burow and Wittstock, 2009).

Genetic Factors Influencing Glucosinolates in *Brassica* Vegetables

In brassicaceous vegetables, plant organs consumed include inflorescences (e.g. broccoli and cauliflower), leaves (e.g. kale and pak choi), heads (e.g. white and red cabbage), as well as roots and bulbs (e.g. radish and turnip). Thus, varying glucosinolate profiles and concentrations can be expected. Higher concentrations of glucoiberin and glucoraphanin, the precursor of sulforaphane (4-[methylsulfinyl]butyl isothiocyanate), are favored in plant breeding programs due to their cancer preventive potential (Abercrombie *et al.*, 2005; Sarikamis *et al.*, 2006; Traka and Mithen, 2009). Glucoraphanin was up to 50-fold higher in broccoli compared to cauliflower (Schonhof *et al.*, 2004; Hanschen and Schreiner, 2017). Indian mustard had high concentrations of sinigrin, whereas in *Brassica rapa* species gluconapin, glucobrassicanapin, and progoitrin were predominant (Krumbein *et al.*, 2005). Important *Brassica* vegetables for human nutrition form epithionitriles and nitriles upon hydrolysis of glucosinolates while health-promoting isothiocyanates were often present in low concentrations only (Hanschen, 2016; Hanschen and Schreiner, 2017).

Pak choi cultivars show a great variability in total glucosinolate content and ratios of gluconapin and glucobrassicanapin, as well as progoitrin concentration (Wiesner *et al.*, 2013b). In a study with 11 turnip cultivars, early varieties had higher concentrations of gluconapin, whereas late cultivars had higher concentrations of glucobrassicanapin (Kim *et al.*, 2003). Moreover, among 16 different accessions of turnips, the glucosinolate profiles and those of their degradation products varied in qualitative and

quantitative terms between the different accessions (Klopsch *et al.*, 2017). In leaves, the health-promoting isothiocyanates were found in low concentrations, while in bulbous taproots of some accessions isothiocyanate concentrations were up to 556 times (on average 58 times) higher (Klopsch *et al.*, 2017). In a comparison of 25 kale cultivars, German and Italian cultivars with high concentrations of glucoraphanin could be differentiated from American varieties with high concentrations of progoitrin and gluconapin (Hahn *et al.*, 2016). Finally, 25 kale cultivars from Italy, Portugal, and Turkey presented higher variation in the aliphatic glucosinolate (nine-fold) compared to the indole glucosinolate (fivefold) concentrations (Ferioli *et al.*, 2013). Recently, not only differences in the glucosinolate profile were detected in sprouts and fully developed heads of different cultivars of broccoli, cauliflower, and cabbages, but also in the formation of their respective hydrolysis products (Hanschen and Schreiner, 2017). For example, the health promoting sulforaphane was especially high in broccoli sprouts (Egner *et al.*, 2014). Moreover, the cauliflower cultivar "Graffiti" was also reported to be a good source of isothiocyanates (Hanschen and Schreiner, 2017). In addition, by short heating or by acidifying the vegetables, the isothiocyanate release from these vegetables could be improved enormously (Hanschen *et al.*, 2017, 2018).

The morphology of most brassicaceous vegetables changes dramatically during development—a process that, along with aging, leads to different glucosinolate profiles and concentrations. Typically, inflorescences and seeds contain the highest glucosinolate concentrations. Both of them are valuable reproductive organs and need special protection from feeding insects. Sprouts represent an excellent source of glucosinolates that prevent the tissue from being damaged by insects and are of particular interest for human nutrition (Moreno *et al.*, 2006)—for example, radish sprouts (Hanlon and Barnes, 2011) or broccoli sprouts (Fernandes *et al.*, 2012; López-Cervantes *et al.*, 2013). In pak choi, either sprouts or adult leaves have high concentrations of glucosinolates, especially gluconapin (Wiesner *et al.*, 2013a; Heinze *et al.*, 2018) When studying the effect of ontogeny during the complete life cycle of broccoli, Rangkadilok *et al.* (2002) observed a continuous decrease in glucoraphanin. Sprouts of different *B. oleracea* crops had higher glucosinolate and hydrolysis product levels compared to the fully developed vegetable heads. During head ontogeny, glucobrassicin decreased with head development in broccoli and cauliflower as well as white, red, and savoy cabbages. Moreover, glucoiberin concentrations decreased from mini- to over-mature white cabbage heads. Among *B. oleracea* cultivars, isothiocyanates were usually highest in the mini-heads, while epithionitriles were richest in the over-mature ones (Hanschen and Schreiner, 2017).

Environmental Factors Affecting Glucosinolates in *Brassica* Vegetables

Due to climate change, temperature is expected to increase in the future, while solar radiation is expected to remain relatively unaffected. An increase in the daily mean temperature at moderate global radiation led to an increase of glucoiberin and glucoraphanin in broccoli, while glucobrassicin simultaneously decreased (Schonhof *et al.*, 2007b). The optimum temperature for indole glucosinolate formation was found to be 15°C, whereas alkenyl glucosinolates were produced in higher concentrations at higher temperatures (Schreiner *et al.*, 2002). In general, higher temperatures were associated with higher concentrations of glucosinolates (Pereira *et al.*, 2002; Radovich *et al.*, 2005b; Choi *et al.*, 2014). Among different *Brassica* species, the variation of the glucosinolate concentrations within one year was up to sixfold, whereas over several years this was only twofold (Aires *et al.*, 2011). For *B. rapa*, the highest concentrations of total and alkenyl glucosinolates were induced at medium and high photosynthetically active radiation (PAR) levels and these changes were mainly attributed to gluconapin and glucobrassicanapin (Fallovo *et al.*, 2011). Medium PAR levels increased the levels of alkenyl glucosinolates sinigrin and gluconapin in Indian mustard (Fallovo *et al.*, 2011). Further, Wallsgrove and Bennett (1995) reported that low PAR intensities reduced the glucosinolate concentration of rape leaves due to decreasing flavin-containing monooxygenases that catalyze the formation of the aliphatic aldoxime, a key regulator step in aliphatic

glucosinolate biosynthesis. In contrast, the major indole glucosinolate glucobrassicin was higher at low PAR levels in *B. rapa*—a trend that was also found in a study on broccoli (Schonhof *et al.*, 2007b). In contrast, exposure to narrow-banded blue and red light with low PAR intensities produced little effect on the glucosinolate concentrations of Brussels sprouts (Acharya *et al.*, 2016). However, in broccoli leaves, narrow-banded violet radiation was observed to decrease 4-hydroxyglucobrassicin concentration along with its derivative 4-methoxyglucobrassicin (Rechner *et al.*, 2016). Low, but ecologically-relevant, UV-B levels trigger distinct changes in the accumulation of glucosinolates (Schreiner *et al.*, 2016). For example, low UV-B exposure induced a distinct increase in glucoraphanin in broccoli sprouts (Mewis *et al.*, 2012).

Agronomic Factors Affecting Glucosinolates in *Brassica* Vegetables and Applications in Agriculture

Most studies report a negative correlation between N supply and glucosinolates. In different *Brassica* species, higher glucosinolate concentrations were found in response to conventional farming compared to organic farming approaches (Vicas *et al.*, 2013). These findings were also verified by Schonhof *et al.* (2007a) and Valverde *et al.* (2015) in broccoli. Vallejo and coworkers (2003) reported an increase of glucoraphanin during broccoli head development as well as indole glucosinolates after S fertilization. Fertilization with S was also observed to induce the biosynthesis of glucosinolates in *B. rapa* (De Pascale *et al.*, 2007; Li *et al.*, 2007). In broccoli, the highest concentrations of alkyl glucosinolates, such as glucoraphanin, and indole glucosinolates, such as glucobrassicin, were found at N to S fertilization ratios below 10:1 (Schonhof *et al.*, 2007a). In turnips, comparable results were found for gluconapin and glucobrassicanapin (Li *et al.*, 2007) and in cabbage (Rosen *et al.*, 2005) and kale (Groenbaek *et al.*, 2014, 2016), for glucobrassicin. Consequently, in *Brassica* species, N fertilization should be combined with S fertilization as the glucosinolates are S-containing compounds (Schreiner, 2005).

Water is a limited resource and current climate scenarios forecast longer periods of drought followed by heavy rainfalls (e.g. Dutta and Maity, 2018). Being part of the plant's defense, limited water supply results in an increase of glucosinolate concentrations in white cabbage (Radovich *et al.*, 2005a), broccoli (Paschold *et al.*, 2000), *B. rapa* (Zhang *et al.*, 2008), and Ethiopian kale (Schreiner *et al.*, 2009). However, under mild drought stress, glucosinolate concentrations were unchanged in rapeseed plants (Jensen *et al.*, 1996) and Ethiopian kale (Ngwene *et al.*, 2017) and decreased in broccoli (Robbins *et al.*, 2005). Tong *et al.* (2014) demonstrated that partial root zone drying also led to a drought-induced increase in glucosinolate concentrations in Indian mustard, particularly of the predominant sinigrin in leaves and roots, but without any biomass loss.

Intercropping systems are still actively used in African and Asian countries. When grown together with sesame plants, the concentration of glucoraphanin in broccoli was not affected by the neighboring plant species (Tong *et al.*, 2015). Further, Stavridou *et al.* (2012) determined in broccoli intercropped with lettuce that there was no change in the overall aliphatic glucosinolate concentration, but there was a reduction in neoglucobrassicin. In contrast, increased glucosinolate concentrations were reported for white cabbage intercropped with red clover (Björkman *et al.*, 2008) as well as for Indian mustard intercropped with lettuce (Stavridou *et al.*, 2012). Moreover, the concentration of 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin was increased by intercropping Ethiopian kale with garden huckleberry compared to sole cropping (Ngwene *et al.*, 2017). Therefore, the response of the glucosinolate metabolism to interspecific competition seems to depend on the *Brassica* species as well as on the neighboring plant species.

Glucosinolate-rich plants are not only of interest for human nutrition, but also as a soil amendment. Glucosinolate-rich plant material is mulched and incorporated into agricultural soils to suppress pathogens, nematodes, and weeds, a procedure named biofumigation (Halkier and Gershenzon, 2006). Biofumigation crops are usually selected for their high glucosinolate content as well as differing glucosinolate concentrations in different plant organs (Kirkegaard and

Sarwar, 1998). During the biofumigation process, glucosinolate-rich brassicaceaous plants or seed-meal, such as rapeseed, Indian mustard, black mustard, white mustard, or radish, are crushed and mixed into the soil and covered with plastic mulch to prevent the release of volatiles if applicable, after which glucosinolates hydrolyze and the volatile and toxic isothiocyanates are released (Bangarwa *et al.*, 2017; Ntalli and Caboni, 2017). The released isothiocyanate concentrations often range between 1 and 100 nmol per gram soil. The variation in isothiocyanate concentrations can be attributed to different extents of tissue disruption and soil parameters (Morra and Kirkegaard, 2002; Dungan *et al.*, 2003; Gabler *et al.*, 2006; Gimsing *et al.*, 2009; Hanschen *et al.*, 2015), as well as chemical degradation (Borek *et al.*, 1995; Dungan *et al.*, 2003; Hanschen *et al.*, 2015). Indian mustard, associated with its allyl isothiocyanate concentrations, was most effective in bioassay screenings on *Brassicaceae* cultivars (Neubauer *et al.*, 2014; Kruger *et al.*, 2015; Ríos *et al.*, 2016). In biofumigation field experiments using Indian mustard and radish, growth of apple rootstocks were positively affected in a site-dependent manner and the fungal community was more affected than soil bacterial composition (Yim *et al.*, 2016).

Conclusion

In brassicaceous vegetables, genetic factors are the most influential in affecting glucosinolate profiles and concentrations. However, environmental and agronomical factors also change the concentration of glucosinolates enormously with up to 556-fold for specific compounds (e.g. isothiocyanates). We now have a better understanding of the abiotic and biotic factors that affect the glucosinolates and this newly generated knowledge should be integrated into future breeding schemes for the production of brassicaceous vegetables that improve human health. Specifically, the health-beneficial effects of isothiocyanates should be augmented in specific plant organs and plant development stages for improved human nutrition.

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16 The Root Vegetables: Beet, Carrot, Parsnip, and Turnip

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Introduction to the Crops

The main aim of this chapter is to identify key physiological factors underlying the initiation and growth of the storage root of beet, carrot, parsnip, and turnip. Additionally, life processes including dormancy, vernalization requirement, flowering, the production of secondary metabolites, and various aspects of growth are examined for each of these crops. Substantial advancements in a number of areas have been recorded since the first edition of this volume was published 20 years ago. Application of molecular biological techniques to horticultural crops has allowed for the identification of a number of genes underlying key plant processes such as root elongation and growth, biosynthesis of secondary metabolites, and flowering.

Beets are members of the *Amaranthaceae*, carrots and parsnips are members of the *Apiaceae*, and turnips are members of the *Brassicaceae.* Despite belonging to different families, they have many features in common. Each of these four crops was domesticated from an annual plant species in an effort to produce a succulent vegetable. As such, they possess derived biennial life cycles. Each of these crops is colloquially known as a "root crop" but is actually comprised of a combination of root and hypocotyl tissue. All four of these species arise from supernumerary cambia; that is, they possess additional swollen and expanded xylem and phloem tissues beyond the primary xylem and phloem. It is this unique feature that allows for their girth and therefore their success as vegetables. Finally, they are economically important crops in many parts of the world. In 2014, 38,835,235 tons of carrots and turnips were produced worldwide (FAO, 2015). In North America alone, this figure was 1,744,131 tons (USDA, 2015 2016). These crops form an important part of the diet for many people, contributing unique colors, flavors, and to the caloric and nutrient needs of humans including, importantly, the provitamin A contributed by carrot. The pigments produced by these crops, including carotenoids from carrot and betalains from beet, have no functional significance in the root and were selected in those organs almost certainly for their vibrant colors and attractiveness to humans. The serendipitous finding that these pigments also possess healthful properties has been an unanticipated outcome of crop domestication.

Perhaps most strikingly, these four crops possess swollen storage organs that are a unique product of domestication. In their first year of vegetative growth, these crops form a rosette of leaves, a swollen hypocotyl/upper tap root, and fibrous roots. These four crops have a similar topology from a functional point of view (Benjamin *et al.*, 1997). Photosynthate produced in the leaves and destined for the fibrous roots and shoot apical meristem are translocated via the storage organ. Similarly, water and nutrients are

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translocated from the fibrous roots to the storage root and eventually to the leaves. In species without swollen storage organs, photosynthate, water, and nutrients are often transferred directly from root to stem. Thus, in these crops, some unique physiological responses exist as a function of the existence of the storage root. Abelenda and Prat (2013) comment that underground storage organs contain starch and soluble sugars, and supply carbon and energy for the growth of the new shoot. Thus, these organs are capable of providing calories to the human diet. They argue that hominids fed on these storage organs during periods when other foods were scarce, and that domestication of tuber-bearing species likely preceded that of cereals and legumes. Continuous selection for root and propagule size gave rise to modern storage root crops.

Beet

The genus *Beta* in the family Amaranthaceae contains several important biennial crop species, including sugarbeet, Swiss chard, mangel, and table beet. All are *Beta vulgaris*. Wild *Beta* species from the Mediterranean region, including *Beta maritima*, appear to possess roots with supernumerary cambia. It is therefore not surprising that selection for increased girth in swollen rooted forms would be successful. Wild *Beta* species include annual forms. The domesticated *Beta* crops are biennials but cultivated as annuals and used as leaf and root vegetables in both fresh market and processing applications, as well as for animal feed and as sources of sucrose and pigments for food coloring.

It appears that the first cultivated forms of this species were leaf vegetables, as described by the Romans, followed by swollen rooted forms that exhibited a biennial life cycle (Ford-Lloyd, 2005). Selection in more recent periods resulted in fodder types, with the most recent selections developed for sucrose exclusively. Today the modern sugarbeet is responsible for nearly 50% of world sugar. Interestingly, *Beta vulgaris* stores exclusively sucrose in its roots as a storage carbohydrate.

The red pigment characteristic of table beet and some Swiss chard cultivars is due to betalain pigments. Betalains are unique to the order Caryophyllales, and are synthesized from the amino acid tyrosine (Wang *et al.*, 2017), in contrast to the much more common anthocyanins, which are synthesized from phenylalanine. There is no apparent functional significance of betalains in beet root tissues, other than to provide color and interest for consumers. Betalains may have evolved in lineages that produced anthocyanins when an excess of tyrosine and a relaxation of the accumulation of tyrosine in plant cells occurred (Wang *et al.*, 2017).

Parsnip

The genus *Pastinaca* is a member of the Apiaceae family and is native to central Asia. Parsnip, *Pastinaca sativa,* was an important cultivated vegetable by Roman times. Today it is a minor but much appreciated root vegetable grown in many temperate regions of the world. Parsnips are white rooted. Closely related to carrot, the parsnip is not consumed as a fresh vegetable but is often cooked, roasted, fried, and prepared similarly to other root vegetables. They deliver a strong and rich flavor to cooked food.

Parsnip is cultivated in a manner similar to carrot, but typically is harvested much later than carrot. Parsnip eating quality improves with later harvest, and cold sweetens following frost. Many members of the Apiaceae family, including parsnip, produce furanocoumarins as defensive compounds. These compounds, when brought into contact with skin in the presence of sunlight, cause phytophotodermatitis, which can cause a severe chemical burn. Furanocoumarins in wild parsnip, which co-exists with cultivated parsnip in many temperate regions of the world, can cause even more severe skin injury.

Carrot

The genus *Daucus* is also from the family Apiaceae and native to central Asia. Wild carrot exists throughout many parts of the world, including Europe, western Asia, north Africa, and Afghanistan. The original domesticates of carrot were yellow and purple rooted (Banga, 1957a, 1957b, 1963), although Stolarczyk and Janick (2011) have suggested that orange rooted carrots may also have existed 1500 years before the present. Regardless, orange-rooted types appear in abundance in the 16th century in Europe.

Daucus carota has been bred for large, succulent roots with deep orange color, though recently there has been heightened consumer interest in other pigmented forms, including yellow, purple, white, and red. Carotenoids are abundant in carrot roots and synthesized in chromoplasts. The presence of carotenoids in root tissue is a function of domestication and does not appear in wild carrot. Fortuitously, carotenoids like beta (β-) carotene deliver provitamin A activity in the diet. Carrots also possess simple sugars and terpenoids, both of which are important components of flavor. The crop is produced for fresh market and processing uses. Processing of carrot includes canning, freezing, juice production, and baby carrot production. This latter market class has dramatically influenced the U.S. market, playing an important role in increasing carrot consumption.

Turnip

The genus *Brassica* contains a number of important crop species of worldwide importance as vegetables and oil seeds. *Brassica rapa*, which includes turnip, Chinese cabbage, bok choy, choy sum, tatsoi, and mustard, evolved from a process of whole genome triplication, which occurred approximately 9–15 million years ago (Cheng *et al.*, 2014). Following this triplication event, additional chromosome rearrangement occurred, resulting in the production of stable diploids. Turnip has two primary centers of diversity, in the Mediterranean and in Central Asia.

Turnip was an important crop plant for the Greeks, Romans, and Byzantines, and appears in a picture in the famous Dioscorides herbal in 512 (Reiner *et al.*, 1995). McNaughton (1995) argued that selection for swollen roots predated the selection of this species as an oilseed crop. The wild type turnip is a slender-rooted branching annual plant. The subspecies *oleifera*, which is known as turnip rape, has been grown as an oilseed crop and is morphologically quite similar to this wild plant.

One of the reasons turnip may have been important in temperate regions of Europe was that it is highly suitable to fermentation. Clearly, though, the crop was grown both as a vegetable and as an oilseed crop. DeCandolle (1824) describes how the turnip was grown for oil in places where other *Brassica* crops were not able to grow because it was too cold. Turnip production and consumption expanded considerably in Europe during the 15th–18th centuries. During this period, the stubble turnip was developed, which was planted in the fall in the stubble of rye, overwintered, and harvested the following season.

Uniqueness of the Storage Root

Storage roots are characterized by cell division and expansion throughout their development (Milford, 1973; Ting and Wren, 1980; Hole *et al.*, 1984). In contrast, the storage organs of other species, for example apple, are characterized by an initial phase predominated by cell division, followed by a phase of cell expansion with no cell division. The storage organs of all four of these root crops develop by the formation of supernumerary cambia. In all four of these species, there is no distinct limit to the distance down the tap root these cambia can extend. These features have implications for the chemical composition, maturity, and mechanical properties of the storage roots.

The storage of carbohydrate in these roots is made possible by storage parenchyma cells that are living, allowing the root to accumulate carbohydrate throughout its growth period. In all storage root crops like beet, carrot, parsnip, and turnip, the storage parenchyma cells are produced by secondary tissues, which arise from division of the vascular cambium. We might refer to these growth processes as anomalous growth, or growth that does not follow recognizable patterns occurring commonly in the majority of vascular plants. Growth in the girth of beet, carrot, parsnip, and turnip is due to meristematic activity in the vascular cambium, producing xylem on the inner side and phloem on the outer side of the stem (Robert *et al.*, 2011). Thus the growth of the economic product of these vegetable species is due to secondary growth produced by secondary xylem and phloem (Fig. 16.1). Robert *et al.* (2011) argue that growth via successive cambia provides an advantage to plants under water stressed conditions, and that species with successive cambia were common in drought or salt conditions. Successive or supernumerary cambia may provide clues as to specific ecological adaptations and geographical

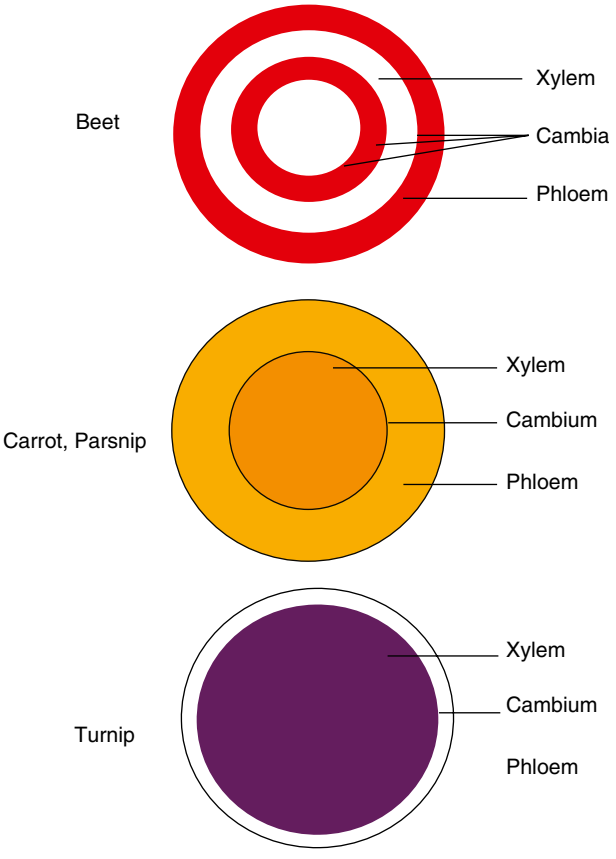

Fig. 16.1. Xylem, phloem, and vascular cambium in root cross sections of beet, carrot/parsnip, and turnip.

distributions of plants. In addition, they represent a distinct growth form. Many vegetables are derived from extensive growth in leaf, stem, petiole, ovary, and fruit tissues, whereas storage roots are characterized by this unique form of secondary growth in the root and hypocotyl.

More than 75 genera form successive cambial layers, each of which produce secondary xylem and phloem (Spicer and Groover, 2010). This trait is very common in the order Caryophyllales, which contains the Amaranthaceae family and table beet. The first cambial layer forms between the primary xylem and phloem and produces secondary xylem and phloem. The second cambial layer forms from the parenchyma tissue within the stem cortex and produces conjunctive tissue to the inside. Sometimes this cambial tissue produces secondary xylem and phloem directly, with new cambia coming from the oldest phloem tissue. Sometimes it functions as a master cambium, producing conjunctive tissue and new cambia on the inside. Regardless, there are repeating increments of secondary xylem and phloem in between conjunctive tissue. These tissues are of functional significance because the additional parenchyma can assist with both carbohydrate and water storage.

In carrot and parsnip, the vascular cambium produces a large amount of storage parenchyma in its secondary phloem tissue. This is where carbohydrate will be stored. This tissue also contains normal conducting cells typical of phloem, but they represent a small amount of this secondary phloem. Beet forms many concentric cambial layers, each of which produces xylem inwardly and phloem outwardly. Groups of lignified cells in the phloem give rise to the zoning or rings typical in a cross section of a beet root (Forbes and Watson, 1996). The more phloem tissue, the more carbohydrate that can be stored. The red rings consist of storage parenchyma, the

lighter colored rings are composed of xylem and phloem. The alternation of the lighter and darker bands shows how successive cambia represent a successful mechanism for the interspersing of vascular tissue (which input and remove stored sugars) between cylinders of storage tissue. In turnip, a small amount of secondary phloem is formed initially, but the secondary cambia in the xylem parenchyma divide to give rise to secondary phloem that are scattered through the xylem, and this helps distribute carbohydrate throughout the root (Forbes and Watson, 1996).

Many key transcriptional regulators of developmental processes associated with the shoot apical meristem are also expressed in the cambial zone during secondary growth (Schrader *et al.*, 2004; Chen and Tang, 2017). Groover (2005) hypothesized that genes involved in shoot apical meristem growth were co-opted during the evolution of cambia and secondary vascular growth. This represents a fertile area for future research in the evolution of storage root vegetables. Despite the potential source for the origin of these genes, there are fundamental differences between the radially organized cambial zone and the three-dimensional organization of the shoot apical meristem.

Function and Evolution of Storage Roots

Storage roots provide for storage of photosynthates during dormancy or during the interval between vegetative growth and reproductive growth. In addition, stored photosynthates are critical for the initiation of reproductive growth in the second season of biennial growth. During the first year of growth, the storage root may also serve other purposes. Benjamin *et al.* (1997) have hypothesized that the demand for photosynthates caused by the formation of a large storage organ may prevent the feedback inhibition of photosynthesis in beet (Thorne and Evans, 1964; Das Gupta, 1972), but that the prevention of feedback inhibition does not occur in carrots (Benjamin and Wren, 1978; Steingrover, 1981). The storage root may also act as a water reservoir, maintaining a constant water supply to the leaves (Olymbios, 1973).

Benjamin *et al.* (1997) described how the storage roots of carrot, beet, parsnip, and turnip are derived from the formation and activity of a cylindrical vascular cambium in the hypocotyl and tap root. This vascular cambium consists initially of separate strips formed by the divisions of cells between the primary xylem and the primary phloem. Subsequently, the strips join to encircle the primary xylem. This pattern of secondary root growth is typical for dicots. In carrots, the initiation of this secondary cambium occurs before the development of the foliage leaves (Esau, 1940). In beet, additional supernumerary cambia are also formed in quick succession at a very early developmental stage. Consequently, a beet very early in its development may contain all the cambial rings it will have at maturity, all of which will develop simultaneously (Hayward, 1938).

The signal for cambial development originates in shoot tissues. Benjamin *et al.* (1997) reviewed a number of studies that demonstrated how excising shoots prevents the development of the cambium, and replacing an excised shoot with capsules containing hormones, such as auxin or gibberellic acid, stimulated root growth. A widely cited pair of studies by Hole *et al.* (1984, 1987) examined shoot–root ratios of different carrot cultivars. They found that the smaller the ratio, the smaller the cross section of xylem parenchyma tissue. Their results pointed to the importance of shoot growth early in the development of carrot.

Hole *et al.* (1984) also determined that despite the presence of cambial tissues, high density plantings of radish and beet can result in stunted roots while carrots and parsnips seemed relatively unaffected. They proposed that once the vascular cambium forms, a stimulus is required for development of the storage root. They further suggested that increasing density was associated with a reduced stimulus for storage root formation.

Many studies have proposed that hormones play an important role in the elongation, formation, and thickening of storage roots. The largest contributor to our understanding of these phenomena has been sweet potato (*Ipomoea batatas*), which is a major crop in many parts of the world and therefore of tremendous economic and nutritional importance. Cytokinins and auxins appear to be important in the early stages of

storage root development, while cytokinins and ABA seem to be important in secondary thickening of these roots (Li *et al.*, 2015; Huang *et al.*, 2017). Additionally, many environmental factors appear to impact the growth of secondary roots, including light, photoperiod, water, temperature, and CO_2 (Loretan *et al.*, 1994; Hill *et al.*, 1996; Mortley *et al.*, 1996; Pardales *et al.* 1999; Kano and Ming, 2000; van Heerden and Laurie, 2008).

Wang *et al.* (2015) examined the role of gibberellins in carrot root growth and development. They found that gibberellin levels in the roots initially increased and then decreased, but these levels were lower than those in the petioles and leaves. They found that gibberellin level may play a vital role in carrot elongation and expansion, and that carrot growth and development may be influenced by gibberellin biosynthesis genes.

The cultivated sweet potato evolved from the wild tetraploid *I. trifida* and diploid *I. trifida*/*I. tabascana* species, which do not form storage roots (Ponniah *et al.*, 2017). In sweet potato, the differentiation of vascular cambia causes cell division and expansion of parenchyma cells for storage of starch granules, which leads to rapid bulking and starchy root formation. Theory suggests that storage root initiation is influenced by cambium propagation and lignification. Three class I *knotted*-like homeobox (*KNOX1*) genes—*SRF1*, *SRF5*, and *SRF6* modulate carbohydrate metabolism and cell division in sweet potato and play a primary role in storage root development (Ravi *et al.*, 2014).

Noh *et al.* (2013) examined the role of an expansin gene (*IbEXP1*) in the formation of the storage root in sweet potato. They hypothesized that *IbEXP1* plays a negative role in the formation of storage roots by suppressing the proliferation of metaxylem and cambium cells. This in turn inhibits the initial thickening growth of storage roots. Eviatar-Ribak *et al.* (2013) found that the cytokinin biosynthesis gene *LONELY GUY 1* changes axillary meristems to minitubers in tomato. Transcriptomic analysis revealed that the minitubers have an altered hormonal balance. Eviatar-Ribak *et al.* concluded that cytokinins may function as universal regulators of storage organ formation in plants.

The *MADS* box gene *IbMADS1* (*Ipomoea batatas MADS-box 1*) has been implicated in hormonal regulation of root formation in sweet potato (Ku *et al.*, 2008). The *MADS* box gene *SRD1* has also been implicated in thickening of storage roots. Noh *et al.* (2012; 2013) found that *SRD1* was involved in the auxin-mediated thickening of storage roots by affecting cell growth in the cambium and metaxlyem. Ravi *et al.* (2014) described that the genes *Ibkn1* and *Ibkn2* activate cytokinin biosynthesis, which are involved in storage root development. Transcription factors derived from *MADS* box genes *IbMADS1*, *IbMADS3*, *IbMADS4*, and *IbAGL17* induce a signal transduction pathway leading to storage root formation and development.

Ebener *et al.* (1993) examined a gene *DcPRP1* and found it was associated with the formation of storage roots in carrot, particularly in response to wounding. They found that *DcPRP1* is linked to secondary root growth and that it can be induced in carrot roots by auxin. Wang *et al.* (2015) found 87 hormone-related differentially expressed genes at different stages of carrot root growth. Despite these results, much more work must be conducted in carrot to identify key regulators of storage root growth; indeed this is one of the major research gaps for this critically important root crop. In contrast, thousands of genes have been identified as being associated with root development in radish and sweet potato. Li *et al.* (2015) studied microRNAs (miRNAs) to understand their potential influence in root development. They found that many miRNAs were differentially expressed during different developmental stages of tuberous roots in turnip. Two miRNAs, miR156 and miR172, that had been shown to be involved in tuberization of potatoes, were also important in tuberous root growth of turnip.

Gancheva *et al.* (2016) examined CLE peptides, which are a group of peptide phytohormones that play an important role in the regulation of meristems. They identified 18 CLE genes in radish and measured their expression in both radish and a related species that does not form storage roots, identifying large increases in expression for two of these genes during secondary root growth in radish but not in the related non-storage root forming species (*raphanistrum*). Two CLE peptides, CLE19 and CLE2, increased the number of xylem elements, and CEL41 induced the formation of extra cambium in secondary xylem. Auxin caused large increases in CLE19 expression levels and decreases of CLE41

expression. Thus, they concluded that CLE19 may play a critical role in the auxin-dependent process of xylem differentiation and that CLE41 stimulates cambium activity in radish.

Sucrose is also important in the development of storage roots. Sucrose synthase appears to regulate tuberous root development in radish. Tao *et al.* (2012) found that the gene sucrose phosphate synthase, which functions in sucrose metabolism, is highly expressed in sweet potato storage roots. Firon *et al.* (2013) found that phenylpropanoid pathway genes were downregulated during the formation of sweet potato storage roots, whereas starch metabolism genes, such as those encoding ADP-glucose pyrophosphorylase and starch synthase, are upregulated. *IbAGPase* that encodes AGPase for ADP-glucose production in starch biosynthesis, is also upregulated during the early period of storage root development. Genes encoding ADP-glucose pyrophosphorylase, granule-bound starch synthase, starch synthase, and phosphoglucomutase are up-regulated, whereas genes encoding pyruvate decarboxylase and lactate dehydrogenase are down-regulated during storage root development.

Transcription profiling of storage roots and fibrous roots also shows the down-regulation of lignin biosynthesis and upregulation of starch biosynthesis during storage root formation. Similarly, during storage root formation in cassava (*Manihot esculenta*), carbon flux moves from the phenylpropanoid pathway to carbohydrate metabolism and starch biosynthesis (Wang *et al.*, 2016). Taken together, recent studies indicate that storage root formation may involve the regulation of lignin and starch biosynthesis.

Important Secondary Metabolites

Carotenoids

Carotenoids in chloroplasts are a critical component of plant cells, serving as key pigments involved in light harvesting and protecting chlorophyll from photo-oxidative damage. They are synthesized in the isoprenoid pathway from geranyl geranyl pyrophosphate (Fig. 16.2). Carotenoids in fruits and flowers, which are present in chromoplasts, deliver colors that attract frugivores and thus confer important fitness traits. Carotenoids are also associated with plant defense and plant development, and of course they play an important role in human nutrition as provitamins, particularly with respect to vitamin A. Modern carrot breeding has focused on improving carotenoid concentration, which has had a positive effect on vitamin A nutrition (Simon, 1990).

Wild carrot is white rooted and the original domesticates of carrot were purple and yellow rooted (Banga, 1963). These have been cultivated since at least the 10th century in Iran and Persia. Stolarczyk and Janick (2011) presented evidence that orange carrot may have arisen more than 1500 years before present, but consistent evidence of orange rooted carrot did not appear until about the 16th century in Europe (Banga, 1957a and 1957b).

The two genes primarily responsible for the differences in carrot root color between orange, yellow, and white are known as *Y* and Y_2, (Buishand and Gabelman, 1979). However, research in the past decade has revealed that these genes are not directly involved in carotenoid biosynthesis. Iorizzo *et al.* (2016) identified *DCAR_032551* as a potential candidate for the *Y* gene, and Arango *et al.* (2014) found a carotene hydroxylase called *CYP97A3* that increased the amount of alpha carotene in carrot roots. However, neither of these genes is able to explain why carrot roots accumulate such high levels of carotenoids.

Transcriptional regulation is primarily responsible for carotenoid accumulation in flowers and fruits, but root tissue carotenoids have received less attention by researchers. Maass *et al.* (2009) overexpressed phytoene synthase (*PSY*), the first step in the carotenoid pathway, in both green and non-green tissues of transgenic *Arabidopsis* plants. They found similarities to the accumulation of carotenoids found in carrot roots. When overexpressing *PSY* in *Arabidopsis* roots, they found 100-fold increases in carotenoid concentration. This increase coincided with a decreased amount of xanthophylls compared to β-carotene. Interestingly, carotenoids were found deposited in crystalline formation. Overexpression of *PSY* in carrot displayed similar increases in crystalline carotenoids. They concluded that *PSY* expression plays a major, rate-limiting role in the transition from white to orange-colored carrots.

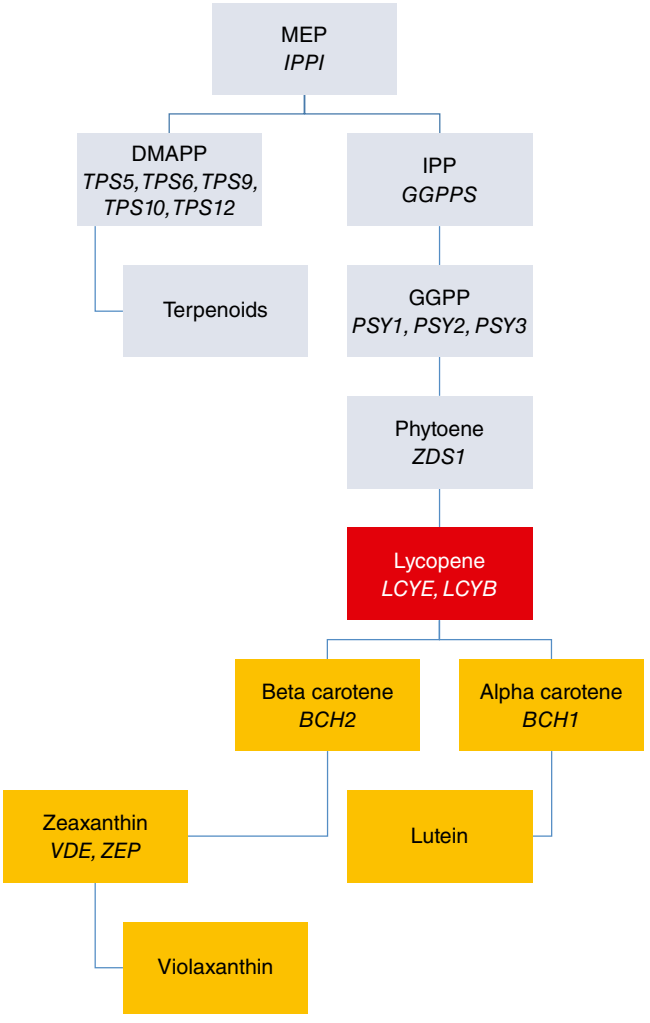

Fig. 16.2. Carotenoid biosynthetic pathway in carrot. Known enzymes are in italics below each substrate, substrate and enzyme identifications are from Iorizzo *et al*. (2016). Redrawn from Iorizzo *et al*. (2016) A high quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. Nature Genetics. 48:657–666.

Clotault *et al*. (2008) studied the expression of eight genes encoding carotenoid biosynthesis enzymes during the development of white, yellow, orange, and red carrot roots: two distinct *PSY* loci coding for phytoene synthase (*PSY1* and *PSY2*), phytoene desaturase (*PDS*), z-carotene desaturase (*ZDS1* and *ZDS2*), lycopene e-cyclase (*LCYE*), lycopene b-cyclase (*LCYB1*), and zeaxanthin epoxidase (*ZEP*). They found that all eight genes were expressed in white rooted carrot cultivars despite the absence of carotenoids. In contrast to the expression pattern of carotenoid biosynthetic genes during fruit development, the expression of carotenogenic genes began during the early stages of plant development and then progressively increased for most of these genes during root development as the total carotenoid level increased in colored carrots.

Recently, Ellison *et al*. (2018) used an association analysis to show that a genomic region containing the gene *Or* was responsible for the differences in carotenoid accumulation among 700 wild and cultivated carrot accessions. This finding suggests that *Or* may be the reason why carrot roots are able to accumulate such large amounts of carotenoids. The *Or* gene forms chromoplasts, which are involved in the biosynthesis and storage of carotenoids, as well as post-transcriptionally regulated *PSY*. Mutations in the *Or* gene are associated with high levels of carotenoids in *Arabidopsis*, cauliflower, and sweet potato (Yuan *et al*., 2015). Perhaps most interestingly,

it seems that the major determinants of carotenoids in carrot roots, *Y, Y2,* and *Or,* are genes that are not part of the carotenoid pathway per se. Thus, in a scenario that seems to repeat itself in many examples of natural and artificial selection, the regulation of existing genes may have provided the key variants upon which the modern carrot crop was selected.

Carotene content increases with age (Nilsson, 1987; Evers, 1989). Carotene concentration is higher at low soil water contents, at temperatures between 10 and 20°C (Bradley *et al.*, 1967) and with applications of mineral fertilizers (Evers, 1989). Excessive irrigation, however, may reduce β-carotene production (Nortjé and Henrico, 1986) and the ratio of β:α carotene is decreased by high soil temperatures before harvest (Bradley and Dyck, 1968). Total carotene content is greater at higher plant densities when comparing roots of similar weight (Banga and De Bruyn, 1964). Carotenoid content increases with increasing nitrogen fertilization but doesn't increase beyond nitrogen rates of approximately 180 kg/ha (Hochmuth *et al.*, 1999). These workers found that nitrogen fertilization rates that maximized yield maximized carotenoid concentration.

Betalains

Betalains are a class of pigments that exist in plants in the order Caryophyllales with functions including pollinator and frugivore attraction. These pigments include red-violet betacyanins and yellow betaxanthins (Clement *et al.*, 1992). Betalains are important food colorants. They are produced from the amino acid tyrosine, which is converted to *L*-3,4-dihydroxyphenylalanine (DOPA) via hydroxylation. DOPA is then either converted to betalamic acid or to *L*-dopaquinone, which is further cyclized to *cyclo*-DOPA. Betalamic acid is then conjugated with *cyclo*-DOPA and various amines to produce betacyanins and betaxanthins, respectively (Fig. 16.3). Betalain production appears to have evolved from anthocyanin production as tyrosine accumulated in certain plant lineages. Lopez-Nieves *et al.* (2017) demonstrated that relaxed sensitivity to tyrosine inhibition may explain the evolution of betalain biosynthesis in the Caryophyllales. Interestingly, two families in that order have reverted to anthocyanin production as they lost their insensitivity to tyrosine accumulation.

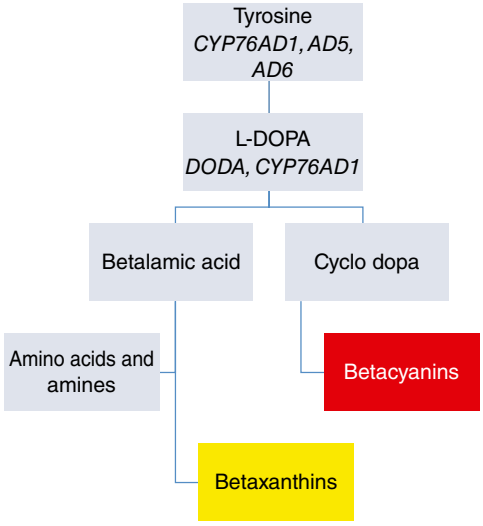

Fig. 16.3. Betalain biosynthetic pathway in beet. Known enzymes are in italics below each substrate, substrate and enzyme identifications are from Wang *et al*. (2017). Redrawn from Wang *et al*., (2017) Limited tyrosine utilization explains lower betalain contents in yellow than red table beet genotypes. J. Agric. Food Chem. 65:4305–4213.

Recent studies showed that betalains have antioxidant activities (Kanner *et al.*, 2001; Tesoriere *et al.*, 2009) and a number of beneficial health-related properties, such as induction of Phase II enzymes that offer protection against certain cancers (Lee *et al.*, 2005).

The presence of dominant alleles at two linked loci, *R* and *Y* (Goldman and Austin, 2000) are responsible for the production of betalain pigment in beet. Red-pigmented roots are observed only in the presence of dominant alleles at both the *R* and *Y* loci, while white rooted plants have recessive alleles at the *Y* locus, and yellow rooted plants have the genotype *rrY-*. Thus, the *R* locus controls the red vs. yellow pigmentation, while the dominant *Y* locus is required for overall betalain production in table beet. Recently, the *R* locus was identified as a novel cytochrome P450, *CYP76AD1*, which catalyzes both tyrosine hydroxylation and L-DOPA oxidation to provide the *cyclo*-DOPA moiety required for all betacyanin synthesis (Hatlestad *et al.*, 2012), although *CYP76AD5* and *CYP76AD6* also catalyze the

first tyrosine hydroxylation step redundantly with *CYP76AD1*. The *Y* locus encodes the *MYB1* transcription factor controlling the expression of DODA and *CYP76AD1* (Hatlestad *et al*., 2015).

Wang *et al*. (2017) conducted a comparative analysis of betalains and their precursor, tyrosine, in beet cultivars producing different kinds and levels of betalain pigments. Consistent with previous studies, red beets generally have five to eightfold higher betalain concentrations than yellow beets. Interestingly, the levels of tyrosine negatively correlated with those of betalains and were higher in yellow than red beets, suggesting that yellow beets are not efficiently utilizing tyrosine for pigment production. Similar results were obtained in red and yellow Swiss chard. Sugar beet, which accumulates very little betalain pigments, showed low levels of tyrosine, suggesting that the supply of tyrosine is reduced in beet cultivars producing high levels of sugars. Based on the observations of Wang *et al*. (2017), increased production of the tyrosine precursor will be required to further increase betacyanin production in red beets, whereas better utilization of the accumulated tyrosine can further improve betaxanthin production in yellow beets. Betalain concentration in table beet has been increased through various selection approaches (Gaertner and Goldman, 2005). Recurrent half-sib family recurrent selection has been effective at modifying betalain pigment levels in table beet.

Anthocyanins

Anthocyanins are responsible for red, pink, purple, and blue colors in many plant species (Grotewold, 2006) and have been identified with a number of potentially health-promoting properties (Landi *et al*., 2015). Despite their promise, dietary anthocyanins have varying levels of bioavailability depending on the nature of the sugar conjugate associated with each molecule, the degree of acylation, and the phenolic aglycone (Cavagnaro *et al*., 2014). Anthocyanins accumulate in the vacuoles of a wide range of cells and tissues and are present in carrot roots, petioles, leaves, and flowers, as well as in turnip roots and petioles.

The anthocyanins are synthesized by the phenyl-propanoid pathway and the amino acid phenylalanine is the substrate that is used (Fig. 16.4). A number of different types of anthocyanins exist, varying in the degree of hydroxylation and number of substituted groups.

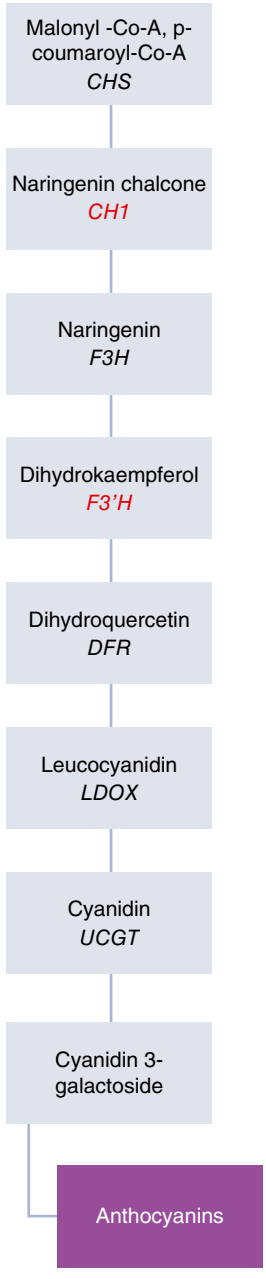

Fig. 16.4. Anthocyanin biosynthetic pathway in carrot. Known enzymes from carrot are in italics below each substrate in black, substrate and enzyme identifications are from Xu *et al*. (2014). Enzymes not identified in carrot but identified in other plants are below substrate in red. Redrawn from Xu *et al*. (2014) Transcript profiling of structural genes involved in cyanidin-based anthocyanin biosynthesis between purple and non-purple carrot (*Daucus carota* L.) cultivars reveals distinct patterns. BMC Plant Biology 14:262.

These include the anthocyanidins, pelargonidin, cyanidin peonidin, delphinidin, petunidin, and malvidin (Andersen and Jordheim, 2006).

Carrot can accumulate substantial quantities of anthocyanins in root, petiole, and leaf tissue, most of which is composed of cyanidin glycosides. Both the acylated and non-acylated forms exist in carrot, though the acylated forms are preferred for food coloring uses while the non-acylated forms seem to have higher levels of bioavailability. Levels of pigmentation in carrot roots differ greatly among carrot genotypes, with some possessing roots that are colored throughout and others with only a few cell layers containing pigmentation (Cavagnaro *et al.*, 2014).

A dominant gene, *P1,* has been associated with anthocyanin accumulation in carrot roots. Five structural genes associated with purple root pigmentation, CHS_1, DFR_1, *F3H*, $LDOX_2$, PAL_3 also seem to play a role in pigment accumulation. The gene *P2* has been found to control purple versus green pigmentation in carrot petioles, with suggested linkage to *P1*. Cavagnaro *et al.* (2014) found purple root pigmentation in carrot was associated with a two-gene model. Purple petiole pigmentation was conditioned by a single dominant gene that co-segregated with one of the genes conditioning root pigmentation. They also found 15 significant QTL for all anthocyanin pigments mapped to six chromosomes. Eight QTL with the largest phenotypic effects mapped to two regions of chromosome 3. Through comparative mapping with two other carrot populations segregating for purple pigmentation, they found that carrot root anthocyanin pigmentation is controlled by at least three genes, in contrast to monogenic control reported previously.

The presence of anthocyanins in root tissue raises the question of the location of their biosynthesis. Many of the enzymes associated with anthocyanin production are light dependent. Some flavonoids are mobile in plants, which suggests the possibility that these compounds are synthesized in plant organs that are exposed to light and then transported to roots. Studies suggest that anthocyanin precursors can be transported from shoots to roots (Buer *et al.*, 2007).

Glucosinolates

Glucosinolates are secondary metabolites that serve a defensive role in plants in the Brassicaceae family. There are three primary classes of glucosinolates, each of which is derived from a different set of amino acids, however the primary glucosinolates 4-Hydroxy-indol-3-ylmethyl glucosinolate, 4-Methoxy-indol-3-ylmethyl glucosinolate, 1-Hydroxy-indol-3-ylmethyl glucosinolate, and 1-Methoxy-indol-3-ylmethyl glucosinolate are made from tryptophan (Fig. 16.5).

Similar to the situation with plants in the Amaryllidaceae family and their cysteine-sulfoxide/allinase system, the glucosinolates are physically separated from the enzyme myrosinase in plant cells. When tissues are disrupted, myrosinase catalyzes changes in glucosinolates that result in isothiocyanates, thiocyanates, and nitriles. Isothiocyanates are toxic to many insect pests and pathogens. Isothiocyanates may also offer protective health benefits for humans who consume *Brassica* vegetables. A number of studies show that consumption of *Brassica* vegetables may reduce certain types of cancer, including those in the colon, stomach, and prostate. This protective effect seems to be associated with the electrophilic properties of isothiocyanates. One of the most widely studied isothiocyanates is sulforaphane, which has been shown to induce protective phase II enzymes.

Padilla *et al.* (2007) examined glucosinolate levels in leaves in a collection of 113 varieties of turnip greens from northwestern Spain grown at two sites. Sixteen glucosinolates were identified, including the aliphatic glucosinolates, gluconapin, and glucobrassicanapin. Other aliphatic glucosinolates, such as progoitrin, glucoalyssin, and gluconapoleiferin were also relatively abundant. Indolic and aromatic glucosinolate concentrations were low and showed few differences among varieties. Differences in total glucosinolate content, glucosinolate profile, and bitterness were found among varieties and between environments. Yang and Quiros (2010) identified and quantified 14 different glucosinolates present in the young leaves of 82 different varieties of *Brassica rapa*, including Chinese cabbage, broccoleto, Pak choi, turnip, sarson, and rapeseed. Gluconapin, glucobrassicanapin (aliphatic), neoglucobrassicin, glucobrassicin (indolic), and gluconaturtiin (aromatic) were the predominant glucinolates in most of the varieties surveyed. While most Chinese cabbage varieties contain lower amount of aliphatic than indolic glucosinolates, broccoleto, turnip, and rapeseed all have much higher aliphatic glucosinolate content than indolic glucosinolate content. Lee *et al.* (2013) evaluated glucosinolate concentration

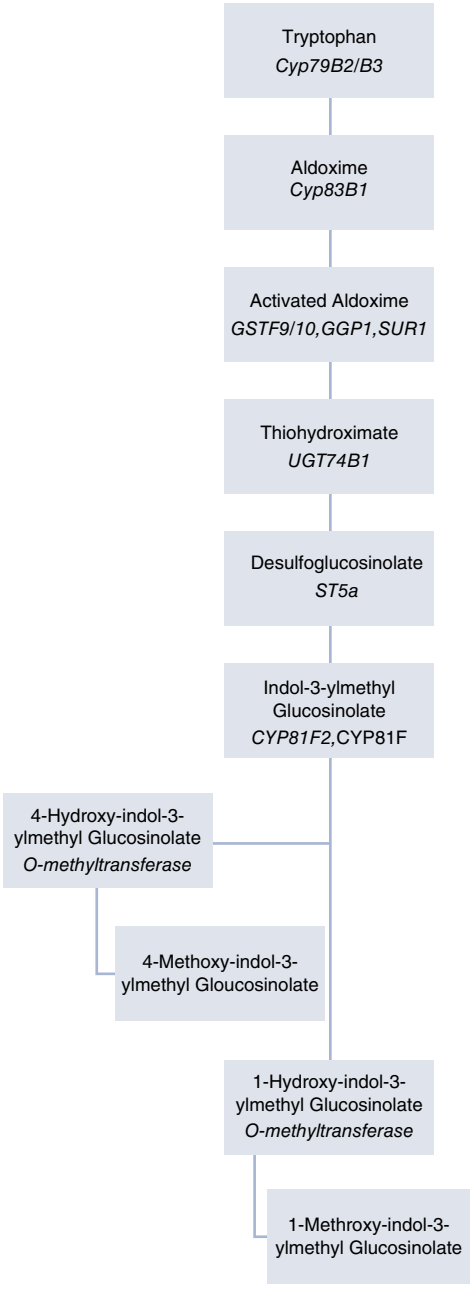

Fig. 16.5. Glucosinolate biosynthetic pathway in *Brassica rapa*. Known enzymes are in italics below each substrate, substrate and enzyme identifications are in Weisner *et al*. (2014). Redrawn from Weisner *et al*., (2014) Functional identification of genes responsible for the biosynthesis of 1-methoxy-indol-3-ylmethyl-glucosinolate in *Brassica rapa* ssp. BMC Plant Biology 14:124.

in 48 turnip accessions from different geographic origin. They used two different methods: high-performance liquid chromatography with a photodiode array detector, and liquid chromatography–mass spectrometry. These two methods were well correlated. Four clusters of accessions could be clearly distinguished based on glucosinolate content, and this clustering was quite different than the clustering based on leaf glucosinolates.

Glucosinolates are responsible for reducing the nutritional quality of oilseed *Brassica* crops, such as *Brassica rapa*. Nour-Eldein *et al*. (2017) reduced glucosinolate levels in *Brassica rapa* seeds through the introduction of loss-of-function mutations for the two primary glucosinolate transporters, which are responsible for bringing glucosinolates to the seeds. Glucosinolate type and concentration in *Brassica* species are also influenced by environmental conditions. Sulfur fertility has a strong influence on glucosinolate content in *Brassica* species and an increase in sulfur supply resulted in significant increases in glucosinolate content (Barickman *et al*., 2013).

Geosmin

The unique scent of soil after a rainstorm and the undesirable smell of musty water is due to a volatile terpene derivative called geosmin (Lu *et al*., 2003a, 2003b). Geosmin is one of the primary flavor determinants in table beet, delivering an earthy aroma and taste. Geosmin (*trans*-1,10-dimethyl-*trans*-9 decalol) is produced by *Streptomyces* spp. in the soil and in turn these bacteria produce the characteristic "earthy" smell of soil (Jiang *et al*., 2006), however up until recently it appeared that the geosmin in table beet was produced from this association. Recent evidence from Lu *et al*. (2003a, 2003b), Friedig and Goldman (2014), and Maher and Goldman (2017) strongly suggests that beet is responsible for endogenous production of geosmin through an as yet unknown pathway.

In bacteria, geosmin is formed during the Mg^{2+} dependent cyclization of farnesyl diphosphate (FPP or FDP). FPP is an intermediate in the HMG-CoA reductase pathway used as a building block in sterol and terpene biosynthesis (Miller and Allemann, 2012). The chemistry of

this reaction was discovered in *Streptomyces coelicolor* in which a bifunctional sesquiterpene synthase called geosmin synthase was found to possess two functionally different catalytic N and C domains (Jiang *et al.*, 2006). The N terminal half of geosmin synthase converts FPP into an 85:15 mix of germacradianol and germacrene D. This is followed by the conversion of germacradianol to geosmin by the C terminal half of geosmin synthase (Jiang *et al.*, 2007). Overall sequence homology in the general class of sesquiterpene synthases found in plants, insects, fungi, and bacteria is lacking, but these synthases share a general chemical outcome resulting in more than 300 sesquiterpene derivatives (Miller and Allemann, 2012). To date geosmin has been isolated from Gram-positive bacterial species, cyanobacteria, fungi, and liverworts (Jiang *et al.*, 2006). The functionality of geosmin in *Streptomyces* spp. is unknown.

Freidig and Goldman (2014) screened beet and related sub-species cultivars such as Swiss chard and mangel in three different environments (field, greenhouse in non-autoclaved soil, greenhouse in autoclaved soil) to evaluate the effect of cultivar and environment on geosmin level. There was no significant difference detected between years or between cultivars grown in autoclaved and non-autoclaved soil, suggesting geosmin content may not be primarily attributable to microbial associations. A significant interaction between cultivar and environment was found but generalizations could be made for high- or low-producing cultivars, demonstrating that geosmin levels were cultivar-specific. In other words, cultivars exhibited specific and repeatable geosmin signatures. "Bull's Blood," "Chioggia," and sugar beet exhibited the highest geosmin levels, while cultivars like "Blankoma" and "Touchstone Gold" were lowest. The high degree of consistency in cultivar performance across years, in ranking for geosmin levels across environments, and the lack of a significant difference between plants grown in autoclaved and non-autoclaved soil suggested characteristic levels of geosmin may be present in and produced endogenously by cultivars of table beet. These findings led to the establishment of breeding populations with defined geosmin levels that were then subjected to recurrent selection.

Maher and Goldman (2017) sought to determine if these table beet populations were responsive to selection for geosmin concentration. Four cycles of bidirectional half-sib recurrent selection for geosmin concentration were conducted over a period of four years, resulting in low (LGC) and high (HGC) geosmin populations. The LGC mean shifted from 17.3 μg geosmin kg^{-1} tissue in year 1 to 4.3 μg geosmin kg^{-1} tissue in year 3. The HGC mean shifted from 22.3 μg geosmin kg^{-1} tissue in year 1 to 33.8 μg geosmin kg^{-1} tissue in year 3. Highly significant positive and negative response to bi-directional recurrent selection for geosmin concentration is one indication that geosmin may be endogenously produced by beets.

Maher and Goldman (2018) grew beet plants in sterile tissue cultures and found that nearly all produced geosmins, and in some cases these levels were higher than those observed for greenhouse-grown plants. This finding added further evidence that beet is indeed capable of endogenous geosmin synthesis. They also sequenced 16s ribosomal RNA from different cultivars of tissue-culture grown beet plants and found no trace of geosmin producing prokaryotic organisms. Taken together, these findings from tissue culture, from diverse growing environments, and from four cycles of recurrent selection, contribute to the evidence that suggests beet produces geosmin endogenously and that it can be manipulated as a breeding trait. The functional significance of geosmin *in planta* remains unknown.

Furanocoumarins

Parsnip produces furanocoumarins as a defense against insect pests, along with other members of the families Apiaceae and Rutaceae. These compounds are produced via the phenylpropanoid and mevalonic acid pathways and consist of a furan ring and a coumarin. These compounds are associated with phytodermatitis and have significant toxicity for insects and humans. Their toxicity increases significantly in the presence of light, and they are particularly abundant in wild parsnip, which grows in many areas of the United States. Lombaert *et al.* (2001) quantified furanocoumarins in celery and parsnip samples. Nearly all parsnip samples and 77% of the celery samples tested in their study showed quantifiable levels of furanocoumarins.

Xanthotoxin and bergapten were the most commonly detected furanocoumarins in both celery and parsnip.

The parsnip webworm, *Depressaria pastinacella*, has some resistance to furanocoumarin toxins, though it also preferentially feeds on parthenocarpic fruits which are lower in furanocoumarins than non-parthenocarpic fruits. Cianfrogna *et al.* (2001) compared the chemical profile of parthenocarpic fruits to that of non-parthenocarpic fruit. Octyl butyrate, a known deterrent to webworms, was highly correlated with furanocoumarin content and differed significantly among normal and parthenocarpic fruit, suggesting that webworms may be able to avoid furanocoumarins by virtue of their behavioral response to octyl butyrate.

Flowering

In their domesticated form, beet, carrot, parsnip, and turnip are biennials, requiring a vernalization period in order to transition from vegetative to reproductive growth. In some crops there is a juvenile period during which plants are insensitive to vernalization stimuli. In carrots, depending on cultivar, this period may be absent or last until at least eight leaves are formed (Atherton *et al.*, 1990). The rates of flowering increase linearly with temperature from −1 to 5°C, and then decrease linearly with temperature from 7 to 16°C (Atherton *et al.*, 1990). The rate of growth of reproductive plants increased from 14 to 26°C, and plants matured sooner at the higher temperature, and consequently, the final size of plants was lower at the higher temperatures (Quagliotti, 1967). Short days (12 h) before or during vernalization induced earlier and more flowering, but long days (18 h) following vernalization stimulated flowering (Fisher, 1956; Atherton *et al.*, 1984). After vernalization, flowering can be suppressed by continuous low light. The apical meristem is the region of sensitivity to this inhibitory effect of low light (Fisher, 1956). This is also the site of synthesis of a zearalenone-like substance during chilling that may control vernalization (Meng *et al.*, 1986).

Sugarbeet and table beet are obligate long-day plants, and vernalization increases the competence of leaves to produce the floral stimulus that is transported to the shoot apex in response to inductive long days (Crosthwaite and Jenkins, 1993). Jaggard *et al.* (1983) reviewed the vernalization requirement of sugarbeet, which may be quite similar to that of table beet. The minimum and maximum temperature for sugarbeet vernalization was 0 and 15°C, respectively, with the fastest rate at 12°C. In extensive field experiments, flowering occurred in about 50% of plants when the air temperature was less than 12°C for 60 days. The receptive site for the vernalization stimulus is thought to be the shoot apical meristem, but it is still uncertain whether the young expanding leaves are receptive to vernalization, too (Crosthwaite and Jenkins, 1993). Turnips have a cold requirement of about three weeks at 5°C for flowering, but photoperiod has only a slight effect on flowering (van der Meer and van Dam, 1984). There is little information on the vernalization requirement for parsnips.

In brief, the gene *Flowering Locus C* (*FLC*) has been identified as the major floral regulator in many plants and its function is to repress flowering through its control of the gene *FT* (Sheldon *et al.* 2000), which is responsible for production of the flowering hormone florigen. FLC is a *MADS*-box transcription factor that represses the expression of *FT*. *CONSTANS* (*CO*) is a transcription factor that is antagonistic to *FLC* and activates *FT*. The *CO* protein is at high levels only when certain environmental conditions exist. The *CO* protein is stabilized in the light, so *FT* is only promoted when winter has passed and there is light in the evening (Hepworth and Dean, 2015). *CONSTANS* orthologs have been identified in many plant species. Chia *et al.* (2008) found a family of *CONSTANS-LIKE (COL)* genes. *BvCOL1* was identified as part of the photoperiod pathway in beet but is not an ortholog of *CO*.

FLC expression is reduced under cold temperatures and is silenced in proportion to the duration of the cold period. When warm conditions return, the silencing is maintained epigenetically, which allows other environmental stimuli to promote the switch to flowering. The epigenetic silencing is caused by post-translational modifications of the histones at the *FLC* locus. This form of epigenetic silencing is stable throughout development and is reset during embryogenesis by the *FRIGIDA* protein, so that each new generation will begin with high levels of *FLC* expression.

Beet has evolved a different strategy than *Arabidopsis* and cereals for regulation of vernalization. An ortholog of *FT* known as *BvFT2* is a floral activator that is expressed in biennial beets after vernalization and in annual beets. Surprisingly, the beet genome has two *FT* like genes, with antagonistic functions (Pin *et al.*, 2013). *BvFT1* is a repressor of flowering that is expressed prior to vernalization. This gene is repressed in annual beets by the gene *BTC1*, and *BTC1* activates *BvFT2*. *BTC1* is synonymous with the B locus, which provides early bolting without vernalization. Dally *et al.* (2014) identified *BvBBX19* as a regulator of flowering time that is responsible for the *B2* locus. *B2* is epistatic to *B*. These authors hypothesize that *BTC1* and *BvBBX19* complement each other and function similarly to *CONSTANS* to regulate *BvFT1* and *BvFT2*. Pin *et al.* (2012) demonstrated that BvBTC1 is essential for flowering and mediation of the response to long days and vernalization. They suggested that beet domestication included selection of a rare partial loss-of-function *BvBTC1* allele that imparts reduced sensitivity to photoperiod that is restored by vernalization, thus conferring bienniality (Fig. 16.6).

Alessandro and Galmarini (2007) identified an early-flowering trait of the carrot cultivar "Criolla INTA" as a dominant allele that mapped to the *vrn1* locus on chromosome 2. It is possible that this dominant allele is similar to the *B* allele in sugarbeet which causes flowering during vegetative growth without vernalization. Ou *et al.* (2017) studied the carrot transcriptome in an attempt to identify flowering time genes. They found homologs of many of the key genes involved in flowering including *CONSTANS* and *FLC* were differentially expressed between a wild species sensitive to vernalization and a cultivated accession that requires vernalization. They also found some overlap between genes involved in the carrot and beet vernalization response.

Flowering carrot plants develop multiple umbels in which seed is produced. The primary umbel is usually the largest contributor to overall seed yield. Merfield *et al.* (2008) examined the hypothesis that increasing density of flowering carrot plants would cause an increase in the contribution to seed yield by the primary umbels and thereby increase overall seed yield. They found that seed yield increased with increasing density. While the primary umbel produces the largest contribution to overall seed yield, it was also the source of the highest quality seed in terms of germination and vigor. They recommended densities greater than 20 plants per square meter for best results.

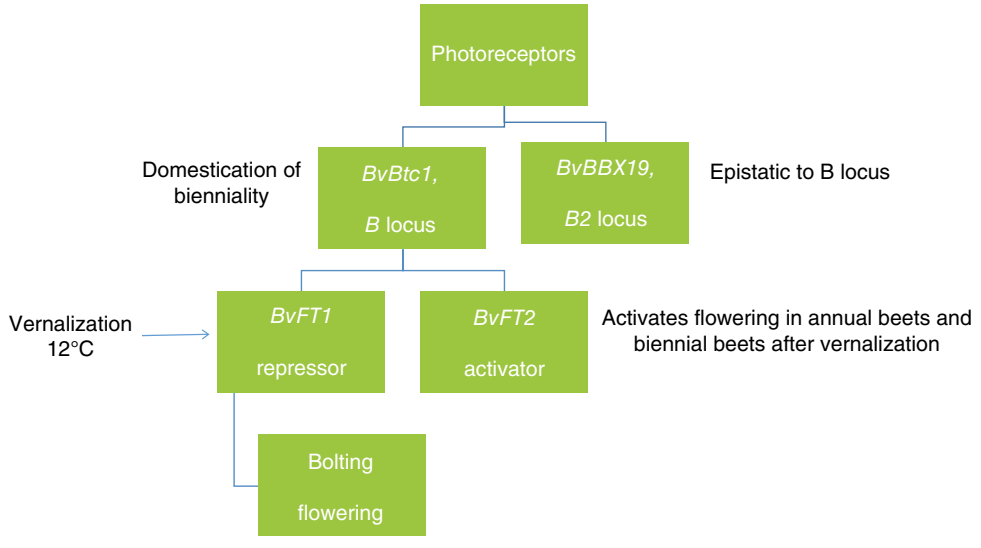

Fig. 16.6. Flowering pathway in *Beta vulgaris*. Loci are italicized.

Carbohydrate Storage

The high sucrose content of sugarbeet is the primary reason for its popularity as a crop. Benjamin *et al.* (1997) indicated the sucrose accumulation in sugarbeet storage roots is subject to rigorous internal plant controls, and is not simply a consequence of a super-abundance of photosynthates. Replacing the shoot system of sugarbeet plants with those of spinach beet by grafting, reduces storage root size but does not affect total sugar concentration in the storage roots (Thorne and Evans, 1964).

In contrast to sugarbeet, environmental factors do influence sugar accumulation in carrot (Barnes, 1936; Nilsson, 1987). This difference may be because carrots cannot adjust their average cell volume by the production of additional supernumerary cambia. Carrot cultivars differ substantially in their ability to accumulate sugars in the storage root and those that have a capacity for high sugar yield have higher net assimilation rates (Lester *et al.*, 1982).

Enzyme activity also determines the rate and species of sugar accumulation in storage roots (Hole, 1994). Sucrose synthase has been associated with sucrose storage in sugarbeet (Giaquinta, 1979). Sugar accumulation in carrots is controlled, at least in part by acid invertase activity, and the expression of this activity is dependent upon concentrations of growth regulators (Ricardo, 1976). Acid invertase is thought to prevent storage of sucrose by its inversion at the tonoplast, and alkaline invertase is thought to control the balance between reducing sugars and non-reducing sugars and thus control the availability of non-reducing sugars for storage (Hole and McKee, 1988). However, Hole and McKee found that there was no effect of cultivar or duration of growth on the activity of any of these three enzymes in carrot storage roots.

The predominant sugars in the storage root of carrots are sucrose, glucose, and fructose (Goris, 1969a,1969b; Alabran and Mabrouk, 1973), with small amounts of starch (Platenius, 1934; Goris, 1969b; Nilsson, 1987). The ability of carrot storage root parenchyma to store sucrose develops within a few days of its formation and the concentration of sucrose increases with time. In contrast, the concentration of the reducing sugars increases slightly or remains constant (Barnes, 1936; Steingrover, 1981; Lester *et al.*, 1982; Nilsson, 1987; Hole and McKee, 1988). However, the maximum sucrose concentration in carrots is substantially less than that of sugarbeet (Hole and McKee, 1988). Phan and Hsu (1973) estimated that the sugar content in carrots reached a maximum about three months after sowing, but roots continued to grow well after this biochemical maturity had been achieved. In contrast, Nilsson (1987) found that sucrose concentration increased with time up to 137 days and claimed that there is no well-defined stage of biochemical maturity for carrot.

Mitsui *et al.* (2015) conducted transcriptomic analysis that revealed that genes in carbohydrate metabolism were activated in thickening roots. In particular, sucrose synthase was correlated with the rate of root thickening. Sucrose synthase activity increases as roots develop. As the concentration of sucrose is lowered in the root with sucrose breakdown, a gradient allows the unloading of more sucrose from the phloem. As leaves provide sucrose into the phloem, a pressure difference between source and sink drives mass flow of water and nutrients into the phloem.

Turesson *et al.* (2014) studied taproots of sugarbeet and compared them to parsnip roots. They found no starch in beet roots, both in cultivated sugarbeet as well as in wild sea beet (*Beta maritima*), but found both starch and sugars in parsnip. While starch is stored in plastids known as amyloplasts, the storage of sucrose takes place in vacuoles. This storage requires continuous energy input to maintain the higher concentration in the vacuole compared to the cytosol. To maintain this difference, proton pumps are utilized. The activity of four starch biosynthetic enzymes, phosphoglucomutase, ADP-glucose pyrophosphorylase, starch synthase and starch branching enzyme, were similar in the roots of sugar beet and parsnip. The expression of starch accumulation genes was detected in both crops. Turesson *et al.* (2014) concluded that the exclusivity of sucrose as a storage carbohydrate in sugarbeet was enigmatic.

Conclusions and Opportunities

It is indeed striking how humans, beginning with wild annual plants without swollen roots, were able to domesticate and select large, succulent phloem and xylem tissues in carrot, beet, parsnip, and

turnip that turned them into important crops on which many people depend. The degree to which our food is dependent on the anomalous growth of these crops is a testament to the power of domestication and crop evolution. As these crops turned into biennial plants, they adopted traits associated with cultivated crops, including cold hardiness, vernalization requirements, the ability to store for lengthy periods during the postharvest period, increases in sugars and decreases in astringent secondary metabolites, and increases in nutritional value. While these crops do not benefit from an extremely large research community as do other staple crops, significant advances in our understanding of their form and function have taken place in the last two decades since the publication of this original volume. Yet much remains to be done.

In their chapter from 1997, Benjamin *et al.* noted that "there is scope to elucidate which genes are involved in cambial initiation by using molecular genetical techniques in combination with growth regulator or surgical methods that stimulate storage organ development." This statement was highly prescient, and in fact the intervening 20 years has revealed tremendous gains in our understanding of the hormonal signals and the genes that produce them, in the formation of crop storage roots. While some patterns have emerged, each crop species has its own idiosyncratic set of genes that underlie its response to environmental stimuli. Carrot, parsnip, turnip, and table beet have had relatively little effort in this area compared to sweet potato, which is a model for our understanding of the genetic and hormonal control of storage root formation. Overall, however, these 20 years have seen tremendous advancements in our understanding of how signals in the shoot, which were predicted by Benjamin *et al.*, manifest themselves in the plant and control the formation of the storage root. Elucidation of specific genes and hormones that control storage root formation in carrot, parsnip, turnip, and beet should be a promising area of research for the future.

Many of the important pathways controlling secondary metabolites such as carotenoids, anthocyanins, betalains, and glucosinolates have been dissected. To some extent, key genes involved in the biosynthesis of these compounds have been identified, but more work must be done to fully elucidate these pathways that produce the compounds that are responsible for nutrition, color, and flavor. Likewise, much progress has been made on understanding the genes that control flowering in *Beta vulgaris*, but much remains to be learned about genes influencing flowering in parsnip, carrot, and turnip. It is possible that the identification of rare alleles conferring sensitivity to daylength and temperature may have been responsible for the domestication of the biennial carrot, turnip, and parsnip, such as has been described in beet (Pin *et al.*, 2012).

Benjamin *et al.* (1997) also noted an additional research opportunity that has not yet been fulfilled. This concerned the dearth of information to explain how and why carrot roots adopt their shapes. Their observation is also true for beet, parsnip, and turnip. Benjamin *et al.* speculated in 1997 that the shape of the storage root might relate to the spatial pattern of cross-linking of hydroxyproline-rich glycoproteins of cell walls (Stafstrom and Staehelin, 1988). While this area still remains a mystery, it represents a highly fertile area for future research.

The shapes of carrot and beet roots vary tremendously with cultivar. Carrots are classified into market classes based on shape and end use. Luby *et al.* (2016) have clearly demonstrated this fact in a recent study that shows significant genetic overlap between market classes. They found that while market classes demonstrate some phenotypic and some genetic differences, they are largely a construct of breeders and are therefore malleable. Thus, there is a tremendous opportunity to gain an understanding of root shape and its control. Such work should help in our understanding of the genetic and hormonal control of root shape in other root vegetable crops.

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17 Allium Crops[1]

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Introduction

The genus *Allium* L. (Amaryllidaceae: Allieae) includes ~ 750–800 species (Block, 2010; Fritsch *et al.*, 2010; Li *et al.*, 2010; Merrow, 2012) of geophytes with bulbs or rhyzomes. Many *Allium*s were domesticated millennia ago (Fritsch and Friesen, 2002) as important foods (Peterson, 2000; Table 17.1), and today > 20 species, characterized by onion-garlic-like flavor due to cysteine sulfoxides, are consumed worldwide as condiments, green vegetables, and nutraceutical foods (van der Meer, 1997; Table 17.2).

All *Allium* spp bear multiple flower head-like (umbel) inflorescences. Each flower has six tepals, six anthers, and a superior ovary with three locules each containing two ovules. The capsule (fruit) shatters when ripe and sheds the firm irregular black seeds.

The common basic chromosome number is $x = 8$, but other numbers ($x = 7, 9, 10, 11$) and variation in ploidy occur (Traub, 1968; Friesen, 1992; Huang *et al.*, 1995; Xu *et al.*, 1998; Zhou *et al.*, 2007).

Centers of Origin and Evolution

The main center of *Allium* evolution stretches along the Irano-Turanian bio-geographical region (Fritsch and Friesen, 2002; Li *et al.*, 2010), where wild *Allium*s grow on dry mountain slopes, and stony open sites with scrubby vegetation (Hanelt, 1990; Fritsch and Friesen, 2002). Species of summer-dry regions may enter summer dormancy, while winter-dormant types are adapted to cold regions. Growth and development of some *Allium*s from arid regions is limited to the spring and early summer; others grow from fall to spring.

In the center of origin, intra- and interspecies cross-pollinations led to gene exchange, and both natural and human selections yielded the genotypic diversity known today (Table 17.2). Hence, onion and shallots, relatives of the rhizomatous *A. vavilovii* and *A. oschaninii* (subgenus *Cepa:* Hanelt, 1990; Havey, 1992b; Fritsch *et al.*, 2010; Van Raamsdonk *et al.*, 2003) resemble a true bulbous plant (Brewster 1990 a, 1994; De Mason 1990; Krontal *et al.* 1998). Selections of leek and kurrat from the bulbous *A. ampeloprasum* resulted in fleshy false-stems made of successive storage leaf sheaths (Poulsen, 1990; van der Meer and Hanelt, 1990; De Clercq and Van Bockstaele 2002). The clustered garlic bulb consists of one or more whorls of lateral buds transformed into cloves, while the biennial rakkyo develops rhizomes and false bulbs made of sheaths (Mann, 1952; De Mason, 1990; Toyama and Wakamiya, 1990).

[1]An updated revision of the chapter by James L. Brewster, 1997. Onions and Garlic. In: Wien, C. (ed.) *The Physiology of Vegetable Crops*. CABI, Wallingford, UK. pp. 581–619.

Table 17.1. Dry and green onions, shallot, garlic and leek[1, 2] productions in top 10 countries (2014).

Crop	Area (1000 ha)	Production (1000 ton)
Dry onions	5300	88,500
Onion, shallot, greens	220	4165
Garlic	1550	26,000
Leek and other alliums	135	2250

[1] http://www.fao.org/faostat/en/#data (October 2017).
[2] https://www.mapsofworld.com/world-top-ten/leek-producing-countries.html.

The wide spread of domesticated *Allium* outside the center of origin (Hanelt, 1990; Engeland, 1991) resulted in a diminution of new introgressions from close relatives, and millennia of genetic shifts and unbalanced selection pressures by growers worldwide resulted in local adaptation, qualitative variation, and loss of useful traits for crop improvement (Voss-Fels *et al.*, 2019).

An example of varietal adaptation is the qualitative differential response to long photoperiod (LP[1]), namely, differential response when the duration of daylight is in excess of a critical value. Other examples include vernalization requirements; earliness and lateness; lengthened leek shafts; fast and slow leaf growth in chives, in Chinese chives, and in Japanese bunching onion; sweetness and pungency; single-heart, medium and large bulbs which vary in shape, and in skin colors; high and low dry matter content for the processing and fresh markets, respectively. Shallots selected for maximum doubling and specific flavor were propagated vegetatively, yet recent releases of seed-propagated hybrids resulted in significant improvements in yield and quality (Brewster and Rabinowitch, 1990; Rabinowitch and Brewster, 1990; Rabinowitch and Currah, 2002).

Seedling Development

Allium seed germination depends mainly on moisture and temperature (Dalezkaya and Nikiforova 1984; Finch-Savage and Phelps, 1993; Specht and Keller, 1997; Kamenetsky and Gutterman, 2000). Hydration brings about enzyme activation, mitochondria biogenesis, cell division, cell differentiation (Brewster, 1997) and epigeal germination. Following radicle emergence, the crooked cotyledon elongates above the surface and turns green upon illumination (Jones and Mann 1963; De Mason 1990).

Onion seeds germinate over a wide range of temperatures. Quantified as the reciprocal of the time for 50% seed germination of the final total, germination rate increases linearly within the range of 5–25°C (Fig. 17.1). Its interception with the temperature axis defines the minimum temperature (T_{min}) for germination, and the reciprocal of the slope is the day-degrees (S) above T_{min} for 50% germination. T_{min} of 1.4−3.5°C prevails irrespective of seed-lot viability but the S value increased with seed deterioration (Dearman *et al.*, 1986; Ellis and Butcher, 1988; equation 1).

$$\text{Time } (d) \text{ to } 50\% \text{ emergence} = S/(T-T)_{\min} \quad (1)$$

Equation 1: Applied over a temperature range of 3–17°C; S was 219 °C·d and $T_{min.}$ (°C) was 1.4 °C.

This model predicts the time to emergence when the mean temperature is < 20°C, as common in spring and winter sowings in temperate and subtropical regions, respectively (Finch-Savage, 1987; Wheeler and Ellis, 1992). It was validated for both constant (Bierhuizen and Wagenvoort, 1974) and fluctuating temperatures within the range 3–21°C, and >70% onion seedlings emerged between 13 and 28°C (Wagenvoort and Bierhuizen, 1977).

T_{min} for onion seed germination is similar to that of many temperate zone vegetables, but the S value ranked fourth out of 31 species, and leek had higher values (Brewster, 1997). High S means that emergence is slow in reaching an equivalent post-emergence stage of development compared to crops with lower S values (Bierhuizen and Wagenvoort, 1974).

Seed genetics and quality, seed treatments, soil temperature, soil water potential, and sowing depth are the main factors determining the percentage and rate of seed emergence. Seed quality and priming (Basra *et al.*, 1994) affect

[1] Photoperiod long enough to induce and stimulate bulb formation.

Table 17.2. Taxonomy of main edible *Allium* species and a number of wild plants which may be considered for domestication (based on information published by van der Meer, 1997; Gregory *et al*., 1998; Fritsch and Friesen, 2002; Li *et al*., 2010).

Species name	Common (vernacular) name	Origin	Sub-genus	Section
Popular crops				
A. ampeloprasum L.	Leek, kurrat, great-headed garlic, pearl onion	Mediterranean	*Allium*	*Allium*
A. cepa L.	Bulb onion	Unknown, possibly Asia	*Cepa*	*Cepa*
A. cepa, Aggregatum group	Shallot, potato onion, aggregatum onion	Unknown, possibly Asia	*Cepa*	*Cepa*
A. chinense G. Don	Rakkyo	Asia	*Cepa*	*Sacculiferum*
A. fistulosum L.	Japanese bunching onion, Welsh onion	Unknown, possibly Asia	*Cepa*	*Cepa*
A. ramosum L. (=*A. odorum* L.)	Fragrant-flowered garlic, Chinese chive	Asia	*Butomissa*	*Butomissa*
A. sativum L.	Garlic	Unknown, possibly Central Asia	*Allium*	*Allium*
A. schoenoprasum L	Chives	Europe		
A. tuberosum Rott. ex Spreng.	Chinese chives, Nira (Japan)	China, Japan, Korea	*Butomissa*	*Butomissa*
New and potential crops, also used from the wild				
Species name	Common (vernacular) name	Origin	Sub-genus	Section
A. altaicum Pall.	Altai onion	Asia	*Cepa*	*Cepa*
A. canadense L.	Canada garlic, meadow leek	North America	*Amerallium*	*Amerallium*
A. galanthum Kar.et Kir	Snowdrop onion	Central Asia	*Cepa*	*Cepa*
A. macrostemon Bunge	Chinese or Japanese garlic	Asia	*Allium*	*Allium*
A. nutans L.	Siberian chives, blue chives	Asia	*Rhizirideum*	*Rhizirideum*
A. obliquum L.	Oblique onion	Asia	*Polyprason*	*Oreiprason*
A. oschaninii O. Fedtsch.	French shallot	Asia	*Cepa*	*Cepa*
A. pskemense B. Fedtsch.	Pskem onion	Central Asia	*Cepa*	*Cepa*
A. scorodoprasum L.	Rocambole, sand leek	Europe	*Allium*	*Allium*
A. senescens L.	German garlic, broadleaf chive	Europe, Asia	*Rhizirideum*	*Rhizirideum*
A. sphaerocephalon L.	Round-headed leek	Europe	*Allium*	*Allium*
A. tricoccum Solander	Ramps	North America	*Anguinum*	*Anguinum*
A. vavilovii M. Pop. et Vved.		Asia	*Cepa*	*Cepa*
A. victorialis L.	Long-rooted garlic	Asia	*Anguinum*	*Anguinum*
A. ursinum L.	Ramsons, wood garlic	Europe	*Amerallium*	*Arctoprasum*

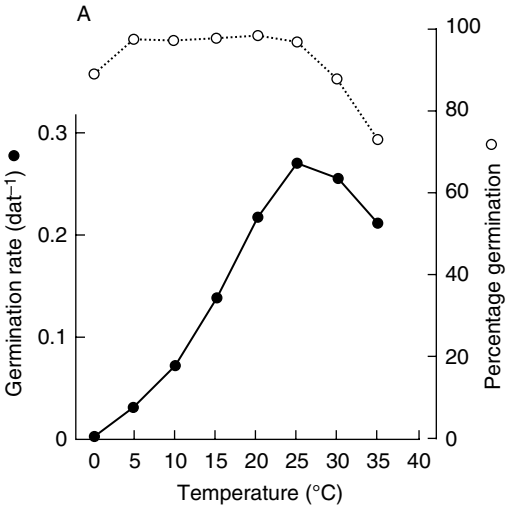

Fig. 17.1. Temperature effect on the rate and percentage of *in vitro* onion seed germination. Rates are reciprocals of the number of days for 50% of viable seeds to germinate (data: Harrington, 1962).

germination rates rather than subsequent growth either pre- or post-emergence (Ellis, 1989; Wheeler and Ellis, 1991). Usually, small advancements in seed emergence do not affect the yield of marketable bulbs; but increase earliness slightly (Brewster *et al.*, 1992).

Allium seedlings grow slowly, at about half the rate of spring cabbage or lettuce (Brewster, 1997). Leaf initiation rates of onion seedlings have a low T_{min} and both its relative growth rates (RGR) and relative leaf growth (RLGR) increase linearly over the range 6–20°C (Brewster 1977, 1979). Emergence speed, synchronization, and stand uniformity, facilitate subsequent agro-management—for example, fast emergence maximizes the growing time where pre-emergence herbicides remain effective, and larger seedlings are more resilient to post-emergence herbicides. Some benefits of good emergence are sporadic, as in rain-fed agriculture. Rapid emergence also reduces the risk of population loss by entrapment under a surface crust. Moreover, in onion, yield and grade depend among other things also on stand (Frappell, 1973) as within certain limits the higher the density the smaller the bulb size (Nasir *et al.*, 2007; Jilani *et al.*, 2009; Geremew *et al.*, 2010; Kahsay *et al.*, 2013; Khokhar, 2017). Hence, the importance of seed and seedbed qualities for the establishment of uniform and proper population density, and consequently on yield and bulb size.

Onion taproot is quickly replaced by a hair-lacking, shallow, sparse adventitious system (Bhat and Nye, 1974; Greenwood *et al.*, 1982; De Mason 1990; Thorup-Kristensen, 2006), and uptake of water and mineral nutrients thus occurs mainly in the soil's top layer (Goltz *et al.*, 1971). Some genotypes, however, especially hybrid cultivars, develop a deeper, vigorous, and denser system than many open-pollinated cultivars, with immediate impact on fertigation management (Rabinowitch, personal observation).

Onions require higher P and K levels for maximum yields than most crops (Greenwood *et al.*, 1980a, 1980b), and their recovery from N fertilizer deficiency is poor (Greenwood *et al.*, 1992). Coupled with the fact that nutrient requirement per unit root length is highest just after emergence (Brewster *et al.*, 1975) there is an immediate response to $(NH_4)_3PO_4$ "starter" solutions expressed in accelerated early growth (Brewster *et al.*, 1992).

Allium plants are more sensitive to water and nutrient deficiencies than many crops. In onions, a decline in leaf water potential and the associated turgor pressure are accompanied by a decrease in RLGR, in stomatal conductance and in plant growth (Millar *et al.*, 1971). Yet, they survive long water stress, and ultimately recover when water becomes available (Levy *et al.*, 1981). In these and other traits, like the inherently low RGR, they exhibit a typical stress-tolerance strategy (Grime, 1979).

Onions are more sensitive to salinity than most crops (Gale et al., 1967). Salt damage

(Wannamaker and Pike, 1987) and low soil water potential due to added nitrates (Hegarty, 1976) led to a low percentage and slow rates of emergence, and the effects on yield were greater in hot dry climates than under humid conditions (Magistad *et al.*, 1943).

Bulbing Physiology

*Allium*s have a determinate growth habit and their bulbs form a resting stage in their annual cycle. The plants' vegetative organs consist of a condensed stem (Figs 17.2, 17.3) with basal roots (Figs 17.2, 17.6) and alternate leaves (Fig. 17.4), each made of a tubular colorless sheath and a green blade whose shape differ with species (De Mason, 1990). Every leaf base envelops the sheath of the newer developing younger leaf, thus forming a cylindrical false stem (aka, neck) (Figs. 17.2–7). This pattern proceeds in onion, shallots and other alliums as long as the environmental cues (e.g., in onions: short photoperiod [SP], temperature, moisture, nutrients) enhance and support leaf production.

When bulbing of onions, shallots and garlic begins, visible leaves keep growing, while leaf bud initiation, elongation, and leaf emergence stop. These buds transform into bladeless true scales (prophylls) (Figs 17.2, 17.3); in garlic, axillary buds become axillary bulbs named cloves (Fig. 17.8).

Assimilation continues during bulbing even after lodging, and green blades export much of their soluble contents down to their respective sheaths, and through the condensed true stem to the prophylls (Davis and Jones, 1944). The receiving cells swell to accommodate the incoming flow of assimilates thus forming the respective false and true scales that serve as an energy source and supply the needs required during dormancy and rest to function properly, for cell divisions, differentiation, and elongation of

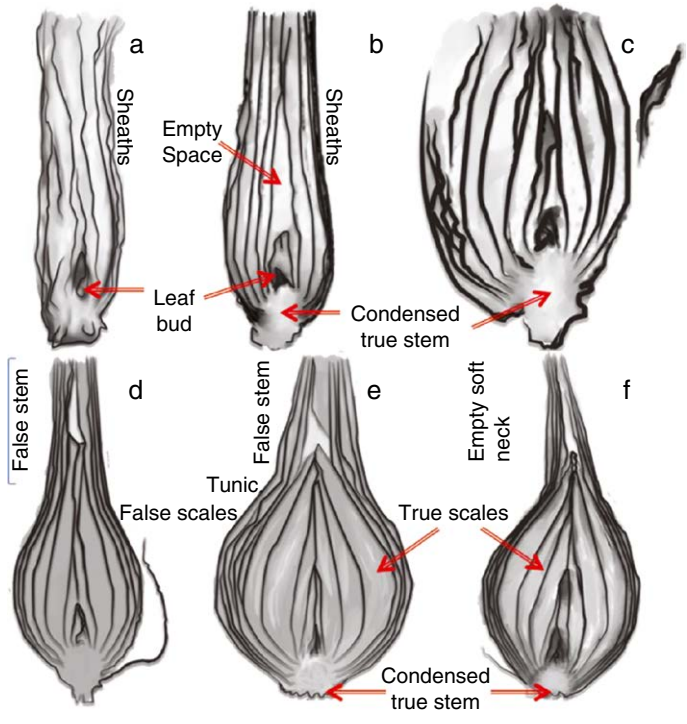

Fig. 17.2. Bulb onion development (adapted from Heath and Holdsworth, 1948 a) Green plant; b) Leaf sheaths and buds accumulate assimilates to become false and true scales. Neck becomes hollow; c–e) Basipetal transportation of assimilates continues, scale sizes increase; f) Outer sheaths dry out, scale sizes increase. False-stem loses turgor and mechanical strength before lodging (original drawings by Asaf Silner).

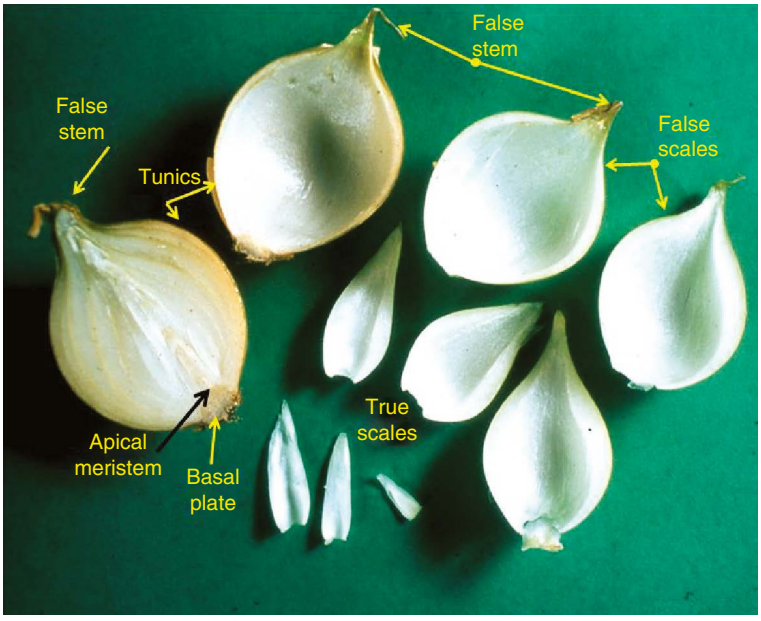

Fig. 17.3. Dissected onion bulb (photographed by J. Brewster. Legends and explanations by authors).

Fig. 17.4. Alternate leaf development of onions. Sheaths form a false stem ("neck").

Fig. 17.5. Soft neck.

developing buds, and for sprouting. A few of the oldest sheaths empty their contents to become protective skins (tunics) (Figs 17.2–5).

When new leaf buds stop growing, the center of the false stem (made of older leaf sheaths) becomes hollow, turgor is lost due to both root death (Figs 17.2, 17.5) and basipetal transport of assimilates. Hence, the neck's mechanical

Fig. 17.6. Falling tops. Winds from the nearby sea made them all lodge to the east.

Fig. 17.7. Leek (left) and spring onion.

strength weakens, and the green tops collapse (Figs 17.5–6).

Scale swelling leads to increase in "bulbing ratio" (*syn.* "bulbing rate"; BR; respective bulb-to-pseudostem's maximum and minimum diameters ratio; e.g., Brewster, 1990; Figs 17.5, 17.12). It is generally accepted that in onions, bulbing begins only when BR ≥ 2 and that BR = 8 represents maturation. Alternatively, "leaf ratio" (LR; leaf blade length : sheath length) of one or less (Fig. 17.9) was proposed as a more refined criterion for initial bulbing. Leaf initials with a decreased ratio of the blade (B) and sheath (S) B/S unity are termed "scales" (Heath and Hollies, 1965; Fig. 17.9).

BR and LR are not always linked, as scale initiation at 400 plants m^{-2} occurred approximately a fortnight earlier than at 25 plants m^{-2}, but BR readings indicated an almost simultaneous bulbing in both populations (Brewster, 1997). Additionally, under conditions favorable for carbohydrate accumulation, sheath thickening, and BR increase did not necessarily coincide (Wiles, 1994), and under N-deficiency BR ≥ 2 can occur without scale development. This stress effect occurs also under SP, as swollen sheaths do not turn into mature bulbs (Brewster and Butler, 1989).

Because of simplicity, rapidity, and non-destructive readings, the BR is the criterion of choice for bulbing characterization, commonly applied to large-scale studies, breeding-work, production, and other extensive research.

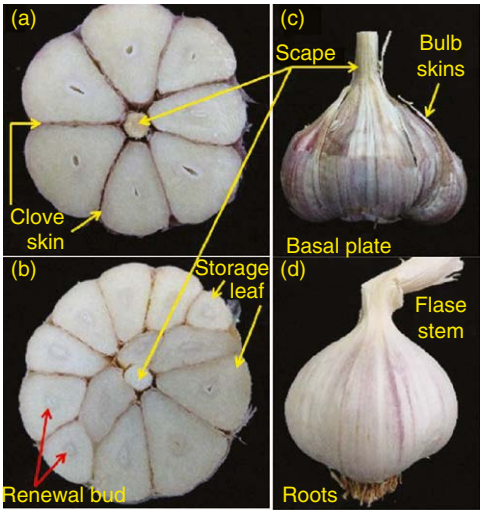

Fig. 17.8. Garlic bulb, cloves, and scape. a and b) Single and double whorled bulbs; c and d) Hard and soft neck, respectively (photograph: T. Ben Michael).

Physiological age and bulbing

No evidence for onion juvenility exists, and sensitivity to long photoperiod (LP) increases with

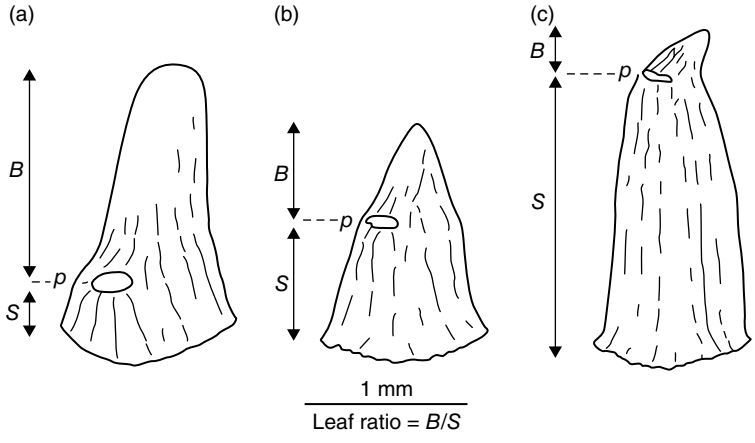

Fig. 17.9. Morphological differences between developing leaf initials at (a) leaf growth, (b) early bulbing; (c) main bulbing processes. p—the pore through which the new leaf emerges from its encasing sheath. A decreased ratio of the blade (B) and sheath (S) lengths characterize bulbing (Brewster, 1997, 2008).

age. In bulb onion, the first true leaf is initiated within the embryo and differentiation occurs during germination (Hoffman, 1933), hence there is no photoperiod effect on its presence and development. When exposed to 8 d × 24 h and then to 10 d × 8 h photoperiods, emerging-onion-seedlings formed bulbs and scales (Terabun, 1971). In another experiment with four-leaf seedlings, three of the leaves were removed and the plants exposed to 14 d × 24 h photoperiods, yet bulbing was similar in all cases.

Under LP, the rate of bulbing increased with plant age (Sobeih and Wright, 1986): 4.5-month-old plants defoliated to the two youngest leaves, bulbed faster than younger plants with greater leaf area; and onions raised from sets bulbed faster than seedlings (Aura, 1963).

In practice, emerging seedlings can produce small sets under appropriate LP (OSU, 2010), which sometimes serve as an alternative for pearl onions (*A. ampeloprasum*).

Environmental and growth regulators control of bulbing

The main plant and environmental factors that control bulbing in onion are depicted in Fig. 17.10. Garner and Allard (1920) first showed LP effect on the transition from green plants to bulbs, and later works demonstrated that onion cultivars differ in the specific minimum LP required for bulbing.

Under adequate LP, however, bulbing is inhibited by cold temperatures (van Kampen, 1970); enhanced by warm growth temperatures (Heath, 1945; Kato, 1964; Magruder and Allard, 1937; Thompson and Smith, 1938), and slows down at high temperatures (38–49°C) compared to 27–30°C (Abdalla and Mann, 1963; Brewster, 1990a; Fig. 17.10). These photo-thermal responses are governed by interactions with the genotype (Steer, 1980) and other factors at optimal, supra- and sub-optimal levels (e.g., fertigation).

For bulbing to be completed, a continuous LP treatment is required for as long as a sizable part of the last green blade functions, whereas a transfer from LP to SP ends the bulbing processes and side shoots sprout (Fig. 17.11) at the expense of energy stored in the swelling scales (Kedar *et al.*, 1975).

The rate of bulbing in a particular photoperiod depends strongly upon the red: far-red (R:FR) ratio of the light. Hence, a photoperiod, which is non-inductive at high R: FR, can be inductive at lower R:FR (Austin, 1972; Mondal *et al.*, 1986b). Leaves absorb more red than far-red wavelengths, therefore the light spectrum below the canopy changes with the consequent drop on R:FR ratio, and bulbing quickens with leaf area index (LAI) (Mondal *et al.*, 1986c). Therefore, shade by weeds/wind-breakers may enhance bulb initiation when photoperiods approach the required threshold (Brewster, 2008).

The action spectrum for 3 h irradiation in the middle of the LP treatment peaks at 714 nm (Lercari, 1983), and bulbing depends on photon

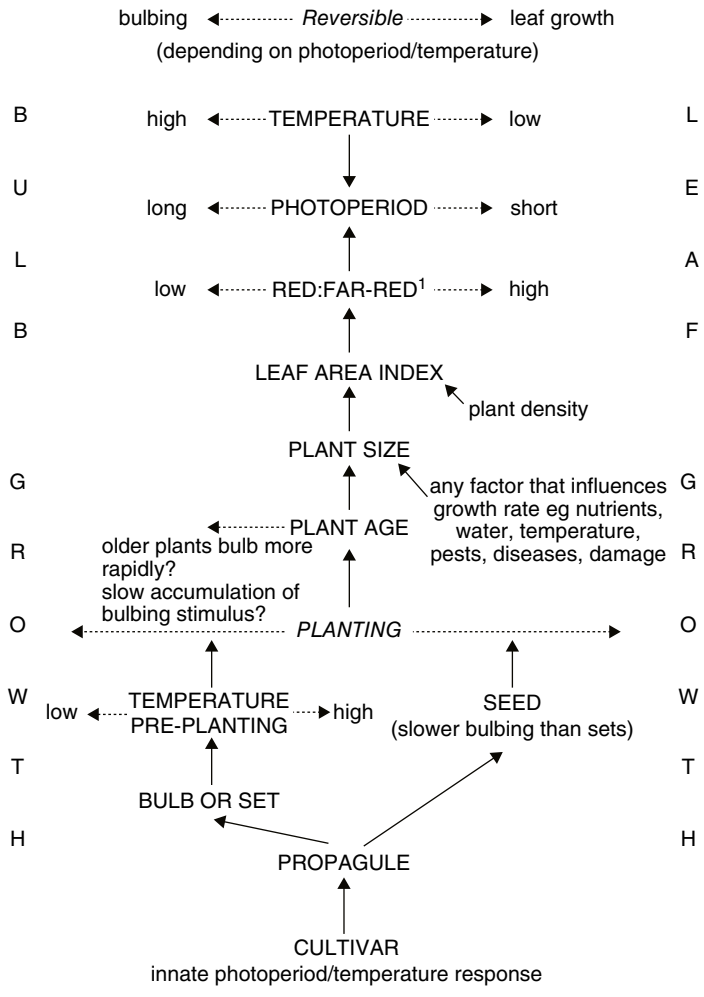

Fig. 17.10. The main plant and environmental factors that control bulbing in onion (Brewster, 1997). Plants are sensitive to spectral composition, specifically to the red: far-red (660:730 nm, Bewley and Black, 1994). On a clear sunny day R:FR in natural daylight is close to 1.

fluence rate ($E_{p,\,o}$).[1] The process is nullified, however, by simultaneous exposure to red light—a typical "high irradiance" phytochrome response. Some rhythmicity in the response of onion bulbing to R:FR is indicated by the temporal change in R: FR optimum during the inductive irradiation and by the strongest promoting effect of FR during the middle of LP light period (Lercari, 1982). Neither exposure to LP without FR, nor the addition of FR to an SP, however, induced bulbing (Lercari, 1982).

Effect of plant growth regulators on bulbing

Daylength provides a faultless signal to monitor and accurately sense the time of the year, and evolution benefitted from the concomitant changes in daylength and temperature, with the consequent adaptation to extreme environments. In many *Allium* spp. the physiological and/or developmental responses to the appropriate length of the day or the night, photoperiodism, resulted in the development of a survival mechanism under the

[1]Photon fluence rate ($E_{p,o}$) = total number of photons incident from all directions on a small sphere divided by the cross-sectional area of the sphere and per time interval https://goldbook.iupac.org/html/P/P04635.html.

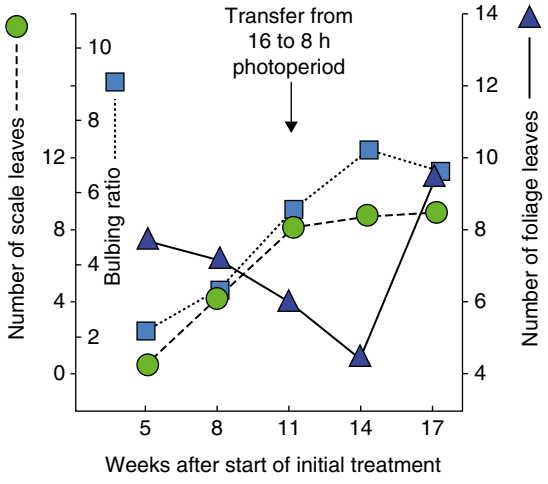

Fig. 17.11. Effect of transfer from 16 to 8 h photoperiods at 135 E m^2s^{-1} PAR on bulbing reversal and re-sprouting in onion cv. "Rocket" (redrawn from Sobeih and Wright, 1986).

scorching summers in Central Asia. This includes the loss of foliage and the formation of underground dormant storage organs as an adaptive strategy to water shortage and evasion of extreme heat. The ability to predict future environmental events requires endogenous mechanisms to permit physiological anticipation of the forthcoming conditions and to build up the defense processes.

Little is known about what the nature of these mechanisms is. The following summarizes the available information: Onions that grow from sets, bulb and mature faster than seedlings, but long storage of sets at ~ 30°C nullified the differential response (Aura, 1963), thus indicating that there is a heat-susceptible bulbing stimulus carried over from the previous season in sets (Heath and Holdsworth, 1948).

Ethylene plays an important role in plant developmental processes, both by stimulation and inhibition of growth. Under naturally increasing photoperiod (which coincides with the transition from spring to summer), the initial bulbing in four onion cultivars was associated with ethylene evolution followed by its decline towards maturation (Levy *et al.*, 1979), and exogenous ethylene (Ethrel,® Bayer) treatment induced temporal bulbing under non-inductive conditions (Levy and Kedar, 1970; Lercari, 1983; Fig. 17.12). Similarly, Ethrel-treated leek plants showed temporal sheath swelling, but never developed into mature bulbs (Levy and Kedar, 1970).

Silver nitrate (Levy *et al.*, 1979) and silver thiosulphate inhibited ethylene-induced bulbing, but neither it nor the ethylene synthesis

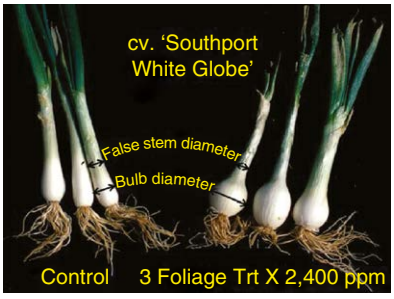

Fig. 17.12. Effect of foliar application of Ethrel® on bulbing of "Southport White Globe" onion. Three treatments at 2400 ppm sufficed to induce a significant increase in BR as compared with control (photography: D. Levy, with permission).

inhibitor aminoethoxyvinylglycine halted the photoperiod-induced processes, indicating that stimulation/inhibition by external ethylene does not necessarily represent the entire endogenous bulbing control mechanism (Lercari, 1983).

Gibberellic acids (GAs) stimulate plant growth and development (Gupta and Chakrabarty, 2013). In culture, GA_3 inhibited leaf and root growth and bulbing in shallot, while inhibitors of gibberellin biosynthesis promoted bulb formation (Le Guen-Le Saos *et al.*, 2002), and jasmonic acid, an important stress-signaling molecule in plants (including growth inhibition), promoted bulbing of garlic (Žel *et al.*, 1997).

Matsubara and Kimura (1991) showed an increase in endogenous ABA level with the onset of bulbing in all tissues, with a maximum at

lodging. However, a causal relationship between ABA content and bulbing was not supported by their *in vitro* culture findings, reemphasizing the complexity of the endogenous control mechanism of bulbing.

While the regulating mechanism of bulbing is far from being known/understood, it is clear that the promoting effects of environmental stress and the role of growth regulators involved in growth inhibition may indicate a direction, but more clear insight is required.

Photothermal response models for the rate of bulbing

Photoperiod and temperature are the most important environmental factors in determining the rate of bulbing; de Ruiter (1986) proposed a bulb growth model, in which under adequate LP, temperature becomes the dominant effector. Initially, the bulb growth rate increases exponentially but slows down with maturation. Therefore, a linear relationship was assumed for log-transformed changes in bulb diameter versus thermal time accumulation (base 5°C).

An equation, commonly used for describing the flowering responses of a number of long- and short-day species (Summerfield *et al.*, 1991), was employed by Brewster (1997) to quantify the temperature and photoperiod effects on the rates of bulbing (Fig. 17.13) as the reciprocal of the interval from the start of LP induction until the formation of the first leaf scale (equation 2). Bulbing can be predicted by models that combine the effects of time, temperature, and photoperiod as photo-thermal time (Searle and Reid, 2016, details in Chapter 5), and a fair agreement between predictions and reality was obtained experimentally (NIAB, 1982).

$$1/\text{time to bulb} = \text{rate of bulbing} = C + A \cdot \text{photoperiod} + B \cdot \text{temperature} \quad (2)$$

Photoperiod is measured in hours and temperature in °C. *A*, *B* and *C* are constants.

Adoption of such an approach implies that photoperiod induction of bulbing is quantitative and that when it crosses the genotype-specific threshold—the stimulus accumulates gradually.

In real life, bulbing and productivity are much more complicated processes than the above description as a strong genotype by environment interaction governs each developmental step involved in the processes. Rate of bulbing depends mainly on LP, temperature, and cultivar, but also on light intensity, actual spectrum, plant stand (Khan *et al.*, 2002), LAI, fertilizers (Brewster and Butler, 1989), water (Ortolá and Knox, 2015), salinity (Bernstein and Ayers, 1953), synergistic microorganisms (e.g., mycorrhiza: Stribley, 1990; Makus, 2004), and more. Additionally, factors affecting foliage development and growth

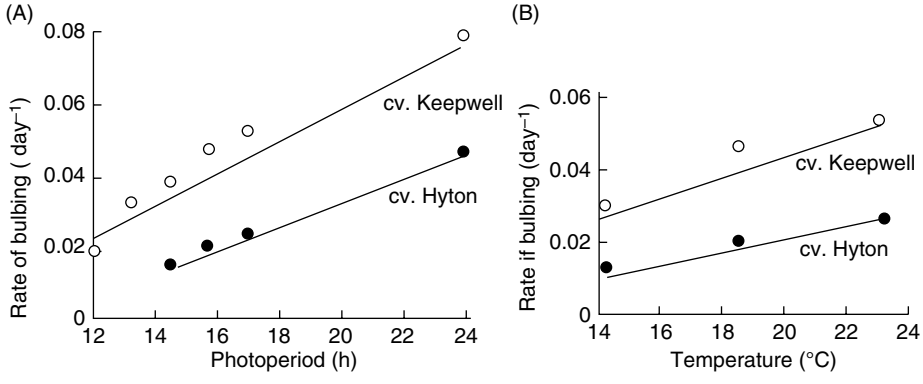

Fig. 17.13. Photoperiodic and thermal effects on bulbing of two onion cultivars (Brewster, 1997). A). The effect of photoperiod on the rate of bulbing at a mean temperature of 18.6°C. The rate of bulbing was derived as the reciprocal of the time in days for 50% of the plants to initiate bulb scales. B). The effect of temperature on the rate of bulbing under a constant 15.75 h photoperiod.

(e.g., heat, nitrogen, stand, weed competition, and stress conditions) play a significant role in both cases. Hence, knowledgeable skilled growers can control growth and manipulate productivity, earliness, and quality in a given field, by proper application of knowledge and agro-techniques.

Agronomic implications

Irrespective of seasonal photo-thermal changes, bulbing proceeds as long as LP and temperatures remain above the respective cultivar-specific thresholds, until maturation. This applies for temperate regions, where spring sown onions are exposed to increase and then decrease in daylengths in the spring, summer, and fall, respectively; and in the tropics, where little photo-thermal fluctuations occur (Jones and Mann, 1962; Currah and Proctor, 1990; Currah, 2002). In the case of fall production of cv. "Autumn Beit Alpha" in Israel, the rate of bulbing decreases with LP and temperature, yet the accumulation of reserves proceeds slowly and bulbing is complete before daylength crosses the line to become SP (Kedar *et al.*, 1975; Kedar, 1988; Corgan and Kedar, 1990).

The increase in the rate of bulbing under a given photoperiod with decrease in R:FR, and the decrease in R:FR within the canopy as LAI increases, opens the way for manipulation of the photo-thermal response by growth promoting and retarding factors (Mondal *et al.*, 1986c), such as nitrogen availability (Brewster and Butler, 1989).

Where the LAI effect is important, e.g., at fall maturation in temperate zones, agro-management can promote uniform bulbing. In Israel, sets planted in the fall quickly develop high LAI, thus advancing the accumulation of reserves in tandem with differentiation of true scales and BR increase, until maturation. In contrast, fall development of seedlings is slow, thus under the same environmental conditions the low-LAI-plants revert early in December to sprouting, and bulbs shrink (Rabinowitch, 1979; Fig. 17.11).

Under increasing photoperiods and temperatures, as with autumn-sown onions, LAI influence is of lesser significance, since assimilation in the bright lengthening spring days is high and together with the increasing temperatures enhances the rate of bulbing (Mondal *et al.*, 1986a, 1986c). LP cultivars from temperate zones, however, do not commence bulbing under these long days in the tropics and subtropics, due to insufficient stimulation.

Virtually all agronomic operations influence the rate of the bulbing, maturity date, yield, and quality, by their impact on plants' health, vigor, and LAI. This is especially true where bulbing would otherwise be slow, for example, when photoperiod shortens, or when cropping late-maturing genotypes in the summer or early fall.

Agro-manipulations, therefore, have a strong impact on earliness, yield, and quality. Examples of stress are shortages of nitrogen and/or water (Woldetsadik, 2003), high temperatures (Brewster, 1990b; Wu *et al.*, 2016a), stand (McGeary, 1985; Mondal *et al.*, 1986c; Bosekeng, 2012), and weed competition (Brewster, 2008) all lead to early bulbing, and the reverse is also true.

These agronomic parameters are affected by the following factors.

1. Stand: seed rate, viability and vigor; seedbed composition, fertility, moisture, and salinity; herbicide damage; temperature, and biotic injuries during germination and emergence.
2. Growth and development: daylength; light intensity and spectrum (e.g., shade by canopy, windbreakers, weeds); temperature; competition from weeds, water stress, and salinity; nutrients availability and root damage by biotic factors.
3. Biotic and abiotic factors: pests and disease; soil and/or water salinity; winds; sandstorms; saline-water drifts; acid rain; sunscald; herbicides or mechanical damage.

Plant factors influencing bulb yields

Bulb onions have a high "harvest index" = the proportion of the harvested product weight in the aboveground biomass. When necks of 80% of the plants soften then ~ 80% of the biomass is in the bulb, yet both bulb weight and harvest index keep on growing as long as conditions that support photosynthesis prevail (Davis and Jones, 1944; Brewster *et al.*, 1977; Fig. 17.14). Hence, for highest yields and longest storability, onion bulbs harvest should take place after foliage senescence and the necks sealing. Yet, for skins adhesion, a somewhat earlier harvest is beneficial.

Alliums suffer badly from inbreeding depression (Jones and Davis, 1944; Khan *et al.*, 2001),

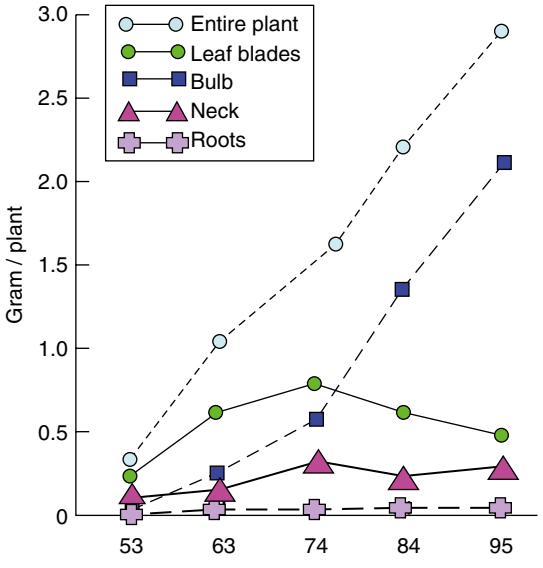

Fig. 17.14. Change in dry weight of the onion plant and its parts under continuous LP. (redrawn from Butt, 1968, with permission).

hence the marked increase in bulb yields and quality with the introduction of F_1 hybrids (Jones and Perry, 1951; Jones *et al.*, 1956; Dowker and Gordon, 1983; Doruchowski, 1986; Aghora and Pathak, 1991). In Israel, the mean increase in yields of original hybrids was twofold compared with the best commercial open-pollinated (OP) cultivars, from ~ 40–50 to ~ 80–100 t/ha, and quality was much improved (Rabinowitch, 1996). These long-keeping high-quality brown skinned hybrids excelled also in Kenya, Thailand (Rabinowitch, 1996), West Africa (Currah and Proctor, 1990), and elsewhere.

In contrast, Cramer (2003) argued that in New Mexico trials, most OP varieties perform as well or better than most of the hybrid varieties. It should be emphasized that hybridization itself is not necessarily accompanied by hybrid vigor, as only a few specific combinations exhibit superior features, and that the advantages in qualities and yield require advanced agromanagement (e.g., fertigation) and higher yielding agroecosystems than the ones common in fields of OP varieties.

Bulb maturation, dormancy, rest, and sprouting

During bulbing, respiration slows down but apical cell divisions remain unchanged until curing commences (Abdalla and Mann, 1963) or earlier, towards maturation (Pak *et al.*, 1995). Yet, initiation and growth of leaves and roots stops and the existing blades lodge, and gradually die back (Figs. 17.6, 17.14). Translocation of assimilates and growth inhibitors from the blades to the scales continues up to the beginning of dormancy (Davis and Jones, 1944; Butt, 1968; Tsukomoto *et al.*, 1969; Thomas and Isenberg, 1972; Stow, 1976; Komochi, 1990; Matsubara and Kimura, 1991; Chope *et al.*, 2007). Hence, defoliation during bulbing resulted in early sprouting (Stow, 1976), and premature harvest (e.g., for early marketing) resulted in low bulb weight and yield (Davis and Jones, 1944), and in short dormancy (Thomas and Isenberg, 1972).

Curing aims at drying the tunics, and tight sealing of the neck, thus lowering water losses, physical protection and mechanically blocking the entry of pathogens (Gubb and MacTavish, 2002; Petropoulos *et al.*, 2017). On the other hand, skin stripping resulted in an increase in respiration rates by nearly twofold (Apeland, 1971) and early sprouting (Tanaka *et al.*, 1985). Wounding may enhance sprouting by the same mechanism, and wax sealing can halt the process (Boswell, 1924).

In the UK, curing begins with 30°C treatment for 3 d followed by 10 d at 24°C and RH < 75%, and thereafter ventilation and cooling to ~ 15°C until necks are tight and dry (Chope and Terry, 2010). A fast curing method of heating at 30°C

for 3–9 d and 98% RH followed by immediate storage at 2°C and 70% RH was recently suggested (Eshel *et al*., 2014).

Information on both presence and length of dormancy is inconsistent (e.g. Abdalla and Mann, 1963; Kato, 1966a, 1966b; Mahotiere *et al*., 1976; Brewster, 1987; Bertaud, 1990; Matejko and Dahlbelm, 1991; Pak *et al*., 1995). Dormancy length is governed by genotype and environment, and for most cultivars true dormancy is short (60–70 days or less; Pak *et al*., 1995; Tanaka *et al*., 1996). Its gradual loss is characterized by an increase in respiration rate (Ward and Tucker, 1976); decrease in dry weight; changes in non-structural carbohydrates (Pak *et al*., 1995), in flavor precursors, and in phytohormones; resumption of cell division and differentiation, and fast leaf buds and roots elongation (Abdalla and Mann, 1963; Tanaka *et al*., 1985; Brewster, 1987; Miedema and Kamminga 1994; Pak *et al*., 1995; Chope *et al*., 2006; Chope and Terry, 2010).

Time to sprout is significantly affected by genetics, pre-storage (field environment, maturation, harvest, bulb size and health, bruises, curing) (Petropoulos *et al*., 2017), and storage conditions (temperature) (Chope *et al*., 2006; Komochi, 1990); RH, ventilation and controlled atmosphere (CA) (Tanaka *et al*., 1996); N_2O (Benkeblia and Varoquaux, 2003; Park *et al*., 2006)], that markedly influence the expression of the genetic potential of a given genotype (Petropoulos *et al*., 2017).

Many long-day (LD) and high dry-matter cultivars store better than those recognizing 11-13 h of light as LP (named "short-day cultivars" [SD]) low dry-matter types (Gubb and MacTavish, 2002; Suzuki and Cutcliff, 1989), yet some SD cultivars store very well (Currah and Proctor, 1990; Rabinowitch, 1996).

When aiming at long storage, growers apply sprout-suppressing maleic hydrazide (MH) to green plants (Brewster 1987; Salama and Hicks, 1987; Smittle and Maw 1988; Wall and Corgan 1994). Harvest is later than fresh market bulbs and storage under 0–4°C, CA and ~ 70% RH slows down water (Smittle 1988; Rajapakse *et al*., 1992), respiration, and dry matter losses (Hong and Kim, 2001; Ladeinde and Hicks 1988; Salama and Hicks, 1987). Ventilation and intermediate RH also slow down the development of storage pests (Matson and Haack) with the consequently extended storage-life of onion (Petropoulos *et al*., 2017; Table 17.3) and garlic (Põldma *et al*., 2012) bulbs; as well as slower sprouting, and in quality preservation.

Dormancy-length is genotype dependent and the rate of sprouting increases from a minimum at ~ 0°C to a maximum of 10–20°C (Miedema, 1994a). Under 10–20°C, mitosis resumes quickly and sprouting is fast (Abdalla and Mann, 1963; Brewster, 1977; Miedema, 1994a; Pak *et al*., 1995; Ernst *et al*., 1999; Gubb and MacTavish, 2002; Fig. 17.15). Dormancy is long at 25–30°C (Abdalla and Mann, 1963; Miedema, 1994a; Gubb and MacTavish, 2002; Brewster, 2008) due to inactivation of enzymes associated with growth (Ward, 1976) yet high evapotranspiration and respiration rates and severe quality losses due to dehydration and rotting incidence, are common (Petropoulos *et al*., 2017). Warm storage, therefore, serves for either short keeping period or where no cold facilities are available, but hot storage at 35°C for one to three weeks may shorten storage-life (Miedema, 1994a). Under ambient conditions, modern simple-to-use edible-coating technologies can markedly reduce storage-losses, (e.g. Nussinovitch *et al*., 2001; Nussinovitch and Rabinowitch, 2016).

Table 17.3. Effect of controlled atmosphere (1% O_2, 1% CO_2, 65-70 % RH) and 1°C storage temperature on bulb onion fresh weight losses, rotting, rooting, growth, and sprouting (adapted from Tanaka *et al*., 1996). N/A = not applicable.

	Control		
Trait	Mean room temp., 24°C	Cold storage, 1°C	The controlled atmosphere, 1°C
Fresh weight loss, %	13.0 in 7 months	13.4 in 12 months	6.6 in 12 months
Rotting, %	20.0	3.3	0.0
Rooting, %	100.0	100.0	0.0
Sprouting, mm	N/A	69.6	44.6

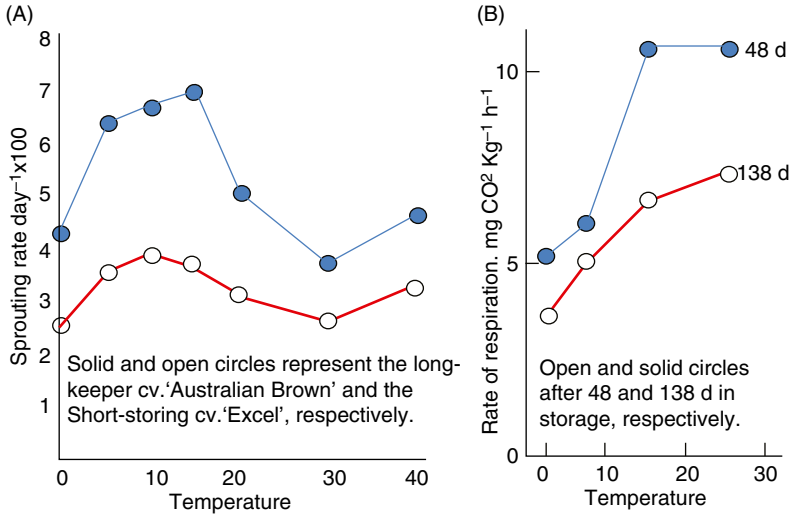

Fig. 17.15. A) Effects of temperature during four weeks of storage on the rate of field-sprouting of two onion cultivars at 15°C (data from Abdalla and Mann, 1963). Rates of sprouting calculated as the reciprocals of the number of days for 50% of the bulbs to sprout; B) Effects of constant storage temperature on the respiration rate of onion cv. "Rijnsburger"'(data from Ward, 1976).

The onion plant's response to 25–30°C has three phases: maturation, dormancy, and sprouting. Only in the dormant phase are such temperatures suppressive of sprouting. When dormancy breaks down and sprouting begins, the rate of growth peaks at around 25°C.

The effects of endogenous and exogenous conditions (including agro-technology) on dormancy, rest and sprouting are summarized in Fig. 17.16.

Effect of plant growth regulators on storage

Antagonism between promoting and inhibitory hormones, such as gibberellins, cytokinins, and abscisic acid (ABA) regulates dormancy (Wareing and Saunders, 1971).

Synthesized in the leaves and translocated to the developing onion bulb's growing-points (Thomas, 1969; Kato, 1996b) ABA concentration varies with genotype, development, tissue, environment, dormancy (Matsubara and Kimura, 1991) and with post-harvest conditions (Chope *et al.*, 2006). Treating green onions with ABA enhanced senescence and prolonged dormancy of the smaller bulbs (Kato 1966a). Moreover, the natural decrease in ABA content well correlated with initial sprout growth or the time until 50% sprouting (Chope *et al.*, 2007).

For a given genotype, one reason for early bulb sprouting is low ABA content, due to poor agronomy, for example, foliage damage or premature harvest. Indeed, early sprouting was evident in bulbs developed following defoliation. In contrast, maximizing pre-storage ABA or reduced degradation by cultural, environmental, or genetic means, delayed sprouting (Sharma *et al.*, 2016). Hence, Chope *et al.* (2006) proposed that the ABA level at harvest better indicates onion bulbs' storability than its decline-rate in storage.

A sharp decline in ABA was observed in the first six weeks of bulb onion storage, whereas post curing increases were first measured in cytokinins (Cools *et al.*, 2016), followed by auxin and gibberellin activities (Isenberg *et al.*, 1974; Miedema and Kamminga, 1994), and changes in auxin activity well correlated with the actual bulbs' dormancy-break.

Synthesized in sprouting roots, cytokinins move upwards and peak before bulbs sprout, hence, root removal retarded sprout elongation (Miedema, 1994b) and benzyladenine injection accelerated sprouting in warm storage. Miedema and Kamminga (1994) thus proposed that cytokinins' induction with ABA depletion is involved in sprouting regulation.

Fig. 17.16. Effect of genotype, plant, and pre- and post-harvest conditions on dormancy and sprouting in onion (adapted from Sharma *et al*., 2016). Dark blue represents factors involved during field development; turquoise represents the post-harvest phase.

In conclusion, the bulbs' thermos-dormancy (Yoo *et al.* 1997) is probably regulated by low cytokinins at > 25°C (Miedema and Kamminga, 1994), or by reduced metabolism under cold (Miedema, 1994a; Ramin, 1999) and/or CA conditions (Weichmann, 1986).

Onion sensitivity to ethylene is low (Kubo *et al*., 1990). Low post-harvest ethylene levels gradually increased during storage and peaked toward the end of dormancy, depending on cultivar and health, and thus may indicate ethylene involvement in sprouting (Abdel-Rahman and Isenberg, 1974; Benkeblia and Selselet-Attou, 1999).

Foliar ethylene application resulted in leaf senescence (Levy and Kedar, 1970), small bulbs and lower yields than control (Corgan and Izquierdo, 1979; Cantliffe, 1980; Thomas and Rankin, 1982), and in sprout inhibition (Thomas and Rankin, 1982; Adamicki, 2005). A continuous exposure in cold storage to ethylene (Johnson, 2006) or to 1-MCP (Chope *et al*., 2007; Downes *et al*., 2010) reduced sprout growth without affecting dormancy length (Bufler, 2009). However, treating onion bulbs with ethephon (Ethrel) (Abdel-Rahman and Isenberg, 1974; Miedema and Kamminga, 1994; Benkeblia and Selselett-Attou, 1999) enhanced sprouting, indicating its regulatory role in dormancy. In conclusion, ethylene probably fulfills at least two functions in stored bulbs: inhibition of sprout-elongation and interference with dormancy (Bufler, 2009).

Combined post-curing treatment with ethylene and 1-MCP resulted in sprouts shorter than in untreated bulbs, thus suggesting the treatment as alternative means for sprout control to MH (Cools *et al*., 2011).

Foliage application of maleic hydrazide (MH) to field growing onion and garlic is commonly used for storage extension. MH moves basipetally to the apex (Stallknecht *et al.*, 1982) and inhibits mitotic divisions and cell elongation (Greulach and Haesloop, 1954; Isenberg *et al.*, 1974), as well as hindering RNA biosynthesis (Pendergrass, 1969; Lercari, 1983), thus delaying sprouting.

Reproductive development

Under non-inductive conditions, the monopodial *Allium* shoot apical meristem (SAM) continuously initiates leaf primordia for bulb onion: (van Kampen, 1970). The combined number of leaves and primordia serves as a reliable time-independent measure of plant physiological state (e.g., juvenile state, Table 17.4).

Alliums sense environmental cues for flowering only at the post-juvenile phase, in the first or second season of development from seed. Interactions between endogenous and environmental factors induce SAM transition to the reproductive state with consequent distinct changes in architecture and in the differentiation of individual flowers (Fig. 17.17). Thereafter, the sympodial plants flower annually and produce renewal bulbs (Rabinowitch, 1990a, 1990b; Kamenetsky and Fritsch 2002).

Following meristem differentiation, the scape elongates and flower buds develop within the subulate spathe-like bract closed-umbel that finally breaks open and sequential anthesis occurs. Each flower consists of five whorls with three organs each: two outer whorls of tepals, two inner whorls of stamens and a gynoecium (Jones and Emsweller, 1936a; DeMason, 1990). The developed inflorescence consists of numerous cymes; each develops several flower buds (De Mason 1990, Kamenetsky and Rabinowitch, 2002). Hence, a single inflorescence blooms for weeks and self-pollination between the protandrous flowers of the same inflorescence occurs frequently in fertile populations.

Many edible *Allium* require vernalization for flowering (e.g., onions: Rabinowitch, 1985, 1990a; Kamenetsky and Rabinowitch, 2002; Khokhar *et al.*, 2007; chives: Poulsen, 1990; De Clercq and van Bockstaele, 2002; Japanese bunching onion: Inden and Asahira, 1990, Yamasaki, 2000a; Dong *et al.*, 2013; shallot: Rabinowitch and Kamenetsky, 2002; garlic: Kamenetsky *et al.*, 2004; Rotem *et al.*, 2011; Wu *et al.*, 2015, 2016b). Pre-vernalization LP induces bulbing thus may prevent flowering (Yamasaki, 2000a), whereas post-vernalization LP promotes scape elongation (e.g., onions: Rabinowitch, 1985, 1990a; garlic: Kamenetsky *et al.*, 2004, Mathew *et al.*, 2011; Japanese bunching onion: Yamasaki, 2000a, 2000b; rakkyo: Toyama and Wakamiya 1990; Chinese chives: Saito, 1990, Kawagishi *et al.*, 2009).

Temperature effects

For most onions, optimum vernalization temperatures range between 7 and 12°C (Brewster, 1987). Some genotypes from temperate regions require long vernalization, probably due to selection pressure against bolting (Rabinowitch 1990a;

Table 17.4. Minimum physiological age required for the transition from vegetative to reproductive state in edible alliums. Leaf numbers vary with genotype between and within species.

Allium spp.	Crop	Minimum leaf number[1] prior to the transition	Source
A. cepa	Bulb onion	4–14	Rabinowitch, 1985, 1990a; Khokhar, 2014
A. cepa Aggregatum group	Shallot	6–7	Krontal *et al.*, 1998, 2000
A. sativum	Garlic	6–30	Shemesh *et al.*, 2008
A. fistulosum	Japanese bunching onion; Welsh onion	10–14	Inden and Asahira, 1990
A ampeloprasum	Leek	6–7	van der Meer and Hanelt, 1990; De Clercq and Van Bockstaele, 2002

[1]Leaf and leaf buds combined.

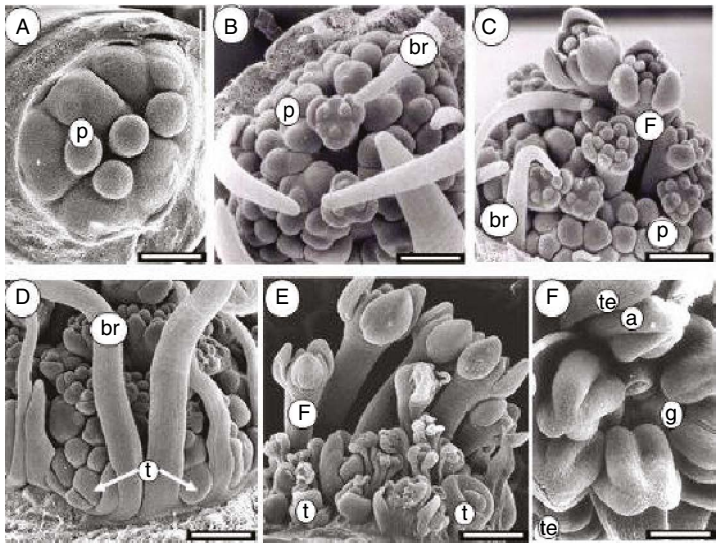

Fig. 17.17. SEM studies of garlic florogenesis. A) SAM transition from vegetative to reproductive. Meristem dome produced flower initials. Bar = 0.3 mm. B) Differentiation of floral primordia. Floral parts are visible in the oldest floral primordia (p), while younger ones appear as undifferentiated meristematic domes. Bracts (br) are visible. Bar = 0.4 mm. C) Non-synchronous uneven flower buds' differentiation in a single inflorescence. Six anthers and six perianth lobes are visible in older buds. Bar = 0.6 mm. D) Newly developed meristems develop at the periphery, near leaf-like bracts (br). They quickly differentiate and form small inflorescence bulbs: topsets (t). Bar = 0.8 mm. E) Inflorescence abortion and drying at the early stages of development. Topsets (t) develop between aborted flower buds. Bar = 1 mm. F) Fully differentiated flowers of fertile garlic. Tepals (te) and anthers (a) and central gynoecium (g) are formed in each flower bud. Bar = 0.3 mm.

Messian *et al.*, 1993; Kamenetsky and Rabinowitch, 2002), while some warm climate types bolt under marginal winter conditions (Sinnadurai, 1970).

Heat may retard blooming, hence long exposure to 28–30°C inhibited inflorescence initiation, delayed (Heath and Mathur, 1944; Aoba, 1960), and reduced (Jones and Emsweller 1936a; van Kampen, 1970) flowering in onions. The further florogenesis progressed, the longer the warm-treatment required for halting the process (Heath and Mathur, 1944).

Storage and/or field vernalization promote SAM transition in many garlic genotypes, some of which develop visible scapes (Takagi, 1990; Etoh and Simon, 2002). Concomitantly, leaf initiation ceases, but emerged leaves continue to elongate until spathe break (Takagi, 1990; Kamenetsky *et al.*, 2004; Rotem *et al.*, 2007; Wu *et al.*, 2015, 2016b). Bolting genotypes vary in cold requirements, time of meristem transition and stalk elongation (Mathew *et al.*, 2011; Rohkin-Shalom *et al.*, 2015). Short induction by LP promotes stalk elongation and flower differentiation, while warm temperatures lead to inflorescence degeneration (Kamenetsky *et al.*, 2004). Indeed, all commercial garlic genotypes suffer from physiological infertility, thus fertility restoration by environmental manipulations is possible (Kamenetsky *et al.*, 2004; Mathew *et al.*, 2011).

Japanese bunching onion genotypes differ in juvenility, and both floral initiation (Nakamura, 1985b) and scape elongation (Nakamura, 1985b; Inden and Asahira, 1990; Yamasaki *et al.*, 2000a, 2000b) require a combined induction by photoperiod and vernalization, as plants under SD produce fewer florets and short seed stalk than those under LD (Yamasaki *et al.*, 2000a, 2000b).

Chives and leek require vernalization for blooming even under SP (Poulsen, 1990; van der Meer and Hanelt, 1990; Brewster, 1994; De Clercq and Van Bockstaele, 2002). In leek, the post-juvenile age varies with genotype and environment (Dragland, 1972; van der Meer and Hanelt, 1990) and in the absence of cold; LP

induces flower initiation and visible bolting. In chives, SP promotes florogenesis and scape elongation (Brewster, 1994) and field temperatures > 18°C prevent flowering (Poulsen, 1990).

Rakkyo and Chinese chives subdivide continuously to form a clump of shoots. Both drought (Mann and Stearn, 1960) and SP inhibit differentiation and inflorescence growth, and LP promotes these processes (Nakamura, 1985a, 1985c; Saito, 1990; Toyama and Wakamiya, 1990). Hence, both species flower in the late summer.

Genetic control and molecular biology

In onions, *FLOWERING LOCUS T (FT)* family of genes controls the induction of both flowering and bulbing (Lee *et al.*, 2013). Flowering promotion by vernalization correlates with *AcFT2* up-regulation whereas bulbing is regulated by two antagonistic *FT*-like genes (Fig. 17.18). Under SP, *AcFT4* products prevent up-regulation of *AcFT1* and bulbing, LP induces its down-regulation, and the *AcFT1* up-regulation promotes bulbing.

Homologs of *Arabidopsis* flowering genes, *GI* (*GIGANTEA*) and *FKF1* (*FLAVIN-BINDING*) associated with photoperiodic response are conserved in onion. Genotypes differing in photoperiod responses showed similar *AcGI*- but varied in *AcFKF1* expression patterns. Hence, the varietal photoperiod response is attributed to *AcFKF1* (Taylor *et al.*, 2010).

In garlic, a single-copy of *gaLFY* (homolog to *Arabidopsis LFY* and to *Antirrhinum majus FLO*:

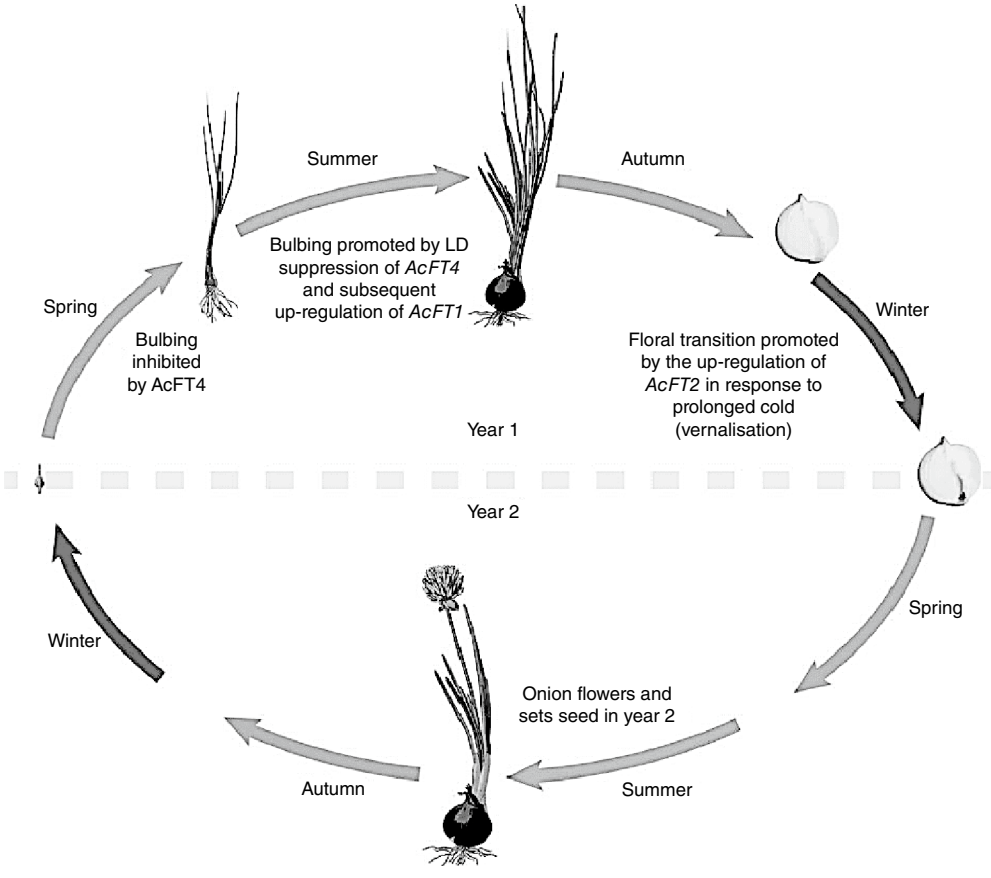

Fig. 17.18. In doubled haploid onion seedlings, *AcFT4* expression under SP prevents bulbing. Under LP, *AcFT4* down-regulation promotes bulbing by up-regulation *AcFT1*. *AcFT2* up-regulation by vernalization leads to floral induction in the spring/summer (Lee *et al.*, 2013, with permission)

Coen *et al*., 1990; Weigel *et al*., 1992) undergoes alternative splicing (Rotem *et al*., 2007). The two mRNAs are differentially expressed during meristem transition, flower differentiation and in mature anthers and ovules (Rotem *et al*., 2011).

Transcriptome analysis of flowering garlic exhibits floral-induction pathways similar to those common in model plants. Orthologs of *CONSTANS* (*CO*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), *LEAFY* (*LFY*), *APETALA1* (*AP1*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), *SEPALLATA1* (*SEP1*), *SEPALLATA3* (*SEP3*) and *AGAMOUS* (*AG*) are differentially expressed in reproductive tissues, leaves and bulbs, indicative of their involvement in flower-signal transduction and in bulbing processes (Kamenetsky *et al*., 2015).

Male sterility

In *Allium*s, female function is mostly normal, but male-sterility (MS), either under genetic or environment control, is common and includes the absence of stamens, meiosis interference, pollen abortion, no dehiscence, or failure to germinate on a receptive stigma.

Cytoplasmic–genetic male-sterility (CGMS) was studied in onions (Jones and Emsweller, 1936b; Jones and Clarke, 1943; Havey, 1993, 1995, 2000, 2002; Kik, 2002; Engelke *et al*. 2003), in bunching onion (Nishimura and Shibano, 1972; Moue and Uehara, 1985; Yamashita *et al*., 2010), in chives (Tatlioglu, 1982, Engelke and Tatlioglu, 2000) and in leek (Smith and Crowther, 1995; Havey and Lopes Leite, 1999).

Specific nuclear and mitochondrial interactions result in cytoplasmic male sterility (CMS) and nuclear genes restore fertility (Jones and Mann, 1963). In onion, normal (N) and two sterile (S and T) mitochondrial genes were identified (Berninger, 1965; De Courcel *et al*., 1989; Holford *et al*. 1991; Havey, 1993, 1995; Satoh *et al*. 1993; Sato, 1998). Irregular tapetum development precedes microspore abortion in S-, while meiosis interruption occurs in T-cytoplasm plants (Monosmith, 1928; Tatebe, 1952; Peterson and Foskett, 1953; Yen, 1959; Dyki, 1973; Patil *et al*., 1973).

The S coded sterility (Jones and Clarke, 1943) is stable and simple to use. Hence, it is commonly employed in commercial hybrid seed production (Havey 1994, 1995; 2004). The T coded sterility (Berninger 1965; Schweisguth, 1973) requires three independently segregating loci for fertility restoration and therefore is hardly used in practice.

In garlic, three MS types were described (Shemesh Mayer *et al*., 2013, 2015): 1) Modified callase function interrupts metabolism and degradation of the tetrades' callose walls (Winiarczyk *et al*., 2012; Tchórzewska *et al*., 2017); 2) Lack of normal cortical cytoskeleton leads to progressive cytoplasm microspores' degeneration (Tchórzewska *et al*., 2015). Here, microspore separation is followed by anthers' degeneration and enlarged tapetal cells, and pollen differentiation ceases on release of post-meiotic microspores (Novak, 1972; Etoh, 1979, 1980; Gori and Ferri, 1982); 3) Complete sterility (Winiarczyk *et al*., 2012): morphologically normal pollen grains do not germinate on receptive stigmas (Shemesh Mayer *et al*. 2013).

Fertilization and seed development

In bulb onion, fastest pollen tube growth, highest seed set, and best embryo development occurred at 35/18°C (day/night), compared with 24/18°C or 43/18°C (Chang and Struckmeyer, 1976). In production fields, insolated ovaries might reach temperatures above 40°C (Tanner and Goltz, 1972), with consequent abortion and low seed-set (Peterson and Trammell, 1976). Under normal conditions, fertilization starts within 12 h of pollination and completes in three to four days (Rabinowitch, 1990a, 1990b). When fertilization fails, ovary shrinkage and color fading occur about three weeks after anthesis, making an early assessment of seed-set difficult (Currah, 1990).

Following fertilization, endosperm nuclei divide first, and embryo cell division and expansion begin only five to six days later (Chang and Struckmeyer, 1976; Dolezel *et al*., 1980). The developing embryo becomes oval, then tubular, and finally a coiled-tubular structure embedded within the endosperm (Rabinowitch, 1990b).

In onion and leek endosperm, free nuclear divisions continued for 17–22 and 31–35 DAF, but only at 45 and 66 DAF, respectively, the embryo and endosperm filled the entire seed coat. At ~ 330 degree-days > 0 °C after flowering

(DDAF) the liquid endosperm develops cell walls (Brewster, 2008), and becomes pasty at ~ 450 DDAF.

Onion and leek germination occurs first between 24 and 31 DAF (Gray and Ward, 1987) just before attaining maximum fresh weight (Steiner and Akintobi, 1986). At 31–45 DAF (~ 570 DDAF) seed coats turn black (Brewster, 1977) and capsules split open (Ogawa, 1961; Gray and Ward, 1987).

Seed dry weight increases exponentially for 31 DAF before slowing down, reaching a maximum at 45 and 59–66 DAF, respectively with a concomitant decline in oxygen uptake (Gray and Ward, 1987). Then, capsules begin to shatter and shed seed, and the seeds' food-reserve oil globules and protein bodies become visible.

Seed storage

On maturation, the seed of most *Allium*s become dormant (Hanelt, 1990; Brewster 1994; Phillips, 2010). In comparison to other crops, however, deterioration of onion seed due to metabolic processes (Priestley, 1986) is fast (Toole *et al.*, 1948; Sijbring, 1963; Mackay and Tonkin, 1967; Ellis and Roberts, 1977), and leek's shelf-life is somewhat longer (Romer, 1999).

Information on seed deterioration at high RH and temperature is of high practical value (Ellis and Roberts, 1977). Aging is associated with peroxidation of unsaturated fatty acids (Chiu *et al.*, 1995; Bailly *et al.*, 1996; Hsu and Sung, 1997; Rao *et al.*, 2006) with consequent damages to membranes, nucleic acids, and proteins (Fujikura and Karssen, 1995). The degradation products of thermo-labile lipid peroxidation accumulate in the senescing seeds, thus resulting in loss of membrane integrity and seed viability.

Among the factors affecting seed vigor and viability, storage conditions are of paramount importance. Fluctuating temperatures and high temperatures and RH accelerate aging (Delouche and Baskin, 1973) while keeping either factor low during storage, slow down degradation processes and aging (Roberts, 1972; Khanal, 1990). Vigor can be alleviated to some extent by limited hydration techniques generally termed priming (Kepczynska *et al.*, 2003; Parena and Cantliffe, 1994) which contribute to repairing membrane integrity (Dawidowicz-Grzegorzewska, 1997) (Chapter 1) with the consequent enhancement and synchronization of field emergence (Khan *et al.*, 1995), especially under suboptimal conditions.

Physiological Disorders

Incomplete bulbing: thick-necking/bull necks

Bulbing onion plants sometimes continue to produce new leaves during bulbing Their necks thicken and remain firm, and BR remains low (bull necks; Fig. 17.19). Firmness remains because the inner pseudostem space is continuously filled and packed with newly-formed leaves. Thick-necking occurs mainly under marginal conditions, in seasons, sites and cultivars where crop maturity is late (Brewster *et al.*, 1987), e.g., due to slow emergence in cold spring, late development of high LAI (Brewster *et al.*, 1987); cool summers (Brewster, 1990a), or/and excessive nitrogen nutrition. The incidence of bull-necking and no-lodging increases when maturation is not complete before daylength shortens to LP threshold, and fall temperatures decline below optimum for bulbing (Scully et al., 1945; Brewster, 1977). Potentially, foliage treatment with ethylene may reduce bull-necked bulbs (Thomas and Rankin, 1982). A better solution is the

Fig. 17.19. Normal (right) and thick-neck bulbing (left), in a genotype requiring LP close to the maximum photoperiod common near the equator.

Fig. 17.20. Inner doubling (left) and splitting (right) in bulb onion and shallot (center).

introduction of cultivars characterized by a shorter LP than the one used.

Premature bulbing

Bulbing, soon after emergence, results in dormancy and zero growth for a significant duration. It happens with summer transplanting or, in sowings when temperatures and photoperiods that promote bulbing prevail. Similarly, bulbing occurs in dense nurseries in tropical countries, in response to low R: FR (Robinson, 1971; Kedar, 1988; Rabinowitch and Zig, 1989; Currah and Proctor, 1990).

Splitting/doubling

Doubling, the development of multiple growing points is under strong genetic control, yet high temperatures and short days promote laterals' development (Robinson, 1971; Chipman and Thorpe, 1977; Rabinowitch, 1979; Steer, 1980; Kamenetsky and Rabinowitch, 2006; Fig. 17.20). In both, shallot and bulb onion doubling is more common in plants raised from sets than from seeds (Rabinowitch, pers. observations) and agro-management has a significant effect (Rabinowitch, 1979), for example, deep transplanting results in lower visible expression of doubling (Chipman and Thorpe, 1977); excessive soil moisture during bulb ripening or excessive nitrogen fertilization during vegetative growth phase (Valenzuela *et al.*, 1999; Abdissa *et al.*, 2011), or temperature and day length (Steer, 1980). For single-heart bulbs, strong selection and direct seeding are recommended.

Bolting

Blooming in the first growing season impairs the bulb's quality and yield. Early sowing/planting of overwintered crops may end up in bolting, due to vernalization of post-juvenile plants by winter temperatures (Brewster and Salter, 1980; Rabinowitch, 1985; Brewster, 1994). Then, the combinations of increasing daylength and cool spring temperatures promote scape elongation. Seasonal changes in field environment result in differences in bolting between years of a single cultivar in the same area (Brewster *et al.*, 1977; Brewster and Salter, 1980).

Early sowing/planting may bear high yields of big bulbs if winter is mild, but is prone to bolting after cold winters. Alternatively, late sowing/planting may result in low bolting rates, but low yields of small bulbs are expected. Hence, decisions on sowing/planting date of a given cultivar are based on regional multiyear experience. A fair compromise accommodates 1–5% bolting as optimum.

Inflorescence suppression

In addition to insufficient vernalization, failure to flower in the second season may result from suppression of inflorescence buds by the thermo-photoperiod promotion of bulbing during the "competition" phase (Kampen, 1970). Favorable agro-management for bolting, for example, cold storage and early planting, offers a solution for flourishing seed production. However, early flowering may result in small umbels (few flowers) and poor pollination due to poor pollinators' activity.

Watery scales

Watery scales is a complex problem caused by a number of environmental factors (Shock *et al.*, 2007), mainly under very hot conditions (Lipton and Harris, 1965), such as insolated bulbs (Oregon State University, 2004). Internal increase in CO_2 concentration (Hoftun, 1993) due to overheating during curing is another major cause of the disorder. In storage, translucent scales developed under either −1 or 49°C for 20 h (Shock *et al.*, 2003) and bulbs' exposure to CA of 10% CO_2 but less so by the low O_2 atmosphere (Komochi, 1990). Symptoms generally develop in the middle and upper parts of the false scales but could include the entire bulb.

Injured bulbs develop thick leathery skin(s) and some watery glassy scales with the consequent losses of yield and quality (Ceponis *et al.*, 1986; Lancaster, 1988; Hale *et al.*, 1990; Weichmann, 1990; Solberg, 2015).

Weak symptoms are sometimes reversible (Shock *et al.*, 2003) and infrequently might disappear (Chen *et al.*, 2013), but usually worsen in storage (Solberg, 2015) and affected bulbs are prone to attack by microorganisms and rot.

Garlic waxy breakdown

In storage, small light-yellow areas occasionally develop and expand to give shrunken, somewhat translucent, amber garlic cloves, waxy to the touch. The disorder might be associated with inadequate ventilation and low oxygen during storage (Anon, 1976), thus suggesting that it may be similar in cause to the "watery scale" disorder of onions, namely CO_2 toxicity (Brewster, 1997).

Garlic rough bulbs and spherical cloves

Often, the differentiation of garlic axillary buds depends on cold induction (Rahim and Fordham, 1988; Kamenetsky *et al.*, 2004). Rough bulbs develop after long propagules' storage at 0–5°C, and/or a long extra-cold winter, before LP and warmth, inducing bulbing (Mann and Minges, 1958; Messiaen *et al.*, 1993). Then, sprouting axillary buds develop cloves that form irregular peripheral bulges on the main bulb.

In contrast, insufficient cold induction, as in spring planting, may result in lack of axillary bud development, thus producing single terminal round bulbs aka "round clove" (Messiaen *et al.*, 1993). Round bulbs are also formed when very small cloves or topsets serve as planting stock (Takagi, 1990).

Concluding Remarks

The genus *Allium* comprises more than 800 species (Li *et al.*, 2010) including a large and variable group of economically important species, common worldwide. All studied species exhibit strong interactions between genotypes and environment, which considerably affect growth, foliage development, yield and quality of storage organs, flowering, seed production, and seed quality. Very little is known about the roots and the endogenous mechanism that controls the formation of storage organs. The complex physiology (especially with regard to competition over resources between generative and storage organs) is far from being understood and molecular knowledge is rather poor. Utilization of both classical and novel tools for genetic and physiological studies, and improved knowledge of inherent control mechanisms of the initiation and development of vital processes, will facilitate improvements in terms of distribution, yield, and quality of these important crops, and provide the tools required for cultivation of these cold-requiring plants under the present and future warming of our planet.

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18 Asparagus

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> At no time cut too severely, but bear in mind that the copious and healthy foliage during the summer, the stronger the produce next spring. The strength of roots depends on the quantity of foliage, … enough left to maintain them healthy and vigorous.
>
> (Barnes and Robinson, 1881: 8)

Asparagus is arguably one of the most important perennial vegetables grown in the world (FAO, 2017). Significant production areas in the world include Asia (96,800 ha; mainly China and Japan), Europe (53,550 ha; Germany and Spain), South America (37,000 ha; mostly Peru), and North America (31,800 ha; Mexico and United States). Minor production regions include Australasia (2250 ha; Australia and New Zealand) and Africa (< 500 ha). Increased production in regions that produce asparagus year-around meets the demand for fresh asparagus in the off-season. Over the last 25 years, significant increases in acreage have centered in Asia, Europe, and South America. It has been estimated that asparagus has a worldwide value in excess of 1.12 billion US dollars (FAO, 2017).

Edible asparagus is an ancient vegetable and has been cultivated since the times of the Greeks and Romans (Hexamer, 1914; Tutin *et al*., 1980). Throughout ancient Europe, asparagus was used for both culinary and medicinal purposes. Roots, shoots, and seeds were used as diuretics, sedatives, pain killers, and liniments (Hexamer, 1914). Today, asparagus is grown for its edible shoots (spears) which are marketed fresh or processed.

Asparagus belongs in the family Asparagaceae (formerly: Liliaceae; Tutin *et al*., 1980) with approximately 300 species spread throughout the world. Norup *et al*. (2015) showed that the genus *Asparagus* is widely distributed, common to the arid and semi-arid regions of Africa and Eurasia, with southern Africa being the ancestral home to most species. *Asparagus* species commonly found in Africa generally have perfect flowers, otherwise most species have similar anatomical features. A second unique center is located in central Asia (Western China and Mongolia) which is home of the dioecious species, *A. officinalis* L. and *A. maritimus*. These are herbaceous perennials with a unique anatomy and growth habit.

In addition to the widely grown edible asparagus (*A. officinalis*) and regionally unique *A. maritimus* (Falavigna *et al*., 2008), there are numerous important ornamental species including *A. sprengeri* (asparagus fern) and *A. plumosus*, *A. laricinus*, and *A. racemosus* (used in the florist trade). Most of the information on the physiology of asparagus has been gleaned from the edible form. This chapter will focus on what we know about *A. officinalis* L. bud, spear, and root growth, carbohydrate (CHO) production and accumulation, and their relation to crop productivity.

 Physiology of Vegetable Crops 2nd edition (eds H.C. Wien and H. Stützel)

Asparagus Plant

The underground portion of the asparagus plant is known collectively as the crown and the foliage as the fern (Fig. 18.1). The crown consists of a rhizome (underground stem) with numerous adventitious roots. The rhizome has at its apex several large and many smaller buds which collectively form a bud cluster (Blasberg, 1932). Accessory buds (lateral buds) form new clusters on the side of the rhizome producing a new axis and direction of growth (Mullendore, 1935). New buds are initiated in the axil of the first scale leaf of the preceding bud. Buds arise alternately and oppositely on either side of the growth axis, extending the rhizome 2–4 cm per year. As the seminal (seed) root senesces, large diameter, adventitious fleshy storage roots are initiated from below or less commonly the side of the elongating rhizome. Storage roots are 2–6 mm in diameter, initiated in pairs on either side of the rhizome, grow for several years, and over time form a crowded mass of roots connected to the rhizome. Fine, fibrous feeder roots grow off the storage roots in an irregular pattern. Fibrous roots primarily absorb water and nutrients, live for one to two months, are moderately branched, small in diameter (< 1 mm), and grow 15–20 cm long.

The aerial foliage of asparagus consists of many individual stems arising from buds on the underground rhizome. Each stem (commonly called fern) grows to a height of 1–2 m (Blasberg, 1932) with many sessile scale leaves (Mullendore, 1935). Lateral branches arise in the axils of these scale leaves, producing secondary branches which have sessile scale leaves of their own with third and fourth order branching quite common. Scale leaves are triangular in shape in a 2:5 phyllotaxy arrangement and nearly all scale leaves are initiated in the bud prior to primary stem elongation. Small, cylindrical, leaf-like structures called cladophylls develop in the whorl of the scale leaves at nodes near the apex of the main stem and along the secondary branches (Blasberg, 1932). Cladophylls are the chief photosynthetic organs of the asparagus plant. Cladophylls are not leaves but rather modified branches with a cuticularized epidermis, sunken stomata, and small guard cells.

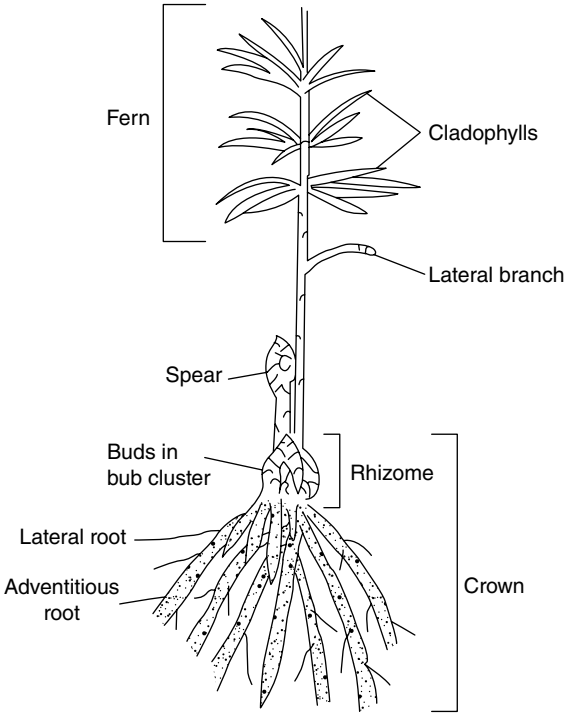

Fig. 18.1. The asparagus plant with important plant parts (adapted and redrawn from Mullendore, 1935).

Shoot growth

During a production season, several stages of fern growth take place (Fig. 18.2). In temperate production areas, bud break, spear growth, and harvest occurs in spring (8–12 weeks); fern expansion, new buds, and storage root growth happens in summer (12–16 weeks); and ferns senesce and plants go dormant in fall as temperatures decrease. In temperate regions, growth is initiated by temperature increases but may require applications of water in drier areas. In tropical areas, growth commonly continues unabated throughout the year. Dormancy is not required but plants may have rest periods which are commonly initiated by drought. A thorough understanding of plant growth and development is required to optimize productivity.

Buds and spears

The initiation of bud growth and spear elongation is primarily controlled by temperature and apical dominance (Kretschmer and Hartmann, 1979; Ku *et al.*, 2007; Graefe *et al.*, 2010), with dominance increasing in the bud cluster as the season progresses (Tiedjens, 1926; Feller *et al.*, 2012). Spears allowed to grow beyond marketable size (> 20 cm) suppress adjacent buds more strongly than shorter-length spears. The longer the interval between growth of the first spear and its harvest, the longer is the suppression of growth of the second spear (Wilson *et al.*, 1999a; Feller *et al.*, 2012). Therefore, timely, regular harvests tend to increase spear productivity, provided temperatures are favorable. Removal of spears or fern allows smaller buds to grow, which accounts for the sequential production of spears throughout the growing season.

It was thought that inhibition of bud break by apical dominance extends only to buds within that bud cluster (Tiedjens, 1926; Feller *et al.*, 2012). However, strong interactions between spears growing from different bud clusters in seedling asparagus occur (Nichols and Woolley, 1985). A linear decrease in relative growth rate of spears emerging sequentially from different bud clusters has been noted, and this effect remained until the earlier emerging spears were harvested. However, when spears from different bud clusters began to grow at the same time, relative growth rates of all spears were similar but subsequent spear emergence was severely inhibited. These patterns may not exist in older plants since connectivity breaks down as the plant ages and diseases disrupt rhizome integrity.

Wilson *et al.* (2002b) and Graefe *et al.* (2010) formulated spear growth and yield models for asparagus. Models investigated the effects of varying climatic and management strategies on

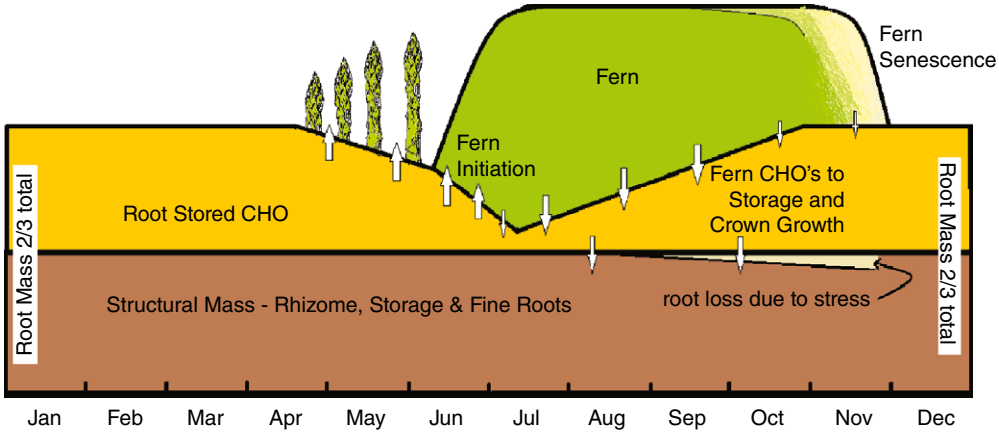

Fig. 18.2. Seasonal pattern of spear, fern, and root biomass accumulation in mature asparagus plant in a temperate production region. Arrows indicate direction and amount of carbohydrate (CHO) movement from roots to spears/fern (spring/summer) and from fern to roots (summer/fall). Some CHO are required to maintain root/rhizome growth. Early fern senescence will reduce CHO storage and contribute to root loss.

green asparagus crop performance (Wilson *et al.* 2002b). They noted that soluble carbohydrate (CHO) concentration and air temperature influence spear growth rate, while solar radiation during fern growth drove CHO accumulation and bud replacement. Graefe *et al.* (2010) used this model to assess daily estimates of spear appearance, size, and fresh weight from white asparagus. Due to apical dominance between spears within a bud cluster, there is a partly temperature-independent rhythmic emergence pattern in early harvest, which smoothed out as the simulation advanced. These models clearly demonstrated that older modeling approaches used to predict yield were inadequate (Blumenfield *et al.*, 1963; Lampert *et al.*, 1980; Liebig and Wiebe, 1982).

Large, healthy fern is required to produce large buds. Bud size is positively correlated with spear size (Tiedjens, 1926; Blasberg, 1932; Drost and Wilcox-Lee, 1997) and spear fresh weight (Nichols and Woolley, 1985). Large buds (diameter > 5 mm) may develop into small spears (Tiedjens, 1926), especially after a number of large diameter spears have been harvested. Small spear size or limited spear growth late in the harvest season is the result of decreased root CHO reserves (Wilson *et al.*, 1999b, 2008), fewer viable buds (Feller *et al.*, 2012), and/or a reduction in remaining bud size (Drost and Wilcox-Lee, 1997).

Fern

After harvest is completed, spears are allowed to grow and develop fern (Fig. 18.1). A balance between harvest length and fern growth is needed to maximize yields (Takatori *et al.*, 1970b; Shelton and Lacy, 1980; Wilson *et al.*, 1999b). This balance depends on the length of the growing season, harvest duration, plant size and age, and any factor that limits growth (Robb, 1984). Fern growth must remain vigorous throughout the fern growing season if bud and root development, growth, and CHO accumulation are to be maximized (Scott, 1954; Wilson *et al.*, 2009b). Fern vigor in one year and yield in the following year are correlated (Moon, 1976; Hartmann, 1985; Knaflewski, 1994), therefore, conditions that limit fern (insect and disease pressure, nutrient deficiency, drought, weed competition, etc.) limit future plant performance (Fig. 18.2).

During each growing period, shoots (spears and fern) are produced first, and only after this is nearly completed are new buds initiated (Tiedjens, 1926; Blasberg, 1932). Bud development is believed to be genetically controlled and not dependent on the growing environment and/or CHO availability of the plant (Feller *et al.*, 2012). Buds are initiated steadily throughout the growing season until the time of peak fern number in young (Haynes, 1987) and mature asparagus plantings (Wilcox-Lee and Drost, 1991). This suggests that new and existing fern growth directly or indirectly influences bud initiation. Some buds may be formed during the harvest season (Tiedjens, 1926; Woolley *et al.*, 2008) but it is more likely that this is continued enlargement of buds initiated in the previous season (Wilcox-Lee and Drost, 1991; Paschold *et al.*, 2008; Feller *et al.*, 2012).

As asparagus grows, more dry weight is partitioned to the crown and less to the fern (Dufault and Greig, 1983; Hughes *et al.*, 1990; Wilcox-Lee and Drost, 1990). The development of large crowns is desirable since it produces more spears, bigger fern, stores more CHO, and initiates more buds (Wilson *et al.*, 2002a; Drost and Wilson, 2003). Benson and Takatori (1980) reported that hybrid asparagus partitioned more dry weight to the crown than open pollinated cultivar even though both had similar leaf area. This suggests improved growth efficiency either by reduced respiration, higher photosynthetic rate, better canopy architecture to process available light, or improved quality of storage CHOs.

Root growth

While asparagus fern growth is well documented, access to roots has limited our understanding of their unique growth patterns. In establishing transplanted asparagus, root growth began six weeks after initial spear emergence (Dufault and Greig, 1983). In older established plantings, root production did not start until after the fern establishment and stopped when the fern began to senesce (Drost and Wilcox-Lee, 2000; Drost and Wilson, 2003; Paschold *et al.*, 2008).

There are two distinct root types, the large, unbranched fleshy adventitious and smaller fine lateral roots (Weaver and Bruner, 1927; Blasberg, 1932; Mullendore, 1935). The conventional

terminology of fleshy and fibrous roots will be used to distinguish between root types (Fig. 18.1). Fleshy roots are formed near young, actively growing buds, are unbranched, grow to 1–2 m long over several seasons, and vary in diameter from 2–6 mm. New fleshy roots are often initiated above older roots on the crown, which forces the crown upward in the soil (Young, 1940; Lindgren, 1990). Jones and Robbins (1928) identified three years of growth on fleshy roots while Scott *et al.* (1939) estimated fleshy roots survive for six years.

Fibrous roots are primarily nutrient and water absorbing organs (Blasberg, 1932). Fibrous roots originate from the pericycle of fleshy roots before wall thickening and epidermal suberization occurs (Mullendore, 1935). Old fleshy roots initiate only a few new fibrous roots and only on younger root parts (Tiedjens, 1926; Reijmerink, 1973). Fibrous roots may be branched or unbranched, grow 15–20 cm long, and are up to 2 mm in diameter (Blasberg, 1932). In asparagus, 60–90% of fibrous roots are in the upper 30–60 cm of soil (Reijmerink, 1973; Drost and Wilson, 2003). Continued fleshy and fibrous root initiation is important for long-term asparagus productivity.

Fibrous root growth occurs before and after harvest (Scott *et al.*, 1939; Drost and Wilcox-Lee, 2000; Drost and Wilson, 2003) and roots grow for one or two months before senescing (Drost and Wilcox-Lee, 2000; Drost and Wilson, 2003). Fibrous root growth near the soil surface is limited and may be due to fleshy root suberization (Tiedjens, 1926; Reijmerink, 1973), a build-up of soil toxins (Yang, 1982), a reduction in nutrient levels (Reijmerink, 1973), physical deterioration of the soil micro-structure, compaction or damage associated with tillage operations (Putnam, 1972; Wilcox-Lee and Drost, 1991; Drost and Wilson, 2003), or general aging of the fleshy roots.

Soil structure strongly influences asparagus root development, rooting depth, and growth. Open, porous soils allow good root development while densely packed or massive soils allow little root penetration (Reijmerink, 1973). Plant performance (yield) is closely correlated with soil type and texture (Drost and Wilson, 2003). Shallow soil disturbance is used to alleviate compaction (Niziolomski *et al.*, 2016), however, tillage increases the risk of root damage and diseases (*Phytophthora* and *Fusarium*) and leads to "asparagus decline" (Elmer *et al.*, 1996). Tillage also alters rooting patterns (Putnam, 1972; Wilcox-Lee and Drost, 1991; Drost and Wilcox-Lee, 2000) and root mass (Drost and Wilson, 2003).

Asparagus Physiology

Most asparagus research has focused on fern or spear growth and root CHO accumulation. Recent studies have related that information back to the assimilation capabilities of the spear or fern. Net photosynthesis in asparagus is a function of plant physiological age, light intensity, photosynthetic rate, temperature, water supply, and genetics. Each impacts photosynthesis and will be examined and related to asparagus performance. In addition, the biochemistry of CHO production and accumulation, redistribution and utilization, and how these interact with the environment and cultural practices, will be discussed.

Photosynthesis

All green tissues of asparagus are capable of photosynthesis. Cladophylls (Fig. 18.1) are the main assimilation sites (Downton and Torokfalvy, 1975; Lin and Hung, 1978; Faville *et al.*, 1999b) as they make up most of the plant surface area. Spears and stems have lower chlorophyll contents and reduced stomatal density compared to cladophylls (Schaller and Paschold, 2009). Spears fix a limited amount of CO_2 (Downton and Torokfalvy, 1975; Lin and Hung, 1978), but can re-fix respired CO_2 (Downton and Torokfalvy, 1975). Canopy net assimilation rates increase as more canopy is established and then decrease as fern ages or senesces (Bai and Kelly, 1999; Faville *et al.*, 1999b) and highest assimilation rates occur in summer and early fall in temperate regions (Fig. 18.3a). New fern needs to be continually initiated or periodically completely replaced in regions where there is no dormant period to ensure photosynthesis remains high.

Net photosynthesis in cladophylls increases as light levels increase up to the light saturation point of 400–500 μmol m^{-2} s^{-1} (Downton and

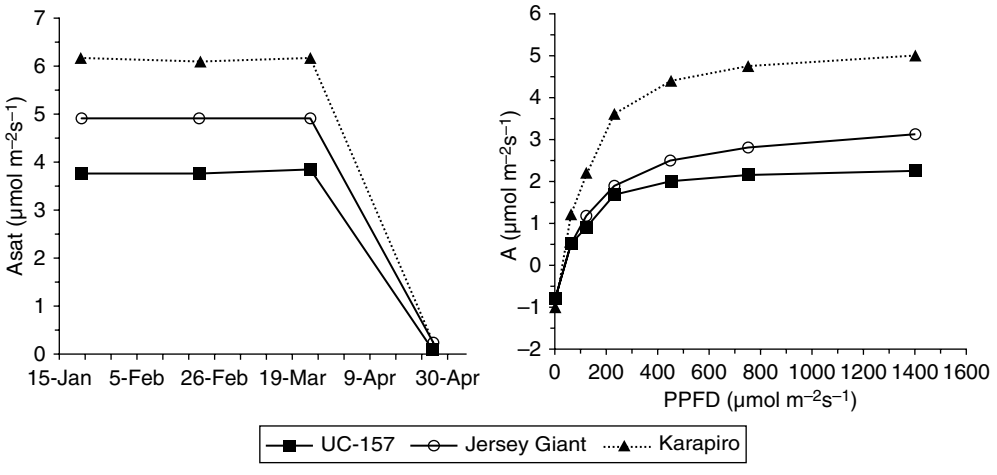

Fig. 18.3. Seasonal changes in light saturated fern photosynthesis (left) and fern photosynthetic rate (right) response to changes in photosynthetic photon flux density of three asparagus cultivars (Faville *et al*., 1999b).

Torokfalvy, 1975; Hills, 1986; Faville *et al*., 1999b) and has a quite low light compensation point (Yue *et al*., 1992; Faville *et al*., 1999b). Low light compensation and saturation points (leaf and canopy levels) suggest asparagus has characteristics of shade-tolerant plants (Fig. 18.3b). Cladophyll morphology (Schaller and Paschold, 2009) and fern architecture limit the number of cladophylls exposed to full sun conditions which maintains photosynthetic efficiency throughout the canopy under most growing conditions (Hills, 1986).

Maximum net assimilation rate in asparagus is quite variable with significant genotypic variation (Wilcox-Lee and Drost, 1990; Bai and Kelly, 1999; Faville *et al*., 1999b; Guo *et al*., 2002) and often decreases during the day (Drost and Wilcox-Lee, 1990; Guo *et al*., 2002). Sawada *et al*. (1962) and Pressman *et al*. (1989) reported fern CHO levels increase continually throughout the day suggesting assimilation exceeds export. Assimilate accumulation and export may respond to environmental conditions. Sucrose accumulation in cladophylls in the afternoon would maintain export in the dark or may buffer changing soil/plant water status, or temperature effects (Guo *et al*., 2002). However, photosynthates are often stored in fern during the day before being transported to the roots at night. In ^{13}C-labeling studies, Faville *et al*. (1999a) found that most of the label was translocated from treated fern to adjoining storage roots with smaller amount deposited in the associated buds. Very little labeled assimilate was detected in other bud clusters connected to the rhizome, confirming the independence of each bud unit on the plant (Feller *et al*., 2012).

Yue *et al*. (1992) reported low photosynthetic rates in asparagus plants compared to in vitro plantlets was due to stomatal closure brought on by water stress. Small changes in soil water potential caused large reductions in gas exchange (Drost and Wilcox-Lee, 1990; Schaller and Paschold, 2009) where assimilation rates increase in the morning and decrease in the afternoon when asparagus was grown in wetter soils but in dry soils assimilation decreased throughout the day (Drost and Wilcox-Lee, 1990). Schaller and Paschold (2009) reported genetic differences in drought tolerance in asparagus. In addition to stomatal pore length differences, variety "Grolim" used a drought avoidance mechanism to regulate water loss while variety "Gijnlim" employed a drought tolerance strategy. These differences may influence net photosynthesis under optimal conditions which could impact productivity.

Stomatal closure, decreasing fern water potentials, high temperatures, and high light levels could all be responsible for the reduction in assimilation that occurs during the afternoon (Drost and Wilcox-Lee, 1990; Bai and Kelly, 1999;

Guo *et al.*, 2002). The optimum temperature for photosynthesis in asparagus is less than 20°C (Sawada *et al.*, 1962; Yen *et al.*, 1993). At higher temperatures, assimilation rate drops off rapidly (Yue *et al.*, 1992; Yen *et al.*, 1993). However, in isolated asparagus mesophyll cells, photosynthetic rate continued to rise as temperature increased up to 35°C (Colman *et al.*, 1979; Hills, 1986).

Vigorous summer fern growth has a positive influence on asparagus spear yield in the following spring (Ellison *et al.*, 1960; Moon, 1976; Wolyn, 1993). Findings suggest genetic variation between cultivars may result from differences in photosynthetic efficiency (Benson and Takatori, 1980). Faville *et al.* (1999b), Bai and Kelly (1999), and Guo *et al.* (2002) noted a positive correlation between photosynthetic assimilation and spear yield among a variety of asparagus cultivars. Since genetic variability exists among asparagus genotypes for fern assimilation rates and this is correlated with yield, evaluation of photosynthesis may be another method to select for improved productivity.

Carbohydrate production

Asparagus accumulates fructans (fructose-yielding sugars when hydrolyzed) as stored CHOs (Meier and Reid, 1982; Pollock, 1986). Fleshy asparagus roots contain 35–40% non-reducing sugars, 5–7% reducing sugars, and < 5% starch (Scott *et al.*, 1939) and these make up 45–50% of storage root mass (Fig. 18.2). Pressman *et al.* (1989) noted that asparagus fern manufactures sucrose, glucose, and fructose but no fructans or starch. Guo *et al.* (2002) showed that starch levels increase in cladophyll during the day and decrease at night. However, only after sugars are transferred to fleshy roots does fructan synthesis occur. Besides fructans, root also contain small amounts of glucose, fructose, and sucrose (Pressman *et al.*, 1993).

Asparagus storage CHOs are fructo-oligo or polysaccharides, with the parent oligosaccharides consisting of 10% glucose and 90% fructose (Shelton and Lacy, 1980). Some shorter chain fructans with degree of polymerization less than 4 (DP ≤ 4) have been characterized for asparagus (Shiomi *et al.*, 1976, 1979; Shiomi, 1992). Several frutosyl transferase enzymes have been extracted, their properties detailed, and substrate specificities determined. This work has provided a comprehensive description of the in vitro synthesis of fructans found in asparagus root tissue (Pollock, 1986; Pollock and Chatterton, 1988).

Cairns (1992) reported that 81–98% of the total soluble fructans had a DP ≥ 5. This is consistent with data of Shelton and Lacy (1980) but higher than values found by others (Shiomi, 1992). The difference between these studies was attributed to when the samples were taken, the difficulty in quantifying the larger fructans (DP ≥ 9), and the methods used to measure them.

Seasonal changes in root CHOs (Fig. 18.2) have been known for more than a century (Morse, 1916; Haber, 1932; Scott *et al.*, 1939). Others built on this work and suggested that root CHO concentration could serve as an indicator of plant vigor (Shelton and Lacy, 1980; Haynes, 1987; Wilcox-Lee and Drost, 1991). Fleshy root CHOs levels decrease slowly during harvest (Fig. 18.2) as stored sugars are used for spear growth (Haynes, 1987; Wilson *et al.*, 1999a). Following harvest, spears grow rapidly into ferns, which depletes root CHOs quickly. After fern is fully established, CHOs increase steadily, reaching pre-harvest levels by late summer. Over-irrigation (or excessive rain) during summer can promote new fern growth and establishment at the expense of CHO storage (Wilson *et al.*, 1996) which adversely affects spear yield the following spring. Faville *et al.* (1999a) noted that when new fern growth in summer was minimal, existing root CHO levels were not depleted. Excessive pathogen pressure (insects or diseases) can also adversely affect asparagus fern which would severely impact stored CHOs (Elmer *et al.*, 1996).

Wilson and his team used this early work and focused considerable attention on how root systems and stored CHOs impact crop performance (Wilson *et al.*, 1999b; Wilson *et al.*, 2002a, 2008). They argued that for high production, one needs to manage both fern and root growth in the current year and over the life of the crop. Since asparagus growth is driven by CHOs which have known patterns of loss and gain (Fig. 18.2), benchmarked CHO levels at key times during growth helps maintain long-term crop productivity. Deviations from the "ideal" pattern identifies problems in production (Wilson *et al.*, 2008).

Most CHO information is expressed as a percentage of total sugars (Robb, 1984). However, percentage data makes interpretation of CHO content difficult as root mass is ignored. Haynes (1987) noted root mass changes affect total stored CHO. Therefore, both root mass and CHO content are better indicators of plant vigor (Scott *et al.*, 1939; Drost and Wilson, 2003).

Wilson *et al.* (2002a, 2008) efforts led to the development of a simple method to assess root CHO content. The "*Aspire*" system first evaluated storage root sap (BRIX%) as a proxy for analytical root CHO concentration. Second, it compared root CHO content patterns in different countries, with different climates and management systems. Third, it weighed the physiological capacity of asparagus to produce CHO during fern growth. These then helped system users understand how common management and production issues influence seasonal CHO patterns. Finally, it began to address the need to track root system size (Drost and Wilson, 2003). Ultimately it is variations in root CHO content that are most important. Recent work showed that root changes during the first five to six years after planting are quite substantial (Drost, 1999a, 2018; Paschold *et al.*, 2008), so tracking root CHO content and mass may address variations in early yield.

As temperatures decrease in fall, fern begin to senesce and dormancy is induced. Initially, fern sucrose levels increase as low temperatures decrease photosynthate translocation (Pressman *et al.*, 1993). Eventually, fern sucrose levels fall as shoots die and photosynthetic rates decline (Bai and Kelly, 1999; Landry and Wolyn, 2011; Panjtandoust and Wolyn, 2016). In roots, low temperatures also slow fructan accumulation as photosynthate translocation from fern slows. Root sucrose levels increase during the dormant period as fructans are broken down. Increasing sucrose levels may serve as a sprouting signal for dormant buds when temperatures rise (Landry and Wolyn, 2011).

Storage roots formed in the previous year supply most of the CHOs used for spear growth the following year (Robb, 1984), since new storage roots are in close proximity to the buds they supply. However, these new fleshy roots are small relative to older roots on the rhizome, suggesting all roots near the bud cluster contribute CHOs to fuel spear growth. Faville *et al.* (1999a) showed that the bulk of ^{13}C-label applied to an individual fern was translocated to buds, new fleshy roots and older storage roots, located near the active, growing end of the rhizome. Although all bud clusters were physically connected by the rhizome, ^{13}C-label did not move to other bud clusters on the rhizome. This indicated each rhizome segment with associated bud clusters and storage roots is an independent unit.

Increasing the harvest length of young asparagus significantly lowered root CHO levels during fern development and contributed to lower future spear yields (Haber, 1932; Takatori *et al.*, 1970b; Shelton and Lacy, 1980; Wilson *et al.*, 1999b; Paschold *et al.*, 2002). The effect of longer harvests is to increase the severity of CHO depletion and to decrease the time available for future CHO storage. Extended harvests also reduce total dry weight partitioned to the crown compared to shorter harvest intervals (Scott *et al.*, 1939). Therefore, fern growth must be maintained for a sufficient duration if CHO recharge is to be maximized. Future yields also decrease when harvests occur at other times of the year (Farish, 1937; Brasher, 1956; Dufault, 1991; Thomas *et al.*, 2011), thus careful plant management is needed to maintain productivity.

Crop Productivity

Asparagus growth and performance is influenced by environmental conditions, plant nutrition, harvest duration, plant growth regulators, genetics, and differences between male and female plants. While some information is available on the effects of cultural practices on crop yield, how these interact with asparagus physiology is not fully understood.

Temperature

Soil and air temperature play important roles in bud break and spear growth. A soil temperature of approximately 5°C is required for bud break (Bouwkamp and McCully, 1972; Nichols and Woolley, 1985; Heißner *et al.*, 2006) though a low of 0°C (Liebig and Wiebe, 1982) and a high of 11°C (Culpepper and Moon, 1939a) have been reported. The temperature threshold for

bud break varies with plant age, since one- and two-year-old plants often produce spears earlier in the spring than older plants (Robb, 1984). There are also strong cultivar differences in early season growth (Heißner *et al.*, 2006). Cultivar evaluations are used to identify cultivars that perform well in specific environments, and knowledge of emergence differences can be used to schedule production.

Since bud break and spear growth is initially a function of soil temperature, increased planting depths delay emergence, slow the rate of spear development (Takatori *et al.*, 1974; Lindgren, 1990; McCormick and Thomsen, 1990), and decrease total yield but increase the number and weight of large diameter spears (McCormick and Thomsen, 1990). In many production areas, soil is mounded over crowns to form a raised or ridged bed (Franklin *et al.*, 1981). Raised beds warm faster in the spring, provide better drainage in heavy soils, tend to have lower root disease incidence, but may expose early emerging spears to damaging low temperature conditions (Arora *et al.*, 1992) or increase runoff and erosion potential (Niziolomski *et al.*, 2016).

Plastic mulches are increasingly important in the production of white asparagus and help control soil temperature, used to alter harvest frequency, and alter plant development (Heißner *et al.*, 2005). Plastic mulches increased marketable and total yield but not spear number when used to grow white asparagus (Makus and Gonzalez, 1991) but create spear emergence and harvesting problems for green asparagus. In white asparagus production systems, ridge temperature significantly influences spear yield and quality (Heißner *et al.*, 2006) and cultivar selection used in these systems can be important for yield optimization. Using information on those factors affecting spear growth and yield, Graefe *et al.* (2010) developed a process-oriented, stochastic model for white asparagus grown under soil ridges. The model adequately predicted daily spear number, diameter, and yield over the whole harvest season across several production sites with various soil temperatures induced by different types of ridge coverings. These yield/quality models can provide useful applications for producers aiming to better schedule on-farm production.

Spear growth is most active and responsive to temperature in the area just below the tip (Tiedjens, 1924; Culpepper and Moon, 1939a, 1939b). Consequently, for green asparagus, spear growth is influenced more by air temperature than soil temperature after spear emergence from the soil (Bouwkamp and McCully, 1972; Wilson *et al.*, 1999a). Early emergence from shallow planted crowns exposes spears (Lindgren, 1990; Arora *et al.*, 1992) and crowns (Lindgren, 1990) to injury from spring frosts. Since early emerging spears are often the largest, frosts often reduce crop yields. Where conditions are not as harsh, the crop is marketed earlier in the spring though spear size is often reduced (Takatori *et al.*, 1974; Lindgren, 1990).

Spear growth rate increases linearly as temperature increases from 10 to 32°C (Blumenfield *et al.*, 1963; Nichols and Woolley, 1985; Dean, 1999) but growth is curvilinear at temperatures below 10°C. Spears and ferns of different heights differ in their response to temperature (Culpepper and Moon, 1939b). At low air temperatures, spear growth rate is slow (Fig. 18.4) which delays the harvest of the elongating spear and further delays the growth of the next bud in the bud cluster (Wilson *et al.*, 1999a; Ku *et al.*, 2007). Regardless of the temperature, the growth increase during a 24-h period was greatest for short spears (5 cm) and slowed as spear height increased (Culpepper and Moon, 1939b). Rapid growth of young spears grown at high temperatures requires more frequent harvests to maintain spear quality, since high temperatures promotes "feathering" (lateral branch growth) which decreases marketability (Roth and Gardner, 1989). Cultivars vary in lateral branch formation height which is a selection criterion for cultivar adaptation to specific growing areas (Ellison, 1986). Fern age also influences stalk sensitivity to temperature, as old and young stalks are less sensitive than recently mature stalks to changes in temperature (Culpepper and Moon, 1939b).

Decreasing fall temperatures result in cold acclimation and freezing tolerance acquisition in asparagus (Landry and Wolyn, 2011; Kim and Wolyn, 2015; Panjtandoust and Wolyn, 2016). Cultivars vary in their adaptation to cold and level of winter hardiness. Cultivars with increased freezing tolerance generally had high concentrations of low-molecular-weight fructans, proteins, and proline and low sucrose concentrations in the rhizome, and high sucrose and

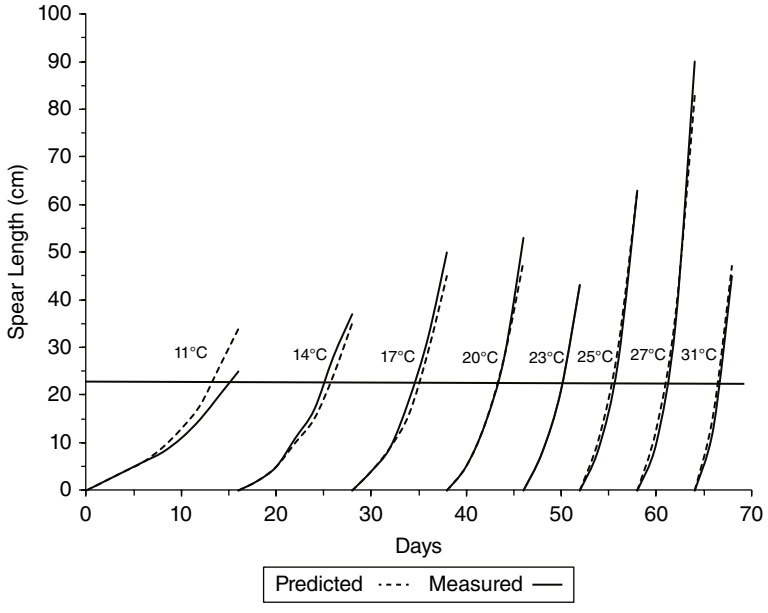

Fig. 18.4. Measured (solid lines) and predicted (dashed lines) spear lengths (cm) over time (days) for different air temperatures (Culpepper and Moon, 1939a; Wilson *et al*., 1999a). Lines can be used to estimate number of days needed to grow a spear to harvest length (23 cm) for different air temperatures.

proline concentrations and low concentrations of low-molecular-weight fructans in storage roots. Winter hardiness in adapted cultivars was associated with early acclimation and acquisition of freezing tolerance in fall and late de-acclimation/de-hardening in spring. Understanding these responses provides clues to those factors involved in bud/crown dormancy, bud break, and spear growth rates.

Moisture

Asparagus has been classified as a drought-tolerant plant but most research shows growth improves when water is supplied under moisture deficit situations. The best growth of seedling asparagus was when soils were maintained close to field capacity as better irrigation management during establishment improved plant survival (Sterrett *et al*., 1990), increased root growth and distribution (Drost, 1999a) and enhanced yields during early harvest years (Sterrett *et al*., 1990; Drost, 1999b).

Irrigation is usually not necessary during harvest since spear water use is low (Roth and Gardner, 1990) and moisture can cool soils resulting in slow spear elongation and decreased yields (Cannell and Takatori, 1970). Dry soils in spring reduce the number of times the crop is harvested, which also reduces yield (Brasher, 1956). Irrigation may be applied during harvest to reduce spear damage from blowing sand, firm the soil to improve foot traffic for harvest, and to reduce feathering in hot weather (Roth and Gardner, 1989).

Water shortages during summer can result in fern wilting (Van Bakel and Kerstens, 1971), limit fern and root growth (Takatori *et al*., 1970a; Wilcox-Lee and Drost, 1990), and decrease photosynthesis and CHO storage (Pressman *et al*., 1989). Total yield decreased in the year following extremely dry seasons (Farish, 1937), and drought stress decreased the number of buds initiated (Drost, 1999b). Irrigation increases yield by increasing the number (Takatori *et al*., 1970a; Sterrett *et al*., 1990; Drost, 1999b) or the size of spears (Drost and Wilcox-Lee, 1997). When precipitation levels increased during summer, asparagus yields were high the following year (Hartmann, 1985; Hartmann *et al*., 1990). However, over-irrigation/precipitation

may stimulate excessive fern growth or late season spear emergence which reduces storage root CHO accumulation and may decrease yield the following year.

Low soil moisture levels also influence root CHO content. Drought induces early fern senescence and decreases glucose, sucrose and fructose content of the fern. At the same time, fructan levels decrease in the roots as respiration rates increase (Pressman *et al.*, 1989). Since photosynthetic efficiency is reduced under drought stress, changes in fern and root CHO balance may be expected.

In production areas where low temperature does not restrict growth (e.g. Arizona, California, Mexico, Peru), asparagus dormancy induction and growth resumption are often imposed by regulating water supply (Cannell and Takatori, 1970; Roth and Gardner, 1989, 1990). In Peru, commercial fields are brought into production by stimulating spear growth with irrigation (Toledo, 1990). After a four- to six-week harvest period, the fern is allowed to grow for up to four months. Dormancy is then induced by withholding water. In the humid tropics, asparagus is produced by the "mother stalk" technique. Several ferns per plant are allowed to grow and the remaining spears are harvested. New fern is generated every three or four months as the older fern senesce and efficiency of assimilation decreases (Lin and Hung, 1978). With proper scheduling, asparagus spears could be produced every month of the year with either of these systems.

Nutrition

Much of the work on asparagus nutrition has focused on young plants (Fisher and Benson, 1983; Adler *et al.*, 1984). Few studies address the long-term nutritional needs of the crop. Brown and Carolus (1965) reported that the annual spear removal of nitrogen (N), phosphorus (P), and potassium (K) amounted to 7, 2 and 5%, respectively, of applied fertilizer. The rest of the NPK in the fern is recycled to the rhizome and roots during senescence or added back to the soil when the fern is mowed. Since spear growth is initiated from stored CHOs, nutrients applied and stored in the prior year have the most effect on future plant performance. Some nutrients are absorbed in the spring (Morse, 1916), however most of the nutrient utilization occurs after harvest.

Nitrogen has the greatest effect on yield when it is applied after harvest (Hartmann and Wuchner, 1979; Sanders and Bandele, 1985; Ledgard *et al.*, 1992). In temperate areas, N applications average 50–100 kg ha^{-1} (Franklin *et al.*, 1981; Sandsted *et al.*, 1985; Paschold *et al.*, 2001). In arid irrigated areas, 350–550 kg N ha^{-1} are often applied (Cannell and Takatori, 1970; Roth and Gardner, 1989, 1990). Smaller, frequent applications of nitrogen are applied where growing seasons are long and irrigation frequency and rates are high.

Ledgard *et al.* (1992, 1994) used ^{15}N-labelled nitrogen to study the uptake and distribution of N within mature asparagus. They estimated 700 kg N ha^{-1} was stored in asparagus rhizome and roots. Less than 6% (30–40 kg N ha^{-1}) of this N was later removed by the harvested spear and N uptake during harvest was minimal. Nitrogen applied before or during harvest was not utilized until fern growth occurred (Ledgard *et al.*, 1992) since there is very little root growth during that time (Wilcox-Lee and Drost, 1991). Nitrogen uptake from the soil increased as fern developed and N accumulated in fern equivalent to 5 kg N ha^{-1} day^{-1} during an eight-week period (Ledgard *et al.*, 1994) with accumulated N coming from root N reserves and soil applied N fertilizer. Timing N applications to meet this high demand period is critical if performance is to be optimized and leaching losses minimized. As fern senesced, approximately 90% of the ^{15}N was remobilized and translocated to the crown (rhizome and fleshy roots).

Arginine and asparagine are the major free amino acids involved in nitrogen storage in asparagus (Fiala and Jolivet, 1982). Amino acids, like CHOs, decreased rapidly as new shoots developed in the spring (Fig. 18.2). However, accumulation of amino acids in fleshy roots to their pre-harvest levels required most of the fern growing period (Haynes, 1987). Increases in root amino acid levels are associated with the senescence of the shoots and a re-mobilization of nitrogen out of senescing fern.

Asparagus has low P and K requirements (Morse, 1916) though yield responses to added P have been reported (Hartmann and Wuchner, 1979; Drost, 2018). Phosphorus additions are made prior to planting the crop (Franklin *et al.*, 1981;

Dean *et al.*, 1993) with additional P applied as warranted. Sommerville and Whalen (2005) noted that increasing P applications during early establishment and production years did not improve productivity, but led to a buildup of soil phosphorus. Annual P removal of 2–3% of applied fertilizer has been reported and studies estimate asparagus extracts only 5–15 $kg \cdot ha^{-1}$ $P \cdot year^{-1}$ (Brown and Carolus, 1965; Hartmann and Wuchner, 1979; Sommerville and Whalen, 2005). Given the low removal values, a single application of P at planting was sufficient to supply the long-term P needs of asparagus (Drost, 2018). Sanders and Bandele (1985) reported yields increase as K levels increase from 0–93 kg ha^{-1}. Additional K applications should be made based on a soil test (Franklin *et al.*, 1981; Sandsted *et al.*, 1985). Trace minerals needs vary and depend on local soil conditions (Brown and Carolus, 1965; Dean *et al.*, 1993). Generally, nutrient deficiencies are not common because the large root system stores large quantities of nutrients.

Harvest duration

Asparagus is traditionally harvested in the spring in temperate climates. Harvesting is labor-intensive (Brown, 1984) requiring daily or twice daily harvests for green spears to meet market standards. Harvest duration extends for 2–12 weeks depending on plant age. Prolonged harvests increased asparagus yield in the harvest year but decreased the percentage of large-sized spears (Jones, 1932; Paschold *et al.*, 2002). Others note yield decreases in the year following the extended harvest season (Haber, 1932; Takatori *et al.*, 1970b; Williams and Garthwaite, 1973; Shelton and Lacy, 1980). Paschold *et al.* (2002) recommended that as crop age increases, harvest season should be shortened since thicker, higher-value spears appears early in a short harvest season and more CHOs are preserved. Feller *et al.* (2012) observed that yield was limited by a lack of viable buds rather than low root CHO status when harvest was extended.

Asparagus yield depends on stored CHO (Paschold *et al.*, 2002) and the number of available buds produced (Woolley *et al.*, 2008; Feller *et al.*, 2012) in the season before harvest. In extended harvest production systems (Shelton and Lacy, 1980; Dufault, 1991; Wilson *et al.*, 1999b), fern growth duration after harvest is often insufficient to replace used CHOs which contributes to asparagus decline (Fig. 18.2). Thomas *et al.* (2011) demonstrated that repetitive off-season asparagus harvest was not a viable production practice because post-harvest fern growth was insufficient to replenish CHO reserves. Shelton and Lacy (1980) and Wilson *et al.* (1999b) in extended harvests noted that yield was limited more by a lack of stored CHOs in the storage roots while Feller *et al.* (2012) in controlled studies identified a lack of viable buds as the yield limiting factor. Ideally, harvest should be appropriate for the environment and allow sufficient time after harvest to grow the fern needed to recharge root CHOs and replace harvested buds (Paschold *et al.*, 2002; Fig. 18.5).

Plant growth regulators

Asparagus is generally hand harvested though different mechanical harvesters have been used (Mears *et al.*, 1969; Cemballi *et al.*, 2007). Since harvesting can account for more than half of the cost of production (Kepner, 1971), efforts to synchronize spear emergence for mechanical harvest becomes economically viable (Brown, 1984). Several plant growth substances influence bud break and spear growth (Dedolph *et al.*, 1963) and may be useful in asparagus production systems.

Abscisic acid (ABA) often acts as a growth inhibiting substance and in asparagus buds, ABA levels are high during summer and peak during dormancy (winter). ABA is presumably produced in fern and transported to the roots (Matsubara, 1980). Kojima *et al.* (1993) speculated that ABA regulates assimilate partitioning thus maintaining apical dominance and restricting bud elongation while high levels in winter control dormancy. As ABA levels decrease in the spring, apical dominance is released and buds begin to grow. Matsubara (1980) noted that dormant buds contain three times more ABA than sprouting buds, with concentrations highest near the tip and decreasing basipetally within the spear (Kojima *et al.*, 1993).

Gibberellic acid (GA) promotes asparagus bud elongation since apical dominance is reduced by applied GA and increases spear number (Kretschmer and Hartmann, 1979) and growth

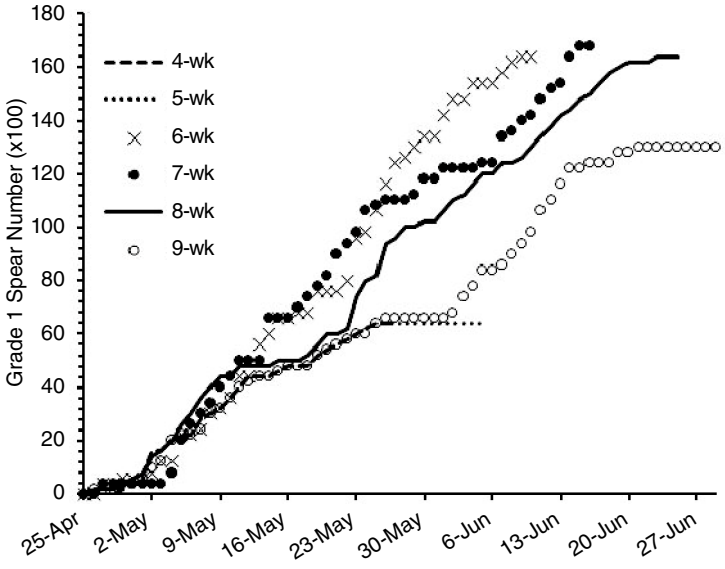

Fig. 18.5. The seasonal accumulation of grade 1 spears after six years of various harvest durations (four to nine weeks). Harvests were terminated each year from late May to late June. As asparagus fields age, harvests should be progressively shorter, as higher value grade 1 spear production (six- and seven-week treatments) is concentrated (Paschold *et al*., 2002).

(Tiburcio, 1961). In other studies, GA had little effect on spear growth (Mahotiere, 1976). Ancymidol, a GA synthesis inhibitor, promoted root and shoot growth in tissue-cultured plants (Chin, 1982), while in seedling asparagus, ancymidol decreased bud number and fern dry weight (Adler *et al*., 1984).

Although auxins are known to be involved in promoting plant growth, indoleacetic acid (IAA) inhibits spear emergence (Tiburcio, 1961). When GA is applied after IAA, spear elongation occurs where it previously had been inhibited. Application of napthaleneacetic acid to crowns had no effect on spear number or growth (Kretschmer and Hartmann, 1979) but did increase crown dry weight. Ethephon, an ethylene precursor, applied to crowns did not affect spear emergence or diameter (Mahotiere, 1976) but did increase spear number and fresh weight.

Other plant growth regulators have been tried in an attempt to stimulate spear growth. Chloroflurenol increased spear growth but caused spear deformation while benzyladenine reportedly stimulated spear emergence in spring when applied to healthy fern in fall (Mahotiere *et al*., 1993). While some of the plant growth regulators may be useful in asparagus production systems, difficulty in applying the materials and variable results limits their effectiveness.

Genetics

Cultivated asparagus is dioecious (2n = 2x = 20) with a haploid genome size of approximately 1308 Mb (Ellison, 1986). Historically breeding efforts focused on high yields, disease resistance, all-male hybrids, and improved spear quality. Early research tried to identify highly productive breeding lines during the first years after planting (Falloon, 1982; Ellison, 1986). This approach was not always satisfactory but selection could be improved by identifying high-yielding plants after the second or third harvest year (Moon, 1976). Falloon and Nikoloff (1986) noted there was no correlation between fern vigor, fern number, or early market yield in early evaluation years and mean market yield over longer time periods. Often breeding line selection is based on simple plant characteristics (fern number, fern vigor, early yield) which commonly correlate with early yield (Coyne, 1967; Moon, 1976; Ellison, 1986) but rarely correlate with high long-term productivity and crop longevity.

Sex in asparagus is determined by the Y chromosome in males which dominantly suppresses female organogenesis and promotes development of fertile anthers. There is known variation in floral development in asparagus where some staminate flowers on male plants produce viable pistils and produce seed (Rick and Hanna, 1943; Sneep, 1953). When seed from these berry-producing andromonoecious males is grown out, it segregates at 3Y:1X suggesting sex is inherited by a simple Mendelian factor. Of those males produced, one-third are homozygous YY and produce only male plants when crossed with females. These "super-males" are sought after and are the foundation of most existing asparagus breeding programs. The viability of the YY "super-male" genotypes is then derived through anther culture or by selfing andromonoecious plants (Franken, 1970; Peng and Wolyn, 1999; Falavigna and Casali, 2002).

Genomic molecular markers have been used for surveys of plant parentage variation (Khandka *et al.*, 1996; Caruso *et al.*, 2008), sex (Jiang and Sink, 1997; Kanno *et al.*, 2014) and development of genetic (Jiang and Sink, 1997; Spada *et al.*, 1998) and physical maps (Telgmann-Rauber *et al.*, 2007). Expressed Sequence Tags (EST) and RNA sequence data are further used to develop gene-linked and often more informative molecular markers. With next-generation sequencing, Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphisms (SNP) will help identify diagnostic alleles in asparagus germplasm.

EST data have been used to develop large SSR and SNPs marker panels for analyses of ancestry and marker-assisted breeding in asparagus (Caruso *et al.*, 2008; Mercati *et al.*, 2013). SNP markers help identify parental relationships within anther donor and double haploids lines used in several international breeding programs (Riccardi *et al.*, 2011; Mercati *et al.*, 2015). Findings show that double haploids collections were derived from early French and German parentages, important ancestral lines of many modern asparagus cultivars.

Molecular markers in *A. officinalis* have the potential to improve breeding through use in Marker-Assisted Selection but abundant genome-wide molecular markers are necessary for this effort. Currently the number of molecular markers for *A. officinalis* is still limited (Caruso *et al.*, 2008). Asparagus is a model plant for the study of sex determination and the evolution of sex chromosomes due to its dioecious nature. Microsatellites play important roles in the evolution of sex chromosomes (Li *et al.*, 2014) and variation in microsatellites in the asparagus genome provide a platform for the evolution of sex chromosomes. While the genetic diversity of *A. officinalis* cultivars is small (Li *et al.*, 2016) and asparagus has low heterogeneity and a relatively limited gene pool (Stajner *et al.*, 2002), there is evidence of single sex clustering within some cultivars (Li *et al.*, 2014, 2016). This research is unraveling the molecular basis of dioecy and early sex determination genes (Harkness *et al.*, 2015).

Developmentally, the chronological separation of male and female organ abortion in *A. officinalis* suggests two genes are involved in sex determination (Caporali *et al.*, 1994). Early in flower development, floral meristems of female, male and super-male genotypes are indistinguishable. However, observable gender differences occur earlier in XY males and super-males when the gynoecium fails to elongate and fuse to form a mature stylar tube. In female flowers, anther degeneration occurs when the tapetum breaks down before the completion of microsporogenesis, ultimately leading to anther sterility (Caporali *et al.*, 1994).

RNA-Sequence transcriptome assembly and expression analysis was used to investigate gender determination in asparagus (Harkness *et al.*, 2015). While many genes exhibit sex-based expression, few actually regulate gender determination. While hundreds of differentially expressed genes were identified, more genes exhibited male-biased than female-biased expression in asparagus. Interestingly, genes exhibiting male or female expression function downstream of gender specification in the developing flower bud. Male-specific expression for known pollen development genes and organ-specifying genes and several tapetum development genes were identified, corroborating Caporali *et al.* (1994) findings. With these recent developments and continued efforts to sequence the asparagus genome, we are getting a better understanding of how to improve asparagus.

Sex differences

One important advance in asparagus research and production has been the development of all male hybrids. Male plants are more desirable

because they have greater marketable yields, produce more spears, have earlier season production, less lag time between emergence of individual spears, longer production seasons, and a lower mortality rate compared to female plants (Ellison *et al.*, 1960; Moon, 1976; Ellison, 1986).

The reason for low yields and poor longevity of female plants in dioecious asparagus cultivars has never been clearly identified (Bouwkamp and McCully, 1972; Fiala and Joliet, 1979 Jolivet in refs). In dioecious lines, a 1:1 ratio of males to females exists at planting (Moon, 1976) which increases to 2.5:1 in older plantings (Yeager and Scott, 1938). Factors other than competition are responsible for the high mortality in females (Bouwkamp and McCully, 1972) though what these factors are is unknown.

Sex differences play a role in spear growth suppression and apical dominance signal strength. Tiedjens (1926) noted that the interval between first bud harvest and growth of the next bud in a bud cluster is longer for pistillate than staminate plants. Staminate plants have higher CHO content than pistillate plants and utilize a greater part of it in spear production (Fiala and Joliet, 1979). Bai and Kelly (1999) and Faville *et al.* (1999b) both reported that all-male varieties consistently had higher photosynthetic rates when compared to dioecious open-pollinated varieties (Fig. 18.3). Thus, increased CHO production may partly explain the higher yield of male plants. Sinton and Wilson (1999) confirmed much of the earlier work on productivity differences between male and female plants in mixed populations. Male plants grew larger root systems, accumulated more CHO, and initiated more buds than female plants.

Benson (1982) noted that female plants had greater fern area than male plants, which may relate to differences in assimilation rate, CHO accumulation, and productivity. Others found a 28% decrease in the assimilation rate of female compared to male plants (Sawada *et al.*, 1962), suggesting photosynthetic production may not be as efficient even though leaf areas may be greater. Lower assimilation rates would decrease CHO production which may lower productivity (Bai and Kelly, 1999; Faville *et al.*, 1999b), and possibly shorten longevity. Male and female performance differences may be due to berry and seed production by female plants (Jones and Robbins, 1928; Jones, 1932), though no studies have specifically looked at this. Sinton and Wilson (1999) did note that berry production caused stem breakage which contributes to reduced CHO accumulation.

Physiological Disorders

Allelopathy

Yield reductions commonly occur as asparagus plantations age (Grogan and Kimball, 1959; Johnston *et al.*, 1979) and most reductions are attributed to stand loss (Wilson *et al.*, 2008). Replanting new seedlings into these fields is often unsuccessful. Seed treatments and soil fumigation have been used in old asparagus fields to improve stands but stunting and wilting still occurs (Wiebe, 1967). Evidence shows that asparagus plants produce substances that are both autotoxic (self-toxic) and allelopathic (Yang, 1982; Young, 1984; Shafer and Garrison, 1986; Hartung *et al.*, 1990). Growth suppression is often exclusive of any disease problems (Yeasmin *et al.*, 2013) though in the field, rhizome and root rot organisms were isolated from infected fields (Hartung *et al.*, 1990).

Rhizome and root infection by *Fusarium* contributes to production decline in established asparagus fields (Cohen and Heald, 1941; Grogan and Kimball, 1959; Johnston *et al.*, 1979). Identified allelopathic compounds produced by asparagus interact with *Fusarium* (Peirce and Colby, 1987; Hartung *et al.*, 1989) and those allelochemicals have direct physiological and biochemical effects that predispose asparagus to *Fusarium* infection (Hartung *et al.*, 1989). Damaged root tissue had higher levels of electrolyte efflux (more permeable membranes) and decreased peroxidase activity which increased their susceptibility to disease. Root cells also had decreased respiration rates suggesting reduced biochemical activity. Thus, *Fusarium* infection increased because *Fusarium* growth was stimulated by the autotoxic materials leaking from asparagus root. Blok and Bollen (1996) question reported allelopathic and autotoxic effects since they found no appreciable increase of *Fusarium* root rot or *Fusarium* populations in soils due to substances present in asparagus root residues.

Allelopathic compounds produced by growing roots and released by dead asparagus roots persist in soil for many years (Blok and Bollen, 1993). In the laboratory, toxins begin to decline in

three to eight months (Yang, 1982; Shafer and Garrison, 1986; Hartung *et al.*, 1989). With adequate time between terminating the old planting and replanting the new asparagus crop, soil effects can be alleviated. There are varietal differences in allelochemical production and allelopathic and autotoxic activity which could be exploited when addressing replant intervals (Yeasmin *et al.*, 2014). The loss of toxicity in soil may be due to leaching of the allelochemicals or by microbial breakdown. The length of time necessary to detoxify the soil ultimately depends upon the amount of root tissue present (Shafer and Garrison, 1986) with most production guides recommending more than ten years before re-planting.

Early work on allelopathy found that asparagusic acid and related compounds isolated from asparagus inhibited the germination and growth of other crop species (Yanagawa *et al.*, 1972, 1973) but asparagusic acid was not toxic to asparagus. Cinnamic acids, including caffeic, ferulic or methylenedioxycinnamic acid, are autotoxic or allelopathic to asparagus (Hartung *et al.*, 1990; Miller *et al.*, 1991). Cinnamic acids have been isolated from asparagus roots and are known to inhibit seed germination of other crops (Rice, 1984; Hartung *et al.*, 1990) and asparagus (Miller *et al.*, 1991; Peirce and Miller, 1993). Root tips and epidermal cells of asparagus radicles are altered after exposure to cinnamic acids (Peirce and Miller, 1993) which may enhance *Fusarium* infection of asparagus root. Other identified allelochemicals toxic to asparagus were oxalic, succinic and tartaric acids (Yeasmin *et al.*, 2013) and some cultivars produced higher concentrations of total allelochemicals (Yeasmin *et al.*, 2014).

Concluding Remarks

Growers are "pushing" production (earlier in the season and life cycle) which leads to an early return on investment but a shorting of field life expectancy. Ideally, the length of the harvest should balance with the time required to grow roots and buds and store CHOs. Thus, in temperate production areas, asparagus is harvested in the spring, fern grows in summer, and the plant becomes dormant in winter. How these time periods are managed over many years will affect plant productivity. In tropical areas, these patterns also exist but harvest can in theory occur at any time of the year because temperature is not the growth limiting input.

Productivity in asparagus is tied to the length of the harvest period, fern vigor during summer growth, and conditions that stress the plant at any time. Environmental and cultural conditions imposed on the plant in one year strongly impact plant performance in subsequent years. Stress effects are commonly manifested by a decrease in fern, root, and crown growth, reduced storage of CHOs, and/or a reduction in number or size of buds. Since these growth processes all occur simultaneously each year, proper management of the crop during the fern growth period is critical.

While significant advances have been made in breeding productive asparagus, we are just beginning to understand the long-term implications of stresses on future productivity. As our understanding of the physiology of asparagus increases (CHO accumulation and utilization) and we learn more about the genetic controls of these events, this information will be used to control bud break and standardize spear growth. Additional efforts are needed to exploit the genetic potential of asparagus in ways that increase productivity, decrease pest pressures, and enhance longevity, which will significantly increase the sustainability of asparagus.

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