Biostimulants for Crop Production and Sustainable Agriculture

Edited by
Mirza Hasanuzzaman,
Barbara Hawrylak-Nowak,
Tofazzal Islam and **Masayuki Fujita**

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Preface

Humanity in the twenty-first century is facing numerous challenges due to the accelerated rate of global climate change. Agricultural productivity is highly vulnerable to climate change-associated erratic changes in meteorologic and edaphic parameters such as temperature, light intensity, precipitation, drought and soil salinity. On the other hand, the world population is projected to reach around 9.7 billion by 2050. To keep up with the pace of population growth, food production will need to increase by 70–100% by 2050 for a well-fed world population. Improved crop production technologies are urgently needed to meet the growing demand for food for the ever-increasing population in the world by addressing the impacts of changing climate on agriculture.

In modern agriculture, new solutions alternative to the currently used hazardous agrochemicals are sought that would help to obtain high crop productivity without affecting the environment. One such promising alternative is plant biostimulant substances. The biostimulants are non-fertilizer exogenous substances, compounds, or microorganisms applied to plants or to the rhizosphere that can stimulate physiological and biochemical processes or can enrich the soil microbiome to improve plant growth, nutrition, nutrient use efficiency, abiotic and/or biotic stress tolerance, yield and quality of crop plants. Plant biostimulants cover a wide range of products, including both single compound and complex formulations with various bioactive components and/or microorganisms. The application of biostimulants is generally safe for the environment. Therefore, they are considered novel solutions in sustainable agricultural practices with the maintenance of the ecological balance.

A large body of literature is available on the impacts of various groups of biostimulants on improved crop productivity and enhanced tolerance of crop plants to various biotic and abiotic stresses. This book updates current knowledge and application of biostimulants in different cropping systems. Written by the leading experts, this book provides useful experimental data, critical discussion and insights on recent progress in the fascinating roles of various groups of biostimulants in increasing the quantity and quality of crop production. A total of 31 chapters cover the various aspects of the effects and functional mechanisms of a wide range of natural substances and their derivatives, natural and synthetic compounds, and valuable microorganisms on crop plants. Although a diverse group of biostimulants cataloged in the literature is covered in this book, there is also a discussion on new, promising biostimulants with a novel mode of action. There is an intense research initiative covering discovery of new biostimulants that are useful for sustainable crop production in the changing climate.

The first chapter provides an in-depth introduction to the concept of biostimulants and their roles in sustainable agriculture. The remaining chapters cover plant growth-stimulating effects and the mechanism of actions of diverse groups of biostimulants, such as trace elements, plant and seaweed extracts, humic substances, polyamines, osmolytes, vitamins, nanoparticles, microorganisms, and their roles in plant stress tolerance. A large number of illustrations show the principles of biostimulants outlined in the text. This book is an important contribution to the flow of knowledge and critical discussion among researchers, commercial enterprises, policy makers, and practitioners, including organic farmers. Moreover, the information and experimental evidence presented in each chapter should appeal to a wide range of readers, including researchers, teachers, students, entrepreneurs, environmentalists, policy-makers and end-user farmers.

We sincerely appreciate the authors for sharing their knowledge, experience, critical discussion for preparing and timely revising their interesting chapters. We are thankful to the knowledgeable reviewers for valuable suggestions and critical remarks for the improvement of each chapter. We also thank Rebecca Stubbs, Commissioning Editor, and Emma McCann of the CABI for excellent editorial assistance and support during the editorial process of this book. Prof. Kamrun Nahar, Dr. Khursheda Parvin, Abdul Awal Chowdhury Masud, Taufika Islam Anee, Farzana Nowroz and Md. Rakib Hossain Raihan deserve our profound appreciation for their valuable suggestion, style editing, and proofreading.

We hope that this volume will be a reference book for all readers who are interested in biostimulants. Happy reading!

Mirza Hasanuzzaman, Barbara Hawrylak-Nowak, Tofazzal Islam and Masayuki Fujita

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1 Biostimulants in Sustainable Agriculture

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Abstract

In an attempt to understand the role of biostimulants in sustainable agriculture, the concerns that arise are matters regarding the possible need for sustainable agriculture and their exact efficacy in promoting sustainable agriculture. Although conventional agricultural practices were initially aimed at increasing plant growth, it gradually became a reason for soil ecology exploitation as a result of the heavy application of fertilizers and various synthetic chemicals such as pesticides (e.g. DDT, carbamate), herbicides (e.g. atrazine), insecticides (e.g. endosulfan, phorate), fungicides (e.g. mancozeb), etc. This very reason led to the introduction of sustainable agriculture that has, so far, proven to be beneficial in balancing the soil ecology by utilizing minimal fertilizers and synthetic chemicals. Hence, sustainable agriculture can be thought of as a promise for a better future for eco-friendly farming. The introduction of sustainable agriculture allowed biostimulants to bring about changes in physiological, structural and metabolic processes to influence plant growth via reduced use of synthetic chemicals and fertilizers, improved tolerance to various biotic and abiotic stresses, efficacy in nutrient uptake and use, and improved, good quality yield. Moreover, biostimulants have also increased plant resistance to pests, biological contaminants or diseases by stimulating their natural defense systems. This chapter focuses on the definition of biostimulants, types of biostimulants, formulations of biostimulants, effects of biostimulants on plant physiology and metabolism, advantages and disadvantages of biostimulant application, challenges faced in this field and the future of biostimulants in sustainable agriculture.

1.1 Introduction

From the beginning of human civilization, agriculture, in disguise, has potentially served as a mere means of survival for living beings walking on this planet. Even though the practice of 'agriculture' existed from a long time, the term itself came into existence much later. The history of basic agriculture dates back to times when people lived in caves. In other words, it has been a developmental criterion in the advancement of human civilization. For decades, development in the agricultural field has been quite slow; however, farmers from Asia, Africa and Europe came up with new techniques, including open field cultivation, which allowed them to harvest edible crops (Vasey, 2002). Crop cultivation began independently in South and North America. While the initial evidence of crop cultivation has been found to be approximately 10,000 years before present in Mexico and South America, earliest evidence of crop production in North

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America has been observed to have begun between 5000 and 4000 years before present.

However, with time, many hurdles baffled traditional farmers which eventually led to the discovery of machines having potential to perform processes, farming tools, use of natural fertilizers, and pesticides and water pumps accompanied by electricity. By the 1900s, a farmer was able to harvest enough food crop to sustain a family of five members and another hundred population. All of this was possible after a major discovery that took place in 1866, when Gregor Mendel paved the way to major breakthroughs in the agricultural sector through the discovery of genetics. However, the real issue occurred with the invasion of the agricultural fields by pests that ranged from insects to animals such as mice and rabbits, disease-causing organisms including viruses, bacteria and fungi along with the presence of weeds in the fields. For a long time, farmers have traditionally tried to get rid of these issues by physically handpicking the insects from the plants, using natural poisons to kill the insects, cultivating the crops in an alternative manner to reduce the presence of insects and by producing high-quality bred crops. Though these physical methods helped to protect crops from the pests, it did not take long before the need to develop some kind of chemical that could control these crop-destroying pests and provide nutrition to increase crop yield. Farmers continued to rely on natural fertilizers including manures, ground bones, animal debris such as bird and bat waste known as guano, wood ash, and fish or fish parts, for providing and replenishing soil with essential nutrients. However, the scientists soon discovered the essential elements and their importance that influence the effective and rapid crop growth and that was when they started producing chemical fertilizers and pesticides containing phosphorous, nitrogen and potassium, considered as the essential elements. There was a large-scale production of these chemicals in the US and Europe by the end of 20th century. Thereafter came the production of chemical fertilizers and pesticides containing nitrates and phosphates which initially helped to increase the total crop yield but over years of usage, they lead to a decline in soil quality and degradation of the environment (Vasey, 2002). Gradually, the world began to face several challenges leading to a global demand to increase the annual crop productivity rate, while maintaining the quality, nutrition, efficacy and shelf-life of the crops to feed the growing population adequately (Rouphael and Colla, 2020).

All of these challenging circumstances impelled scientists to divert from the conventional methods and bring about holistic changes in the field of biotechnology and agricultural sciences laterally. This eventually led to the concept of sustainable agriculture, which required the invention of such eco-friendly substances that could support the idea of sustainability, alongside proving beneficial to the environment and maintaining the ecological balance. Scientists discovered one such group of substances that was not only capable of replacing chemical fertilizers, but could also promote plant growth and development, and increase nutrient uptake efficiency, annual crop productivity, overall plant metabolism and crop resistance to environmental stresses. This group are now called biostimulants (Calvo *et al.*, 2014).

1.2 Biostimulants

The discovery of biostimulants had a positive impact for farmers who, previously, had suffered from crop loss, production of low-quality crops and destruction of crops by pests. Moreover, the biostimulants helped to minimize global hunger and brought about serious changes in the world economy. Although it might appear that the concept of biostimulants was clear from the beginning, it was quite the opposite. Biostimulants, also known as natural plant biostimulants, were initially described as a substance without some of its essential functions such as fertilizers, growth factors and products of plant protection (du Jardin, 2012). Following this, many scientists attempted to provide a proper definition of biostimulants until 2011, when the European Biostimulant Industry Council (EBIC) was formed. According to EBIC, ‘biostimulants can be defined as the substance(s) or compound(s) and/or micro-organisms which, when administered on plants or the rhizosphere, function as a stimulant in the naturally occurring physiological processes in plants that improve nutrient uptake and efficiency, increase tolerance of the plants to abiotic stresses along with quality and yield of the plants' (du Jardin, 2015). The Association of American Plant Food Control Officials also defined biostimulants as 'any compound or substance except the primary, secondary or micronutrients, which proved to be beneficial by scientific approach, when naturally administered to one or more species of plants exogenously' (Gupta *et al.*, 2021). An impromptu study was conducted by the European Commission in 2012 to understand the nature of substances and materials involved in the formation of plant biostimulants for a better definition. This study was published by du Jardin in 2012 as an ad hoc study report for the European Commission, entitled 'The Science of Plant Biostimulants-A Bibliographic Analysis', from where it could be concluded that plant biostimulants are, rather, simply heterogenous substances. He also proposed in his study that there are eight categories of substances that have the ability to act as biostimulants:

- organic materials that are commonly procured from urban and agro-industrial wastes, composts, sewage sludge end products and manures;
- substances derived from humus;
- extracts of seaweeds, mainly obtained from brown, red and green macroalgae, and phosphite that form the inorganic salts;
- chemically beneficial elements, such as Al, Co, Na, Se and Si;
- substances like kaolin and polyacrylamide that act as anti-transpirants;
- chitin and its derivatives;
- free amino acids; and
- substances containing nitrogen, such as polyamines, peptides and betaines.

However, that meant that none of the existing microbial components were considered to be biostimulants (Rouphael and Colla, 2020). After three years of extensive work, a special issue on 'biostimulants in horticulture' was in *Scientia Horticulturae*, and plant biostimulants were given a more specific definition that included the nature, mode of action and type of effects on horticultural and agricultural crops with the support of scientific evidence. Du Jardin then again modified the definition of plant biostimulants as 'micro-organism(s) or substance(s) which, when applied to plants, effectively enhances crop quality, nutrient-uptake efficiency, tolerance to abiotic stress, irrespective of the nutrient content.' The addition to the definition to include 'plant biostimulants also form commercial products that consist of such micro-organisms or substances' provides a complete and meaningful definition (du Jardin, 2015). In *Scientia Horticulturae* special issue, Colla and Rouphael (2015) proposed categorizing plant biostimulants into six non-microbial and three microbial categories, which are as follows:

- humic and fulvic acids (Canellas *et al.*, 2015);
- phosphites (Gómez-Merino and Trejo-Téllez, 2015);
- seaweed extracts (Battacharyya *et al.*, 2015);
- beneficial bacteria, such as plant growth-promoting rhizobacteria (PGPR) (Ruzzi and Aroca, 2015);
- beneficial fungi, such as arbuscular mycorrhizal fungi (Giovannini *et al.*, 2020);
- inorganic compounds, such as silicon (Savvas and Ntatsi, 2015);
- chitosan and its derivatives (Pichyangkura and Chadchawan, 2015);
- protein hydrolysates and other compounds containing nitrogen (Colla *et al.*, 2015); and
- *Trichoderma* spp. (López-Bucio *et al.*, 2015).

Over the years, there have been a lot of arguments regarding a proper description that could define plant biostimulants in the best way possible. Recently, a new definition has been formulated pertaining to plant biostimulants, under a new regulation (EW) 2019/1009, that not only defines biostimulants, but also provides a specification about its functions. According to this new regulation, 'Plant biostimulants are EU fertilizing products which, when administered on the rhizosphere or the plants, functions to stimulate the nutrition processes of the plants irrespective of the nutrient content of the product with only the sole purpose of enhancing one or more characteristics of the plants or rhizosphere that are as follows: (1) resistance tolerant to (a)biotic stress, (2) efficiency of nutrient use and uptake, (3) availability of nutrients confined in the rhizosphere or the soil, or (4) characteristics of the quality of the soil.' Following this definition, several naturally occurring bioactive substances and chemically active derivatives of synthetic and natural substances along with the beneficial micro-organisms (bacteria and fungi) were categorized under plant biostimulants that include:

- environmentally derived substances such as humic and fulvic acids;
- products of seaweed extracts obtained from macroalgae;
- animal- and vegetal-based protein hydrolysates;
- chemically beneficial elements such as silicon;
- beneficial micro-organisms:
  - fungi that includes arbuscular mycorrhizal fungi (AMF); and
  - bacteria including strains of N-fixing bacteria that belongs to the genera of *Azotobacter*, *Azospirillum* and *Rhizobium*.

1.3 Why biostimulants in sustainable agriculture?

Various studies have indicated that biostimulants have positive impacts and advantages on crop quality and yield. Biostimulants, as defined earlier, are substances that improve the quality traits, vitality, physiological processes, metabolic pathways, root and shoot growth and development, protection against biotic and abiotic stresses and also diseases, thus improving the annual crop productivity. The various substances cataloged under plant biostimulants such as humic substances (including humin, humic acid and fulvic acid) help in turning minerals into organic compounds for easy absorption by plants along with providing protection against toxic substances. These substances regulate water infiltration and induce the growth of healthy roots.

Amino acids belong to major phytohormone origin that influences the metabolic activity of plant systems. These stimulate early germination and division of seeds, and cell division rate. They enhance mature development of fruits, flowering, fruit setting and pollination in crop plants.

Seaweed extracts are widely extracted from red, green and brown algae. Among all the types of algae, brown algae such as *Sargassum*, *Turbinaria*, *Laminaria*, *Ascophyllum nodosum* and *Fucus* contribute greater advantages to crops, including nutrient-uptake efficiency, faster and quicker germination of seeds, fruit setting, development of quality fruits and flowering, resulting in the formation of healthy crops. These are applied as biostimulants for the presence of natural phytohormones such as auxins, gibberellins, and cytokinins, along with metal elements like nitrogen, aluminum, potassium, iron and manganese.

Looking at all the advantages provided by natural biostimulants in improving quality, quantity, desirable characteristics and productivity of crops, it is now believed by biotechnologists that utilization of biostimulants can, indeed, bring about a new dimension in sustainable agriculture.

1.4 Microbial and non-microbial biostimulants

1.4.1 Humic and fulvic acids

Humic and fulvic acids come under the broad category of humic substances. Humic substances can be defined as any substance that is the end product of natural decomposition of microbes, plants, animals and degradation of dead biota that are present in the soil, chemically (Lavkulich *et al.*, 2019). Humic substances have been reported to have huge effects on the crop yield, quality characteristics, nutrient-uptake efficiency, effectiveness of gas exchange, plant physiology and biological factors. These are naturally occurring heterogenous substances that are inherently classified on the basis of their molecular weights into three major types:

- *Humic acids* are natural, alkali-soluble humic substances that are present in soil. These are derived from soil by addition of a dilute alkali, followed by precipitation in acidic solution.
- *Fulvic acids* are soluble in alkaline as well as acidic solutions.
- *Humins* are humic substances that cannot be extracted from soil.

The function of humic substances in various plant species is greatly influenced by their structural differences. The variations can be observed in acidity, extent of polymerization, content of

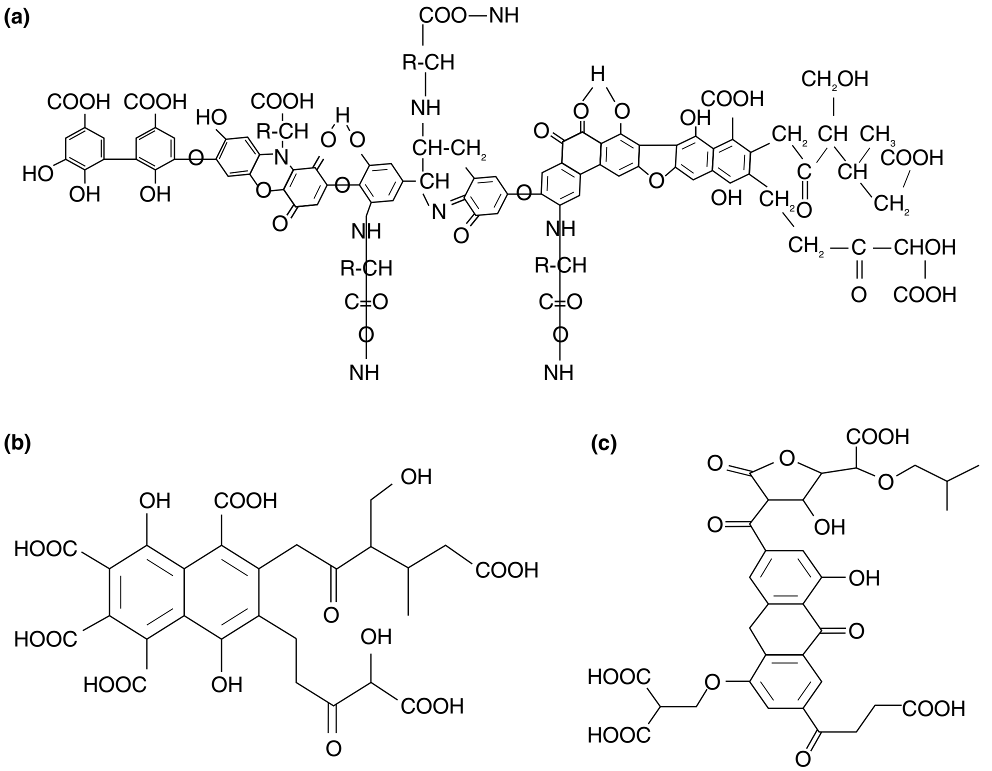

Fig. 1.1. **(a)** Humic acid. Molecular size range from 10,000 to 100,000 with a combination of complex carbon chains and rings that radiate a dark color. Adapted (with permission) from de Melo (2016). **(b)** Fulvic acid. Molecular size range from 1,000 to 10,000, consisting of nitrogen, hydrogen, oxygen and carbon. **(c)** Suwannee River fulvic acid model. Reprinted (adapted) with permission from Atalay, Y.B., Carbonaro, R.F. and Di Toro, D.M. (2009). Distribution of proton dissociation constants for model humic and fulvic acid molecules. *Environmental Science & Technology* 43(10), 3626–3631. © 2009 American Chemical Society, used with permission.

carbon and oxygen, color and molecular weight. These can be accounted for by the difference in the structure of humic and fulvic acids (Fig. 1.1).

The differences in structure, function and effects on plant species are as follows (Canellas *et al.*, 2015):

- Humic acids are large molecules with molecular size ranging from 10,000 to 100,000 Da with a complex combination of carbon chains and rings and nitrogen, oxygen, hydrogen and phosphorous. Fulvic acids are smaller molecules ranging from 1000 to 10,000 that consists of nitrogen, hydrogen, oxygen and carbon.
- Humic acids often act as chelating agents that bind to toxic metals, thus preventing their entry into the plant biome. Moreover, it helps in stimulating the activity of microbes in the soil, increasing the water retention capacity of the plants and enhancing the growth and development of roots and shoots. On the other hand, fulvic acids are the best chelating agents ever known that work by binding to the essential nutrients present in the soil and transports them into the plants for efficient physiological processes.
- Humic acids radiate a dark color whereas fulvic acids emits a golden color.
- While humic acids act as dilators to increase the permeability of the cell wall for better absorption of nutrients, fulvic acids act as railcars as they help in transportation

of essential macronutrients and micronutrients from the soil to the plants.
- Due to the presence of a high content of oxygen, fulvic acids are twice as biologically active as compared to humic acids.

Humic acids are the ‘all-stars’ of the biochemical, biophysical and physiological processes of plants as they help in setting the optimum environment for optimum functioning, whereas fulvic acids act as carriers to provide vital nutrients and minerals to the plants.

According to research conducted by Iowa State University on the regulation of humic and fulvic acids on soybean, it was found that humic and fulvic acids help in enhancing the root development. Along with that, the effects of these substances on soybean plant were also investigated when a soybean cyst nematode was present as it leads to approximately $1.5 billion in loss of crop annually (McGrath *et al.*, 2013). Early research had also shown that exposure of soybean plants to humic substances had led to an increase in the dry weight of roots, shoots and nodule; however, it was also noted that the nodule weight was inversely proportional to the amount of humic substances administered.

From Table 1.1, we can conclude that the initial effects of humic substances (humic and fulvic substances) on any crop species is initiating growth in the primary and secondary roots, followed by shoot growth. Moreover, it has enhanced the uptake efficacy of various macronutrients and micronutrients by the plants and has thus increased the rate of flowering of buds and development of fruits with high levels of carbohydrates, soluble sugars and other nutrients. It has also increased the H^+-ATPase activity along with providing protection against abiotic stress. Therefore, it can be concluded that administration of humic substances on various crop species has, indeed, proved to govern a positive effect on the biological, biochemical and physiological processes of the plants along with increasing the total crop yield.

1.4.2 Seaweed extracts obtained from microalgae

To begin with, seaweeds can be defined as the brown, green and red marine algae that are mostly found anchored to a solid support, near the seashore, by means of a rootlike ‘holdfast.’ These extracts of seaweeds are chemically composed of some very important constituents that mainly include complex polysaccharides, phytohormones, vitamins, minerals, essential nutrients and fatty acids that play an integral role in traditional agriculture. These extracts impart valuable inputs as plant biostimulants, and their application extends to horticultural crops where they mainly serve as plant-growth promoters and as an ameliorating factor to induce tolerance against abiotic stress, such as extreme temperatures, drought, flood, salinity, nutrient deficiency, etc. Recent research has highlighted the effects of seaweed extracts on activation of certain mechanisms involved in plant processes that are still being studied. Moreover, they have also been observed to increase shelf-life, productivity and yield of the crops on administration (Battacharyya *et al.*, 2015).

It has been reported by several scientists that certain seaweeds such as *Ecklonia maxima*, *Ascophyllum nodosum* or *Pterocladia capillacea* have the ability to (Kocira *et al.*, 2019):

- improve the settling of fruits in eggplants (Pohl *et al.*, 2019); and
- enhance the agronomically evolved performance of potato (Wadas and Dzuigiel 2020), bean (Kocira *et al.*, 2020), and Jew’s mallow (Ashour *et al.*, 2020).

The effects of seaweed extracts on various plant species are still being studied. One such example is the Bio4Safe project an ongoing project that is funded through the European Interreg 2 Seas Programme. It is being conducted in the 2-Seas region coordinated by PCS Ornamental Plant Research (Belgium) and other six partners: Ghent University (BE), North Sea Farmers (The Netherlands), Vertify (The Netherlands), NIAB-EMR (UK), Junia (France) and Pôle Légumes (France) (Interreg 2 Seas Mers Zeeën Bio4safe, https://bio4safe.eu/about. accessed 9 April 2022). This project is based on a tenure of four years with a total budget of €3.2. This project was started with the aim of increasing the efficiency of water and nutrient use by crops by the

Table 1.1. Different effects of humic substances on various species of plant or crops. Adapted (open access CC-BY) from Calvo *et al.* (2014).

Crop species	Classification of humic substances	Effect on crop species
Corn	Humic acid	Results showed that humic acid application on corn stimulates shoot and root growth
Tomato	Humic and fulvic acids	Significant shoot and root growth with an improved nutrient uptake of N, P, Fe, and Cu Ascorbic acid and total soluble solid content increase in fruits Humic substance regulation increased the annual crop yield
Grape	Humic substance	Experiments have shown that administering grape crops with humic substances increases efficiency of P and Fe uptake It also decreases the efficient Na absorption
Potato	Humic acid	It increased ascorbic acid content and percent protein in tubers, further enhancing total tuber yield
Wheat	Fulvic acid	Some studies have shown that fulvic acid improves plant growth, bioaccumulation of GD^{3+}, Y^{3+} and La^{3+} and nutrient uptake of ^{32}P in the roots and shoots It also enhanced the chlorophyll content in the leaves Results have shown that low levels of Se enhanced growth of the roots of the seedlings and significantly reduces toxicity The influence of fulvic acid notably reduces cell membrane permeability induced by Se and free-proline content It activates glutamic oxaloacetic transaminase enzyme
Soybean	Humic and fulvic acids	Administration of soybean with humic and fulvic acids showed to increase soil plant analysis development chlorophyll meter readings
Maize	Humic and fulvic acids	It increases primary root elongation followed by secondary root proliferation and increase in root surface area It increases the total leaf area, the total content of chlorophyll (a and b) pigment, dry weight of the crop along with an increased yield under both drought and non-drought conditions Humic substance use enhanced phenylpropanoid pathway mechanism, decreased amino acids, phenylalanine and tyrosine contents, and increased certain phenolic compound concentration It also stimulates H^+-ATPase activity in the plasma membrane and the mitotic sites covering the developmental regions of lateral roots Assimilation rate of proline and atmospheric CO_2 increased
Cucumber	Humic and fulvic acids	Humic substance presence increased the N, P, Mg, K, Ca, Fe, Cu and Zn uptake efficiency It has also enhanced shoot growth NO_3 concentration and decreased amount of NO_3 in the roots It has improved total fruit yield, total reducing and soluble sugar content, dry weight of shoots and roots and blooming of more quantity of flowers in each plant along with chlorophyll (b) content increase It has increased Fe(II) root transporter (CsIRT1) and Fe(III) chelate-reductase (CsFR01) gene transcription H^+-ATPase activity increased along with a change in root-to-shoot ratio of NO_3 presence and certain growth hormones like cytokinins and polyamines, have been observed Enhancement in the total plant growth and development and a significant increase in the crop yield observed

Continued

Table 1.1. Continued.

Crop species	Classification of humic substances	Effect on crop species
Arabidopsis	Humic and fulvic acids	Humic substances stimulate the emergence and growth of lateral roots in *Arabidopsis* It showed an active mechanism of DR5: GUS - a synthetic auxin reporter with an improved H^+-ATPase activity in the root vesicles
Pepper	Humic and fulvic acids	Nutrient uptake of N, Ca, K, P, Mg, Cu, Mn and S increased under moderate salinity stress Dry weights of shoots, roots and fruits increased, accompanied by enhanced total reducing and soluble sugar yield, chlorophyll content. It also increased carbohydrate, phenol, carotenoid levels, followed by antioxidant activity in fruits. An overall growth in the crop species yield was observed
Sunflower	Fulvic acid	Fulvic acid acts as a chelating agent which formed a bond with Fe^{3+}, thus enabling an effective nutrient uptake It also releases and mobilizes Fe from iron chelates, thus allowing the crops to absorb free Fe
Rice	Humic and fulvic acids	Under water stress conditions, lipid peroxidation was lower with humic acid as compared to the ones without humic acids. However, no change in abscisic acid (ABA) was seen in plants with or/and without humic acid under water-stressed conditions. This suggested that mechanisms involving ABA independence like regulating the genes encoding for tonoplast aquaporin can protect the crops under water-stressed conditions. It also reduces oxidative stress in water-stressed plants Water deficiency led to a considerable growth in plants and biomass Fe absorption was more when a combination of Fe-fulvic acid was applied rather than $FeCl_3$ alone. This suggested that fulvic acid overcome the rate-limiting step barrier in Fe absorption from the soil to the roots through diffusion An increase in proline along with a reduction of H_2O_2 content in the plants as a result of induced peroxidase action, have been observed. This maintains cell membrane permeability
Chrysanthemum	Humic acid	Administering these plants with humic acids increases in the vegetative growth and flowering Increased the amounts of carbohydrates and N, P, K under conditions of salinity stress
Pistachio	Humic acid	Application of humic acid increases shoot growth with an improved nutrient uptake Proline and abscisic acid decreased in humic acid presence
Basil	Humic acid along with or/and without PGPR	Humic acid alone or/and combined with PGPR helped in enhancing oil yield

application of biostimulants in the form of seaweed extracts. The objectives of this project are as follows:

- Reduction of the water consumption by horticultural crops by 20%.
- Reduction of the fertilizer consumption by horticultural crops by 10%.
- Development of a protocol dedicated to the policy makers for tracking the impacts and effects of biostimulants on fertilizers and water uptake efficacy of plants.
- Provide a detailed market study for the seaweed-based companies of the region that would include the calculation defining the economic potentiality of seaweed-derived biostimulants.

The trials under this project are performed by the North Sea farmers in the four countries within the 2 Seas region – France, Netherlands, United Kingdom, and Belgium. They are using commercially available seaweed-derived biostimulants on various economically viable crops that include strawberry, lettuce, tulip, tomato, raspberry, hydrangea, chrysanthemum and surfinia. This project aims to determine the effect of seaweed extracts on the horticulture and agriculture industries in Europe. The following impacts have been observed so far:

- There has been an improvement in the water retention capacity of the soil, water uptake by the plants and increased content of chlorophyll in the leaves.
- Foliar application of seaweed extracts has helped to increase the depth and 50% of the surface area of the root system, thus allowing crops to absorb more water and nutrients and increasing the resilience of the crops.
- A 10–30% increase in the dry weight of the plant system has been observed.
- There has been an average increase of 30% of the leaf sizes that has enabled an increase of the chlorophyll content. With the increase of chlorophyll content, the fresh look of floral crops is preserved.
- The increase in the chlorophyll content also regulates the doubled production of flowers in each plant along with an increased vase life.
- Results have shown a remarkable increase of approximately 43% of the total crop yield, even under stressful conditions such as high salinity or drought.

It has also helped in increasing the production of flavonoids, carbohydrates, phenols, antioxidants and proteins, hence improving the overall quality of the crops.

It is predicted that the application of seaweed extract-based plant biostimulants will, indeed, bring about a major shift in the ecological provision, safeguarding the food requirement for the future, and lessen a farmer's fight against odds.

1.4.3 Animal-based and vegetable-based protein hydrolysates and other compounds containing nitrogen

Protein hydrolysates can be broadly categorized as protein-derived products that, on application, can stimulate plant growth and resistance to (a)biotic stresses. These protein-derived products are classified into two types of protein hydrolysates: (i) a combination of amino acids and peptides originating from plants and animals as its constituent; or (ii) those composed of amino acids alone such as proline, glutamine and glutamate (Kauffman *et al.*, 2007; Ertani *et al.*, 2009; Kunicki *et al.*, 2013; Cavani *et al.*, 2021), along with some major amino acids, including glycine, alanine, arginine, valine and leucine (Ertani *et al.*, 2009). Chemical, enzymatic and preparation of protein hydrolysates involve hydrolysis of a wide range of plant and animal residues, such as connective tissues and epithelial cells (Ertani *et al.*, 2009; Cavani *et al.*, 2021), alfalfa residues (Schiavon *et al.*, 2008), elastin and collagen fibers (Cavani *et al.*, 2021), glycoproteins present in the cell wall of *Nicotiana* (Apone *et al.*, 2010), proteins derived from carob seeds and protein obtained from algae (Lucia and Vecchietti, 2012).

Application of protein hydrolysates has a direct influence on the microbial activity and biomass, cellular respiration and fertility of the soil. It is also evident from experiments that certain amino acids serve as chelating agents (e.g. proline) that can bind heavy metals within the soil, thus preventing plant damage. Protein hydrolysates also help in increasing the mobility and the addition of micronutrients to the plants. This indirectly contributes to the development of roots and nutrient availability to all the parts of a particular plant (Lavkulich *et al.*, 2019).

In an experiment conducted on tomato plants at extreme climatic conditions, regulation of peptide-derived products or amino acids in the form of non-microbial protein hydrolysates have provided tolerance against a wide range of stresses, which mainly include hypo-toxic, salinity, excessive nutrient and heat stresses and unfavorable environmental conditions (Francesca *et al.*, 2020). Administration of protein hydrolysates on maize seedlings that were grown under hydroponic conditions alleviated the effects of multiple (hypoxia + salinity + nutrient stress) or single (hypoxia or salt deficiency) stresses that could have been detrimental to the health of the maize plants. It also upregulated the primary genes responsible for transport of nitrates and detoxification or oxygen reactive species which consistently improved the growth of the shoot system, thus eliciting the structure and architecture of the newborn maize plants

(Trevisan *et al.,* 2019). Application of vegetal-based biostimulants, especially those that are legume based or tropical plant-derived protein hydrolysates, has significantly increased the functional and nutritional quality of tomato (Caruso *et al.*, 2019) as well as lettuce (Cozzolino *et al.*, 2020).

Some amino acids (e.g. glycine betaine, which is the substituted N-methyl derivative of glycine and proline) are known to act as osmolytes or osmo-protectants, enzymes, stabilizing proteins and membranes as a result of denaturing effects of high salinity conditions and extreme temperatures (Chen and Murata, 2008; dos Reis *et al.,* 2012; Ahmad *et al.,* 2013;). Hence, proline and glycine betaine have come to be related to stressful conditions since administration of these have shown to induce increased abiotic stress tolerance in a wide variety of crop plants such as maize, soybean, barley, rice and alfalfa (Chen and Murata, 2008; dos Reis *et al.*, 2012; Ahmad *et al.*, 2013). Along with certain other amino acids (e.g. ornithine and/or glutamate), proline precursors, when exogenously applied, can increase the resistance against abiotic stress (Chang *et al.*, 2010; da Rocha *et al.*, 2012). When plants are exposed to stressful conditions, accumulation of arginine within the plant has also been observed (Lea *et al.*, 2007). Some non-protein amino acids, including gamma-aminobutyric acid (GABA) and beta-aminobutyric acid (BABA), function as endogenous molecules of signaling and enhancers of stress tolerance (Zimmerli *et al.*, 2008). Regulation of GABA has, indeed, helped in curing the postharvest chilling injury in *Prunus persica* (peach) (Schwartz, 1978).

1.4.4 Chemically beneficial elements and inorganic compounds

There are certain chemicals that are not required by all crops for normal sustainability; however, the presence of such essential elements can enhance crop development. There are five important compounds that are particularly beneficial as biostimulants: Na, Si, Al, Co and Se. These elements are present in the form of inorganic salts in the plants, as well as in the gramineous form of non-crystalline silica ($SiO_2.nH_2O$) in insoluble forms. These inorganic salts have constitutive functions, including strengthening the cell wall or expressing themselves under stressful environment, such as Na^+ osmotic stress or during attacks of Se. Hence, the functions of these inorganic salts are not only dependent on their unique chemical structures, but also dependent on the external environmental conditions. Under such conditions, they have been observed to have promoted plant growth and development along with resistance to such stress. On the other hand, the activity of some biostimulants such as residues of crop or crop waste, or seaweed extracts, may provoke the expression of physiological functions of these inorganic elements.

The derivatives of these inorganic salts or beneficial compounds such as phosphite anions, phosphates, chlorides, silicates and carbonates, have been known to have functioned as fungicides under certain conditions. Although all the functions of these compounds are not yet known, it has been evident that they help in maintaining the redox homeostasis and pH, hormonal signaling, and act as enzymes. Moreover, they improve the activity of the co-factor–enzyme complex, osmoregulation, enhance the nutrient uptake, symbiont interactions, and provide protection against toxic heavy metals (Lavkulich *et al.*, 2019).

1.4.5 Chitosan and its derivatives

Chitosan can be defined as a de-acetylated polysaccharide that is naturally and industrially derived from chitin, one of the world's most abundant polysaccharides. It contains amino acids in its structure which makes it viable for getting easily protonated, thus converting it into an acid-soluble polysaccharide. It is known that the cell membranes are negatively charged, so characterization of chitosan by the presence of positive charges allows it to interact with the cell membranes efficiently. This character also allows it to bind to various components of cells, for example DNA and constituents of the cell wall and membrane. It also has the ability to bind to particular receptors, thus, regulating cell signaling.

Due to the characteristic properties of a chitosan, including natural origin, abundant availability, biodegradable nature, reactivity, etc., it has been broadly associated with plant growth, abiotic stress tolerance (drought, salinity, drought, waterfall) and production of primary and secondary metabolites (Lavkulich *et al.*, 2019).

1.4.6 Beneficial fungi

Over the ages, fungi have interacted with the plant system by colonizing around the roots of the host plant. They have been either directly involved through symbiotic relationship or indirectly involved through parasitism. In either way, they have shown to have increased the capability of nutrient and water absorption by the host plant system, while on the other hand, the plants provide shelter and food in the form of carbohydrates (produced from photosynthesis) to the fungi. Mycorrhizae is one such symbiotic relationship between plants and fungi. It has been predicted that almost 90% of land plants live in close association with the mycorrhizal fungi (Herbarium and Matthew, 2021).

Among all the forms of mycorrhizal fungi, the most essential and popular form of endomycorrhiza that is related to crop and horticultural plants is the arbuscule-forming mycorrhiza (Rouphael *et al.*, 2015). This type of tripartite association allows the unique species of fungal hyphae to invade the cortical root cells, thus forming branch-like structures called arbuscules. Considering the known facts about the increased efficiency of water, mineral and nutrient (micro- and macro-) uptake by the plant system in the presence of AMF, they have been considered to function as biostimulants. Through experiments it has been evident that AMF has helped in balancing the water content and increased resistance against (a)biotic stress. Application of AMF on plants has shown responses that include promotion of morphogenesis and organ growth, efficient usage of nutrients and overall enhancement in the crop yield (Lavkulich *et al.*, 2019).

Studies have also inferred that the activity of AMF can be maximized by adopting agriculturally beneficial practices, involving various useful strains of AMF and, most importantly, careful selection of the host plant. One such experiment was the application of four types of microbial biostimulants (AMF, *Trichoderma*, and enriched rhizosphere with seaweed extracts and amino acids) on common green bean. The results showed an elevated yield of seeds and pods, along with increased chemical and nutritional composition (Petropoulos *et al.*, 2020).

1.4.7 Beneficial bacteria

There are two major groups of beneficial bacteria: (i) plant growth-promoting bacteria (PGPR) rhizobacteria; and (ii) *Rhizobium* of endosymbionts. *Rhizobium* and its associated taxa are commercially applied as biofertilizers, whereas the PGPRs are used for its multifunctional properties that influence each and every physiological process of plants, including the morphogenesis and organ development, plant growth and nutrient-uptake efficacy, tolerance against biotic and abiotic stress, and interaction with the ecosystem, including organisms and external environment. *Bacillus thuringiensis*, an important PGPR, is also used as an efficient biostimulant to boost the crop yield and pave a way for sustainable agriculture. Concerning the improvement of quality traits of crops, application of *Bacillus subtilis* CBR05, a strain of PGPR, to tomato plants significantly increased the quality and content of carotenoids (lycopene and β-carotene) (Chandrasekaran *et al.*, 2019). Another experiment conducted by inoculating *Rhizophagus intraradices,* or a combination of *R. intraradices* and *Funneliformis mosseae* into saffron, grown without soil, showed an improvement in the antioxidant activity, synthesis and accumulation of bioactive elements, such as picrocrocin, crocin II and quercitrin, and molecules like polyphenols, anthocyanins and vitamin C that promote plant health (Table 1.2; Caser *et al.*, 2019a, 2019b).

Although biostimulants are natural-derivates and environmentally beneficial, sometimes these may not be effective in its native state. There is therefore a need to formulate these natural biostimulants to produce more economically and environmentally beneficial product forms.

1.5 Biostimulant formulations

Biostimulant formulation can be simply defined as the processed forms of those natural substances considered as biostimulants, that can be

Table 1.2. The effect of various biostimulants on crop species. Source (with permission): du Jardin (2015).

Mechanisms and pathways	Humic and fulvic acids	Seaweed extracts	Protein hydrolysates	Glycine betaine	Plant growth-promoting rhizobium
Physiological function (effect on the entire plant processes)	Enhanced root biomass and encouragement of liner growth of roots	Improved the efficiency of transport of nutrients from roots to shoots and increased the concentration of tissues	Flavonoids provided protection against oxidative damage and ultraviolet rays	Under salinity stress, the photosynthetic activity of leaves increased	Increased the surface area of roots for absorption and enhanced the lateral growth of roots
Environmental and economic benefits (improvement in quality of products, yield and economic benefits)	Reduced usage of fertilizers and harmful environmental effects along with higher yield of crops	Improved plant tissue biofortification enhanced nutrient (S, Fe, Mg, Zn, Cu) uptake	Under high salinity conditions, there was a higher yield of crop	Under high salinity conditions, there was a higher yield of crop	Reduction of environmental loss and use of fertilizers, thus increasing the crop yield
Cellular mechanism (cellular interaction)	In maize crops (*Zea mays*), it promoted elongation and wall loosening of the cell along with activation of proton-pumping ATPases present in the plasma membrane	In oilseed rape (*Brassica napus*), seaweed extract (*Ascophyllum nodosum*) led to expression of micronutrient transporter genes	Under high saline conditions, alfalfa hydrolysate (*Medicaco sativa*) helps in gene expression, stimulation of phenylalanine ammonia-lyase (PAL) and produced flavonoids	In quinoa, it leads to activation of reactive oxygen species to save the photosystem II from photodamage under saline conditions	In winter wheat (Triticum aestivum), PGPR *Azospirillum brasilense* induced release of auxin to activate the auxin-signaling pathways that regulate the morphogenesis of roots
Horticultural and agricultural benefits (enhancement in crop production)	Increased the efficacy of nutrient use and foraging capacity of roots	Expanded the mineral content in plant tissues	Increased crop resistance to abiotic stress	Tolerance of crops to abiotic stress increased	Improvised the foraging capacity of roots and nutrient use efficacy

easily stored, transported, produced and be more efficient in its function, therefore, creating a safe and convenient path for practical use. These are generally produced based on the physiological characteristics of the already existing materials in respect to market demand. Biostimulants, based on different principles, are of various formulations including a diverse range of molecules like amino acids (Colla *et al.*, 2017), seaweed extracts (Battacharyya *et al.*, 2015), microbial elements (Mire *et al.*, 2016), phytohormones (cytokinins, gibberellins, auxins, ethylene, brassinosteriods and abscisic acid) (Pacifici *et al.*, 2015), polyamine (Fuell *et al.*, 2010), protein hydrolysates (Colla *et al.*, 2017), nitrobenzene, etc. They are also available in the form of granules, powders, or solutions given to soil or as foliar applications in liquid or dried form.

1.6 Types of biostimulant formulations

The various types of biostimulants in use, e.g. fitofortificants, supplements, plant strengtheners and conditions, soil improvers, depends on individual countries and their regulatory laws.

1.6.1 Old formulations

As this type of biostimulant formulation has been used time and again for various purposes, including the increase in bio-efficacy, rapid growth of crops within a stipulated time and others, it is termed an 'old', 'traditional', 'classical' or 'conventional' biostimulant formulation. Biostimulants in the developing countries of the Pacific region and Asia, exist in the form of solutions or wettable powder, dust or emulsifiable concentrations.

Over decades, old formulations of biostimulants have been strictly restricted to natural, homemade preparations that had significant incorporation of extracts derived from organic solvents and crude oil for improving the crop yield and efficacy. There have been many such natural formulations of biostimulants that have been prepared by various scientists and have proved to be effective in various ways.

For the regulation of plant growth, a composition of abscisic acid and a plant-growth regulator such as 1-naphthyl acetic acid, triacontanol, gibberellic acid, nitrohumic acid, maleic hydrazide, fulvic acid, brassinolide, nitrohumic acid, oligosaccharins, salicylic acid, chitosan, etc. was formulated that claimed to be suitable for certain crop plants such as rice, soybean, cotton, tobacco, corn, sugarcane, cereals, sugar beet, corn and rape (Tan *et al.*, 2002).

Recently, a novel plant-growth stimulating composition consisting of heteroauxin indole-3-acetic acid, gibberellins and cytokinin 6-(4-hydroxy-3-methyl-2-trans-betenylamino) purine, has been developed by Jones and Gates (2019). All the components of this composition act actively thus producing a synergistic effect on the plants that regulate shortening of the dormancy period in seeds and early seed germination, increasing the fruiting and flowering period, and improving crop yield. Not only this, but it has also been observed to prevent lodging, help in the recovery of damaged crops, and has enhanced root and shoot proliferation (Jones and Gates, 2019).

There are four main types of old formulations of biostimulants:

- Wettable powders (WP) are a form of old formulations that can be made by using 50% or more dry concentrates of micronized active biostimulant that is mixed with a dispersing agent, a wetting agent and a finely ground diluent. The dispersing agent acts by inhibiting the agglomeration of biostimulant particles, while the wetting agent provides assurance of effective wetting of the active biostimulant in water. The most commonly used solid diluent in the production of wettable powders is clay because of its certain unique properties. The properties are as follows:
    - It has a naturally occurring small and fine particle size ranging from 5 to 10 μm.
    - It has the innate ability to inhibit the biostimulant particles inside the spray tank.
    - It has immense compatibility with the actives present in the biostimulants.
- The wettable powder is added to water enclosed in a spray tank before using it as

foliar spray or direct application on target crop species. This achieves the minimum effective concentration. The wettable powder is quite often applied as a dilute aqueous suspension because it is easier to control its reach to the non-target areas, which is difficult in dustable powders. Even after all these advantages, there is still a major concern regarding exposure of this type of respirable biostimulant. In India, an approximate of registered biostimulant in wettable powdered form is sulfur 80% WP, TSS containing marine plant (*Ascopyllum nodosum*) extract 32% WP, etc. (Knowles, 2008).
- Dustable powder is a form of old biostimulant formulation that is prepared by the sorption of plant extracts or any other active particles onto a fine, inert, solid ground, for example, clay, talc or chalk. As these biostimulant particles are dry and not wetted prior to its application on the crops, so their sizes are generally higher, ranging from 25 to 35 µm. A very innovative formulation including water–water or dry flowable dispersible granules was formulated with Trichoderma strains. This formulation increased micronutrients and phosphorous metabolization which, in turn, promoted plant growth and enhanced shelf-life (Knowles, 2008).
- Emulsifiable concentrates (EC) is a blend of emulsifiers, adjuvants and biostimulants mixed in a volatile oil. This formulation is stable only when it is dissolved in water inside the spraying tank. The emulsifying agents used here are usually chemicals with long chains that face toward the oil droplets and form a water–oil complex that does not allow the oil and water to get separated. Traditionally, liquid biostimulants or biostimulants with low-melting point have been formulated to produce ECs. In India, the registered concentration of emulsifiable biostimulant is nitrobenzene 20% EC (Knowles, 2008).
- Soluble liquids (SL) are the simplest form among all the types of biostimulant formulations. A soluble liquid or concentrate is a biostimulant formulation that is clear in appearance and is applied only after dilution in water. This type of formulation is mainly based on either water alone or any other solvent that is completely miscible in water. A soluble concentrate or soluble liquid is a clear solution to be applied as a solution after dilution with water. Soluble concentrates are based on either water or a solvent that is completely miscible in water. Few polar compounds like humic acids, polar plant extracts, amino acids and others are very useful in the production of this formulation. Here, the process rarely needs agitation in water inside the spray tank. SLs are very effective in containing the salt form of biostimulant, which leads to an increased salt concentration in the spray tank as compared to other biostimulant formulations. Even after having advantages, this formulation is not dynamic because of the limited hydrolytic solubility and water solubility of the formulated biostimulant. This might, sometimes, give rise to flocculation of other materials that are being dissolved inside the tank such as ECs. In India, the registered soluble liquid biostimulant is Ethephon 39% SL (Knowles, 2008).

As the new-age generations emerge along with their productive and innovative ideas, these formulation types are becoming ancient and conventional that notably utilize organic solvents and petroleum for their production. So, there is a speedy shift from the older formulations to newer, environment-friendly biostimulant formulations.

1.6.2 New formulations

Over the years, it has been observed that implementation of older, conventional methods of formulation (mentioned above) has caused harm to environment and human health. As an alternative, biostimulant formulations are being extensively studied and researched worldwide so that new versions of biostimulant formulations can be invented. These newer formulations are produced with the intention of reducing toxicity, increasing efficacy and safety, minimizing harmful effects on the environment and human health, ease of application, decreased need of labor and better cost effectiveness. The areas of attention include concentrated emulsions – controlled release, microemulsion, etc. and water dispersible granules.

Water dispersible granules (WDG), also termed as dry flowables, are modified forms of wettable powders that are produced by aggregation to form uniform granules. This increases the ease of handling and is also efficient in eliminating respirable particles. WDG are an alternative to wettable powders as the use of the same ingredients, including clay and dispersants, but usually have lower levels of diluent and higher levels of activity. The application process of WDG is also similar to that of WP. The ingredients used in this process allows optimum efficacy of the active particles due to their fine particle sizes, which also prevents clogging in the nozzle.

The formation of uniform granules and powder blend uses various methods, including fluid bed granulation, high-speed mixer agglomeration, spray drying, extrusion granulation and pan granulation. Out of all these methods, extrusion granulation is the most preferred method because of its versatility, safety and economy. The uniform granules produced are dissolved in water already present in the spray tank and then administered as dilute suspensions, alike wettable powders.

WDG formulations comprise materials, such as dispersing agents 5–15% (e.g. naphthalene sulphonate-formaldehyde condensates, lignosulphonates), active ingredients (e.g. seaweed, humic acid) and binder (e.g. lactone). Other components include disintegrating agents or fillers (e.g. precipitated silica, China clay), where the active ingredients constitute 50–90% of the total mass.

This latest type of biostimulant formulation is now becoming quite popular due to the advantages it provides that involve convenience in packaging and ease of use, and free-flowing, dust-free granules that have the potential to disperse immediately on adding to water in the spray tank. Some of the properties are as follows:

- There is a uniformity in particle size that ranges from 1–2 mm and is relatively hard.
- It easily disperses and disintegrates in water to result in the formation of a homogenous stable suspension.

In India, registered WDG biostimulant includes seaweed extract (*Ascopyllum nodosum*) 25% WG and sulfur 80% WG. When WDG formulations are compared to WP formulations, it is seen that WDG provides better outcomes, including ease of handling and measuring, complete evacuation from the container and probability of less spillage (Tan *et al.*, 2002).

1.7 Selection of the type of biostimulant formulation

The most important factors when determining the biostimulant formulation includes the following:

- potentiality;
- biological effectiveness;
- convenience of manufacturing;
- ease of handling;
- cost effective;
- environment-friendly;
- ability to enhance crop productivity and yield; and
- develop crop tolerance against (a)biotic stress.

To choose the most appropriate biostimulant formulation for a given crop species, there are certain factors that are to be taken into account. These include:

- Design of the formulation:
  - compound inputs – chemical, physical, and biological properties;
  - marketing inputs – safety, attractiveness, economy, user friendly, durability;
  - application inputs – plant species, climatic conditions, and various ingredients; and
  - manufacturing inputs – QC facilities and production equipment.
- Development of biostimulant formulation:
  - preliminary studies – this includes preparation of the lab, and collection of all the chemical and physical tests;
  - investigational stage – shelf-life, performing small scale trials of the field, development of analytical methods, bio-efficacy and phytotoxicity; and
  - commercial – process of formulation, development of packaging, and compatibility of tank mixing.

- Requirements to design an ideal formulation of biostimulant:
    - it should be biologically effective on application with no undesirable side effects;
    - it should contain higher levels of active ingredients in its composition to have maximum biological effects at the minimum expense;
    - it should be capable of providing reliable and effective dispersion;
    - it must be favorable for large scale manufacture at a minimum cost;
    - it should provide safety during manufacture, packaging, storage and transportation;
    - it must have an adequate shelf-life; and
    - it should be produced in such a way that it is acceptable by the registration authorities and consumer (Lavkulich *et al.*, 2019).

1.8 Advantages of administration of biostimulants in sustainable agriculture

According to EBIC, biostimulants influence the growth, differentiation and development of a crop plant beginning from seed germination through to the entire maturity of the crop. It regulates a lot of physiological, chemical and biological functions and plant metabolism in various ways.

- It influences nodulation and nutrient assimilation which allows improvement in the efficacy of nutrient uptake and better utilization of nutrients and minerals by the crop plants.
- It increases soil fertility by nurturing the growth of soil microbes around the crop plants which also replenishes the soil with nutrients from where the crop plants absorb nutrients for themselves.
- It provides crops with enhanced tolerance to biotic and abiotic stresses. This prevents damage of the economically viable crops at the growth stage.
- It improves the efficiency of the metabolic pathways that occur in the plant systems.
- Administration of biostimulants have shown results of crops with improved quality traits, such as improved fruit seeding and color, increased carbohydrate content, vitamins, proteins and many others (Lavkulich *et al.*, 2019).
- It has also decreased the harmful effects on the environment and consumption of biostimulant-based crops have proved to be safe for the human health.

1.9 Challenges in biostimulant administration

Biostimulants are an emerging concept which, until now, has not been socially or widely accepted by all nations. Researchers are still determining the exact effects of biostimulants on various crop species. It has not been universally defined as of yet. This involves certain challenges that include:

- Regulatory challenges – since it is an emerging concept and is not universally defined, it applications become limited to certain extent. It is an ongoing researched subject which has limited market data and availability. Due to all these reasons, this becomes very less reliable which restricts the regulatory bodies to formulate any regulatory framework for biostimulant administration specifically.
- Scientific challenges – since the physiological and metabolic effects of biostimulants on the plant species are still being researched, the extent of complexity is still not entirely known to us. Hence, this challenge really becomes a topic of immense attention. Even though we have certain results to show the response of plants toward itself and the external environment on application of biostimulants, we cannot be sure of it entirely because environmental changes are subjective and it evolves every day. Only further studies on this field can help to clarify the ongoing doubts.
- Technical challenges – these challenges mainly cover the formulation of biostimulants with other organic and inorganic compounds and plant protection products.

The technical issues include the study of methods of application and their outcomes under different climatic conditions which still requires a lot of research.

1.10 Conclusion

Biostimulants are considered as an effective weapon that can bring about valuable changes in the conventional methods of agriculture and have the potential to carve a path that leads to sustainability. Today, the world is dominated by food insecurity, hunger and insufficient crop products to feed the stomachs of millions of humans and animals on this planet. Year after year, the soil quality has been observed to have been degraded by the overuse of chemical fertilizers, pesticides, fungicides and other such substances under the need to increase crop yield. However, despite giving positive results, these methods ultimately reduced the crop quality and yield. At this time of need, biostimulants have emerged as an alternative method that can provide several benefits to farmers and can help satisfy the global food crisis. So far, the research on biostimulants have reported quite good results, including improved resistance to abiotic and biotic stress, increased efficacy of nutrient uptake and utilization, increased carbohydrate content, mature flowering, improved seed germination and fruiting, and enhanced good quality traits and crop yield. They show potential in reducing harmful and hazardous effects on the environment and human health. They have helped increased nodulation that has allowed the accumulation of soil microbiome, which in turn has replenished the soil with nutrients and fertility. Biostimulants also provide the opportunity to evolve the formulations in a way that encourages the development of more efficient and resilient agricultural technologies. The studies on biostimulants have so far proved that it can bring about a major change in the field of sustainable agriculture. However, there is a long way to go for biostimulants to get widely and universally accepted within the global market. Following a systematic study and focusing on the constant evolution of biostimulant formulations, the physiological effects and plant metabolism can help in shaping a more secure future in sustainable agriculture. Moreover, trying out new formulations can help in creating a synergistic and resilient effect on crop plants in the near future.

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2 Diversity and Functions of Biostimulants in Crop Plants

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Abstract

External factors that affect the growth and proliferation of plants are known as plant stressors and can be of major two types, abiotic and biotic. Depending on the type of plant stressor, it can affect the plant in several ways including its growth, development and yield. Various biostimulants such as humate-based biostimulants, protein hydrolysates, seaweed extracts, beneficial microbes, industrial waste-based biostimulants, designed nanoparticles, etc. are known to enhance tolerance of crop plants in various ways. Although the mechanisms are not fully elucidated, these substances aid the plant to tolerate abiotic and biotic stresses in diverse environmental conditions. Plant biostimulants can be of several categories. Each category can promote growth and development of the crop and/or enhance resistance against stressors and improve yield and quality of crop. The functional mechanisms of the biostimulants are also diverse. In this chapter, we review various types and sources of biostimulants and their mechanism of actions in crop plants.

2.1 Introduction

Stress is a phenomenon that may lead to a decrease in crop yield during the production of crops and is therefore an external factor that contributes to crop productivity (Liliane and Charles, 2020). There are two types of stresses: biotic and abiotic stresses (Gull *et al.*, 2019). The biotic stresses consist of competition from and among other plants, attacks by pests and pathogens such as fungi (Islam *et al.*, 2016; Hussain and Usman, 2019), bacteria (Gull *et al.*, 2019), viruses (Pandey *et al.*, 2017) and nematodes (Suzuki *et al.*, 2014). The abiotic stress factors stem from unfavorable environmental conditions such as drought, radiation, salinity, floods, extreme temperature and heavy metals. The biotic and abiotic stresses limit crop production

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worldwide and the severity of these factors is increasing due to global climate change (Onyekachi *et al*., 2019). Some examples of major plant stresses include an excess of trace elements in soils, soil salinity, infestation by insects, and diseases (Wang *et al*., 2013; Enez *et al*., 2018). Several other factors are not usually labeled as direct stress-causing factors as they may promote plant growth, such as plant nutrients (Wallace, 1986), growth regulators or genetic limitations (Wallace, 1986). However, an imbalance in the optimum amount or a deficiency of these factors may decrease crop yield just as other stress factors may be present (Wallace, 1986). Similarly, the removal of a stress factor may cause the yield of the crop to increase (Wallace, 1986; Liliane and Charles, 2020).

Plants have developed mechanisms of their own to tolerate these stress factors and overcome them. Once they detect a stress factor, a stimulus is generated from the sensors present in the cytoplasm or cell surface that then, with the help of several signal transduction pathways, is transferred to the nucleus where the transcriptional machinery is located (Gull *et al*., 2019). This process allows the plant to become tolerant against the stress, and these signaling pathways are an excellent linker in between sensing the stress factor and generating an appropriate biochemical and physiological response (Gull *et al*., 2019). Stress factors influence a number of plant responses, including gene expression, growth rates, cellular metabolism, crop yields (Verma *et al*., 2013). Novel approaches are needed for sustainable crop production for ensuring food and nutritional security of an ever-increasing global population (Qaim, 2020). Various biostimulants with diverse functional mechanisms have been discovered as solutions for mitigating the abiotic and abiotic stresses in crop production (Vasconcelos and Chaves, 2019). This chapter reviews various types and sources of biostimulants and their mechanisms of action in crop plants.

2.2 The concept of biostimulants

Biostimulants, or more specifically plant biostimulants, are substances that not only benefit the plant in general, but also offer resistance against abiotic and biotic stresses (Du Jardin, 2015; Vasconcelos and Chaves, 2019). They are not nutrients and are, in fact, substances that aid the uptake of nutrients or contribute to the promotion of growth in plants or the resistance to stress factors (Table 2.1; Brown and Saa, 2015; Rouphael and Colla, 2020). As plants are no longer viewed as a singular entity in relation to the environment, they are now being associated as hosts and partners with pathogens such as bacteria and fungi, both outside and inside the tissues, thus allowing plants to adapt and respond to stress factors (Vandenkoornhuyse *et al*., 2015; Choi *et al*., 2020).

The theory of ‘biogenic stimulants’ was first discussed by Professor V.P. Filatov in 1933 in the USSR (Filatov, 1944, 1951a, 1951b; Gordon, 1947; Sukhoverkhov, 1967). Filatov’s concept of derived biological substances from species, particularly plants, that have been subjected to stress factors impacting metabolic and energetic processes in people, animals, and plants was subsequently developed, and a more precise concept regarding biogenic stimulants was presented as ‘organic acids with stimulating effects owing to their dibasic characteristics that might improve the enzymatic activity in plants’ (Blagoveshchensky, 1945, 1955). Herve was the first researcher to display the first proper conceptual approach to biostimulants where he suggested the development of ‘bio-rational products’ should follow a systematic approach including the chemical synthesis, biotechnology and biochemistry as imposed on the actual plant physiological, ecological and agricultural constraints (Herve, 1994). The need for an empirical and detailed analysis of these products specifically focusing on hormonal and antioxidant systems to discover the many important benefits of biostimulants along with an idea of ‘prestress conditioners’ causing an improved photosynthetic efficiency, reduction of spread and intensity of several diseases and resulting in better yields was suggested by Zhang and Schmidt (2000). Finally, biostimulant research and development officially began when Basak launched a conversation on biostimulants and Du Jardin gave the first in-depth examination of plant biostimulant science, concentrating on biostimulant systematization and classification depending on biochemical and physiological role, mechanism of action and origin, (Basak, 2008;

Table 2.1. Sources of biostimulants with their functions.

Source of biostimulants	Functions	References
Humate-based biostimulants	Promote root growth of the root Improve soil cation exchange capability Neutralizes soil pH Produce secondary metabolites Reduce the effect of stress on the plants Form complexes with Pb^{2+}	Wally *et al.* (2012); Pereira *et al*. (2021); Wire (2021)
Protein hydrolysates	Improve soil quality Enhance the growth of soil microbiota Chelate soil micro- and macronutrients Stimulate root growth in crops and fruits Improve enzymes activity	Schiavon *et al.* (2008); Marfà, *et al*. (2009); Schaafsma (2009); Lucini *et al.* (2015); Colla *et al*. (2017)
Seaweed extract	Enhances plant root growth Increases biomass of the hydroponic plants Promotes plant growth Increases yield of fruits Triggers nitrogen assimilation enzymes	Vernieri *et al*. (2006); Zhang *et al*. (2010); Begum *et al.* (2018)
Bacterial and fungal biostimulants	Controls gray mold formation in strawberries B*acillus lentimorbus, B. megaterium, B. pumilis, B. subtilis* inhibits the growth of *B. cinerea.* Prevents conidia budding LABs produce bacteriocins or organic acids Prevent bacterial plant pathogen Produces siderophores which chelate iron ions Releases phosphatase and other compounds making P available in the soil for plants *Trichoderma* spp. prevents surrounding fungi hyphae growth Releases lytic enzymes to degrade the cell wall Produce proteases which inhibits the pathogen enzymes *Aureobasidium pullulans* compete with other pathogenic fungi Produce antimicrobial enzymes and antibiotics	Chi *et al*. (2009); Brito *et al.* (2014); Gloria *et al.* (2017); Klechkovskiy *et al.* (2018); Vieco-Saiz *et al.* (2019); Jaiswal *et al.* (2021)
Industrial and other wastes	Produce secondary metabolites Facilitates phenylalanine ammonia-lyase activity	Ertani *et al*. (2011)
Nanoparticles and nanomaterials	Facilitates the transportation of ions and metabolites	Lei *et al.* (2007); Qi *et al.* (2013); Khan (2015); Raliya *et al.* (2015)

Du Jardin, 2012, 2015; Vasconcelos and Chaves, 2019; Rouphael and Colla, 2020).

Since the proposed use of plant biostimulants in industry in 2012, it has been defined as substances and/or microorganisms that, when applied to the plants or the rhizosphere, can enhance the nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality by stimulating the natural processes in a plant. As biostimulants do not directly reduce pests, they cannot be classified as pesticides. As defined by Du Jardin (2015), a plant biostimulant is 'any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content'.

The definition of biostimulants has always sparked debates as it is extremely broad and diverse to be simply defined, but Yakhin *et al.* (2017) proposed a definition that seems to be most fitting, 'a formulated product of biological origin that improves plant productivity as a consequence of the novel, or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds'.

2.3 Sources of biostimulants

Several lines of evidence indicate that the yield in organic farming is 5–32% lower compared to yield in conventional farming (Ponisio *et al.*, 2015). The difference in yields exists due to the presence of fungal or bacterial pathogens and nutrient deficiency (de Ponti *et al.*, 2012). Although pelleted chicken manure, fish, meat meal, seabird, bat guano and abattoir waste were used as organic fertilizers, it has been seen that these organic fertilizers often fail to supply the nutrients required for rapidly growing crop plants. Hence, substances that promote nutrient uptake increases abiotic stress tolerance, improve the activity of soil both microbiologically and enzymatically, change root structure for better uptake of water and nutrients, and improve the solubility and transportability of micronutrients, act as the most efficient plant biostimulants (Table 2.2). Various sources of biostimulants have been discovered over the years of investigation for eco-friendly agriculture. Seaweed extracts (Colla *et al.*, 2015), protein hydrolysates (Battacharyya *et al.*, 2015), humic and fulvic acids (Savvas and Ntatsi, 2015), silicon (Pichyangkura and Chadchawan, 2015), chitosan (Canellas *et al.*, 2015; Chakraborty *et al.* 2020), phosphite (Gómez-Merino and Trejo-Téllez, 2015), arbuscular mycorrhizal fungi (Rouphael *et al.*, 2015), *Trichoderma* (López-Bucio *et al.*, 2015), plant growth-promoting rhizobacteria (Ruzzi and Aroca, 2015) are some sources of plant biostimulants. Several studies have been conducted regarding these specific biostimulants where the experiments were carried out in greenhouses or in the field (Rahman *et al.*, 2018a, 2018b; Carletti *et al.*, 2021).

2.3.1 Humate-based biostimulants

Humic compounds are a source of plant biostimulants produced from the breakdown of chemical and biological organic materials. These substances promote the growth of the root, which facilitates the uptake of water and nutrients from deep inside the soil, improves soil cation exchange capability and neutralizes soil pH (Pereira *et al.*, 2021). It facilitates microbial activity in the soil, thus improving its quality and helps to fight against several pests. It has been shown in potato crops that humic-based biostimulants are involved in an increment in the tuber count as well as the size of the crop (Wire, 2021). Humic compounds make insoluble elements available to the plants that are otherwise very difficult to be absorbed by the plants. The substances facilitate the production of secondary metabolites, reduce the effect of stress on the plants and can also form complexes with Pb^{2+}, a toxic compound, hence preventing its uptake by the soil. Recent studies show that amino acids, aromatic carboxylic acids and linear carboxylic acids present in humic acids function as auxin thus enhancing plant root growth (Wally *et al.*, 2012).

2.3.2 Protein hydrolysates

Protein hydrolysates function as plant biostimulants (Schaafsma, 2009) by helping to improve soil quality, enhance the growth of soil microbiota and chelate soil micronutrients and macronutrients making them available for the plants (Colla *et al.*, 2017). The peptides and amino acids that act as hormones stimulate root growth in crops as well as in fruits such as strawberries (Marfà *et al.*, 2009; Lucini *et al.*, 2015). Studies have shown that protein hydrolysates improve enzyme activity required mainly for carbon metabolism and nitrate assimilation (Schiavon *et al.*, 2008).

2.3.3 Seaweed extract

Seaweed extract (SWE), a mixture of multiple growth regulators such as cytokinin, auxins, gibberellins, betaines, macronutrients (Ca, K, P)

Table 2.2. Biostimulant products or substances with a biostimulant effect to counteract specific abiotic stress conditions.

Abiotic stresses	Name and function of biostimulant	Doses	References
Cold or chilling stress	Psychrotolerant soil bacteria applied in tomatoes caused higher seeds germination, reduced membrane damage as well as antioxidants system stimulation	2 L ha^{-1}	Lisiecka *et al*. (2011); Botta (2013); Subramanian *et al*. (2015, 2016); Wang *et al.* (2021)
	Application of enzymatic hydrolysates from animal hemoglobin caused an increase in root biomass and an early production in strawberry plants grown in cold temperature	2 L ha^{-1}	
	Application of enzymatic hydrolysates from animal hemoglobin caused an increase in fresh weight, dry weight, specific leaf area, and relative growth in lettuce plants grown in cold temperature	4 L ha^{-1}	
	Application of amino acid biostimulant (Terra-Sorb® Foliar) exhibited an increase in fresh weight and an elevation in stomatal conductance on lettuce plants grown in cold temperature	3 mL L^{-1}	
	5-Aminolevulinic acid applied on pepper (*Capsicum annuum*) seedlings exhibited an increase in fresh biomass, elevated content of proline, sucrose, and water whereas membrane permeability decreased	0, 1, 10, 25, 50 ppm (15 mL for soaking the seed and 25 mL for soil drenching)	
Heat stress	Brassinosteroids applied on tomatoes and snap beans resulted in higher biomass, increase in the rate of photosynthesis, increased growth and quality of snap bean pod in terms of NPK content	0.01, 0.1, and 1.0 mg L^{-1}	Yildirim *et al.* (2002); El-Bassiony *et al.* (2012); Vendruscolo *et al.* (2016)
	Stimulate® biostimulant applied on melon showed an increase in germination rate		
	Humic acid and biozyme applied on parsley, leek, celery, tomato, onion, lettuce, basil, radish, and garden cress seed was shown to increase seed germination		
	Product-based protein applied on Perennial ryegrass plants exhibited improved photochemical efficiency and membrane thermostability	0, 4, 8, and 12 mL L^{-1}	
Salinity stress	Biostimulants from humic substances and *Moringa oleifera* leaf extract showed improved physical and chemical properties of soil after application.	–	Azevedo and Lea (2011); Aydin (2012); García *et al.* (2012); Canellas *et al.* (2020)
	Humic acids applied to common beans resulted in increased endogenous proline levels and reduced membrane leakages	–	
	Humic acids applied on rice caused activation in anti-oxidative enzymatic function and an increased reactive oxygen species (ROS) scavenging enzymes	–	
	Bee-honey based biostimulant applied on onion plants improved its salinity stress tolerance	–	

Continued

Table 2.2. Continued.

Abiotic stresses	Name and function of biostimulant	Doses	References
Drought stress	*Ascophyllum nodosum* applied on broccoli and spinach improved gas exchange by increasing stomatal closure hence making the plants resistant to water stress *Azospirillum brasilense* applied on water-stressed plants showed high exposed xylem vessels area and thus an improved flow of water from the soil to the leaves *Achromobacter piechaudii* applied on tomato seedlings were activated to accumulate biomass during the stress period Seaweed extracts containing plant growth hormones such as auxins, abscisic acid, cytokinins, gibberellins, polyamines, oligosaccharides, betaines, and brassinosteroids are used for treating cultivated plants Microalgae-based biostimulant applied on water-stressed tomato plants were shown to reduce the damaging effects of stress, increase plant growth and increase the area of the leaves	0.50%	Mayak *et al.* (2004); Xu and Leskovar (2015)

and micronutrients (Fe, Cu, Zn, B, Mn, Co, and Mo) enhances plant root growth. Seaweed extracts also aid in early seed germination along with better crop performance and yield (Begum *et al.*, 2018). Researchers have shown that the biomass of the hydroponic plants grown in low-nutrient concentration increases after the addition of SWE (Vernieri *et al.*, 2006). It is also seen that the foliar application of SWE tends to promote plant growth and yield of fruits. Nitrogen assimilation enzymes are also triggered by SWE (Zhang *et al.*, 2010).

2.3.4 Bacterial and fungal biostimulants

Bacteria and fungi also function as plant biostimulants. Mutualistic species such as *Azorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mezorhizobium*, *Rhizobium* and *Sinorhizobium*, as well as *Azospirillum*, *Azotobacter*, *Bacillus* and *Klebsiella* promote plant growth and development by making nitrogen, phosphorus, and other micronutrients available in the soil (Hayat *et al.*, 2010; Bhardwaj *et al.*, 2014; Calvo *et al.*, 2014; Jaiswal *et al.*, 2021). These microbes can reuse organic matter along with fixing N_2 and also make nitrate available for it to be absorbed by the plants (Miransari, 2010). Gray mold caused in strawberries is a disease that can be controlled by some bacteria. Research shows that *Bacillus lentimorbus*, *B. megaterium*, *B. pumilis* and *B. subtilis* can inhibit the growth of *B. cinerea.* Apart from this, it also prevents the conidia budding on the strawberries (Klechkovskiy *et al.*, 2018). Some lactic acid bacteria (LAB) produce bacteriocins or organic acids. It works by producing an arsenal or through a competitive exclusion mechanism which tends to prevent the growth of harmful microbes (Vieco-Saiz *et al.*, 2019). Research showed that sugarcanes planted in the presence of *A. dizaotrophicus* increased the amount of nitrogen in the soil by 60–80% compared to conventional methods of plantation (Gloria *et al.*, 2017). Species such as *B. megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa* and *B. sircalmous* can also solubilize phosphates.

Bacteria and fungi promote plant growth through the production of plant hormones: auxins (stimulate root growth, water, and nutrient uptake), cytokinins (elevates mitotic cell division in shoots and roots), gibberellins (produces flower and fruit and facilitated germination of seed) and abscisic acid (helps to control the effects of environmental stresses) (Sah *et al.*, 2016).

Siderophore-producing fungi chelates iron ions and releases phosphatase and other compounds, which make P available in the soil for plants (Rouphael *et al.*, 2015). *Trichoderma* spp. produces antibiotics – a mechanism known as antibiosis – which prevents the growth of plant pathogens. *Trichoderma* spp. can also produce toxic enzymes such as viriden, peptaboils, gliotoxins, sesquiterpenes and isonitriles that prevent the growth of other competitors nearby. They promote plant growth by competing for nutrients and space, releasing inhibitory compounds, inactivating the pathogen enzymes and inducing resistance (Brito *et al.*, 2014). *Trichoderma* spp. prevents the growth of surrounding fungi hyphae by secreting lytic enzymes that tend to degrade the cell walls of pathogenic fungi, thus preventing its growth, a process known as mycoparasitism. Specific strains of *Trichoderma* also produce proteases that inhibit the pathogen enzymes. *Aureobasidium pullulans* can also compete with other pathogenic fungi for nutrients and space. They also produce antimicrobial enzymes and antibiotics (Chi *et al.*, 2009).

2.3.5 Biostimulants from industrial and other wastes

Biostimulants derived from food waste, industrial waste, compost, manure and aquaculture remains are shown to effectively promote growth and development in plants. Agro-industrial bi-products are reported to act as potential biostimulants through the production of secondary metabolites that are responsible for plant responses. It also facilitates phenylalanine ammonia-lyase activity (Ertani *et al.*, 2011). These products used as biofertilizers are environment friendly and can be afforded by farmers as well thus lowering the product price to produce crops with better quality and yield.

2.3.6 Nanoparticles as biostimulants

Nanoparticles and nanomaterials, particles between the range 1 nm and 100 nm, can efficiently improve the quality of the crops and tolerance to abiotic stresses once applied (Lei *et al.*, 2007; Qi *et al.*, 2013; Khan, 2015). The communication between nanoparticles and nanomaterial with plants facilitates the transportation of ions and metabolites. It also improves receptor activity by changing the environment with respect to energy and charges. A study showed that zinc oxide nanoparticles applied on tomato plants greatly improved plant height, chlorophyll content, and overall soluble protein content (Raliya *et al.*, 2015).

2.4 Biostimulants and abiotic stresses in plants

Abiotic stresses include drought, salt, cold, heat and water that tends to decrease the growth and development of the plants. Plants are subjected to extreme abiotic stresses, along with climate changes due to human activities, which puts food security under great threat. To overcome these issues, biostimulants with anti-stress compounds can be applied to plants at different stages of their growth: on seeds, during early stages of growth or on completely developed plants depending on the preferred result (Parađiković *et al.*, 2018). For instance, biostimulants with anti-stress compounds such as proline or glutamic acid are applied during stressed conditions, whereas biostimulants that are involved in the activation of bioactive compounds must be applied before any stress occurs. The proper timing of biostimulant application as well as optimum dosage ensures better productivity of the crop, avoids waste of product, high cost of production, and unwanted results.

2.4.1 Biostimulants and cold or chilling stress

Plant metabolism is reduced due to low temperature, which in turn leads to photoinhibition and reduced photosystem II activity. Cell membranes are destroyed and the phosphor lipids are destabilized. According to the studies conducted, it has been shown that the effect of cold stress in tomatoes is overcome by the application of psychrotolerant soil bacteria. The results show higher seed germination, reduced membrane damage as well as antioxidants system stimulation in tomato plants when exposed to lower temperatures (Subramanian *et al.*, 2015, 2016). Another study on the strawberry plant was carried out to determine the effect of an enzymatic hydrolysate from animal hemoglobin in the initial stage of its growth. An increase in root biomass and an early production of fruit was observed. Moreover, an increase in fresh weight, dry weight, specific leaf area and relative growth rate was observed when the experiment was done using lettuce plants (Lisiecka *et al.*, 2011). Amino acid biostimulant (Terra-Sorb® Foliar) applied to lettuce plants grown in cold temperature shows an increase in fresh weight and an elevation in stomatal conductance (Botta, 2013). Biostimulants that are composed of amino acids tend to store compatible osmolytes, which improves the plant's stress tolerance by preventing protein breakdown and saving energy resources in plants. 5-Aminolevulinic acid was applied to pepper (*Capsicum annuum*) seedlings to determine their chilling tolerance. Positive results were exhibited by the plants – an increase in fresh biomass, elevated content of proline, sucrose, and water, whereas membrane permeability decreased (Wang *et al.*, 2021). Several studies conducted to investigate the effect of the biostimulant on the cold tolerance level of plants showed an increase in the accumulation of osmotic molecules through the activation of biosynthetic pathways which eventually led to the production of cold protectant substances. These are also shown to induce increased membrane thermostability and hence reduce the chilling effect.

2.4.2 Biostimulants and heat stress

A rise in the temperature as a result of global warming has negative impacts on plant growth and development. High temperature leads to disruption in protein synthesis and activity thus deactivating enzymes and damaging membranes. The optimum temperature for desired structural integrity and enzyme activity is between the range 30°C and 45°C, but once it increases beyond 60°C, the proteins start to get denatured and enzyme activity is lost. This affects the rate of photosynthesis and respiration and produces toxic reactive oxygen species, which causes oxidative stress. High temperature leads to inhibition of seed germination, decreases plant growth, decreases pollen activity and germination, prevents flower differentiation and development, and also reduces fruit growth and yield. Brassinosteroids applied to tomatoes and snap beans resulted in higher biomass, an increase in the rate of photosynthesis, and an increased growth and quality of snap bean pod in terms of NPK content (El-Bassiony *et al.*, 2012). Although the exact mechanism is not yet known, it is thought that brassinosteroids must have an advantageous role in the photosynthetic apparatus. Stimulate® biostimulant was applied in an increasing concentration (0, 4, 8 and 12 mL L^{-1}) on melon as a thermal stress reliever at 25°C and 40°C. Results showed an increase in germination rate as the concentration of the biostimulant was increased at both temperatures (Vendruscolo *et al.*, 2016). Research to examine the effect of two biostimulants (humic acid and biozyme) on three different concentrations of salt on parsley, leek, celery, tomato, onion, lettuce, basil, radish and garden cress seed germination at 10, 15, 20, and 25°C was carried out. Increased seed germination was observed in the case of all vegetables (Yildirim *et al.*, 2002). Product-based protein was used to treat perennial ryegrass plants followed by exposure to lengthened high air temperature stress. Results showed an improved photochemical efficiency and membrane thermostability than the plants which were untreated (Vendruscolo *et al.*, 2016). Thus, it can be concluded that biostimulants can lead to improved germination of crops even when exposed to high temperatures. Protein hydrolysates can also positively affect plant tolerance to heat stress (Fig. 2.1).

2.4.3 Biostimulants improve salinity stress tolerance

High concentration of salt can cause osmotic stress to develop on plants and eventually affect plant growth and metabolism. Most of the plants grown near the coast are subjected to this stress since saltwater is used to irrigate the crops. Lettuce cultivation, as well as fresh weight and chlorophyll content, are reduced due to salinity stress. Chlorophyll is a major factor in green leafy vegetables, especially when the edible parts

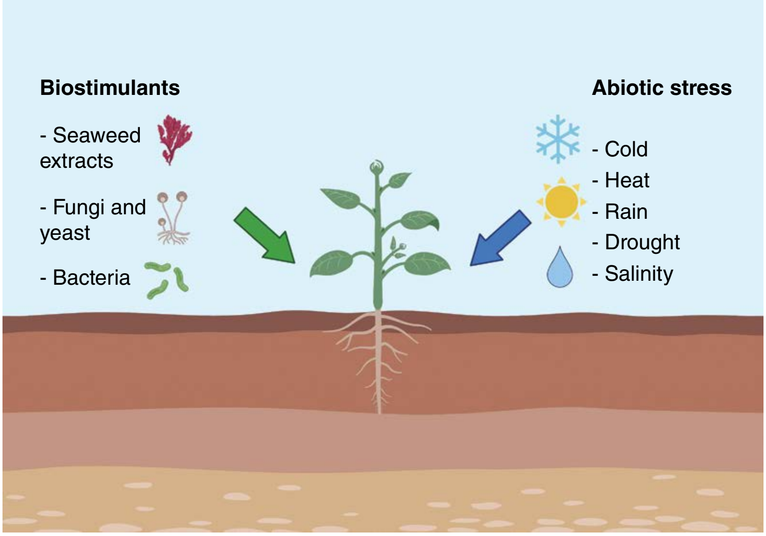

Fig. 2.1. Biostimulants enhance plant tolerance to various abiotic stressors.

are leaves, sprouts or flower buds. Consumers tend to only buy those that appear to be the best. Biostimulant composed of humic substances and *Moringa oleifera* leaf extract showed improved physical and chemical properties of soil after application. Such biostimulants can control osmosis by maintaining water absorption and cell turgidity (Azevedo and Lea, 2011; Canellas *et al.*, 2020). Humic acids applied to common beans (*Phaseolus vulgaris* L.) under high salinity (120 mM NaCl) have also been shown to increase endogenous proline levels and reduce membrane leakages (Aydin, 2012). Activation in anti-oxidative enzymatic function and an increased reactive oxygen species (ROS) scavenging enzymes were observed when rice (*Oryza sativa* L.) was applied with humic acids (García *et al.*, 2012). In addition to these, biostimulants with algae and arbuscular mycorrhizal fungi, fungi and bacteria are shown to increase the germination rate, growth of shoots and roots, improve plant quality, productivity and yield. According to a recent study, an improved salinity stress tolerance on onion plants was shown after the application of bee-honey-based biostimulants. The biostimulants applied in the case of salinity stress tend to provide advantageous effects by enhancing the cell osmotic potential and the level of protective molecules against oxidative stress.

2.4.4 Biostimulants improve drought stress tolerance in plants

There is a high possibility of the fertile land becoming sterile due to the negative impacts of global climate change on water resources. Drought stress influences the rate of photosynthesis and transpiration, which ultimately affects the yield of the crop. *Ascophyllum nodosum* applied to broccoli and spinach improved gas exchange by increasing stomatal closure hence making the plants resistant to water stress (Xu and Leskovar, 2015). In certain cases, it is seen that bacterial infection causes closed xylem vessels and hence prevents water flow. *Azospirillum brasilense* can be used to treat water-stressed plants. The treated plants showed a high exposed xylem vessel area and thus an improved flow of water from the soil to the leaves. *Achromobacter piechaudii* applied to tomato seedlings were activated to accumulate biomass during the stress period and the ethylene concentration, which has a negative impact on the membrane, was shown to decrease when compared to controls (Mayak *et al.*, 2004). Seaweed extracts containing plant growth hormones such as auxins, abscisic acid, cytokinins, gibberellins, polyamines, oligosaccharides, betaines and brassinosteroids are used for treating cultivated plants. Water-stressed tomato plants treated with microalgae-based biostimulant with a known composition were shown to reduce the damaging effects of stress, increase plant growth and increase the area of the leaves (Oancea *et al.* 2013).

2.5 Effect of biostimulants on various factors

Biostimulants tend to improve productivity and yield, as well as promote good health and stress tolerance in plants. Experiments show that plant-based biostimulants and seaweed extract have a positive effect on the chlorophyll content and as a result, an appealing green color on the leaves is observed which tends to attract more consumers (Khan *et al*., 2009). Biostimulant treatments also increase the level of chlorophyll and carotenoids in rocket, lettuce and endive (Vernieri *et al*., 2006). An elevated level of photosynthetic pigments, P and K content, fresh and dry weight of celeriac leaves is detected after the use of amino acids or seaweed extract (Shehata *et al*., 2016). Plant growth-inducing bacteria (PGPR) exhibit similar results in broccoli (e.g. *Bacillus cereus*, *Brevibacillus reuszeri* and *Rhizobium rubi*), tomato (e.g. *Bacillus*, *Pseudomonas* and *Azotobacter*) and strawberry (*B. subtilis, B. atrophaeus, B. spharicus* subgroup, *Staphylococcus kloosii* and *Kocuria erythromyxa*) under non-stressed condition (Yildirim *et al*., 2011; Karlidag *et al*., 2013; Babu *et al*., 2015). A recent study used a phenolic compound from *Ecklonia maxima* on cabbage plants and results showed improved phytochemicals and myrosinase activity (Rengasamy *et al*., 2016). A rise in vegetative growth, chlorophyll content, total sugars, phenols, ascorbic acid, and the photosynthetic rate was observed when rocket salad was treated with moringa leaf extracts (Mona, 2013). Fennel and squash under water-stressed conditions also show similar results (Abd El-Mageed *et al*., 2017; Abdel-Rahman and Abdel-Kader, 2020). Increased fruit weight, chlorophyll and ascorbic acid content were obtained from tomato plants when treated with biostimulants. Experiments led by Kulkarni *et al.* (2019) observed improved growth, chlorophyll and carotenoid content in spinach when treated with smoke and seaweed. Reports suggest that the application of Goemar BM86 and Seasol in broccoli plants increased micronutrients and macronutrients (Mattner *et al*., 2013). Biostimulant also increases the ability of the plants to uptake nutrients from the soil. They do this by altering the soil structure and by improving root structure directly in plants, which facilitates the absorption of nutrients. A biostimulant product named Viva® applied to tomato plants subjected to reduced NPK nutrition shows improved results on plant growth (Koleška *et al*., 2017). Biostimulants also maintain cell homeostasis and avoid oxidative stress to build up. A seaweed-based product (Kelpak®) treatment was applied on nutrient-deficient okra seedlings and was compared with seedlings subjected to polyamine solution treatment. Plants that were treated with biostimulants showed an increase in shoot length, stem thickness, leaves and roots numbers (Papenfus *et al*., 2013). Plant probiotic biostimulants and chitosan biopolymers improved growth, yield and quality of strawberry fruits (Rahman *et al.* 2018a, b). All these experiments prove that biostimulants help reduce the effect of nutrient deficiencies on plants by increasing the nutrient uptake ability through root biomass, nutrient transport and enzyme activities involved in nutrient correlation.

2.6 Conclusion

Abiotic stressors such as drought, salinity, heat and cold can cause adverse effects on the growth of the crop plant such as altering its development as well as yield. To mitigate these effects of stressors on the plants, biostimulants are considered as eco-friendly powerful agents for addressing these stressors for promoting sustainable crop production. There is huge diversity in sources and kinds of biostimulants such as humate-based biostimulants, protein hydrolysates, seaweed/plant extracts, secondary metabolites, environmental microorganisms and nanoparticles. Although the mechanisms of all these bioagents are not fully understood, the biostimulants are considered important alternatives to sustainable agriculture. Recently developed genomics and post-genomics approaches would facilitate elucidation of the underlying molecular mechanisms of the effects of biostimulants on crop plants.

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3 Biostimulants for Promoting Eco-friendly Sustainable Agriculture

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Abstract

As promoters of plant growth and health, biostimulants have garnered attention in research for the myriad benefits they provide. Higher yields, enhanced plant growth, greater fruit quality, protection against pathogens, a boost of antioxidants, rapid germination and improved photosynthetic ability are among the numerous beneficial traits provided by biostimulants. Besides, as the plants constantly battle with the negative effects of climate change, biostimulants can provide relief by enhancing environmental stress tolerance in plants. While they are increasingly being integrated for agricultural uses, specific and targeted uses of these active compounds for other plant species need to be addressed. Importantly, biostimulants can provide solutions to provide more sustainable agricultural practices. Globally, the agriculture sector accounts for a large portion of greenhouse gas emissions and other environment-degrading consequences. Through reduced dependence on chemical fertilizers, lowered CO_2 emissions and the formation of healthier ecosystems, biostimulants can provide eco-friendly alternatives to farming practices. Depending on the necessity, biostimulants could be commercially produced to address different needs of the plants but lack of adequate data. In addition, fear of unpredictable results is still a barrier in this field. In this chapter, we review the main categories of biostimulants and various formulations. Importantly, we summarize the role of biostimulants in abiotic stress management and their contributions to particular plant traits. Finally, we explain the role of biostimulants in establishing eco-friendly sustainable agriculture for coping with depleting resources and uncertain environmental conditions in the changing global climate.

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3.1 Introduction

Biostimulants are any substances, materials or microorganisms that improve plant growth and crop productivity and reduce the need for synthetic chemicals (Malik *et al.*, 2020). Plant biostimulants (PBs), also known as bioeffectors, are administered to plants to improve nutrient uptake and utilization, build a tolerance to abiotic stress and enhance the quality of crops (Zulfiqar *et al.*, 2020). These substances or mixtures of substances, obtained from natural or microbial sources, can enhance the plant quality without causing any adverse side effects (Juárez-Maldonado *et al.*, 2019). Although these biostimulants do not provide any additional nutrients to the plants (unlike fertilizers), they greatly facilitate plant nutrient uptake by supporting their metabolic and enzymatic processes (Van Oosten *et al.*, 2017; Madende and Hayes, 2020). Some of the vital categories of PBs are protein hydrolysates, humic and fulvic acids, seaweed extracts, chitosan, silicon, plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), and other beneficial microorganisms (Van Oosten *et al.*, 2017; Madende and Hayes, 2020; Nephali *et al.*, 2020). The use of biostimulants in the agricultural sector has rapidly increased over the past few decades (Egamberdieva, 2016; Kumar *et al.*, 2017; Ansary *et al.*, 2018; de Moraes *et al.*, 2018; Rahman *et al.*, 2018a, 2018b). To effectively respond to the ever-changing environmental challenges as well as the biotic and abiotic stress conditions that the plants are continuously exposed to, plants have developed their own multi-layered dynamic defense systems. These defense systems can be enhanced by techniques such as biological and chemical priming (Nephali *et al.*, 2020). Biostimulants are considered vital plant priming agents due to their effectiveness in enhancing and sensitizing plant defense against different stress conditions and their capability of altering the biochemical, physical, and epigenetic states of the plants (Zhu, 2016; Bäurle, 2018; Fleming *et al.*, 2019). Besides, the use of biostimulants is a rapid and cost-effective approach toward the goal of sustainable agriculture.

At all stages of agricultural production, biostimulants can be used to stimulate germination and enhance plant metabolism, photosynthesis and nutrient absorption, resulting in better productivity and yield. Therefore, these PBs are increasingly being integrated into agriculture (Yakhin *et al.*, 2017; Fleming *et al.*, 2019; Nephali *et al.*, 2020). Moreover, biostimulants are like ‘pre-stress conditioners’ that can mitigate the spread and intensity of some diseases, and also reduce the negative effects of abiotic stresses on plants (Yakhin *et al.*, 2017). Biostimulants are natural, eco-friendly, biodegradable and non-hazardous to humans and other organisms. These agents can be mixed with different fertilizers and may offer an alternative to the harmful synthetic plant stimulants (Maignan *et al.*, 2020). Furthermore, biostimulants can be utilized as bioremediants to boost the degradation rate of xenobiotics and enhance phosphatase and urease activity, thereby improving the beneficial microbial communities in soil. Henceforth, biostimulants have shown great potential as a novel approach for the modification and/or regulation of physiological processes in plants to enhance growth and alleviate stress-induced challenges, and overall increase yield. This chapter discusses the importance and impacts of the application of biostimulants for promoting eco-friendly sustainable agriculture.

3.2 Main categories of plant biostimulants

Biostimulants can be classified based on their function, source of active ingredient and type of activity or mode of action (Yakhin *et al.*, 2017). There are many categories of biostimulants with their unique functions (Table 3.1) such as biofertilizers, humic acids, fulvic acids, protein hydrolysates, seaweed extracts, nitrogen-containing metabolites, chitosan, inorganic compounds and beneficial microbes.

Biofertilizers are products containing natural substances or living organisms like fungi, algae or bacteria that can stimulate plant growth (Kumar, 2018; Ronga *et al.*, 2019). There are three categories of biofertilizers (i) nitrogen-fixing bacteria; (ii) phosphate-solubilizing microorganisms; and (iii) organic matter decomposers. Nitrogen-fixing bacteria are involved in reducing atmospheric nitrogen into ammonia to improve its uptake in plants by using nitrogenase enzyme (Kumar, 2018). Phosphorus, an essential plant

Table 3.1. Main categories of biostimulants, their sources, and potential functions.

Main categories	Sources	Functions	References
Humic acids	Organic matter of the soil, mostly consisting of decomposed animal and plant residues	Induce plant growth and differentiation Inhibit chloride ion uptake Stimulate nutrient uptake and release of phytohormones Alter primary and secondary plant metabolism linked to abiotic stress tolerance	Wang *et al.* (2019); Zanin *et al.* (2019); Canellas *et al.* (2020)
Fulvic acids	The organic fraction of the soil, mostly derived from coal using acid-base reaction, and solvent extraction	Stimulate nutrient absorption in plants Induce beneficial metabolic arrangements Induce anti-stress effects Reduce salt stress Improves nutrient content in plant tissues Regulate stomatal openings Stimulate soil enzymatic activities Reduce lead uptake and lead phytotoxicity	Li *et al.* (2019); Wang *et al.* (2019); Elrys *et al.* (2020); Gong *et al.* (2020); Del Buono (2021); Yao *et al.* (2021)
Protein hydrolysates	Products of plants (soy extracts, alfalfa plants), or animal residues (animal connective tissues, etc.) that have undergone enzymatic, chemical, or thermal hydrolysis	Improve germination and growth of seedlings Improve productivity and quality of crops Increase uptake of small peptides and amino acids Enhances soil respiration and microbial activity Improve nutrient uptake and use-efficiency	Saju *et al.* (2019); Francesca *et al.* (2020); Rouphael and Colla (2020); Consentino *et al.* (2020)
Macroalgal and microalgal extracts	Macroalgae consist of multicellular brown, green, and red algae while microalgae consist of mostly green and blue algae	Improve natural stress response to abiotic stress Increase nutrient uptake and nutritional quality Enhance resistance to pathogens Enhance biomass accumulation and crop yield Improve root system Enhance contents of proteins, osmoprotective molecules and antioxidant enzymes	Fleming *et al.* (2019); Saju *et al.* (2019); Ronga *et al.* (2019); Carillo *et al.* (2020); Madende and Hayes (2020)
Chitosan and other biopolymers	These biopolymers are found in the outer shell of shellfish, lobster, shrimp, or crabs (crustaceans), and also in fungal cell walls	Stimulate various defensive genes scavenging system Stimulate various enzymes of the reactive oxygen species Promote plant growth Promote environmental stress tolerance Promote resistance to several pathogens	Mehebub *et al.* (2019); Zohara *et al.* (2019); Chakraborty *et al.* (2020); Ren *et al.* (2021)
Inorganic compounds	Inorganic salts of Al, Co, Na, Se and Si are found in soil and plants; insoluble forms of these elements (amorphous silica, etc.) are found in graminaceous species	Reduce several fungal diseases Stimulate plant's antimicrobial defense Inhibit sporulation and hyphal growth Improves crop yield, floral intensity, and fruit size	Lagogianni and Tsitsigiannis (2019); Bhupenchandra *et al.* (2020); Havlin and Schlegel (2021); Hossain *et al.* (2021b)

Continued

Table 3.1. Continued.

Main categories	Sources	Functions	References
Beneficial microbes	Beneficial fungi such as AMF; beneficial bacteria – PGPR such as *Parabukholderia, Bacillus, Rhizobium, Azospirillum, Lysobacter, Pseudomonas* etc.	Inhibit pathogenic growth Produce volatile organic compounds Increase abiotic stress tolerance Improve nutrient availability Promote growth, yield, and quality of crop plants	Rahman *et al.* (2018a); Rajput *et al.* (2019); Chakraborty *et al.* (2020)

macro-element, often forms complexes in soil that can be prevented or solubilized by phosphate-solubilizing bacteria, making it available for uptake by plants (Kumar, 2018; Abawari *et al.*, 2020). Biofertilizers can be made by using an effective microorganism solution (a mixture of various bacterial species) and waste materials that can secrete useful substances to improve the quality of plant and soil (Ma and Liu, 2019; Mahmud *et al.*, 2021). Straw compost-based biofertilizers improve soil health and biodiversity of beneficial microbes in the rhizosphere, increase the crop productivity and induce systemic resistance in plants (Simarmata *et al.*, 2016).

3.2.1 Humic acids (HAs)

Humic acids are complex mixtures mainly made from hydrophobic compounds such as fatty acids that remain stable at neutral pH, precipitate in acidic solutions and remain soluble in aqueous alkaline solutions (Mahler *et al.*, 2021). Biostimulants can be derived from humic substances, which represent the major components of organic carbon on the earth's surface as they account for around 70–90% of soil organic matter (Mahler *et al.*, 2021; Nardi *et al.*, 2021). Through various chemical and biochemical reactions, humic substances are synthesized from the transformation of organic matter residues by microbial decomposition (Mahler *et al.*, 2021). These humic substances consist of nutrients such as nitrogen and sulfur that makes them ideal for use as organic fertilizers (Nardi *et al.*, 2021). The resulting metabolites can induce plant growth and root initiation, stimulate nutrient uptake, activate the plasma membrane H^+-ATPase activity and signal transduction pathways that regulate the growth and development of the plants (Tavares *et al.*, 2016; Galambos *et al.*, 2020; Cozzolino *et al.*, 2021). Moreover, humic substances can increase proline levels and lower membrane leakage which can allow plants to adapt better to salt stress (Van Oosten *et al.*, 2017). In addition, humic substances act like signaling molecules in the rhizosphere, resulting in the production and release of phytohormones (Canellas *et al.*, 2015).

3.2.2 Fulvic acid (FA)

Fulvic acid is a source of organic matter in the soil that can stimulate nutrient absorption in plants and induces beneficial metabolic arrangements (Del Buono, 2021). It is an important component of soil organic matter and has an anti-stress effect on plants. Soil application of FA reduces salt stress and improves nutrient content in plant tissues (Elrys *et al.*, 2020). It regulates stomatal opening, which is a useful trait for reducing the transpiration rate during drought stress (Fang *et al.*, 2020; Del Buono, 2021). Depending on the amount of FA added to the soil, it can reduce lead uptake and lead phytotoxicity in plants (Canellas *et al.*, 2015).

3.2.3 Protein hydrolysates (PHs)

Protein hydrolysates are a combination of several polypeptides, oligopeptides, free amino acids, carbohydrates, negligible amounts of minerals, phytohormones, phenols and some other organic compounds produced from the partial hydrolysis of protein derived from animal or plant sources (Colla *et al.*, 2017; Van Oosten *et al.*, 2017). PHs improve germination and growth of seedlings, and also the productivity and quality of the crops even in the presence of abiotic stresses (Rouphael and Colla, 2020). Plants absorb amino acids and peptides via their roots and leaves, which is often hampered due to the soil microbial activity. With the foliar application of PHs, the competition for amino acids with the microorganisms can be minimized, and therefore plants can uptake peptides and amino acids properly (Colla *et al.*, 2015). Besides, PHs can form complexes by chelating between soil nutrients and amino acids, resulting in enhanced soil respiration and microbial activity. Moreover, these metabolites can stimulate carbon and nitrogen metabolism in plants, and thus increase nutrient availability, uptake, and nutrient use-efficiency (Francesca *et al.*, 2020).

3.2.4 Macroalgal and microalgal extracts

Seaweed extracts affect the natural stress response and molecular priming mechanisms of the plant, resulting in improved resistance to abiotic stresses (Fleming *et al.*, 2019). These extracts have rich polysaccharide contents like fucoidan, alginate and laminaran, along with minerals and proteins that vary according to the type of the variants. The presence of phytohormones is considered to be the bioactive ingredient in biostimulants (Xu and Geelen, 2018; Du Jardin *et al.*, 2020). Seaweed extracts or macroalgae can also enhance nutritional quality and improve resistance against plant pathogens (Madende and Hayes, 2020).

Microalgae are unicellular autotroph organisms that synthesize various bioactive compounds using light energy (Tang *et al.*, 2020). Microalgae can be used to recover the excess nutrients when they are cultivated in wastewater or agricultural runoff, allowing the water to be reused. The application of microalgae can significantly boost nutrient uptake, biomass accumulation and crop yield, and in some cases, can increase the rate of seed germination (Garcia-Gonzalez and Sommerfeld, 2016). Moreover, plants have a better root system and improved content of proteins, osmoprotective molecules and antioxidant enzymes after the application of microalgae (Del Buono, 2021). This is mainly due to the bio-elicitor effect that microalgal polysaccharides have on the biochemical and metabolic pathways of plants (Rachidi *et al.*, 2021).

3.2.5 Nitrogen-containing metabolites

Organic nitrogenous molecules or nitrogen-containing metabolites (N-containing metabolites) are a group of peptides, polyamines, betaines, free amino acids and similar substances that can be synthesized chemically or by the chemical and enzymatic hydrolysis of agro-industrial by-products. The mixture of these molecules can be used as a biostimulant, as these are important constituents of several cellular, physiological and metabolic processes. In addition, these amino acids can also form chelates and complexes with nutrients present in the soil, which influences plant nutrition (Du Jardin, 2012). Free amino acids like histidine, taurine and proline regulate nitrogen accumulation through roots enhance nutrient uptake and improve yields. In addition, many precursors and derivatives of these amino acids work as important osmoprotectants that can protect the plant membranes and enzymes from disruption in cases of abiotic stresses. Simultaneously, some amino acids such as asparagine and cysteine act as metal chelators that significantly reduce heavy metal toxicity in plants (Madende and Hayes, 2020).

3.2.6 Chitosan and other biopolymers

Chitin and chitosan are bioactive co-polymers of N-acetyl-ᴅ-glucosamine and ᴅ-glucosamine, which can stimulate various defensive genes and several enzymes of the reactive oxygen species scavenging system in plants. As a biostimulant, it can promote plant growth (Mukta *et al.*, 2017), quality (Rahman *et al.*, 2018b), environmental stress tolerance and resistance to several pathogens (Mehebub *et al.*, 2019; Chakraborty *et al.,* 2020). As chitosan is the deacetylated form of chitin, it is not easily found in nature. Chitosan can be produced by the heterogeneous deacetylation of chitin. Chitin is usually found in all marine and terrestrial ecosystems, for instance in shrimp shells, crab shells, squid pins and in some filamentous fungi as well (Chakraborty *et al.,* 2020). The production procedure is very crucial since slight differences can greatly affect the physical properties of chitosan (Du Jardin, 2012; Pichyangkura and Chadchawan, 2015).

3.2.7 Inorganic compounds

Many plant beneficial elements like Al, Co, Se and Si are available in the soil as inorganic compounds. These components can directly protect the plant from osmotic stress or microbial damage, or harden the plant cell walls due to silica deposition. Moreover, these components can greatly improve the growth and quality, while also improving the plant's defense against abiotic (Bhupenchandra*et al.*, 2020) and microbial stresses (Du Jardin, 2012; Lagogianni and Tsitsigiannis, 2019). Phosphite-based products have been widely used in agriculture due to their antifungal properties. In addition, phosphites serve as a source of phosphorus, and their application has resulted in significant improvement in the quality and yield of various crops, such as rice, celery, cherry, cotton, etc. (Havlin and Schlegel, 2021).

3.2.8 Beneficial microbes (bacteria, fungi)

Microbial inoculants consisting of beneficial microorganisms, bacteria and/or fungi can be used to inhibit pathogenic growth and improve plant health (Ansary *et al.*, 2018; Rahman *et al.*, 2018a). Depending on the primary effects of these microorganisms, these can be divided into two different groups: (i) plant growth-promoting microbes (PGPM); and (ii) biological control agents (BCA). PGPR are a group of microorganisms that inhabit the plant rhizosphere and rhizoplane. These organisms can promote plant growth by producing volatile organic compounds, increasing abiotic stress tolerance and improving nutrient availability by either causing hormone secretions or by inducing hormonal changes within the plants. Both PGPM and BCA play an important role in controlling plant diseases, whereas BCA can directly promote plant growth when the plants are not affected by microbes (Prasad *et al.*, 2019; Huang *et al.*, 2021; Javeed *et al.*, 2021).

3.3 A brief outline of biostimulant formulation

Biostimulant formulation refers to the development of commercial PBs from natural substances, so these can be effectively stored, transported and applied for use without any inconveniences or drawbacks. Depending on the requirements of the consumer, the product can be created in either dry or liquid form, while maintaining all the required physicochemical properties of the biostimulant (Kumar and Aloke, 2020). Successful formulations result in an end product that has a long shelf-life, intact biological activity, easy application procedure and retains its activity long after application (Fig. 3.1; Oancea *et al.*, 2016).

3.3.1 Design inputs

At first, the biological and chemical characteristics of the raw materials for biostimulant production have to be identified; this helps to characterize the active ingredients in the raw materials required for the specific physiological responses. Then, the correct procedure for the isolation of these bioactive ingredients is decided to isolate the correct ratio of each of the ingredients without hampering their efficacy and quality. Next, the qualitative analysis for these ingredients is carried out using either liquid or gas chromatography, followed by quantification to make sure that the separated ingredients are appropriately carried out so that variations can be avoided in the different batches (Kumar and Aloke, 2020).

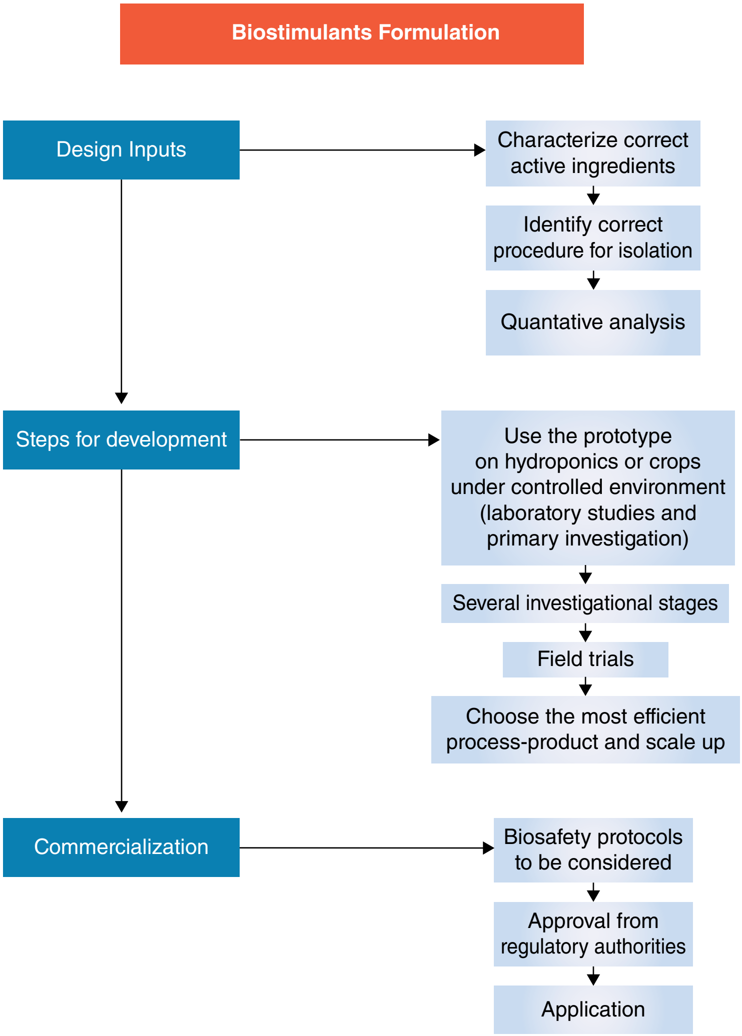

Fig. 3.1. A schematic diagram of the outline of biostimulant formulation.

3.3.2 Steps for development

The obtained product or prototype is first tested on hydroponics or crops in a controlled environment to determine the appropriate application techniques, times and rates. Following the first stages of investigation, many field trials are conducted to validate the prototypes' efficacy in distinct crops under various conditions. Finally, a consistent, efficient and cost-effective manufacturing method is developed. This manufacturing method is carried out in different labs and pilot plant facilities to obtain a commercial scale-up protocol (Povero *et al*., 2016; Kumar and Aloke, 2020).

3.3.3 Commercialization

Although commercial biostimulants increase plant yield and productivity, their application can lead to unexpected outcomes due to variances in the mechanism of action mostly affected by the soil type, treatment timing, species and cultivar (cv.) (Nephali *et al*., 2020). Notably, microbial biostimulants within the same species may have different strains with different genetic variations. Therefore, those microbial strains can be either harmless or pathogenic depending upon their genetic characters. Moreover, the horizontal gene transfer can also turn safe microorganisms into pathogenic strains (Barros-Rodríguez *et al.*, 2020).

3.4 Mechanism of biostimulants in abiotic stress management

3.4.1 Water stress

Food demand is rising as the world's population continues to grow. To meet this demand and boost crop yield, different harmful synthetic fertilizers and chemicals are being used in agricultural fields. In this case, the use of biostimulants can serve as a better alternative (Shukla *et al.*, 2019). Under water stresses, plants undergo several physiological changes such as stomatal closure, reduced turgidity, decrease in water potential of cells, and disruption of cellular components and metabolic functions by the accumulation of reactive oxygen species (ROS) (Zargar *et al.*, 2017). In this state, biostimulants can improve the photosynthetic efficiency, water content, as well as metabolic activity resulting in greater plant biomass (Van Oosten *et al.*, 2017). Biostimulants can improve the antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxide (APX) to protect the plant cells, and reduce the toxicity of free radicals under stress (de Vasconcelos *et al.*, 2009). In soils with low water content, treating seeds or seedlings with biostimulants can stimulate root production and growth, reduce the need for fertilizers and enhance productivity along with water stress resistance. Moreover, the increased antioxidant levels in plants enhance the root and shoot growth by retaining high water content in leaves and reducing the leakage of cellular electrolytes under stress. PBs promote root versus shoot growth so that the root can penetrate deeper to uptake water even in the water stress condition. Additionally, roots can uptake more water at a time due to the enlargement of root surface area stimulated by the microbial biostimulant (Van Oosten *et al.*, 2017; de Vasconcelos and Chaves, 2019).

3.4.2 Salt stress

Salt stress or a high level of salts in the environment is toxic for the plants, causing nutrient or ion imbalance, disruption of metabolic processes such as nitrogen assimilation, protein synthesis and photosynthesis, followed by cell injury during transpiration due to less water uptake. As a result, overall plant productivity is greatly hampered (Khalid *et al.*, 2019). Worldwide, about 800 million hectares of land, especially the arid and semiarid areas, are affected by salinity (Hernández, 2019). The application of humic substance-based biostimulants can improve the osmotic balance by improving water absorption and cell turgidity. The biostimulants containing algal extracts induce salt-stress tolerance in plants by targeting different pathways, improving the total chlorophyll and antioxidant content. Moreover, the bioactive compounds in biostimulants can enhance the germination rate, growth of shoots and roots, and improve the overall plant quality, productivity and yield under salt stress (de Vasconcelos and Chaves, 2019). Application of microalgal extracts from *Dunaliella* spp. and *Phaeodactylum* spp. in bell pepper significantly reduced the production of superoxide radicals and improved salt-tolerant ability during seed germination (Ronga *et al.*, 2019). Under salt stress, bioactive compounds in *Ascophyllumnodosum*-based biostimulants can improve tolerance by acting as a hydration buffer and sequestering ions to protect other proteins in plant cells (Shukla *et al.*, 2019). Beneficial microorganisms in the rhizosphere can also induce salt tolerance in plants such as *Piriformospora indica* in wheat grain (Hossain *et al.*, 2021a).

3.4.3 Temperature stress

Germination rate, growth, development, yield and metabolic processes are greatly affected by temperature stress (EL Sabagh *et al.*, 2020). In plants, heat stress causes inhibition of chlorophyll biosynthesis and photosystem II activity (Francesca *et al.*, 2020) and affects the production of secondary metabolites (Wozniak *et al.*, 2020). At higher temperatures, electrolyte leakage, disruptions of photosynthetic processes, inactivation of enzymes and loss of water-splitting activity is observed in plants (Khalid *et al*., 2019). Biostimulants can act as thermal stress relievers and can improve the germination and early stage growth of melons and a few vegetables such as leek, parsley and celery during high temperature stress (de Vasconcelos and Chaves, 2019). Heat tolerance in plants is usually achieved by the accumulation of several antioxidants, heat-shock proteins and osmoprotectants (ElBasyoni *et al*., 2017). Therefore, the application of amino acid-based biostimulants results in better photosynthetic efficiency, stomatal conductance and plant growth under temperature stress, as these are full of nitrogen-rich solutes which are accumulated by plants for thermal acclimation (Van Oosten *et al*., 2017; de Vasconcelos and Chaves, 2019). Besides, the application of biostimulants improves the stability of plant cell membranes at elevated temperatures and minimizes the accumulation of ROS (Bulgari *et al*., 2019).

3.5 Biostimulants for beneficial agronomic and physiological traits of plant

Biostimulants offer a potentially novel approach for sustainable agriculture. Biostimulants can improve overall plant growth and health in different ways (Fig. 3.2). Biostimulants such as seaweed extracts can promote germination of seeds and seedling vigor (Fayzi *et al*., 2020). However, the selection of appropriate biostimulants is necessary. For example, *Trichoderma* spp. significantly improve germination energy of scarlet sage seeds but reduces the germination energy in common zinnia seeds (Majkowska-Gadomska *et al*., 2017). Similarly, biostimulants can improve germination energy but not the germination capacity of the same species (Majkowska-Gadomska *et al*., 2017). Thus, an ideal biostimulant should be developed and selected to treat specific crops. Since biostimulants enhance the ability of plants to uptake nutrients and assimilate them, the resulting improvement in plants' quality and their yields are seen. Moreover, greater germination of quality seeds and seedlings results in a greater yield of the plants themselves. For instance, AMF inoculation can facilitate greater build-up of nutrients such as soluble sugars in plants and build a tolerance to abiotic stresses while also supporting seedling survival (Emmanuel and Babalola, 2020). Consequently, AMF inoculation can result in greater yields of crops (Emmanuel and Babalola, 2020). Similarly, the yield of crops such as winter wheat is improved through the application of amino acid formulations (Popko *et al*., 2018). While they do not directly provide nutrients to plants, biostimulants assist metabolic processes in the soil and plants which eases the absorption of nutrients and improves root system architecture (Drobek *et al*., 2019; Rouphael and Colla, 2020; Cozzolino *et al*., 2021). For instance, AMF helps plants to more effectively absorb phosphorus for growth (Tavarini *et al*., 2018). Similarly, the application of rosemary essential oil increases the uptake of nitrogen, potassium, magnesium, iron, and zinc in tomato seedlings (Souri and Bakhtiarizade, 2019). Alongside, biostimulants increase the concentrations of primary metabolites such as soluble carbohydrates and chlorophyll (Souri and Bakhtiarizade, 2019; Kulkarni *et al*., 2019). Interestingly, they can also induce plant stress conditions to amplify the production of secondary metabolites including carotenoids, phenylalanine ammonia-lyase and phenolic compounds (Drobek *et al*., 2019; Kulkarni *et al*., 2019).

Furthermore, biostimulants could be devised to provide disease resistance to many crops which is crucial to tackling rising pathogens. To illustrate, PSP1 provides wide-ranging protection against many pathogens including *Corynespora cassiicola*, *Acidovorax avenae* and *Fusarium graminearum* (Chalfoun *et al*., 2018). Different defense responses such as accumulation of superoxide radicals alongside hydrogen peroxide and induction of pathogenesis-related genes are induced by PSP1. In another instance, the application of chitosan could suppress *Phytophthora capsici* infection in cucumber seedlings while also improving seed germination and growth (Zohara *et al*., 2019). In addition, biostimulants enhance the photosynthetic ability of plants. Greater amounts of chlorophyll are caused by increases in leaf surface area, which biostimulants can contribute to. Extracts of *Moringa oleifera* leaf and seaweed have been shown to enlarge leaf surface area alongside chlorophyll content (Drobek *et al*., 2019). As a result, increased chlorophyll content makes the photosynthetic process more efficient. Treatment of maize with *Azotobacter chroococcum* shows an increase of

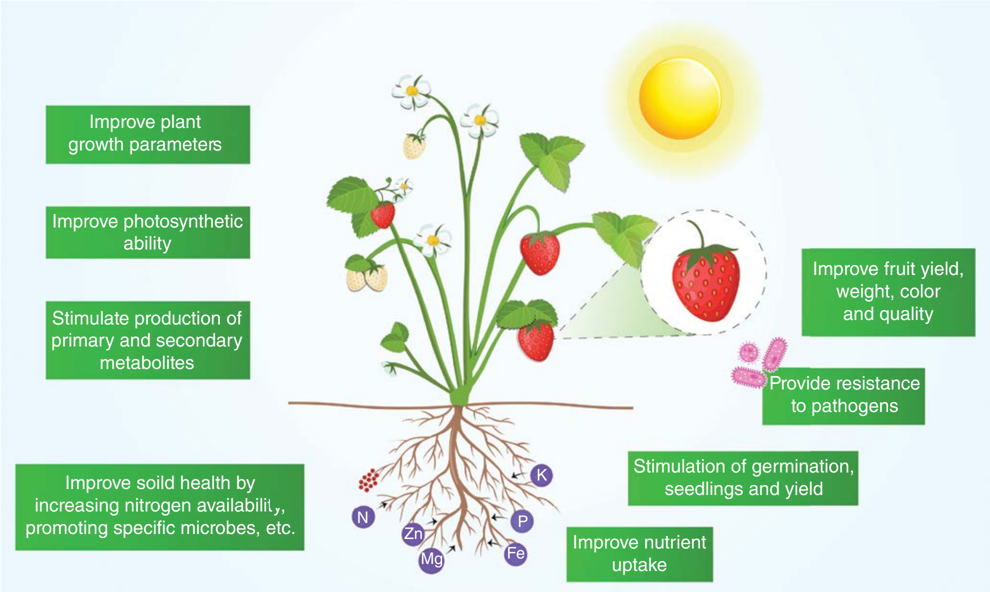

Fig. 3.2. A schematic diagram outlining the effects of biostimulants on plant growth and health.

5.3% in chlorophyll content, which coincides with an increase of 18.4% in photosynthesis rate (Efthimiadou *et al.*, 2020).

Accordingly, greater nutrient absorption and higher production of metabolites lead to produce of better quality (Table 3.2) (Chakraborty *et al.*, 2020). Growth parameters including plant height, dry weight and stem diameter are also positively affected by specific biostimulants (Szöke *et al.*, 2021). Besides the weight and length of fruits, biostimulants increase the shelf-life for many fruits and vegetables (Arnao and Hernández-Ruiz, 2021). For instance, the application of chitosan at 500 ppm and 1000 ppm could prevent 100% decay in tomatoes until the 8th day of treatment at room temperature (Sakif *et al.*, 2016). Biostimulants also have an impact on the color of the fruit which largely depends on the anthocyanin content. Redder strawberries and darker cherries were obtained after treatment (Weber *et al.*, 2018; Correia *et al.*, 2019). Importantly, altering color will boost consumer preference, sales and profit for farmers. Varying modifications in acidity arose from applying biostimulants which did not necessarily provide any concrete evidence of positive or negative influence on taste (Drobek *et al.*, 2019). Also, levels of glucose, fructose and sucrose rise in treated fruits. In squash treated with moringa leaf extract, total soluble sugars concentration saw an increase of 80.6% (Abd El-Mageed *et al.*, 2017). Alternatively, berry skins treated with an *Ascophyllum nodosum* extract had a lower sugar content due to delay in ripening (Salvi *et al.*, 2019). Therefore, the overall effect on the sugar level in fruit seems to be specific to each biostimulant. Besides benefiting plants, biostimulants improve soil quality by affecting specific microbial communities, stimulating mineralization of organic matter, and increasing nitrogen availability (Hellequin *et al.*, 2018; Yousfi *et al.*, 2021). By promoting soil bacterial growth and greater soil enzyme activity, biostimulants contribute to a greater impact on soil biological activity (Sadeghi and Taban, 2021). Lastly, long-term benefits of soil treated with biostimulants in semi-arid climatic conditions are the production of vegetation, protection against erosion, and contribution to soil restoration. Biostimulants such as *Ascophyllum nodosum* extracts provide chelation in soil that increases soil aeration, water-retention capacity, and minerals (Shukla *et al.*, 2019). Several benefits of selected biostimulants can be summarized using a few examples in Table 3.2.

Table 3.2. Advantages of selected biostimulants on the growth of plant species at particular experimental doses.

Biostimulant	Treated species	Doses	Benefits	References
6-Benzyladenine	Northern highbush blueberry (*Vaccinium corymbosum* L.)	100 mg L^{-1}	Increase fruit weight (in Duke) by 33.6% in the 2nd year compared to the control and (in Bluecrop) by 33.1% in the 2nd year compared to the control Higher fruit yield (in Duke) at 2714 g/bush in 2nd year compared to control at 1546 g/bush and (in Blue crop) at 2344 kg/bush in 2nd year compared to control at 1134 kg/bush	Milić *et al.* (2018)
α-Naphthaleneacetic acid	Northern highbush blueberry (*V. corymbosum* L.)	20 mg L^{-1}	Increase fruit weight (in Duke) by 41.9% in the 1st year compared to control and (in Blue crop) by 55% in the 1st year compared to control Higher average fruit diameter (in Blue crop) at 15.3 mm in the 1st year compared to 12.6 mm in control	Milić *et al.* (2018)
Humic acid	Yarrow (*Achillea millefolium* L.)	20 kg ha^{-1}	Increase plant height by 23% compared to the control in the greenhouse Increase the shoot dry weight by 50% compared to the control in the greenhouse Increase the antioxidant activity of the leaves by 17% compared to the control in the field	Bayat *et al.* (2021)
Fulvic acid	Yarrow (*Achillea millefolium* L.)	20 kg ha^{-1}	Increase the shoot dry weight by 60% compared to the control in the greenhouse Increase the total chlorophyll content by 45% compared to the control in the field Increase the total flavonoid content by 91% compared to the control in the field	Bayat *et al.* (2021)
CycoFlow (sugar cane molasses mixed with yeast extract)	Tomato (*Solanum lycopersicum* L.)	Initial concentration: 400 mL per plant, final concentration: 3 g L^{-1}	Increase above ground fresh biomass by 68.4% in genotype PIDVIT compared to control Increase pollen viability of all genotypes (particularly E107) compared to respective controls Increase maximal PSII photochemical efficiency (Fv/Fm) in genotypes PDVIT and E107 compared to respective controls Increase reduced ascorbic acid (in E17 and E36), total ascorbic acid (in E36 and PIDVIT), phenols content (in PIDVIT), carotenoid content (in E36 and E107), chlorophylls a and b (in E36), and total lipophilic antioxidant activities (in PIDVIT and E107) compared to respective controls	Francesca *et al.* (2020)
Plant probiotic bacteria (*Bacillus amylolequefaciens* BChi1 and *Paraburkholderia fungorum* BRRh-4)	Strawberry (*Fragaria* × *annanasa*)	approx. 1×10^9 CFU mL^{-1}	Increase leaf width at 14.15 cm for BRRh-4 treated plants and at 13.54 cm for BChi1 treated plants compared to 10.78 cm for control	Rahman *et al.* (2018a)

			Increase shoot fresh weight found at 220 g/plant for BRRh-4 treated plants and 193.2 g/plant for BChi1 treated plants compared to only 134.5 g/plant in control Increase root fresh weight at 21 g/plant for BRRh-4 treated plants and 20.5 g/plant for BChi1 treated plants compared to 12.6 g/plant in control Increase fruit yield by 48% in BRRh-4 treated plants and by 43% in BChi1 treated plants compared to control Increase anthocyanin content of fruits at 222 mg cyanidine-3-O-glucoside/100g fruit for BRRh-4 treated plants and at 187.47 mg cyanidine-3-O-glucoside/100 g fruit for BChi1 treated plants compared to 81.11 mg cyanidine-3-O-glucoside/100 g in control Significantly increase total carotenoid content, phenolic content, flavonoids content, and antioxidant activity for both BRRh-4 treated plants and BChi1 treated plants compared to control	
Carboxylic acids: N-acetiltiazolidin-4-carboxylic acid (AATC) and triazolidine carboxylic acid (ATC)	Apricot (*Prunu sarmeniaca* L.)	200 mL/100 L of water	Increase initial fruit set by 13.5% compared to control in 2015 and 0.3% compared to control in 2016 Increase yield per tree by 6.3 kg compared to control in 2016 Fruit length, width, and thickness increased in 2015 where phenolic content improved in 2016 compared to control. On the other hand, antioxidant capacities enhanced in both 2015 and 2016 compared to respective controls	Tarantino *et al.* (2018)
Ascophyllum nodosum (brown seaweed) extract	Grapevines (*Vitis vinifera* L. cv. Sangiovese and cv. Pinot Noir and Cabernet Franc)	1.5 kg/ha	Increase yield, cluster weight, skin total anthocyanins, and phenolics compared to controls (cv. Sangiovese) Increase shoot lengths and leaf soluble sugars compared to controls (cv. Pinot Noir & Cabernet Franc) Increase net photosynthesis and stomatal conductance compared to controls (cv. Cabernet Franc) Increase transpiration and photosystems efficiency compared to controls (cv. Pinot Noir)	Frioni *et al.* (2018)
Protein hydrolysate-based biostimulant	Tomato (*Solanum lycopersicum L.*)	3 g/L of water	Increase pollen viability by 51% compared to non-treated plants under water deficit Increase stomatal conductance by 84% compared to control under full irrigation Increase total antioxidant activity in leaves by 98% compared to control under water deficit	Francesca *et al.* (2021)
Chitosan	Strawberry (*Fragaria × annanasa*)	500 ppm	Increase the fruit yield by 42% compared to the control Increase total fruit weight at 19.25 g/fruit compared to 15.7 g/fruit compared to untreated control Increase the carotenoid content of fruit by 2.4-fold compared to the control Increase the anthocyanin content of fruit by 2.3-fold compared to the control	Rahman *et al.* (2018b)

3.6 Role of biostimulants in sustainable agriculture

Sustainable agriculture has drawn the interest of researchers with limited farming land, increasing deforestation and rapid environmental destruction. To combat the disadvantages of scarce resources in the world, biostimulants could be the answer to efficient utilization of such resources without sacrificing food quality and quantity. Currently, many biostimulants are being developed as active compounds derived from wastes or raw organic material. The role of biostimulants in sustainable agriculture is shown in Fig. 3.3. To illustrate, composted urban waste, sewage sludge, vermicompost, protein hydrolysate and chitin derivatives are few classes of waste-derived biostimulants or organic matter with biostimulants that have proven to be effective in agriculture (Xu and Geelen, 2018). While preservation of bioactive compounds in such origins is critical, the abundance of waste resources and low costs highlight the advantages of waste biostimulants (Colantoni *et al.*, 2017). Besides, biostimulants derived from waste will help in recycling and reducing landfills, particularly from industrial waste. Most protein hydrolysate-based biostimulants are derived from cattle leather wastes (Colantoni *et al.*, 2017). Coupled with promoting soil health mentioned earlier, biostimulants can help establish a more environment-friendly agroecosystem.

Another crucial factor of sustainable agriculture depends on the use of organic fertilizers. Since chemical fertilizers are more harmful to the environment, organic fertilizers are being considered as an eco-friendlier alternative. Although organic fertilizers improve soil fertility, increase soil nutrient content and boost the growth of beneficial soil microorganisms, the yields of organic farming in many cases are much lower compared to conventional farming (Xu and Geelen, 2018; Cheng *et al.*, 2020). To compensate for the lower yield, biostimulants can complement organic fertilizers. Vermicomposts not only increase yields, but also improve plant growth parameters and photosynthetic activity (Younas *et al.*, 2021). Thus, utilizing a combination of biofertilizers and biostimulants can set up a sustainable food production system

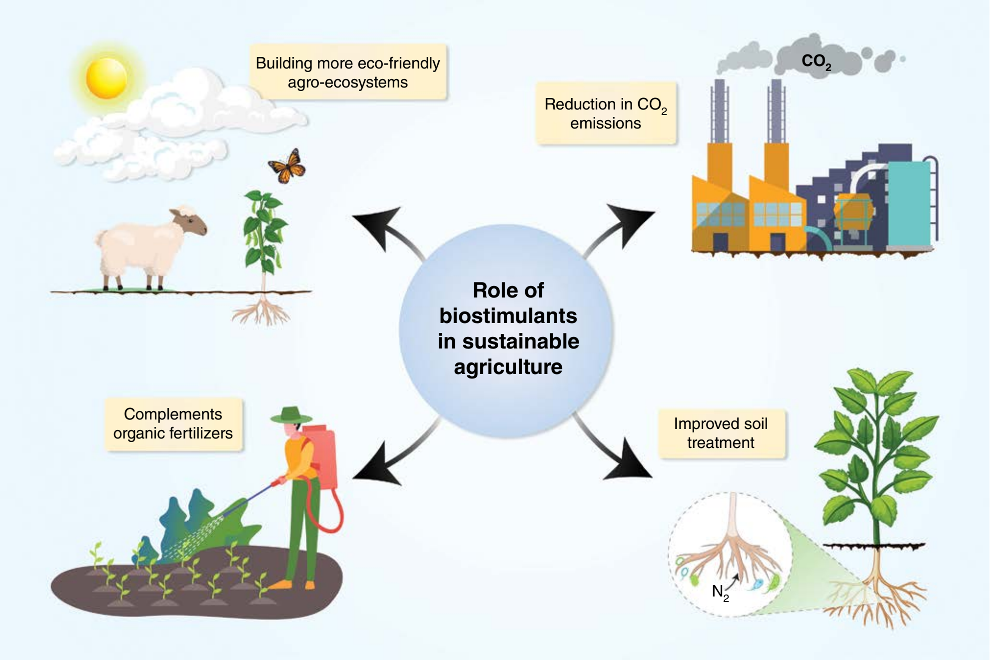

Fig. 3.3. A schematic diagram that shows biostimulants' contribution to building sustainable agricultural practices.

with lower harmful impacts on the environment. Finally, biostimulants can be used as a way of mitigating climate change through the use of reducing greenhouse gas emissions. As the production and utilization of chemical fertilizers emit large amounts of greenhouse gases, replacement with biostimulants could reduce such emissions while also increasing photosynthetic activity which has an overall positive effect on carbon dioxide emissions (Del Buono, 2021). Accordingly, the application of *Kappaphycus* seaweed extract (KSWE) at just 5% saved at least 260 kg CO_2 equivalents (Mg cane production) ha^{-1} (Singh *et al.*, 2018). Thus, this field of research warrants immediate attention as the agricultural sector is a large contributor to global warming.

3.7 Conclusion

Biostimulants are developed as key players in boosting plant health and growth that are available in a myriad of formulations with various ingredients. Tackling abiotic stresses, these beneficial agents have proven to be effective for numerous positive agronomic traits and establishing eco-friendlier farming practices. Therefore, biostimulants have immense potential in the future for providing more sustainable agriculture with minimal environmental impact and an efficient food production system. Nevertheless, certain factors need to be addressed before turning this into a reality. Firstly, monitoring the physiological effects on plant processes requires careful consideration since these processes have been tightly regulated for millions of years. Filtering and perfecting a specific compound that noticeably impacts these processes require long-term studies and considerable capital. Similarly, the design and formulation of biostimulants face technical challenges and difficulties. Importantly, the production of biostimulants from animal origins has a worse impact on the environment than those from plant origins. Therefore, an efficient, cost-effective and eco-friendly method of production for these bio-agents must be determined. If the application of biostimulants is in conjunction with organic farming, it will take many years to achieve the targeted yield. Finally, it is vital to establish regulatory protocols concerning the utilization of different biostimulant formulations on specific plants to ensure the prioritization of a healthy ecosystem and environment. When these factors are addressed, the dependence on chemical fertilizers and emissions of greenhouse gases will be depleted which can reverse global warming. To conclude, biostimulants may be one of the only solutions to provide food security without destroying the environment and the future of the human population.

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4 Biofortification for Crop Quality Enhancement

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Abstract

In the developing world, micronutrient malnutrition has emerged as a major health problem. Insufficient supply of various types of vitamins, minerals and amino acids leads to poor body growth and major economic losses. Globally, 2 billion people (or one in three individuals) suffer micronutrient deficiency. These deficiencies can be treated through biofortification, a process which is used to enhance crop yield through transgenic techniques, plant breeding and agronomic practices. Biofortified crops are grown and used by around 20 million people in the world and will be used by 2 billion people in 2030. A number of elements, including carbon, nitrogen, hydrogen, selenium, zinc and iron, are essential for daily life and are found in major biofortified staple food. Non- staple food prices have also increased which makes their availability difficult to poor communities. Therefore, biofortification is a feasible and cost-effective way to improve crop quality. This chapter discusses the role of biofortification in quality enhancement of crops and nutritional security of the common people.

4.1 Introduction

Malnutrition has caused serious problems worldwide, particularly in underdeveloped countries (Biesalski and Birner, 2018). The main reasons for this serious problem may include improper balanced diet, lack of immunity and susceptibility to diseases. Most important above all is the loss of gross domestic product per year – its rate is 11% in Africa and Asia (Harimbola and Mizumoto 2018). It is estimated that lack of proper nutrition affects 2 billion people all over the world. Generally, under the age of 5, around 151 million children have impaired growth and 51 million have low and improper height (Yadav *et al.*, 2018). A balanced diet generally comprises the proper ratio of both micro and macronutrients. Macronutrients include carbohydrates, proteins and lipids, whereas micronutrients include iron, zinc, selenium, cobalt, vitamin A and nickel (Prashanth *et al.*, 2015). Besides the fact that these are required in minute amounts, micronutrients have evolved to play a vital role in our bodies individually and in collaboration with other nutrients as a cofactor (Welch and Graham, 2004). Agricultural products are considered as

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the main source of food and mainly survival particularly in developing countries (Schneeman, 2001).

Pregnant women and pre-school children experience increased severity of malnutrition than any other groups. Hidden hunger is faced by 30% population across the world (Stevens *et al.*, 2013). So, malnutrition of micronutrients is a major factor of many of the problems. Unequal distribution of micronutrients in plants is another factor related to malnutrition (Zhu *et al.*, 2007). Poverty and lack of awareness about health are some other problems associated with malnutrition (Gilani and Nasim, 2007). The only solution to resolve the problem of malnutrition is to make food rich in micronutrients. Scientists are devising new strategies for overcoming the shortage of nutrients. Biofortification is the process of enhancing quality of crops and minerals by using transgenic techniques, plant breeding and agronomic practices. The crops that are produced from such techniques are called biofortified crops. Scientists put force on the main purpose for producing such crops which is improved quality (Bouis *et al.*, 2011). The main focus is to improve nutrition in staple food at low cost.

4.2 Valuable micronutrients

The main reason behind biofortification is the prevalent rate of diseases due to malnutrition in underdeveloped countries. Micronutrients have made a revolution in science – the so-called green revolution is key to the biofortification process.

Zinc plays a vital role in our bodies, including carrying signals across the cells and other functions (MacDonald, 2000). It lowers the risk of cell destruction from reactive species (Prasad *et al.*, 2004). For instance, zinc availability may have a preventive role against various types of cancer, including prostate and pancreatic cancer. (Costello and Franklin, 2017). Zinc has a significant role in cell division, so its deficiency can cause serious problems during pregnancy. Zinc deficiency affects both physical and mental health. As a result, our body fails to respond to the changes in our surroundings (Roohani *et al.*, 2013).

Iron is the fourth most abundant element in the Earth's crust. Iron has significant role in the electron transport chain and synthesis of nucleic acids like DNA (Abbaspour *et al.*, 2014). Iron is the central atom of hemoglobin (McDowell, 2003). Around 2 billion people in the world are affected with iron-deficiency anemia (De Benoist *et al.*, 2008). Stunted growth in children and loss of mental skills are basic symptoms correlated with iron deficiency (Lozoff *et al.*, 2008).

Iodine is considered to be an important part of the human endocrine system as it is responsible for the production of thyroxin in the thyroid gland, deficiency of which causes goiter and other diseases in which the brain fails to respond to stimulus and even leads to death. Iodine deficiency in pregnant women causes maternal and fetal hypothyroidism and other serious cognitive and neurologic disorders in both mother and child (Pearce *et al.*, 2016).

Carotenoid deficiency affects the part of the retina called macula, which is responsible for central vision. A special type of carotenoid called lutein has a role in treatment of cataracts and it also normalizes blood pressure that may cause heart problems (Moeller *et al.*, 2000). Vegetables, especially carrots, are a rich source of beta-carotene, and recent studies have determined that carotenoids may have properties that are helpful in the treatment of cancer (Tanaka *et al.*, 2012).

Selenium has a key role in body as it fights against cancer and protects cell organelles from damage (Rayman, 2005). Diseases like Keshinbeck and Keshan develop (Tinggi, 2008) when selenium availability is reduced (Zengand and Combs Jr 2008). Vitamin B plays an important role in the synthesis of proteins, DNA and RNA (Scott *et al.*, 2000). Many types of secondary metabolites are formed through folic acids like alkaloids and lignin, and chlorophyll in plants is also likely to be synthesized from vitamin B (Hanson and Roje, 2001). As humans cannot synthesize folates, they need to eat plant material as a source of folates (Basset *et al.*, 2005). Unavailability of vitamin B may affect mental health including depression (Moorthy *et al.*, 2012) and loss of memory (Ramos *et al.*, 2005), as well as heart problems (McCully, 2007) and uncontrolled cell division (Jang *et al.*, 2005). Folic acid is also suggested as a supplement for pregnant women (Pitkin, 2007) as it can prevent certain development disorders in the fetus (Scholl and Johnson, 2000). If the folate level is low, it can be lethal to newborns (Wallock *et al.*, 2001). Vitamin B plays a vital role in the reproduction cycle (Tamura and Picciano 2006).

4.3 Methods used for enhancing nutritional values

Depending upon the demands of food and its security, various methods have been used for enhancing crop quality and quantity that are also cost effective. These may include dietary diversification, food supplements and biofortification (Fig. 4.1).

Different crops are fortified with micronutrients to increase the quality and quantity of food with minimum risks (Hefni *et al.*, 2010). The best example is the addition of vitamin B containing folic acid, which is the primary source of folates (Shohag *et al.*, 2012). Zinc and vitamin A levels can be easily improved through biofortification (Wiltgren *et al.*, 2015). In dietary diversification, all kinds of plant-based foods are grown with proper domestic level techniques, which may include fermentation or soaking, through which the availability of nutrients is increased (Gibson and Hotz, 2001), for example with better iron content (WHO, 2004). In cereals, other methods like germination or fermentation may also be used that make easy access of nutrients by hydrolyzing the acids through enzymatic actions (Cook, 2005).

The main target of biofortification is increasing the crop quantity and reducing the poor quality of food due to nutrient deficiency (Saltzman *et al.*, 2013). Depending on requirements, one or several methods may be used in a single crop. For examples, cereals, legumes and vegetables may involve all three approaches (agronomic, transgenic and conventional) for synthesis (Fig. 4.2).

4.4 Agronomic means of biofortification

Agronomic approaches use various methods to enrich crops nutrients, including mineral fertilizers or foliar fertilization and inoculation of microorganisms into soil (White and Broadley, 2009).

In foliar application, nutrients are directly applied to the aerial parts of the plant including shoots and leaves (Garg *et al.*, 2018). Different pulse crops have been fortified through foliar sprays containing nutrients like iron, selenium or zinc. Recent studies have shown that iron concentration was increased in cowpeas (Márquez-Quiroz *et al.*, 2015) and peanut when iron was administrated by foliar application. Foliar application of iron and zinc increased their concentrations in cowpea (*Vigna unguiculata* L.) (Salih, 2013) and chickpea seeds, and also enhanced protein levels (Nandan *et al.*, 2018). Foliar application of zinc not only enhances its supply and transport in chickpea (*Cicer arietinum* L.), but also increases grain yield (Shivay *et al.*, 2015). Beans are important source of

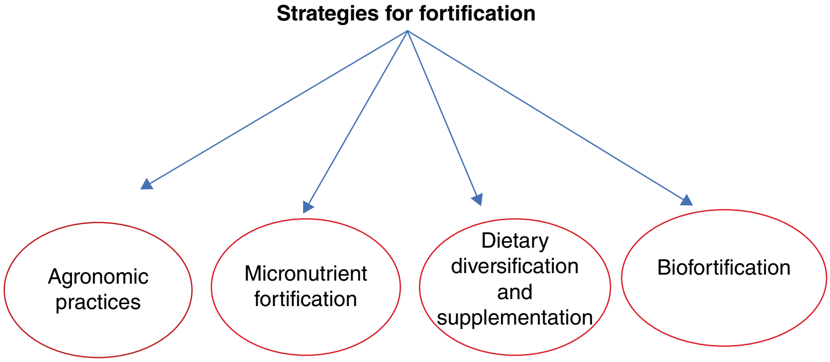

Fig. 4.1. Strategies for biofortification.

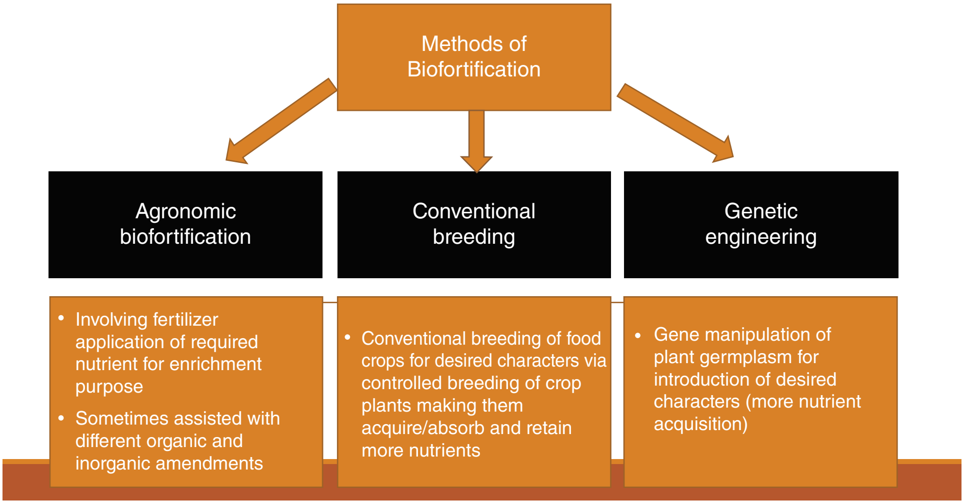

Fig. 4.2. Approaches for biofortification.

nutrients (Ibrahim and Ramadan, 2015) and are also fortified with zinc (Ram *et al.*, 2016), which shows that zinc fortification through foliar spray is helpful in beans and also in peas (Poblaciones and Rengel, 2016). Foliar application increased selenium in peas (Smrkolj *et al.*, 2006), chickpeas (Poblaciones *et al.*, 2014), beans and lentils (Rahman *et al.*, 2015) and iodine in plants (Çakmak *et al.*, 2017).

Plants usually absorb essential nutrients from the rhizosphere. If the soil is nutrient deficient, essential nutrients can be applied through fertilizers (Dai *et al.*, 2004). Zinc and iodine levels are commonly improved through mineral fertilization (Winkler, 2011), but iron levels are not improved in saline soils. Zinc has found diverse importance through mineral application (Poblaciones and Rengel, 2016). However, as this application method requires a continuous supply it can become an environmental hazard (Hefferon, 2015). Furthermore, different types of soil (Frossard *et al.*, 2000) and the location of soil also affect the application and results (Ismail *et al.*, 2007).

Bacteria play an important role in plants by providing them with nutrients and aiding in the production of hormones. Mineralization also takes place through bacteria (Panhwar *et al.*, 2012). Phytosiderphores increase the iron availability in soils by making chelates of iron (Sreevidya *et al.*, 2016). The association between plants and fungi is important because studies have revealed that it has a role in increasing Zn concentrations (Smith and Read, 2010). In the root nodules of chickpea and soybeans, increased quantities of nutrients, yield, growth (Valverde *et al.*, 2007) and rate of nitrogen fixation was found to be increased (Minorsky, 2008) because of the presence of various types of bacteria in the roots, for example *Bacillus* spp. and *Pseudomonas* spp. (Gopalakrishnan *et al.*, 2015). Actinobacteria in chickpeas has been shown to be responsible for increased concentrations of iron and zinc (Sathya *et al.*, 2016), as does the presence of mycorrhizal fungi (Pellegrino and Bedini, 2014).

4.5 Agronomical biofortification in cereals

Agronomic approaches include direct applications of nutrients by physical means (Çakmak and Kutman, 2018) and through microorganisms (Table 4.1).

Spraying zinc on rice aerial parts increased the concentration of zinc in the plants (Boonchuay *et al.*, 2013) and also improved the yield (Shivay *et al.*, 2008), which becomes responsible for availability of zinc for plant growth (Ram *et al.*, 2016). Zinc in excess quantities (Jiang

Table 4.1. Agronomic approach for cereals and legumes.

Cereal/legume	Biofortification	References
Rice	Iron	Fang *et al.* (2008)
	Zinc	Wei *et al.* (2012)
	Selenium	Chen *et al.* (2002)
Wheat	Zinc	Yang *et al.* (2011)
	Chemical fertilizer	Ramzani *et al.* (2016)
	Mycorrhiza	Noori *et al.* (2014)
Sorghum	Bacteria	Dhawi *et al.* (2016)
Soybean	Selenium	Yang *et al.* (2003)
Chickpea	Calcium	Sathya *et al.* (2016)
	Zinc	Shivay *et al.* (2015)
Pea	Zinc	Poblaciones and Rengel (2016)
Common bean	Zinc	Ram *et al.* (2016)

et al., 2008) gives plant a proper growth period (Hamidou *et al.*, 2014). Selenium (Chen *et al.*, 2002) plays an important role in the (Ros *et al.*, 2016) against antioxidants (Premarathna *et al.*, 2012) when produced in large quantities in the plant of rice (Giacosa *et al.*, 2014). In brown rice, selenium accumulation was the evidence of successful foliar application (Liu and Gu, 2009). Spray on the aerial parts of wheat resulted in strong growth of the plant with increased accumulation of nutrients (Aciksoz *et al.*, 2011). For instance, foliar application of different nutrients solutions on rice significantly mitigates seawater salinity and improved the dry biomass, grain quality, yield and nutrient accumulation (Sultana *et al.*, 2001). Moreover, zinc foliar spray has also resulted in growth of wheat and hydrolyzes different acids like phytic acid (Yang *et al.*, 2011). Another study reveals that the combined use of humic acids, chitosan with foliar application of zinc could be the effective approach in improving *Phaseolus vulgaris* L. growth, yield and nutrient uptake (Ibrahim and Ramadan, 2015).Use of fungal association with plants is another key process through which fortification is done to ensure nutrient availability (Noori *et al.*, 2014). Chemicals and fertilizer application makes the iron fortification possible (Ramzani *et al.*, 2016), and zinc is introduced via bacteria (Ramesh *et al.*, 2014).

Both the fertilizers (Fahad *et al.*, 2015) and zinc spray onto leaves have been applied to maize plants (Zhang *et al.*, 2013). Biofortification using rhizobacteria to provide efficient nutrient availability of zinc to plants has been reported (Prasanna *et al.*, 2015). A similar process has been used for Se fortification (Ros *et al.*, 2016). Sorghum, which is grown as fodder and also used as a source of food for human beings, has also been fortified through agronomic approaches to improve its nutrition profile. Both organic and inorganic fertilizers are used in biofortification (Table 4.1 and Table 4.2). Recent studies have shown that the combined use of bacteria and fungi has a positive effect on sorghum, which is key source of fodder (Dhawi *et al.*, 2016). The co-inoculation of phosphate-solubilizing bacteria and *Azospirillum* increases the phosphate and nitrogen in the soil, which improves the growth and yield of the sorghum via increasing the production of amino acids (Patidar and Mali, 2004).

Complex salts of Se are applied on soybean to minimize the deficiency of Se. This application has been done through foliar spray. Mineral deficiencies like Fe, Zn, Ca, Mg and Mn have been reduced by the use of microorganisms in chickpea (Sathya *et al.*, 2016), resulting in increased growth and yield (Pellegrino and Bedini, 2014). Peas (*Pisum sativum*) are well known for their enriched protein content, and have been fortified through foliar spray of Zn or through soil microorganisms (Poblaciones and Rengel, 2016). Foliar spray of Zn has been reported in common beans (*Phaseolus vulgaris* L.) (Ram *et al.*, 2016).

Foliar spray of zinc sulfate effectively increased Zn (White *et al.*, 2017) and Se content in potato tubers (Cuderman *et al.*, 2008). Beta-carotene content increased in sweet potato (*Ipomea batatas* L.) by application of fertilizers (Laurie *et al.*, 2012). Iodine biofortification has shown positive results in tomato with foliar application of potassium iodide in tomato (Landini *et al.*, 2011). Transgenic or genetic engineering is generally applied when there is a limitation in genetic variation (Zhu *et al.*, 2007). A specific gene is identified for required trait and is then inserted into the plant (Christou and Twyman, 2004). Use of bacteria is common in this technique (Newell-McGloughlin, 2008) (Table 4.2).

4.5.1 Transgenic cereals

To overcome color blindness, genetically modified golden rice was introduced (Beyer *et al.*, 2002). The gene for carotene desaturase has been encoded and expressed (Paine *et al.*, 2005). Rice crops have been modified to increase the folic acid content as these are key elements of vitamin B9 and helpful for women during pregnancy (Bibbins-Domingo *et al.*, 2017). Genes responsible for the high iron ratio in soybean have been inserted into wheat (Xiaoyan *et al.*, 2012). To improve the production of carotene, the provitamin A gene has been inserted in wheat (Wang *et al.*, 2014; Zhu *et al.*, 2008). A similar procedure has been done for carotenoids concentration in wheat (Zhu *et al.*, 2008; Cong *et al.*, 2009). Zinc transporter genes have been encoded and expressed to enhance zinc productivity in barley (Ramesh *et al.*, 2004). Moreover, beta-glucan genes have been inserted into barley to increase its concentration, which has benefits for preventing heart diseases (Burton *et al.*, 2011). Sorghum, by the introduction of the lysin protein gene, has been modified to produce more amino acids (Zhao *et al.*, 2003). The bacterial *PSY* gene has been inserted in soybean to enhance the content of oleic acid as it is the best source of vegetable oil (Schmidt *et al.*, 2015) (Table 4.3).

4.5.2 Transgenic vegetables and oilseeds

Potatoes have been modified in order to improve production by inoculating the *PSY* gene to increase carotenoid production (Ducreux *et al.*, 2004) (Table 4.4). The gene which converts the beta-carotene into zeaxanthin and hence results in deficiency of carotene, is made

Table 4.2. Agronomic biofortification in vegetables and fruits.

Vegetables/fruits	Biofortification	References
Carrot	Iodine	Smoleń *et al.* (2016)
Potato	Zinc	White *et al.* (2017)
Sweet potato	Beta-carotene	Laurie *et al.* (2012)
Tomato	Iodine	Landini *et al.* (2011)
Canola	Selenium	Nosheen *et al.* (2011)

Table 4.3. Cereal biofortification through transgenic means.

Cereals	Biofortification	References
Rice	Zinc	Lee and An (2009)
	Folate	Storozhenko *et al.* (2007)
	Iron	Lee and An (2009)
	Beta-carotene	Ye *et al*. (2000)
Wheat	Amino acids	Tamás *et al.* (2009)
	Anthocyanin	Doshi *et al.* (2006)
	Iron	Xiaoyan *et al.* (2012)
Sorghum	Lysin	Zhao *et al.* (2003)
	Provitamin A	Lipkie *et al.* (2013)
Barley	Zinc	Ramesh *et al.* (2004)
	Lysin	Ohnoutkova *et al*. (2012)
Maize	Provitamin A	Zhu *et al.* (2007)
	Vitamin E	Cahoon *et al.* (2003)

Table 4.4. Transgenic vegetables and fruits.

Vegetables/fruits	Biofortification	References
Cassava	Beta-carotene	Den Herder *et al.* (2012)
Potato	Amino acid	Chakraborty *et al.* (2010)
	Beta-carotene	Lopez *et al.* (2008)
Linseed	Flavonoids	Lorenc-Kukuła *et al.* (2007)
	Carotenoids	Fujisawa *et al.* (2008)
Tomato	Beta-carotene and lycopene	Fraser *et al.* (2007)
Banana	Beta-carotene	Waltz (2014)

silent by introducing RNAi (Van Eck *et al.*, 2007). *IbOr-Ins* gene from oranges has been inserted into sweet potatoes (*Ipomea batatas* L.) to increase the ratios of various nutrients like anthocyanin and carotenoids (Kim *et al.*, 2013). The *Arabidopsis* H^+/Ca^{2+} transporter gene has been inserted in carrot (*Daucus carota* L.) to increase the calcium content (Morris *et al.*, 2008). The ferritin gene from soybean has been introduced into lettuce (*Lactuca sativa* L.) to improve iron production (Goto *et al.*, 2000). Flax seeds (*Linum usitatissimum* L.) are good oilseeds and have unsaturated fatty acids, which are healthy, but there is a problem that this oil can auto-oxidize, which results in toxicity. To negate this toxicity, a gene that plays a key role in process, the Chalcone synthase gene (CHS), has been silenced to make flax seeds more stable (Lorenc-Kukuła *et al.*, 2007). In canola, genetic modifications via bacterial genes have enhanced carotene production (Newell-McGloughlin, 2008). Transgenic tomato (growth hormone regulator and provide immunity) is available worldwide. 3-Hydroxymethylglutaryl CoA has been added to tomato to enhance sterol production (Enfissi *et al.*, 2005). Banana (*Musa acuminata* L.) is an important fruit in developing countries and its carotenoid content has been increased by expressing bacterial *PSY* gene (Waltz, 2014) (Table 4.4).

4.6 Conventional breeding approach of biofortification

Generally, plant breeding is considered to be the result of the coevolution between plants and humans, and modern civilization is not possible without conventional breeding. Even though genetic engineering has been used for a long time, it is still viewed as suspicious by some people (Winkler, 2011). Even though genetically engineered crops have eased hunger in most the regions of the world, as it often involves genetically modified organisms people are still reluctant to accept the methodology (Watanabe *et al.*, 2005). Some countries, for example Japan, still place emphasis and work with conventional breeding (Inaba and Macer, 2004).

The controversy between genetic engineering and conventional breeding can be summarized by the golden rice example. The genetically engineered plant has a 50% increase in vitamin A concentration (Wesseler and Zilberman, 2014), but consumers are hesitant to buy the product because it does not have the approval of regulatory agencies and it is perceived as potentially unsafe (Bouis and Saltzman, 2017). Hence conventional plant breeding (Nestel *et al.*, 2006) is still preferred over genetic engineering (Bouis *et al.*, 2011). The major advantage of plant breeding is that it is cost effective and accessible to everyone regardless of wealth (Blancquaert *et al.*, 2014). Various types of trace element deficiencies (White and Broadley, 2005) have been covered through this technique (Welch and Graham, 2005). The Health Grain Project was started with collaboration among different countries to make safe availability of food (Fardet, 2010) (Table 4.5).

4.6.1 Conventional breeding in cereals

New high mineral rice varieties have been modified through conventional breeding. A traditional variety of rice that contains more iron and zinc than common varieties, known as jalmanga, has been used for breeding purposes (Gregorio *et al.*, 2000). Several varieties with

Table 4.5. Plant breeding in cereals and pulses.

Cereals/ pulses	Biofortification	References
Wheat	Zinc	CIAT, HarvestPlus
	Lutein	Ficco *et al.* (2014)
Rice	Zinc	HarvestPlus, CIAT
	Iron	IRRI
Maize	Vitamin A	CIMMYT, HarvestPlus
	Tryptophan	CIMMYT
Millets	Iron	ICRISAT, HarvestPlus
Cowpea	Iron	HarvestPlus
Beans	Zinc	Gelin *et al.* (2007)

improved iron and other nutrient contents (Çakmak *et al.*, 2004) have been used for crop quality enhancement (Ortiz-Monasterio *et al.*, 2007) (Table 4.5).

The conventional breeding resulted in increased trace element contents especially for zinc and iron (Welch *et al.*, 2005). A lot of wheat varieties have been released from past few years (Digesù *et al.*, 2009). Based on color varieties, a black cultivar of wheat has been used for many years in China with encouraging results (Li *et al.*, 2006). This cultivar is generally considered to have higher concentrations of trace elements like Se and also proteins. Similarly, Austria has released a purple cultivar (Eticha *et al.*, 2011). Maize fortified with vitamin A that has been introduced into Zambia has had significant results in improving child health (Pixley *et al.*, 2013). Fortified maize proVA, is a good source of phenolic compounds (Muzhingi *et al.*, 2017). Maize has been biofortified to provide maximum ratio of carotenoids (Lago *et al.*, 2014), phenolics and vitamin E (Goffman and Bohme, 2001) (Table 4.5). Multiple varieties of sorghum have been identified as having important nutritious features (Reddy *et al.*, 2005) like beta carotenes, proteins, lutein and other trace elements (Waters and Pedersen, 2009). Biofortified millet is a good food source with additional benefits of being cost effective (Velu *et al.*, 2007).

4.6.2 Conventional breeding in legumes and pulses

Lentils that are grown in drylands are easy to cook and because of having important nutrients, breeding has also been reported in them. Several genes are responsible for seed accumulation of zinc and iron in common beans via oligogenically or multigenically (Blair *et al.*, 2009). It has been reported that nutrient availability is an inheritable trait (Beebe *et al.*, 2000) (Table 4.6).

Table 4.6. Transgenic pulses and beans.

Legumes/pulses	Biofortification	References
Soybean	Oleic acid	Zhang *et al.* (2014)
	Flavonoids	Yu *et al.* (2003)

4.6.3 Breeding in vegetables and fruits

Breeders put focus on antioxidant properties (Brown *et al.*, 2010). Furthermore, genes for trace elements have also been identified that can play a key role in breeding to enhance quality (Burgos *et al.*, 2007) (Table 4.7). Natural variation in zinc content has been found in cauliflower which is helpful for further processes (Broadley *et al.*, 2010). Cassava as a vegetable has a lot of nutrients, including protein, iron and zinc, and is a good candidate for future breeding programs (Chávez *et al.*, 2005). The Sun Black tomato has fortification of purple pigment in its peel that was introduced through conventional plant breeding methods (Mazzucato *et al.*, 2008). Mangoes have also been suggested for breeding as they have maximum quantity of phenolics like gallic acid (Lauricella *et al.*, 2017). Grape cultivars with strong traits of vitamins and phenolics have also been identified and are used for breeding (Xu *et al.*, 2010).

4.6.4 Release of biofortified crops

Before the biofortified crops are released, they need to be modified and the synthesized products need to be tested to ensure they produce the correct modified trait. Conventional breeding has been used to increase their trace elements ratios in varieties of cassava, maize, wheat, rice and pearl millet, among others (Table 4.8).

Table 4.7. Plant breeding in vegetables and fruits.

Vegetables/fruits	Biofortification	References
Cassava	Iron	Maziya-Dixon *et al.* (2000)
	Carotenes	Maziya-Dixon *et al.* (2000)
Potatoes	Antioxidants	Lachman *et al.* (2005)
	Zinc and iron	Brown *et al.* (2010)
	Zinc	Burgos *et al.* (2007)
Cauliflower	Beta-carotene	IARI, India
Tomato	Anthocyanin	Mazzucato *et al.* (2008)
Mango	Beta-carotene	IARI, India
Grapes	Phenolics	Xu *et al.* (2010)

Table 4.8. Release schedule of biofortified crops.

Crop	Nutrient	Country released	Specific trait	Year
Bean	Zinc, iron	Rwanda	Drought resistant	2007
Cassava	Provitamin A	Nigeria	Disease resistant	2011
Sweet potato	Provitamin A	Uganda	Drought resistant	2007
Pearl millet	Iron, zinc	India	Mildew resistant	2013
Wheat	Iron, zinc	Pakistan, India	Disease resistant	2013
Rice	Zinc, iron	Bangladesh, India	Cold resistant	2013
Maize	Provitamin A	Zambia	Disease resistant	2012

4.7 Future challenges in biofortification

The products synthesized by using biofortification approaches sometimes share the same promoters as other micronutrients, for example cadmium and iron have the same promotors. So, all the products should be carefully evaluated. Biofortification may also have side effects as when transporting the important trace elements, other heavy metals may also be transported. Compared to other crops, the biofortification research of pearl millet is relatively limited (Shivran, 2016). Overall, more research needs to be done to increase our knowledge in crop metabolic processes, their ecological attributes and rhizosphere interaction.

There is a need to understand all these perspectives at the molecular level. Species with best genetic variations and traits should be taken as an exemplary model for those that are not well developed. The advancement of molecular technology has helped to identify the key genes and their traits in different crops. Reduced variation in crop genetics and the minimum time available for crop development are some limitations in biofortification. The key focus should be on the increase in micronutrient requirements and concentration (White and Broadley., 2009).

4.8 Conclusion

Biofortification is considered as a key source to overcome the shortage of food. Millions of people are affected every year due to lack of food and nutrient supply. The deficiency of food and nutrients not only causes hidden hunger, but also results in diseases that can be fatal. So, by using the different approaches like dietary supplements and food fortification, scientists have devised new ways to overcome the food shortage. Biofortification can be categorized into three main approaches: agronomic practices, plant breeding and genetic engineering. By fortifying the crops with key trace elements, the food shortage can be alleviated and the quality of nutrients can also be enhanced. There is generally more emphasis on the conventional breeding compared to genetic engineering and agronomic practices. Overall, biofortification is not only the source of food for developing countries, but it is also big advantage for economists.

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5 Biostimulant-induced Improvement of Soil Health and Water-use Efficiency in Plants

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Abstract

Soil is a vital and sensitive resource for improved production of crops that can be used for food, medicinal and aesthetic purposes. Soil health is the main factor to achieve high-quality crops. The management of soil to sustain its health is very important for crop production. Intensive use of chemical fertilizers and rapid growth of industries have been the major environmental issues for degradation of soil health. In order to boost crop production and food supply, the availability and affordability of fertilizers (nutrients) are the main concern of farmers and investors. Recently, application of biostimulants (protein hydrolysates, humic and fulvic acids, seaweed extracts and botanicals, chitosan, and beneficial microbes) has been gaining attention in sustainable agriculture as a prominent soil management strategy. Application of different types of biostimulants enhances the quantities of amino acids, growth regulators (auxins, cytokinins and gibberellins), macronutrients, micronutrients and vitamins in the soil. These supplements improve soil health by increasing nutrient- and water-use efficiency, enhancing plant growth and reduction in fertilizer application. Commercial application of biostimulant products is being widely adopted by farmers due to the fact that these products not only fulfill the basic requirements of plants, but also efficiently improve the physical and chemical properties of soil. This chapter presents our current understanding with respect to application of biostimulants to improve soil health and water-use efficiency, recognizing their conceivable contribution to more sustainable agricultural productivity but moreover highlighting problems that must be addressed before this technology can be evaluated as a substitute for conventional techniques.

5.1 Introduction

Soil is one of the important resources for better production of plants to fulfill the human demands related to food, medicine and aesthetic gratification. Soil health refers to the biological, chemical and physical characteristics of soil that are vital to long-lasting, sustainable agricultural productivity with minimal environmental impact (Haney *et al.*, 2018). Healthy soil is integral for suitable plant growth to achieve high-quality crops, so the management of soils to sustain its health is very important. For suitable growth, a healthy soil must fulfill four functions: (i) provision of nutrients; (ii) constant water supply; (iii) permit the exchange of gases to and from the roots; and

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(iv) provide anchorage for the plants (Tariq *et al.*, 2012). Soil is also the habitat for various microorganisms; it is where these microorganisms live, flourish and sustain nutrient availability. Considering all the aspects of soil health, the idea of soil health is described as 'the continued capacity of a specific kind of soil to function as a vital living system within natural or managed ecosystem boundaries, to sustain animal and plant productivity, to maintain or enhance the quality of air and water environment, and to support human health and habitation' (Doran and Zeiss, 2000). Currently, the concomitant challenges of uplifting the crop production to feed the increasing population around the globe (Lichtfouse *et al.*, 2009) and enhancing the resources use efficiency, while decreasing the environmental impact on the ecosystems and soil health, are being faced by agricultural sector. In order to cope with the demands of a growing population, the agricultural industries have developed as a major environmental issue for degradation of soil health by activities like intensive application of chemical fertilizers (to improve plant growth), pesticides (to defend against insects and diseases) and other cultural practices (Tahat *et al.*, 2020; Ngatia *et al.*, 2021).

Salient approaches should be adopted to improve soil health for the high-quality crops that can fulfill the demands of a growing global population. Recently, a prominent soil management strategy that uses different materials like chitosan, beneficial microbes, humic and fulvic acids, protein hydrolysates, seaweed extracts and botanicals as biostimulants has been raised in sustainable agriculture production (Chen *et al.*, 2003; Mueller and Kussow, 2005; Maini, 2006; Khan *et al.*, 2009; Brown and Saa, 2015; Du Jardin, 2015; Yakhin *et al.*, 2016, Zulfiqar *et al.*, 2019). The improvement of soil health with application of biostimulants triggers numerous physiological processes that increase the efficiency of nutrient utilization, accelerating plant development and reducing consumption of fertilizers (Kunicki *et al.*, 2010; Bulgari *et al.*, 2015). Beside the higher nutrient uptake by plants, soil treated with biostimulants also (i) improves moisture-holding capacity; (ii) increases soil microbial and enzymatic activities; (iii) increases micronutrient mobility and solubility, in particular Fe, Zn, Mn and Cu; (iv) alters the root architecture of plants, particularly root density, length and number of lateral roots; and, (v) enhances Fe(III)-chelate reductase, glutamine synthetase and nitrate reductase activities (García-Martínez *et al.*, 2010; Colla *et al.*, 2014, 2015; Kaur, 2020). Beside all these characteristics, the biostimulants may also contribute to the decrease in the negative consequences of various environmental stresses. The adaptation of this modern technique to resolve soil health issues is a wise approach to increase agricultural food production. The current understanding regarding the application of biostimulants to improve soil health and water-use efficiency as an alternative approach to conventional techniques are summarized and discussed in this chapter.

5.2 Contribution of biostimulants in improving soil health

The health of soil plays a significant role in plant growth, development and production under both natural and managed ecosystems. The biological (microbes), physical (particles) and chemical (nutrients) properties are the indicators of health, fertility and productivity of the soil (Kumar *et al.*, 2014; Adekiya *et al.*, 2020). Since 1970, approximately one-third of the soils around the world have been facing a reduction or complete depletion in productivity (Blanco-Canqui and Lal, 2010). Therefore, there is an urgent need to protect the soil health by adopting organic, sustainable or environmentally friendly techniques. In this regard, biostimulants can play an integral part in improving soil health by enhancing fertility and productivity of the soil (Fig. 5.1). The contribution of different types of biostimulants in improving soil health is described below.

5.2.1 Protein hydrolysates

Protein hydrolysates (PHs) are developed by chemical, enzymatic or thermal hydrolysis of different animal and plant residues and are considered an essential group of plant biostimulants based on a mixture of amino acids and peptides (Kunicki *et al.*, 2010; Calvo *et al.*, 2014; Rouphael *et al.*, 2021). These biostimulants have attained an increasing trend in the recent years due to their beneficial properties that can sustainably enhance resource-use efficiency in different crops, reduce

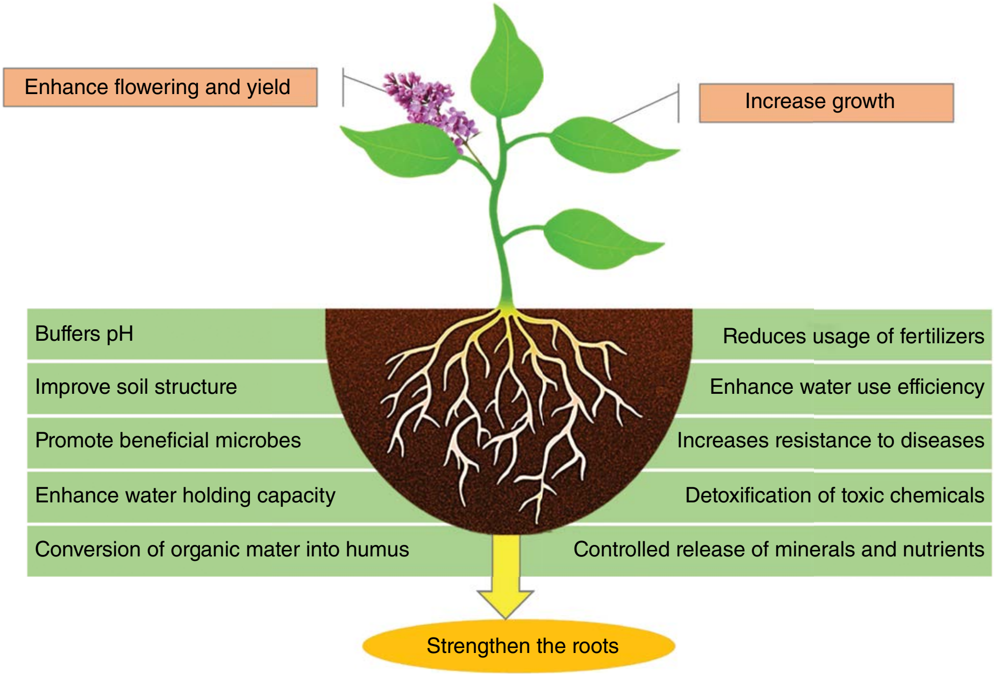

Fig. 5.1. Impact of biostimulants on soil health and plant growth.

the frequency of abiotic stresses and increase the quality of produce (Bulgari *et al*., 2019; Del Buono, 2021; Rouphael *et al*., 2021). Protein hydrolysate substances can be effectively applied to plants as foliar application or in soil (growth substrate). The application technique may vary for different crops; for example, in tomato, drenching (soil application) was found more efficient in improving photosynthetic activity, plant biomass and stomatal conductance of the plant (Paul *et al*., 2019). The improvements are mainly due to increased carbon and nitrogen metabolism, nutrient availability and nutrient-use efficiency by plants in treated soils. Apart from these beneficial impacts, improvement in soil health by upgrading its composition and structure, and stimulating microbial community are other important benefits of PH.

5.2.2 Humic compounds

Humic compounds such as humic acid and fulvic acid are other potential biostimulants that are produced by biological and chemical transformations of animal and plant matter, mainly from microbial metabolism. The humic substances represent the major pool of organic carbon in all types of soils around the globe (Canellas *et al*., 2015). These substances have a positive impact on soil health as the addition of humic compounds not only improve soil conditions, but also establishes a balance between various plant nutrients essential for enhancing productivity of soil. The improved soil health has positive impacts on plant growth and yield, and it also contributes to reduce the adverse effect of stresses (particularly soil stresses) through various mechanisms inside plants and in soil (Unlu *et al*., 2011; Abdellatif *et al*., 2017). All the above advantages of humic substances for plants and soil health are mainly due to the contribution of these substance to soil chemistry. These remarkable brown to black products act as redox reagents and pH buffers, sorb organic solutes, hold water, bind metal ions, photosensitize soil reactions, enhance plant growth and bio-transform toxic pollutants (Davies and Ghabbour, 2001). Treating soil with humic substances is helpful in improving soil health by combating water and soil pollution, reducing the impact of biotic and abiotic stresses, and minimizing our dependency on chemical fertilizers.

5.2.3 Seaweed extracts

Seaweed extracts (marine algal seaweed species) are often considered as organic matter sources and organic fertilizer (source of nutrient), which is why seaweed extracts have been used as soil conditioners for centuries (Temple and Bomke, 1988; Khan *et al.*, 2009). Seaweed extracts can also contain countless plant-bioactive inorganic and organic compounds such as oligosaccharides, polysaccharides, mannitol, phytohormones (e.g. auxins, betaine, cytokinins, and gibberellins), antioxidants and vitamins. They also contain minerals such as B, Ca, Mn, P, K, Na, Mg and Zn, and several other trace elements in a low concentration (Sunarpi *et al.*, 2020; Singh *et al.*, 2016; Patel and Mukherjee, 2021). It has been widely used as soil amendment and fertilizer in agriculture and therefore, a large variety of seaweed and seaweed-derived products are available in market (Khan *et al.*, 2009; Nayak *et al.*, 2020; Kaur, 2020). Seaweed and seaweed-derived products enhance soil health by improving the biological, chemical, and physical characteristics of the soil, which in turn positively affects plant growth. The application of seaweeds and their products is much better than chemical fertilizer to improve soil fertility, because the high quantity of organic matter present in the seaweed enhances the moisture-holding capacity of the soil by promoting the growth of beneficial soil microbes (Ramarajan *et al.*, 2012; Nabti *et al.*, 2017). The improvement in soil health creates a suitable environment for root growth and enhances biological activities like nitrogen mobilization, mineralization of nutrients and respiration, which provides ideal conditions for plant growth and development (Battacharyya *et al.*, 2015; Ronga *et al.*, 2019; Malik *et al.*, 2021). Seaweed products also enhance soil health as its application has a supplementary advantage of protecting plants against unfavorable environmental conditions such as excess nutrients, water stress or extreme temperatures (Anderson, 2009).

5.2.4 Other botanicals

Botanicals are the substances extracted from plants and these can be a rich source of bioactive compounds that can stimulate plant growth. Biostimulants are extracted from plants using alcohol, water or other solvents from either fresh or dried parts of the plant (Gurjar *et al.*, 2012; Isman and Grieneisen, 2014; Younis *et al.*, 2018; Mulugeta *et al.*, 2020). The application of leaf extracts of neem (*Azadirachta indica)*, chinaberry (*Melia azedarach*) and tobacco (*Nicotiana tabacum*) to control soil-borne diseases, insects and nematodes for better plant growth and development is being practiced (Regnault-Roger, 1997; Hassanein *et al.*, 2008; Taniwiryono *et al.*, 2009; Asogwa *et al.*, 2010; Li *et al.*, 2014). The diluted solution of *Moringa oleifera* contains plant growth-promoting substances that enhances the growth, yield, content of the photosynthetic pigments, proteins, and nutrients in pea plants (Merwad, 2018). Similarly, botanicals extracted from leaves of red grape, blueberry fruits and hawthorn increase the biomass of roots and leaves, as well as the content of chlorophyll and sugars in maize (Ertani *et al.*, 2016). The aqueous extract of garlic has shown a stimulatory effect on the vegetative growth, root length and spread, fresh and dry biomass, and the metabolite content of plants, for example chlorophyll, carotenoid pigments and soluble sugars in eggplant and pepper (Hayat *et al.*, 2018). Botanical stimulants (plant extracts) facilitate the uptake, transport and distribution of macro and micro elements from soil to plants (Godlewska *et al.*, 2019). *M. oleifera* (extract from moringa leaves) is another source of botanical biostimulant that gained attention in recent years due to its positive effect on plant growth and productivity (Yasmeen *et al.*, 2014). Moringa leaf extracts have been shown to improve nutrient use efficiency, germination of seed, plant growth and production, pre- and post-harvest quality traits of crops and products, as well as tolerance to abiotic stresses (Zulfiqar *et al.*, 2020). The use of botanicals as a biostimulant in agriculture is a vital, sustainable and environmentally friendly technology for reshaping a productive and efficient cropping systems.

5.2.5 Chitosan

Chitosan is a linear polysaccharide composed of N-acetyl-D-glucosamine and β-(1-4)-linked D-glucosamine that is produced from the chitin of fungal cell walls, exoskeletons of insects and shells of crustaceans (crabs, lobster, and shrimp) through the process of heterogeneous deacetylation (Nwe *et al.*, 2011; Muñoz *et al.,* 2015; Pichyangkura and Chadchawan, 2015). It was originally reported as an elicitor of plant responses under stress conditions, and further research proved that it could stimulate plant protection responses, resistance inducers to abiotic stresses and growth enhancers in various crops (Mondal *et al.*, 2012; Van *et al.*, 2013; Ali *et al.*, 2013; Jail *et al.*, 2014; Hidangmayum *et al.*, 2019). The soil amendment with chitosan has a positive impact on soil health, most importantly suppression of soil-borne plant diseases, insects and nematodes. Application of chitosan as a biostimulant is a better option to improve soil fertility as compared to chemical fertilizers as it contains high nitrogen and calcium content and it also contributes to an increase in biomass (without raising the C:N ratio) and activities of beneficial microbes in the soil (Ohta *et al.*, 2004). The status is further ameliorated with improvement in the physical structure of soil and nutrient availability, so all these soil factors have a direct role in plant growth stimulation (Xu and Mou, 2018).

5.2.6 Beneficial microbes

Beneficial microbes, such as plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi present in the rhizosphere can provide plants with essential nutrients, defend them from insects and pathogens, help plants to acclimatize to environmental deviations and degrade toxic pollutant (Ahmad *et al.*, 2018; Hirt, 2020). These soil microbes perform a vital role in enhancing soil health by nitrogen fixation, producing enzymes like 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, solubilization of nutrients (e.g. phosphorus) and activities of chitinase and catalase (Khan *et al.*, 2009; Zhang *et al.*, 2011; Xiao *et al.*, 2017; Ahmad *et al.*, 2018). In addition, these beneficial microorganisms maintain the quality of water and soil by suppression of pathogens, decay of soil organic matter and detoxification of poisonous chemicals (Dubey *et al.*, 2016). The positive attributes to enhance soil health along with an improvement in crop yield increase the interest in using these microbes as biostimulants (biofertilizers, biocontrol agents or biopesticides) in agriculture as an organic substitute to agrochemicals. Currently, the commercial microbial biostimulant products, mainly those containing PGPR, are increasingly being used (Egamberdiyeva, 2007; Berg, 2009; Bhattacharyya and Jha, 2012; Berg *et al.*, 2020). The application of microbial biostimulants to soil further enhances the soil biological activities without disturbing the existing communities of beneficial microorganisms. These biostimulants also regulate the physical and chemical attributes of soil and contribute to boost soil health (Rawat *et al.*, 2020). The improved and healthy soil where microbial biostimulants (biofertilizers) in sustainable module are being used, helps to enhance plant growth, productivity and eliminate the issues regarding food availability for the growing population (Ansari *et al.*, 2017).

5.3 Applications of biostimulants to enhance water-use efficiency

Agricultural production is measured by the biomass harvested per unit of land area, and this biomass production depends on water-use efficiency (WUE) which is the ratio of carbon uptake to water use by vegetation (Sinclair *et al.*, 1984; Farooq *et al.*, 2019; Tarin *et al.*, 2020). Water-use efficiency is a valuable index for representing the efficiency of water used in plant production, which may enable the preservation of irrigation water (Battilani *et al.*, 2009). There are three aspects of soil health that impact water-use efficiency: organic carbon, soil physical indices (including aggregation, bulk density, infiltration) and soil biological indices (Rice *et al.*, 2019). The improved soil health by the application of biostimulants can play an integral part in the growth and architectural development of root systems which have been reported to influence WUE more than canopy architecture (Hammer *et al.*, 2009). Seaweed extracts improve root growth (maize) mainly due to exogenous auxins as well as other compounds in the extracts (Crouch and Van Staden, 1993). The root system development improves water- and nutrient-use efficiency in such a way that the overall increase in growth and vigor of plant can be observed. Similarly, the application of various humic substances positively impacts soil health by improving the soil's physical, chemical and biological dynamics, ultimately enhancing the fertilizer and WUE in potato crop (Seyedbagheri, 2010). The application of chitosan-coated fertilizer (75% CNK) improved the physical attributes of soil by increasing porosity, higher water conductivity and enhanced friability that helped root growth and developed double the root biomass as compared to control plants. Therefore, the better WUE led to increase productivity of *Zea mays* L. by elevating the total biomass production (Kubavat *et al.*, 2020). Apart from root growth, the leaf growth and physiology also play an essential role in water and nutrient use and CO_2 fixation. Caulet *et al.* (2014) reported the capacity of biostimulants (marine origin) to ameliorate yield of strawberry and *Lycopersicon* spp. under low water regimes, by increasing stomata closure and maintaining higher WUE.

Beneficial microorganisms (PGPR and mycorrhizal fungi) can provide plants with essential conditions for plants growth especially improving plant WUE. *Pseudomonas aeruginosa* inoculated plants tend to improve the WUE of plants (Nadeem *et al.*, 2014). Therefore, these bacterial species can be valuable to the environment as these bacteria contribute to reducing unnecessary water consumption by the plants (Ansari *et al.*, 2017). The combined application always had better results; for example, co-inoculated (mycorrhizal regimes and *P. fluorescen*) Arizona cypress seedlings had enhanced WUE compared to those inoculated with either mycorrhizae or rhizobacteria (Aalipour *et al.*, 2021). The mycorrhizae inoculation positively contributed to enhancing WUE by the addition of glomalin-related soil protein (GRSP) in the rhizosphere. These GRSPs (soil portions partially made from arbuscular mycorrhizal fungi) are the glycoproteins that provide a protective layer over fungal hyphae to conserve water and nutrients for mycorrhizae (Rillig, 2004). GRSPs also play a significant role in improving soil quality and health as they are an important component of soil organic matter and act to bind mineral particles (Comis, 2002). The improvement in soil–plant water relations by decreasing water loss from soil is due to ability of GRSPs to alter the relative distribution of water-stable aggregate sizes (Miransari, 2010; Aalipour *et al.*, 2021). The major indicator of improved WUE is an increase in biomass, and the application microbial biostimulants (such as mycorrhizae and PGPR) can perform this job very well for different plant species. There are several published studies in this regard that have been reported by researchers from all over the world (Vafadar *et al.*, 2014; Visen *et al.*, 2017; Aalipour *et al.*, 2019).

5.4 Role of biostimulants in agriculture

5.4.1 Biostimulants as biofertilizers

Biostimulants, as mentioned earlier, are the substances or microorganisms that when applied to plants or the soil, stimulate existing biological and chemical processes in the soil and associated microbes (e.g. rhizobacteria and mycorrhizal fungi) to increase plant growth, quality and yield by improving nutrient uptake, nutrient and WUE and tolerance to abiotic stress. The biostimulants that contain sufficient levels of plant nutrients (N, P, K, Ca, Mn, etc.) are also categories of biofertilizers. The composition of commercial biostimulants that are being used as biofertilizers is presented in Table 5.1.

5.4.2 Biostimulants as plant growth-promoting agents

Biostimulants that are sometimes referred to as agricultural biostimulants contain the right amount of plant growth-promoting hormones like auxin, abscisic acid, cytokinin, ethylene and gibberellin. These growth-promoting substances in the form of biostimulants, when applied exogenously, contribute to various physiological processes related to plant growth, development and, in particular, response under stress conditions (Ali *et al.*, 2020). Beneficial effects of seaweed-based biostimulants have been reported in several plant growth bioassays that have led to the assumption of the presence of growth-regulatory substances (Hong *et al.*, 2007; Khan *et al.*, 2009). The microbial biostimulants, particularly PGPR, are another source of exogenous plant growth-promoting substances that effect all characteristics of plant life, including morphogenesis, growth

Table 5.1. Composition of different biostimulants available on the market.

Product	Composition	References
Commercial protein hydrolysates		
Amino16®	25% organic matter, 4% total N and 11.3% L-amino acids	Koukounaras *et al*. (2013); Tsouvaltzis *et al*. (2020)
Trainer®	5% total N, 35.5% organic matter and 27% amino acids and soluble peptides	Colla *et al*. (2014); Carillo *et al*. (2019); Rouphael *et al*. (2021)
Siapton®	9.1% total N, 8.7% organic N, 0.4% ammonium nitrogen, 25% organic carbon 63% dry matter content, 10% free amino acids, 55% short chain peptide bound amino acids	Mladenova *et al*. (1998); Colla *et al*. (2017); Mineva *et al*. (2020)
Commercial humic compounds		
Diamond Grow ® (Humi[K] WSG- Water Soluble Granule and Humi[K] WSP- Water Soluble Powder)	95% humic acid, 10-15% fulvic acid, 1% N, 14% K, water soluble	Zhang *et al*. (2021)
HumiTop 85	80-85% potassium humate (dry basis), 10% K_2O (dry basis)	Castañeda Sarmiento (2014); Arreaga and Florentino (2018)
Commercial seaweed products		
AgroKelp	2.94% organic matter, 1.31% K, 1.12% total carbohydrates, 0.25% total S, 0.026% N, 0.018% Ca, 0.0054% P, 0.0024% Mg, 0.0012% Zn, 0.00093% B, 0.0005% Fe, 0.00006% Cu, 0.00005% Mn	Khan *et al*. (2009); Chatzissavvidis and Therios (2014); Jayaraman and Ali (2015); Mishra *et al*. (2020)
High Tide Bio-Genesis™	4.5% soluble potash (K_2O), 6.0% non-plant food ingredients	Chatzissavvidis and Therios (2014); Dapper *et al*. (2014)
Kelp Meal	1.0% total NN, 2.0% soluble potash (K_2O), 0.1% available Phosphate (P_2O_5)	Appleby-Jones (2014)
Stimplex®	0.1-0.5% total N, 3.0% soluble potash (K_2O), 2.5% available phosphate (P_2O_5), 0.2% total S, 0.08% Ca, 0.04% Mg, 0.01% cytokinin (as kinetin)	Khan *et al*. (2011); Sidhu and Nandwani (2017); Moreno-Reséndez *et al*. (2017); Ozbay and Demirkiran (2019)

and development, water and nutrient uptake, biotic and abiotic stress response and interactions with other organisms in the agroecosystems (Berendsen *et al*., 2012; Philippot *et al*., 2013; Du Jardin, 2015). Phytohormone production by soil bacteria is a key signaling molecule used in improvement of production in different crops. Auxin (IAA) in a major quantity, along with other hormones such as abscisic acid, cytokinin (CK) and ethylene (ET), proved beneficial by stabilizing plant immunity, biocontrol and productivity of the crop (Smaill *et al*., 2010; Maheshwari *et al*., 2015). Apart from hormones, different types of organic and inorganic compounds (e.g. amino acids, mineral constituents [Ca, K, S, Mn and P] and vitamins) are present in these biostimulants, which can also act as plant growth-promoting agents (Ali *et al*., 2020).

5.5 Conclusion and future prospects

Soil health deterioration due to anthropogenic activities and natural means is becoming a major concerns for agriculture production systems and soil ecology. Biostimulants as soil application serve as a better management approach by maintaining soil physiochemical (soil fertility restoration and maintenance) and biological ecosystem (stimulating microbial activity) to improve soil health for the sake of ecofriendly and sustainable crop production. These biostimulants and their products serve as a natural reservoir of a complex mixture of amino acids, polysaccharides, phytohormones (auxins, cytokinins and gibberellins), macronutrients, micronutrients and vitamins. These substances can increase nutrient and water efficiency, decrease the application of fertilizer and replace synthetic plant growth regulators. The application of biostimulants can lead to increased plant growth, flowering and productivity and, more importantly, helps the plants to withstand both biotic and abiotic stresses. This is a cost-effective, ecofriendly, renewable, and non-intrusive technique for harvesting a quality product without damaging the soil health and its biological ecosystem. In the past few decades, plant researchers have reported the effective use of biostimulants for plant growth promotion and enhancement of soil health, but a major challenge is to develop products at commercial levels with the provision and promotion of these product globally. To gain the benefits from biostimulants, restrictions on the use of the available products must be determined in future studies.

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6 Silicon-based Biostimulators for Sustainable Agriculture

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Abstract

Enhancement of plant defense against multiple abiotic stresses such as low or high temperature, water deficiency, salinity and heavy metal toxicity is crucial for providing sustainable agricultural production. Introduction into agricultural practice of silicon (Si)-rich substances as soil amendments, fertilizers and biostimulators has demonstrated their prospects for the plant protection under stress. Silicon-based biostimulators are more cost efficient in comparison with other types of Si agrochemicals. Today, liquid and solid forms of Si biostimulators are available to considerably enhance the plant resistance to abiotic and biotic stresses when applied at a low rate (0.1–10 kg ha^{-1}). Several mechanisms responsible for the Si biostimulator benefits for plant immune system have been proposed. Among them, activation of the plant signaling system and additional synthesis of stress proteins, reduced transport of toxic elements, enhanced antioxidant activity and others are notable. Silicon-based biostimulators accelerate the mineral fortification of cultivated plants, thus contributing to the crop quality. In greenhouse conditions, solid (fine amorphous silica) and liquid (monosilicic acid) types of Si biostimulators were tested on chilling-exposed wheat and sugar beet. Both Si substances, when applied to foliar material at low rates (2–5 kg ha^{-1} Si), increased the wheat and sugar beet tolerance to cold stress. Silicon treatments benefited the antioxidant enzyme activities. Silicon is assumed to be directly involved in the synthesis of stress proteins and enzymes with a key role of polysilicic acid. Polysilicic acid can form Si gel, which has surface properties facilitating the synthesis of organic molecules. However, this hypothesis needs to be experimentally confirmed.

6.1 Introduction

Over the last decade, the use of different types of biostimulators has increased remarkably (Posmyk and Szafrańska, 2016; Xu and Geelen, 2018). Although a precise definition of biostimulators is still absent, they include substances able to regulate and modify vital metabolic processes, thus providing enhanced plant growth and yield, improved crop quality, and increased plant defense against biotic and abiotic stresses (Rouphael and Colla, 2020). Biostimulators improve plant metabolic processes without changing their natural pathway, which differentiates them from bioregulators or hormones (Posmyk and Szafrańska, 2016). Biostimulators show efficiency when applied in small amounts. According to the available data, rates of biostimulators are generally about a few hundred g ha^{-1} and do not exceed several kg ha^{-1} (Du Jardin 2015; Rouphael and Colla, 2020).

The proper use of biostimulators requires understanding the mechanisms responsible for the stimulant effect on plant viability and

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development. Any stress enhances the synthesis of reactive oxygen species (ROS), which serve as a non-specific plant response to stressors (Choudhury *et al.*, 2017). The imbalanced generation of ROS triggers oxidative damage to metabolically essential cell compounds and organelles. The balance between production and scavenging of ROS is of crucial importance in plant defense under stress. The antioxidant system composed of enzymes and low-molecular compounds like glutathione and ascorbic acid plays a key role in the ROS regulation. Generally, highly efficient biostimulators are directly involved in the stimulation of antioxidant activities (Posmyk and Szafrańska, 2016).

Silicon is recognized as a 'beneficial' element. However, most cultivated plants absorb Si more than other macronutrients (nitrogen, N; phosphorus, P and potassium, K). The term Si fertilizer had appeared in agriculture since 1842 (Liebigh, 1842). Until the early twentieth century, Si fertilizers were investigated together with N, P and K fertilizers. Since the introduction of pesticides, Si-related studies remarkably decreased compared to those of N, P and K (Ma and Takahashi, 2002; Tubana *et al.*, 2016).

The results of numerous current investigations demonstrated that active forms of Si have a positive influence on plant growth and protection against biotic and abiotic stresses (Ma and Takahashi, 2002; Vivancos *et al.*, 2015; Verma *et al.*, 2020). Several mechanisms underlying Si-induced plant defense have been suggested: (i) mechanical protection via Si accumulation in epidermal tissue and formation of a thick epidermal layer that protects plants against fungi and insect attacks (Alhousari and Greger, 2018); (ii) physiological protection due to increasing plant viability through optimization of root development and improvement of photosynthesis (Zhang *et al.*, 2018; Frazão *et al.*, 2020); (iii) chemical protection via chemical interaction between monosilicic acid and toxic compounds in plant tissue (Ji *et al.*, 2016; Stevic *et al.*, 2016); and (iv) impact on the transport of elements (Imtiaz *et al.*, 2016). These mechanisms are indirectly supported by high concentrations of monosilicic and polysilicic acids to be observed in the plant sap (Matichenkov *et al.*, 2008; Wei *et al.*, 2021).

Three main groups can be distinguished among the Si-rich substances being used in agriculture today.

- Silicon soil amendments that mostly influence soil properties (adsorption capacity, pH, structure, and others) and are commonly applied at rates more than 500 kg ha^{-1}. Considering high application rates, these materials also provide plant Si nutrition. This group includes calcium silicate slag, zeolite and diatomite (Chaiyaraksa and Tumtong, 2019; Matichenkov *et al.*, 2020).
- Silicon fertilizers, the main purpose of which is to provide plant Si nutrition. Their application rates range between 50 and 500 kg ha^{-1}. Amorphous silicon dioxide (microsilica, fumed silica), silicon gel and sodium or potassium silicate can be recognized as fertilizer (Ma and Takahashi, 2002; Rao *et al.*, 2017).
- Silicon biostimulator is a new class of Si-based agrochemical that is foliar applied at rates less than 10 kg ha^{-1} (Gugała *et al.*, 2019; Quinonez *et al.*, 2020; Artyszak *et al.*, 2021).

To assess the protective function of Si in the plant defense system under cold stress, it is important to determine the Si contribution in the plant's ability to alleviate oxidative destruction. The main aim of this study was to investigate the impact of Si-based biostimulators on the physiological state of cold-exposed plants of wheat and sugar beet using the indicators of the antioxidant system and the content of plastid pigments.

6.2 Materials and methods

Wheat (*Triticum aestivum* L. cv Novosibirskaja; monocot) and sugar beet (*Beta vulgaris L.* cv Bogema; dicot) were used as test materials under greenhouse growth conditions. Before planting, seeds were treated with 3% H_2O_2 and washed in distilled water (DW). Ten seeds per pot for wheat and five seeds per pot for sugar beet were planted in 1-L plastic pots filled with organic muck soil (pH 6, 60% organic matter; 10% sand, 10% $CaCO_3$). Plants were grown for 25 days at the following conditions: air temperature 26 ± 4°C during the day and 22 ± 2°C during the night; the light period was 12 hours at intensity of 200 lmol photons m^{-2} s^{-1} by UV/Vis lighting, and the relative air humidity was 85 ± 5% during the day and 78 ± 5% during the night. Plants were irrigated with DW to maintain soil moisture between 20% and 40%. Half of the pots were placed into the cold room at +6 °C for 12 hours to simulate short cold stress. After that, plants were returned to the greenhouse at the conditions described above for 1 more week.

Two types of Si biostimulators were used: (i) solid Si biostimulator (SiB) with total Si about 47% (LSD Apatit, Russia); and (ii) liquid Si biostimulator Ecosil – stabilized monosilicic acid with 15% of Si and 15% of Na (Beijing Plum Agrochemical Trading Co. Ltd., China). Each SiB was diluted by DW at a proportion of 1:500 and foliar applied at 1 and 2.5 kg ha^{-1} twice 3 days before and 1 hour after chilling. Total rates of each biostimulator were 2 and 5 kg ha^{-1}. Control plants (without exposure to chilling) were treated in parallel at the same rates. The experimental scheme was as follows:

- control;
- control + chilling;
- SiB, 2 kg ha^{-1};
- SiB, 5 kg ha^{-1};
- Ecosil, 2 kg ha^{-1};
- Ecosil, 5 kg ha^{-1};
- SiB, 2 kg ha^{-1} + chilling;
- SiB, 5 kg ha^{-1} + chilling;
- Ecosil, 2 kg ha^{-1} + chilling; and
- Ecosil, 5 kg ha^{-1} + chilling.

Leaves of wheat and sugar beet were sampled 24 hours after chilling for testing the antioxidant activity. After 1 week, both the soil and plant were sampled. Soil samples were analyzed for water- and acid-extractable Si by the following methods. To analyze water-soluble Si: (i) 6.0 ± 0.1 g of fresh soil was placed into a 100 mL plastic vessel; (ii) 30 mL of water was added to each vessel; (iii) after 1 hour shaking, the sample was filtered and a clean extract was analyzed for monosilicic acid using the method described by Mullin and Riley (1955). The acid extraction procedure was as follows: (i) two 2.0 ± 0.1 g of an air-dried soil sample was placed in a 100 mL polyethylene cup; (ii) 20 mL of 0.1 utesM HCl was added and the mixture was agitated at 200 rpm for 30 minutes; (iii) after standing overnight, the mixture was agitated again for 30 minutes, then the supernatant was centrifuged at 3000 *g* for 15 minutes. Silicon was analyzed in cleaned extract by the method mentioned above.

Besides measuring biomass, plant samples were analyzed for Si after microwave digestion (CEM MARS 6 MS5181) of dried plant tissues. Fresh leaves of wheat and sugar beet were also analyzed for pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) using standard methods (Lichtenthaler and Wellburn, 1985).

To measure the stress response, leaves collected after chilling were evaluated by the concentration of thiobarbituric acid reactive substances (TBARs) as a marker for lipid peroxidation and by the activity of antioxidant enzymes: ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX).

The TBAR content was assessed using the method described by Uchiyama and Mihara (1978). A reaction mixture containing 3 mL of 1% phosphoric acid, 1 mL of 0.6% aqueous solution of thiobarbituric acid and 0.1 ml of aqueous $FeSO_4 \times 7 H_2O$ (2.8 mg mL^{-1}) was added to 0.3 mL of a crude homogenate. All the samples were heated in a water bath for 1 hour. After cooling and the addition of 4 mL of butanol-1, each reaction mixture was mixed vigorously and centrifuged at 3000 *g* for 10 minutes. The absorbance of TBARs was measured at 532 and 600 nm using Shimadzu UV-VIS 160A (Kyoto, Japan) spectrophotometer. The concentration of TBARs was calculated using the coefficient of the extinction equal to 1.56×10^{-5} M cm^{-1}.

The activity of APX (EC 1.11.1.11) was estimated by measuring the decrease in absorption at 300 nm during ascorbate oxidation and expressed as μmol of ascorbate oxidized g^{-1} FW min^{-1} (extinction coefficient of 2.8 mM^{-1} cm^{-1}) (Nakano and Asada, 1981).

The activity of GR (EC 1.6.4.2) was determined by the glutathione-dependent oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) (Foyer and Halliwell, 1976). The reaction mixture contained 30 mM K/Na phosphate buffer, pH 7.8, 0.15 mM NADPH, 3 mM $MgCl_2$ and 0.5 mM oxidized glutathione (GSSG). Corrections were made for NADPH oxidation in the absence of GSSG. The reaction was initiated by the addition of enzyme extract. The measurements were performed at 340 nm; 6.22 mM cm^{-1} coefficient of extinction was used for the calculations.

The activity of GPX (EC 1.11.1.9) was measured spectrophotometrically (Gavrilenko *et al.*, 1975) on the basis of guaiacol oxidation; 26.6 mM^{-1} cm^{-1} coefficient of extinction for tetraguaiacol at 470 nm was used for the calculations. All the results were calculated per 1 g of fresh weight (FW).

Soil was sampled in three replications and plants were sampled in five replications. All data obtained were subjected to a statistical analysis based on comparative methods using Duncan's multiple range tests for mean separation at the 5% level of significance (Duncan, 1955).

6.3 Results

Chilling stress considerably reduced the wheat biomass but had insignificant effect on the biomass of sugar beet (Table 6.1). Both the SiBs increased the biomass of wheat by 41% and 40% and of sugar beet by 27% and 32% with and without stress, respectively. The effects of tested Si biostimulators were similar.

The application of Si biostimulators had no influence on the content of water- and acid-extractable Si in the soil (Table 6.2) as well as on the Si content in roots and shoots of wheat and sugar beet (Table 6.3).

The content of pigments in the leaves is presented in Table 6.4. Chilling stress significantly reduced the leaf content of all tested pigments in both wheat (by 2.4–7.1%) and sugar beet (by 5.2–6.8%). Silicon treatment increased chlorophyll *a*, chlorophyll *b* and carotenoids in both the plant species under and without chilling. The difference between the efficiency of Si substances was insignificant. Higher application rate provided more increase in the pigment contents.

In Si-untreated plants, chilling stress did not change TBARs in wheat and resulted in its insignificant increase (from 56 to 59 nmol g^{-1} FW) in sugar beet. Both the Si materials substantially reduced TBARs in the leaves of wheat and sugar beet (Tables 6.5 and 6.6). For both plant species, higher reductions in TBARs were provided by Ecosil at a higher rate.

Chilling as well as Si treatment had low or variable effects on the leaf GP activity. In wheat plants, chilling led to a decrease in the GP content from 0.88 to 0.68 μmol g^{-1} FW. The Si treatment caused increasing GP only in chilling-exposed wheat plants. In sugar beet plants, the Si treatment considerably increased the GP activity under and without stress. Higher efficiency was shown by Ecosil at a higher rate, providing increases from 0.20 to 0.29 μmol g^{-1} FW and from 0.22 to 0.33 μmol g^{-1} FW without and with chilling, respectively.

As regards APX, wheat plants were more responsive to the Si treatment as compared to sugar beet. Chilling reduced the APX content from 5.8 to 4.6 μmol g^{-1} FW in wheat and increased it from 3.1 to 4.1 μmol g^{-1} FW in sugar beet. The treatment with Ecosil resulted in increasing the APX content in unstressed wheat up to 10.4 μmol g^{-1} FW and up to 8.2 μmol g^{-1} FW under stress. By this means, the Si treatment provided doubled activity of APX, whereas in sugar beet, the APX content increased up to 3.7

Table 6.1. The effect of Si biostimulators on dry weight of wheat and sugar beet (g $plant^{-1}$).

	Wheat		Sugar beet	
Treatment	Roots	Shoots	Roots	Shoots
Without chilling				
Control	0.11	0.23	0.09	0.076
SiB 2 kg ha^{-1}	0.15	0.29	0.10	0.081
SiB 5 kg ha^{-1}	0.17	0.31	0.11	0.093
Ecosil 2 kg ha^{-1}	0.14	0.26	0.11	0.080
Ecosil 5 kg ha^{-1}	0.15	0.32	0.12	0.092
Chilling stress				
Control	0.09	0.21	0.09	0.077
SiB 2 kg ha^{-1}	0.12	0.25	0.11	0.083
SiB 5 kg ha^{-1}	0.13	0.27	0.13	0.092
Ecosil 2 kg ha^{-1}	0.13	0.24	0.12	0.084
Ecosil 5 kg ha^{-1}	0.14	0.28	0.13	0.091
LSD_{05}	0.02	0.02	0.02	0.002

Table 6.2. Water- and acid-extractable Si in the soil after experiment (mg kg^{-1}).

	Wheat		Sugar beet	
Treatment	Water-extractable Si	Acid- extractable Si	Water-extractable Si	Acid- extractable Si
Without chilling				
Control	8.3	181	8.5	190
SiB 2 kg ha^{-1}	8.4	185	8.4	186
SiB 5 kg ha^{-1}	8.2	182	8.3	185
Ecosil 2 kg ha^{-1}	8.3	184	8.4	188
Ecosil 5 kg ha^{-1}	8.3	185	8.5	190
Chilling stress				
Control	8.2	190	8.4	192
SiB 2 kg ha^{-1}	8.3	191	8.4	190
SiB 5 kg ha^{-1}	8.2	188	8.5	188
Ecosil 2 kg ha^{-1}	8.4	184	8.5	189
Ecosil 5 kg ha^{-1}	8.3	188	8.5	192
LSD$_{05}$	0,2	15	0,2	15

Table 6.3. The content of Si in the roots and shoots of wheat and sugar beet (%).

	Wheat		Sugar beet	
Treatment	Roots	Shoots	Roots	Shoots
Without chilling				
Control	0.85	0.80	0.43	0.32
SiB 2 kg ha^{-1}	0.85	0.79	0.42	0.32
SiB 5 kg ha^{-1}	0.84	0.80	0.42	0.31
Ecosil 2 kg ha^{-1}	0.85	0.80	0.43	0.32
Ecosil 5 kg ha^{-1}	0.85	0.80	0.43	0.31
Chilling stress				
Control	0.85	0.78	0.41	0.33
SiB 2 kg ha^{-1}	0.84	0.79	0.42	0.31
SiB 5 kg ha^{-1}	0.85	0.80	0.43	0.32
Ecosil 2 kg ha^{-1}	0.84	0.79	0.44	0.31
Ecosil 5 kg ha^{-1}	0.85	0.78	0.43	0.32
LSD$_{05}$	0.05	0.04	0.03	0.03

Table 6.4. The content of chlorophyll a, chlorophyll b and carotenoids in the leaves of wheat and sugar beet (mg g^{-1} FW).

	Wheat			Sugar beet		
Treat-ment	Chlorophyll a	Chlorophyll b	Carotenoids	Chlorophyll a	Chlorophyll b	Carotenoids
Without chilling						
Control	0.903	0.802	0.604	0.745	0.586	0.804
SiB 2 kg ha^{-1}	0.917	0.814	0.613	0.759	0.593	0.824
SiB 5 kg ha^{-1}	0.922	0.816	0.622	0.775	0.603	0.829
Ecosil 2 kg ha^{-1}	0.918	0.813	0.620	0.760	0.605	0.823
Ecosil 5 kg ha^{-1}	0.925	0.817	0.621	0.783	0.604	0.828
Chilling stress						
Control	0.845	0.745	0.589	0.706	0.546	0.756
SiB 2 kg ha^{-1}	0.900	0.783	0.585	0.735	0.548	0.778
SiB 5 kg ha^{-1}	0.911	0.811	0.603	0.754	0.559	0.784
Ecosil 2 kg ha^{-1}	0.905	0.794	0.594	0.730	0.550	0.779
Ecosil 5 kg ha^{-1}	0.913	0.814	0.602	0.759	0.559	0.785
LSD05	0.025	0.025	0.20	0.020	0.025	0.020

Table 6.5. The content of TBARs in the leaves of wheat.

Treatment	TBARs (nmol g^{-1} FW)	GP (μmol g^{-1} FW)	APX (μmol g^{-1} FW)	GPX (μmol g^{-1} FW)
Without chilling				
Control	69	0.88	5.8	15.6
SiB 2 kg ha^{-1}	54	0.75	6.8	17.5
SiB 5 kg ha^{-1}	35	0.75	7.9	19.4
Ecosil 2 kg ha^{-1}	35	0.82	8.5	20.4
Ecosil 5 kg ha^{-1}	32	0.79	10.4	21.6
Chilling stress				
Control	70	0.68	4.6	17.5
SiB 2 kg ha^{-1}	78	0.61	5.4	22.5
SiB 5 kg ha^{-1}	70	0.72	7.9	20.5
Ecosil 2 kg ha^{-1}	58	0.68	6.4	23.2
Ecosil 5 kg ha^{-1}	69	0.83	8.2	24.6
LSD$_{05}$	3	0.08	0.3	0.6

Table 6.6. The content of TBARs in the leaves of sugar beet.

Treatment	TBARs (nmol g^{-1} FW)	GP (μmol g^{-1} FW)	APX (μmol g^{-1} FW)	GPX (μmol g^{-1} FW)
Without chilling				
Control	56	0.20	3.1	0.58
SiB 2 kg ha^{-1}	47	0.25	3.5	0.63
SiB 5 kg ha^{-1}	42	0.28	3.6	0.72
Ecosil 2 kg ha^{-1}	45	0.26	3.4	0.62
Ecosil 5 kg ha^{-1}	41	0.29	3.7	0.73
Chilling stress				
Control	59	0.22	4.1	0.68
SiB 2 kg ha^{-1}	50	0.28	3.9	0.78
SiB 5 kg ha^{-1}	48	0.32	4.0	0.83
Ecosil 2 kg ha^{-1}	48	0.27	4.2	0.76
Ecosil 5 kg ha^{-1}	45	0.33	4.5	0.82
LSD$_{05}$	3	0.03	0.3	0.05

and 4.5 μmol g^{-1} FW without and under stress, respectively, which is only 19 and 9.7%.

Changing the GPX activity was also greater and clearer in wheat than that in sugar beet. Chilling activated GPX in wheat leaves from 15.6 to 17.5 μmol g^{-1} FW and in sugar beet from 0.58 to 0.68 μmol g^{-1} FW. The treatment with Ecosil resulted in increasing the GPX content in unstressed wheat up to 21.6 μmol g^{-1} FW (by 38.4%) and in stressed wheat up to 24.6 μmol g^{-1} FW (by 40.6%), while the corresponding increases in sugar beet were by 25.8 and 22.0%, respectively.

6.4 Discussion

The results of the current study showed that the tested Si substances can be classified as highly effective biostimulators according to their ability to regulate the balance of the oxidation-reduction processes in plant cells. In general, the effects of both Si biostimulators were similar. We suggest that both the products provide the release of monosilicic acid as active compounds able to stimulate the antioxidant system, thus enhancing the plant tolerance to chilling. The mechanisms responsible for the efficiency of both products could be the same. The reduction in the leaf TBARs in both plant species suggests that Si biostimulators alleviated a detrimental impact of external stress.

Our data showed that Si-induced activation of the antioxidants depended on the plant species. In wheat, chilling and Si treatment had low or ambiguous influence on the GP activity. whereas both factors led to its significant increase in sugar beet. In contrast, Si-induced change in the APX activity was more pronounced in wheat than in sugar beet. This is perhaps related to the biochemical features of the protective mechanisms in wheat and sugar beet.

The mechanisms underlying the stimulant effect of Si on the plant defense system are very poorly investigated. Recent studies report that Si-enhanced plant tolerance is attributed to increasing the activity of defense-related enzymes, but detailed mechanisms of Si action remain unknown (Wang *et al.*, 2017; Yin *et al.*, 2019). Silicon-mediated plant stress tolerance can be due to: (i) indirect influence via inducing the stimulation of the plant signaling system resulting in the formation or activation of defense-related enzymes; or (ii) direct influence on the additional synthesis of enzymes and proteins involving in scavenging ROS. The involvement of active forms of Si in modulating the plant signaling system is very difficult to verify directly by existing chemical or physical methods. Silicon-induced alterations in the antioxidant potential of stressed plant indirectly demonstrate a role of Si in the plant signaling system. According to the data obtained, active components of Si biostimulators, such as monosilicic acid in liquid form or amorphous fine silica in solid form, are supposed to participate in regulating the plant defense mechanisms.

It is well documented that any stress initiates enhanced Si uptake and redistribution within the plant, providing Si accumulation in the stressed sites. We hypothesize that active forms of Si can participate in the synthesis of enzymes or stress proteins directly. This mechanism may include the following steps.

- Step 1. *Initiation.* Stress or Si biostimulator activate the plant signaling system resulting in Si translocation to the stressed or treated site.
- Step 2. *Silicon uptake.* Soil- or foliar-applied monosilicic acid penetrates through the root plasmalemma (cell 'sluice') or leaf epidermal tissue inside the cell and forms polysilicic acids. Monosilicic and polysilicic acids move within plant (Matichenkov *et al.*, 2008; Wei *et al.*, 2021).
- Step 3. *Silicon distribution.* Silicon compounds partly translocate into epidermal layer, root caps, cell walls, and other organs and tissues where they form Si-containing structures like phytholites. Some of Si compounds return into cell to form Si gel – the basis for further low-temperature synthesis of organic compounds. Some Si is stored 'in reserve' as polysilicic acid or gel within the cells or in the intercellular space (Wei *et al.*, 2021).
- Step 4. *Synthesis of organic compounds on the polysilicic acid matrix at non-stress conditions.* Inside the cell, newly formed Si gel is able to adsorb any organic molecule (Banerjee *et al.*, 2001). It is important that the organic molecule adsorption on the Si gel surface involves certain surface alterations with the formation of a special matrix that 'remembers' the structure of adsorbed molecule (Fig. 6.1). After 'printing' and moving out of the replicating organic molecule, a modified Si gel-plate provides the catalytic synthesis of copies of the former-molecule (Banerjee *et al.*, 2001). This process is widely used in organic chemistry and pharmacology (Miao and Wang, 2008; Mendes *et al.*, 2012; Maurya *et al.*, 2016).
- Step 5. *Silicon-dependent synthesis of protective compounds at stressful conditions.* Stress activates the plant signaling system initiating additional synthesis of the stress proteins and antioxidants. Simultaneously, the stressed plant provides additional Si uptake from the environment and translocation of the stored Si to the stress-exposed site. Having received information about stress, the cell nuclear response is to modulate the additional synthesis of the defense-related compounds such as stress proteins, antioxidant enzymes and low-molecular antioxidants (Fig. 6.1). Then the molecules synthesized in response to stress are transported to damaged targets. However, under high stress conditions, the rate of synthesis and quantity of synthesizing compounds may be insufficient because of the necessity to resolve other problems vitally important to the plant. As a result of escalating energy and time deficiencies, the process of synthesis of 'routine' compounds essential for cell functioning slows down or even ceases.

We propose that some of protective compounds are translocated within the cell to the newly formed Si gel where they are synthesized as former-molecules. Then, former-molecules move to stressed zone leaving their prints on the Si gel surface, thus

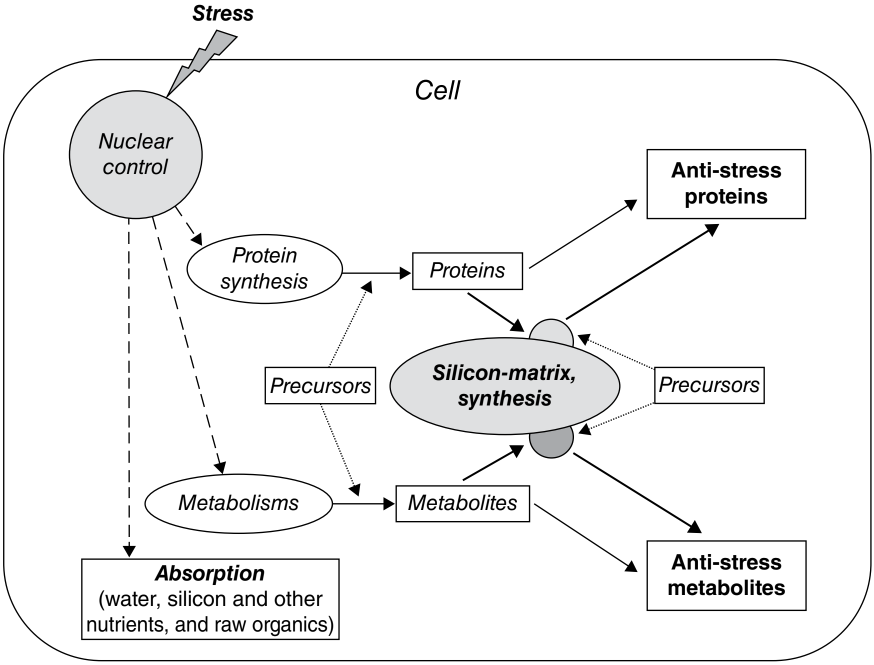

Fig. 6.1. Scheme of Si gel-mediated synthesis of organic molecules in a plant cell.

facilitating synthesis of the same molecules. So, the Si gel matrix provides the formation of defense-related compounds before stress without direct participation of the genetic apparatus.

6.5 Conclusions

The results of our study showed that both liquid (monosilicic acid) and solid (fine amorphous silica) forms of foliar-applied Si at low rates (2–5 kg ha^{-1} Si) promote a significant increase in wheat and sugar beet tolerance to cold stress. Both Si substances can be classified as effective biostimulators due to the ability to benefit the activity of antioxidant enzymes, such as APX, GR and GPX, as well as to promote the reduction in TBARs. The antioxidant systems of tested plants varied in the response to cold stress and Si treatment. In wheat, cold stress mostly enhanced the APX and GPX activities, while in sugar beet it resulted in the activation of GPX. Both Si biostimulators benefited the activity of all tested antioxidant enzymes. Silicon substances are thought to be directly involved in the synthesis of stress proteins and enzymes with a key role of polysilicic acid. Polysilicic acids can form Si gel that possesses surface properties facilitating the synthesis of organic molecules. However, further studies are required to make this confirm this hypothesis.

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7 Application of Iodine, Selenium and Silicon for Promotion of Crop Production

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Abstract

Food quality and resistance to pests and diseases have significant impacts on food security. One of the potential options to increase crop tolerance to stressors is the addition of bio-stimulating elements. Plants need at least 14 macronutrients and micronutrients for normal functioning; however, a lot of elements are non-essential, although they can have positive effects on plant growth. Research into fertilization with certain elements has attracted a lot of attention recently. Three microelements, that is, iodine (I), selenium (Se) and silicon (Si), can provide plants benefits under various biotic and abiotic stressed conditions. These elements also play essential roles against antioxidant activities in plants exposed to unfavorable environmental conditions. This chapter discusses the effects of applied I, Si and Se on the enhancement of abiotic stress tolerance and promotion of growth of crop plants.

7.1 Introduction

In terms of the iodine (I), selenium (Se) and silicon (Si) in the Earth's crust, I and Se are known as trace elements, while Si as the second most abundant element after oxygen (Medrano-Macías *et al.*, 2018). Iodine, Se and Si can have positive effects on plant growth and antioxidant activities, as wells as on the quality of their edible parts. Climate change is expected to result in more frequent extreme weather events, which will significantly affect crop production. Changes in crop yield, food quality and resistance to pests and diseases can have significant impacts on food security. One of the potential options is the addition of such bio-stimulating elements to crops to increase their tolerance to stressors.

The three elements I, Se and Si can provide plants with positive benefits under various biotic and abiotic stress conditions. For example, application of iodate (IO_3^-) to lettuce plants under salinity stress has significant beneficial effects on their biomass (Leyva *et al.*, 2011; Blasco *et al.*, 2012). Supplemental Se improves wheat grain yield under water-deficient conditions (Nawaz *et al.*, 2015). Se ameliorates arsenic-induced oxidative stress in rice through the modulation of antioxidant enzymes and thiols (Kumar *et al.*, 2014). Calero Hurtado *et al.* (2019) reported that Si application can neutralize the deleterious effects of salt stress and can increase yield in sorghum and sunflower plants. High Si concentrations in various plants prevents photoinhibition by maintaining the leaves in an erect position, and also increases plant resistance

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to pathogens and pest attacks, and mitigates adverse effects of drought. Silicon can alleviate chemical stresses caused by toxicities of heavy metals like aluminum, cadmium, manganese and iron (Rout and Swain, 2018). As well as this increased tolerance to stress conditions, the addition of I, Se and Si to plants can provide biofortification of these elements in the crops, and increased production of antioxidants in their edible parts (Medrano-Macías *et al.*, 2018).

7.2 Iodine

Micronutrients are important growth-promoting elements that have key roles not only for crops, but also for humans. The elimination of iodine deficiency and iodine supplementation are serious health problems, mainly in European countries (Duborská *et al.*, 2020). The possibility of increasing I levels in different crop plants has not been widely studied, except for over the last decade or so (Ledwożyw-Smoleń *et al.*, 2020). It has been reported that I is a 'beneficial element' that stimulates growth and development of higher plants (Grzanka *et al.*, 2020). However, if I is applied in excess, this can lower the plant biomass and cause leaf chlorosis and necrosis, and even plant death (Smoleń and Sady, 2012). Although I is not an essential element for plants, its use in agriculture might bring benefits through biofortification, as it has antioxidant capacity and can potentially induce tolerance to stress (Dávila-Rangel *et al.*, 2019).

7.2.1 Effects of iodine on plants depend on dose and form

In plants, the responses to I fortification in terms of biomass and yield show great variability depending on the plant species and the growing conditions (Dávila-Rangel *et al.*, 2019). It has been shown that the addition of I does not affect the plant biomass. Indeed, a significant reduction in the aerial biomass was seen in lettuce at I application rates >40 μM (in the growth solution) when the applied form was I^-, while IO_3^- did not have any negative effects on biomass (Blasco *et al.*, 2008). For chicory plants, foliar spraying with 1000 mg L^{-1} I (as I^-, IO_3^-) at the onset of head formation showed no effects on yield or above-ground mass (Germ *et al.*, 2020). Similarly, for buckwheat, kohlrabi and pumpkin, where foliar treatments with I^- and IO_3^- had no effect on yield or biomass (Germ *et al.*, 2019; Golob *et al.*, 2020a, 2020b).

In agreement with this literature, it was also recently shown in a 3-year study of potato that soil application of potassium iodide (KI) and foliar application of potassium iodate (KIO_3) at up to 2.0 kg I ha^{-1} did not decrease total yield, marketable yield, mean tuber weight or dry matter content. This therefore indicated no harmful effects of this agronomic practice (Ledwożyw-Smoleń *et al.*, 2020). Biomass is one of the most important characteristics of crops, and biofortification of plants with any elements should not lower the biomass. Thus, it is very important to reach a steady-state increase in the accumulation of I in plants without lowering the biomass.

7.2.2 Addition of iodine for better crop performance

As well as the effective accumulation of I in the edible parts of crops being safe for human nutrition, positive effects of I on plants should bring benefits for the producers. Dobosy *et al.* (2020) showed that irrigation water that contained I at 0.1 mg L^{-1} and 0.5 mg L^{-1} did not significantly influence the photosynthetic efficiency and chlorophyll levels of cabbage and tomato. They also showed that the growth of cabbage leaves was slightly stimulated, while for tomato plants, the dry mass did not change. The consumption of 100 g fresh vegetables would cover about 80% and 15% of the recommended I intake as cabbage leaves and tomato fruit, respectively. The presence of I led to reductions in iron and phosphorus levels in the tomato fruit, while the magnesium, copper, manganese, zinc and boron levels remained unchanged.

On the other hand, significantly greater root length and mass, and above-ground mass, were reported for sweetcorn with the addition of 10 μM I (KI, KIO_3) applied four times (Grzanka *et al.*, 2020). Indeed, the effects of I on the mineral composition of crops differ greatly in terms of dose, the I form (e.g. I^-, IO_3^-) and method of addition, and the plant species. Iodine can also act antagonistically or synergistically on the uptake of macroelements and microelements. For example, Smoleń and Sady (2012) studied the effect of different iodine forms and the method of I fertilization on I accumulation and the level on mineral nutrients in spinach plants. No effect of iodine fertilization and fertigation in spinach plants was found regarding to yield and the content of phosphorus, potassium, magnesium, sulfur, boron, copper, manganese, molybdenum and cadmium.

Iodine can also affect the sugar levels in plants, thereby greatly enhancing the quality of the fruit. In a study by Ledwożyw-Smoleń *et al.* (2020) on potato, soil application of KI and foliar application of KIO_3 at up to 2.0 kg I ha^{-1} only had small effects on the tuber sugar levels. However, I applied at low-to-moderate levels (0.25–1.0 mg L^{-1}) was shown to promote significant increases in sugar accumulation in the fruit of pepper (Li *et al.*, 2017a) and strawberry (Li *et al.*, 2017b). The I level improved the strawberry fruit quality through significant increases in the levels of vitamin c.

Biofortification with I can also have an impact on the total plant antioxidants and the plant tolerance to different stresses (Gonzali *et al.*, 2017; Dávila-Rangel *et al.*, 2019). In pepper leaves, the chlorophyll-*a* levels and catalase, peroxidase and superoxide dismutase activities increased, while the malondialdehyde levels decreased. The I-induced changes in the photosynthetic and antioxidant capacities of the plants increased the pepper fruit quality (Li *et al.*, 2017a). In a study by Blasco *et al.* (2008), iodine-treated lettuce plants showed significant increases in antioxidant compounds after application of I. They suggested the use of I in the form of I^-, as the antioxidants increased more compared to IO_3^-. Application of 7.9 μM IO_3^- increased the contents of ascorbic acid and total phenolic compounds in tomato (Smoleń *et al.*, 2015). Addition of IO_3^- to lettuce plants under salinity stress showed significantly positive effects on their biomass (Blasco *et al.*, 2012; Leyva *et al.*, 2011). Medrano-Macías *et al.* (2016) indicated that I is an inducer of tolerance to certain pathogens.

Kieferle *et al.* (2021) recently showed that very low amounts of iodine (between 0.20 and 10 mM) improved plant growth and development thus promoting both biomass production and early flowering in *Arabidopsis thaliana*. The authors also found that iodine was able to modulate gene expression and that it can be a structural component of several different proteins thus showed that iodine might have nutritional role in plants.

7.2.3 Leafy vegetables are an effective medium for iodine biofortification

Many studies have indicated marginal I mobility in the plant phloem (Blasco *et al.*, 2008; Voogt *et al.*, 2010; Lawson *et al.*, 2015). Similarly, Humphrey *et al.* (2019) showed that I uptake is predominantly passive; however, I^- can be absorbed actively through the symplast. Spinach leaves absorbed I under foliar fertilization, but the I translocation within the plant was strongly limited. They therefore indicated that foliar I application is not likely to significantly increase the I content of fruit, grain or tubers via phloem translocation. However, even though translocation of I relies mainly on the xylem, and thus less on the phloem, this is not always the case, with phloem important in, for example, tomato (Landini *et al.*, 2011), as also recently shown in the kohlrabi *Brassica oleracea* L. var. *gongylodes* (Golob *et al.*, 2020b). Thus, accumulation of added I in fruit and tubers is also seen, although I appears to be accumulated more effective in leafy vegetables (Humphrey *et al.*, 2019) (Table 7.1).

7.3 Selenium

Selenium is a naturally occurring element in soils and minerals. It is not generally considered to be essential to plants (White, 2016), but numerous studies have shown that although Se is toxic at high concentrations, it can have beneficial effects on plants at low concentrations (Cartes *et al.*, 2005). The uneven distribution of Se over the Earth's surface causes wide Se-deficient areas, leading to increased risk of health problems in humans (Golubkina *et al.*, 2019). The interest in the biological impact of Se on the environment and the food chain has increased more recently because Se is essential for humans and animals as it is an important component of various Se-dependent enzymes (Ventura *et al.*, 2017). Selenium enters the food chain through plants, and therefore the availability of Se for higher organisms in the food chain depends not only on the total Se levels in the soil, but also on the soil properties, such as pH, redox potential, organic matter composition and competitive ions (Zhao *et al.*, 2005; Hawrylak-Nowak, 2013).

Table 7.1. Some biofortification studies with iodine, selenium, and silicon in different crop species.

		Silicon				Selenium			Iodine	
		Biotic stress	Heavy metal	Salinity	Water loss	General	Drought	Heat Stress	General	Salinity
Minerals	Nutrient uptake			I (Sahebi *et al.*, 2015)		I (Golob *et al.*, 2018a)				
	Na concentration			d (Matoh *et al.*, 1986)						
	Heavy metal accumulation		d (Feng *et al.*, 2010)							
	Antioxidant capacity		I (Vega *et al.* 2019)			I (Ríos *et al.*, 2009; Saeedi *et al.*, 2021)			I (Dávila-Rangel *et al.*, 2019; Blasco *et al.*, 2008)	
Secondary metabolites (absorbing UV)	Anthocyanin	I (Resende *et al.*, 2013)				I (Liu *et al.*, 2017)				
Antioxidants (non-enzymatic system)	AsA					I (Ríos *et al.*, 2009)			I (Li *et al.*, 2017; Smoleń *et al.*, 2015)	
	GSH (glutathione)					I (Ríos *et al.*, 2009)				
	Phenolic substances		I (Vega *et al.* 2019)			I (Saeedi *et al.* 2021).	I (Saffaryazdi *et al.*, 2012; Xu and Hu, 2004; Walaa *et al.*, 2010)		I (Smoleń *et al.*, 2015)	
	Soluble sugars								I (Li *et al.*, 2017)	
Antioxidants (enzymatic system)	CAT (catalase)		d (Song *et al.*, 2009)					I (Djanaguiraman *et al.*, 2010)	I (Li *et al.*, 2017)	
	SOD (superoxide dismutase)		d (Song *et al.*, 2009)	I (Zhu, 2004)				I (Djanaguiraman *et al.*, 2010)	I (Li *et al.*, 2017)	
	POD (peroxidase)	I (Resende *et al.*, 2013)						I (Djanaguiraman *et al.*, 2010)	I (Li *et al.*, 2017)	

	DHAR (dehydroascorbate reductase)			I (Zhu, 2004)					
	GR (glutathione reductase)			I (Zhu, 2004)					d (Jerše *et al*., 2017)
	GPX (guaiacol peroxidase)			I (Zhu, 2004)		I (Xue *et al*., 2001; Ríos *et al*., 2009)			
	APX (ascorbate peroxidase)		d (Song *et al*., 2009)	I (Zhu, 2004)		I (Ríos *et al*., 2009)			
	PPO (polyphenol oxidase)	I (Resende *et al*., 2013)							
Membrane damage	MDA/TBARS (lipid peroxidation)					d (Xue *et al*., 2001)			
	Osmolyte leakage			d (Zhu *et al*., 2004)					
	Ethylene					I (Freeman *et al*., 2010)			
	Salicylic acid					I (Freeman *et al*., 2010)			
	Methyl jasmonate					I (Freeman *et al*., 2010)			
	JA (jasmonic acid)					I (Tamaoki *et al*., 2008)			
Oxidative stress	ROS							d (indirectly: Djanaguiraman *et al*., 2010)	
	H_2O_2			d (Zhu *et al*., 2004)		d (indirectly: Ríos *et al*., 2009)			
Photosynthesis	photosynthetic rate				I (Hattori *et al.*, 2005)				
	Chlorophyll		I (Matichenkov and Bocharnikova, 2001)			I (Saeedi *et al*. 2021; Saffaryazdi *et al*., 2012; Golob *et al*., 2018b)	I (Yao *et al.*, 2009)		
Morphology	Growth rate					I (Hartikainen *et al.*, 1997; Xue *et al.*, 2001; Seppänen *et al.*, 2003; Djanaguiraman *et al.*, 2005)			

Continued

Table 7.1. Continued.

		Silicon				Selenium			Iodine	
		Biotic stress	Heavy metal	Salinity	Water loss	General	Drought	Heat Stress	General	Salinity
	Total biomass					d (Jerše *et al.*, 2017; Germ *et al.*, 2019)			d (Jerše *et al.*, 2017, Germ *et al.*, 2019)	I (Blasco *et al.*, 2012; Leyva *et al.*, 2011; Kiferle *et al.*, 2021)
	Root–shoot ratio				I (Hattori *et al.*, 2005)					
	Dry weight of shoots			I (Ahmad *et al.*, 1992)						
	Dry matter yield			I (Liang *et al.*, 1996)						
	Leaf area				I (Gong *et al.*, 2003)					
	Fruit quality (sugar accumulation)								I (Li *et al.*, 2017)	
Water content	Relative water content				I (Gong *et al.,* 2003)					
	Water potential				I (Gong *et al.,* 2003)					
Cell structure	Lignification		I (Vega *et al.,* 2019)							

7.3.1 Selenium uptake and accumulation in plants

Plants absorb Se from the soil predominantly in the form of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), with selenite less bioavailable because it is strongly absorbed to iron and aluminum oxides, clay particles and organic matter (Lima *et al.*, 2018). Selenium levels in cultivated plants depend on the Se concentrations in the soil, which are usually below 0.1 μg Se g^{-1} dry weight soil. Typical agricultural crops are classified as non-accumulators of Se, and they can tolerate up to 50 μg Se g^{-1} dry weight soil. Only a few plant species can accumulate higher levels of Se; e.g. garlic (*Allium sativum* L.), onion (*Allium cepa* L.), broccoli (*Brassica oleracea* L.), wild leek (*Allium tricoccum* L.), Indian mustard (*Brassica juncea* L.) and canola (*Brassica napus* sp. *oleifera* L.) (Hasanuzzaman *et al.*, 2010).

7.3.2 Molecular mechanisms of selenium-induced tolerance in plants

Plants do not require Se for growth, but many studies have shown that plants can benefit from addition of Se. Recent studies have shown that Se application to plants can improve their tolerance to drought (Nawaz *et al.*, 2015; Bocchini *et al.*, 2018), salt stress (Hasanuzzaman *et al.*, 2011), high temperature stress (Djanaguiraman *et al.*, 2010), heavy metal toxicity (Li *et al.*, 2020) and UV-B radiation stress (Germ *et al.*, 2005; Yao *et al.*, 2011). Selenium may reduce the uptake of toxic metals by immobilizing them in the growth medium, especially in the rhizosphere (Rizwan *et al.*, 2020). Numerous studies have contributed to the possible mechanisms of Se promotion of improved stress tolerance, although the specific physiological and molecular mechanisms that underlie the positive effects of Se in plants have not been fully elucidated.

Selenium applied at low concentrations enhances the growth and antioxidative capacities of different plant species, and this can also delay senescence and promote growth of aging seedlings. The anti-aging effects in Se-treated plants might be related to decreased lipid peroxidation and enhanced glutathione peroxidase activity (Xue *et al.*, 2001). A stimulatory effect of Se on growth and crop yield has been demonstrated for ryegrass, lettuce, potato and soybean (Hartikainen *et al.*, 1997; Xue *et al.*, 2001; Seppänen *et al.*, 2003; Djanaguiraman *et al.*, 2005). These growth-promoting effects of Se are at least partly related to the improved antioxidative capacity triggered by Se addition. Selenium increases the activity of the enzymes that detoxify hydrogen peroxide (H_2O_2), as ascorbate peroxidase and glutathione peroxidase in particular, and also increases the foliar levels of antioxidant compounds, such as ascorbate and glutathione (Ríos *et al.*, 2009). Addition of Se also increases the activity of catalase, peroxidase and superoxide dismutase in sorghum leaves exposed to heat stress (Djanaguiraman *et al.*, 2010). These antioxidant enzymes are involved in scavenging of the reactive oxygen species that are generated during the exposure of plants to different environmental stressors.

The effects of Se on the increased resistance of plants to stress might be related in part to the influence of Se on the metabolism of certain plant hormones. Jasmonic acid and its precursors and derivatives are important hormones in the mediation of plant responses to biotic and abiotic stresses. They can activate the plant defense mechanisms, which mainly involve the antioxidative enzymes and other defensive compounds (Ali and Baek, 2020; Wang *et al.*, 2020). Studies on *Arabidopsis* have shown that Se increased the content of jasmonic acid and ethylene, through promotion of increased expression of the enzymes involved in their synthesis (Tamaoki *et al.*, 2008). The involvement of Se in upregulation of the genes for the enzymes involved in the biosynthesis of the phytohormones methyl jasmonate, jasmonic acid, salicylic acid and ethylene was also shown for *Stanleya pinnata* (prince's plume), a Se hyperaccumulator (Freeman *et al.*, 2010).

One of the most consistent responses of plants to environmental stress is synthesis and accumulation of secondary metabolites (Ashraf *et al.*, 2018). Liu *et al.* (2017) confirmed that low Se levels can stimulate accumulation of anthocyanin in purple lettuce. Increased anthocyanin accumulation might be a consequence of variations in gene expression of UDP glycose flavonoid glycosyltransferase and flavanone 3-hydroxylase, the enzymes involved in anthocyanin production in many plants (Zhao *et al.*, 2012). Foliar application of Se significantly increased the levels of phenolic compounds in spinach (Saffaryazdi *et al*., 2012), green tea leaves (Xu and Hu, 2004) and cucumber grown under NaCl stress (Walaa *et al*., 2010). At a concentration of 10 mg/L sodium selenate, photosynthetic pigments, total phenolic compounds and antioxidant capacity were enhanced in cauliflower (Saeedi *et al*., 2021). All of these indicate that these Se treatments result in increased phenylalanine ammonia lyase activity (Saffaryazdi *et al*., 2012).

Another mechanism of the positive activity of Se in plants is through promotion of the biosynthesis of photosynthetic pigments, as has been reported for spinach. Spinach plants treated with low Se levels showed increased chlorophyll-a and chlorophyll-b contents, which were instead decreased at higher Se levels (Saffaryazdi *et al*., 2012). The initial increase in the chlorophyll content of spinach leaves might be a consequence of increased protection of the chloroplast enzymes. This is in line with the delayed loss of chlorophyll seen in senescent *Vicia faba* (broad bean) under Se treatment (Moussa and Ahmed, 2010), and also in wheat plants exposed to drought (Yao *et al*., 2009). Se application enhanced the chlorophyll content of tall fescue leaves, but only when they were under cadmium treatment. Se enhanced the transcription level of the *psbC* and *psbB* genes that encode the core antenna protein complexes of the reaction center of photosystem II, which would explain the increased content of the chlorophylls (Li *et al*., 2020).

Application of Se can also stimulate pathogen resistance responses through disruption of the cellular redox balance, which might induce defense-related genes (Freeman *et al*., 2010) (Table 7.1).

7.4 Silicon

Silicon is an element that can occur in various forms, and it is found in clay, granite, quartz and sand as Si dioxide (SiO_2) and as silicates (Chen and Lewin, 1969). At 28.8%, Si is the second most abundant element in the continental crust, after oxygen at 47.2% (Wedepohl, 1995; Song *et al*., 2009). In combination with oxygen atoms, Si forms different silicates and silicate minerals. Weathering of silicates and their transformation to carbonate rock is a process that consumes atmospheric carbon dioxide and forms dissolved silicates. This can thus contribute to lowering atmospheric CO_2 levels, especially over the long term (Berner *et al*., 1983).

Silicon is found in the various soil fractions in both the solid and liquid phases, and it can be absorbed onto soil particles (Matichenkov and Bocharnikova, 2001). The solid phase forms can be amorphous (e.g. amorphous silica, Si-rich organisms) or crystalline (e.g. quartz, clay, minerals). The liquid phase of the Si dissolved in the soil contains monosilicic and polysilicic acids and complexes with inorganic, organic and organosilicon compounds (Matichenkov and Bocharnikova, 2001).

Silicon is essential for some organisms. For example, it is an essential element for the diatom (Bacillariophyceae) algae. In the plant kingdom, Si is considered an essential element for horsetail (Equisetaceae) (Chen and Lewin, 1969).

7.4.1 Silicon content in different plant species

Incorporation of Si into living organisms is called biosilicification. However, although Si is found everywhere, plants cannot take it up in just any form. Plants take up silica in the form of dissolved Si, as monosilicic acid (H_4SiO_4). Once absorbed, the monosilicic acid is transported from the roots through the xylem to the upper parts of the plant (Hartley and Jones, 1972). When the monosilicic acid concentrations in plant tissues exceeds 2 mM, it transforms into polymers of H_4SiO_4, and then eventually into hydrated silica ($SiO_2 \times H_2O$) (Exley, 2009, 2015). Silica accumulates in the epidermis of different plant tissues and forms phytoliths (Sangster and Hodson, 1986; Ma, 2004). In cucumber plants, once Si is precipitated in the tissues, it cannot be remobilized to different parts of the plant (Samuels *et al*., 1991).

The Si levels in different plant species vary from 0.1% to 10% Si by dry shoot weight (Ma and Takahashi, 2002). Some plants are Si accumulators, which means that their tissues contain >1% Si and the Si:Ca ratio is >1.0. Specific groups of Si accumulators include some monocotyledons, pteridophytes and bryophytes, with most of the Si-accumulating monocotyledons from the families Cyperaceae and Poaceae. There are no dicotyledons that are Si accumulators, although some belong to intermediate types, where the Si:Ca ratio is between 0.5 and 1.0, such as cucurbits (e.g. melons, pumpkins, cucumbers) and nettle (*Urticaceae*). Si excluders have a Si:Ca ratio of <0.5 (Ma and Takahashi, 2002).

7.4.2 Importance of silicon for plants

Silica in plant tissue provides better structural support and helps in plant defense against biological stress (e.g. pathogens, herbivory) and abiotic stress (e.g. salinity, drought, UV, nutrient deficiency, metal toxicity). Under stressed conditions, Si boosts the antioxidative responses, osmolyte levels and induction of stress-related signaling responses within plants (Souri *et al.*, 2021).

Silicon increases protection against pests and diseases induced by fungi, bacteria and viruses (Rodrigues *et al.*, 2015). It has been shown that immediately after infection of cucumber plants with powdery mildew (*Sphaerotheca fuliginea* (Schlecht, ex Fr.) Poll.), siliceous rings can form around the pathogens, which results in milder disease symptoms (Samuels *et al.*, 1991). Sorghum with 2 mM Si in the nutrient solution was less affected by the fungal disease anthracnose (*Colletotrichum sublineolum*) compared to the control without Si. Anthocyanin levels and the activities of peroxidases and polyphenol oxidases were also higher in these Si-treated plants. The conclusions were thus that the precipitation of Si establishes a physical barrier that prevents penetration of the pathogens deeper into the leaf (Leusch and Buchenauer, 1970; Liang *et al.*, 2005; Resende *et al.*, 2013).

Silicon stimulates the formation and activity of proteins that are important for the synthesis of secondary metabolites (Singh *et al.*, 2020). It thus represents a possible factor in the defense of plants against being eaten by animals, through the accumulation of monosilicic acid in shoots and SiO_2 in the form of phytoliths. A recent study also showed that the grazing of rescue grass (*Bromus catharticus*) by the grasshopper *Oxya grandis* was lower for the plants with higher Si levels (Mir *et al.*, 2019).

Heavy metals are naturally present in the soil, but at higher concentrations they can have harmful effects on plants. Silicates can increase the pH of the soil and reduce the availability of heavy metals. Silicates can also regulate metal transporters in cells and root excretion that can chelate heavy metal ions (Wu *et al.*, 2013). In barley plants, silicon decreases Al uptake by providing reductions in oxidative damage and improvements in antioxidant activity. It also stimulates phenolic metabolism in Al stressed plants, shown by the increase in the phenol concentrations and the lignin accumulation at the root level (Vega *et al.*, 2019). Silicon addition can reduce cadmium accumulation in leaves of cucumber (*Cucumis sativus* L.) (Feng *et al.*, 2010). Here, Si reverses chlorosis induced by cadmium stress, protects chloroplasts from disorganization, and increases the content of pigments important for biomass accumulation (Matichenkov and Bocharnikova, 2001). Treatment with Si during cadmium stress in pak choi (*Brassica chinensis* L.) decreases activities of the antioxidant enzymes catalase, superoxide dismutase and ascorbate peroxidase (Song *et al.*, 2009).

Furthermore, when Si was applied to shoots of rice growing under high salinity conditions, the plants contained 54% less sodium (Matoh *et al.*, 1986). Silicon also increased potassium levels and suppressed sodium uptake in barley (Liang *et al.*, 1996), such that the dry matter yield was increased when Si was added to the plants under salt stress. Cucumber (*Cucumis sativus* L.) treated with Si in a saline environment showed increased activity of the enzymes involved in protection against oxidative damage of the cell membrane under salt stress (Zhu *et al.*, 2004). In the same study, decreased electrolytic leakage and H_2O_2 levels were observed with Si addition. Addition of Si to wheat (*Triticum aestivum*) under salt stress can increase the dry weight of shoots (Ahmad *et al.*, 1992). Also, application of Si to plants under salt stress reduces their transpiration, which reduces the osmotic stress in the cells (Sahebi *et al.*, 2015). This Si also increased the root activity, which was reflected in higher nutrient uptake and reduced salt toxicity.

Silicon is known to activate defense mechanisms against the negative effects of UV-B of rice plants (Goto *et al.*, 2003). This study also showed that when these rice plants were fertilized with Si, they had lower UV absorbance between 280 nm and 320 nm than the control plants.

Silicon can reduce the effects of water loss by reducing cuticular transpiration through the formation of a double layer of silica under the epidermis of the leaves, which also makes the leaves thicker. Under drought conditions for wheat, when Si is added, the relative water content, water potential and leaf area are increased (Gong *et al.*, 2003). Silicon reduces leaf transpiration and the water flow rate in the xylem (Gao *et al.*, 2004). Si-treated sorghum showed lower shoot to root ratios under drought stress, which indicated promotion of root growth and maintenance of high stomatal conductance and photosynthetic rate. This enhances the plant absorption of water from the soil (Hattori *et al.*, 2005).

7.4.3 Silicon and crop improvements

According to calculations by Matichenkov and Bocharnikova (2001), considerable amounts of Si are removed from the soil by plants that accumulate high levels of Si, such as sugarcane, rice and wheat (Tubana *et al.*, 2016). Due to this removal of large amounts of plant-available Si from the soil, fertilization of such plants with Si is recommended. This is needed for all soil types, except for those with already atypically high Si levels (Matichenkov and Bocharnikova, 2001). Increased levels of available Si can be achieved through soil or foliar fertilization. Some studies have also shown that Si improves the growth of various crops, such as maize (Jawahar *et al.*, 2019) and wheat (Neu *et al.*, 2016) (Table 7.1).

7.5 Conclusion

Iodine and Se are known as trace elements, while Si is the second most abundant element after oxygen. Iodine, selenium and silicon have important roles in plants exposed to various biotic and abiotic stress conditions. Climate change implies fluctuating and extreme environmental conditions, such as long periods with clear skies and high UV levels, combined with severe drought. Extreme weather events will significantly affect crop production. Changes in crop yield, food quality and resistance to pests and diseases can have significant impacts on food security. One of the potential options is the addition of such bio-stimulating elements to crops to increase their tolerance to stressors. Crops might thus benefit from I, Se and Si application, as they enhance the production of protective phenolic compounds and increase tolerance to stress conditions. The addition of I, Se and Si to plants can provide biofortification of these elements in the crops, and increased yield and quality of crops (Fig. 7.1).

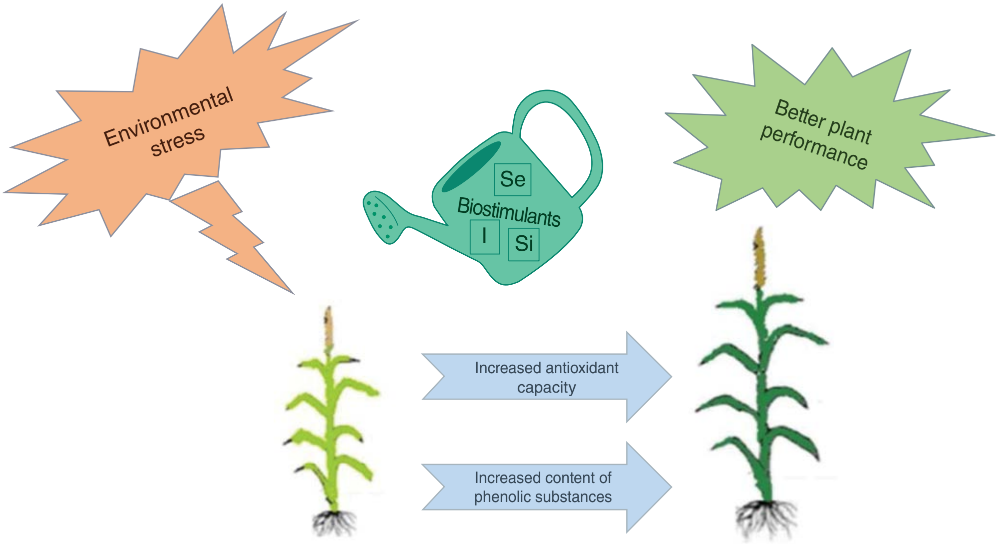

Fig. 7.1. Response of plants to the addition of I, Se and Si.

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8 Organic Manure for Promoting Sustainable Agriculture

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Abstract

Worldwide, most agricultural soils are less fertile because of low organic matter content. In addition, increased dependency on chemical fertilizers for improving crop yield leads to disturbance of soil health and its natural environment, as well as human health, and becomes the reason for high expenses for crop production in the world. In such environments, use of organic supplements or fertilizers for crop production may produce an environmentally friendly approach for ideal crop production. Maintaining the soil organic matter content results in enhancement of organic carbon level, improved biotic activities performed in the soil and increased nutrient availability in the soil, which ultimately maintains the physical, biological and chemical properties of the soil. Use of organic amendments is also a useful method for improving soil water-holding capacity, and its structure also ensures the minimum leaching. A range of organic amendments are available for improving soil environments to meet different requirements, which are obtained through a variety of sources of plants and animals such as cattle manure, chicken manure and compost. The organic matter raw material is nutritious but can be less effective when compared to the processed form (i.e., compost). When the organic matter has attained its maximum maturity and stability, it becomes a highly valuable compost in agricultural fields. Different organic wastes which vary in quality and stability are used from different sources for the preparation of compost. Under the current situation of soil organic content, it is necessary to maintain the regular incorporation and recycling of organic wastes into the soil for the maintenance of the optimum level of organic matter and other essential nutrients. This chapter aims to review the current knowledge on agronomical uses of organic manures and their important roles in better crop production and agricultural development.

8.1 Introduction

The characteristics of soil determines the fertilizer requirement for crops. It is important to understand these characteristics when seeking solutions for resolving soil problems. For example, some soils have low concentrations of accessible phosphorus due to the unavailability of innate phosphorus (Ahmed *et al.*, 2016) and some have a low cation exchange capacity (Mango and Hebinck, 2016) or low content of nitrogen and microelements. Soil deficiency is a serious problem (Phullan *et al.*, 2017). Use of organic matter can increase the soil productivity, water-holding

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capacity and reduce the loss due to erosion. Organic matter has a reflective effect on the physical and chemical features of soil (Al Amin *et al.*, 2017), and is frequently considered as soil quality. Sustainable agriculture production and soil fertility depend on organic matter (Delate *et al.*, 2017). Organic matter has a positive effect on plant development as due to its micronutrient and macronutrient contents (Mukai, 2018).

The introduction of high productive varieties and monoculture have shown to lessen the soil productivity by repeatedly giving the soil the same environment. Low soil fertility and organic content can also occur in the absence of reliable crop sequencing and the removal of crop cover from the soil, combined with the fast decay due to high temperatures (Henneron *et al.*, 2015). This low crop production is not influenced by the volume of inorganic fertilizers used. The addition of organic wastes can boost the level of productivity and the quality of the degraded soil can be improved. The amount of organic material already in the soil determines the volume of organic matter/fertilizers that needs to be added to soil to improve the root structure of plants, crop productivity (Gill *et al.*, 2019).

In the past few years the use of inorganic fertilizer has increase in agriculture, and this has badly affected the environment, food security and value, and human and animal well-being. Hence, there has been a move to use organic content to increase soil health by sustainable practices. Soil fertility and properties can be improved and increase crop growth and quality through the development of root growth and nutrient uptake (Erdal and Ekinci., 2020). Therefore, there has been a rising interest in the use of organic manure. Ahmed *et al.* (2016) reported that if organic manure is applied at the accurate rates, most crop yields would not be reduced. To counteract the progressive loss of organic matter and nutrients over time, techniques such as composting can be used to recycle agricultural and animal wastes to produce organic amendments required for sustainable productivity of soil (Fanish, 2017).

The organic content and nitrogen in soil is reduced by the overuse of inorganic fertilizers in comparison to organic fertilizers. This can be attributed partially to the lack of information such as the techniques of fertilizer production and application, absence of periodical analysis of soil needs, using the traditional cultivation methods, unilateral development of crop and animal systems of production, and lack of appreciation of the value of organic fertilizers in the maintenance of soil fertility (Fanish, 2017). This chapter aims to review the local experiences on the use of organic manures, with an emphasis on compost production, and its potential importance in sustainable agriculture.

8.2 Types of organic manures and their source

Manures are organic product obtained from animal and plant waste, and they comprise plant nutrients in compound organic form (Khandaker *et al.*, 2017). The addition of manure increases the amount of organic matter in soil, the number of soil microorganisms (particularly bacteria), the activity of some soil enzymes such as urease, and the ability of the soil to hold humidity and reduce insects and weeds. Henneron *et al.* (2015) reported that organic manure can act as a pool of plant nutrients and stop nutrient leakage by upholding a high cation exchange capacity, as well as buffering growing plants against sudden changes in their chemical environment.

Nutrient release is slow in organic manure, which can be beneficial for current and future crops, and the is no loss of nutrients through leaching (Leithold *et al.*, 2015). Due to its slow release and low nutrient content, organic manure should be applied in large quantities (e.g. in tons per hectare). The ever-increasing demand for food combined with the high costs and accessibility and pollution issues of inorganic fertilizers means that the exclusive use of these fertilizers is not practical. Complementary use of organic with inorganic fertilizers can be employed in an ecologically safe and sustainable manner based on research outcomes.

Organic manures vary widely in contents of plant nutrients, hence are divided into the several categories.

8.2.1 Concentrated organic manures

Major plant nutrients are found in these organic manures that are richest sources of plant nutrients of all organic manures. Cakes of groundnut, sesame, mustard, castor, neem, cottonseed and sunflower are examples of concentrated organic manure.

8.2.2 Bulky organic manures

Bulky organic manures (e.g. farmyard manure and compost) are a source of small quantities of plant nutrients and are rich source of micronutrients.

8.2.3 Green manure

Green manure is a cover crop that is grown for plowing its whole mass in the soil before blooming. This leads to an increase in the soil organic matter and fertility. It can be grown as a primary or secondary crop. Any plant is favorable for green manure if it gives enough biomass for the climate and soil requirements. The seeds are not too expensive. Soil organic content can be increase by legumes crops as well as soil nitrogen content by rhizobial nodulation. Farmyard and animal manure are more efficient in improving organic content of the soil, due to its higher decomposability; still this practice has several benefits to the soil and the growing crop if properly used.

Green manure often serves to protect loose or slope soil from erosion. Soil stability is needed for optimal plant growth and yield. The green manure can also stimulate the activity of soil microorganisms as a result of changes in the soil biochemistry (De Jesus Souza *et al.*, 2019).

8.2.4 Sewage and sludge

The development of the sanitation sewage system led to the production of sewage and sludge. Sewage has a solid portion, known as sludge, and a liquid portion, commonly known as sewage water. Sludge can be further categorized as settle sludge, digested sludge, activated sludge, digested activated sludge and chemically precipitated sludge. Sludge has a high nutrient content, making it an ideal biological fertilizer without requiring composting. Due to health reasons it cannot be applied to edible crops (i.e. possibility of occurrence of heavy metals and harmful microbes).

It contains (approximately) 1.5–3.5% nitrogen, 0.75–4.0% phosphorus and 0.3–0.6% potassium (Table 8.1). Therefore, it is a valuable source of organic contents for use on selected crops (Dawi., 2014).

8.2.5 Animal manure

The species of animal and the different types of food they eat have an impact on composition of animal manure. Controlled-release nitrogen, phosphorus and sulfur, plus some chelates and micronutrients, are supplied by animal manure. It can improve soil health, productivity, physiochemical and biological properties of soil. However, it is difficult to store, handle and transport manure because of its large volume and repulsive smell; and expensive to store. In addition, elements such as copper or zinc may accumulate over several years and reach very high levels. Holik *et al.* (2018) reported that animal manure comprises more concentrated nutrients than that of plant manure. Much of initial N content can be lost unless manure is well stored and composted. Cattle manure is the most commonly used organic fertilizer and is used to increase the productivity of soils that contain inadequate levels of organic carbon (Bajgai *et al.*, 2016). The chemical composition and nutrient percentage of animal manure are presented in Table 8.1.

8.2.6 Chicken manure

With an increase in the demand for chicken meat, there is an increasing use of chicken manure as a fertilizer. Chicken manure is commonly applied before planting crops. Factors that affect the nutrient level, production and decomposition of the chicken manure includes the breed and age of chickens, the type and quantity of feed, the climatic conditions at the time of collection, and the storage process. The improvement of soil and in crop production are well documented (Ahmed *et al.*, 2016). Chemical composition of poultry manure is given in Table 8.1.

Fresh chicken manure contains twice as much nitrogen compared to cattle manure. It is rich is phosphorous and has more potassium

Table 8.1. Chemical composition of different manures (data taken from Ozdemir *et al.* (2004), Punjab State Council for Science and Technology (2010), Zayed *et al.* (2013) and George and Duke (1994)).

Nutrients	Sewage sludge	Animal manure	Poultry manure	Compost
Dry matter	75	70	–	–
Organic matter	35	–	–	–
pH	6.03	–	–	7.80
EC (dS m^{-1})	2.14	–	–	3.60
N	2.7	0.5	3.52	156.5
P	1.25	0.2	0.86	0.35
K	0.41	0.5	1.83	0.48
Ca	3.76	0.9	0.45	2.27
Mg	1.76	0.2	0.51	0.01
Na	0.27	–	–	0.57
Fe (ppm)	41	146.5	1050	11690
Cu (ppm)	83	2.8	60.4	17
Zn (ppm)	1200	14.5	230	128
Mn (ppm)	402	69.0	240	414

when compared to cattle manure. Ahmed *et al.* (2016) reported that poultry manure is rich in nitrogen and phosphorus, and it is an excellent source that can be combined with other components in most fertilizer plans. According to Ahmed *et al.* (2016), chicken manure mineralizes faster than other animal manure such as cattle or pig dung.

8.2.7 Compost

Composting is the bio-oxidative process of organic waste, including the mineralization and partial humification of the organic matter, leading to a stabilized final product (compost), free of phytotoxicity and pathogens and with certain humic properties. As a result, compost is a dark crumbly and earthy smelling form. Sustainable development is linked to the process of composting, and highlights the circular flow of production where waste is re-used as energy or raw material for another product or process (Ball *et al.*, 2014). The chemical composition of compost is presented in Table 8.1.

8.2.8 Organic sources of plant nutrients

Supplementation of entire crops through the use of farmyard manure sustains crop productivity at a higher rate compared the use of conventional N fertilizers. The estimate of NPK availability for plant from organic source is based on the presence of nutrients in these organic wastes. The efficiency of these resources is not guaranteed to meet the nutrient requirement of crop as compared to mineral fertilizers, but the combined use of organic sources with chemical fertilizers is capable of sustaining higher crop productivity, improving soil quality, and productivity on a long-term basis (Muhieldeen *et al.*, 2014).

These organic sources enable the conversion of unavailable sources of elemental nitrogen, bound phosphates, micronutrients and decomposed plant residues into an available form of nutrients that the plant can absorb. The application of organic sources encourages the growth and activity of mycorrhizae and other useful organisms in the soil, and is also helpful in alleviating the increasing incidence or deficiency of secondary and micronutrients. It is also capable of sustaining high crop productivity and soil health (Muhieldeen *et al.*, 2014).

8.3 Sustainable crop production with use of organic manure

8.3.1 Effect of organic nutrition on crop productivity

Crop yield can be increased by the addition of organic matter into the soil. Grain and straw yield of rice can be increased by the application of organic material as reported by Ram *et al.* (2016). They noted that rice grain yields was enhanced by 20% after application of a combination of NPK and spent mushroom and rice straw compost, a result that was similar to farmyard manure. Ram *et al.* (2016) reported that significantly more grain and straw yields can be obtained by the application of 7.5 t ha^{-1} farmyard manure produced over unfertilized fields. By increasing the rate of farmyard manure the entire yield attributing character of rice can be enhanced. Naturally managed field activity of earth worm is higher than in inorganic agriculture (Gill *et al.,* 2019) reported by many researchers.

Vermicompost is produced by biodegradation process in which microbes and earthworms work together, which is the worm fecal matter with worm casts. N, P, K, Ca and Mg, and microelements such as Fe, Mo, Zn and Cu, are provided by vermicompost (Gill *et al.,* 2019). Nitrogen, phosphorus and potassium are found in vermicompost at concentrations of 0.74%, 0.97% and 0.45%, respectively (Erdal and Ekinci, 2020). The productivity of crops in organically managed systems is low during the initial years, but productivity in later year's increases due to the cumulative addition of organic matter in the system. Similarly, Lairon and Huber (2014) noted that grain yield increases over a period of due to the cumulative addition of organic fertilizer.

Ma *et al.* (2019) showed that conventional rice production is cheaper than organic production; the input cost of organic rice production in three different regions was 46%, 25% and 22% higher than conventional farming, but rice yield was only 55%, 94% and 82% of conventional rice production, respectively. However, the cost of lower yield with higher inputs is compensated by the higher premium prices of organically crops in the markets (Ma *et al.,* 2019). Farmers can increase profit by organically vegetable productions as vegetable are more responsive to organic nutrients.

8.3.2 Effect of organic nutrition on quality parameters of crops

Zhi-Wei *et al.* (2019) carried out an experiment to assess the effect of vermicompost vegetable waste on the biochemical characters of chili and found that the protein was higher at 60 DAS (113 mg g^{-1}) and 90 DAS (79 mg g^{-1}). The carbohydrate content was higher in vermicompost treatment at 60 DAS (15.34 mg g^{-1}). Chlorophyll (2.61 mg g^{-1}) and total chlorophyll (3.62 mg g^{-1}) contents were observed at 60 DAS, while chlorophyll a (1.01 mg g^{-1}) was higher at 90 DAS as compared to inorganic fertilizers. In another experiment, Zhi-Wei *et al.* (2019) suggested that tubers from organic potato cropping may be expected to have sufficiently high tuber dry matter concentrations (19%) for processing into French fries without impairing the texture of the fries when concentrations exceed 23%. Similarly, application of farmyard manure at 10 t ha^{-1} alone increased the economic yield and quality parameters like hulling percentage, milling percentage, and protein and amylose content of rice (Karmegam *et al.,* 2019). Although foliage nitrogen content was increased for the conventional crops, difference between N content of organic and conventional tubers were not significant, as well as for K, Ca and Mg.

8.3.3 Effect of organic nutrition on soil fertility

Mango and Hebinck (2016) also reported that macronutrients and micronutrients are released into the soil solution by the decomposition of organic wastes, which becomes available to the plants, resulting in higher uptake. Higher crop productivity can be obtained by organic farming and improving soil quality and productivity by manipulating the soil properties on a long-term basis. CO_2 does not contribute to the organic pools and normal composting takes a long time, leading to considerable loss for organic material (Mahaly *et al.,* 2018).

Mahaly *et al.* (2018) reported that compost reduced the broadleaf weed population by 29% and grassy weed population by 78% as the use of compost raised soil pH from 6.0 without compost to 6.5. The nutrient-supplying capacity is reduced by degradation of soil, especially, on soils with high initial soil organic matter content in rice-wheat cropping system (Ram *et al.,* 2016). Organic matter content can be improved by organic farming and labile status of nutrients and soil physicochemical properties. The composting characteristics of manure can be improved by the addition of carbon aqueous materials such as straw, wood, bark, sawdust or corn cobs. These materials raised the C:N ratio and reduced water content. However, under Indian climate conditions, separate composting is not more beneficial than joint composting of the manure slurries with plant remains. Use of farmyard manure and green manure maintained high levels of Zn, Fe, Cu and Mn in rice-wheat rotation (Fanish *et al.,* 2017).

8.3.4 Effect of organic nutrition on soil biological properties

Soil biophysiochemical properties are affected by agricultural practices. Bacteria, protozoa, nematode and arthropod populations are higher in soils under organic farming compared to conventional farming. Holik *et al.* (2018) noted that the population of advantageous soil microorganisms can be increased by organic fertility amendments, reduced pathogen population, increased total carbon, and cation exchange capacity, and lowered bulk densities, thus better soil quality. In low-input agriculture, conventional farming is comparable to the crop productivity under organic farming.

8.4 Environmental and economic benefits

8.4.1 Effects of organic manure on the environment

The world faces many environmental difficulties like climate change, loss of biodiversity, soil depletion as a result of agriculture production and water pollution (Lee *et al.,* 2015). Worldwide, it is thought that organic farming has less negative environmental exogenous factors as compared to conventional agriculture, which is why governments are promoting organic practices. In this segment we will analysis the different environmental features which effected on organic farming.

8.4.2 Land potency

The total land that is used for agricultural purposes is 40% (Muller *et al.,* 2017). If a land is used continuously, it creates changes and difficulties in the environment, mainly the loss of soil carbon into the atmosphere and loss of biodiversity. If we want to create balance in our production and environment, we should use our land and our natural resources systematically. Conventional methods show higher land-use efficiency as compared to organic systems – we noted above that conventional agriculture has higher yields than organic farming. In addition, in rotation of organic crops, most of the crops that are used are not required for human utilization.

Simply put, this means that an increased volume of fodder and extra land area is required to produce organic meat (Treu *et al.,* 2017). In estimating the outcomes of organic and conventional farming, sustainability of land use is also applicable.

8.4.3 Utilization of energy and greenhouse gas discharge

The total human-made greenhouse gas discharge that is accountable for the purpose of food production is 25% (Edenhofer *et al.,* 2014). Carbon dioxide, methane and nitrogen oxides are released from the burning of fossil fuels. They are also released throughout the production process, for example during the processing of fertilizer and minerals, the use of agriculture equipment, processing of soil chemicals, and in livestock digestive systems (Meier *et al.,* 2015). Use of energy and discharge of greenhouse gasses is a continuous process until the product leaves the farm (Smith *et al.,* 2015). It has been suggested that organic farming per unit of land requires lower energy and less area as compared to the per unit output of conventional farming. Regarding greenhouse gasses release, mostly studies report that organic farming has less impact per sown unit of area, but the same is not applicable to the unit of production. Normally, organic farming has lower nitrogen inputs, which reduces the nitrogen oxide discharge capacity. Stability in the application of nitrogen is quite difficult and challenging in an organic system. If we apply organic fertilizer, it may cause normal discharge of nitrous oxide (N_2O).

The lower supply of nutrient may lead to reduced yields (Clark and Tilman, 2017). Carbon stock rate and sequestration rates are high in organic systems as compared to conventional systems in crop production. As in animal production, lower concentrated organic husbandry systems conduct a higher amount of manure for each unit of meat. So, the more release of methane and nitrogen oxide will happen (Skinner *et al.*, 2014).

8.4.4 Leaching of nutrient and water classification

In concentrated agriculture land, nutrient leaching is a serious problem – it is the reason for the accumulation of nutrients in water bodies and in marine ecosystem (Meier *et al.*, 2015). Leaching of nitrate (NO_3^-) is reduced in organic management of each unit of area, but not the output of each unit. The eutrophication potential of each unit output is calculated in the form of phosphate (PO_4) and the potency of acidification is calculated in the form of sulfur dioxide (SO_2). Both are higher in organic farming (Clark and Tilman, 2017). If we do not use artificial fertilizer, it leads to low leaching potential. The challenge in organic systems is the increase in nutrient losses. As the use of artificial pesticides is restricted in organic farming, the risk of water pollution is lower. Howe, some non-synthetic defoliants that are widely used by organic farmers may have negative effects in water bodies (Lori *et al.*, 2017). For example, in the production of organic horticulture a solution of copper is used to prevent fungal diseases (Niggli, 2015).

8.4.5 Soil quality

Due to the lack of management, many fertile lands become infertile and unsuitable for agriculture production, mainly due to erosion (Niggli, 2015). Soil erosion can be reduced by adopting organic practices, for example by adding manure and crop rotation by cover and catch crop. These practices also help in reducing the decline of soil fertility. Many studies have confirmed that fields that are managed according to organic systems have more organic content and matter, and increase the microbial populations, resulting in an increase of soil quality (Lori *et al.*, 2017).

8.4.6 Biodiversity

The rise in agriculture and urbanization has contributed to the loss in biodiversity. The reduced use of pesticides, and introducing crop rotation and natural landscaping elements organic farms are more biodiverse (Schneider *et al.*, 2014; Tuck *et al.*, 2014). Using metal analysis methods, organic farming showed an increased species richness and uniformity of species when compared to conventional farming. However, the benefits we are getting from organic farming start to decrease if the intensity of organic agriculture increases (Niggli, 2015).

8.4.7 Economic benefits

The organic system of farming just is a way of sustainable agriculture. It is important that farmers get the same or similar income from selling organic produce as compared to conventional methods. A recent study across 14 countries revealed that 22–35% organic farming is more profitable as compared to conventional farming. Although the yield we get from organic farming is reduced compared to conventional methods, farmers are getting a higher price of their products from a certified market (Crowder and Reganold, 2015).

Basically, the magnitude of price increase at farm level is 30%. Without any increase in price, it might be low beneficial for us as compared to conventional farming. Meta analysis include details from many countries are mostly from the US and developed countries (Crowder and Reganold 2015).

8.4.8 Small farmers and organic production

In the previous section we said that the yield from organic production is reduced when compared to the yield we get from conventional methods (Jena *et al.*, 2017). These differences are a result of specific circumstances. In this condition farmers have less access to contemporary production techniques and they also apply fewer amounts of inputs to their fields, and so the yield of organic farming might be close to conventional yield (Parvathi and Waibel, 2016). This is not a practical farming method for smallholder farmers, especially those in developing countries (Ibanez and Blackman, 2016).

By increasing the training and use of organic fertilizer, there may be a notable increase in the practice of organic management if it can be shown to reduce the mutability and susceptibility against water stress and other environmental stresses. Organic farming has low yield where new inputs are available and are commonly used as compared to conventional farmers. The difference between yields enhanced throughout the economic development (Niggli, 2015).

8.4.9 Price premiums

Most farmers in developing countries grow cash crops like coffee, tea and tropical fruits etc. for exporting to developed countries, where consumers have a high awareness of certified organic products. However, the price increase at the market level is not always reflected in the prices farmers get from organic products, because different actors within value chain also take some profits from the selling price. Also, the farmers in developing countries often do not get the same profits as farmers in developed countries (Chiputwa *et al.*, 2015). Despite this, the study indicates that at the farmer level the organic premium ranges between 6% and 44%. Some organic farmers do not sell their products in certified markets – this may be because it is difficult to meet the requirements for certified organic products (Parvathi and Waibel, 2016).

Another reason is the difficultly in managing the delivery of certified crops in a timely fashion to certified markets due to lack of capacity (Ibanez and Blackman 2016). The price fluctuations may also be a factor (Kleemann *et al.*, 2014), and there may also be a delay in payment processing via certified markets. Some farmers need fast cash turnover, which is why they often sell their organic products in conventional markets at lower prices. All these factors combined mean that smallholder farmers get lower prices as compared the amount they have possibly planned for (Mitiku *et al.*, 2017).

8.4.10 Production costs

Associated costs of contract farming (CF) and certification fees for smallholder farmers are normally paid by farmer supportive traders or development organizations. The additional investment costs required for new tools is a cost that the smallholder must meet (Kleemann *et al.*, 2014). Organic certification requires a transition period of 3 years, which is considered to be a sunk cost. Farmers cannot sell the produce as the organic premium price, and the yield is reduced due to experimentation of new methods in this transition period (Caldwell *et al.*, 2014).

Variable production cost is also affected by organic certification. The organic material already present at the farm is not sufficient, so additional material needs to be purchase to fulfill the nutrient requirements. If the organic material/fertilizer is limited, the cost saved from inorganic fertilizers can be used to purchase high-price organic fertilizers to increase profitability (Kloos and Renaud, 2014).

8.4.11 Indirect economic benefits

Organic certification is also directly or indirectly related with economic benefits. Usually, services are offered by certified farmer organizations in developing countries like price information, training, credit or value addition etc. These are used to aid smallholders to meet credential demands and best quality production by farmers, which is demanded in international organic markets (Parvathi and Waibel, 2016).

8.5 Limitation of organic manure

The preceding section discussed the productivity, cost and production value in organic agriculture under defined conditions. Due to the above mentioned reasons, various researchers reported a low outcome from organic farming. The high-end cost is not enough to recompense for lower yield or for higher production costs (Mitiku *et al.*, 2017) and higher costs is added by organic certification in the small farm sector (Kleemann *et al.*, 2014; Crowder and Reganold, 2015).

Despite this, a minor pivot on the benefit from one verified crop, such as efficiency of yield, is an imperfect measurement of the whole effect of organic agriculture (Jena *et al.*, 2017). In particular, in the small agriculture area it is true that the family circle is usually busy in multiple farms and off-farm activities (Ayuya *et al.*, 2015).

The price comes from economic activities are also affected by resource allotment and excess of different types due to less inputs at family level. The impact of organic certification on total family costs is also important (Mitiku *et al.*, 2017). In some research it is given that organic farming systems have no effect or even negative effects on the outcomes and livelihood of smallholders.

8.5 Opportunities and challenges

Organic farming faces both positive and negative constraints. In organic farming, both finance and cultivation are considered to be important factors. In this farming system, we know how to manage it under favorable natural conditions (Reganold and Wachter, 2016).

The system of organic farming is not complicated. It is based on common sense: compassionate treatment of animals, using less dangerous methods, e.g. reduced use of chemicals, and natural plant breeding for favorable genetic traits. Food security is dependent on the unity and trust between farmers, farmworkers, suppliers, consumers and other actors in the supply chain. Unity and trust are the basic principle of organic farming system's success (Willer *et al.*, 2020). There are various other agriculture methods that can be used as a role model in farming system, for example, traditional farming systems, growth of crops under sufficient conditions and conservation tillage. Organic agriculture has been shown to be one of the best substitutes of conventional farming systems (Skinner *et al.*, 2014).

There may be some conflict between worldwide harmonization versus local adaptation of farming systems. Some of the challenges faced by organic farming system are also opportunities, for example policies and standards reviews. Defined agronomic demands can also provide pressure to meet standards. Such as under the certification standards, phosphorus fertilizer is allowed to give limited amount of P in the inherently insufficient in South Australia's different parts.

Sometimes the yield of organic farming is not as high or good when compared to traditional farming due of insufficient nutrients, weed issues, non-cash crop rotation phases or management with less experience (Kloos and Renaud, 2014).Reduced pesticide contamination of food is a positive factor of organic agriculture. It is too early to say that organic farming is completely environmentally sustainable. Some negative impact has occurred on certain organic farms due to some soil nutrients and exhaustion of soil (Lynch, 2015). It is important to maintain sustainability in worldwide economy and balance the organic principles with commercial requirements (Adekiya *et al.*, 2019)

8.6 Conclusion

Biological fertilization techniques are strategies used to efficiently manage the agriculture resources with less negative impacts on environment. They may be affecting various things like water assets, biome and ultimately, the human life. Moreover, in different geographic, economic and cultural backgrounds, possibilities of developing a conservative agriculture are also provided by biological fertilization. To limit the inorganic fertilization, proper research is required on finding the possibilities to handle the problems and provide useful opportunities or framework to develop energy-saving practices and enhancement on the productivity. There are many options for biofertilization available. Some techniques are already used in agriculture like animal dung, crop residues, composting, microorganism inoculants and algae extracts but, their application still needs more research, capital and technological expansion to properly determine their effects on soil, plants, livestock and humans as well.

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9 Use of Organic Substrates in Sustainable Horticulture

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Abstract

The optimum selection of potting mix is one of the most important factors for quality potted plant production as it holds up plant roots, maintains the plant growth and allows free water and air movement to the plant root zone. Under field conditions, due to economic limitations, it is challenging and rather impossible to ensure the desired characteristics of the soil, but in container-grown plants it is feasible to provide the potting mix with desired properties for optimum growth. Optimal potting mix characteristics comprise porosity and density of the medium to facilitate good aeration and water-holding capacity; also, optimum pH and cation exchange capacity synchronize nutrient uptake and absorption, enabling vigorous growth of the plants. This chapter discusses the effect of different organic potting media like sphagnum peat moss, peat moss, shredded bark, leaf compost, coir (shredded coconut husk), farmyard manure, rice/peanut hulls, composted cotton gin waste, spent mushroom compost, agricultural by-products, municipal compost, sawdust, etc., on the growth and development of potted plants. Physical and chemical properties of the medium play a vital role in the availability of food and nutrition to plants. Various physical and chemical properties such as texture, particle size, structure, pore spaces, water-holding capacity, pH, cation exchange capacity, N, P and K percentage of various potting media are reported in this chapter. An inventory regarding various organic materials suitable as a growing substrate for container-grown plants is well-elaborated. Also, the effect of different organic potting media on growth and the morphological characteristics of different ornamental plants are described. Utilization of low-cost renewable materials as growing potting mixes is a significant strategy for sustainable potted plant and seedling production. The nutrient availability is critical when using organic materials as a growing potting mix. Generally, the mineralized nitrogen is present in small quantity in various composts because of the immobilization process and nitrogen loss due to leaching. However, management of composting process and utilizing combination ingredients from different sources can facilitate increased availability of mineralized nitrogen and other essential elements required for optimal plant growth.

9.1 Introduction

Potting medium, potting mix or growing substrate, is any medium that supports plant growth and provides nutrients and moisture to the plant during its growing period in a pot or container. This term was first introduced among American agriculturists in 1861. To grow plants in pots or

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containers, soil is not proved a decent growing medium as it gets either too wet to provide sufficient aeration or too dry to offer sufficient water content for optimal plant growth.

For environmental and ecological sustainability, it is crucial to lessen the usage of chemical fertilizers and amendments in agriculture generally and in container-plant production particularly. Also, increased population is resulting in more agriculture and kitchen waste production both at the farmer and municipality levels (Sabiiti, 2011). Many environmental issues of great concern are due to the huge amounts of organic waste that undergoes the decomposition processes that are inherent in nature. The impact of agricultural waste on our environment is not only determined by the amount produced, but also by disposal management. Burning of agricultural waste is a serious threat to our environment due to release of pollutants like carbon monoxide (CO), nitrous oxide (NO), nitrogen dioxide (NO_2) and particulate matter (Ezcurra *et al.*, 2001). These pollutants also result in ozone and nitric acid formation that causes acid rain (Farooq *et al.*, 2020) and create human and ecological health risks. It is convincing for growers to synchronize the use of various agricultural waste after composting as a source of organic fertilizer (Riaz *et al.*, 2015). They have a great amount of organic matter which can be used as a potting medium without any hazard and also it can decrease the volume of the waste, thereby reduce the environmental risk regarding disposal of waste (Altaf *et al.*, 2021). During the last two decades, there is an increasing concern in using different agricultural wastes and by-products as an organic nutrition source for container-grown plants owing to better awareness about the global environment (Grigatti, 2008).

Organic constituents of the growing media have a positive impact on its chemical, physical and biological properties, and after the decomposition of organic matter, nutrients become available to plant roots. The main benefit of using composted waste as a potting medium is its ability to resist against root-borne diseases and also the potential substitute of peat in the planting medium for potted plants (Younis *et al.*, 2008). Over the last few decades, nursery growers have sought out various potentially economical and disease-free media. Different plants have varying requirements for physical support, water and nutrients, and accordingly need growing media to facilitate the best growing environment. Selection of appropriate media components is critical for successful production of potted plants. In this context, the various commercial potting media brands select different constituents on the basis of their properties, performance, ease in availability, plant requirement and sustainability concerns. Commercial potting mixes are typically a blend of varying proportions of various organic components that include: sphagnum peat moss, peat moss, shredded bark, leaf compost, coir (shredded coconut husk), farmyard manure, rice/peanut hulls, composted cotton gin waste, spent mushroom compost, agricultural by-products, municipal compost and sawdust. The appropriate growing medium offers optimal growth conditions that are predictable, consistent and reliable, hence contributing to better quality enabling farmers to fetch a high price in the market.

9.2. Physical and chemical properties of media

It is important to study the physical and chemical properties of the medium, its structure, texture, density, consistency, saturation percentage, organic matter percentage, and the available nitrogen (N), phosphorus (P) and potassium (K) percentage. These physicochemical properties of growing media play a key role in providing physical support, water availability, nutrient uptake, gaseous exchange and optimal plant and root development. However, these physicochemical properties of organic media may change with the passage of time. This change may be associated with oxidization of organic contents, consolidation of media, leaching nutrients, salt accumulation and reduction in aeration due to root expansion (Caron *et al.*, 2015). However, most media are selected based on their original properties before planting. For most container-grown plants, growing medium represents ~10% of the total production cost; hence, it is essential to select the most appropriate and economical potting ingredients with the optimal combination for quality low-cost plants (Carlile *et al.*, 2015).

9.2.1 Physical properties

The physical properties of potting medium are the important factor for better plant growth in pots/containers as these determine the water availability and aeration in the growth medium (Younis *et al*., 2015). It is essential to know the physical properties before selection of growing media for its better management afterwards, specifically water retention characteristics of the medium. Generally, a growing medium consists of solid, liquid and gaseous constituents. The solid constituents usually occupy ~30–60% of the total media volume. The liquid portion contains water and dissolved nutrients, whereas the gaseous part (air) includes oxygen and carbon dioxide. As air is important for plant roots, a potting medium must constitute 20–25% air (by volume) after excessive water drainage (Havis and Hamilton, 1976; Riaz *et al*., 2008). Different growing media have their own characteristic distribution of particle size. The pore spaces between the solid particles can either be filled with water or air, and this reflects the 'total porosity'. In growing mixes, porosity determines the nutrient absorption, air exchange by the root system and water-holding capacity which depends upon the size of pores and varies with the type of constituents of the growing media. The constituents with smaller pore size can hold water tightly compared to constituents with large pores as these cannot hold against gravity. The pores, if positioned higher in the pot/container, will hold less water against gravity and, consequently, the media in the container top portion will remain dry compared to the bottom. Media constituents with large pore size at the bottom of the pot/container will hold more water, keeping the bottom layer of media saturated.

Oxygen is very important for root growth in the media and 10–25% oxygen concentration is required for proper root growth. The reduction in optimum oxygen will result in root damage or retarded root growth (Younis *et al*., 2014). Potting mixes must be formulated to maintain a balance in solid particle and pore spaces. The water-holding capacity or container capacity is 'when the media have been watered to saturate and filled the total pore spaces with water, then it is allowed to drain excessive water by gravitational pull'. Smaller pores will retain water while larger pores will not hold it and will be filled with air to keep a balance between moisture and air (Fig. 9.1). It is important to note that the available water (absorbed by roots) is present in the pore spaces of the medium (Fig. 9.2). Some of the water in the medium is present in the form of a thin film that binds tightly to medium particles

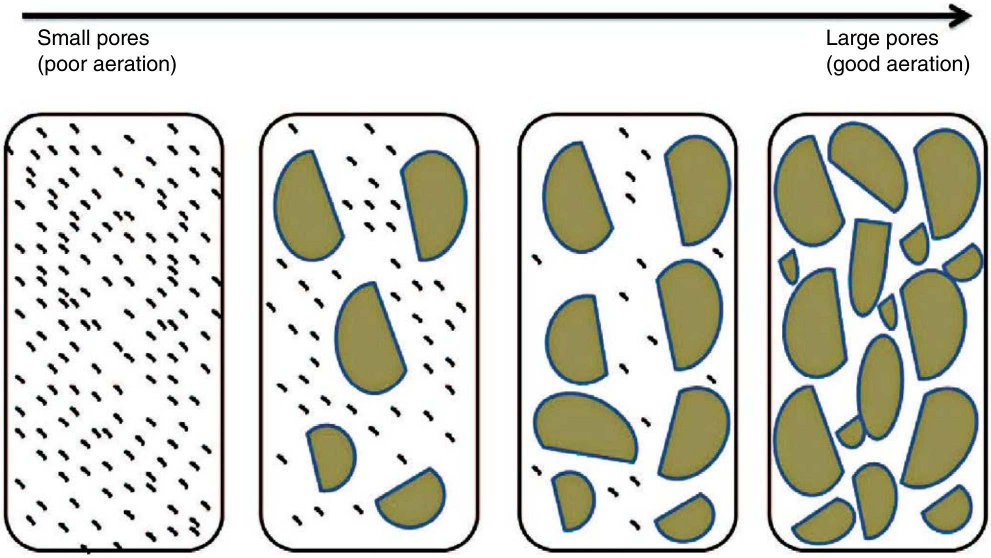

Fig. 9.1. Relationship between media pore size and aeration.

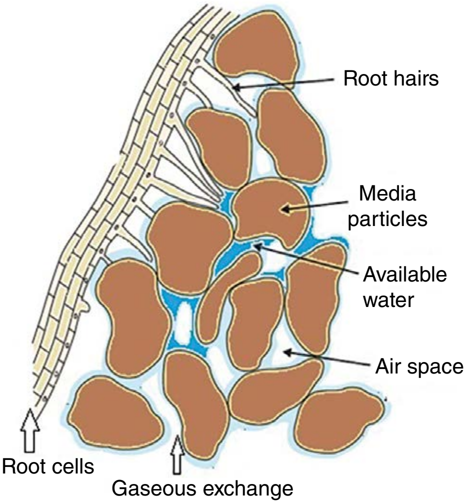

Fig. 9.2. Arrangement of medium particles showing available water to roots and air spaces for gaseous exchange.

and cannot be used by plants – this is called unavailable water.

For quality plant production in pots or containers, it is essential to have a balance in available water and aeration. For the healthy growth of plants, there must be adequate small pore spaces to hold sufficient water for root uptake and sufficient large pore spaces for the exchange of gases in the growing medium to ensure critical oxygen levels. The anaerobic condition does not support the plant roots to get energy from the cellular respiration and such conditions will favor root decay and other diseases. Pore spaces facilitate air pockets within the medium to provide oxygen to convert sugars into energy during the respiration process in root cells and also help in the exchange of carbon dioxide a by-product of respiration which is vital for healthy root growth.

The pore spaces in the media are reliant on the size, shape and distribution of its constituents. Most of the organic media used for container production have pore spaces between 75% and 85%. In potting media, having a greater number of small pore spaces allows increased water retention resulting in the air spaces being filled with water, which may lead to damage/rotting of roots due to water saturation (Younis *et al.*, 2010). However, total porosity and the distribution and size of pores and particles of organic media in containers can alter adversely during plant growth that may lead to restricted gaseous exchange and limited water and nutrient supply to the plant (Caron *et al.*, 2015). Consequently, the plant roots growing in media with poor aeration are weaker, and likely to be more susceptible to root rot pathogens (*Phytophtora* and *Pythium*). Also, plants growing under poor aeration in pots exhibit micronutrient deficiencies more frequently compared with plants growing in well-aerated media.

The volume of the medium can also influence the porosity and aeration. The volume of the medium can decrease because of shrinkage, erosion, compaction, particle degradation and root penetration that will reduce the readily available water and drainable air space. It is suggested to reduce the compaction impact, and media should not be compressed too hard during filling the container.

Bulk density (mass per unit volume) is also one of the important physical properties of any growing medium and is usually expressed in grams per cubic centimeter (g/cc). This property will specify the solid's volume and pore space occupied by the growing medium. A porous, loose growing mix usually has a lesser bulk density compared to heavy, compacted growing media (Yasmeen *et al.*, 2012). Generally, with the increase in bulk density there will be a reduction in total pore space. A higher bulk density of media is desirable if plants are grown in outdoor conditions to avoid wind damage, but media with lower bulk density are good for indoor settings to ease in handling and to support the plant. To adjust the medium bulk density according to plant requirements, organic matter such as peat, manure or compost can be added. Figure 9.3 represents the relationship trend between potting mix organic carbon and bulk density.

9.2.2 Chemical properties

Chemical characteristics of a medium are also important as these will determine the nutrient availability to plants. Among the chemical properties, pH is a significant factor that affects plant

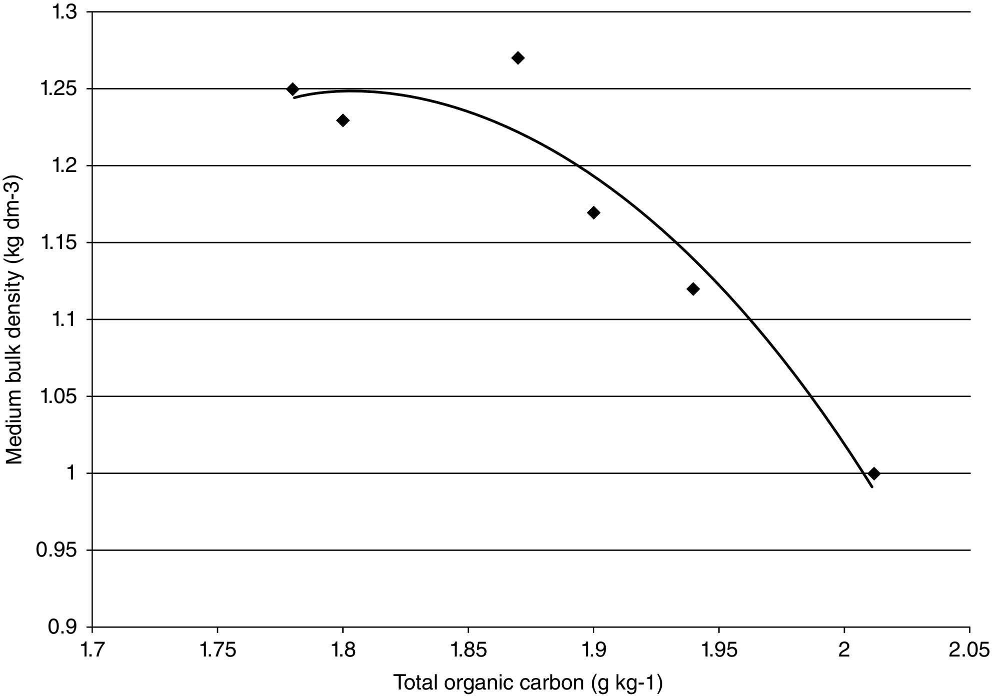

Fig. 9.3. Relationship between media total organic carbon content and bulk density.

growth in containers. Various media constituents, irrigation water and fertilizers can alter the pH of the media. The optimum pH will regulate the nutrient availability to plants either in soil or in pots (Younis *et al.*, 2013a, 2013b). An increase or decrease in pH can restrict the availability of some nutrients and plants show retarded growth due to deficiency of some nutrients. Optimal micronutrient and macronutrient availability occurs at pH 4.5–6.5. Above or below this pH range, nutrient availability to plants becomes constrained due to changes in nutrient chemical properties and plant growth lessens and starts showing deficiency symptoms (Younis *et al.*, 2013c).

Cation exchange capacity (CEC) is another important chemical property for selection of optimum growing substrate for potted plants as it regulates the nutrient-holding capacity of the growing substrate. CEC is ‘the total sum of exchangeable cations, or positively charged ions, that a growing medium can adsorb per unit weight or volume’ and is expressed in milli-equivalents per 100 cubic centimeter (milliequ/100 cc) or gram (milliequ/100 g). The media with high CEC can hold more nutrients even after irrigation compared to media with low CEC. The organic potting mixes such as peat, manures or compost, have a high CEC that will ultimately increase nutrient-holding capacity of the mix. However, sometimes a potting mix that holds too many nutrients can result in salt accumulation at the top of the medium. A good mixture of different components will subsidize the salt accumulation (Tariq *et al.*, 2012). The macronutrient cations that the media can retain on its exchange sites are ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), sodium (Na^+) and magnesium (Mg^{2+}), and micronutrients such as zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) (Fig. 9.4). The amount of all these cations is limited into the specific container volume in the media. To avoid the buildup of these minerals in the media (soluble salt concentration) monitoring is necessary. The increase soluble salt concentration can restrict plant roots to absorb water, due to greater cations gradient concentration in the media (Riaz *et al.*, 2014).

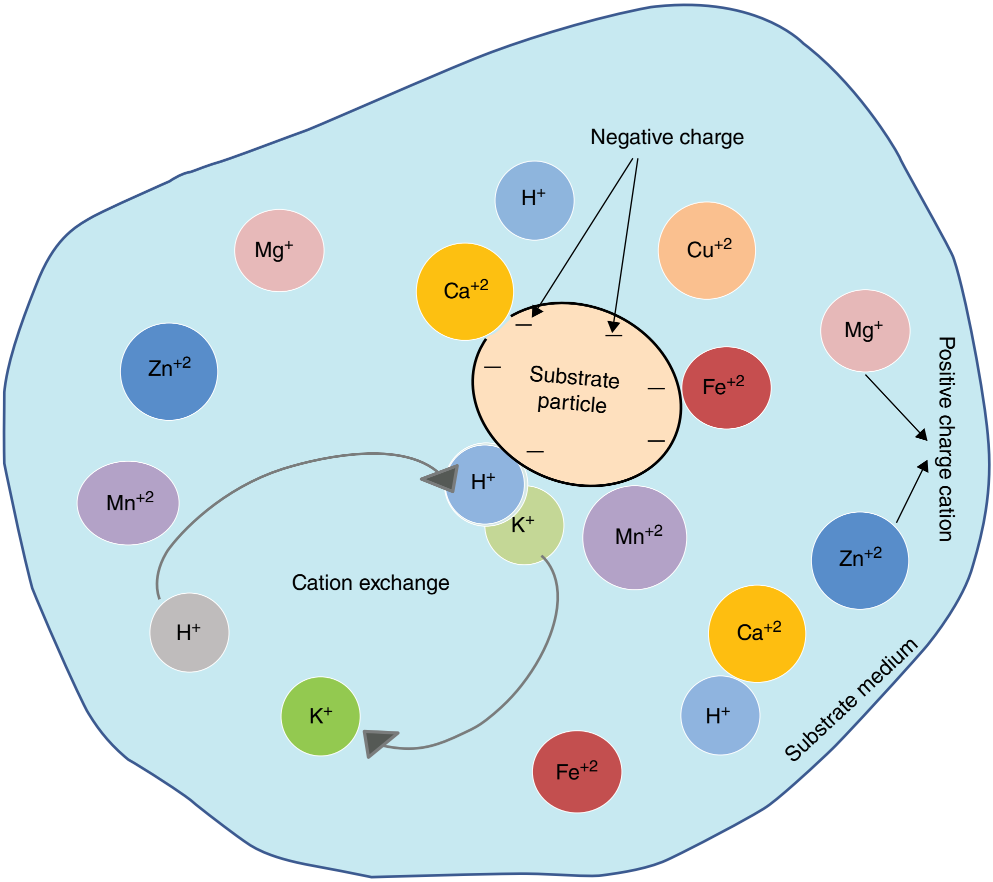

Fig. 9.4. A schematic diagram of cation exchange.

The carbon to nitrogen ratio (C:N) is the ratio of the mass of carbon (C) to the mass of nitrogen (N) in a medium. A high C:N ratio is undesirable as it reveals a trend for the media for quick decomposition and a subsequent decrease in volume and aeration of the media. All organic media contain substantial amount of carbon pooled with a reduced amount of nitrogen. Some media such as sawdust and wood shavings have a high C:N ratio (350:1 and 300:1, respectively). These media exhibit significant decomposition in pots, which may cause the deterioration in physical properties of the media and nitrogen draft, as microorganisms involved in composting can utilize the nitrogen. This can cause leaf yellowing and stunted plant growth. The C:N ratio of some selected organic growing substrates are presented in Table 9.1.

9.3 Organic components of growing media

Organic growing media are usually tailored from a blend of raw organic matter from varying sources to get the optimal balance of physicochemical and biological properties for container-grown plants. The selection and composition of organic materials, as well as the composting process, may change the nutritional status and structural quality of potting media (Dresbell, 2004). Organic waste/residues derived from agriculture and livestock farming, green spaces and forests, kitchen and domestic waste, municipal and industrial residues are excellent for utilization as recycled sources in container-grown plants (Younis *et al*., 2008, 2011). Also, for a certified organic grower, it is obligatory that

Table 9.1. Carbon to nitrogen ratio (C:N) of some selected organic growing substrate.

Substrate	C:N	Substrate	C:N
Composted kitchen waste	30:1	Pruning waste compost	50:1
Wood ashes	30:1	Pine leaves	70:1
Sawdust	350:1	Wood shaving	325:1
Wheat straw	70:1	Peanut shells	40:1
Green manure crops	10–25:1	Seaweed	20:1

potting media should be derived from organic sources and should fulfill the plants' needs for water, nutrients and aeration. It is reported by Bunt (1988) that organic growing media use in container-grown plants has raised the production capacity and quality due to improvements in water, gaseous exchange and nutrient management; there is also better control of soil-borne diseases (Caron *et al*., 2015).

Humus is the black colored organic matter produced in the soil top layer after natural decomposition of plant and animal material that contains many valuable nutrients (Fig. 9.5A). In formulation of organic potting media, management of nitrogen (N) source is a key factor as the N in organic matter is present in a more complex form and needs to be mineralized before its availability to plants. The nitrogen contents in the organic potting media stimulate the soil microbes' activity, which in turn mineralize the nitrogen source and make it in available form for absorption to the plant roots over time. The combination of different ingredients with complementary physicochemical properties are highly recommended to formulate superior quality potting media. Individual potting media components are discussed in detail in this chapter.

9.3.1 Peat

Peat is the most common and extensively used component of potting media for growing plants (Fig. 9.5B). Peat is a heterogeneous organic mixture obtained from decomposition of plant material located in bogs that comprise mosses, reeds and shrubs. Peat bogs are located throughout the world, but mainly in Canada, USA, Russia, UK, Finland and Ireland.

Peat is reliable for growing a wide range of container plants as it possesses all the properties that commercial growers require in a growing medium. Peat-based substrates are stable and consistent in physicochemical and biological properties, making them ideal for horticulture and other container plants. Peat possesses an optimum pH and nutrient status and ideal nutrient buffering with no hazardous materials (Table 9.2). Among the physical properties, peat has a high structural stability and wettability with optimal aeration and water-holding capacity ensuring quality in line with plant requirements. Among the biological properties, it is free of weed seeds and pathogens with uniform constancy attributes, which together offer favorable conditions for plant growth. These outstanding properties of peat are revealed by its top ranking among various growing mixes in terms of the quantity used.

Despite the proven advantages and increasing demand of peat for container-plant production, public and environmental regulatory concerns about the protection of non-renewable peat resources due to its intensive exploitation necessitate the need to look for substitutes for the growing mix. Various waste materials have the potential to be an alternative to peat for sustainable container-plant production. Also, a continued curiosity exists in using different agricultural, livestock and domestic waste as an organic potting mix for container plants due to concerns regarding the increase amount of waste produced (Riaz *et al*., 2015). New potting components are now sourced from agriculture waste, kitchen composts and manures of different origin, as well as coconut coir, wood shaving and pruning waste. For commercial media, it is mandatory that these components should be derived from quality stock and must possess the characteristics such as being lightweight, inexpensive, easy availability, free from weeds and pathogens, eco-friendly, and consistent and stable in its physicochemical properties.

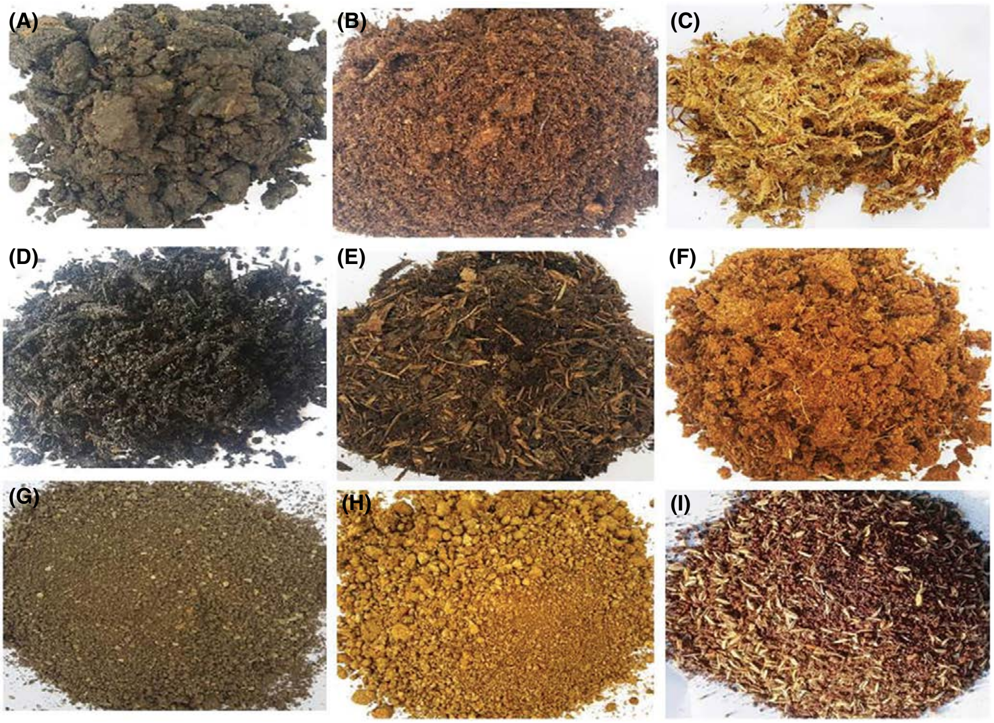

Fig. 9.5. Various organic potting mixes: **(A)** humus**; (B)** peat**; (C)** sphagnum moss; **(D) compost; (E)** leaf mold/compost; **(F)** coir; **(G)** vermicompost; **(H)** poultry manure; **(I)** rice hulls**.**

9.3.2 Sphagnum moss

Sphagnum moss (Fig. 9.5C) is a popular component used for making soilless substrates due to its characteristics like excellent air circulation, having 70–80% fiber, absorption ~20 times more water than its weight, low bulk density, higher surface charge density, low decomposition rate and reduced leaching of nutrients. The length of individual moss strands, and length and thickness of fibers, can determine the absorption and sponginess of the medium. The porosity of sphagnum moss ranges from 15–25%, which is comparable to bark as a medium and greater than peat moss. This high air-filled porosity favors epiphytic orchids' growth as it facilitates air circulation around the roots. It has a high CEC and can hold positively charged nutrients such as potassium, calcium, ammonium and magnesium for later use rather than leached them out (Table 9.2).

Sphagnum moss is a versatile potting mix used for growing of *Cymbidium*, *Dendrobium*, *Phalaenopsis* bonsai plants, and so on. Sphagnum moss grows in swamps or bogs that are distributed in the northern hemisphere and sub-alpine zone of Australia and New Zealand. *Sphagnum cristatum* is the most popular species in New Zealand used for this purpose. This species is considered best for epiphytic orchid growing due to its premium quality, as it has soft, clean, long fibers to hold sufficient moisture without staying too wet and provides good air circulation around cut surfaces when using the layering method to prevent rotting and encourage rooting. In 1994, Hand Picked called sphagnum moss 'green gold' due to its attribute of sustainability as it continues to regrow and reproduce for many years with proper care and management (Picked, 1994).

Sphagnum moss has phenolic compounds in its cell walls that avoid it to decay or decompose. Generally, the sphagnum cells absorb selected mineral ions from water and release hydrogen ions in the surrounding water and this can acidify its surroundings; therefore, it is recommended that this medium should not be used for growing plants that like alkaline

Table 9.2. Comparison of some characteristics of organic growing mixes.

	Peat	Sphagnum moss	Coir	Compost	Sawdust	Vermicompost	Leaf compost	Bark compost	Spent mushroom compost	Rice hulls	Cotton gin trash	Animal manure	Poultry manure
pH	4.5–5.5	4–5	5–6.5	6–7.5	4–5.5	6–6.5	6–7	5–6.5	7–8	6–6.5	5.5–6.5	6.5–7.5	6.5–7.5
N (%)	1–2	2	1–1.5	1.5–2.5	2–2.5	1.5–2	0.5–1	0.5–1	0.3–0.5	0.5–0.8	1–1.6	0.5–1.5	2–2.5
P (%)	0.04–0.5	0.5–1	1	0.2–0.5	2	1–1.5	0.2–0.5	0.2–0.5	0.1–0.3	0.3–0.5	0.1–0.3	0.2–1	1.2–1.5
K (%)	0.1–0.5	0.5–1	1.2	0.2–0.5	0.5–0.8	0.5	0.5–1.5	0.5–1.4	0.5–1	0.2–0.5	0.5–0.8	0.5–1.5	0.5–1
Bulk density	Low	Very low	Low	Moderate	Low	Moderate	Moderate-high	Moderate	Moderate low	Low	Moderate	High	High
Porosity: water	High	Very high	High	Low–moderate	High	Moderate	Moderate	High	Moderate	High	Moderate	Low–moderate	Low–moderate
Porosity: air	High	Very high	High	Moderate	Moderate	Moderate	Moderate	Very high	Moderate	High	Low	Moderate	Moderate

conditions. This acidic nature of the medium and high zinc content assists in creating a root zone environment hostile to harmful fungi and bacteria, which helps to protect the roots from root diseases. If the irrigation water contains salts, sphagnum moss will retain those salts which are detrimental to plants.

9.3.3 Compost

Compost can be used as an alternative to peat moss in potting mixes on a fairly large scale as an independent medium or mixed with other materials for making inexpensive and quality potting media. Compost can be prepared by using kitchen waste or garden waste by adopting a series of steps in a process termed composting (Fig. 9.5D). The composting process is an anaerobic thermophilic treatment carried out mainly in the presence of microbes. The microbial activity during composting is mainly reliant on the temperature, pH and moisture content. The nutrient availability is very important when plant-based compost is used as a potting medium. The main benefit of compost is to provide mechanical (or physical) support to plants and essential nutrients for plant growth. The nutritional status and properties are dependent on the material used for composting. Microbes present in the compost also convert unavailable forms of nutrients to available forms. Generally, mineralized nitrogen in compost is present only in minute quantities due to nitrogen losses as a result of leaching and the immobilization process. The other disadvantages associated with this medium include: it is not uniform in appearance; the particle size varies greatly and it may be too large or very small in size; it gets very saturated after watering; it has insufficient air spaces; it may carry different root disease pathogens; the presence of weed seeds; and the presence of toxic heavy metals, if derived from industrial waste materials. Therefore, management of the composting process is necessary in order to get more efficient and quality compost as a growing medium.

9.3.4 Leaf mold/leaf compost

Leaf mold/compost has an earthy, dark brown texture, comprising decaying leaves, pruning waste, grass clippings, wood waste, fallen branches as common components that are digested by oxygen-loving microorganisms (Fig. 9.5E). This proves to be a valuable soil conditioner. The leaf compost takes a longer time for its preparation due to slow decomposition of leaves. The amount of lignin, nitrogen and calcium in the leaves are directly associated with the time required for leaf breakdown and decomposition. The leaf compost is rich in carbon contents, with a C:N ratio ranging from 20–100. Leaf compost contains nearly all the essential nutrients required for good plant growth and provides an excellent sphagnum peat moss substitute, which has lower amount of nutrients.

9.3.5 Coir

Coir is the natural fiber derived from the coconut husk and processed to make fibrous material for potential use as a growing potting mix (Fig. 9.5F). The coco fibers appear to have potential use as a substrate of peat moss due to many common qualities. Coir is a renewable resource that is stable and consistent in quality and contributes to the environmental sustainability, which makes it a better choice over other media. Coir's major characteristics include: high water-holding capacity (can hold water up to ten times its weight); ideal pH (5–6.5); good drainage; excellent aeration; the ability to re-hydrated easily; biodegradable (requires 4–5 years); low in electrical conductivity (EC), high CEC; possess antifungal properties; inhibits soil-borne diseases (*Pythium*); and contains a significant amount of phosphorus and potassium. Furthermore, it has lignin (a natural polymer) in its composition, which encourages growth of bacteria for better plant growth in potting mix (Table 9.2).

Coir is mainly produced in India, Sri Lanka and the Philippines. The use of coir as a growing potting medium started in the late 1980s, and soon received appreciation and became commercially important in the early 1990s. Since then, its consumption has increased to include the growing of house plants, annual flowering plants, vegetable seedlings and fruit transplants. Also, due to its excellent wettability property, it is often mixed with other constituents and used for sowing, propagating and potting of flowers and vegetables.

Coir sometimes needs to be treated with calcium nitrate buffer solution to neutralize sodium. However, more focused research is required to establish a comprehensive set of standards to confirm the physical and chemical characteristics of the coir. Although coir has no significant nutrient value, it can be employed as a media amendment to lighten the potting mix, increase water-holding capacity and improve the media texture and structure. The research trials on coir indicated that this is a variable ingredient and to formulate a uniform, consistent and stable substrate, it is desirable to test each batch of coir before blending the substrate (Lowry, 2015). Likewise, comprehensive information regarding particle size and grade fractions needs to be established on medium aeration, water-holding characteristics, and the ratio of coir mixing with other media components for best growth of plants in containers.

9.3.6 Vermicompost

Vermicompost is the earthworm processed organic waste that is produced after accelerated bio-oxidation of organic matter (Singh, 2014). This medium has fine peat-like pellets with high aeration, drainage, porosity and water-holding capacity (Fig. 9.5G). The most common earthworm species used for vermicomposting are *Eisenia foetida*, *E. veneta* and *Lumbricus rubellus*. These worms consume various types of organic materials and 1 kg of worms can eat 1 kg of residue each day. The excreta (castings) of the earthworms are rich in nitrate and other water-soluble nutrients.

There are different factors that may affect the worms' efficiency in producing vermicompost. The key factor is the adequate moisture supply (60–70%) for efficient worm activity. High humidity may cause anaerobic conditions that can be fatal to worms. Aeration is important for increase activities of the worms as improper aeration may cause suffocation. Temperature is also an important factor as it greatly affects the earthworms' activities, growth, metabolism and reproduction. Vermicompost appears to be superior compared to other conventionally produced composts as it is produced under moderate temperature range, i.e., 10–35°C and has different microbial communities (Singh, 2014). This is considered as a stable form of organic matter because of its more humified nature and there is a reduced possibility of tying up nutrients with a minimum chance of salt accumulation in pots compared to thermogenic composts. It is valued as a potting mix due to its excellent characteristics that include: a diverse community of microorganisms; good water-holding capacity, it retains important nutrients such as N, K, P, Ca, Mg; it contains a considerable amount of humic acid and plant growth hormones; and it has the ability to resist diseases and different pests. Overall, it has proved to be an efficient nutrient-rich media additive for improved seed germination and vigorous seedling growth and development. Also, vermicomposting is an environmentally friendly process and has a great potential as a component of sustainable agriculture.

9.3.7 Animal manure (farmyard manure)

Animal manure is a common type of organic amendment for organic potting media. It is biodegradable, renewable and eco-friendly, and also has no risk of toxic chemicals. Manure contains well-decomposed valuable organic fertilizer used in potting mix, as it contributes to the potting medium fertility with the addition of plant nutrients and organic matter. Organic manures perform as a mixed fertilizer and provide almost all the major nutrients (N [0.5%], P [0.25%], K [0.4%], Mg [0.05%], S [0.02%] and Ca [0.8%]) as well as micronutrients (Fe [0.45%], Cu [0.0005%], Zn [0.005%], and Mn [0.007%]) essential for optimal plant growth. Manure also improves soil physicochemical and biological properties as well as soil structure, thus providing a better root environment. Organic manures also help in maintaining a suitable C:N ratio that facilitates the microbial population and their activities. The manure is a complex mixture of many chemical compounds and act as precursors of soil humus. The addition of manure in potting mix leads to its decomposition and conversion into carbon dioxide, nitrogen, sulfur oxide and retains the carbon, nitrogen and sulfur levels in container-grown plants.

Selecting well-decomposed and cured manure is critical for use in potting media as undecomposed or partially decomposed manure has adverse effects on plant growth and development. If the manure is not fully decomposed, then the heat produced and strong biological activities during decomposition may harm the plant roots. Also, partially decomposed manure has inconsistent results when using in organic potting mixes as its performance can change with handling and management. Proper management during composting ensures optimum physical and chemical properties and appropriate stability and maturity (Benito *et al.*, 2003). Also, the nutrients in manure can substitute substantial amounts of mineral fertilizers, if used appropriately, and the organic matter can increase the fertility status.

9.3.8 Poultry manure

Poultry manure is a valuable organic fertilizer that is not only a potential source of N, P and K, but it can also increase the organic matter of the soil to improve texture, structure, aeration and water-holding capacity of the growing substrates. Unprocessed poultry manure is more concentrated compared to farmyard manures, and it comprises nearly 50% water. Raw poultry manure is recommended in potting mixes for container-growing plants as it does not go through any sort of heat treatment to overcome the chances of undesirable pathogens, bacteria, weed seeds and unstable nitrogen availability.

Processed poultry manure has excellent value as it contains more macro and micro plant nutrients compared to other manures (Fig. 9.5H). The availability of nutrients is mostly fairly consistent, with the exception of nitrogen. Nitrogen is present in various forms – the predominant form in poultry manure is uric acid, which can readily convert to free gaseous NH_3 (ammonia). However, when poultry manure is thoroughly mixed with organic matter, the NH_3 (ammonia) converts to NH_4^+ (ammonium), which has the ability to hold on to organic matter. Therefore, mixing with other organic material can minimize the nitrogen loss and more nitrogen will be available to plants. But care should be taken while selecting this manure as potting mix, particularly for seed germination and seedling growth, as excessive nitrogen may be disastrous. The best recommendation is to use in small amounts in combination with other potting mix to gain the best results from it.

9.3.9 Sawdust

For the last decade, sawdust has been extensively used in potting mix for commercial potted plant production. Sawdust is a by-product of cutting, piercing, grinding or pulverizing wood with a saw or other tool, mainly comprising fine wood particles. This is a very cheap material with high moisture retention and is available almost all year round. Usually, it is used in combination with other constituents to formulate a good-quality potting mix. Poole and co-workers suggested that in potting mix, sawdust proportion should not exceed 20% for optimal plant growth. The sawdust quality used as a potting mix also depends on the plant wood species. The larch sawdust is excellent for mushroom species *Sparassis crispa*, and its highest mycelial growth was recorded in this medium (Lee at al., 2004). Sawdust produced from cedar, redwood and walnut is toxic to plants, whereas oak and maple sawdust may tie-up soil nitrogen more quickly than the evergreen trees. In sawdust, the lignin content is lower but the C:N ratio is greater when the bark is used as a potting mix. Sawdust is heavier than peat and holds slightly more water. Sawdust proved to be an excellent peat substitute for growing seedlings, foliage plants and flowering plants (Worrall, 1981). As potting mix, it undergoes a gradual decomposition which is undesirable as a substrate because its physical properties convert from dry to wet condition with poor aeration. The use of sawdust in the growing potting mix without proper composting is not recommended because it undergoes rapid breakdown in the medium, and also may cause phytotoxicity. However, this problem can be overcome by composting wood residues before using in a potting mix.

Although sawdust proved to very effective for growing of plants in pots, this medium has some disadvantages. For example, it is non-renewable and has an uneven, unstable and inconsistent quality; it can be over-saturated with water, causing little air-filled spaces for oxygen supply to roots; and it has a higher C:N ratio that reduces the chances of nitrogen availability to plants and may carry salts that can cause phytotoxicity.

9.3.10 Bark compost

Bark is the outer protective sheath of the trunk, twigs and branches of trees or woody shrubs. It is often used as a potting mix to increase the air porosity, lighten the mix and to impart disease resistance. Pine bark in potting mix resists water loss through evaporation (São José *et al*., 2021). Composted bark has a high lignin content with slow degradation capacity, a pH range 5.0–6.5, low soluble salt content, a higher CEC and low bulk density (van den Berg *et al*., 2018). It is low in fertility and requires more nitrogen application as it is deficient in N content (Hayden, 2005). Deficiency of nitrogen may be attributed to decomposition of large amounts of degradable carbon present in cellulose, or due to the nitrogen fixation physically or chemically within the bark particles. Its application as potting mix can sometimes contribute to toxicity due to the presence of organic or inorganic toxins, e.g. phenols and manganese or volatile monoterpenes. Bark-based potting mixes are excellent for flower nursery seedling production, greenhouse tomatoes, chrysanthemum, orchid, potted plants and woody ornamentals.

Generally, its use in potting mix is to improve the aeration or to replace peat due to high cost. Bark is a variable material naturally and it is not recommended to use it fresh, but it can exhibit good results if utilized efficiently and wisely in potting media. The tree species the bark is sourced from, the type of wood (softwood or hardwood) and age of tree can affect the efficiency of bark as potting mix. The characteristics of bark obtained from softwood and hardwood are greatly divergent and must be used after careful consideration, otherwise its effects may be devastating. Important softwood plants for bark production are *Pinus ponderosa*, *Pinus pinea*, *Pinus radiate*, *Pinus pinaster*, *Pinus sylvestris*, *Pseudotsuga menziesii*, *Picea* spp., *Sequoia sempervirens*, *Cypressus macrocarpa* and *Larix* spp., whereas, hardwood trees important for bark production are *Fagus* spp., *Quercus* spp. and *Betula* spp.

9.3.11 Spent mushroom compost

Spent mushroom compost (SMC) is the composted leftover organic material of mushroom farming. This compost contains a blend of various natural products such as poultry manure, horse-bedded straw, wheat straw, hay, cottonseed hulls, rice hulls, ground corn cobs and other materials (Stamets, 2000). This compost has a pH range of 7–8, high salt content, high phosphorus and potassium content, low to moderate nitrogen content (1.0–1.5%), high levels of calcium (C:N ratio 13:1) and high organic matter contents (26%) which makes it a significant candidate for potting media (Fidanza *et al.*, 2010). Generally, in the fresh form this compost looks like partially decomposed material with reduced water-holding capacity. Its application in containers, however, resulted in further rapid decomposition and shrinkage followed by an increase in water retention but reduced porosity. It is strongly suggested that this material should be composted for at least 6–9 months before using as a potting mix. SMC with 20–30% moisture contents are ideal for potting mix.

Spent mushroom compost has many advantages: it improves soil/media texture and structure, provides essential plant nutrients (P, K and minor elements), increases plant nutrient mobility and availability, increases soil aeration, increases soil microbial activities, higher CEC, improves root growth and development, improves water retention and reduces compaction. Mushroom compost proved to be a viable, recycled agricultural product valuable as a natural organic fertilizer, and can be potentially incorporated into plant growth media (Fidanza *et al.*, 2010).

9.3.12 Rice hulls

Rice hulls (or rice husks) are thin, hard, protective coatings of rice grains (Fig. 9.5I). It has also become a common ingredient of soilless growing substrates as it is light in weight, very cheap, carbonaceous, renewable, and helps to improve drainage, aeration and water retention. Fresh rice hulls should be avoided as container substrate or potting mix because it contains high cutin, lignin and insoluble silica contents that can make it very slow to decompose, and there is also a chance of weed seeds being present. Before using as a potting mix, it must be composted or treated. After the milling process, rice hulls are subjected to a steaming and drying process to produce parboiled rice hulls, which is free from viable weed or rice seeds. The pH of parboiled rice hulls ranges from 6.0 to 6.5 with nearly 30% porosity. This potting mix appears to be a good buffering agent in potting substrates for growing short-term crops like annual flowers and vegetables. Substrates containing rice hulls as potting mix are less susceptible to fungus gnats. Rice hulls in pots may cause rapid drying of the surface layer even though there is ample moisture in the layers below the surface; care should therefore be taken to avoid over-watering.

Rice hull substrate is considered to be a suitable perlite alternative for container production in greenhouse growing. Terra *et al.* (2011) reported that rice hulls could be used as a potential sole potting mix or part of a media for growing of potted chrysanthemums. Another research project showed that rice hull ash has the potential to be used as a potting mix for bedding plant production if added in 50% or less (Tatum and Winter, 1997). Management of substrate pH must be carefully observed to ensure there is no excessive tie-up of nutrients.

9.3.13 Cotton Gin Trash

Cotton gin trash is a natural material that can be composted and turned into a rich source of nutrients. It has high nitrogen levels and also contains low to moderate levels of P and K. The high nitrogen contents are particularly valuable, as it is one of the primary nutrients for plant growth and development. Composted cotton gin trash is an excellent potting amendment when assorted with other composted organic materials. It has the potential to increase the water and nutrient-holding properties of substrate. It has a pH range from 5.5 to 6.5. Although, high soluble salts can be a problem, this can be lowered with an ample supply of water. The addition of this in other organic potting mixes can increase the pH, EC and water-holding capacity of the substrate.

Generally, this proved a valuable candidate for growing of various potted plants either used solely or mixed with other potting mixes. As a potting mix it has medium to high CEC, high porosity, medium water-holding capacity and high organic nitrogen content that is preserved during the composting and slowly released after maturity (Rizfotiou *et al.*, 2001). Cotton gin trash has the ability to produce vigorous root growth for container-grown plants and this can be further explored to achieve quality plant production. However, more research needs to be conducted to turn composted cotton gin trash into the appropriate commercial substrate for the production of potted plants.

9.4 Conclusion and future prospects

Utilization of low-cost renewable materials as growing potting mixes is a significant strategy for sustainable potted plant and nursery production. The nutrient availability is critical while using organic materials as a growing potting mix. Generally, the mineralized nitrogen is present in small quantity in various compost types because of the immobilization process and nitrogen loss due to leaching. However, management of composting process and utilizing combination ingredients from different sources can facilitate getting more mineralized nitrogen and other essential elements required for optimal plant growth.

Various organic materials to be used as a growing mix should be selected judiciously to ensure sufficient nutrients supply, its stability and suitable physiochemical properties. The physical, chemical and biological properties are greatly influenced by the proportions of the individual components, and the selection of suitable potting mix will have a great impact on potted plant production. It is important to note that grower success, production cost, profit, techniques and consumer acceptability will be greatly affected by modifications in substrate formulations. The medium stability is also important for potted plants and it is primarily dependent on the material selection. When using organic materials as a growing substrate for potted plants, it is very critical as a decrease in volume can occur due to decomposition and degradation of organic matter. The structure of the organic matter after the composting process can disturb the medium volume, as the distribution of particles affects the way the substrate is compressed. Effective potted plant management involves continuous focus and monitoring to understand the plant's requirement as it relates to moisture, aeration, nutrition and plant physical support that are directly interrelated with particular substrate blending and environmental conditions.

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10 Biostimulants: An Alternative to Chemical Pesticides for Crop Protection

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Abstract
Due to the increased use of chemical pesticides and their adverse effect on the environment, the requirement for biologically originated pesticide has also increased with time. This requirement led to the emergence of biostimulants as pesticide. Different biostimulants such as humic substances, N-containing compounds, seaweed extract, biopolymers, bacteria and fungi are positively influencing the plant growth and abiotic stress resistance. Some of the biostimulants can also be used as a pesticide due to their antipathogenic property or favorable impact toward the plants. Different types of products (e.g. pyrethrum, azadirachtin, rotenone, ryanodine) are extracted from plants and these products can be used as an effective substitute to chemical pesticides and also do not harm the physical and the morphological nature of the crop plant. These substances are sourced from fungi and rhizospheric bacteria and are used as pesticides because they are easily available. They do not poison the habitat of plants and are not harmful to animals when compared to chemical pesticides. This chapter gives a detailed account of how the biostimulants can successfully be substituted for chemical pesticides in the crop fields.

10.1 Introduction

Pesticides are in continuous in use to control the various kinds of diseases, but in agricultural practices, increased use of chemical pesticides can create resistance in crop varieties and lead to environmental hazards. These adverse effects generate the need to develop pest management practices and formation of environmentally safer compounds to manage agriculture diseases and combat adverse impacts on the climate (Forget, 1993). Our natural surrounding is continuously facing issues with the increment in agriculture sector industrialization. Pesticides not only cause environmental issues, but they also affect animal health; in other words, they harm the whole ecosystem (Nicolopoulou-Stamati *et al.*, 2016). In many parts of the world, different approaches are used to develop biological control against plant diseases (Pilkington *et al.*, 2010). China has the largest area of greenhouse vegetation comparatively, and they effectively use biological control to manage disease rather than chemical pesticides (Yang *et al.*, 2014). Due to the rising concerns to the environment, chemical pest strategies are continuously being shifted more toward the biological control (Khachatourians, 1986).

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This biological approach is known as biostimulants. These substances are known to stimulate plant growth and their abiotic resistance. They also reduce the need or dependency of crop plant for chemical fertilizers (Du Jardin, 2015; Xu and Geelen, 2018). Biostimulants can be used as an effective alternative against different types of plant diseases (Sultana *et al.*, 2012). Bio-based pesticides provide a number of benefits: they are biodegradable in nature, less harmful and also less applications are required (Gupta and Dikshit, 2010).

10.2 What are biostimulants?

Biostimulants are organic substances or microorganisms that are applied to plants to stimulate growth. Over the past decades the interest in plant biostimulants has increased due their ability to enhance the crop quality by increasing their nutrient content and make the plants more tolerant to different stress conditions (Du Jardin, 2015; Van Oosten *et al.*, 2017). Biostimulants can also be considered to be biological substances or products of bacteria, fungi, seaweeds, higher plants, etc. that contribute to increasing plant productivity. The wide range of bioactive compounds found in biostimulants are still unknown. They do not have adverse effects on plant yield and quality and they can reduce the use of fertilizers. Biostimulants are also proven to increase chlorophyll and carotenoids in the leaf, stimulate root growth and enhance the antioxidant potential in plants (Bulgari*et al.*,, 2014). Different biostimulant substances such as humic substances, amino acids, peptides and microbial inoculants (PGPR, arbuscular mycorrhizal fungi and *Trichoderma* spp.) can improve the physiological activity of the plant without the need for phytohormonal activation – this action of biostimulants is known as ‘biostimulation’ (Tagliavini and Kubiskin, 2006; De Pascale *et al.*, 2017). In agriculture, biostimulants are used as bioactive products and also categorized as ‘biopesticides’ or ‘biofertilizers’ due to their beneficial properties similar to pesticides and fertilizers (Halpern *et al.*, 2015; Ertani *et al.*, 2012).

Nowadays, fertilizers and pesticides are continuously used to boost crop production without concern about their negative impact and environmental harm (Chakrabarty *et al.*, 2014; Gupta *et al.*, 2021). The continuous use of chemical fertilizers can cause eutrophication and also lead to the reduction of organic matter and microbiotic activity in the soil (Sahoo *et al.*, 2012). It has become necessary to practice eco-friendly agriculture to overcome their harmful impact on the environment (Mishra, 2013). Biostimulants are natural substances that have favorable impact on the plants. They are environmentally friendly substances, and their physiological effects depend upon their composition (Parad¯ikovic´ *et al.*, 2019). Biostimulants have been shown to be useful substances in forestry and agriculture practice, and provide a wide range of opportunities for farmers, including aiding the crops to deal with environmental stress. Abiotic stress is a big concern; there are various management practices available to farmers, including using genetic manipulation to improvement in stress tolerance in crops. However, these methods are time consuming and species specific. Biostimulants have become a new agronomic tool to overcome abiotic stress issues (Bulgari *et al.*, 2014, 2019). Biostimulants are used as plant growth stimulators in both optimal as well as in stressful conditions. They can lower the nitrate accumulation in leafy vegetables and also stimulate the secondary metabolism, which is required in stress conditions. These can be beneficial for human health as well. Biostimulant application on plants increases the plant efficiency to cope with injuries and adverse effects of herbicides (Panfili *et al.*, 2019). They can influence reactive oxygen species (ROS) production in both normal or stressed conditions, which impacts on photosynthesis and photosynthetic pigment (Wozniak *et al.*, 2020). Alves and colleagues proved in their experiment that biostimulants associated with micronutrients shows favorable impact on *Acacia mangium* seedlings (Alves *et al.*, 2019). The application of plant biostimulants improves organic horticulture (De Pascale *et al.*, 2017). It is expected that biostimulants can also contribute in the discovery of novel biological molecules, pathways and processes (Yakhin *et al.*, 2017). Nowadays, biostimulant-coated products are available for increasing fertilizer efficiency (McGehan, 2020). Figure 10.1 represents of the beneficial aspects of biostimulants use.

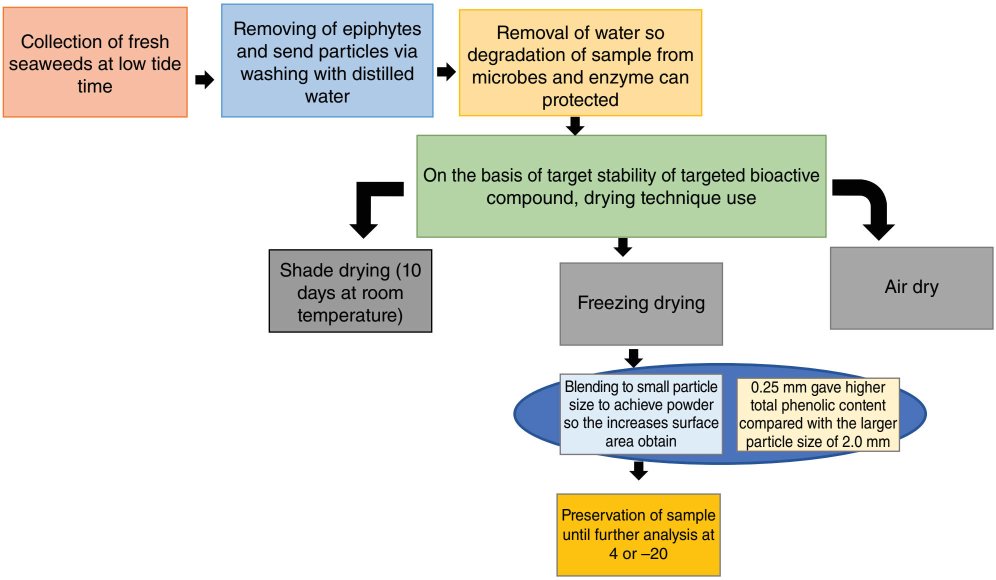

Fig. 10.1. Representation of diverse beneficial aspect of biostimulants is used in agriculture.

10.3 Categorization of biostimulants

There is no clear categorization of biostimulants, but some scientists group biostimulants on the basis of their source, chemical composition, or their mode of action (Halpern *et al.*, 2015; Yakhin *et al.*, 2017; Du Jardin *et al.*, 2015). The categories of biostimulants and their effects on plant are described below.

10.3.1 Humic substances as biostimulants

Humic substances (HS) are organic constituents of soil and sediments generally present in almost all terrestrial as well as aquatic environments such as soil, water, lake sediments, peat and brown coal. Humic substances represent about 25% of the total organic carbon on the earth. Humic substances are formed due to chemical and biological degradation of plant and animals via biotic or abiotic pathway (Schnitzer, 1978; Weber *et al.*, 2018). Humic substances are the collection of low-molecular weight compound combined through hydrophobic interaction and hydrogen bonds; biomolecules are also strongly bonded within the HS (Sutton and Sposito, 2005).

Humic substances positively stimulate plant growth by increasing root and shoot length if the concentration of HS increases; growth of the plant also increases. The physiological effects of HS on plants depends on the source, concentration and molecular weight of the humic fraction. These substances are generally divided into fractions on the basis of their molecular mass; it has been observed that low-molecular weight fraction easily enters into the plasma membrane of the higher plant cell rather than high-molecular weight fraction (Nardi *et al.*, 2002). Humic substances remains an area of research interest due to their phytohormone-like activity, making them ecologically important (Pizzeghello *et al.*, 2002). These substances may modify the structure as well as the activity of microorganisms in a rhizosphere (Canellas and Olivares, 2014). It has been proven that HS are beneficial for increasing nitrogen, phosphorus and potassium uptake; on the other hand, sodium and magnesium uptake is not affected (Verlinden *et al.*, 2009). HS increases nitrogen, phosphorus and potassium uptake in barley plants and also boosts the production of organic tomatoes (Olivares *et al.*, 2015; El-Sheshtawy *et al.*, 2019). They tend to improve the iron uptake in grape (Sánchez-Sánchez *et al.*, 2006) and significantly increases the fruits and flower quantity (Arancon *et al.*, 2006).

10.3.2 Amino acid, peptides and other N-containing compounds

There is a new group of biostimulants that is based on amino acids. These types of biostimulants stimulate the crop yield and growth, and also enhances nutrient uptake (Popko *et al.*, 2018). Biostimulants containing amino acids are generally obtained by chemical or enzymatic hydrolysis. The content of free amino acids and their enantiomeric purity is determined by the type of hydrolysis (Sierras *et al.*, 2016). Amino acids and amide play crucial roles in the regulation of nitrate and ammonium uptake, nitrate reduction, ammonium incorporation, protein metabolism and remobilization (Atilio and Causin, 1996). Plant-derived protein hydrolysate used as biostimulants are known for their potential to increase the germination, productivity and quality. These protein hydrolysates also reduce the impact of abiotic stress (Colla *et al.*, 2017).

10.3.3 Seaweed extract and plant protection products

Seaweeds are cheaply available marine resources that are a rich source of lipid, protein, carbohydrates, amino acids, antimicrobial compounds, etc. (Raghunandan *et al.*, 2019). Seaweeds have been used as a source of food, industrial raw material, bioresource, as well as a therapeutic or botanical source, for centuries. Seaweed extracts contain a large number of plant growth-stimulating compounds (Khan *et al.*, 2009). The use of seaweeds and seaweed extract can enhance the performance of plants through different physiological and biochemical pathways, but the actual mechanism of seaweed extract action is not completely known (Craigie, 2010). Rathore and colleagues conducted a field experiment and found that seaweed extract shows yield enhancement in soybean production (Rathore *et al.*, 2009). The rapid increase in the world's population has triggered the need for liquid seaweed extract use in agriculture – their growth-enhancing properties, such as shoot and root elongation, also enhances biotic and abiotic stress tolerance such as saline resistance, resistance toward phytopathogenic organisms, etc. (Arioli *et al.*, 2015; Nabti *et al.*, 2017). Seaweed-treated plants showed increased concentrations of carbohydrates, proteins, free amino acids, polyphenols and nitrogen (Pise and Sabale, 2010). It has been reported that seaweed liquid fertilizer of *Codium decorticatum* provides maximum seed germination, leaf area, number of pods and increased total chlorophyll content (Vijayakumar *et al.*, 2019). Seaweed extract can be used for industrial and agricultural purposes due to the presence of high-quality materials such as macro- and micro-elements (Hong *et al.*, 2007). Kumar and Sahoo conclude that liquid seaweed extract positively stimulates the growth and yield of *Triticum aestivum* var. Pusa Gold (Kumar and Sahoo, 2011).

10.3.4 Biopolymers as biostimulants

Biopolymers are materials that can be produced by natural or synthetic process from material that can be obtained from biological sources. These natural and synthetic biopolymers can be sub-divided on the basis of their composition as biodegradable and non-biodegradable. Biopolymers are known for properties such as seed protection, yield enhancement and plant growth regulators; they can also minimize the use of fungicides so they also provide protection from plant diseases. It has been observed that the application of biopolymer-based biostimulants significantly enhanced the dry weight and total biomass of plants, and also stimulates the defense mechanisms against biotic and abiotic stress. Synthetic biopolymers can be made from plants, microbes, agriculture waste and feed stocks (Raj *et al.*, 2011; Lucini *et al.*, 2018). Nanotechnology and biotechnology are both used in the production of biopolymers. The biodegradable, biopolymer-based nanoplatforms are also used for seed coating that enhances agrochemical delivery and seedling development (Raj *et al.*, 2011; Xu *et al.*, 2020). Chitosan-based biopolymers are increasingly being used in agriculture (Qu and Luo, 2020). They are actually a de-acetylated chitin derivative that is present in the shells of shellfish (e.g. shrimp, crab and lobster) and fungal cell walls. These chitosan-based biopolymers are well known in influencing plant physiology (Chakraborty *et al.*, 2020). Biodegradable

Cu-chitosan nanoparticles are known to boost the defense response in some crops (Choudhary *et al.*, 2017); on the other hand, seaweed biopolymer films are used in plasticulture (Chong *et al.*, 2019). Plant seed mucilage is also a kind of biopolymer that is known for its application in food industries (Soukoulis *et al.*, 2011). Functional biopolymers are those produced by biochemical technology and also used in food technology (Khan *et al.*, 2009).

10.3.5 Plant growth-promoting bacteria

Biostimulants that are of biological origin are known as 'elicitors'. Bacterial biostimulants used in agriculture increase agronomic efficiency by improving growth and productivity. There are number of bacteria that are known for their growth-promoting activity, disease resistance, and abiotic and biotic stress tolerance (Vargas-Hernandez *et al.*, 2017; Souza *et al.*, 2015). All these activities occur due to the plant–microbial interaction that is affected by the surrounding environment and ecology (Ishaq, 2017). This plant–microbial interaction can be positive or negative (Singh *et al.*, 2019). There are different kinds of bacteria in the rhizosphere that can be classed on the basis of their mechanism of action, such as phosphate-solubilizing bacteria, ammonifiers and nitrifiers, which are responsible for the conversion of organic N-compounds into inorganic forms (NH_4^+ and NO_3^-). The bacteria present in the rhizosphere known for their positive effects on plants are known as PGPR (e.g. *Rhizobium*, *Azospirillum*, *Acetobacter*, etc.). PGPR also enhances the presence of mycorrhizal fungi (Osorio Vega, 2007). Plant growth-promoting bacteria have been proven to be effective in abiotic stress resistance. For example, Kasim and colleagues suggested that the plant growth-promoting bacteria can control drought stress in wheat (Kasim *et al.*, 2012). Plant growth-promoting bacteria also enhance the nutrient uptake and contribute to making soil healthy, for example in *Amaranthus hypochondriacus* seeds (Negi *et al.*, 2019). Plant growth-promoting bacteria also produce growth regulators, for example 'siderophore', which is known for fixing nitrogen and solubilizing organic and inorganic phosphates. All these activities provide enhanced salt tolerance in plants (Numan *et al.*, 2018).

10.3.6 Fungi as biostimulants

Fungi often work as biostimulants due to their plant growth-promoting and stress-resistance ability (Xavier and Boyetchko, 2002). Some strains such as *Trichoderma* provide biocontrol as well as plant growth-promoting capacity. Some strains of rhizofungi in the genus *Trichoderma* directly affect the plant pathogens and also influence the phytohormone network of the host plant (Martínez-Medina *et al.*, 2014). Endophytic fungi are known for their ability to synthesis biostimulants. These symbiotic fungi have been proven to be effective influencers on *Cymbidium aloifolium.* Some other endophytic fungi such as *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 also play a significant role in plant growth promotion (Chand *et al.*, 2020). Fungi also influence seed germination and seedling growth (Mishra and Sinha, 2000). *Arbuscular mycorrhiza* acts as a biostimulant and is known for its ability to perform as a biofertilizer, bioregulator and bioprotector (Rouphael *et al.*, 2015).

10.3.7 Algae as biostimulants

To reduce the use of chemicals in agriculture, use of algae as biostimulant is becoming a suitable alternative. Algae is known to increase yield, biomass and quality of the crop. Algae can be used as a biostimulant pesticide and organic fertilizer (Dmytryk and Chojnacka, 2018; Carillo *et al.*, 2020). Algae as biostimulants have become the focus of research due to their high nutrient production and their ability to increase seed germination, seedling growth, plant growth and tolerance to abiotic stress. Algae also produce a vast variety of complex macromolecules and signaling molecules (Chiaiese *et al.*, 2018; Colla and Rouphael, 2020). For instance, application of microalgae, *Cynobacteria*, *Aphanothece* spp. and *C. ellipsoidea* significantly increases root and shoot length of tomato plants. They also enhance the nitrogen, phosphorus and potassium uptake by the plants. Baltic algae (macroalgae) are becoming a cheap source of naturally occurring bioactive compounds (Michalak *et al.,* 2016; Mutale-Joan *et al.,* 2020).

10.4 Biostimulants as pesticides

Pesticides are used to kill pests and insects that harm crop yield and quality; however, pesticides also have some adverse impact on human health and environment. Pesticides are frequently used to control number the of insects, weeds, rodents, fungi or other pests and diseases. The use of pesticides in agriculture is quite challenging when they are applied with chemical fertilizer, as the increased use of pesticides also increases the risk of toxicity (Forget, 1993; Mahmood *et al.,* 2016). Modern agriculture is one of the main factors for environmental pollution, specifically the use of chemical pesticides increases the concern of environmental pollution. Here, it can be said that the use of chemical pesticides produces undesirable effects on biotic as well as abiotic factors (Kabir and Rainis, 2012; Kumar, 2012). As the resistance of many insect pest species toward chemical insecticides has increased with time, pest control strategies have shifted toward more eco-friendly and sustainable approaches (Chidawanyika *et al.,* 2012). As mentioned above, the biostimulants are derived from microorganisms or natural products that are applied to land, so biostimulant pesticides are those products that are made from natural products or microorganisms to resist pests. Therefore, biostimulants as pesticides are considered as biopesticides.

With passing time, the use of new alternative tools for pest management has increased, for example plant-incorporated products and botanicals, as well as new microbial-derived chemicals (Seiber *et al.,* 2014). For pest management, various types of microorganisms and their derived products (e.g. secondary metabolites) have been used as next-generation pesticides (Chanika *et al.,* 2011; Cheba, 2011; Mnif and Ghribi, 2015; Kachhawa, 2017; Keswani *et al.,* 2020). Botanical and plant-derived essential oil-based pesticides have also been used as a new approach in pest management. Many plants produce compounds that known for their action against pests; for example, *Azadirachta indica*is known for its inhibition activity on the digestive alpha-amylase, which is present in insect pests (Isman, 2000, 2020; Sami, 2014). Botanical-derived pesticides such aspyrethrum, rotenone, nicotine, sabadilla and quassin have been used as pesticides; out of these, pyrethrum, which is derived from chrysanthemum flowers, is one of the most rapidly used botanical pesticides known for rapid action against crawling and flying insects. By contrast, the use of rotenoneis has decreased due to its adverse effects in plants (Jacobson, 1989).

10.4.1 Seaweeds: emerging future pesticides

Due to the continuous increment in organic farming, the use of seaweeds as a fertilizer is also increasing. They can also protect our climate via chemical-free disease suppression. Seaweeds are considered as natural biostimulant to plants and also protects plants from abiotic stress. Ethanol and water extracts of number of seaweeds show significant hostile activity toward *Meloidogyne javanica.* Seaweeds have similar antipathogenic effects toward root pathogen of tomato and sunflower plants and have suppressed fungal root and nematode galls infections (Sultana *et al.,* 2012). Viqar *et al.* (2009) conducted a greenhouse experiment to test the potential of brown seaweed *Sargassum swartzii* and *S. asperum* against pathogenic fungi and nematodes. They found that the seaweeds have very good efficacy against root-rotting fungi, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium solani* and root knot nematode *Meloidogyne javanica* in tomato roots. When seaweeds are used with fungicides, they show higher potential effects in comparison to their individual use (Viqar *et al.,* 2009). In the marine environment, different types of pesticides can be extracted as solid residues from seaweeds samples. A variety of technical approaches have been used for extractions; for example, matrix solid-phase dispersion where graphitized carbon black and florisil are used as clean-up adsorbents, followed by gas chromatography-mass spectrometry (GC-MS) (García-Rodríguez *et al.,* 2012). Seaweeds can be considered as a safer substitute to chemical pesticides because of their capacity to produce a broad spectrum of chemically active secondary metabolites that have antipathogenic, antioxidant and anti-inflammatory activities. To use seaweeds as pesticides, their processing is necessary which is followed by extraction of antipathogenic bioactive compounds (O'Keeffe *et al.*, 2019). The processing of seaweed shown in Fig. 10.2. Spirulina can also be useful because of its antipathogenic activity (Sivakumar and Santhanam, 2011).

10.4.2 Biopolymers: effective alternatives to pesticides

Some biopolymers possess antioxidant and antipathogenic activity, and the biopolymers that lack these activities can generally work as a carriers for biopolymer in which antioxidant and antimicrobial properties are present (Sivakanthan *et al.*, 2020). Biostimulant and biodegradable polymers (e.g. chitin obtained from crustacean shells) effective in plant disease management are defined by their mechanism of action, which is determined by their chemical structure. Chitin is a natural enemy of plant pathogens (Ramírez *et al.*, 2010). A chitin derivate known as chitosan is an effective substitute to pesticides. It is generally composed of poly β-(1–4)-D-glucosamine and isolated from the cell walls of fungus and crustaceans. Chitosan is known for the formation of phytoalexins, which are generally antiparasitic in nature. It is highly effective against fungal germination, sporulation and fungal formation (Ebinesar *et al.*, 2021). Coating chitosan on plants enhances the plant resistance toward pathogen (Chakraborty *et al.*, 2020). Figure 10.3 shows how chitosan suppresses the pathogen activity. Nowadays, chitosan is also used for antimicrobial food packaging with the input of nanotechnology (Kumar *et al.*, 2020).

10.4.3 Cysteine-rich plant peptides: antimicrobial nature

Antimicrobial peptides have been isolated from some plants with antimicrobial properties. These peptides generally contain an even number of cysteine molecules connected pair-wise by disulfide bonds. These peptides can be extracted from all unstressed parts of the plant, but mostly from the outer cell lining. The antimicrobial peptide (AMP) genes are also used for inducible defense response via expression in transgenic plants (Broekaert *et al.*, 1997). To ensure the purity of AMPs, it is important to use appropriate isolation and purification techniques, such as reversed-phase C18 solid phase extraction, reversed-phase high-performance liquid chromatography (RP-HPLC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and ion-exchange application (Tang *et al.*, 2018). AMPs show a variety of amino acid composition and structure. They have a high affinity against a broad spectrum of microbes, which facilitates

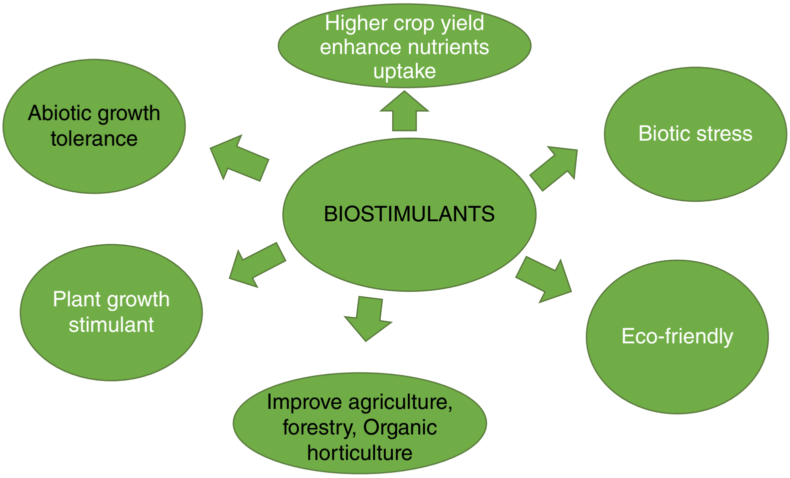

Fig. 10.2. Required processing of seaweeds for their antifungal activity extraction.

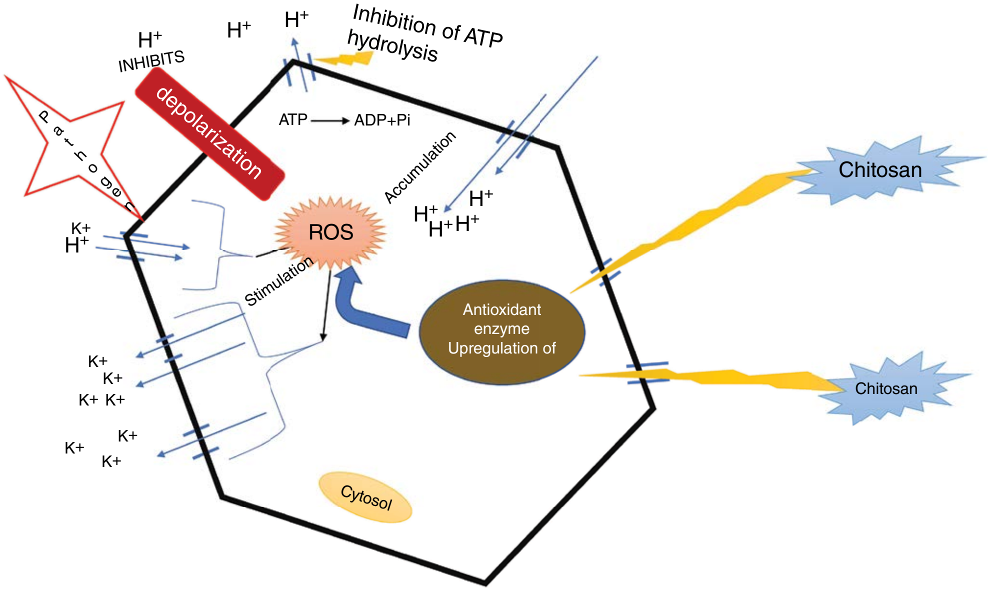

Fig. 10.3. Chitosan antipathogenic mechanism. Pathogenic attack that stimulates the ROS production is cytosol and depolarization of the cell membrane. Due to the ROS production, the K^+ efflux occurs from ROS sensitive channels, chitosan inhibits the K^+ efflux via antioxidant enzyme activation and higher H^+-ATPase reduces the depolarization of cell membrane. Chitosan reduces the ATP hydrolysis and increase the H^+ ions concentration within the cytosol so the H^+ and K^+ transport decreases.

their use in agriculture (Maróti *et al.*, 2011). AMPs of small amino acid sequence length, i.e. 22 amino acid residues, have been developed to conquer the pathogenic resistance in plants (Powell *et al.*, 2000). The PhytAMP database provides details of the biological activities of plant peptides in agriculture and pharmaceuticals (Hammami *et al.*, 2009). Antimicrobial peptides generally present in flowers has enough potential to develop long-term plant genetic improvement by enhancing the resistance toward the pathogen via genetic modification of plants (Tavares, 2008).

10.4.4 Pyrethrum

Pyrethrum is naturally derived from *Tanacetum* or *Chrysanthemum cinerariifolium*. It is a naturally derived insecticide with a low toxicity to mammals. Pyrethrums are derived from successive isosteric modifications (Casida, 1980). According to environmental agencies and a forest survey of poison control centers, pyrethrums are more effective insecticides than any other class except organophosphate (Soni and Anjikar, 2014). Nowadays, compounds such as organochlorines, carbonates and organophosphate are being rapidly replaced by the pyrethrums, pyrethroids and other sort of natural pesticides due to their low toxic effect, particularly in the consumer pesticide market (Shannon *et al.*, 2007). It works by a contact insecticide action, which results in toxicity of the nervous system, causing knockdown or death of the insect. This chemical is generally effective against mosquitoes, fleas, ticks, lice, flies and chigger (Fradin, 2001). They are mostly used in powder form, but in some cases they also used in spray form (Evans, 2009). It is generally applied in combination with synergists (such as piperonyl, butoxide) that are known to inhibit toxification (Copping and Menn, 2000).

10.4.5 *Azadirachta indica*-based pesticides

Leaves from *Azadirachta indica* (neem) can also be used as biostimulants. The addition of neem leaves on earthworm vermicompost enhances the reproduction and growth of earthworms, and when this mixture is used with fertilizers it leads to enhanced plant growth. Neem compost has an amazing capacity to convert 7% of the feed to vermicompost every day, which is known to enhance yield (Gajalakshmi and Abbasi, 2004). Due to the increased fear of bioaccumulation of chemical pesticides in the climate, which lead to enhanced resistance and decrease biodiversity of ground, the use of *A. indica* for the development of eco-friendly pesticide has been investigated. *A. indica* contains different active constituents, for example azadirachtin, salannin (insecticidal property), and nimbolide B and nimbic acid B that possess herbicidal activity. *Azadirachta* generally works as repellent, anti-feedant and repugnant agent and also induces sterility in insect (Chaudhary *et al.*,2017). The structure of azadirachtin shows similarity with '*ecodysone*'(an insect hormone that controls the metamorphosis of the insect) (Mordue *et al.*, 1998). The herbicidal ingredients of neem, nimbolide B and nimbic acid B, are known to inhibit the growth of jungle rice, crabgrass, alfalfa, lettuce and barnyard grass. These inhibitory observations have proven effective in determining their allopathic and phytotoxic mechanisms (Kato-Noguchi *et al.*, 2014). Salannin is known to deter the feeding and also expand the larval duration, which leads to delayed molting and mortality of larvae and pupae (Govindachari *et al.*, 1996). Various extraction procedures can be used to extract the active components from neem, for example alcohol extraction (methanol and ethanol extraction), hexane extraction, NLGP (extraction of neem leaf glycoprotein) extraction and water extraction (Chaudhary *et al.*, 2017).

10.4.6 Rotenone

Rotenone is the naturally occurring ketone widely used as fish poison. Rotenone is generally extracted from *Derris elliptica* Benth. and *Derris malaccensis* (Sae-Yun *et al.*, 2006; Dalu *et al.*, 2015). For the extraction of rotenone, both conventional and non-conventional methods can be used (Zubairi *et al.*, 2016). Rotenone is considered to be the most widely used pesticide and insecticide throughout the world (Lawana and Cannon, 2020). It is involved in the autophagy in pathogens due to toxicity induced by rotenone. It has been proven that under optimal conditions, the application of rotenone can be toxic for insects such as aphids, fly larvae, thrips, leaf hoppers, caterpillars, beetles' larvae and culicine mosquitoes (Davidson, 1930).

10.4.7 Ryanodine and related alkaloids

Alkaloids obtained from plant extracts have insecticidal properties, but their direct application is harmful. Ryanoid is an alkaloid that is obtained from the *Ryania speciosa.* The root and stem material of *R. speciosa* is used for the insecticidal preparation by extraction with water and many other inorganic solvents (Rogers *et al.*, 1948; Ujvary, 1999). The *Ryania* extract is not widely used as an insecticide; it provides good control when it is used for specific species. *Ryania* extract is available in the form of wettable powder as Natur-Gro R-50 and Natur-Gro triple plus. Ryan 50, which is also known as Dunhill chemical, has a comparatively low range of mammalian toxicity (Copping and Menn, 2000).

10.5 Rhizosphere microbes as pesticides

The loss of organic material from the root leads to the development of an active microbial population in the rhizosphere environment. As a result of the loss, mycorrhizal fungi grow in the rhizosphere and subsequently allow the growth of disease-causing bacteria in rhizosphere environment. Various types of microbe interactions take place, including their use as biocontrol agents that can contribute to plant defense mechanisms application as pesticides (Whipps, 2001; Ab Rahman *et al.*, 2018). Microbial pesticides have precise host ranges and can also be integrated with other novel crop protection strategies (Butt *et al.*, 1999). The microbial agents that are applied to plants against disease are referred to as microbial biological control agents (MBCA). MBCA provide disease control through a variety of strategies, for example inducing resistance to plant pathogen or providing resistance by generating adverse conditions for pathogen growth (Köhl *et al.*, 2019). Biological control against pathogens can be achieved by four strategies: classic biological control, inoculation biological control, inundation biological control and conservation biological control (Eilenberg *et al.*, 2001). MBCA is a key element of an integrated pest management program (Kiewnick, 2007). The PGPR group provides a defense against disease and can be used as pesticides, for example Azotobacter is a PGPR known to produce antifungal substances (Gurikar *et al.*, 2016; Besset-Manzoni *et al.*, 2018).

Insect pathogenic microorganisms such as bacteria (*Bacillus thuringiensis*), fungi (*Beauveria bassiana*), nematodes (*Steinernema*) and protozoans (*Nosema locustae*) are known for their function against insects and can be used as microbial insecticides that provide potential benefits to agriculture. These microbial insecticides generally release specific metabolites that induce changes in metabolism, increasing the likelihood of insect death (Sarwar, 2015). Nowadays, several microorganism-derived products are available as pesticides (Copping, 2000). Table 10.1 lists different natural product that are generally derived from microorganism and their specific use as pesticides.

10.5.1 Bacterial pesticides

The use of bacterial pesticides increased in the 20th century out of concern for use of chemical pesticides in the quest for improved quality food and fiber production. As we know from the above discussion, environmental concern regarding the use of chemical pesticides has increased, and therefore the search for natural pest control is underway. Nowadays, various biological control strategies are continuously introduced and tested for commercial purpose (Gerhardson, 2002). Bacteria have immense capacity against insects that are generally known to damage food crops, defoliate trees and are vectors of disease. Conventionally, these bacteria are generally isolated from the soil and sometimes also from the dead insects. Martin and colleagues used *Manduca sexta* L. (order Lepidoptera) as the insect model for toxicity testing of bacterial agents extracted from soil samples. They found that most of the insecticidal bacteria generally belong to *Bacillus cereus* group, which includes three crystal-forming strains of *B. thuringienisis* and three other spore-forming organisms (*Psychrotrophic, B. weihenstephanensisand Lysinibacillus fusiformis*) (Martin *et al.*, 2008). Most of the commercial bacterial insecticidal products are based on *B. thuringiensis*, which accounts for 90% insecticidal bacterial pesticides. Known for its insecticidal proteins, the Cry protein kills insects, but its effectiveness is mostly restricted to lepidopteran spp., and sometimes mosquitoes and blackflies. The first Cry protein gene encoding was reported in 1981 – this led to the development of commercial bacterial-based insecticides that are comparatively safe for the environment. Nishiwaki and colleagues determined the action of the gene against *Plutella xylostella, Aedes japonicus, Spodoptera litura* and *Bombyx mori* insect models. Nine *cryI* genes that show activity against lepidopteran species genes have been isolated: *cryI-Ac*, *cryIB*, *cryIAa*, *cryIAb*, *cryIAc*, *cryIB*, c*ryIC*, *cryIE* and *cryID* (Chattopadhyay *et al.*, 2004; Federici, 2005). *B. sphaericus* is also known for its insecticidal properties. It is used for pest management and produces protein crystals during the sporulation process. Some other bacteria also used in pest management include *Paenibacillus popilliae* (form toxic crystals) known for its activity against beetle larvae, *Clostridium bifermentans* and *Brevibacillus laterosporus* (form crystalline inclusions) used against mosquitoes. Some microbial activities are also known for the degradation of pesticides. *Flavobacterium, Alcaligenes, Pseudomonas* and *Rhodococcus* are known for their ability to metabolize pesticides (Aislabie and Lloyd-Jones, 1995).

In addition to Cry proteins, other types of proteins are also present in bacteria, for example Vip proteins, which are further divided into four groups (Vip1, Vip2, Vip3 and Vip3A), that are known for their toxicity in Coleoptera, Lepidoptera and Hemiptera. Vip1 damages the midgut of the pest and Vip2 resists microfilament formation (Chakroun *et al.*, 2016). Surfactants of microbial origin are also used in integrated pest management. These biosurfactants are eco-friendly and biodegradable in nature (Yang *et al.*, 2017). A number of bacteria and fungi are commercially available for use against pathogen attack. The combined effect of microorganism also seems to be effective against insect attack, for example the combination of *Yersinia entomophaga*

Table 10.1. Commercially available natural products as pesticides that are derived from microorganism.

Natural products	Use as	Isolated from	References
Blasticidin	Fungicides-bactericides	Actinomycete, *Streptomyces griseochromogenes*	Takeuchi (1958); Misato *et al.* (1959)
Kasugamycin	Fungicides-bactericides	Actinomycete, *Streptomyces Kasugaensis*	Umezawa (1965)
Mildiomycin	Fungicides-bactericides	Actinomycetes, Streptoverticillium	Harada and Kishi (1978)
Natamycin	Fungicides-bactericides	Actinomycetes, *Streptomyces*	Pedersen (1992); Atta *et al.* (2015)
Streptomycin	Fungicides-bactericides	Actinomycete, *Streptomyces griseus*	Ingham and Coleman (1984)
Polyoxins	Fungicides-bactericides	Actinomycetes, *Stretomyces cacaoi* var. asoensis	Mamiev *et al.* (2013); Chen and Deng (2009)
Validamycin	Fungicides-bactericides	Actinomycete, *Streptomyces hygroscopicus*	Kaur and Singh, (2016); Wei *et al.* (2012)
Milbemectin	Insecticides	Actinomycete *Streptomyces hygroscopicus, Streptomyces avermitilis*	Pluschkell *et al.* (1999); Wang *et al.* (2010); Copping and Menn (2000)
Avermectins	Insecticides	*Actinomycete Streptomyces avermitilis*	Putter *et al*. (1981); Zhuo *et al.* (2010)
Bacillus thuringiensis d-endotoxins	Insecticides	*Bacillus thuringiensis*	Knowles (1994)
Spinosad	Insecticides	Actinomycete *Saccharopolyspora spinosa*	Williams (2003); Tao (2019)
Polynactins	Insecticides	Actinomycete *Streptomyces aureus*	Yoon *et al.* (1993); Shishlyannikova, *et al.* (2017)
Bilanofos	Herbicides	Actinomycete *Streptomyces hygroscopicus*	Copping and Menn (2000)

MH96 and *Brevibacillus laterosporus* and with *Beauveria bassiana* J18 fungus is effective against larvae of diamond black moth (Narciso *et al.*, 2021). Bowen and colleagues reported that a specific type of toxic substance is secreted by entomophagous nematode gut bacterium, *Photorhabdus luminescens*. The toxic substance is secreted in the hemocoel of the insect, and is responsible for the insect death (Bowen *et al.*, 1998). Endospore-forming Gram-positive rod-shaped bacteria have been shown to be insecticidal (Srivastava and Kumar, 2019). *Photorhabdus* and *Xenorhabdus* show symbiosis with entomopathogenic nematodes and are also considered as a potent alternative to BT (*Bacillus thuringienesis*) product (Dowling and Waterfield, 2007). Chiriboga and colleagues conducted an experiment to evaluate how insecticidal and growth-promoting bacteria colonize in the root. *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas protegens* CHA0 are known for their suppressing nature toward pathogens and also enhance systemic resistance (Chiriboga *et al.*, 2018).

10.5.2 Fungal substitutes for chemical pesticides

Synthetic pesticides and chemical pesticides are known for their adverse effect on the ecosystem. Also, the pest develops resistance against these synthetic or chemical pesticides. So, the synthetic as well as chemical pesticides are continuously being replaced with biopesticides due to which the production and commercialization of biopesticides is continuously rising. Biopesticides can be produced with different strategies such as submerged fermentation which is a conventional method to develop biopesticides. Technologies such as solid-state fermentation and agro-industrial waste are considered as an alternative to conventional methods (De la Cruz Quiroz *et al.*, 2015). Fungi and fungal-based products can be used as pesticides, as suggested by exploratory research regarding the field application and their production and formulation for the development of fungal-based pesticides (McCoy, 1990). Some fungi, including *Beauveria bassiana* and *Metarrhizium anisopliae*, have been shown to be effective against the larvae of *Spodoptera litura* (Borkar *et al.*, 2013). The sporulization and growing ability in submerged fermentation in 60% humid conditions reduces powdery mildew. If this process is combined with mineral oil adjuvants, it is more effective (Hofstein and Fridlender, 1994; Daoust and Hofstein, 1996). The extract produced from medicinal plants and fungi, and fungi with yeast, proves effective against plant disease (Punja and Utkhede, 2003; Stangarlin, *et al.*, 2011). Aq10 from *Ampelomyces quisquali* is the first formulation developed against disease resistance used against powdery mildew in plants like tomato, strawberry, tree fruits, grapes and ornamentals (Dik *et al.*, 1998). *Phlebiopsis gigantea* is a wood-rooting fungi that is applied to freshly cut pine stumps as a growth controlling agent against the colonization of the root-rotting fungus *Heterobasidio nannosum*. *Phlebiopsis gigantea* in suspension is considered to be the first fungal disease control agent in Britain (Pratt *et al.*, 1999). Isolated *Trichoderma harzianum*, T39 is commercially available as a biocontrol agent generally known for its action against *Botritis cinerea* (gray mold), specifically in green house crops and vineyards (Elad *et al.*, 2000). A combination of *T. harzianum* and *T. polysporum* is commercially available as BINAB-T. This combination uses the IMI 206040 and IMI 306039 strains of *T. harzianum*, and is mostly used for the prevention of gray mold and silver-leaf disease even though it is effective against numerous diseases. The combination of *T. harzianum* with *T. viride* led to the formation of Teichoject and Trichoseal, which are used against silver-leaf of Pip and stone-fruit trees (Whipps and Lumsden, 2001). *Candida oleophila*, a yeast used as a biopesticide, is known to decrease the effect of green and blue mold and rot diseases that are caused by different species of *Penicillium* (e.g. *P. digitatum* and *P. italicum*) (Wilson *et al.*, 1993; Droby *et al.*, 1998). Fungi are also beneficial for use in bioremediation and biodegradation of pesticides (e.g. lindane, endosulfan, atrazine, cryypermethrin, methamidophos, dieldrin, heptachlor, methyl parathion, etc.) by their naturally secreted enzymes such as aslaccase, peroxidase, hydrolase, esterase, lignin peroxidase, dehydrogenase and manganese peroxidase (Maqbool *et al.*, 2016). Jauregui and colleagues studied the transformation of organophosphorus pesticides by different white-rot fungi strains (Jauregui *et al.*, 2003).

10.6 Conclusion and future prospects of biostimulant use as pesticides

From the above discussion, it is clear that the continuously increasing use of pesticide generates ecosystem hazards. Scientists are focusing on alternative strategies to overcome the adverse impact to the ecosystem. They have identified some natural products that will enhance the yield and growth of crop plants, which can be used as an effective alternative to harmful chemical pesticides. Biostimulants can be considered as an effective alternative or substitute to pesticides as they are natural products and also stimulate the growth of the plant even in stressed conditions. They are antipathogenic and can be used to protect the plant from harmful pests. Various biostimulant-based pesticides are also commercially available in the market. Nowadays, biostimulants are also integrated with nanotechnology and biotechnological approaches. Continued research is required in this field to enhance the applications across a wider range of plants.

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11 Role of Humic Substances on Growth and Yield of Crop plant

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Abstract

Sustainable agriculture inputs require effective fertilizers that contain macroelements and microelements and depend upon the plant growth biostimulants, which are a rich source of biologically active compounds whose function is to stimulate natural processes. Natural inputs are the back-bone of clean farming, including a wide range of products to support soil fertility, biological activity and plant growth. Humic substances (HS) are natural, organic compounds that are variably sourced from decayed plants, peat, soil, composts and coals such as lignite and leonardite. Humates are the active form of humic substances. Numerous studies have demonstrated significant crop responses to humates. However, there is some spatial and temporal variability in the field efficacy compared with that under controlled conditions. The mechanistic explanations of HS effects are most commonly grouped into the soil nutrient availability, other soil properties, direct plant biostimulation and/or microbial processes. Humates have been shown to stimulate plant growth in terms of increasing plant height and dry or fresh weight as well as enhancing nutrient uptake. Multiple plant processes have been affected by HS, including enzyme activity, protein metabolism, photosynthesis, respiration, and water and nutrients uptake. The underlying mechanisms involve hormone fluxes, cell membrane permeability, electron chain transport components, free radical activity within the humic structure as well as reactive oxygen species in plants. The two most useful quality tests for a humate product are the humic or fulvic quantities in the product and biological activity. The functions of HS for the enhancement of plant growth differ widely depending on the application mode, plant stage and rate. There are five application types of HS in the field: direct application in the soil as liquid status, direct application in the soil as solid status, foliar application, fertigation and soaking. This chapter summarizes the role of humic substances on growth and yield of crop plants.

11.1 Introduction

The agricultural sector is encountering an unprecedented challenge toward secure food for humanity because of the rapid and progressive increments in the human populations beside their attendant adverse threats on the ecosystem. Nowadays, organic farming has been proposed as one of the alternative approaches that is attempting to overcome such challenges concerning sustainability issues. Despite an argument whether organic farming is synonymous with sustainable agriculture (Rigby and Cáceres, 2001), organic farming is a technique that paved the way toward the concept of sustainability in agriculture. Organically grown

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crops are favored by most consumers worldwide as a resource of fiber and nutraceutical products because they are perceived as more nutritious and healthier (Scotti *et al.*, 2016). In context, Aertsens *et al.* (2009) revealed that most consumers who are well informed about organic agriculture in most countries appreciate it as the best option for food and the environment. Such a tendency toward healthy food considering the environmental issues enthuses the farmers and stakeholders toward a sustainable agriculture that is based on naturally derived practices parallel to the reduction/elimination of synthetic inputs. So, there is a common interest and universal desire to develop and implement sustainable agricultural practices (Gazzola *et al.*, 2019; Rigby and Cáceres, 2001). On the other hand, conventional agriculture and its disruptive human activities triggers manifold threats in terms of environmental, economic and social concerns, including pesticide and minerals pollution, pest adaptation and resistance, reduced soil health and organic forms of carbon, soil erosion and desertification, losing biodiversity as well as overpriced food and starving people in poor nations (Lichtfouse *et al.*, 2009; Imadi *et al.*, 2016).

Despite the valuable advantages of organic farming, the lower yield comparing the conventional one remains the major obstacle to those who adopt it (Dorais, 2015). The reduction in productivity of organically grown crops ranged from 20% to 34% lower than conventional crops (de Ponti *et al.*, 2012; Seufert *et al.*, 2012). The unbalanced nutrients bioavailability and/or unfavorable soil characteristics appeared to be the significant yield-limiting factors in many organic production systems (Berry *et al.*, 2006; De Pascale *et al.*, 2018). The unbalanced nutrients bioavailability is mostly due to the slow release of nutrients, which mismatch the plants uptake during their growing stages (Pang and Letey, 2000; Zhao *et al.*, 2009; Lester and Saftner, 2011) and/or unfavorable soil characteristics like adverse soil pH that cause insoluble forms of nutrients (Niemiec *et al.*, 2020).

Accordingly, there is an urgent need to minimize the gap between organic and conventional outcomes by increasing nutrient bioavailability, improving plant uptake and/or assimilation (Barbieri *et al.*, 2015; De Pascale *et al.*, 2016). So, the interest in soil health and its quality is being reawakened in parallel with the emergence of sustainable agriculture, emphasizing the importance of soil organic matter (SOM) into the arable soil for better crops (Manlay *et al.*, 2007). Soil scientists are recognizing SOM as a major factor controlling the magnitude of soil fertility (Fig. 11.1) and environmental health, by mitigation of atmospheric carbon emissions, which contribute to sustaining human societies (Fageria, 2012; Valenzuela and Cervantes, 2021).

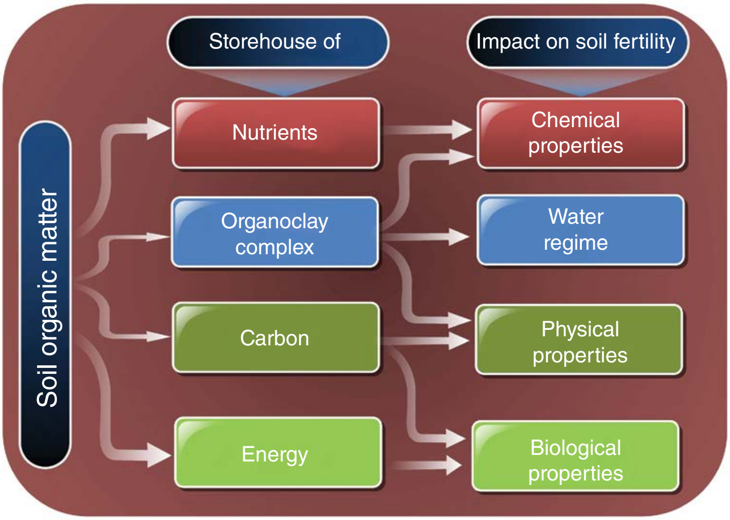

Fig. 11.1. Role of soil organic matter in the fertility of soil.

Furthermore, plants' biostimulation using naturally derived biostimulants globally have gained interest as a promising tool and sustainable approach (De Pascale *et al.*, 2018; Pylak *et al.*, 2019). Humic substances (HS), sometimes a synonym of SOM (Waksman, 1925), are natural, organic compounds that are variably sourced from decayed plants, peat, soil, composts and coals such as lignite and leonardite. HS are occasionally recognized as an essential contributor to the physicochemical and biological properties of soils, as well as biostimulation of plant growth beside others to raise the popularity for HS to be used in various agricultural practices (du Jardin, 2015; Halpern *et al.*, 2015). The exogenous application of HS within agronomic systems can be used to aid the development of sustainable intensification (Canellas *et al.*, 2015). Furthermore, HS are crucial in ecosystems because they regulate the global carbon and nitrogen cycles, the growth of plants and microorganisms, the fate and transport of anthropogenic compounds and heavy metals, and the stabilization of soil structure (Nebbioso and Piccolo, 2011). When comparing various carbon-based substances for soil health improvement, humic-based soil amendments are accessible due to the industrial request for efficient and environmentally friendly soil supplements (Pukalchik *et al.*, 2019). The specific properties of humic products (HP) enables their ubiquitous application to be worldwide in cropland agriculture as soil conditioners in industry, the environment and biomedicine (Peña-Méndez *et al.*, 2005; Olk *et al.*, 2018). Practically, commercial HP usually contain between 15% and 85% of HS (Yakimenko and Terekhova, 2011; Yakimenko *et al.*, 2018). This chapter discusses the role of humic acid substances on growth and yield of crop plants.

11.2 Historical perspective

Across ancient eras until the present time, the term ‘humus’ has had various meanings. The Roman writers coined it as ‘soil’ or ‘earth’ as a whole. The ‘humus’ was replaced by ‘*terra*’ in the 1st century BCE, sourced from French ‘*terre*’ (earth), ‘*terreau*’ or ‘*terre vegetale*’ (mold) which survived until the 18th century. During the 1700s, the term ‘humus’ was brought back in European literature meaning ‘loam’ or ‘mold’ (Feller, 1997). The great botanist Linnaeus (1707–1778) used ‘humus’ in a soil classification system similar to the one he developed for plants (Waksman, 1936). In 1761, Johan Gottschalk Wallerius (1709–1785), who is regarded as the founder of agricultural chemistry, first introduced the word ‘*humus*’ in his book ‘*Agriculturae fundamenta chemica*’ with the definition and the description referred to as decomposed organic matter, which is still accepted until today, with minor revisions (Abbt-Braun, 2012). From the early 1800s, humus became the subject of debate among various disciplines including pedologists, microbiologists, plant physiologists, agronomists and chemists, etc.

Over the last 300 years, the humus entity in the sustainability of cropping systems was developed and distinguished into three periods: the humic period; the mineralist period; and the ecological period (Manlay *et al.*, 2007). The first and second periods are closely concerned with theories about plant nutrition, while the third is characterized by a widening of the perception of SOM's contribution to ecosystem function and human well-being.

The humic period started before 1840 and believed that plant dry matter was mainly derived from the uptake of matter supplied by SOM. At this time, SOM was termed ‘humus’ and the agriculturists thought the management of cropping systems fertility was coupled with the management of SOM through organic inputs. In 1809, Albrecht Thaer (1752–1828) laid the groundwork for a ‘humus theory’ that is based on the assimilation of soluble humus by plant roots or chemical heterotrophy as we know it today. Although his humus theory was largely incorrect, the humus theory remained very influential for 30 years, and Thaer's system of rational agriculture was brilliant because it tackled the question of agricultural sustainability (Feller *et al.*, 2003; Manlay *et al.*, 2007).

During the mineralist period (1840–1940), Liebig's ‘mineral nutrition theory’ had been developed based on the theory that carbon comes from carbon dioxide, hydrogen from water, and other nutrients from solubilized salts in soil and water, providing the basis of modern agricultural science (Feller *et al.*, 2003). The mineralist theory of plant nutrition began looking forward as a substitute for the humus theory and paved the way to establish the revolution of the artificial fertilizer industry.

By entering the ecological period (from 1940 up to the present time), interest in the sustainability of intensive agriculture, especially where soils were continuously cropped without organic inputs, had already been raised which resulted in the manifest of biodynamic farming (Merrill, 1983; Manlay *et al.*, 2007). In the 1940s, Sir Albert Howard founded the modern organic movement and proposed his 'law of return' theory, whereby all organic waste materials should be returned via composts to the soil to build humus and fertility, taking a hardline position against the use of chemical fertilizers (Heckman, 2007). SOM has been gaining recognition as a complex bio-organo-mineral system and a key indicator for soil quality and agroecosystems fertility (Manlay *et al.*, 2007).

11.3 Humic substances origin and sources

Humic substances ultimately originate from organic matter. The concept of organic matter has been varied across scientific disciplines. However, it is simply recognized as a matter arising from living matter. In chemical science specifically, it refers to carbon-containing compounds. Basically, in soil science, the organic matter term is devoted to a mixture of fresh and dead organisms, composed of carbonaceous remains of organisms that once occupied the surface of the earth (Weil and Brady, 2017). As a general term for various ecosystems and environments, it can be described as 'natural organic matter' or NOM (Fig. 11.2), which includes a complex mixture of a variety of materials from all organisms, mainly of plant origin, on planet Earth (Tan, 2014). Terrestrial or soil organic matter is formed in the lithosphere, whereas aquatic organic matter is formed within the hydrosphere (Kosobucki and Buszewski, 2014).

Natural organic matter is differentiated into living and dead biomass. Living or fresh biomass undergoes a physical, chemical and biological breakdown to form the dead biomass at various decomposition stages. The partial decomposition by which the original material's

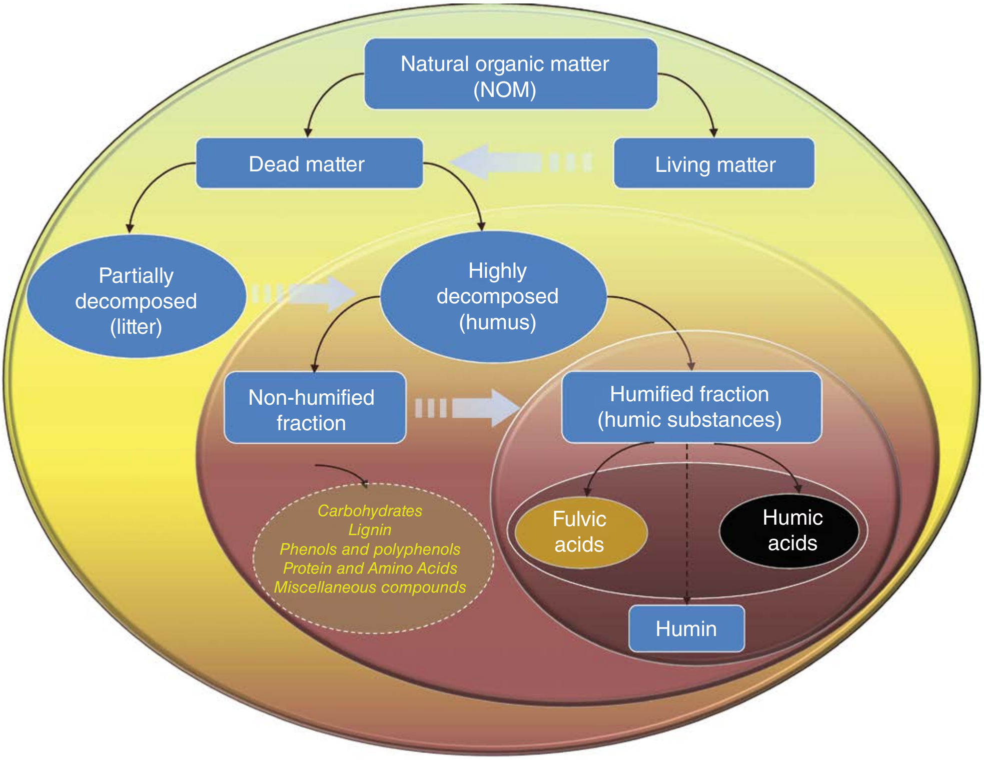

Fig. 11.2. Origin of humic substances.

morphology is still visible, often called ‘litter’ when it lies above the ground or on the surface of soils (Tan, 2014). The highly decomposed biomass or humus commonly appears as a brown to black amorphous material with no traces of the cellular structures of original materials.

Globally, two major categories of humus are recognized: ‘terrestrial humus’, which is characterized by high lignin contents, and ‘aquatic humus’, which is characterized by the presence of more carbohydrates. Terrestrial matter was also called ‘soil humus’ by many scientists previously and is believed to be the ‘soil humic matter’ (Whitehead and Tinsley, 1963). The two names are used interchangeably, and many scientists consider soil humus to be soil humic matter (Stevenson, 1994).

The humus in soil is grouped into non-humified and a humified fractions (Stevenson, 1994; Tan, 2011). The non-humified matter includes most of all biochemical compounds synthesized by plants and other soil organisms and it being released due to decomposition. Such substances have definite physical and chemical characteristics (i.e. colorless and chemically identifiable substances) that differentiate them from the humified fraction, e.g. carbohydrates, amino acids, protein, lipids, waxes, nucleic acids, lignin, and many other organic compounds (Page, 1930). After their release, it may be involved in the formation or synthesis of the humified fraction by a process called humification or it is adsorbed by the inorganic soil components, such as clay, which temporarily protects it from further decomposition reactions, enabling its accumulation in soils (Tan, 2014).

In contrast to the non-humified fraction, which is present in small quantities, the humified fraction makes up the bulk of humus (Tan, 2014). Humic substances are the most active fraction of humus (Hayes *et al.*, 1989). They have a wide range of molecular properties: different molecular weights, functional groups, elemental composition, etc. There are four methods of fractionation (summarized by Fig. 11.3): solubility and precipitation, molecular weight, differences in charge or adsorption (Sánchez-Andréu *et al.*, 1994). The more frequent fractionation method is based on differences in the solubility of the organic compounds with pH variation (Fig. 11.4). Humic substances include fulvic acid (FA), humic acid (HA) and humin. Fulvic acid has the lowest molecular weight and least resistance to decomposition by microorganisms. Humin has the highest molecular weight and greatest resistance to decomposition (Fig. 11.5). Because it has a large number of negative charges per unit mass, it has a very high cation exchange capacity (CEC).

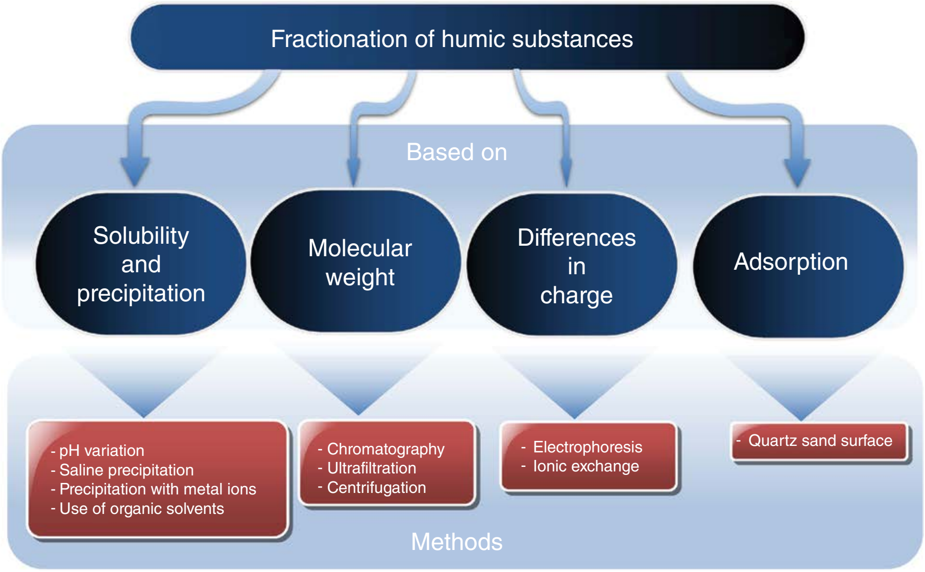

Fig. 11.3. The basis and methods of humic substance fractionation.

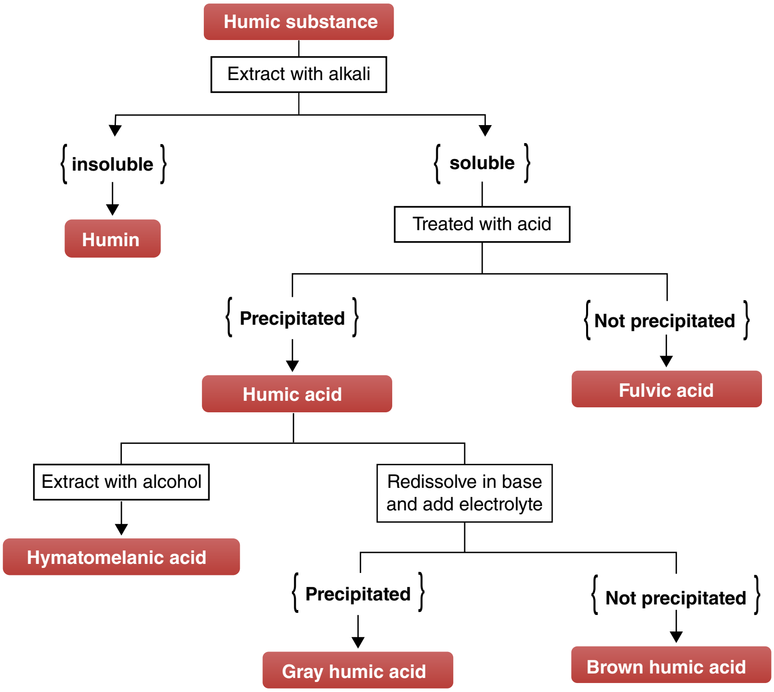

Fig. 11.4. Flow sheet for fractionation of humic substance based on pH variation.

11.4 Role and action of humic substances on plant growth

Regarding plant-soil systems, HS have an indirect role and/or direct action by which HS affects plants' growth and thereby crop productivity (Chen and Aviad, 1990; Mora *et al.*, 2014). The indirect role comes through their actions on the soil properties to improve its fertility. On the other hand, the direct action of HS emerges from influencing several physiological processes, including hormonal biostimulation, photosynthesis, enzyme activation and nutrient uptake.

11.4.1 Indirect role of humic substances on plant growth

The HS act on plant growth medium (soils, substrates) either by changing the physical characters (e.g. soil porosity, soil aggregation, water permeation, gas exchanger; to change chemical character mainly associated with nutrient bioavailability) or by changing the biological character and soil microbiota activity (Erro *et al.*, 2016).

11.4.1.1 Action on soil physical properties

The HS possess specific physical characteristics to give the soils certain physical and physicochemical properties which makes it a more favorable and more balanced medium for plant growth. Some of these characteristics are mentioned by Waksman (1936) as the following: (i) color; (ii) characteristic structure; (iii) volume weight; (iv) properties of cohesion and adhesion; (v) specific weight; (vi) high water-holding capacity; (vii) high permeability; (viii) heat capacity and heat absorption; (ix) shrinkage on drying; (x) coagulation by electrolytes; (xi) absorption of salts and gases (high base exchange capacity); (xii) high buffering power; and (xiii) specific odor.

Traditionally, color is a simple indicator for soil fertility (Brown and O’Neal, 1923). Black/dark brown soil is usually fertile, indicating a high content of organic matter. On the other hand, a pale/yellow soil often indicates low organic

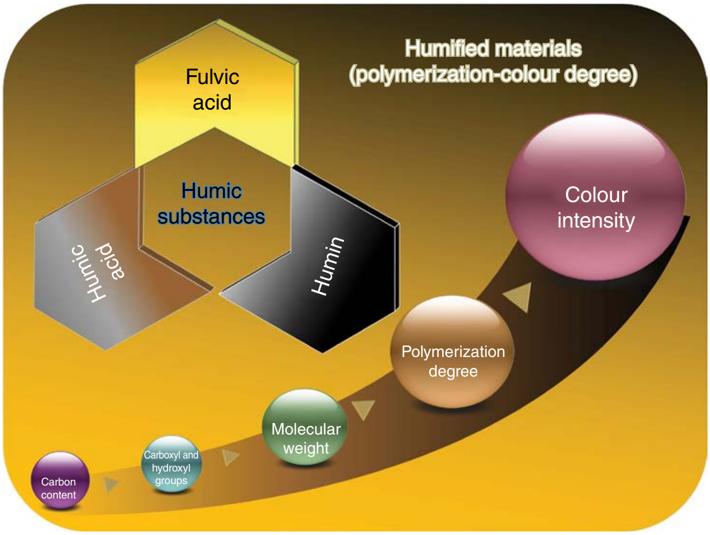

Fig. 11.5. Classification and chemical properties of humic substances.

matter and nutrients, which generally means poor fertility and requires plenty of organic matter. According to Schulze *et al.* (1993), the black HA was responsible for the dark color of the soil organic matter. The dark brown color of soil that is imparted by HS facilitates warming and hence plant growth and yield (Vaughan and Ord, 1985). This aspect is particularly important in the temperate regions of the world.

It is noted that the addition of HS to the soils usually facilitates interrelated changes to their physical properties, and the change in one physical property will often be followed by changes in other physical properties. Any change in soil structure and/or soil texture will be followed by changes in pore spaces, aeration, water flow, percolation and leaching. So, the addition of HS into soils with a clay texture normally improves soil porosity, permeability and aeration, transforming at the same time the high plasticity into friable conditions. Also, the adsorption capacity of soils with sandy textures is usually increased significantly and the formation of soil structure enhanced by application of HS. Such improvement in soil textures and soil structure will consequently be followed by high water-holding capacities, low bulk density values and are often fluffy or friable in consistency (Tan, 2014).

The most widely acknowledged function of HS, as an organic amendment, is improvement of soil structural condition (Quilty and Cattle, 2011). The addition of HS is potentially effective as a soil conditioner and improves aggregation in soils with a range of texture grades and mineral suites because the refractory nature of their chemical structures renders them more resistant to degradation by soil microorganisms (Piccolo *et al.*, 1997; Imbufe *et al.*, 2005; Margherita *et al.*, 2006). The micromorphological techniques and image analysis by Fortun *et al.* (1990) on the shape, size and numerical density of aggregates in a sandy loam and a clay soil treated with HS, extracted from manure or peat, revealed changes in the structure. The differences in the molecular structures within HS, depending on the source of HS, resulted in an increment of the number of small aggregates (<1000 μm) in the soil treated with a peat-extracted HS, while application of a manure-extracted HS led to the formation of larger aggregates (1500–2500 μm). In addition, the improvement in aggregation resulting from the application of HS was greater in the clay soil than the sandy loam due to the greater number of binding sites available on clay minerals in the clay soil.

11.4.1.2 Effect on soil chemical properties

Humic matter can affect the soil chemical properties in various ways since it can generate a variety of chemical reactions. As noted by Tan (2014), the chemical behavior of humic matter is, in general, controlled by two functional groups: the carboxyl and phenolic-OH groups. The development of the negative charge is pH dependent; this charge is called pH-dependent charge or variable charge (Tan, 1965). A number of reactions can take place because of the presence of these charges. At low pH values, the humic molecule is capable of attracting cations, and such electrostatic attraction leads to cation exchange reactions. This kind of reaction will no doubt affect the CEC in soils. The CEC of humic matter can be estimated from its total acidity values, which are usually very high. Humic acid shows CEC values, in terms of total acidity values, ranging from 500 to 1200 cmol kg^{-1}, whereas FA exhibits a somewhat higher range of 600 to 1500 cmol kg^{-1} (Schnitzer and Khan, 1972).

Humic acid can act as a buffer in alleviating the adverse effects of heavy metals and toxic substances such as pesticides and other xenobiotics. HA tends to produce more insoluble metal chelates and the humo-metal chelate is considered to serve as a sink for toxic metals (Tan, 2014). Chemical chelation with humic acid preventing Al toxicity in sand soil containing Al increased dry matter production of corn from 32.5% to 42.5% compared to those with no HA, and the plants appeared healthy and green. The beneficial effect of HA was also reflected in the Al and P content of the corn leaves. Leaf-Al concentrations increased linearly with increased Al treatments at 0 units HA but decreased from a high of 86.6 μg g^{-1} at 50 units Al and 0 unit HA to 60.5 and 57.4 μg g^{-1} with 100 and 350 units HA, respectively (Tan and Binger, 1986).

The indirect role of HS on plant growth mainly results from their ability to form stable natural chelates or complexes with metals in soil (Stevenson, 1994). In general, actions of HS on soil result from a singular ability of HS to form stable complexes with multivalent metals like Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+} or Mn^{2+} among others (Senesi, 1992). The ability of HS to form stable natural chelates (or complexes) with multivalent cations has been studied for many years and its relevance in the transport and dynamics of metals in natural ecosystems has been well established (Tipping, 2002).

11.4.1.3 Effect on soil biological properties

A large number of organisms live in the soil that perform a variety of functions for their growth and reproduction, which make soil behave like a living entity able to photosynthesize, respire and reproduce. So, soil becomes a dynamic body for the activity of soil organisms and any changes caused by soil organisms have their impact on soil fertility and productivity. Although the soil biota occupies a very small fraction of the total soil volume (<0.5 %), it has tremendous influence on soil properties and soil processes (Osman, 2013). Soil heterotrophic organisms play a crucial role in decomposing the organic matter, retaining C and N in their biomass and releasing CO_2, CH_4, NO_2 back to the atmosphere (Le Mer and Roger, 2001; Trumbore, 2006; Zhu *et al.*, 2013). Due to their gradual decomposition kinetics, inputs of HS continuously provide nutrients utilized by microbial communities (Murphy *et al.*, 2007). For that reason, HP tend to act as slow-release biostimulants for microorganisms. The review by Pukalchik *et al.* (2019) summarized some examples of how HP impact specific soil microbial and enzyme activities, with associated soil processes given and a link to the impact on soil health.

The mechanism of how the HP influences the biological properties may be linked to the redox active functional groups of HP, which can be readily oxidized and subsequently act as electron donors for bacterial respiration, with subsequent changes in CO_2 and CH_4 production (Lovley *et al.*, 1999; Coates *et al.*, 2002). HP can have either stimulating or toxic effects on microorganisms, fungi and soil fauna (Pukalchik *et al.*, 2019). When applied as soil amendments, HP provide a certain influence on the biological activity of the soil as a whole.

A study by Bhuma and Selvakumari (2003) on the effect of HA on the biological properties of soil, with and without fertilizers on green gram (mung bean), clearly indicated a significant effect of application of HA with 100% recommended dose of fertilizer on enhancing the microbial population of the soil, which is linked to an a marked increase in the phosphatase activity, as compared to the recommended dose of fertilizer alone. They attribute a favorable effect of HA on microbial population to the stimulating effect of HA on the growth of microorganisms.

The crude humate application stimulates microbial count and dehydrogenase activity in the rhizosphere of lentil plants, compared to a full dose of mineral fertilizers (El-Tahlawy and Hassanen, 2021).

The results reported by Gryndler *et al.* (2005) indicate that HA may represent a stimulatory component of the soil environment with respect to arbuscular mycorrhizal fungi because HS stimulated root colonization of maize and production of extraradical mycelium by the mycorrhizal fungus. In a 3-year consecutive experiment to investigate the continuous effects of HA on the microbial diversity and enzyme activities of soil under continuous cropping peanut was conducted by Li *et al.* (2019), the HA changed the community structure of soil microorganisms, increasing beneficial microorganisms and reducing harmful microorganisms, which is favorable for plant growth. In addition, HA changed the activities of sucrase, urease and phosphatase in the soil and enhanced the metabolism of substances in the soil and affected soil microbial activity.

Due to the complexity of microbial communities in soils to specify the effects of HS, artificial pure cultures of bacteria and fungi for growing individual species are usually used to elucidate the interaction between HS and fungal and bacterial metabolism, as well as the biological effects produced by HS themselves (Crowther *et al.*, 2018). Overall, HS produce both beneficial and non-beneficial effects on the microorganisms isolated from soil habitats and cultivated on different artificial media, such as Czapek's medium with sugars (Tikhonov *et al.*, 2011) and potato dextrose agar (Loffredo *et al.*, 2008) and under other artificial conditions. Humic acids at a concentration of 0.1 g l^{-1} stimulated the growth of the soil and intestinal bacteria strains on Czapek's medium, probably, acting as a regulator of the cell metabolism (Tikhonov *et al.*, 2010).

Some authors propose that humic acid is a source of food and energy for microorganisms (Burges and Latter, 1960; Prát, 1960). This is supported by Mathur and Paul (1966) who indicate that *Pseudomonas sinuosa*, *Actinomyces* spp., and some bacteria can use HA as a source of C and N. Neelankantan *et al.* (1970) reported similar findings with *Aspergillus* spp. and *Streptomyces* spp. Using ^{15}N-labeled humic acid, Andreyuk *et al.* (1973) found HA to be a source of N for *Bacillus megaterium*, *Pseudomonas fluorescens*, *A. globisporus* and *Mycobacterium citreum*.

Humic acid in the form of sodium humate and FA markedly increased the growth and efficiency of nitrogen fixation of *Azotobacter chrooeoccum*. Fulvic acid proved more effective than the sodium humate. The effectiveness of these substances was more pronounced in increasing the number of cells than affecting the nitrogen fixation. The increases in growth and nitrogen fixation were in direct proportion to the quantities of sodium humate and FA applied up to 500 and 700 ppm, respectively (Bhardwaj and Gaur, 1970).

Experiments with two species of legume nodule bacteria, *Rh. trifolii* and *Rh. meliloti*, show that the addition of natural HA to a synthetic (sugar-inorganic salts-nitrate) medium produces a marked increase in bacterial numbers and oxygen consumption. The growth obtained, over the concentration range of 0–600 ppm dry matter, was nearly proportional to the quantity added. In the absence of HA, and where a very pure sugar was used, no appreciable growth occurred (Allison and Hoover, 1936).

Studies on the effect of FA on the growth of an ectomycorrhizal fungus, *Pisolithus tinctorius*, as conducted by Tan and Nopamornbodi (1979) show evidence of definite absorption of FA. Moderate amounts of FA (640 ppm) seem to have stimulated the growth and dry weight content of the ectomycorrhiza, cultivated at pH 7.0 and 4.0.

Visser (1985) investigated the influence of HS on numbers and activities of microorganisms belonging to various physiological groups and found higher microbial counts in the media containing HA, indicating a stimulatory effect, particularly for the groups of denitrifiers, aerobic cellulose decomposers and starch decomposers, proteolytic organisms, pectinolytic organisms and anaerobic cellulose decomposers. They suggest the effect could have implications on the activity of organisms in environments in which HA are normally present, such as soils and natural waters.

11.4.2 Direct effects of humic substances on plants growth and yield

The knowledge of the mechanism of action of HS is rather fragmentary and it has not been integrated in a comprehensive model yet (Mora *et al.*, 2014), although it has been extensively studied for many years. In general, all findings reported so far clearly show that direct effects mediated by HS involve several different, but probably interconnected, mechanisms integrated into a complex network of events occurring at both transcriptional and post-transcriptional levels (Mora *et al.*, 2014).

A plant biostimulant is any substance or microorganism applied to plants to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content (du Jardin, 2015). Biostimulants are available in a variety of formulations with varying ingredients but are generally classified into three major groups based on their source and content (Kauffman *et al.*, 2007). These groups include HS, hormone-containing products and an amino acid-containing products. Humic substances are biostimulants and many reports in the literature reveal that HS can, under certain conditions, improve plant growth, physiological processes and yield (Table 11.1).

Direct effects require uptake of HS-macromolecules into the plant tissue resulting in various biochemical impacts at the cell wall, membrane or in the cytoplasm (Chen *et al.*, 2004). Changes in color of certain organs in the plant were observed to detect the absorption and incorporation of HS to plant tissues (Chen and Aviad, 1990; Sánchez-Andréu *et al.*, 1994). The study by Prát and Pospíšil (1959) demonstrated that if radioactive HA is added to water or to nutrient solution of sugarcane and corn, a radioactivity appears in the roots and later weakly in the leaves, assuming it penetrates into the plant slowly and it is not accumulated but spreads throughout the plant slowly from the roots. Aso and Sakai (1963) conducted an experiment to obtain information about absorption of HA through plant roots, and to clarify the role of HA in the nutrition of plants. They found that the homogenates of seedling parts of mulberry tree previously immersed in ammonium humate were colored brown and so it was claimed that humate had penetrated the tissue. Furthermore, they found that growth of treated mulberry trees is substantially improved when they are as compared those trees treated with ordinary nitrogen fertilizers.

One of the major impacts of HS on plant growth is the reinforcement in nutrient uptake and the elongation of the lateral root growth, often recognized as 'auxin-like effect', which is a result of the induction of ATPase activity according to the acid growth theory in the plasma membrane (Maggioni *et al.*, 1987; Zandonadi *et al.*, 2007). The stimulating action of HS is attributed to hormone-like activity (Canellas *et al.*, 2008; Nardi *et al.*, 2016). The hormone-like feature (auximones) was first reported by Bottomley (1917). Such a characteristic has subsequently been assigned by many authors (Trevisan *et al.*, 2010, 2011; Jindo *et al.*, 2011), confirming the presence of physiologically active indoleacetic acid (IAA) concentrations in HS.

Root growth and function play a fundamental role in nutrient uptake especially in organic farming where nutrients are often available in soil solution at relatively low concentrations. Therefore, an extensive root system is a pre-requisite to guarantee a sufficient absorption of nutrients to meet crop demand in organic farming. In this respect, HS could stimulate the expression of the early auxin-responsive genes IAA5 and IAA19 and induce plasma membrane H^+-ATPase synthesis, activity and expression promoting lateral root induction and root hair growth (Canellas *et al.*, 2008; Zandonadi *et al.*, 2010). It has been proposed that HS could promote root growth in a way similar to auxin by modulating not only the plasmalemma (Canellas *et al.*, 2002; Quaggiotti *et al.*, 2004), but also the tonoplast proton pumps (Zandonadi *et al.*, 2010). The elongation and differentiation zone of roots include small, dense meristematic cells that are in continuous metabolic activity and are susceptible to lateral root formation. HS were found to have marked effect on the emergence of lateral roots and the hyper-induction of sites of lateral root emergence upon HS treatments have been observed (Canellas *et al.*, 2002; Zandonadi *et al.*, 2007). The availability of micronutrients such as iron can be improved with HS, not only by chelation, but also by promoting the root capability to uptake nutrients from the soil solution (Aguirre *et al.*, 2009; Zanin *et al.*, 2019).

Humic substances could make changes to primary metabolism that are essential for plant survival, growth and reproduction such as glycolysis. HS affected the enzyme activities

Table 11.1. Response of some crops to application of humic substances (HS).

Crop	HS treatment	Response	Reference
Corn (*Zea mays*)	Humic acid (HA), fulvic acid (FA) and water-soluble fractions	Defense mechanisms against biotic and abiotic stress from heavy metals Xanthine and xanthine-oxidase system	Cordeiro *et al.* (2011); García *et al.* (2016)
Wheat (*Triticum aestivum* L.)	HA in presence and absence of nitrogen	Significant increase in the growth of roots and shoots as well as moisture uptake and N content of the seedlings	Malik and Azam (1985)
Winter wheat (*Triticum aestivum* L.)	The humic preparation prepared from vermicompost by alkaline extraction	Decrease the toxic effect of herbicide, improve the supply of soil with mineral nutrients, and increase the crop yield	Bezuglova *et al.* (2019)
Faba Bean (*Vicia faba* L.)	HA or FA in combination with compost tea as soil drench	The combination positively affected several metabolic processes, enhancing plant growth and development via playing a major role in increasing photosynthesis, endogenous hormones, nutrients uptake and protein synthesis	Ali (2015)
Faba bean (*Vicia faba* L.)	Commercial HA plus amino acids were used as foliar treatments in a field experiment	Increased growth and mineral content, 100-seed weight (by 26%) and decreased the damage by chocolate spot and rust diseases	El-Ghamry *et al.* (2009)
Dry bean (*Phaseolus vulgaris* L.)	Potassium humate combined with micronutrients and chitosan; foliar spray. field experiment	Increased yield by 25–35% Foliar application with zinc combined with HA and chitosan is the best treatment for dry bean production on normal or delayed sowing	Ibrahim and Ramadan (2015)
Lentils (*Lens culinaris* Medik)	Rates of crude humate extracted from compost against rhizobacteria or N-fertilization	High rate of humates recorded maximum values with respect to the plant height, shoot dry weight and leaf area	El-Tahlawy and Hassanen (2021)
Cucumber (*Cucumis sativus*)	FA from 20 to 2000 ppm added to Hoagland solution	Increased growth and development, nutrient uptake and flowering	Rauthan and Schnitzer (1981)
Sunflower (*Helianthus annuus* L.)	HA soil application, foliar HA spray and a combination of both	HA application as both soil and foliar spray led to obtain the highest values of plant height, N P K in leaves, chlorophyll content, Proline, seed yield as well as seed oil % and protein HA application ameliorate negative effects of salinity on sunflower	Mourad *et al.* (2020)
Sugar beet (*Beta vulgaris* L.)	Foliar application with HA, FA and potassium humate and nitrogen fertilization rates	Fulvic acid surpassed the other humic substances in the content of sucrose, extractable sugar, and purity percentages; also yield, and lowest juice impurities	El-Hassanin *et al.* (2016)
Tomato, (*Lycopersicon esculentum* L)	Compared concentrations and forms of humic acids (K-, Na- and NH_4-humates) in hydroponic systems	HA positively influence germination of tomato seeds Effects depends on the humate form and material used for the extraction (peat, coal) HA improved plant growth depending on the concentration and frequency of treatments	Thi Lua and Böhme (2001)

related to glycolysis and the tricarboxylic acid cycle in different ways, depending on humic molecular size, molecular characteristic and concentration (Nardi *et al.*, 2007). The high level of transcription of genes involved in primary metabolism in *Arabidopsis thaliana* supported previous studies about the physiological effects of HS on plant metabolic pathways (Trevisan *et al.*, 2011). HS also interfere with secondary metabolism by altering gene expression and changing the content of chemical compounds in plant cells, such as those related to the Krebs cycle, metabolism of nitrate and phosphorus, glycolysis and photosynthesis (Lotfi *et al.*, 2018; Roomi *et al.*, 2018). Plant secondary metabolism produces a large number of specialized compounds that do not directly aid in the growth and development of plants but are required for the plant to survive in its environment and under biotic and abiotic stress. Several reports have been published on the impact of HS on the growth of pepper, common beans, rice, tomato, corn, sorghum and cucumber under these stress conditions (Jindo *et al.*, 2020).

A study on growth, nitrogen metabolism and photosynthesis in maize treated with various HS-products was conducted by Ertani *et al.* (2011). They found that all HS-products increased root and leaf growth. Furthermore, there was also an increase in glutamine synthetase and glutamate-synthase enzyme activities, phenol content and protein content as well as an increase in chlorophyll content, glucose, fructose and rubisco enzyme activity, suggesting a positive role of HS in the photosynthetic process. To evaluate the effect of HS on leaf carbohydrate metabolism. Merlo *et al.* (1991) treated maize plants grown in nutrient solution with or without 100 mg organic carbon L^{-1} of HS and after 14 days of growth, apical and basal segments from the third leaf were collected and analyzed for sugar content and for the activities of enzymes involved in the synthesis and breakdown of starch and sucrose. The results showed that HS may affect both the level and percentage distribution of sugars of maize leaves; these effects seem to be mediated by changes in the activities of enzymes involved in carbohydrate metabolism. Leaf starch content decreased in plants treated with HS, whereas the level of soluble sugars concomitantly increased. The decrease of starch was accompanied by an enhanced activity of amylase, whereas the activity of ADP-glucose pyrophosphorylase was not affected. Activities of invertases and sucrose synthase were stimulated by HS treatment in apical tissues and inhibited when HS were applied to basal tissues.

The N metabolism is the basis of amino acids, proteins, enzymes and nucleotide formation. The enhancement of N uptake/assimilation and N metabolism in plants treated with HS had been well documented (Canellas and Olivares, 2014). The rate of nitrate uptake by barley seedlings as well as activities of nitrate reductase, glutamate dehydrogenase and glutamine synthetase were stimulated by HS (Albuzio *et al.*, 1986; Piccolo *et al.*, 1992). The stimulation of nitrate uptake by HS cannot be explained by an effect on the primary transport of solutes, but it should instead decrease pH at the root surface, thus facilitating the $H^+/NO^{-}{}^{3}$ symport (Nardi *et al.*, 2000). Besides $NO^{-}{}^{3}$ uptake, HS also induce changes on N cell metabolism as indicated by an increase in the activities of glutamine synthetase and glutamate synthase, which are key enzymes involved in N assimilation, in maize treated by HS (Ertani *et al.*, 2011). On the other hand, Panuccio *et al.* (2001) documented that HS stimulate only ammonium uptake and influence ammonium assimilation in two different coniferous species treated with humic fraction, with a high molecular weight extracted from a soil. Combined application of FA or HA with compost tea on faba bean caused a remarkable increase in photosynthetic pigments, flowering duration, endogenous phytohormones (IAA and gibberellin), while abscisic acid content and total abscission were decreased compared to untreated plants (Ali, 2015).

Reactive oxygen species (ROS) are small molecule metabolites of oxygen that tend to participate in redox reactions because of their high reactivity. ROS is being involved in root cell elongation via Ca^{2+} channel activation which is a key step in the regulation of other important processes, including antistress regulation and hormone signaling (Mori and Schroeder, 2004; Miller *et al.*, 2010). ROS can influence the expression of a number of genes and signal transduction pathways, suggesting that cells have evolved strategies to utilize ROS as signals that control various biological programs (Desikan *et al.*, 2005). Several reports of HS direct effects on the generation of ROS and their activity have been documented. Likewise, Cordeiro *et al.* (2011) reported the application of HA was mediated by ROS production, evidenced primarily by increased fresh and dry weight of lateral roots of corn. The increase in ROS production may have induced the expression of antioxidant genes such as catalase, decreasing the oxidative effects of the ROS necessary for root growth. Garcia *et al.* (2016) suggested that the whole effects caused by HS on root phenotype, both at macromorphological and micromorphological levels, may involve two pathways: one hormonal dependent (micromorphological effects) and the other ROS dependent (macromorphological). HS stimulate $O^{2\bullet-}$ production through regulation of the xanthine/xanthine-oxidase system, which is an important defense mechanisms against biotic stress (Berner and Van der Westhuizen, 2010) and abiotic stress from heavy metals (Corpas *et al.*, 2008).

11.5 Factors that impact the beneficial effects of humic substances

The magnitude of the impact of HS is determined by several factors, including the source of the HS, species and age of plants, method and rate of application, the measured parameter and soil type (Vaughan and Malcolm, 1985; Rose *et al.*, 2014).

Various methods are used in application of HS to plants, for example foliar, application to the soil in liquid state, direct application to the soil as solid state, fertigation and soaking (Sánchez-Andréu *et al.*, 1994). Foliar application of HA significantly affected plant height in canola, with the tallest measurement being achieved with 2% foliar application of HA and the shortest plant height being obtained under zero HS addition leading to a decrease nitrogen application in soil, which can be the most important for the non-pollution of soil by nitrogenous fertilizers (Sani, 2014). The application of solutions of HA directly to soil under greenhouse conditions can also enhance the dry matter yields of crops such as *Triticum vulgare*, *Trifolium alexandrinum* and *Sesbania aculeata* (Gaur and Bhardwaj, 1971). Externally supplied HS only have a substantial effect when added to soils of low organic matter content or to a nutrient solution, giving the greatest growth response as compared to a high organic matter soil that give a little growth response or even a slightly negative response (Lee and Bartlett, 1976). More recently, a combined application using more than one method has been studied for crops such as coffee, maize or soybean. These studies demonstrated that soil and foliar HA application leads to improved physiochemical properties of soil, increased the growth, yield and chemical constituents of plants, and also reduced the additional mineral dose requirements by 50% along with a reduction in pollution and costs of production (Mahmoud *et al.*, 2011; El-Shafey and Zen El- Dein, 2016; Kishor *et al.*, 2021). It noted that soil application of HA was better in influencing the microbial population than foliar spray and seed soaking, indicating highly beneficial impact due to soil application of HA (Bhuma and Selvakumari, 2003).

Plant responses to HS also appear to be highly dependent on plant species. Overall, the growth response of monocotyledonous to exogenously applied HS appears to be greater than for dicotyledonous plants (Canellas *et al.*, 2015). Furthermore, plant physiological responses to HS isolated from brown coal (e.g. lignite, leonardite, subbituminous coals) are less than those observed in response to the addition of HS isolated from peat, composts or vermicompost (Canellas and Olivares, 2014). Also, Azcona *et al.* (2011) previously confirmed that a better crop response in terms of growth, phenological development, and photosynthetic activity of pepper was observed using humic-like substances obtained from compost or vermicompost than from humic extracts from leonardite due to the chemical structure of the HS. Mindari *et al.* (2018) found that HA from peat increased plant biomass weight, plant roots, grain number of tillers and chlorophyll content more than from compost, manure and coal. However, HA from manure or compost was more effective in improving plant growth and plant uptake than humic acid from coal.

The studies were conducted with different HS concentrations and showed a bell-shaped dose-response curve. The optimum concentration was dependent on the specific plant and mode of application (Canellas *et al.*, 2015). However, increasing doses of HA significantly affected the neck diameter, the plant height and the number of leaves of lettuce plants but not the plant weight (Tüfenkçi *et al.*, 2006). There were also significant effects from increasing the doses of HA on the N, P, K, Ca, Mg, Fe, Zn, and Cd content of lettuce plants. Their Cd, Cr, and Co content decreased with increasing HA doses. HS action in plants have shown that ROS production, particularly the production of H_2O_2, is dependent on the concentration of HS. It is noted that when rice plants treated with moderate HS concentrations, ROS production does not cause lipid peroxidation, thereby favoring the processes of growth and lateral root formation. On the other hand, when plants are treated with elevated concentrations of HS, a high rate of ROS production can lead to lipid peroxidation and negatively affect the growth and root development (Berbara and García, 2014). Recently, El-Tahlawy and Hassanen (2021) found that a high dose of crude humate, extracted from compost, significantly affected the growth of lentil plants depending on the lentil variety under calcareous soils conditions.

11.6 Conclusion

Humic substances represent a major fraction of the SOM as a final stage of a complex interaction between non-living organic matter and microbial communities as a result of humification processes. Humic-based soil amendments are accessible due to the industrial request for efficient and environmentally friendly soil supplements. The action of HS on the physical, chemical and biological properties of soil has been extensively demonstrated as indirect effect on plant growth beside their direct role in sustaining plant growth. Humic substances are plant tonic and modulate the physiological and biochemical processes of plants by triggering multiple interconnected signaling pathways. Effects on root architecture, such as induction of lateral roots and root hairs, may be accompanied by changes in the biochemistry of energy generation and transport system across plasma membranes. Primary and secondary plant metabolisms of above- and below-ground tissues are recognized to be affected by HS. The impact of HS depends on several factors, such as the extracted material, species and age of plant, method and rate of application, the measured parameter, as well as soil type. Further research is required to clearly understand the stimulatory role of HS on the growth, development and yield of various crop plants.

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12 Growth-stimulating Effects of Chitosan Biopolymer in Plants

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Abstract

In recent years, searching for biological strategies to prevent harmful chemicals from being used in agriculture contributed to the research on the application of biopolymers. Among the biomaterials studied, the most promising findings have been reported from those focused on chitosan. Chitosan is a chitin-modified natural biopolymer that exists in the crustaceans' exoskeleton (e.g. shrimps and crabs) and the cell wall of fungi. Because of the excellent biocompatibility, biodegradability, non-toxicity, antimicrobial properties, plant growth and immunity promoting activities, along with the economic benefits, this biopolymer is a potential solution for sustainable farming systems. Plant growth parameters are significantly enhanced by the application of chitosan. It stimulates plant growth and immunity through regulating various physiological processes such as cell elongation, cell division, protein synthesis, enzymatic activation and uptake of nutrients by roots, which ultimately contribute to an increase in crop yield. The effects and mechanisms of chitosan as a plant growth stimulant as well as the prospects for its use in sustainable agriculture under the changing climate are highlighted in this chapter.

12.1 Introduction

Chitosan is a natural biopolymer. It is a linear amino-polysaccharide containing *N*-acetylglucosamine and glucosamine units, generated by alkaline deacetylation of chitin obtained from the crustacean exoskeletons (e.g. crabs and shrimps) and the cell wall of some fungi (Badawi and Rabea, 2011). Chitin is the second most abundant natural polysaccharide present in the world after cellulose (Rinaudo, 2006). Because of its outstanding biocompatibility, biodegradability, anti-cancer, antioxidant, non-toxic, antimicrobial effects, plant growth-promoting activity, and economic benefits, chitosan is recognized as the most valuable natural material in terms of its potential future applications. The chemical structure of chitosan is readily converted to generate related polymers for specific uses (Dash *et al.*, 2011; Shukla *et al.* 2013). Chitosan research has increased in the recent decade as a result of its wide range of applications in various fields, including plant sciences. Chitosan is mainly employed to control abiotic and biotic stresses in plants. The very first study of the efficacy of chitosan as an anti-pathogen in plants, which described the fungicidal activity of chitosan of several fungi, was demonstrated by Allan and Hadwiger (1979). Using both dicotyledonous and monocotyledonous plants, the application

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of chitosan to enhance the defense system of plants is the main focus of this biopolymer in multiple research fields (Barber *et al.*, 1989). Chitosan induces the plant defense pathways against the pathogen, thereby stimulating plant immune responses (Allan and Hadwiger, 1979; Chirkov, 1994; Li *et al.*, 2013; Kaya *et al.*, 2017).

In recent years, the ever-growing global demand for food, the continuing climate change, the risky consumption of agricultural land, and the rising market interest in safe, high-quality, healthy and eco-friendly food commodities have driven the search for alternate biological approaches to satisfy these challenges. Among the current alternatives under research, it is possible to boost the growth and production of agricultural plants by avoiding the application of large quantities of synthetic chemical fertilizers and hazardous agricultural practices which create severe environmental imbalances and have catastrophic consequences on human health. Among the biomaterials investigated, chitosan biopolymer-based compounds produced the best results. The utilization of chitosan as a biostimulant has been investigated in a variety of crop species, including cereals, vegetables, fruits, flowers, and ornamentals (Chakraborty *et al.*, 2020). Several experiments have demonstrated that foliar application and seed treatment with chitosan enhances plant growth, productivity, increases the quality and shelf-life of fruits, and promotes the secondary metabolites synthesis in plant cells such as polyphenols, lignins, terpenoids, flavonoids and phytoalexins (Sakif *et al.*, 2016; Emami Bistgani *et al.*, 2017; Xoca-Orozco *et al.*, 2017; Mukta *et al.*, 2017; Rahman *et al.*, 2018, 2019; Mehebub *et al.*, 2019; Chakraborty *et al.*, 2020). It affects the permeability of seed plasma membranes, raises the concentration of sugar and proline, and improves peroxidase (POD), tyrosine ammonialyase (TAL), phenylalanine ammonia lyase (PAL) and catalase (CAT) activities (Guan *et al.*, 2009). It influences different reactions in plants based on the concentration and structure of chitosan biomolecules (Lin *et al.*, 2005; Limpanavech *et al.*, 2008; Kananont *et al.*, 2010), plant species and their developmental stages (Ohta *et al.*, 2004; Pornpienpakdee *et al.*, 2010). This chapter discusses the role and mode of actions of chitosan as a growth stimulator of plants and discusses its future prospects in agriculture.

12.2 Chitosan and its derivatives

Chemical alterations of chitosan are gradually being investigated as they have the ability to make new applications. It is generally used in areas such as biotechnology, cosmetics, pharmaceuticals and agriculture in relation to its unique characteristics, including biodegradability, biocompatibility and non-toxicity to mammals. Despite its advantages, its porosity, low surface area and limited solubility make it difficult to use. Poor solubility is the major restricting factor for chitosan application in biology. Its solubility is restricted to a pH higher than 6.5 when chitosan begins reducing its cationic nature (deAlvarenga, 2011). If water-soluble chitosan is easily accessible, the biological and physiological abilities are predicted to increase considerably. It is a linear biopolymer comprised of two sub-units: namely D-glucosamine and *N*-acetyl-D-glucosamine, which are bound to each other by 1,4-glycosidic bonds (Muzzarelli, 1973; Rinaudo, 2006). The structure of chitosan contains three rings. Three functional groups, primary and secondary hydroxyl, and several amine groups are seen in chitosan. Chitosan also comprises beta-1,4 glycosidic bonds. Oxygen atoms (O^1 and O_2) are connected to C_6–C_7 and C_{10}–C_{13} atoms (Shahidi and Abuzaytoun, 2005). Such functional groups permit them to easily undergo chemical alterations. Modification may be accomplished using chemical or physical methods like grafting, cross-linking, composite or substituent incorporation. Chitosan has several groups of reactive amino side chains that enhance the applicability of chitosan and offer the potential to develop different derivatives of chitosan.

Oligochitosan is a major water-soluble chitosan derivative. Like other polysaccharides, chitosan may also be hydrolyzed by biodegradants because of its poor glycosidic linkages. Various techniques such as acid hydrolysis (Aljbour *et al.*, 2019), enzymatic hydrolysis (Kaczmarek *et al.*, 2019), oxidative degradation (Ma *et al.*, 2014) and ultrasonic degradation (Kasaai *et al.*, 2008) can be utilized to produce oligochitosan. Due to their amphiphilic properties, chitosan derivatives have significantly improved their solubility and capacity to self-assemble micelles and aggregates from hydrophobic moieties through intermolecular and intramolecular interactions.

It serves as an excellent drug delivery model and enhances the transfection of gene therapy (Dowling *et al.*, 2011).

Chitosan oligosaccharide (COS) may be generated by chitosan depolymerization, allowing it to be used at pH 7. Enzymes, chemicals or irradiation degrade chitosan to produce these low-molecular mass molecules. The bioactivity of COS is primarily determined by its structure and physico-chemical properties (Xu *et al.*, 2014). The COS has many biological properties, such as anti-inflammation (Yousef *et al.*, 2012), immunostimulation (Zhang *et al.*, 2014), anti-tumor (Park *et al.*, 2011), anti-obesity (Huang *et al.*, 2015), anti-diabetic (Kim *et al.*, 2014), anti-hypertension (Park *et al.*, 2003a), anti-Alzheimer's disease (Byun *et al.*, 2005), tissue regeneration promotion (Wang *et al.*, 2016), drug and DNA delivery enhancement (Aied *et al.*, 2013; Sanna *et al.*, 2014), antimicrobial (Kittur *et al.*, 2005; Wang, Y. *et al.*, 2007), anti-oxidation (Fernandes *et al.*, 2010; Park *et al.*, 2003b) and calcium-absorption enhancement (Jung *et al.*, 2006).

Hydroxyalkyl chitosan is produced when the chitosan interacts with epoxides. Self-assembled glycol-based nanoparticles of chitosan have been developed as drug carriers (Park *et al.*, 2006). Another cationic chitosan analog that is water soluble is trimethylchitosan ammonium. It is produced by chitosan quaternization or the reaction of chitosan with methyl iodide and sodium hydroxide in the presence of low acetyl content. It has flocculating properties like kaolin dispersions, making it important for the processing of paper (Zargar *et al.*, 2015). Polymer immobilization of the thiol group has improved the mucoadhesive properties of chitosan. Thiolated chitosan increases permeation and exhibits outstanding coherent characteristics for prolonged and controlled dispersion of embedded therapeutic materials (Kafedjiiski *et al.*, 2005).

N-Carboxymethyl chitosan is a derivative of water-soluble chitosan with various applications in the medical, food and gene therapy fields (Khanjari *et al.*, 2013). It is produced by the reaction of chitosan and glyoxylic acid. It is needed to develop several drug delivery methods of protein such as cross-linked hydrogels, super porous hydrogels, and hydrogels sensitive to pH (Yin *et al.*, 2007). The anionic analog possessing amphoteric properties is N-methylene phosphonic chitosan (NMPC). The NMPC, besides Ca^{2+} and other transition metals (Cu^{2+}, Cd^{2+}, Zn^{2+}), has cation-binding potency (Xiao *et al.*, 2012). At the nitrogen atom, N-arylated chitosan provides hydrophobic aspects and also nucleophilic and hydrophilic characteristics (Sajomsang, 2010).

Nano-chitosan can be produced by precipitation or coagulation, ion cross-linking, covalent cross-linking, and emulsion droplet coalescence processes. Chitosan is degraded to lower molecular weight chitosan utilizing varying concentrations of H_2O_2 (Zhao and Wu, 2006; Wang, X.Y. *et al.*, 2007; Huang *et al.*, 2009). Different tripolyphosphate (TPP) concentrations were then added to develop nano-chitosan. Nano-chitosan is a sustainable component of outstanding physicochemical characteristics, rendering it an eco-friendly material of superior quality. It is also utilized as a drug carrier of regulated release for transferring genes in artificial organelles for conferring antibacterial effects and immune prophylaxis (Ting and Shen, 2005).

Because of their structural resemblance to heparin, chitosan sulfates have been found to exhibit anticoagulant and hemagglutination action, as well as anti-sclerotic, antioxidant, antiviral, antibacterial and enzyme inhibition effects. They also have outstanding capacities of adsorption and are utilized for metal ion recovery. For mercury recovery and uptake of precious metal, sulfur compounds are grafted upon chitosan. Sulphonic chitosans are the flocculants of fine metal oxide (Xing *et al.*, 2005).

The lactic-glycolic acid chitosan hydrogels, another active derivative of chitosan, demonstrate stronger interaction among water and the chains of chitosan. These can be produced without a catalyst through grafting of D,L-glycolic or lactic acid onto the chitosan directly. This has valuable applications in the biomedical field for wound dressings and drug delivery processes (Abd-Alla and Wafaa, 2010). Sugar-bonded chitosan has distinctive receptor-binding properties and is being investigated for its antiviral activity (Morimoto *et al.*, 2001). In order to enhance drug delivery mechanisms, cosmetics, and also textile effluent disinfection, a chitosan-comprising pendant of cyclodextrin has been developed (Prabaharan and Mano, 2006).

Chitosan modifications with phosphorylcholine compounds provide anticoagulant effects. Enzymatic grafting of phenolic compounds on chitosan using tyrosinase to confer water solubility in particular circumstances has been reported (Kumar *et al.*, 1999). Utilizing grafted chitosans like galactosylated chitosan, PEG-chitosan, etc., DNA delivery also takes place (Park *et al.*, 2000). The majority of polyacryl grafted hydrogels are temperature or pH sensitive (Mahdavinia *et al.*, 2004). Salts of chitosan (e.g. format, acetate, lactate, malate, glyoxylate, citrate, pyruvate, tartarate, malonate, glycolate and ascorbate) are water soluble. Chitosan is mostly desirable for its charges and various functional groups which allow it to be utilized in many variants through implementations in various fields (Prashanth and Tharanathan, 2007). Structural properties of chitosan are critical for their stimulating activities on plants and other organisms.

12.3 Plant growth-stimulating effect of chitosan

Chitosan stimulates growth in a variety of crop plants, including cereals, pulses, spices, vegetables, fruits, flowers and ornamentals. It also increases yield as a consequence of plant growth stimulation. Chitosan has a strong impact on the rates of growth of shoots, roots and flowering, as well as a number of flowers (Zohara *et al.*, 2019). Hydrophilic chitosan molecules reduce stress damage in plant cells through decreasing water content and speeding up the activities of multiple biological macromolecules. Its growth-stimulating effects on various crop plants are described in the following sections.

12.3.1 Effect on cereal crops

Chitosan enhances the growth and development indexes of several cereal crops. Phothi and Theerakarunwong (2017) reported that rice yield accelerated substantially after application of a chitosan solution. Application of the formulation of chitosan increased shoot and root length and rice grain yield (Vasudevan *et al.*, 2002). Relative to other approaches, seed treatment with chitosan solution prior to sowing produces more panicles and seeds in rice plants (Boonlertnirun *et al.*, 2008). In broad-scale field trials, spraying of chitosan at various growth phases influences the growth and productivity of wheat by enhancing the yield parameters, including spike number and the grains per spike (Abdel-Aziz *et al.*, 2016, 2018; Samdurkar *et al.*, 2021). Abdel-Aziz *et al.* (2018) observed that nano-chitosan combined with NPK (nitrogen + phosphorous + potassium) fertilizers significantly promoted the crop index, harvest index and yield mobilization index parameters of wheat. The growth and production of wheat plants improved by chitosan-polimethacrylic acid–NPK nanoparticles (Abdel-Aziz *et al.*, 2016). The combined actions of chitosan as well as plant growth-stimulating rhizobacteria enhanced the growth, germination and nutrient absorption of maize plants (Agbodjato *et al.*, 2016). Seed priming with varied chitosan concentrations increases the growth and physiological variances of two inbreed lines of maize, as reported by Guan *et al.* (2009). The seedling growth, development and protease and α-amylase activities of maize were considerably boosted by chitosan-Cu (Copper) nanoparticles. The nano-formulations were speculated to allow seed penetration and consequently increase the seed's metabolism (Saharan *et al.*, 2016). Chitosan oligosaccharides substantially improved the growth and production of rice (Tham *et al.*, 2001), wheat (Zou *et al.*, 2017), barley (Luan *et al.*, 2006) and soybean (Tham *et al.*, 2001; Luan *et al.*, 2006). Chitosan enhanced the microspore embryogenesis and regeneration of plantlets of canola effectively (Ahmadi and Shariatpanahi, 2015). Table 12.1 summarizes the growth-stimulating effects of several types of chitosan on cereal crops.

12.3.2 Effect on vegetable, spice and pulse crops

Chitosan has been studied extensively for its potential application in the growth and development of more than 20 plant species of vegetable, spice and pulse crops (Table 12.2). Falcón-Rodríguez *et al.* (2017) showed that chitosan increases the *in vitro* growth of plantlets and improves potato yield by micropropagation approach. Chitosan improved heights of plants, leaf areas and canopy width of chili pepper (Chookhongkha *et al.*, 2012). The improvement

Table 12.1. Effect of chitosan biopolymer on growth and development of cereal crops.

Species	Chitosan types	Method of application	Effects	References
Rice (*Oryza sativa* L.)	Chitosan	Seed treatment	Enhanced growth parameters	Garude *et al*. (2019)
	Nano-chitosan	*In vivo*	Enhanced growth parameters	Divya *et al*. (2019)
	Chitosan	*In vivo*	Enhanced growth parameters and photosynthesis rate	Phothi and Theerakarunwong (2017)
	Chitosan	*In vivo*	Enhanced growth parameters, photosynthesis rate, and yield	Theerakarunwong and Phothi (2016)
	Oligochitosan	Foliar application	Enhanced growth parameters	Chamnanmanoontham *et al*. (2015)
	Chitosan	*In vivo*	Enhanced growth parameters and yield	Toan *et al*. (2013)
	Chitosan	*In vivo*	Enhanced growth parameters	Pongprayoon *et al*. (2013)
	Chitosan	Seed treatment	Enhanced growth parameters and yield	Boonlertnirun *et al*. (2008)
	Chitosan	Soil amendment	Enhanced growth parameters and yield	Vasudevan *et al*. (2002)
	Chitosan oligosaccharides	Hydroponic	Increased plant growth	Tham *et al*.(2001)
Wheat (*Triticum aestivum*)	Chitosan	Foliar application	Enhanced growth parameters and yield	Samdurkar *et al*. (2021)
	Nano-chitosan	Foliar application	Enhanced growth parameters and yield	Abdel-Aziz *et al*. (2016, 2018)
	Chitosan oligosaccharides	Hydroponic	Enhanced growth parameters	Tham *et al*.(2001)
	Chitosan oligosaccharides	Seed treatment and foliar application	Enhanced growth parameters and yield	Wang *et al*. (2015)
	Nano-chitosan	*In vivo*	Enhanced growth parameters and yield	Abel-Aziz *et al*. (2016)
	Chitosan oligosaccharides	Foliar application	Enhanced growth parameters and yield	Zou *et al*.(2017)
	Nano-chitosan	*In vivo*	Enhanced growth parameters	Li *et al*. (2019)
Maize (*Zea mays* L.)	Nano-chitosan	Seed treatment and foliar application	Enhanced growth parameters and yield	Kumaraswamy *et al*. (2021)
	Nano-chitosan	Seed priming	Enhanced growth parameters	Gomes *et al*. (2021)
	Chitosan	*In vivo*	Enhanced growth parameters	Younas *et al*. (2021)
	Nano-chitosan	*In vivo*	Enhanced growth parameters and grain weight	Choudhary *et al*. (2017)
	Nano-chitosan	Seed treatment	Enhanced seed germination and growth parameters	Khati *et al*. (2017)
	Nano-chitosan	*In vivo*	Enhanced growth parameters	Saharan *et al*. (2016)

Continued

Table 12.1. Continued.

Species	Chitosan types	Method of application	Effects	References
	Chitosan	*In vivo*	Enhanced growth parameters and yield	Agbodjato *et al*. (2016)
	Chitosan	Foliar application	Enhanced growth parameters and yield	Suvannasara *et al*. (2011)
	Chitosan	Seed treatment	Enhanced growth parameters	Guan *et al*. (2009)
	Chitosan	Seed treatment	Enhanced seed germination and growth parameters	Shao *et al*. (2005)
Barley (*Hordeum vulgare* L.)	Chitosan	Foliar application	Enhanced growth parameters, quality, and yield	Al-Tawaha *et al*. (2020)
	Chitosan oligosaccharides	*In vitro*	Enhanced growth parameters and yield	Luan *et al*.(2006)
	Nano-chitosan	Foliar application	Enhanced growth parameters, quality, and yield	Behboudi *et al*. (2018)
Soybean (*Glycine max*)	Chitosan	*In vivo*	Enhanced growth parameters	Chibu *et al*. (2002)
	Chitosan oligosaccharides	Hydroponic	Enhanced growth parameters	Tham *et al*.(2001)
	Chitosan oligosaccharides	*In vitro*	Enhanced growth parameters and yield	Luan *et al*.(2006)
	Oligochitosan	Foliar application	Enhanced yield	Phu *et al*. (2017)
	Chitosan	Seed treatment	Enhanced growth parameters	Costales-Menéndez and Falcón-Rodríguez (2020)
Mustard (*Brassica ra*pa L.)	Chitosan	Foliar application	Enhanced growth parameters and content of leaf chlorophyll	Zong *et al*. (2017)
Canola (*Brassica napus* L.)	Chitosan	*In vitro*	Enhanced growth parameters	Ahmadi and Shariatpanahi (2015)
Finger millet (*Eleusine coracana*)	Nano-chitosan	Foliar application	Enhanced growth parameters, mineral content and yield	Sathiyabama and Manikandan (2021)
Pearl millet (*Pennisetum glaucum*)	Chitosan nano-emulsion	Foliar application	Enhanced growth parameters and yield	Priyaadharshini *et al*. (2019)

Table 12.2. Effect of chitosan biopolymer on growth and development of vegetable, spice and pulse crops.

Plant species	Chitosan type	Method of application	Effect on plant	Reference
Potato (*Solanum tuberosum* L.)	Chitosan	Foliar application	Enhanced growth parameters and yield	Falcón-Rodríguez *et al.* (2017)
	Chitosan	Foliar application	Enhanced growth parameters	Falcón-Rodríguez *et al*. (2017)
	Chitosan	*In vitro*	Enhanced growth parameters and yield	Asghari-Zakaria *et al*. (2009)
	Chitosan	*In vitro* and *in vivo*	Enhanced growth parameters and yield	Kowalski *et al*. (2006)
Tomato (*Solanum lycopersicum*)	Chitosan	*In vivo*	Enhanced fruit quality and yield	Sathiyabama *et al*. (2014); Sathiyabama and Charles (2015)
	Chitosan oligosaccharides	*In vivo*	Enhanced yield	Goñi *et al*. (2016)
	Chitosan	Foliar application	Enhanced growth parameters and yield	Mondal *et al*. (2016)
	Chitosan	Foliar application	Enhanced growth parameters and yield	Saharan *et al*. (2016)
	Oligochitosan	Foliar application	Enhanced growth parameters and phenolic content	Sultana *et al*. (2017)
	Chitosan oligosaccharides	Foliar application	Enhanced growth parameters and yield	Monirul *et al*.(2018)
	Chitosan	In vivo	Enhanced growth parameters and yield	Reyes-Pérez *et al*. (2020)
	Chitosan	Foliar application	Enhanced fruit quality and yield	Hassnain *et al*. (2020)
	Chitosan	Foliar application	Enhanced growth parameters, quality traits, and yield	Hussain *et al*. (2019)
	Chitosan	Foliar application	Enhanced growth parameters, quality traits, and yield	Abd El-Gawad and Bondok (2015)
	Chitosan	Foliar application	Enhanced growth parameters and yield	El Amerany *et al*. (2020)
Carrot (*Daucus carota* subsp. sativus)	Chitosan	Seed treatment and soil amendment	Enhanced growth parameters and yield	Rahman *et al*. (2021)
Cabbage (*Brassica oleracea*)	Chitosan	In vivo	Enhanced growth parameters	Spiegel *et al*. (1988)
Chinese cabbage (*Brassica rapa* subsp. pekinensis)	Chitosan	Seed treatment and foliar application	Enhanced growth parameters	Wang *et al*. (2012)
Chinese kale (*Brassica alboglabra*)	Irradiated Chitosan	Hydroponic	Enhanced growth parameters and yield	Isa *et al*. (2016)
	Chitosan	Hydroponic	Enhanced growth parameters	Hafiz *et al*.(2003)
Okra (*Hibiscus esculentus* L.)	Chitosan	Foliar application	Enhanced growth parameters and yield	Mondal et al. (2012)
	Irradiated Chitosan	Foliar application	Enhanced yield	Win and Lay (2017)
Eggplant (*Solanum melongena*)	Oligochitosan	Foliar application	Enhanced growth parameters and yield	Sultana *et al*. (2017)
	Chitosan	Foliar application	Enhanced growth parameters and yield	Liaqat *et al*. (2019)

Continued

Table 12.2. Continued.

Plant species	Chitosan type	Method of application	Effect on plant	Reference
Cucumber (*Cucumis sativus*)	Chitosan	Foliar application	Enhanced growth parameters and quality traits	Shehata *et al*. (2012)
Daikon radishes (*Raphanus sativus*)	Chitosan	*In vivo*	Enhanced growth parameters	Tsugita *et al*. (1993)
Cassava (*Manihot esculenta* Crantz)	Chitosan	Hydroponic	Enhanced growth parameters and peroxidases activities	Kra *et al*. (2019)
Bean (*Phaseolus vulgaris*)	Chitosan + alginate	*In vitro*	Enhanced growth parameters, carotenoids, and chlorophylls levels	Pereira *et al*. (2017)
	Nano-chitosan	Seed treatment	Enhanced seed germination and growth parameters	Zayed *et al*. (2017)
	Nano-chitosan	Foliar application	Enhanced growth parameters and yield	Hasaneen *et al*. (2016)
Lettuce (*Lactuca sativa*)	Chitosan	Soil amendment	Enhanced growth parameters	Xu and Mou (2018)
Indian spinach (*Basella alba* L.)	Chitosan	Foliar application	Enhanced growth parameters	Mondal *et al*. (2011)
Fenugreek(*Trigonella foenum-graecum* L.)	Irradiated chitosan	Foliar application	Enhanced growth parameters, trigonelline content, and yield	Dar *et al*. (2015)
Basil (*Ocimum ciliatum* and *O. basilicum*)	Nano-chitosan	Foliar application	Enhanced growth parameters and phenol content	Pirbalouti *et al*. (2017)
	Chitosan	Seed treatment and root dipping	Enhanced growth parameters and phenol content	Kim *et al*. (2005)
Garden thyme (*Thymus daenensis*)	Chitosan	Foliar application	Enhanced growth parameters	Bistgani *et al*.(2017)
Chili (*Capsicum frutescence* L.)	Chitosan	*In vivo*	Enhanced growth parameters and yield	Akter *et al*. (2018)
	Nano-chitosan	Seed treatment	Enhanced growth parameters and yield	Asgari-Targhi *et al*. (2018)
	Chitosan	*In vivo*	Enhanced growth parameters	Chookhongkha *et al*. (2012)
	Oligochitosan	Foliar application	Enhanced growth parameters and yield	Dzung *et al*. (2017)
Chili (*Capsicum annuum* L.)	Chitosan	Foliar application	Enhanced growth parameters and chlorophyll content	Fenny *et al*. (2021)
	Oligochitosan	Foliar application	Enhanced growth parameters and yield	Islam *et al*. (2018)
Bell pepper (*Capsicum annuum*)	Chitosan	*In vivo*	Enhanced growth parameters and yield	Mahmood *et al*. (2017)
Turmeric (*Curcuma longa*)	Chitosan	Foliar application	Enhanced growth parameters, yield, and curcumin content	Anusuya and Sathiyabama (2016)

Coriander (*Coriandrum sativum* L.)	Chitosan	Foliar application	Enhanced growth parameters and yield	El-Gamal and Ahmed (2016)
Arugula (*Eruca vesicari*a ssp. sativa)	Chitosan	*In vitro*	Enhanced growth parameters	Acemi *et al*. (2021)
Soybean sprouts (*Glycine max*)	Chitosan	*In vivo*	Enhanced growth parameters	Lee *et al*. (2005)
Ajowan (*Carum copticum*)	Chitosan	Seed treatment	Enhanced seed germination and growth parameters	Batool and Asghar (2013)
	Chitosan	Seed treatment	Enhanced seed germination and growth parameters	Mahdavi and Rahimi (2013)
Faba bean (*Vicia faba* L.)	Chitosan	*In vitro*	Enhanced growth parameters and phenolic compounds	Ahmed *et al*. (2020)
	Nano-chitosan	Seed treatment	Enhanced growth parameters, total phenols, and antioxidant enzyme activities	Abdel-Aziz (2019)
	Chitosan oligosaccharides	Foliar application	Enhanced growth parameters and yield	El-Sawy etal. (2010)
Mung bean (*Vigna radiate*)	Chitosan	Foliar application	Enhanced growth parameters	Mondal *et al*.(2013)
Chickpea (*Cicer arietinum*)	Chitosan	*In vivo*	Enhanced growth parameters	Mahdavi and Safari (2015)
	Nano-chitosan	In vivo	Enhanced growth parameters	Anusuya and Nibiya Banu (2016)
	Nano-chitosan	Seed treatment	Enhanced seed germination and growth parameters	Muthukrishnan *et al*. (2019)

in plant height, branch number, leaf area, leaf number, and dry and fresh weight of Indian spinach results from the foliar application of chitosan. Higher chitosan concentrations are required for optimal okra growth. Foliar spraying of chitosan improves the yield of okra, and also plant height, number of leaves, rate of growth and photosynthesis (Mondal *et al.*, 2012). Foliar spraying of chitosan on tomato plants has enhanced the weight, quality, and production of the fruit (Sathiyabama *et al.*, 2014; Sathiyabama and Charles, 2015). Chitosan can be applied to herbs to improve their phenolic content. Before sweet basil seedlings were transplanted, root dipping and seed soaking in chitosan solution led to enhanced growth and content of secondary metabolites, along with improved levels of rosmarinic acid and eugenol, respectively (Kim *et al.*, 2005). In an experimental investigation, Pereira *et al.* (2017) reported that chitosan nanoparticles significantly enhanced the leaf area, content of chlorophyll, concentration of gibbrellic acid and carotenoids of bean. Seeds of Chinese cabbage mixed with chitosan, accompanied by foliar application, accelerated leaf area and plant height (Wang *et al.*, 2012).

Nano-chitosan supplementation increases seed germination and bean growth under salinity stress conditions (Zayed *et al.*, 2017). Chickpea seeds coated by chitosan-thiamine nanoparticles showed an increase in germination rate (Muthukrishnan *et al.*, 2019). Seed treatment with chitosan increases the yield of anise (Madhavi and Rahimi, 2013). Various crops, such as faba bean (Ahmed *et al.*, 2020), Chinese kale (Isa *et al.*, 2016), eggplant (Liaqat *et al.*, 2019), cucumber (Shehata *et al.*, 2012), cassava (Kra *et al.*, 2019), coriander (El-Gamal and Ahmed, 2016), etc., were treated with chitosan and boosted growth and production.

12.3.3 Effect on fruit crops

Chitosan has been utilized on fruit crops for growth enhancement. Chitosan enhances growth parameters (Zagzog *et al.*, 2017; Mohamed, 2018; Wang *et al.*, 2021), quality features (Ahmed *et al.*, 2016; Uddin *et al.*, 2020) and improves antioxidant functions (Rahman *et al.*, 2018) in several fruits. After transplantation, chitosan application on strawberries improved growth and productivity despite influencing fruit quality (El-Miniawy *et al.*, 2013). Foliar spraying of nano-chitosan on the leaves of robusta coffee seedlings leads to enhanced growth and chlorophyll content (Minh and Anh, 2013). Similarly, chitosan nanoparticles applied to the leaves of coffee plants accelerated their growth (Van *et al.*, 2013). Nano-chitosan spraying over mango trees increased the fruit number, fruit size and weight, and the vegetative growth (Zagzog *et al.*, 2017). Accelerated growth parameters of orange seedlings were reported after spraying with chitosan under field conditions (Ahmed *et al.*, 2016; Mohamed. 2018). Table 12.3 summarizes the growth-stimulating effects of several types of chitosan on fruit crops. Foliar application of chitosan significantly improves yield and quality (higher contents of antioxidants) strawberry fruits in the field conditions (Mukta *et al.*, 2017; Rahman *et al.*, 2018, 2019) (Fig. 12.1).

12.3.4 Effect on flower crops and ornamentals

Chitosan is also useful in enhancing the growth and yield of many flower crops as well as ornamentals. *Dendrobium* treated with chitosan exhibited increased *in vitro* culture growth (Nge *et al.*, 2006; Kananont *et al.*, 2010), whereas the use of suitable concentration and type of chitosan increased the percentage of seed germination as well as growth of *D. formosum* and *D. bigibbum* (Kananont *et al.*, 2010). Applied chitosan increases the quality of *in vitro* plantlets and promotes the eventual plantlets acclimatization under *ex vitro* conditions (Nge *et al.*, 2006). Chitosan usage in soil enhanced lisianthus growth and early flowering (Ohta *et al.*, 1999) as well as Persian violet, wishbone flower, garden lobelia, elatior begonia, gloxinia and monkey-flower (Ohta *et al.*, 2004). Foliar spraying of chitosan promoted growth and raised the number of flowers of marigold (Abdel-Mola and Ayyat, 2020). Likewise, the utilization of chitosan through corm dipping has contributed to the accelerated growth of the freesia plant, along with increased chlorophyll content, early flowering and inflorescence shoot number. In

Table 12.3. Effect of chitosan biopolymer on growth and development of fruit crops.

Plant species	Chitosan type	Method of application	Effect on plant	Reference
Strawberry (*Fragaria × annanasa*)	Chitosan	*In vivo*	Enhanced growth, yield, and total antioxidant activities	Rahman *et al*. (2018)
	Chitosan	*In vivo*	Enhanced growth and yield	Mukta *et al*. (2017)
	Chitosan	Foliar application	Enhanced growth and yield	El-Miniawy *et al*. (2013)
Highbush blueberry (*Vaccinium corymbosum*)	Chitosan	*In vivo*	Enhanced growth parameters and yield	Cabrera *et al*. (2010)
Navel orange (*Citrus sinensis*)	Chitosan	Foliar application	Enhanced growth parameters, quality traits, and yield	Ahmed *et al*. (2016)
Orange (*Citrus sinensis*)	Chitosan	Foliar application	Enhanced growth parameters	Mohamed (2018)
Watermelon (*Citrullus lanatus*)	Nano-chitosan	*In vivo*	Enhanced growth parameters	González Gómez *et al*. (2017)
	Chitosan	*In vivo*	Enhanced growth parameters	Li *et al*. (2013)
Pineapple (*Ananas comosus*)	Irradiated chitosan	*In vivo*	Enhanced growth parameters and quality traits	Uddin *et al*. (2020)
Kiwifruit (*Actinidia deliciosa*)	Chitosan	Foliar application	Enhanced growth parameters and fruit quality	Wang *et al*. (2021)
Peach (*Prunus davidiana*)	Carboxymethyl chitosan	*In vivo*	Enhanced growth parameters and quality traits	Xu *et al*. (2020)
Chestnut rose (*Rosa roxburghii* Tratt)	Chitosan	Foliar application	Enhanced growth parameters and yield	Li *et al*. (2021)
Grapevine (*Vitis vinifera* L)	Chitosan	*In vivo*	Enhanced growth parameters	Barka *et al*. (2004)
Mango (*Mangifera indica*)	Nano-chitosan	Foliar application	Enhanced growth parameters	Zagzog *et al*. (2017)
Robusta coffee (*Coffea canephora*)	Nano-chitosan	*In vivo*	Enhanced growth parameters	Van *et al*. (2013)
	Nano-chitosan	Foliar application	Enhanced growth parameters and chlorophyll content	Minh and Anh (2013)
	Nano-chitosan	*In vivo*	Enhanced growth parameters	Ha *et al*. (2018)
Tea (*Camellia sinensis*)	Irradiated chitosan	Foliar application	Enhanced growth parameters and yield	Hossain *et al*. (2013)

Fig. 12.1. Effect of different doses of chitosan on vegetative and reproductive growth of cv. Strawberry Festival (Adapted from Mukta *et al*., 2017; Rahman *et al*., 2018.) CC0 1.0

addition, it has also increased the corm's weight and number (Salachna and Zawadzi'nska, 2014). Oligomeric chitosan has raised the polyphenol content of Greek oregano enhanced plant growth (Yin *et al.* 2012). Cho *et al.* (2008) documented that chitosan nanoparticles usage increased the seed germination and growth parameters of sunflower plants significantly. Table 12.4 summarizes the growth-stimulating effects of several kinds of chitosan on flower crops and ornamentals.

12.4 Modes of action of chitosan as a growth stimulator

There is a large volume of literature on using chitosan to stimulate plant growth *in vitro* and *in vivo* on a variety of plants (Fig. 12.2). By modifying the cells' osmotic pressure, chitosan promotes plant growth through improving the absorption and supply of water and essential nutrients (Guan *et al.*, 2009). In order to modulate plant growth and development, the signaling pathways of chitosan and its analogs have been studied over the past decade. It has been shown that chitosan leads to the activation of the hydrolytic enzymes, which are required for the mobilization and degradation of the reserve foods like protein and starch (Hameed *et al.*, 2013). Chitosan can facilitate the root cell division of plants by inducing plant hormones, such as cytokinin and auxin, which therefore increase the uptake of nutrients. Chitosan possesses positive ionic charges which chemically bind them to plant nutrients, resulting in a slow-release action in plants that led to enhanced growth and yield (John *et al.*, 1997; Dzung *et al.*, 2011). Chitosan may also accelerate seed germination, boost seedling growth and development, and stimulate antioxidant enzymes to avoid potentially detrimental effects of reactive oxygen species (ROS) during seed germination (Hameed *et al.*, 2013; Saharan *et al.*, 2015; Anusuya and Nibiya Banu, 2016).

Zeng and Luo (2012) reported that chitosan has an outstanding ability to form a semi-permeable coating on the surface of seeds that can hold seed moisture and retain additional soil moisture to promote seed germination. Chitosan significantly enhances the growth indices like germination rate, root activity, root length and fresh weight, as well as impacts on the physiological indices like peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and chlorophyll and malondialdehyde (MDA) content, when coated on seeds (Zeng and Luo 2012). Amin *et al.* (2007) found that the plant growth-stimulating activities of chitosan is directly associated with the effects on the physiological pathways of plants, like nutrient uptake, cell elongation, cell division, protein synthesis and activation of enzymes. Ajowan seeds primed with varying levels of chitosan resulted in an enhancement in the germination percentage, seedling vigor index, radical length and dry weight (Batool and Asghar, 2013). Oligochitosan stimulated the growth of rice seedlings through various complex pathways of photosynthesis, metabolism of carbohydrate and redox homeostasis of the cell (Chamnanmanoontham *et al.*, 2015). Chitosan biostimulant controls infection of cucumber by *Phytophthora capsici* through suppression of asexual reproduction of the pathogen (Zohara *et al.* 2019).

Chitosan is an elicitor that systemically and locally induces plant growth stimulation by triggering signaling cascades in the plants (Fig. 12.2). It is a potent activator of many specific enzymes that are involved in the synthesis of phytoalexins and structural barriers, including lignification (Bhaskara Reddy *et al.*, 1999). Chitosan interferes with upstream processes by increasing the oxidative bursts in response to the induction of NADPH oxidase, resulting in hydrogen peroxide (H_2O_2) production (Kauss and Jeblick, 1996; Rossard *et al.*, 2006). Thus, in a dose-dependent manner, chitosan stimulates the ROS scavenging mechanism (Yusupova *et al.*, 2005). Chitosan-induced H_2O_2 production is documented as a major element in the enhancement of rice growth under stressful conditions (Pongprayoon *et al.*, 2013). In *Dendrobium*, the expression of the *ycf2* gene in young leaves was down-regulated by chitosan application, providing widened chloroplasts. This indicates the essential role of chitosan in enhancing the flowering capacity of ornamental plants by regulating the specific pathways (Limpanavech *et al.*, 2008).

Chitosan nanoparticles (NPs) have been found as a promising nano-carrier for nutrient

Table 12.4. Effect of chitosan biopolymer on growth and development of flower crops and ornamentals.

Plant species	Chitosan type	Method of application	Effect on plant	Reference
Dendrobium aggregatum	Chitosan	*In vitro*	Enhanced growth parameters	Chandrkrachang (2002)
	Chitosan	*In vitro*	Enhanced growth parameters	Uthairatanakij *et al*. (2007)
Dendrobium bigibbum var. Compactum	Chitosan	*In vitro*	Enhanced growth parameters	Kananont *et al*. (2010)
Dendrobium formosum	Chitosan	*In vitro*	Enhanced growth parameters	Kananont *et al*. (2010)
Dendrobium phalaenopsis	Chitosan	*In vitro*	Enhanced growth parameters	Nge *et al*. (2006)
Pineapple lily (*Eucomis autumnalis*)	Chitosan	*In vitro*	Enhanced growth parameters	Salachna and Pietrak (2021)
Roselle (*Hibiscus sabdariffa* L.)	Nano-chitosan	*In vivo*	Enhanced growth parameters	Mirheidari *et al*. (2021)
Oregano (*Origanum vulgare*)	Oligochitosan	Foliar application	Enhanced growth parameters	Yin *et al*. (2012)
Phyla dulcis	Chitosan	*In vitro*	Enhanced growth parameters	Sauerwein *et al*. (1991)
Freesia (*Freesia corymbosa*)	Chitosan	*In vivo*	Enhanced growth parameters	Salachna and Zawadzinska (2014)
Marigold (*Calendula officinalis* L.)	Chitosan	Foliar application	Enhanced growth parameters and yield	Abdel-Mola and Ayyat (2020)
Lavender (*Lavandula officinalis*)	Chitosan	Foliar application	Enhanced growth parameters and yield	Fahmy and Nosir (2021)
Sunflower (*Helianthus annuus*)	Chitosan	Foliar application	Enhanced growth parameters and yield	Sohail *et al*. (2021)
	Chitosan	Seed treatment	Enhanced seed germination and growth parameters	Cho *et al*. (2008)
Gerbera daisy (*Gerbera jamesonii*)	Chitosan	*In vivo*	Enhanced growth parameters	Wanichpongpan *et al*. (2000)
Rieger begonias (*Begonia hiemalis* Fotsch)	Chitosan	Soil amendment	Enhanced growth parameters	Ohta *et al*. (2004)
Garden lobelia (*Lobelia erinus*)	Chitosan	Soil amendment	Enhanced growth parameters	Ohta *et al*. (2004)
Gloxinia (Sinningia *speciose*)	Chitosan	Soil amendment	Enhanced growth parameters	Ohta *et al*. (2004)
Monkey-flower (*Mimulus ringens*)	Chitosan	Soil amendment	Enhanced growth parameters	Ohta *et al*. (2004)
Persian Violet (*Exacum affine*)	Chitosan	Soil amendment	Enhanced growth parameters	Ohta *et al*. (2004)
Wishbone flower (*Torenia fournieri*)	Chitosan	Soil amendment	Enhanced growth parameters	Ohta *et al*. (2004)

Continued

Table 12.4. Continued.

Plant species	Chitosan type	Method of application	Effect on plant	Reference
Lisianthus (*Eustoma grandiflorum*)	Chitosan	*In vivo*	Enhanced growth parameters and quality traits	Ohta *et al*. (1999)
	Chitosan oligosaccharides	*In vitro*	Enhanced growth parameters	Luan *et al*. (2002)
Lavender (*Lavandula angustifolia*)	Chitosan oligosaccharides	*In vitro*	Enhanced growth parameters	Luan *et al*. (2002)
Chrisanthemum (*Chrysanthemum × morifolium*)	Chitosan oligosaccharides	*In vitro*	Enhanced growth parameters	Luan *et al*. (2002)
Cymbidium insigne	Chitosan	*In vitro*	Enhanced growth parameters	Nahar *et al*. (2012)
Milk thistle (*Silybum marianum* L.)	Chitosan	Soil amendment	Enhanced growth parameters	Safikhan *et al*. (2018)
East Indian globe thistle (*Sphaeranthus indicus*)	Nano-chitosan	*In vitro*	Enhanced growth parameters	Thamilarasan *et al*. (2018)
Scots Pine (*Pinus sylvestri*s L.)	Chitosan	*In vivo*	Enhanced growth parameters	Trzcinska *et al*. (2015)
Kemiri sunan (*Reutealis trisperma*)	Chitosan	*In vivo*	Enhanced growth parameters	Irawati *et al*. (2019)

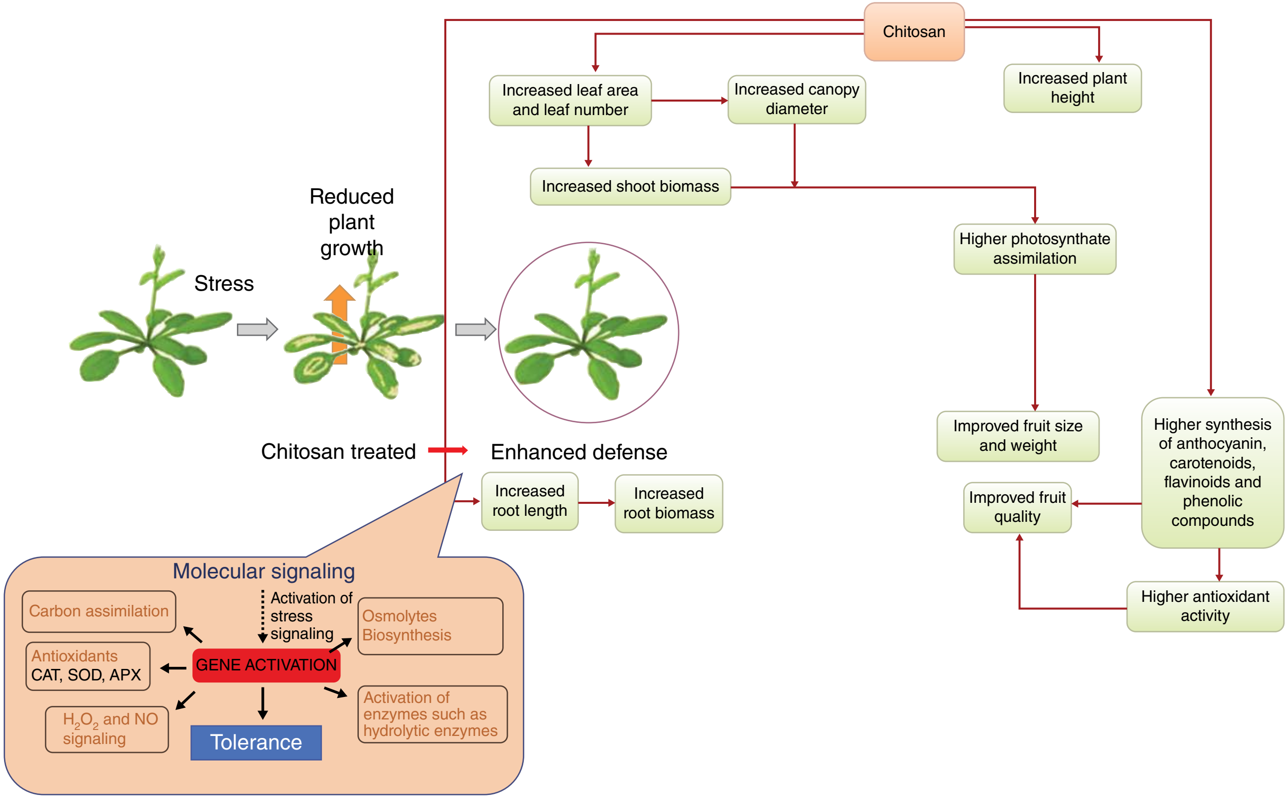

Fig. 12.2. Induction of plant growth and stress tolerance in plants by chitosan under stressful conditions (from Chakraborty *et al*., 2020 Creative Common CC BY license.)

delivery. It has excellent binding and loading capacity values (Kim *et al.*, 2007). Chitosan-soaked maize seeds significantly increased the germination rate as well as the vigor index of coffee, maize, and chickpea, contributing to the early development of seedlings (Minh and Anh, 2013; Anusuya and Nibiya Banu, 2016; Saharan *et al.*, 2016). Maize seed treated with Cu-chitosan NPs regulates hydrolytic enzyme synthesis, including α-amylase and protease, as well as enhances their functions. Improved α-amylase and protease activity resulted in rapid mobilization and degradation of reserved food, leading to increased germination of seed (Saharan *et al.*, 2016). Chitosan NPs also greatly improve biophysical activities like nutrient absorption and net photosynthesis rate, which lead to the growth stimulation of coffee seedlings. The positively charged nano-sized chitosan can easily penetrate into plant cells or bind to plant surfaces, thus enhancing seed germination and biophysical processes. Chitosan also greatly improves crop production by raising photosynthesis via improving stomatal activity and also chlorophyll content. Polycationic chitosan increases the osmotic pressure of the stomatal cells, leading to the accelerated opening of stomata and the integration of CO_2. Moreover, the content of biomass in the leaf region and the nitrogen fixation in the soybean nodules are also enhanced by chitosan (Dzung and Thang, 2002).

12.5 Concluding remarks and future perspectives

Chitosan is a derivative of the second most broadly recognized natural polymer chitin. The utilization of chitosan and its derivatives promotes plant growth, development and stress endurance. The biological and biocompatible activities of chitosan render it a promising agricultural bioactive material. Chitosan is a potent non-toxic material with multiple modes of action that promote plant health. The broader use of synthetic fertilizers can be mitigated, at least partly, through the utilization of chitosan and nanochitosan. There is enough proof to indicate that after the chitosan treatment, plants can gain enhanced growth and development, showing that as a natural inducer, chitosan has an important role in the sustainable agriculture.

Although a plethora of research has been conducted, multiple issues related to the mechanisms of chitosan of increasing plant growth parameters and yield are still unknown. In this respect, research must give consideration to the discovery of new chitosan derivatives, since their successful chemical modifications could greatly boost their chemical and physical properties and increase their field applicability via maintaining low toxicity to living organisms including plants. Most of the functionality and physiological functions of chitosan and its analogs are dependent on their molecular mass. In addition, future research is needed to verify whether chitosan biopolymers have the potential to affect plant physiological processes.

Moreover, further research should also focus on knowing the aspects at molecular level, which may provide insights into chitosan’s unknown biochemical pathways. Combined proteome and transcriptome analysis of known genes and proteins will strengthen our understanding of the complex signal pathways regulated by chitosan and facilitate the stimulation of plant growth by developing biotechnological strategies. Better understanding of chitosan's mode of action in plants will enhance the prospect of its effective application. In addition, the participation and collaboration of research institutes, governmental regulatory officials, and enterprises will be the principal key to the progress of the utilization of chitosan via uncovering its growth and yield promotion in plants and the biotechnological opportunities for sustainable farming. Research is needed for the green production of structurally and chemically diverse chitosan from the shrimp and crab wastes or industrially from fungi. Availability of the low-cost biofunctional chitosan for large-scale use in crop production would promote sustainable agriculture in the changing climate.

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13 Effect of Plant Extracts on Improvement of Crop Yield

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Abstract

Nature is decaying day by day with the application of synthetic fertilizers and pesticides, highlighting the need to sustain both the environment and nature. There is a growing demand for plant protection measures in the agronomy sector because of new and existing plant diseases. There are many beneficial uses of plant extracts/biostimulants as plants oil have antifeedant, antiviral, antimicrobial, antiparasitic, antifungal, antitermitic and antinematical activities. These plant extracts are extracted from various crops and trees like garlic, eucalyptus, onion, neem and many wild plant species. These extract may be used for plant protection measures to control insect pests and weeds and can also be used biostimulants to improve the growth and yield of the crops. Although, these extracts are not a quickly reactive as synthetic chemicals, their long-term use is sustainable and environmentally friendly. In this chapter, we discuss various plant extracts and their use in agriculture for plant protection and enhancement of crop yield.

13.1 Introduction

Plant extraction is a process that aims to extract some components present in plants. It is a process of liquid/solid separation (Zhang *et al.*, 2018). Certain components of plants are separated to use for other purposes. There are two types of plant extracts: water and oil-soluble. The waste products of extraction can also have benefits; for example, components of neem plant left behind after neem oil extraction can be used as organic matter (Agrawal and Mehta, 2008). Neem oil application repels the disease-causing and yield-losing insects from field crops. Plant extracts can be used for yield improvement, weed control, promoting the growth and development of plants and disease control. The chemicals present in medicinal plants (phytochemicals) have health benefits and antimicrobial activity against many pathogenic bacteria (Leitzmann, 2016). Plant species have been developed that have resistance against insect attack and diseases caused by fungal, bacterial and viral infections. For example, general bittersweet apple varieties have been shown to be resistant against aphids (Chuyong *et al.*, 2019). These also help the plant to perform better as compared to weeds for resources like light, moisture, space and nutrients (War *et al.*, 2012). By studying internal structure of plants, the breeders

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have developed many plant biostimulants that are being used as biopesticides, such as *Bacillus thuringiensis* for insect control (Lengai and Muthomi, 2018). In the same way, *Hypericum perforatum* extract can be used to increase the biomass of crop plants (Zobayed and Saxena, 2004). It increases the chlorophyll pigments in cabbage (Godlewska *et al.,* 2020). Similarly, botanical extracts like onion extract, garlic extract and bitter cola extract can be used to improve yield. These also have a great role in avoiding nutrient loss. Neem oil has been shown to be a nitrification inhibitor, which is responsible for the denitrification process in the nitrogen cycle (Rouphael and Colla, 2020). It also has an insecticidal effect (Trebbi *et al.,* 2021). *Chromolaena odorata* extracts also have some pesticide uses (Kesharwani *et al.,* 2018). Onion extracts have activity against scab disease of sweet potatoes (Sumartini, 2014) and is the best alternative to control scab disease in sweet potatoes as compared to synthetic pesticides. Onion also has antimicrobial activity and reduces the growth of bacteria which causes disease in crops (War *et al.,* 2012). Plant botanicals also aid in controlling insect pests. Nettle extract has a repelling smell. It can be used to control spiders (Garmendia *et al.,* 2018). It is also rich in nitrogen content and can be sprayed on the crops to counter the nitrogen deficiency (Caruso *et al.,* 2020). Nettle can be intercropped with the carrot plants, which will benefit as control of carrot fly that is a major pest of carrot plants (Nabrdalik and Grata, 2015).

The growth of plants can also be increased by using plant extracts. *Eucalyptus camaldulensis* extract application in low concentration on mungbean causes an increase in growth and germination (Khan *et al.,* 2007). When it is applied in higher concentrations, it decreases the growth and germination of mungbean and vice versa. Thyme oil has many pesticides uses (Wu *et al.,* 2017). It can be applied on cucurbits, spinach and tomatoes in order to control insect attacks and disease incidence. Thyme oil is an important plant extract that has been used for many field research and laboratory experiments (El-Bouhy *et al.,* 2021). It has no negative effects on human health and environment. It gives significant results as compared to synthetic pesticides (Wu *et al.,* 2017).

Here, we discuss some important points of using botanical extracts, types of botanical extracts, their use in agriculture, and their role in sustaining the environment in order to increase the yield of crop plants. In recent years, much research and many seminars, conferences, and workshops have been conducted in order to introduce environment friendly plant protection measures. This chapter explains the application of plant botanicals/biostimulants on crops for yield improvement and their impacts on the environment. It also explains the positive and negative impact of plant botanicals on ecosystems.

13.2 Types of plant extracts

Plant extracts are divided into two types based on their solubility (Masaki *et al.,* 1995).

- **Water-soluble** plant extracts are those that are easily soluble in water and include propylene glycerol, green tea extract, butylene glycerol, *Aloe vera* extract, glycerol extracts, and various others (Burlando and Cornara, 2017).
- **Oil-soluble** plant extracts are those that are soluble in oil and include vegetable oils such as coconut oil, sunflower oil, essential oil, olive oils, sprayed-dried powder, enzyme-hydrolyzed vegetable protein powder, and various other extracts (Virgilio *et al.,* 2015).

13.2.1 Water-soluble plant extracts

Almost all botanical extracts are water-soluble (Burlando and Cornara, 2017). There are many water-soluble plant extracts that can be used in agriculture and other industries. There are given below:

- green tea extract;
- cucumber extract;
- yucca extracts;
- red clover extract;

- elderflower extract;
- lemon grass extract;
- rosemary extract;
- calendula flower extract;
- nettle extract;
- peppermint extract;
- chamomile extract;
- tamarind seed extract;
- plantago extract;
- willow bark extract;
- beetroot extract;
- bitter apple extract;
- calendula extract; and
- eucalyptus extract.

These botanical extracts have different chemical nature from each other and can be used as antibiotic, antifungal, antiviral, antinematical and antioxidant (Roschek *et al.,* 2009). These extracts can be used in agriculture and other industries (Giordano and Costs, 2013).

13.2.2 Oil-soluble plant extracts

There are many oil-soluble plants extracts that can be used in agriculture and different industries including raspberry seed extract, pomegranate seed extract, jasmine phyto-scented botanical extracts, amaranth seed extract, amica flower seed extract, rosehip seed extract, schisandra fruit extract, rosemary antioxidant CO_2 extract, sunflower oil extract, mineral oil extract, sesame oil extract, amla extract, ajwain extract, ginger extract, neem extract, papaya extract, turmeric extract, tulsi extract, vacha extract, coconut oil extract and moringa extract. These plant extracts can be used as an antioxidant, antiviral, antifungal or antinematical (Karim *et al.,* 2017).

13.2.3 Uses of water-soluble plant extracts

Most of the water-soluble plant botanicals can be used to increase yield and are beneficial for humankind. They can be used in cosmetics, pharmaceutical industries, creams, shampoos, conditioners, lotions and in agro-based pesticides (Barbulova *et al.,* 2015).

13.2.3.1 Uses of water-soluble plant extracts in agriculture

Botanicals have been used for a long time in pest control. In the 19th century, plant-based allelochemicals were used as biocontrol agents and biopesticides. It can be explained in a recent experiment, where herbicidal effects of some plant extracts were tested on durum wheat (Carrubba *et al.,* 2020). In one year, plant botanicals obtained from *Rhus coriaria, Artemisia arborescens, Thymus vulgaris, Euphorbia characias* and *Lantana camara* were used in a durum wheat field. In the second year, only plant extract obtained from *Rhus coriaria* and *Artemisia arborescens* were used. In both years, the incidence of weeds was compared with the yield of wheat. None of these plant extracts could remove weeds from the field completely (Niziol-Lukaszewska *et al.,* 2020). In the first year, dicots were dispersed in those plots that were treated with botanicals of *Euphorbia characias*. On the other hand, monocots were dispersed after treatment with *Rhus coriaria* and *Lantana camara*. In the second year, low biomass and diversity of weeds were observed. Only two weeds (*Phalaris* and *Avena*) were observed at the harvesting stage. Therapy by water-soluble plant extracts affected the economical yield of durum wheat, but it can be said that those effects were not by weeds attack, it was due to some allelochemicals substances (Niziol-Lukaszewska *et al.,* 2020). Thus, water-soluble plant extracts can be used for weed control, pest control and yield improvements.

13.2.3.2 Uses of water-soluble plant extracts in cosmetics, skincare, and pharmaceutical purposes

Beetroot extract can be used in the manufacturing of many formulations including lotions, serums and moisturizers. Beetroot extract also helps in the reduction of irritation associated with cleansing substances. Beets (*Beta vulgaris*), commonly referred to as sugar beets, contain nutrients and phytocompounds that are excellent for skincare applications (Kesharwani *et al.,* 2018). Since beets contain nutrients, polysaccharides and sugars in excess, their extract can be used to moisturize the skin (Srivastava *et al.,* 2010). The sugars in beets draws moisture near the skin surface where it is needed. Beetroot also contains anthocyanin and betalins, which have antioxidants effects (Kesharwani *et al.*, 2018).

Chamomile extracts are also used for skincare and medical formulations. Chamomile extracts can be used for treating stomach upset, fever and muscle damage. Chamomile plant grows up to 20 inches tall; it is a pale green feathery plant that flowers between October and May in northern hemisphere. Chamomile has 30 different kinds of compounds that have anti-inflammatory properties. It has rutin, chlorogenic acid and caffeic acid that are considered to be helpful in sunburn injuries. It aids in the treatment of temperature-actuated burns and induces the synthesis of collagen. The combination of phytochemicals and active properties make the plants the perfect ingredient for antiaging and wound healing (Kesharwani *et al.*, 2018).

Cucumber extract is a powerful antioxidant provider to the human body. It has enzymes, vitamins and natural salts that can promote growth and repair (Akhtar *et al.*, 2011).

Green tea extract can be made by leaves of evergreen shrubs, *Camellia sinensis*. It has antioxidant, anti-inflammatory and astringent qualities (Niziol-Lukaszewska *et al.*, 2020).

Calendula flowers can be used for their healing properties. Its extract can be used for skincare and hair care. It is used in hair conditioners, lotions and shampoos (Ashwlayan, 2018).

Tamarind seed extract improves skin smoothness, elasticity and hydration (Farooq *et al.*, 2017). It has great importance in cosmetics.

Bitter apple extract is a newly discovered extract that can be used for skincare. Apple contains zinc, vitamin A and vitamin C, which are important for glowing and healthy skin. The presence of alpha-hydroxy acid and malic acid in apples assure healthy and smooth skin (Mammen *et al.*, 2010).

Eucalyptus extract has diverse benefits. It can be as antibacterial and anti-inflammatory; it also has a use in cosmetics. Eucalyptus is an evergreen plant has origin in Australia and California (Godlewska *et al.*, 2020).

Rosemary extract is also a common extract that can be used in skincare products, shampoos, hair conditioners and antiaging creams. It can be used to recover the rough skin and increased blood flow in the body (Tsai *et al.*, 2013). Rosemary extract has a great importance in cosmetics.

13.2.4 Role of water-soluble plant extracts in agriculture

Water-soluble plant extracts proved to be beneficial in sustainable agriculture. These can be used as an antiviral, antifungal, anti-insecticidal or antibacterial agent. Recently many reports have been revealed that use of valuable plant extracts have become more popular in modern agriculture. According to recent research, botanically based extracts from *Urtica dioica* L. were applied to cabbage seedlings, which increased root length by 31%. On the other hand, those that were treated with 1% *Polygonum aviculare* L. extract showed an increase in root length of about 72% when compared to control plants (Godlewska *et al.*, 2020). Botanical extracss of *Urtica dioica* L. (0.1%), and *Equisetum arvense* L. (0.5% used) enhanced the newly developing organs of cabbage by 112 and 113% respectively. The maximum root weight was observed with the extracts of *Equisetum arvense* L. (0.5%), *Urtica dioica* L. (2.5%), and *Polygonum aviculare* L. (0.5%) – they were increased by 205%, 206%, and 207%, respectively. Plant extracts increased the chlorophyll pigment of cabbage which is involved in photosynthesis by 156% when treated with 0.1% *Hypericum perforatum* L. extract and showed impacts on an antioxidant property (Godlewska *et al.*, 2020). *Polygonum cuspidatum* extract also has some antioxidant properties and some medicinal uses. Weeds in durum wheat can be controlled due to the application of water-soluble plant extracts obtained through *E. characias* and *L. camara* (Manourova *et al.*, 2019). The application of plant extracts increases the growth of different crops. These are also called biostimulators. Plant extracts are those substances that have a positive impact on plant metabolism, improve the immune system of plants, and can be used at various phases of plants development and growth (Rouphael *et al.*, 2020).

Biostimulants increase stress tolerance in favorable conditions which increase yield, suppress weed growth, bad consequences of drought on crops and environment, freezing, chemical and mechanical damage, as in the case of viral infestations. They are also used to treat the seeds before sowing to prevent seed-borne diseases and insect attacks. Biostimulants increase the growth of crops under stressful conditions. They regulate the biological activity of plants and microflora in soil (Rouphael *et al.*, 2020).

Nettle extract proves to be very effective and can aid in sustainable agriculture. Nettle (*Urtica dioica*) is a perennial plant (Roschek *et al.*, 2009). It is an herbaceous plant. Some species of nettle are used in folk medicine. Extract of nettle has a high nitrogen concentration, which promotes growth and development of cultivated crops. These extracts have many oligo-elements that can be applied as an acaricide to control red spiders. These extracts have a repelling effect on various insects and can be used as a fungicide – they contain a special odor that repels insect attacks and controls brown rot (Roschek *et al.*, 2009).

13.2.4.1 Preparation of nettle extract

For the preparation of nettle extract, only the leaves are needed: 1 kg of apical parts is immersed in water and left to stand in shade for 10–15 days (preferred temperature of 18°C). The solution will become brown and thick. When there are no air bubbles on the surface it is ready to use. It is recommended that the extract is used during the vegetation period. The whole solution is diluted with water before using and put into the sprayers to treat the plants. It is used in the ratio of 1:7. It can be stored in a sealed container in a shady area for up to 12 months (Garmendia *et al.*, 2018).

13.2.4.2 Uses of nettle and other extracts

For making solution against celery fly, 224 g of newly developing parts of nettle is soaked in water for 7 days, then process the solution as described in the previous section. The concentrated solution (i.e. no dilution) is used on the desired field. It can used on different types of fruits and vegetables (Garmendia *et al.*, 2018) to strengthen the plants' immune systems and repel insects. Foliar application of nettle extract yields better results than soil application. Nettle plant extract can affect the development and growth of many plants when applied at a ratio of 1:100. This can also intercropped with carrots to control carrot fly because carrot fly will attack the nettle hence protecting carrot plants (Ekhuemelo and Ekhuemelo, 2012).

Freshly cultivated nettle plants are put in kitchen cabinets to prevent different kinds of flies and moths. The macerate of nettle plants can be used for the control of aphid attack. It can be mixed with macerate from *Equisetum arvense* L. at a ratio of 1:1.5 (Nahla *et al.*, 2018) and then added to 20 L of water. The resulting solution can be applied to the field for the eradication of red spiders (Garmendia *et al.*, 2018).

Equisetum arvense has a thin, long horizontal root and is a perennial plant. The underground part of the stem is like a thread and black in color (Ekhuemelo and Ekhuemelo, 2012). When applied to plants, it can induce resistant to disease. It contains vitamin C, carotenoids, organic acids, microelements and macroelements. The macerate is used to control aphids and acts as an insecticide. The extract of this plant also has some antifungal properties (Garmendia *et al.*, 2018).

13.2.5 Beneficial and adverse effects of water-soluble plant extracts on environment

Everything in nature is valuable because of its beneficial and adverse effects on mankind. Similarly, water-soluble plant botanicals play a role in sustaining the environment. Beneficial effects of water-soluble plant extracts include control of bacterial, fungal and other diseases, control of various weeds, yield improvement, reducing pollution and sustaining the environment (El-Hack *et al.*, 2018).

13.2.5.1 Beneficial effects and yield improvement due to application of water-soluble botanicals

Water-soluble botanicals can be used to control fungal, bacterial and other diseases which ultimately results in the increase of overall yield. The main abiotic stresses include heat and drought which cause huge losses to the economical crop yield when these occur at various developmental stages. In a recent research, drought and heat stresses were observed in wheat at the anthesis stage, post-anthesis stage, heading stage and booting stages with the effect of foliage application of plant botanicals (Ahmad *et al.*, 2017). This was 2 years of research to examine the responses of wheat crops under different stress conditions. The wheat crop was planted in an ideal temperature with 70% moisture-holding capacity until the leaf boot stage in both years. At these three stages (booting, anthesis and post-anthesis stages), foliage application of (3% each) plant extracts of sorghum, sunflower and moringa were carried out. The stresses (heat and drought) were observed at each stage after 7–8 days of application of these plant extracts. Pots were placed in a glass canopy with 2 to 4°C than ambient temperature and 35% (WHC) than recommended (Ahmad *et al.*, 2017). The stresses at the booting stage and heading stage caused severe loss compared to the anthesis and post-anthesis stages. The two wheat varieties (Chakwal-50 and Mairaj-2008) were observed to have better water use efficiency and grain filling ability during drought and heat stresses. Foliar application of brassica extract shows increased grain yield and biomass in bread wheat. It improves the water use efficiency and grain filling during drought conditions (Ahmad *et al.*, 2017).

In a recent research experiment, the application of leaf extract of *Eucalyptus camaldulensis* L. on mungbean improves the yield when applied in low concentration (Trebbi *et al.*, 2021). It enhanced the growth and the germination. Weeds can also be controlled easily by the application of *Eucalyptus camaldulensis* extracts. If these extracts are applied in a high amount, then germination and growth of mungbean will be negatively affected; however weeds will be destroyed (Ekhuemelo and Ekhuemelo, 2012). Some beneficial effects of water-soluble plant botanicals are given below.

- By the application of plant botanicals, the length of shoot and root of white head cabbage can be increased with the application of nettle root and red clover extracts (Ekhuemelo and Ekhuemelo, 2012).
- The weight of fresh shoots and roots can be significantly increased by the application of nettle extract on white head cabbage. It also improves the dry weight of roots and shoots.
- Application of *Eucalyptus camaldulensis* on mungbean increases growth and germination of the seedling. It also reduces weeds growth and aids in weed control (Khan *et al.*, 2007).
- Application of nettle extracts results in insect control and yield improvement. It controls carrot fly which causes yield loss in carrot fields (Garmendia *et al.*, 2018).
- The application of water-soluble plant extracts improves the antioxidant activity in plants, which strengthens the immune system of the plant to fight against various diseases (Selvaraj and Kaliamurthi, 2010).

13.2.5.2 Adverse effects and yield loss due to application of botanical extracts

In a recent study, the application of *Eucalyptus camaldulensis* on maize showed lower grain yield and biomass production (Khan *et al.*, 2007), that is, it caused yield loss. Seed germination severely was affected due to the application of this extract. Shoot and root lengths were also reduced. The dry and fresh weight of maize was also reduced by the application of this botanical extract. Similarly, a new hypothesis reported that germination of maize seeds will be lower where the fields are surrounded by eucalyptus trees. Moreover, history proved the same condition of maize seed germination. It is due to the allelopathic chemicals of eucalyptus trees on maize seeds. These chemicals prevent seed coat rapturing and increase the dormancy period of maize seeds (Khan et al., 2007). These chemicals also destroy the favorable conditions in the soil which aids in seed germination. These chemicals prevent the embryo from developing into a young seedling. It is recommended not to sow maize seeds in fields that are surrounded by eucalyptus trees. Some limitations, side effects, and negative activities of botanical extracts are given below:

- When some botanical extracts are applied in higher concentrations, the growth and germination of some plants are affected, for example in maize.
- Some extracts have an allelochemical impact. This reduces the yield of the crop (Khan *et al.*, 2007).
- Botanical extracts have a slower action as compared to chemical applications (Khan *et al.*, 2007).
- Plant extracts have a short residual duration (Vaisakh and Pandey, 2012).
- The application of eucalyptus extract on maize, millet, sorghum and wheat seeds affects the germination and growth of these cereal crops.

13.2.6 Uses of oil-soluble plant extracts

Oil-soluble plant extracts can be used in cosmetics, creams, shampoos and conditioners, and also in the pharmaceutical and agro-based industries (Ribeiro *et al.*, 2015). Botanical extracts increase yield when compared to synthetic chemicals (Wu *et al.*, 2001). Here, we discuss some uses of oil-soluble plant extracts in different categories.

13.2.6.1 Uses of oil-soluble plant extracts in cosmetics

Pomegranate extract contains anthocyanidins, vitamins and hydrolyzed tannins with strong antioxidant and anti-inflammatory properties. The extracts of a pomegranate can be used in making scents (Giordano and Costs, 2013). Pomegranate extracts contain vitamin C, which can aid in repairing and protecting hairs. It has many benefits to human health and can be used in the food industry (Caruso *et al.*, 2020).

Moringa seed extract has a high volume of vitamin C, which is a powerful antioxidant. It also contains potassium, iron and beta carotene. The extract has antiaging and antiwrinkle properties. It also has some minerals that aid in the normal functioning of the skin. It helps the skin to absorb adequate minerals (Niziol-Lukaszewska *et al.*, 2020). Moringa has several health benefits:

- Early research shows that moringa is effective in controlling asthma (Agrawal and Mehta, 2008).
- Moringa maintains blood cholesterol levels and blood pressure.
- It can aid in controlling cancer, dandruff, diarrhea, headache, heart diseases, kidney stones, obesity, skin infections, snakebite, stomach ulcers and many other diseases (Niziol-Lukaszewska *et al.*, 2020).

13.2.7 Role of oil-soluble botanicals in agriculture

Oil-soluble botanicals and biostimulants play an important role in agriculture, including crop protection, insect management, weed control and yield improvement. Here, we discuss some botanicals that act as fungicides, insecticides, bactericides and ultimately result in an increase in yield (Godlewska *et al.*, 2020).

13.2.7.1 Crop protection

Crop protection is a big challenge in agriculture to increase productivity. It is also a big challenge for the world in order to feed the whole population (Muscolo *et al.*, 2013). In the current scenario, pest control in agriculture is carried out by excessive use of synthetic chemicals which damage the biodiversity and result in a polluted environment. On the other hand, biopesticides are the best alternative to these synthetic chemicals, which enable the safe control of pests (Choudhury *et al.*, 2018). However, there are some drawbacks to biopesticides, including photosensitivity, volatilization and short shelf-life. These restrictions make biopesticides difficult to use in large-scale areas (Virgilio *et al.*, 2015).

Thyme oil, in the form of an essential oil spray, can be used as an insecticide, fungicide and bactericide (Wu *et al.*, 2017). It is very useful in regular application and is a no chemical alternative to commercial pesticides. Thyme oil spray has been observed effectively on cucurbits, citrus, spinach and tomatoes. Thyme oil spray is common in the US (Florida and North California) and countries in South America (Wu *et al.*, 2017). Thyme oil can be successfully used in rotation with insecticides in order to gain the best results. The extract is environmentally friendly and non-toxic. It is better to use these extracts in order to control pests because they have no health issues as compared to synthetic pesticides. Synthetic pesticides can have a negative impact on health, for example dizziness, swelling, breathing difficulties and nausea.

13.2.7.2 Protection from weeds

Weeds cause great loss to the overall yield of any crop. They compete with the crop in order to attain moisture, space, nutrients and sunlight. Weeds also suppress the growth of economic crops due to increased uptake of nutrients and moisture (Duric *et al.*, 2019). Weeds with broadleaves use sunlight efficiently and prepare their food faster than economic crops. Weeds with narrow leaves also use moisture efficiently as compared to our economic crops. Weeds grow rapidly and their population establishment is high as compared to economic crops (Brown *et al.,* 2020). So, there is a great need to control weed infestation in our economic crop field.

13.2.7.3 Protection from diseases

Diseases also cause major losses of our economic crops. Disease in plants is caused by different pathogens (Draz *et al.,* 2019). Major pathogens for plant diseases include bacteria, fungi, viruses and nematodes. Oil-soluble botanical extracts/biostimulants play a significant role in controlling different kinds of plant diseases caused by various pathogens. Like the leaf, rust can be controlled through the application of *Lantana camara* extracts (Draz *et al.,* 2019). For example, onion has antibacterial and antifungal properties.

13.2.7.4 Protection from insect pests

Insects cause a heavy loss in our economic yield. Insects have different types depending upon their feeding habits (Tembo et al., 2018). Chewing and sucking insects are the major pests for any economic crop. *Lantana camara* plant has pesticidal uses. Insect pests cause abnormal growth of crops which ultimately cause yield loss (Tembo *et al.,* 2018). Oil-soluble plant extracts/biostimulants have a great role in order to control insect pest attacks on crops. Like other extracts, neem oil can also be used to control insects.

13.2.8 Beneficial and adverse effects of oil-soluble plant extracts on the environment

In nature, everything has some beneficial and adverse effects on humankind (Werrie *et al.,* 2021). Here, we discuss some positive and negative impacts of oil-soluble botanicals on the yield of crops and the environment.

13.2.8.1 Use of neem oil

Natural compounds extracted from aromatic plants often have a unique odor and are rich in alkaloids, terpenoids and phenolics. They are extracted from plants by different methods. Their mechanism of action may vary from plant to plant (Campos *et al.,* 2016).

Azadirachta indica is a small-to-medium sized plant belonging to the Meliaceae family. Initially, its origin was the Indian sub-continent but it is now planted worldwide. The phytochemicals obtained from the plant can be used in pest control and human health. The neem tree consists of a lamina, petiole and the base (Lokanadhan *et al.,* 2012).

Neem oil consists of 100 biologically active substances. Among these substances, the most important substances are from the triterpene group, known as limonoids (azadirachtin). Limonoids are responsible for the eradication of pests in the field. It has a melting point of 160°C. The molecular weight of azadirachtin is 720 g mol^{-1}. Neem oil also consists of nimbidin, nimbin, nimbinin, meliantriol, salannin, nimbolides and fatty acids (stearic, oleic, and palmitic). The main product of neem trees is neem oil, which can be extracted from neem tree by different methods. The azadirachtin contents in neem seed can be maximized by treating with arbuscular mycorrhiza (Campos *et al.,* 2016).

Neem oil has translaminar activity. It is regarded as a contact insecticide presenting systematic activity. It inhibits feeding, affects hormone function in juvenile stages and reduces ecdysone. It delays development and suppresses fertility. It sterilizes, repels oviposition and disrupts the molting processes (Lokanadhan *et al.,* 2012). Azadirachtin is a feeding inhibitor, causing weakness and pest death. Azadirachtin, limonoids and salannin inhibit the insect metamorphosis process. It causes problems in fertility and fecundity (Campos *et al.,* 2016). Nimbidin and nimbin have antiviral activity. Meliantriol and salannin inhibit the feeding of insects. Neem oil showed action against:

- Hemiptera: Neem oil causes inhibition of development and ecdysis defects which ultimately causes the death of nymphs (Campos *et al.,* 2016).
- Lepidoptera: Neem oil results in maximized larvae mortality and antifeeding effect (Campos *et al.,* 2016). Application of neem oil results in effective control of insects.
- Hymenoptera: Neem oil decreases food intake, reduces pupal and larval development, larval life end during their molting processes (Choudhury *et al.,* 2018).
- Neuropterans: Neem oil affects pupation. It has side effects in the midgut cells of larvae.

Neem plants can be used with organic and inorganic materials as a fertilizer to improve the quality of soil and crops. The remaining waste material after the extraction of oil from the neem can be used as a biofertilizer which provides macro-nutrients (Campos *et al.*, 2016).

Nitrogen is the major nutrient of plants, but it can be used only in available form (NO_3). Loss of nutrients from the soil occurs by the denitrification process. Neem oil has the capacity to inhibit denitrifying bacteria, thus avoiding nitrogen losses. Hence it keeps nitrogen in the soil for a long time (Campos *et al.*, 2016). It also can act as antifeedant, repellent, growth regulator and sterilant. Hence, neem oil can provide an effective pest control. Botanicals are those products or materials that are derived from plants. They can be used in pest, disease and weed control. Botanical extracts are the renewable products that can be prepared from dried products, powders, liquid extracts, cakes and miniporous bags. Plant disease needs to be controlled in order to avoid yield loss and health issues (Kandylis and Kokkinomagoulos, 2020). Different practices may be implemented to control various diseases. Beyond good agronomic practices, farmers also rely on the application of chemical pesticides and botanical extracts derived from plants. So, neem has a positive role in sustaining the environment (Lokanadhan *et al.*, 2012).

13.2.8.2 Use of *Allium cepa* extract

Throughout history, the onion has had great importance as a vegetable. It can be cultivated mostly as a food source of millennia. A number of studies have shown that onion has a great ability to fight against bacterial and fungal diseases of human pathogens. It is a rich source of organic sulfur, saponins, flavonoids, polyphenols and other secondary compounds, which are responsible for its medicinal qualities (Sumartini, 2014). Onion has a great inhibitory effect against the growth and development of Gram-positive and Gram-negative bacteria. It protects humans from dermaphytic fungi. It has a positive role in sustaining the environment. Scab is a fungal disease of sweet potatoes caused by the fungus *Sphaceloma batatas*. This disease can be successfully controlled by applying onion extract (Sumartini, 2014). Onion extract can be prepared in the laboratory. First, onions are washed and then sliced into small pieces. Then grind 50 g of sliced pieces of onion and place it in a blender with 1 L of water. Now incubate this solution overnight. The extract is ready to use the next morning, after filtering. The solution is sprayed onto the plant (Sumartini, 2014). This onion extract application on sweet potatoes results in both disease control and yield improvement. It has no side effects on the environment (Sumartini, 2014).

13.2.8.3 Use of *Allium sativum* extract

Garlic contains allicin, which is an antibiotic compound. It is effective against many bacterial species at dilutions of 1:10. *Allium sativum* extracts have also been used to protect stored foods and plants (Lara *et al.*, 2020). Lara *et al.* (2020) studied the antifungal behavior of various botanical extracts against fungal species *Fusarium pallidoroseum*. They found the best results when garlic bulb extract was applied to plants, observing that spore germination was reduced by garlic bulb extract. In other words, it has antifungal activity against *Fusarium* spp. These observations were also reported by Selvaraj *et al.* (2010) using garlic bulb extracts against many different fungi including *Fusarium* spp. The application of garlic extract on groundnut and cowpea for insect control has some limitations (Adeleke, 2016). If the extract is applied at a high concentration, then nodulation and growth of cowpea and groundnut are greatly affected. Similarly, if the extract is applied at low concentration to control insects, nodulation and growth of cowpea and groundnut is minimally affected. Intercropping of garlic and other crops showed some advantages, but there are great effects due to allelochemicals. Roots of legumes such as cowpea are affected when garlic is applied in higher concentrations (Selvaraj *et al.*, 2010). So, garlic extract proves have beneficial properties and is an economic product.

13.2.8.4 Use of *Carica papaya* extract

Papaya plants belong to Caricaceae family. Papaya possesses a self-supporting stem and is a perennial plant. Papaya plants can be monoecious, dioecious or hermaphroditic, and are widespread in tropical Africa (Ahmad *et al.*, 2018).

The fruit of the papaya is a berry, developing from the syncarpous ovary with parietal placentation. The value of herbal medicines has been emphasized because they are safe, effective and economical for treatment of various diseases. Extracts from papaya fruit and seed have been shown to have antibacterial activity against *Staphylococcus aureus* and many other fatal microorganisms (Ahmad *et al.*, 2018). Mintah *et al.* (2018) studied the fungi toxic activity of crude extract of papaya and neem on *Alternaria solani*, isolated from rotting yam tubers. They reported that the extracts had fungi toxic constituents that suppressed the mycelia disease incidence and growth. According to Mintah *et al.* (2018), the inhibition of mycelia growth and development was higher in plates containing *Carica papaya* leaf extract compared to the plates containing neem extract. The inhibitory action on mycelia growth increased with the increase in the concentration of extracts. Bio-insecticide can also be produced through the extract of *C. papaya*. This bio-insecticide can be produced by NADES solvent using ultrasound-assisted extraction. The use of these bio-insecticides has no side effect on the environment. So, the use of papaya extract is beneficial and economic (Ahmad *et al.*, 2018).

13.2.8.5 Use of *Chromolaena odorata* extract

Chromolaena odorata belong to the family Asteraceae. It is found in America, especially in central and southern regions (Samuel *et al.*, 2019), and Nigeria, where it is commonly called Awolowo, Siam weed, independence weed, bitterbrush, triffid weed and Jack-in-the-bush (Karim *et al.*, 2017). The people of south-eastern Nigeria call it obiara Kara or ahihia eliza. It is known for its antispasmodic, antiprotozoal, antifungal, antihypertensive and antibacterial activities. In Vietnam and Nigeria, fresh leaves of triffid weeds are used for the treatment of skin infections, burn wounds, leech bites, soft tissue wounds, dento-alveolitis and to stop bleeding (Karim *et al.*, 2017). Investigation into the antifungal properties of *C. odorata* and *C. papaya* on mycelial growth showed that the crude extracts of these plants possess some inhibitory constituents which cause a significant reduction in mycelial growth and development of fungi. This agrees with the reports by Tsai *et al.* (2013), who claimed the efficacy of extracts on mycelial growth of *Colletotrichum capsici*, *Protomycopsis phaseoli* and *Erysiphe cichoracearum*, compared with benlate and ridomil that are chemical pesticides. The use of *C. odorata* extract proves to be beneficial in the treatment of wounds and skin diseases, and they are used in traditional medicines (Tsai *et al.*, 2013). Siam weed (*C. odorata*) has some inhibitory effects on seedling growth and seed germination of some crops (Karim *et al.*, 2017). It also has allelopathic effects on certain crops. So, extract of *C. odorata* can be used as environmentally friendly and economical treatment option.

13.2.8.6 Use of *Acalypha ciliata* extract

Acalypha ciliata occurs across Senegal to Ethiopia, and Namibia and South Africa. It belongs to the family Euphorbiaceae. It grows near lakes, wooden grassland, coastal bushland, often in rocky localities, on flood plains from sea level up to 1650 m altitude (Chekuri *et al.*, 2016). *Acalypha ciliata* is a weed in fields and is not common in high rainfall areas. *Acalypha ciliata* is an annual herb and monoecious, growing up to 1 m tall. In an *in vitro* experiment, leaf extract of *A. ciliate* showed a reduction in the growth of *Fusarium moniliforme* on maize grain. In a field experiment, the leaf extract of *A. ciliata* showed some activity against blotch disease (*Colletotrichum capsici*) and insect pests of cowpea including *Clavigralla tomentosicollis*, *Maruca testulalis* and *Nezara viridula* (Chekuri *et al.*, 2016). It is not commonly used as a medicinal plant, therefore its use remains limited. In crop protection, its fungicidal and insecticidal effects are still being researched. Rouphael *et al.* (2020) tested the effects of different concentrations of various extracts including *A. ciliata*, *C. papaya*, and *C. odorata* on the growth of pathogenic fungi. The results showed that mycelial growth was reduced by the application of *Acalypha ciliata* more than by other extracts. Similarly, *A. indica* extract also has an antimicrobial and antioxidant activity. So, the extract of *A. ciliata* can be used as it economically viable and environmentally friendly.

13.2.8.7 Use of *Garcinia kola* (Bitter kola) extract

Garcinia kola belongs to the Clusiaceae family and is found in coastal rainforests in the south-eastern and south-western region of Nigeria. Bitter kola is a good antibacterial agent. Bitter kola also contains dimeric flavonoids, which are beneficial in healing wounds. It is used to treat many diseases in Africa (Manourova *et al.*, 2019). It is referred to as the wonder plant because it is used in the treatment of various diseases in Africa. Manourova *et al.* (2019) studied the antibacterial activities of extracts from *G. kola* and *C. papaya* on eight bacterial strains: *Bacillus subtillis*, *Proteus vulgaris*, *S. aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Pseudomonas fluorescens* and *Bacillus shigelladysenteria*. Bitter kola contains saponins, which are absent in pawpaw. On the other hand, pawpaw contains tannins, which are absent in bitter kola. According to results of the study, *G. kola* extract showed the best inhibitory effects against the test bacteria (Manourova *et al.*, 2019). The mycelial growth of *P. aphanidermatum* was reduced and the highest dry weight was obtained by application of *G. kola* extract.

13.2.8.8 Use of *Moringa oleifera* extract

Moringa oleifera is an important tree as it can be used for food and other purposes. In many countries, it is also used for livestock forage. Moringa micronutrient liquid can be used to kill parasites and other metabolic activities against pandemic diseases in many countries (Niziol-Lukaszewska *et al.*, 2020). The leaf extract of *Moringa oleifera* L. can be used as an insecticide and pesticide. It can be used to repel insects and reduce many fungal diseases. Aqueous moringa seed extract can be used as a fungicide against many fungal diseases. It is cheap and easy to use when compared to synthetic compounds. Moringa leaf extract can be used to repel the larvae and adult of *Trogoderma granarium*. Moringa leaf extract showed fungicidal effects against soilborne fungi such as *Rhizoctonia*, *Fusarium* and *Pythium*. The leaf extract of moringa showed great resistance against *Sclerotium rolfsii* (El-Hack *et al.*, 2018), the causative agent of stem rot and damping-off in cowpea (Niziol-Lukaszewska *et al.*, 2020).

13.2.8.9 Use of *Zingiber officinale* extract

Ginger (*Zingiber officinale*) contains polyphenols and gingerols that have many advantages to crops and the environment (Amuji *et al.*, 2012). The rhizome in the ginger is tolerant against many diseases that affect other cultivated crops. Recently, scientists investigated the control of bacterial leaf spot on *Solanum* by applying different plant extracts, including ginger extract. Ginger extract gave better results as compared to other test extracts for the control of bacterial leaf spot on *Solanum*. Ginger extract can also be used to control okra flea beetle and cowpea bruchid. Its residues are also used to control storage pests. So, ginger has a positive role in sustaining the environment.

- They have more residual effects than chemicals (Makhuvele *et al.*, 2020).
- The botanical extract reduces pollution and is environmentally friendly.
- They control the growth of harmful insects and pests.
- Plant extracts have a major role in the uptake of nutrients and water by the plants.
- They are cheap and easily available (Amuji *et al.*, 2012).

13.2.8.10 Negative impacts of botanical extracts on the environment

The application of plant botanicals/biostimulants has limited adverse effects on the environment (Mishra *et al.*, 2020).

- Neem extract: due to the high rate of photodegradation, it has low stability under field conditions.
- It has slow killing rates and short residence time as compared to traditional pesticides.
- However, extraction techniques and environmental factors also affect the composition of plant extracts.
- Extracts like garlic applied in higher concentration result in decreased growth and nodulation, which also leads to low yield.
- The indigenous use of pesticides causes a number of biological and environmental hazards.
- More time is required in order to produce these pesticides.
- Application of plant botanicals results in less control as compared to chemical application.
- As low power of botanical pesticides, several applications is necessary in order to control pests, which can lead to resistance.

13.3 Conclusion

Plant extracts play a vital role in controlling plant diseases (nematocidal, fungal, viral, and bacterial), weeds and insect pests. Oil-soluble and water-soluble plant extracts play a vital role in sustainable agriculture. The application of plant botanicals is the best way to control weeds, pests and diseases, and is beneficial when compared to manmade chemicals. They benefit the farmers by their low cost and easy availability. Nature sustainability is assured when botanicals are applied as biostimulants. These biostimulants can be used to enhance the yield of agricultural crops. The substances have biologically active compounds that affect the insects which attack plants. They are used in crop protection to control different kinds of diseases. They are also used to control weed density in the crop field. They have a limited effect on the environment. Applications of plant botanicals are eco-friendly and can be used to increase yield in sustainable agriculture.

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14 Use of Seaweed Extracts for Enhancement of Crop Production

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Abstract

Seaweed is a natural bioactive material. It is rich in organic content, micronutrients and macronutrients, vitamins, cytokinins and auxins (plant growth hormones), hence are widely used in agriculture and horticulture. Seaweed extracts have been shown to promote yield of crop plants by enhancing nutrient uptake. Stimulation in development of roots, leaves, flowers and fruits has been noted in plants treated with seaweed extracts. Indirectly, seaweeds exert positive effects on the growth of plants by bringing improvement in soil structure, its productivity and microbiological content. Studies indicated that seaweed application triggers growth of beneficial soil microbes and increase release of soil-conditioning substances produced by them. In addition, liquid formulations of seaweeds are known to provide protection to plants against disease and pests and increase tolerance capacity of plant against abiotic stresses such as cold or drought. Secondary metabolites such as terpenes present in seaweed extracts impart antimicrobial activities and hence benefit growth of plants. Seaweeds can be exploited for its wider application in the agricultural domain to achieve high crop yield with minimum fertilizer application.

14.1 Introduction

The declining land and water resources and the rapidly changing climatic conditions have affected food production all over the world. Excessive use of agrochemicals has deteriorated soil quality and affected the agricultural land. The increasing requirement of food for the growing world population has prompted the search for a technology that enhances soil productivity without harming the environment and reducing dependency on chemical fertilizers. Various materials have been used to supplement the soil with the aim of improving its fertility, productivity and maintaining the sustainability. Seaweed is a material that has shown great potential for restoring soil productive capacity due to its unique composition and properties.

Seaweeds are marine macro algae that are an integral part of marine ecosystems. Seaweeds are a good source of nutrient supplements, biostimulants or biofertilizers (Du Jardin, 2012; Al-JutheryHayyawi *et al.*, 2020; Mishra *et al.*, 2020). The materials that promote growth of plants after application in small quantities are called as 'biostimulants' or 'metabolic enhancers'. Seaweed extracts are applied as fertilizer (Khan *et al.*, 2009; Craigie, 2011; Kumar and Sahoo, 2011; Pramanick *et al.*, 2013; Shah *et al.*, 2013; Zamani *et al.*, 2013; Calvo *et al.*, 2014). Brown algal species such as *Ascophyllum, Fucus* spp., *Laminaria* spp., *Sargassum* spp. and *Turbinaria*spp

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have shown potential to be used as biofertilizers (Hong *et al.*, 2007). Macro-nutrient and microelement nutrients present in seaweed extracts help in restoring soil fertility. Seaweed fertilizers are applied to soil either in the form of a foliar spray, liquid formulations, granules, powder or manure (Kumari *et al.*, 2011). Extracts obtained from various seaweed species are available commercially for their use in agriculture and horticulture. Some of the commercially available seaweed extracts include Maxicrop (Sea born), Goemar GA14, Algifert (marinure), Kelpak 66, Seaspray, Seasol, Cytex, Seacrop 16 (Gandhiyappan and Perumal, 2001).

Use of seaweed as a soil supplement has gained popularity in recent years. This is because it possesses potential in increasing soil sustainability. Application of seaweed extract prevents excessive use of chemical fertilizers. They also help in improving the retention of minerals in soil. Seaweed fertilizers have received wide acceptance because of their superiority to conventional fertilizers in terms of a high level of organic matter, vitamins and fatty acids. Their biodegradability and non-hazardous nature are some other features that establish them as ideal soil supplements. This chapter describes the role of seaweeds in improving soil and plant productivity. The mechanism involved in action of seaweeds has also been discussed.

14.2 Composition of seaweeds

Seaweed extracts (SWE) are a rich source of macronutrients, micronutrients and diverse organic compounds viz. growth hormones, amino acids, vitamins, betaines, sterols and polyamines (Tarakhovskaya*et al.*, 2007; Zhang and Ervin, 2008; Zodape*et al.*, 2008). Seaweeds also contain fiber, fat, cellulose, hemicelluloses, lignin, bromine and iodine (Mohammadi *et al.*, 2013; Shri Devi and Paul, 2014; Heltan *et al.*, 2015; Mirparsa *et al.*, 2016). The presence of betaines and betaine-like compounds has been reported in extracts of *Ascophyllum nodosum, Fucus* and *Laminaria* (Craigie, 2011). The presence of minerals such as F, Ca, Mg, Na, K, Fe, Mn, Zn, Cu, Ni, Co, Cr, Cd in high concentrations have been noted in *Chladophora glomerata, Ulva reticulata, Halimedamacroloba* belonging to Chlorophyceae and *Gelidiellaacerosa, Hypeneamusciformis* belonging to Rhodophyceae (Anantharaman *et al.*, 2010; El-Said and El-Sikaily, 2013; Tuhy *et al.*, 2015). These mineral elements play a role in the stimulation of plant growth (Mancuso *et al.*, 2006).

Extracts of seaweed act as a good source of plant growth substances which include cytokinins (CKs), auxin, gibberellins and abscisic acid (Khan *et al.*, 2009). Indole acetic acid (IAA) and its conjugates, CKs such as zeatin (Z) and isopentenyl (IP) and aromatic CKs such as benzyl amino purine and topolin (6-[3-hydroxybenzylamino] purine) have been found to be present in extracts of seaweeds (Stirk *et al.*, 2003, 2004). Alkaline hydrolyzates of *A. nodosum, Fucus vesiculosus* and other seaweeds have indicated the presence of auxin-like compounds other than IAA. The presence of brassinosteroids has been reported in the extract of kelp (Kelpak™ manufactured from *E. maxima*). The seaweed extract called Seasol™ contains both brassinosteroids and strigolactones (Stirk *et al.*, 2014; Arioli*et al.*, 2015). Brassinosteroids aid in flowering, stress tolerance and immune response in plants (Divi and Krishna, 2009), whereas strigolactonesare stimulates seed germination in certain plants and acts as stress regulator in plants exposed to drought or salinity (Marzec *et al.*, 2013; Ha *et al.*, 2014).

Brown seaweeds such as *A. nodosum, F. vesiculosus* and *Saccharina longicruris* contain polysaccharides such as laminaran, fucoidan and alginate in high quantities (Shevchenko *et al.*, 2007). Alginate, laminaran and fucoidan play a role in various biological activities (Rioux *et al.*, 2007). They help in (i) stimulating growth of root directly or indirectly (González *et al.*, 2013a, 2013b); (ii) triggering defense mechanisms in plants (Subramanian *et al.*, 2011); and (iii) activating genes involved in defense related to pathogens (Vera *et al.*, 2011). Laminarin stimulates induction of genes encoding pathogenesis-related (PR) proteins which possess antimicrobial properties.

Apart from these, fucosterol and fucosterol derivatives have also been found in brown seaweeds and cholesterol and cholesterol derivatives have been reported from red seaweeds. The presence of ergosterol and 24-methylene cholesterol has been noted in green seaweed. Seaweed extracts contain polyphenols, antimicrobial agents and several other compounds of agronomical value.

The composition of seaweed varies with species and environmental conditions such as temperature, light, salinity and nutrient availability (Karthikaidevi *et al.*, 2009; Anantharaman *et al.*, 2010; Hanan and Shimaa, 2013) (Table 14.1).

14.3 Effect of seaweed application on soil health

Seaweeds affect the properties of soil (physical, chemical and biological) to a great extent. Application of seaweed extracts stimulates the growth of beneficial soil microbes and increases the release of soil-conditioning substances produced by them. Seaweed extract increases soil productivity by improving soil properties such as moisture-holding capacity, texture and growth of beneficial soil microbes (Kumari *et al.*, 2013). Seaweeds help in retaining moisture and nutrients in the upper layers of soil. This happens because of high quantities of organic matter, carbohydrates, proteins, fatty acids, nutrients such as N, P, K, ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) and other minerals present in them (Sethi, 2012; Mirparsa *et al.*, 2016).

Polyuronides such as alginates and fucoidans (present in the cell walls) present in brown seaweeds affect properties of soil and support growth of beneficial fungi. Alginate binds to metal ions present in the soil and form high-molecular-weight complexes. These complexes absorb moisture and retain it. Improved aeration of soil stimulates soil microbial activity and growth of roots in plants. Enzymatic degradation of alginic acid produce oligosaccharides that stimulate hyphal growth and elongation of arbuscular mycorrhizal (AM) fungi (Ishii *et al.*, 2000). Extracts of *Laminaria japonica, Undaria pinnatifida* promote growth of AM fungus (Kuwada *et al.*, 2006a, 2006b). An improvement in root colonization of AM fungi has been noted after application of seaweeds. Compounds present in the red and green algae stimulate mycorrhizal development in higher plants. Improvement in development of AM fungi has been noted when roots of papaya (*Carica papaya*) and passion fruit (*Passiflora edulis*) are treated organic fractions of red and green algae (Kuwada *et al.*, 2006b). Alginate has also shown capacity to increase availability of phosphorus in the rhizosphere by 49%.

Table 14.1. Composition of *Ascophyllum nodosum* extract.

Compounds/elements	Range (%)
Alginic acid	15–30
Laminarin	0–10
Mannitol	5–10
Fucoidan	4–10
Protein	5–10
Fats	2–7
Tannins	2–10
Magnesium	0.5–0.9
Iodine	0.01–1.0
Potassium	2–3
Sodium	3–4
Glycine betaine	0.002–0.006
Water	70–85
Other active substances	15–25

Application of commercial seaweed extracts produced from *A. nodosum* alters the bacterial diversity of soil. A study has shown that extract of seaweed, *Macrocystis pyrifera*, enhanced the hydrogenase activity of the soil. An increase in number of beneficial microbes such as fungi and bacteria was noted when seaweed extract of *Lessonia nigrescens* and *Lessonia flavicans* were applied at the rate of 40 g $kg^{-1.}$ This caused growth stimulation in *Malus hupehensis*.

Supplementations of seaweeds directly or in composted form (along with straw, peat or other organic wastes) improve productivity of soil. They also assist in reclamation of alkaline and nutrient deficient soils (Craigie, 2011). This is because seaweeds can act as soil stabilizers (Bhardwaj *et al.*, 2014; Arioli *et al.*, 2015).

14.4 Effects on plant health

Seaweeds exert positive effects on growth of cereals, pulses, flowering and fruit plants (Kavipriya *et al.*, 2011; Ravi *et al.*, 2018; Ali *et al.*, 2019; Özbay and Demirkıran, 2019; Mohammed *et al.*, 2020). Seaweed extracts affect various aspects of plant development which include an increase in nutrient uptake, stimulation in seed germination, development of roots, promotion of vegetative growth (increase in plant height), increase in leaf area index, total dry matter production and yield (Nabti *et al.*, 2010; Aruthur *et al.*, 2013; Pramanick *et al.*, 2013; Pacholczak *et al.*, 2016a) (Fig. 14.1).

Seaweeds help in restoring the growth of plants under stress and control phytopathogenic fungi, bacteria, insects or other pests (Bouhlal *et al.*, 2010; Ravikumar *et al.*, 2011; Asha *et al.*, 2012; Alves *et al.*, 2016a, 2016b).

Application of seaweed extracts (exogenous and foliar) enhance plant productivity (Divi and Krishna, 2009; Ha *et al.*, 2014). Constituents such as organic matter, nutrients, phytohormones (mainly CKs) or betains present in the seaweeds contribute to stimulation of plant growth (Zodape *et al.*, 2010; Alam *et al.*, 2014; Divya *et al.*, 2015a; Michalak and Chojnacka, 2015; Shahbazi *et al.*, 2015; Mirparsa *et al.*, 2016).

14.4.1 Root development

Application of seaweeds promotes root growth and development. Application of seaweed extracts to roots as a foliar spray increases root growth. Improvement in formation of lateral roots and increase in volume of the roots has been noted after seaweed application (Vernieri *et al.*, 2005; Mancuso *et al.*, 2006). Auxins present in the seaweeds promote root development. A well-developed root system improves water and nutrient uptake efficiency, thereby enhancing plant growth and vigor (Vernieri *et al.*, 2005). Seaweed fertilizers promote the growth of roots by increasing microbial diversity and biological activities like respiration, nitrogen mobilization and mineralization of mineral nutrients (Selvaraj *et al.*, 2004; Battacharyya *et al.*, 2015).

Extracts of red algae (e.g. *Neorhodomela larix, Tichocarpu scrinitus*), brown algae (e.g. *Saccharina japonica, Sargassum pallidum*) and green algae (e.g. *Ulva fenestrate, Codium fragile*) exert a positive effect on root length in soybean seedlings. The increase in root length can be attributed to increased expression of an auxin-related gene leading to high auxin concentration. Alginate oligosaccharide-induced gene expression thus promotes formation and elongation of root. An increase in the growth of roots could also be due to increased uptake and utilization of nitrogen and sulfur.

Wheat plants treated with seaweed extract Kelpak exhibited an increase in root:shoot dry mass ratio. An increase in number of rooted cuttings and improvement in vigor of the roots was noted in *Pinus pinea* after treatment of Kelpak (1:100 dilution). A very low concentration of kelp seaweed extract (1:600 seaweed extract: water) stimulated growth of tomato plants. Treatment of marigold (*Tagetuspatula*) with 10% SWC Kelpak for about 18 hours have shown to increase number and dry weight of roots.

14.4.2 Vegetative growth

Foliar application of seaweed extracts significantly enhanced vegetative growth. A significant

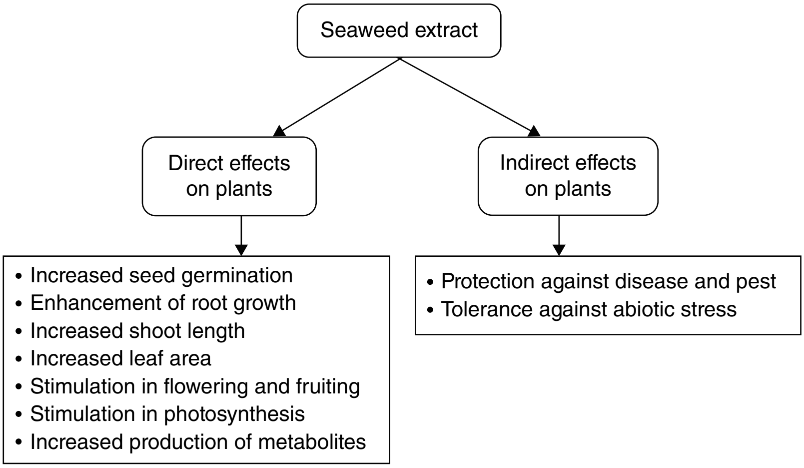

Fig. 14.1. Various ways by which seaweeds affect plant growth.

increase in length of leaf, width of leaf blade and plant height was noted in many species. The increase could be attributed to hormones like auxin and CKs present in seaweeds that exert stimulatory effects on metabolic activities by promoting cell division and elongation.

Plants treated with extracts of *A. nodosum* showed enhancement in growth (Mancuso *et al.*, 2006; Rayorath *et al.*, 2008). An increase in height and number of leaves was noted in *Arabidopsis* treated with extracts of *A. nodosum* (concentrations of 1 g L^{-1} plant). Shoot length showed increase in sweet orange trees treated with extracts of *A. nodosum* (Spann and Little, 2011). *Phaseolus aureus* seeds treated with extracts of red marine algae (*Asparagopsis taxiformis*) showed an enhancement in length of shoot, petiole and leaf surface area (Renuka Bai *et al.*, 2007). The extracts from *Durvillaea potatorum* and *A. nodosum* improved growth in seedlings of broccoli. Such plants showed an increase in leaf number, stem diameter and leaf area (Mattner *et al.*, 2013). The plant growth stimulation resulted due to presence of CKs in high quantities.

14.4.3 Seed germination

Seaweeds stimulate seed germination in plants (Sunarpi*et al.*, 2010; Ramarajan *et al.*, 2012; Godlewska *et al.*, 2016). Application of seaweed extract at low concentration promotes seed germination in plants. An increase in rate of seed germination and seedling vigor was noted in plants exposed to low concentrations of seaweed extracts. This increase in germination and vigor could be due the presence of growth promoting substances (IAA, IBA, gibberellins), micronutrients, vitamins and amino acids (Sivasankari *et al.*, 2006; Layek *et al.*, 2014). A high percentage of seed germination was noted in rice seeds exposed to low concentrations of seaweed extract (Layek *et al.*, 2018). An increase in seedling vigor index and shoot and root length was noted in these plants. Maize seeds treated with low concentrations (5%) of seaweed saps (*Kappaphycus* and *Gracilara* species) showed a high rate of germination (Layek *et al.*, 2016). A high rate of seed germination and increased seedling vigor was also reported in other crop plants such as green gram (*Vigna radiata*), cowpea (*Vigna sinensis*) and fenugreek (Sivasankari *et al.*, 2006; Sabale and Pise, 2010).

Treatment of seeds with liquid fertilizer (60%) prepared from marine algae *Enteromorpha intestinalis* showed increase in seed germination of soybean (Chetna *et al.*, 2015). Wheat seeds treated with *Kappaphyccus* or *Gracilara* seaweed sap (15% concentration) noted an increase in rate of germination (Dilavarnaik *et al.*, 2017). On the contrary, application of seaweed extract at high concentration (15%) inhibits seed germination. Reduction in germination after application of seaweed extracts could be attributed to presence of high concentration of salts, excessive hormones or minerals.

14.4.4 Flowering and fruiting

Application of seaweed concentrates (SWC) trigger early flowering and fruit set in a crop plants (Ali *et al.*, 2019; Dookie *et al.*, 2020). Tomato seedlings treated with SWC showed enhanced flowering in comparison to control plants.

14.4.5 Nutrient absorption efficiency

Application of seaweed extract has shown to increase nutrient uptake efficiency in plants. Enhancement in nutrient uptake could be attributed to up-regulation of genes encoding root nutrients transporters. Increased expression of genes (e.g. BnNRT1.1; BnNRT2.1; BnSultr4.1; BnSultr 4.2) encoding for nitrogen- and sulfur-uptake root transporters has been noted in plants treated with seaweeds.

Brassica napus treated with *A. nodosum* seaweed extract showed increased uptake of nitrogen and sulfur (Jannin *et al.*, 2013). *Laminaria* spp. and *A. nodosum* treated (concentration of 5 mL L^{-1} for 48 hours) maize plants showed an increased ability to absorb Ca, Mg, S, Fe, Cu, Mn, Mo, Zn, and B. Increased uptake of N, P, K, Mg and Ca has been noted in grapevine and cucumber treated with seaweed extract (Turan and Kose, 2004; Mancuso *et al.*, 2006). Application of *A. nodosum* extract significantly enhanced potassium content of leaf of poplar plants grown under greenhouse conditions and nitrogen content of stem of poplar plants under field conditions.

The increase in nutrient uptake noted after seaweed application proves an eco-friendly approach for improving soil fertility as this reduces the use of chemical fertilizers.

14.4.6 Production of chlorophyll and other metabolites

Application of seaweeds extracts induces an increase in plant chlorophyll content (Ramarajan *et al.*, 2013). Betaines present in the seaweed extract reduce chlorophyll degradation and protects thylakoid membrane by regulating osmotic adjustment and enhancing ion homeostasis. Betaine also acts as a source of nitrogen at low concentration but as an osmolyte at higher concentrations. Increased in chlorophyll production could also be due to presence of magnesium which is the supplied by seaweed extracts (Savasangari *et al.*, 2011).

Application of a low concentration of *A. nodosum* extract to foliage produced leaves with higher chlorophyll content in tomato. An increase in chlorophyll content has been noted in crop plants such as tomato, dwarf French bean, wheat, barley and maize treated with extracts of *A. nodosum*.

Application of *Caulerpa scalpelliformis* liquid fertilizers at 20% and *Ulva reticulata* at 2% promoted production of photosynthetic pigments, amino acids and total sugar content in *Vigna mungo* (Kalaivanan *et al.*, 2012; Selvam and Sivakumar, 2013).

14.4.7 Photosynthesis

Oligosaccharides present in the seaweed extract increased uptake and better assimilation of nitrogen leading high rate of photosynthesis and altered metabolic pathways (Abbas *et al.*, 2020).

14.5 Benefits of using seaweed extracts in agriculture

Seaweed extracts act as biostimulants. They improve vegetative growth, nutrient efficiency and tolerance capacity in plants exposed to abiotic stresses and diseases (Khan *et al.*, 2009; Craigie, 2011; Du Jardin, 2012; Calvo *et al.*, 2014; Selvakumari and Venkatesan, 2017; Begum *et al.*, 2018; Yao *et al.*, 2020). Liquid seaweed extracts have proved effective in improving productivity of crops by enhancing growth via promoting germination of seeds, imparting metabolic benefits, triggering disease response pathways, increasing pathogen resistance and stress tolerance (Sathya *et al.*, 2010; Wally *et al.*, 2012; Divya *et al.*, 2015a; Mirparsa *et al.*, 2016) (Table 14.2).

Table 14.2. Effects of some of the seaweed extracts on crop species.

Seaweed species	Crop plant	Reference
Sargassum myriocystum	Vigna mungo	Kalaivanan and Venkatesalu (2012)
Kappaphycusalvarezii	Soybean (Glycine max)	Rathore *et al.* (2009)
Ascophyllum nodosum	Eggplant (Solanum melongena)	Bozorgi (2012)
Sargassum wightii mixed with Ulva lactuca	Peanut (Arachis hypogaea)	Sridhar and Rengasamy(2011)
Sargassum wightii	Brinjal	Divya *et al.* (2015b)
A. nodosum	Grapes (Vitis vinifera)	Norrie and Keathley (2006)
D. potatorum and A. nodosum	Broccoli	Mattner *et al.* (2013)
Ecklonia maxima	Brassica rapa	Jannin *et al.* (2013)
A. nodosum extract	Alfalfa plants	Khan *et al.* (2013)
Kappaphyccus alvarezii	Maize, green gram, black gram	Zodape *et al.* (2009, 2010); Jadhao *et al.* (2015)
Kappaphycus alvarezii Gracilaria edulis	Potato	Prajapati *et al.* (2016)
K. alvarezii G. edulis	Wheat	Shah *et al.* (2013)

Foliar application helps in uptake of nutrients as at a faster rate (Fernandez and Brown, 2013; Shah *et al.,* 2013; Nedumaran and Arulbalachandran, 2015). Cereals, vegetables, fruit plants and horticultural crops showed improvement in growth and yield after application of seaweed extracts (Khan *et al.,* 2009; Hurtado *et al.*, 2009; Elansary *et al.,* 2016). Enhancement in nutrient uptake promotes growth and productivity of crops. The improvement in growth is attributed due to the presence of growth regulators (auxins such as IAA and IBA, gibberellins, CKs), osmoprotectants, betains and micronutrients (Wally *et al.*, 2012; Mathur *et al.*, 2015; Gopalakrishnan and Binumol, 2016; Pacholczak *et al.*, 2016a, 2016b; Reddy *et al.*, 2016; Vyomendra and Kumar, 2016).

Ecklonia maxima and *A. nodosum* extracts proved beneficial in stimulating growth and productivity in many agricultural crops (Rayorath *et al.*, 2008; Sridhar and Rengasmay, 2011; Gireesh *et al.*, 2011; Stirk *et al.*, 2014; Michalak *et al.*, 2016). Foliar application of *A. nodosum* extract (AN) at 0.5% for 10 days increased plant height, the number of leaves, plant dry biomass, root length and chlorophyll content. Plants also showed significant increase in number of flowers, fruits and fruit yield. The presence of high levels of plant growth promoting substances such as CKs, particularly trans-zeatin-type CK, abscisic acid (ABA), gibberellins, brassinosteroids and castasterone help in promoting growth (Wally *et al.*, 2012). Studies have proved that phytohormones stimulate root development, increase plant growth and vigor by improving efficiency of uptake of nutrients and water.

Enhancement in growth of rice, maize, green gram, *Vigna,* okra and corn has been noted after foliar application of seaweeds extracts/sap (Partani, 2013; Pramanick *et al.*, 2013; Singh *et al.*, 2015a, 2015b, 2016). Extract of *Kappaphycus alvarezii* increased growth and straw yield of soybean. vegetative growth, and yield of fig trees (*Ficus carica*) showed an enhancement after treatment of algal extracts (Al-Hameedawi, 2016). *Azotobacter chrocoocum* and *Bacillus megaterium*, when mixed with marine algal extracts, triggered growth of bitter orange seedlings. A greenhouse study showed that fresh and dry yield increased in *Brassica rapa* L. treated (via root application) with *Ecklonia maxima* SWE at 3 mL L^{-1}. An improvement in the nutritional status (higher P and K and lower Na concentrations), photosynthetic rate and chlorophyll content was noted in plants treated with SWE. *Ascophyllum nodosum* exerted positive effects on the yield of 'Thompson seedless' grape (*Vitis vinifera* L.) (Norrie and Keathley, 2006). An increase in the production of berries, size and weight of berry and number of bunches per plant was noted in treated plants. The yield of fruits increased in tomato plants sprayed with SWE during the vegetative stage. Foliar sprays of seaweeds at 5–10 mL L^{-1} promoted growth of petunia and tomato. The parameters such as shoot weight (FW), biomass, root index, chlorophyll index (SPAD) and flower number noted an increase in plants after treatment. Growth, yield attributes in four onion cultivars was positively influenced after foliar application of SWE (Abbas *et al.*, 2020). Uptake and accumulation of elemental nitrogen content was noted in the bulbs. Auxin present in the seaweed enhanced cell division, elongation, and differentiation leading to increase in weight of bulb.

Seasol is commercially available liquid seaweed extract prepared by alkaline hydrolysis of biomass *of D. potatorum* and *A. nodosum*. Stimulatory effect on seed germination, seedling length and weight in crop plants was noted after application of seaweed extract (SM6) at 10%. Liquid extract of *Rosenvingea intricata* increased growth and pigment concentration in *Cyamopsis tetrogonolaba* and *Abelmoschus esculentus* (Thirumaran *et al.*, 2009a, 2009b). Stimplex® 6, a seaweed extract derived from brown algae (*A. nodosum*) when applied at the dose of 3.2 mL L^{-1} showed improvement in vigor, health and yield of lettuce, mustard, kale, Swiss chard, amaranths and collards. Kelpak SL (a water-soluble concentrate) obtained from *Ecklonia maxima* increased yield in *Phaseolus vulgaris* (Kocira *et al.*, 2013).

Algal extracts proved useful in improving quality of fruits and yield in apple trees (Spinelli *et al.*, 2009). Application of LBS6 at 1 ml L^{-1} improved growth and yield in sugarcane. An increase in the yield of wheat grain was noted after application of *Kappaphyccu salvarezii*and *Gracilaria edulis* sap at 5–7.5% (Zodape *et al.*, 2009; Shah *et al.*, 2013). The protein content in rice and wheat grain was significantly increased after application of K or G saps at 15% (Shah *et al.*, 2013; Layek *et al.*, 2018). The application of seaweed extract also increased the level of micronutrients like Cu, Zn, Fe and Mn in rice grains (Layek *et al.*, 2018). Similar results were also reported in okra and wheat treated with seaweed extracts (Zodape *et al.*, 2009; Shah *et al.*, 2013). Seaweeds exert positive effects on root proliferation and increase uptake of nutrients such as N, P and S required for protein synthesis (Shah *et al.*, 2013). Chelating compounds (e.g. mannitol) present in the extract increase availability of some micronutrients to plants. The number of flowers and seeds per flower head showed increase in marigold after Kelpak treatment. Maxicrop treatment increased yield in lettuce and the size of the florets in cauliflower. Similar responses have also been noted in barley and peppers (Arthur *et al.*, 2003). Foliar application of Kelpak 66 improved yield in bean. Treatment of seaweed LBS 6S at 1 ml L^{-1} (LBS) at vegetative and early flowering stages improved bunch weight and number of fingers per bunch in plants. Application of *Kappaphyccus alvarezii* extract at 10% improved the yield in maize. The number of pods, weight of pods, seed yield per plant and 100-seed weight noted increase in treated plants (Zodape *et al.*, 2010).

Application of the recommended dose of fertilizer along with extract of *Kappaphycus alvarezii* (K sap) or *Gracilaria edulis* (G sap) at 10% concentration improved growth and yield in potato (Prajapati *et al.*, 2016). *Sargassum horneri* seaweed extract (at 60 kg hm^{-2}) increased the yield of tomato. The increase in yield could be attributed to improved photosynthetic capacity of the leaves. Seaweed extracts exert a positive effect on growth of the potato plant and total yield in potato by increasing production of tubers, improving nitrogen availability and protein content (Sarhan 2011; Haider *et al.*, 2012). An increase in oil content, oil yield and crude protein in sunflower after seaweed treatment (Osman *et al.,* 2011). An increase in oil content has also been noted in sunflower after application of *Gracilaria dendroides* and *Ulva lactuca* extract at 0.6% (Osman and Salem Olfat, 2011).

Application of seaweed extract enhanced growth and yield attributes in legume plants (Sethi and Adhikary, 2008). Straw yield increased in green gram treated with seaweed extract at 15% (Pramanick *et al.*, 2013). Application of *Kappaphykus* sap at 15% followed by 15% *Gracilaria* along with the recommended dose of fertilizer increased grain yield in green gram. The increase in yield could be attributed to the presence of micro-elements and plant growth regulators in the extract (Zodape *et al.*, 2009). Foliar application of *K. alvarezii* extracts at 10% increased the carbohydrate and protein content in green gram (Anantharaj and Venkatesalu, 2001; Zodape *et al.*, 2010). Application of *Kappaphyccus alvarezii* and *Gracilaria edulis* extracts at 10% concentration increased grain yield in black gram (Jadhao *et al.*, 2015). Application of *Kappaphyccus* and *Gracilaria* sap increased number of panicles, panicle length, number of grains per panicle and 1000-grain weight in rice (Pramanick *et al.*, 2014; Layek *et al.*, 2018). The application *Kappaphycus alvarezii* extract at 15% improved grain yield in soybean. This result was obtained because plant height, number of branches, number of pods per plant and number of grains per plant increased in treated plants (Rathore *et al.*, 2009). *Sargassum wightii*, *Ulva lactuca* and *Enteromorpha intestinalis* extract promoted seed germination and growth in legume plants (Mathur *et al.*, 2015).

Seaweed application increased number of functional nodules in legumes. The bioactive compounds and their organic subfractions present in the seaweed extract affect the legume–rhizobia signaling processes. These processes trigger production of nodules and overall growth of plants (Khan *et al.*, 2013). Cytokinins such as *trans*-zeatin riboside and their dihydro derivatives present in seaweed extracts play a major role in this (Saravanan *et al.*, 2003).

14.6 Other effects of seaweeds on plants

Besides affecting the growth of plants directly by improving the nutrient content in plants, seaweed extracts improve growth of plants indirectly via imparting tolerance against stress and providing protection from disease and pests.

14.6.1 Protection from pathogen

Extracts from seaweeds enhance plant defense against pest and diseases (Allen *et al.*, 2001; Jayaraman *et al.*, 2011). Seaweeds are rich source of elicitors that play a role in the defense of plants (Cluzet *et al.*, 2004). Elicitors are the signal molecules perceived by plants and play a role in protection against pathogen invasion. The cell wall of attacking pathogens contains a wide range of molecules such as oligosaccharides and polysaccharides, peptides, proteins, and lipids. Polysaccharides present in algal extracts act as effective elicitors of plant defense against diseases.

Red algae contain agars and carrageenans, brown algae contain alginates, laminarans, sulfated fucans, and other complex mucilages. Green algae (e.g. *Ulva* spp.) contain mucilages composed of rhamnose, uronic acid and xylose units present in the cell walls (Cluzet *et al*., 2004). Laminaran induces multiple defense responses in plants such as alfalfa and tobacco. Carrageenans act as elicitors and induce defense in tobacco plants (Mercier *et al*., 2001).

Antimicrobial activities have been noted in secondary metabolites, for example terpenes, aromatic-like compounds, acetogenins, amino acid derived products, phlorotannin, polymeric substances fatty acids, phlorotannins, pigments, lectins, alkaloids, terpenoids and halogenated compounds produced by green, brown and red algae (Thinakaran and Sivakumar, 2013; Shri Devi and Paul, 2014; Perez *et al*., 2016).

Seaweed extracts have been found to be effective against nematodes. *Ecklonia maxima* showed reduction in root knot nematode infestation in tomato. Extracts of seaweeds namely *Sargasssum tenerrimum, Padina tetrastromatica* and *Melanothamnus afaqhusainii* showed nematicidal activity against the root knot nematode *Meloidogyne javanica* (Khan *et al*., 2015). Application of liquid seaweed extract showed reduction in primary and secondary infection of *Plasmodiophora brassicae* in broccoli (Wite *et al*., 2015). Seaweed extract (Seasol™) prepared from *D. potatorum* and *A. nodosum* suppressed primary and secondary infection by *P. brassicae* (55 to 84%) in broccoli. Supplementation of *Ulva lactuca* powder in soil at the rate of 5 g kg^{-1} significantly decreased root knot nematode infections in banana plants as the number of galls reduced. Extracts of *Melanothamnus afaqhusainii* showed significant suppressive effect on root rotting fungi *Fusarium solani* and root knot nematode *Meloidogyne incognita* in eggplant. Application of *Spatoglossum variabile, M. afaqhusainii*, and *Halimeda tuna* extracts suppress fungi *Rhizoctonia solani* and *Fusarium solani* found on the roots of tomato. Seaweed extract imparts resistance against nematodes.

Seaweed extracts showed capacity to suppress clubroot infection process. Algal extracts increased resistance to *Colletotrichum* in alfalfa plants. Liquid seaweed extracts stimulated the growth and activity of microbes showing an antagonistic action against *Pythium ultimum.* This fungal pathogen causes damping-off disease in cabbage seedlings (Dixon and Walsh, 2002).

Seaweed extracts suppressed effect of *Albugo candida.* Organic extracts of seaweeds such as *Sargassum vulgare, Cystoseir abarbata, Dictyopteris membranacea, Dictyota dichotoma* and *Colpomenias inuosa* showed antifungal effect on fungal species such as *Alternaria alternata, Cladosporium cladosporioides, Fusarium oxysporum, Epicoccum nigrum, Aspergillus niger, A. ochraceus, A. flavus,* and *Penicillium citrinum* (Khallil *et al*., 2015). Seaweed application significantly reduced white blister disease on broccoli leaves (Mattner *et al*., 2013). Suppression of disease resulted from activities of defense-related enzymes such as phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, chitinase, β-1,3-glucanase and total phenolic compounds. Halogenated terpenes, fatty acids (hexadecanoic and octadecanoic acids), and quercetin of seaweeds contributed to antifungal effect.

Seasol™ suppressed growth of *Sclerotinia minor,* a necrotrophic pathogen in horticultural crops such as lettuce, green bean, cabbage, broccoli and others. Foliar sprays of seaweed extracts inhibited fruit rot and gray mold development. *Ascophyllum* extract proved effective in reducing disease caused by pathogens in crops grown under greenhouse and field conditions. *A. nodosum* treatment induced resistance to *Phytophthora capsici* in pepper.

Extracts of *Ulva fasciata* and *Ulva lactuca* destroyed nymph and adults in cotton pest (*Dysdercusc ingulatus*) (Asha *et al*., 2012). *Ulva* extract inhibited expression of pathogenesis-related genes, namely PR-10. The gene is responsible for initiating defense against diseases caused by pathogen attacks (van Loo *et al*., 2006). The disease resistance in plants improved because of action of defense-related genes (Jayaraj *et al*., 2008). Extract obtained from brown algae species reduced necrotic lesions induced by *Alternaria solani*. Aphids and other sap-feeding insects reduced in number in plants treated with seaweed extracts. Seaweed extracts reduced population of red spider mite present on apple trees. Extract from brown seaweed *Padina pavonica* showed nymphycidal activity and significantly reduced the nymphal development by interfering with physiology of *Dysdersusc ingulatus* (cotton pest).

Terpenes present in seaweeds reduce antimicrobial activities (Paulert *et al.*, 2009; Peres *et al.*, 2012). Seaweeds possess antioxidant polyphenols that show bactericidal properties (Zhang *et al.*, 2003). Suppression of disease resulted from activities of defense-related enzymes including phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, chitinase, β-1,3-glucanase and total phenolic compounds.

14.6.2 Mitigation of abiotic stress

Abiotic stress such as drought, salinity, extreme temperatures and nutrient deficiencies are blocked by seaweed extracts (Sharma *et al.*, 2004). Bioactive substances present in seaweeds impart stress tolerance and enhance performance in plants. Drought and salinity tolerance imparted by seaweeds is mainly due to its mineral composition and growth regulators (CKs and ABA). Polysaccharides extracted from seaweeds enhance adaptability of plants to stress. Polysaccharides contribute to the increase in length of shoot, root length, dry and fresh matter of plants under stress conditions. Polysaccharides increase sugar, proline content, and regulate Na^+/K^+ ratio thereby maintaining the osmotic status in stressed plants.

Seaweeds decrease relative electrolyte leakage and malondialdehyde (MDA) content but increase the activity of antioxidant enzymes such as superoxide dismutase, peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX). These enzymes are involved in scavenging of reactive oxygen species (ROS). Seaweed extracts play a role in scavenging ROS and inhibition of lipid peroxidation.

Seaweed extracts have also shown capacity to improve seed germination under conditions of salt stress (Alalwani *et al.*, 2012; Latique *et al.*, 2014). Extract of *Sargassum latifolium* (SAR) removes the adverse effect of salinity in barley (*Hordeum vulgare* L.). This is done by reducing lipid peroxidation (MDA) but stimulating total antioxidant activity, proline and phenols. Extracts of *A. nodosum* enhanced flowering in *Amaranthus tricolor* exposed to salinity (Abdel Aziz *et al.*, 2011). Liquid extracts of *Ulva lactuca* showed potential to restore growth of durum wheat (*Triticum durum)* under salinity conditions (Nabti *et al.*, 2007, 2010).

Growth and yield showed improvement after application of *Durvillaea plantarum* extract in bean plants exposed to water stress. Extract of *Fucus spiralis* (macroalgae) induced tolerance in water stressed sage plants (*Salvia officinalis*) (Mansori *et al.*, 2019). *A. nodosum* extracts proved useful in improving drought tolerance in creeping bent grass (Zhang and Ervin, 2004). Extracts of *Sargassum* or *Ulva* help in curtailing damaging effects of oxidative stress induced by drought via activation of antioxidative system. These extracts provide phytohormones and micronutrients essential for growth of wheat (Kasim *et al.*, 2015). Total phenolic content increased in sage plants subjected to drought stress after treatment with SWE (25%). Treatment with *Gracilaria dura* increased biomass and yield in wheat plants subjected to drought stress (Sharma *et al.*, 2014). Sap of *Kappaphycus alvarezii* (K sap) showed potential to alleviate salt and drought stress in durum wheat. Morphological parameters such as total chlorophyll, carotenoids levels increased, but electrolyte leakage and lipid peroxidation (MDA) reduced in stressed plants. Treatment with sap reduced the ionic imbalance by decreasing the Na^+/K^+ ratio but increase calcium content and accumulation of osmoprotectants, proline, total protein, and amino acids (Patel *et al.*, 2015).

Zeatin riboside (a cytokinin) present in the seaweed extract proved useful in inducing heat tolerance in creeping bent grass (Zhang and Ervin, 2008). Plants sprayed with seaweed extract enhanced tolerance capacity to freezing (low temperature) (Mancuso *et al.*, 2006). Commercial seaweed extract (Kelpack) enhanced seed germination rate in *Ceratotheca triloba* exposed to low temperatures. Maize plants exposed to cold stress showed restoration after treatment with addition of seaweeds extract (Brandáčová *et al.*, 2016). *Ascophyllum* extracts (Seasol) improved freezing tolerance in grapes. Algafect, a commercial seaweed extract obtained from *A. nodosum*, *Fucus* spp. and *Laminaria* spp. reduced leaf necrosis in maize subjected to low temperatures. Application of seaweed extract (Maxicrop) improved winter hardiness and increased frost resistance in barley (*Hordeum vulgare*) growing in field conditions.

Extracts of *A. nodosum* improved fresh biomass in lettuce, shoot, leaf area and length in almonds plants subjected to potassium deficiency. Extracts obtained from *A. nodosum* and *Durvillea potatorum* have shown capacity to alleviate iron deficiency in tomato plants (Carrasco-Gil *et al.*, 2018).

14.7 Conclusions and future perspectives

Beneficial roles of seaweeds in agriculture and horticulture have been well demonstrated. Seaweeds improve soil fertility by improving organic content, which increases the availability of water and minerals. Lipids, proteins, carbohydrates, phytohormones, amino acids, osmoprotectants, minerals and antimicrobial compounds present in seaweeds contribute to improving growth and yield in plants. The components such as hormones (mainly CKs) and trace nutrients exert a positive effect on growth of crop plants. Seaweed exerts support growth of plants directly by increasing nutrient supply but indirectly by providing protection against abiotic stress conditions such as salinity, drought, high and low temperature, mineral stress and biotic stresses such as phytopathogens. Seaweeds thus can provide an environmentally friendly alternate for restoring soil fertility via nutrient management.

Bioactive molecules present in seaweed extracts enhance growth, uptake of nutrients, photosynthetic capacity and improve antioxidant machinery in plants exposed to stress. The function of molecules present in the seaweed extract and their complex mode of action still remain to be elucidated. Understanding of action of seaweeds in increasing growth and productivity of plants via stimulation in biological activities help in establishing them as materials of immense value.

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15 Biostimulants for Plant Abiotic Stress Tolerance

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Abstract
Plant growth and development have been shown to be significantly affected under environmental stress condition, resulting in poorer yield and quality of output. Increasing crop stress resistance by genetic improvements also needs prolonged breeding plans and diverse growing environments for verification of crop performance. Biostimulants are projected as agricultural tools for resisting abiotic stress. Certainly, these substances with bioactive molecules improve plant growth, physiology and metabolism, which enables plants to survive under stressful environment and improves the yield and quality of the products. Several establishments have invested in the development of novel biostimulant products, as well as in recognizing the bioactive molecules like polysaccharides, amino acids, and short peptides present in various types of extracts with maximum efficiency and ability to promote specific abiotic stress responses in plants. The majority of such substances are unknown, making it nearly impossible to determine the actual composition, thus, they can be categorized depending upon their function in plants. Biostimulants are usually used in high-value crops to offset the most common environmental stresses. Biostimulants regulate plant physiology and signaling cascades under stressful conditions. In this chapter, the major roles of biostimulants in conferring plant abiotic stress are discussed.

15.1 Introduction

Biostimulants are chemicals or microorganisms that are used to enhance plant physiology and productivity by supplementing essential nutrients or improving stress tolerance. In turns it improves crop yield and quality (Du Jardin, 2015). During the last decade, there has been a phenomenal increase in interest in plant biostimulants as a possible alternative to minimize the negative impact of environmental change on agriculture, as well as revolutionized agriculture for eco-friendly crop production. Although the concept of biostimulants was first proposed in 1933, it has only recently been acknowledged, and research from diverse domains has been conducted to clarify, explain and comprehend plant biostimulants and their metabolic pathways (Nephali *et al.*, 2020).

These compounds are effective in minor amounts and enable plants to tolerate adverse environmental conditions, thus improving the overall crop quality and nutritional effectiveness. They act similarly to other plant hormones on external application (Yaronskaya *et al.*, 2006). Being a major issue for plant growth and development in the current period, water stress, salt stress, heavy metal stress as well as extreme

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temperature stress, cause massive losses of crops in the world (Singh and Takhur, 2018). For preventing these losses, biostimulants are widely used in agriculture to change a plant's biochemical processes and boost production (Yakhin *et al.*, 2017). Biostimulants are different from traditional N, P, and K fertilizers, and can have various organic compounds, such as humic substances, algal derivatives, proteins and minerals as well as some other compounds like plant growth hormones (auxin, cytokinin, gibberellin, ethylene and abscisic acid), which may be changed at the will of the maker (Yaronskaya *et al.*, 2006). Biostimulants provide a potentially new method for regulating and/or modifying plant physiological procedures to enhance production, reduce the negative impact of stress and enhance productivity. Biostimulants have a wide range of functions, regardless of the fact that their influence is yet unknown. Some of them can act directly on plant productivity while others affect crop yield indirectly (Yakhin *et al.*, 2017). Several studies have been done to assess how biostimulants enhances plant productivity under stressful environment. In biostimulant formulations, various primary ingredients such as organic acids, seaweed derivatives, plant growth hormones as well as growth-promoting microorganisms are used (Du Jardin 2012; de Vasconcelos and Garófalo Chaves 2019).

15.2 Biostimulants

Plant biostimulants are inorganic or organic compounds derived from plants or microorganisms which are reported to enhance plant growth, production and stress tolerance (Du Jardin, 2015; Rouphael and Colla, 2018). Amino acids, polysaccharides, oligosaccharides, vitamins, minerals and a small number of phytohormones are the major components of biostimulants. However, the activity of biostimulants should not be determined by the quantity of nutrients or phytohormones in the product. Biostimulant-activated processes are usually difficult to recognize and require further studies (Paul *et al.*, 2019). High-throughput phenotyping as well as 'omic' technologies are valuable methods to know how biostimulants work (Bulgari *et al.*, 2017; Dalal *et al.*, 2019; Nardi *et al.*, 2009). Through improving the soil structures, they directly influence the physiological and metabolic activities of plants (Bulgari *et al.*, 2015; Caradonia *et al.*, 2019). Biostimulants are able to induce alteration in the biochemical mechanisms of plants that can enhance the water use efficiency, nutrient uptake, growth and physiology of plant under stressful environment (Nardi *et al.*, 2009; Yakhin *et al.*, 2017; Caradonia *et al.*, 2019; Bulgari, *et al.*, 2019a).

15.3 Types of biostimulants

Although recently a lot of effort has been made to clarify the controlling effect of biostimulants on plants, currently no standard or authentic definition can explain it thoroughly. This condition excludes a detailed listing and classification of the compounds as well as microorganisms covered under this concept. Nonetheless, researchers, policymakers and consumers have broadly identified certain major classes in combination with chemical and microbial components (Calvo *et al.*, 2014; Du Jardin, 2012; Halpern *et al.*, 2015).

During the past few years, various authors have categorized biostimulant products based on the major constituents or their mode of action. In order for these products to be noticed, both types of information must be included in the labels in several parts of the world (Yakhin *et al.*, 2017). Although biostimulant products are currently categorized on the basis of their primary constituents, it may not be a requirement to provide reliable knowledge regarding its biological activity (Du Jardin, 2015). Therefore, biostimulants are classified as given below.

15.3.1 Humic substances (HS)

These substances are humic acids, fulvic acids and humins. They are formed by the continuous biodegradation of organic residues as well as the metabolic activities of soil dwelling microorganisms (Rouphael and Colla, 2018). Humic substances create a favorable environment in the plant root zone that prolongs the physiological activity of roots (Canellas *et al.*, 2002; Trevisan *et al.*, 2010). This result can be noticed by the enhanced nutrient uptake, water use efficiency and tolerance to abiotic stresses (Canellas *et al.*, 2015; Nardi *et al.*, 2016). Humic substances' mechanism and their influence on plant physiology is not very clear. This might be related to the complexity of these chemicals at the molecular level, as well as the amount and variety of responses of plants influenced by their use. Besides, a significant interaction between the medium properties and bioactivity of HS has been observed (Canellas *et al.*, 2012). The positive influences are exerted by these complex substances because they act similarly as plant hormones as well as IAA-independent processes (Trevisan *et al.*, 2011). HS can also stimulate plant growth and development and promote the synthesis of H^+ ATPase in plasma membrane in a similar way as auxins (Nardi *et al.*, 2000, 2002; Dobbss *et al.*, 2010).

15.3.2 Hydrolyzed proteins and amino acid containing products

Commercially produced hydrolyzed phytoprotein products are reported to have positive effects on the growth and physiology of plants compared to commercially produced hydrolyzed animal proteins (Cerdán *et al.*, 2009; Lisiecka, 2011). Compared to the control, lettuce plants treated with an animal-based protein hydrolysate had higher fresh weight as well as dry weight (Botta 2013). Usually, these compounds can activate plant defense responses as well as enhance plant resistance against several abiotic stresses (Lucini *et al.*, 2015; Rouphael *et al.*, 2017; Colla *et al.*, 2017; Trevisan *et al.*, 2019).

15.3.3 Microorganisms

Some of the beneficial microorganisms like plant growth-promoting bacteria, fungi and microalgae are used in soil to enhance crop productivity by metabolic actions. They increase the nutrient uptake by fixing nitrogen as well as nutrients solubilization and modifying the hormonal status through induction of phytohormones biosynthesis. Some of them produce volatile organic compounds and increase resistance against abiotic stresses, which have a direct influence on plants. Plant growth-promoting rhizobacteria (PGPR) can improve plant responses to abiotic stresses by enhancing physical, chemical and biological activity (Ruzzi and Aroca, 2015; Turan *et al.*, 2017). Microorganisms provide positive influences on plants by forming a protective biofilm on the root surface, enhancing nutrient and water uptake.

15.3.4 Extracts of plants of waste products

Plant extracts can contain bioactive compounds which may act as potential biostimulants. For example, seaweeds provide enhanced plant growth and stress tolerance to plants. Application of seaweed extracts could provide antioxidant defense and, therefore, mitigated plant oxidative stress (Hasanuzzaman *et al.*, 2021). Biostimulants may also be produced from food waste extracts, industrial by-products, composts and compost extracts, vermicompost, manures, aquaculture residues and their waste streams, as well as sewage treatments among other things (Xu and Geelen, 2018). Due to the diversity of source materials as well as extraction methods, the mode of action of these products is not very easy to determine (Yakhin *et al.*, 2017). The new fertilizer regulation, as well as the Circular Economy Action Plan, are based on the idea of using by-products as raw materials that may be converted into fertilizing products with an emphasis on sustainable agriculture. The standards for fertilizer regulation, the need to develop environmentally friendly farming practices while retaining the elevated yield and quality of crop, synthetic fertilizer price rise, several agrichemicals withdrawal, and the multidimensional consequences of biostimulants on plants or soil are responsible for the expansion of this market.

15.3.5 Nanoparticles and nanomaterials

Juárez-Maldonado *et al.*, (2019) recently proposed a novel class of biostimulant products, involving nanoparticles and nanomaterials. Nanoparticles and nanomaterials are generally those particles that have dimensions between 1 nm and 100 nm and exhibit characters that are not present in their bulk form. They can alter the yield and quality of the product and resistance to abiotic stresses when used in reduced amount as foliar sprays or nutrient solutions in vegetable crops (Qi *et al.*, 2013; Kiapour *et al.*, 2015; Lei *et al.*, 2008; Khan, 2016). Their biostimulant characteristics are probably related to the structure as well as properties of the substances. The connection between the plant with nanoparticles and nanomaterials surfaces can have a positive impact on ions and metabolites transfer, as well as receptor activity through alteration in the energy and charges of the neighboring environment. This activity is not based upon chemical configuration. Nanoparticles and nanomaterials discharge chemical elements such as Fe or C, which are helpful in plants when metabolized (Bulgari *et al.*, 2019a).

15.4 Role of biostimulants in abiotic stress

Abiotic stress is an environmental state which reduces growth and yield of the plants under the optimum amount (Cramer *et al.*, 2011). Abiotic stress like cold, drought and salt mainly affects plant development, but it also affects crop yield. Abiotic stress is becoming the main risk for food security because of continuous alterations in climate as well as worsening environmental conditions due to human actions. For resisting abiotic stress, plants can start a variety of molecular, cellular and physiological alterations in response to and for adapting to these stresses (Huang *et al.*, 2013). Abiotic stresses can be restricted through optimization of plant growth environments and by providing water, nutrients and plant growth regulators such as auxins, cytokinins, gibberellins, strigolactones and brassinosteroids. Along with these conventional strategies, biostimulants are known for the promotion of optimizing yield through alteration in physiological mechanisms in plants. Biostimulants give a possible new strategy for the control and/or alteration of physiological mechanisms in plants to enhance growth, lessen stress-induced restrictions, as well as to enhance productivity (Yakhin *et al.*, 2017).

Auxin is a plant hormone that mainly controls some characteristics like cell division and expansion, tropisms, differentiation, apical dominance, senescence, abscission along with flowering. The cytokinins are primarily accountable for cell division, in addition to influencing many other mechanisms like vascular development, apical dominance, as well as nutrient transfer, particularly when having interaction with auxins (Teale *et al.*, 2006).

Gibberellic acid has a profound impact on the seed germination procedure, stimulates hydrolytic enzymes activities like α-amylase and protease, which actively participate in the unfolding of the reserve materials, helping in the mobilization of the endosperm. Along with this, they encourage the cessation of dormancy, stem expansion and growth, cell division and, ultimately, leaf enlargement (Taiz and Zeiger, 2009).

According to Santos and Vieira (2005), the biostimulant containing cytokinin, indole butyric acid and gibberellic acid, used in seed, enhanced the percentage of seedling emergence of *Gossypium hirsutum* L., as well as leaf area, height, together with the growth of seedlings. The algal extract which is used on leaf gave maximum seed yield in *Glycine max* L. (Rathore *et al.*, 2009).

It is reported that foliar application of putrescine along with amino acid glutamine enhance the quality and quantity of *Allium cepa* L. bulbs (Amin *et al.*, 2011). L-Glutamic acid is a significant amino acid that functions as a main molecule in the higher plant's metabolism (Forde and Lea, 2007) as the precursor of the chlorophyll synthesis in leaves (Yaronskaya *et al.*, 2006), together with the carbon regulation function as well as nitrogen metabolism (Robinson *et al.*, 1991). Glutamate is also a precursor of arginine as well as ornithine, which later on act to synthesize polyamines, which function in plants for reducing stress conditions (Rhods *et al.*, 1986; Lea *et al.*, 2007).

Along with this, certain other amino acids are also significant in cell metabolism with the expressive variety of biological roles. The use of algae or certain other plant extracts has useful influences on growth as well as stress tolerance. Algal extracts, protein hydrolysates, humic and fulvic acids, together with certain extra compounds, have characters that are beyond basic nutrition which mostly promotes growth as well as stress resistance. Although a majority of plant biostimulants are added to the rhizosphere to enable nutrients uptake, several of them also have roles in defense against environmental stress like water deficit, soil salinity and exposure to adverse growth temperatures (Van Oosten *et al.*, 2017).

15.4.1 Biostimulants and drought stress in plants

Drought is one of the significant and widespread stresses of plants in several areas of the world, particularly in arid as well as semiarid regions. It is a multidimensional stress and usually modifies the physiological, morphological, ecological, biochemical, as well as molecular features of plants. Additionally, it may harm the quantity and quality of plant development as well as productivity. Plant responses differ to water shortage in accordance with the intensity of water scarcity and duration, the plant species, age and growth stage.

Biostimulants, when used in seeds or early plant development, promote root production as well as growth (Lana, 2009), particularly in low fertility soil and low water accessibility, and work upon the enhanced regaining of the seedlings in adverse situations like water scarcity. These substances, particularly organic ones, decrease the requirement of fertilizers in plants and enhance their yield as well as tolerance to water and climatic stress because they work like hormonal and nutritional increments (Russo and Berlyn, 1990). As a result, a range of biostimulants have been manufactured and sold primarily in the agricultural sector. Biostimulants sold under the trade names Generate, Crop Set, Fulcrum, and Redicrop 2000 acted positively in both the root system as well as a foliar spray in three tree species *Quercus rubra*, *Betula pendula* and *Fagus sylvatica*. The biostimulant Yoduo was used in soybean leaves, providing 8.61 bags per hectare in comparison to control.

Plants exposed to water stress face damage to their cells through free radicals; however, through antioxidants which are provided by biostimulants, they reduce the toxicity of these radicals and improve the defense system of plants because of rising in the level of antioxidants.

According to Hamza and Suggars (2001), plants with a high number of antioxidants promote root as well as shoot growth, maintain a high level of water in the leaves, and have less frequency of diseases, both in perfect conditions and in environmental stress. Water scarcity influences many characteristics of plant growth, of which the main possible impact of water stress is shown through the decrease in plant size, leaf area, as well as crop yield (Kramer, 1983). In recent times, those substances that are known as plant biostimulants are being studied and applied in plants in water stress which enhances agricultural productivity. For example, the biostimulant used through the foliar spray in tomatoes in water stress has given the maximum total soluble (brix)/titratable acidity index, which proved that the use of this biostimulant improves these indices in tomato fruits, even under water stress (Peripolli *et al.*, 2018).

According to Morais *et al.*, (2018), the use of the biostimulant through seed treatments and foliar application in water stress enhanced the total chlorophyll index in soybean plants, giving better photosynthetic efficiency to these plants. By contrast, de Carvalho *et al.*, (2013) assessed the impact of the amino acid L-glutamic acid, through seed treatment, on germination as well as the development of *Phaseolus vulgaris* seeds in water scarcity. Various quantities of the amino acid were used in seeds which were kept on PEG hydrated filter paper (PEG 6000) in different osmotic potentials such as 0, 0.2, −0.4, and −0.6 MPa.

Therefore, it has been proved that the amount of this particular amino acid does not support the growth of the seedling and negatively influences the germination at an osmotic potential equal to or less than −0.2 MPa. In addition, seed development was severely influenced in osmotic potential equivalent to or less than −0.2 MPa, showing reduced germination, root length and seedling volume. The impact of kinetin and calcium on the physiological characteristics and productivity of soybean plants under water stress and shading in the flowering phase were assessed (Fioreze *et al.*, 2013) and it was observed that the use of these substances helped in maintaining the relative water content and decreased discharge of cellular electrolytes. The use of calcium and kinetin in soybean plants subjected to water stress and shading has not changed the final grain productivity. Maize (*Zea mays*) is susceptible to water stress and one of the management tools associated with the induction of tolerance in water stress in this plant is the use of biostimulants.

Almeida *et al.,* (2017) have recognized the influences of two different levels of foliar spray of the Carbonsolo® biostimulant upon the physiological reactions of various maize hybrids under as well as without water scarcity. Thirty days after sowing, the Carbonsolo® biostimulant, having 25% fulvic acids, 50% humic acids, 20% amino acids and 2% water-soluble nitrogen, was used on the plant. These scientists proved that the foliar use of this biostimulant, in the early phases of the maize crop, gave greater relative water content in the leaves and there was less difference in leaf temperature with air temperature in a water-stressed environment. A trial was done with Stimulate® biostimulant as well as various water treatments like full, partial and non-irrigated irrigation to assess the function of this biostimulant on leaf water potential, relative water content, liquid photosynthesis, transpiration, stomatal conductance, plant height, main root length, total leaf area, and dry shoot and shoot mass of *Eucalyptus urophylla*. Stimulate® decreased leaf water potential as well as relative water content, but it enhanced transpiration, stomatal conductance and liquid photosynthesis in these plants (Santos, 2015). This helped to promote higher growth, both in plant height as well as in the length of the main root. Stimulate® enhanced thickening of the roots of the non-irrigated plants, which is a significant response in a water-stressed environment, as it permits the arrest of water in deep-seated soil layers, promoting the growth maintenance for more time. Moreover, the Stimulate® biostimulant was applied for assessing the use of biostimulants in the early phase of growth and dehydration resistance of sugarcane plants in mild water stress in a particular study. Photosynthesis, transpiration, as well as stomatal conductance, was maintained at higher rates (Wanderley, 2011). According to Van Oosten *et al.,* (2017), the biostimulants that enhanced plant resistance in water-stressed conditions should induce root versus shoot growth, which might permit plants to discover lower layers of soil in the drought season and activate the synthesis of compatible solutes for re-establishing advantageous water potential gradients as well as water uptake in decreasing soil water. The same type of positive influences can be provided through those microbial biostimulants which make absorption surfaces about the root systems and segregate soil water for supporting the plants.

15.4.2 Biostimulants and salt stress in plants

Salinity stress is an important restrictive issues for crop growth as well as yield. Salts in the soil water may prohibit plant growth by decreasing the capacity of the plant for water uptake, which leads to a decrease in the growth rate. When a large amount of salt gains entry into the plant in the transpiration stream, it damages cells of transpiring leaves which ultimately may result in a decrease of growth. These salts result in ion imbalance and disturb ion homeostasis and cause toxicity. This modified water status then leads to initial growth decrease and, ultimately, restricts plant productivity (Parihar *et al.,* 2015). The management approaches used for cultivation may enhance the yield as well as land use both in saline and non-saline environments. Of these approaches, organic matter and biofertilizer application, mycorrhization, foliar spray of organic and inorganic substances, together with the use of biostimulants are emphasized (Lacerda *et al*, 2010).

Biostimulants containing humic substances have been investigated for stress tolerance against salt due to their stimulatory effects (Türkmen *et al.,* 2004; Paksoy *et al.,* 2010; Aydin *et al.,* 2012). In salt-affected soil, Desoky *et al.,* (2017) observed a marked increase in physical and chemical properties of soil because of humic substances. *Moringa oleifera* leaf extract, which is recognized as a biostimulant, has been applied for plant development in normal as well as in salinity stress. The utilization of humic substance-containing biostimulants in plants under salt stress exhibited the ability of osmotic adjustment through maintenance of water absorption as well as cell turgor (Azevedo and, Lea, 2011). Thus, humic substances-containing biostimulants are identified as strong growth biostimulants because biostimulants in their true nutritive sense defend several crop plants against salinity.

The biostimulant Stimulate® consists of 0.009% cytokinin, 0.005% gibberellin and

0.005% auxin, and is utilized in various studies related to salt stress in plants (Souza *et al.*, 2018; Oliveira *et al.*, 2013, 2015a, 2015b, 2016; Klahold *et al.*, 2006). However, these studies are not very sure about its influences on the improvement of plant tolerance in salinity stress. The use of the commercial biostimulant Retrosal® (containing Ca, Zn and some particular active ingredients) promoted resistance in lettuce plants subjected to NaCl treatments because of its multidimensional action at both biochemical and physiological levels. A prominent biostimulant impact was seen on various parameters like fresh weight, dry biomass, chlorophyll level *in vivo*, nitrate amount, together with certain leaf gas exchange parameters as well as chlorophyll fluorescence parameters (Bulgari *et al.*, 2019b). Algal extracts use a variety of pathways to enhance resistance toward stress (Salehi-Lisar Bakhshayeshan-Agdam, 2016).

Various investigations have revealed that the use of commercial biostimulants containing arbuscular mycorrhizal fungi (AMF) inoculum assists crops subjected to agricultural salt stress environment through support to plant nutrition, affecting plant development (bioregulators) and providing resistance against salt stress (bio protector) (Rouphael *et al.*, 2015). AMF can play a role in defending tomato plants against salt by lessening the salt-induced oxidative stress (Latef and Chaoxing, 2011). This remediating impact of mycorrhizal establishment shows special relations of cultivar with salt exposure. Increased antioxidant enzymes activity, as well as lesser lipid peroxidation in mycorrhizal plants, might play some role in improved maintenance of the ion balance in the photochemical reactions in leaves subjected to salinity.

Plant growth-promoting rhizobacteria containing biostimulants are known as simple to use agroecological tools for activating plant growth and improving plant nutrient uptake as well as salinity stress resistance (Mire *et al.*, 2016). Salt-resistant PGPR can have a considerable effect on the growth as well as yield of wheat crops in saline soil (Upadhyay and Singh, 2015).

The impact of different types of biostimulants in salinity stress on plants is categorized by several workers into direct as well as indirect influences. The indirect influences are related to enhancement in physical, chemical and biological characteristics of soils, whereas the direct influences increase the germination, growth of root and shoot which improve tolerance of plants to salinity stress, as described earlier (Lacerda *et al.*, 2010). Several workers have identified the use of biostimulants as a sustainable tool for plant production as well as a helpful strategy for countering salinity stress in plants. In this manner, the use of biostimulants in agriculture under saline environments has shown the capacity of different varieties of biostimulants in enhancing crop production and reducing salt stress.

15.4.3 Biostimulants and temperature stress in plants

Temperature stress in plants is categorized into three different types based on the stressor: high, cold, and freezing temperature. Plants under temperature stress display lower germination rates, growth reduction, a decrease in photosynthesis, and mostly perish. The temperature stress can be activated through higher or lower temperatures and can be dependent on the length of the exposure, the rate of temperature variations and the developmental stage of plants at which stress exposure takes place. However, plants have several molecular processes that involve proteins, antioxidants, metabolites, regulatory factors and other protectants, as well as membrane lipids to deal with temperature stress (Kai and Koh, 2014). Temperature hinders germination and the initial growth of several horticultural crops. Different investigations have shown that high temperatures have a lethal impact on seed germination in several crops. Thus, biostimulants are good choices for lessening these effects as well as providing protection from abiotic stresses (e.g. drought, salinity, and high difference in temperatures) as they can improve the defense system of plants for such stressors (Du Jardin, 2015). Increasing concentrations of Stimulate® biostimulant (0, 4, 8 and 12 $\mathrm{mL\,L^{-1}}$) for relieving thermal stress at temperatures of 25°C and 40°C on germination and early growth of melon improved the germination rate through enhancement in the biostimulant doses at both temperatures (Vendruscolo *et al.*, 2016). Therefore, biostimulants can be used to enhance the germination in melon at high temperatures as well as for improving the early growth of the melon in high temperatures areas.

In two different experiments on lettuce plants that endured short-term episodes of extreme cold and heat with varied doses of PHH, the efficiency of the enzymatic hydrolysis product of pig hemoglobin (PHH) as a biostimulant that lessens the influence of thermal stress in plants was investigated. It was observed that at the top concentration, the product of PHH counteracted the negative impact of rising temperature on lettuce growth as well as decreased the detrimental influences of the cold, and also activated those responses which lowered the damage produced through severe cold and heat treatments (Polo *et al.,* 2006).

In a study that compared two concentrations of PHH with commercial biostimulant and a control, Marfà *et al.* (2009) investigated PHH, particularly swine blood, on strawberry plants in the early embryonic stages following transplantation and exposure to extreme cold. It was observed that the highest PHH doses formed higher biomass of new roots and both concentrations of PHH promoted early flowering, as well as enhanced the early fruit production in comparison to cold treatment. However, not a single biostimulant treatment increased the survival ratio of the strawberry plants in comparison to controls.

Botta (2013) has observed that the plant thermal acclimatization process stores compatible nitrogen-rich solutes, as amino acids, which provide stress resistance. Therefore, for evaluating the impact of exogenous amino acids treatments, various studies were done by using an amino acid product produced by enzymatic hydrolysis (Terra-Sorb® Foliar) on plants like lettuce and ryegrass exposed to three different types of cold stress. It was observed that treated lettuce plants possess more fresh weight in comparison to control plants, demonstrating greater stomatal conductance, which suggests productive enhancements. Foliar spray of TE caused a substantial increase in turf quality, density and chlorophyll concentration in comparison to controls. Both TurfVigor and CPR substantially enhanced visual quality through improvement in both shoot and root growth. These results were constant and exciting and exhibited that foliar spray of protein hydrolysates can have a positive impact on plant resistance to heat stress (Kauffman *et al.,* 2007).

The bacterial biostimulants defend rainfed field crops from stress and have significant importance in increasing temperatures which was predicted by most prediction models of climate change. Inoculation of thermotolerant *Pseudomonas putida* strain AKMP7 in wheat considerably improved heat resistance. Inoculated plants had more biomass, shoot and root length, as well as seed size (Shaik *et al.,* 2011). Bioactive compounds of seaweed extracts increase the functioning of plants in abiotic stresses. The use of extract sprays enhanced plant resistance in freezing temperature stress. Extract of commercial *Ascophyllum nodosum* enhanced the functioning of lettuce seedlings in high-temperature stress. Besides this, germination of lettuce seeds was affected through priming with extract of *A. nodosum* as germination increased in the high-temperature environment (Battacharyya *et al.,* 2015; de Vasconcelos and Garófalo Chaves, 2019).

15.4.4 Other stresses

Verdenelli *et al.,* (2019) has reported that the use of mineral fertilizer has an important effect on soil properties, a large variety of fungus and bacteria with microbial diversity. Guo *et al.* (2019) has also reported after a long period of fertilization study that soil pH was the major significant feature affecting soil bacterial population, whereas the amount of soil water has great significance in the structure of the fungal community. Beneficial fungi and/or bacteria, together with several functional compounds present in biostimulants, can assist in preserving both plant as well as soil health, therefore adding to the increasing appeals for sustainable food production along with environmental protection.

According to Carvalho *et al.* (2020), the advantages of unbalanced nutrient levels has not been well studied in plant development. Moreover, the mineral level in edible plant parts is a very important character in plants cultivated for food or fodder use, and might have an important influence on food security all over the world. Among metal elements, copper, iron, manganese and zinc are micronutrients that are required in the physiological procedures of living beings and thus are an important part of the soil–plant–food range (Teklic *et al.,* 2013). In the study conducted by Jakovljevic *et al.* (2019) in basil grown for 1 month under nutrient stress, increased synthesis of phenolic compounds with less lipid peroxidation, greater phenylalanine ammonia lyase (PAL) activity and significant antioxidant activity, was observed. The PAL enzyme, the first important enzyme in the phenylpropanoids metabolism, is susceptible to environmental stimulus and plants might counter stress with modifications in PAL activity as well as phenylpropanoid accumulation. The scientists reported that a greater level of plant-derived compounds can be synthesized in a specific stress environment, which then provides plants different odors, tastes and quality along with yield capacity. Consequently, El-Nakhel *et al.* (2019) reported that nutrient stress with suitable cultivar choice is an efficient way to enhance the phytochemical level and improve all-year lettuce production in closed soilless systems.

Commercial biostimulants are often presented as bio-fertilizers, but this is incorrect as they do not possess the needed nutrient level for being considered to be fertilizers. They do, however, possess a specific concentration of macronutrients and micronutrients along with other plant growth-stimulating substances. When root growth is promoted through biostimulants, their growth-inducing effect can affect the uptake of plant nutrients from the soil or other growing substrates together with plant nutrient hormesis within the plant. González-González *et al.* (2020) emphasized that biostimulants have insignificant nutrient levels, but they work upon the plant metabolism which is different from fertilizers. Thus, biostimulants can be regarded as novel, practical and sustainable techniques in the agricultural biofortification of crop plants. However, the study by de Vasconcelos (2019) showed that biostimulants provided in the recommended concentration did not have any impact on a nutrient level in an aerial portion of wheat and maize, which proved that biostimulants cannot substitute normal fertilizers in more nutrient requiring crops. Klokic *et al.* (2020) described different responses of tomato cultivars in the restricted nutrient environment, that were elucidated through cultivar-specific metabolic approaches to counter stress and were compensated through the exogenous use of the chosen compound in the making of biostimulants.

The humic compound is not only regarded as an essential part of soil organic carbon, but also recognized as a biostimulant that can execute phytohormone-like behaviors to activate nutrient uptake, while plants respond differently according to the source of humic acids, amount, the process of treatment and plant developmental stages (Jindo *et al.*, 2020a). According to Jindo *et al.* (2020b), anatomical as well as biochemical modifications in the root system activated through humic compounds are the key factors accountable for enhanced nutrient uptake, with the rise in the accessibility of nutrients by chelation. In the area of phosphorus scarcity, further investigations are required regarding humic compounds to know their connection with plant hormones and other secondary metabolism processes at various levels. The capacity of these compounds to increase root growth through alteration in root architecture is mostly related to their hormonal activities like influences of auxin and production of nitric oxide (Zandonadi *et al.*, 2019), and it was expected that the existence of alkamides in them may provide enhancement in plant cell signaling as well as faster metabolism.

The use of a protein hydrolysate-dependent biostimulant alters the primary and secondary metabolism in plants of baby spinach and lamb's lettuce, which leads to the synthesis as storage of phytochemicals with health-stimulating characters in leaves (Di Mola *et al.*, 2020). They thought the use of protein hydrolysates as the sustainable remedy in intensive greenhouse cropping systems for increasing crop yield, nitrogen utilization proficiency and plant functional value in optimal and suboptimal (low-input conditions) nitrogen systems, can be accredited for improving root architecture. The foliar spray of these biostimulants promoted better physiological and biochemical levels of the soil–plant analysis development (SPAD) index, chlorophyll and carotenoids level, giving better agronomical performance (Di Mola *et al.*, 2019).

Visconti *et al.*, (2020) reported that *Trichoderma* inoculation of rocket can be regarded as a helpful management technique in leafy vegetable cropping systems for the effective application of remaining fertilizers from former crops. Moreover, they have said that increasing product quality through microbial inoculation is a difficult and complex problem to solve, considering the increasing attention of vegetable growers to find fresh crops of greater nutrient value. Carillo *et al.* (2019) reported amino acids engaged in activating carbon and nitrogen metabolism, peptides like root-promoting hair peptides and phytohormones such as auxins and cytokinins as possible inducer substances in the tested commercial biostimulant Auxym® that might be inducing fresh yield production of jute in both a half and a quarter-strength nutrient solution. They reported more photosynthetic activity, SPAD index and increased leaf nutritional level, revealed through a rise in K and Mg and a decrease in Na levels, showing better effective storage as well as translocation of assimilates in photosynthetic sinks, regardless of macronutrient amount in the nutrient solution, therefore permitting better crop functioning.

Paradikovic *et al.* (2011) tested four commercial biostimulants in sweet yellow pepper plants grown in a greenhouse, in a 30% nutrient solution strength in comparison to the recommended formulation. The findings revealed that natural biostimulants positively influence the vitamin C and the total phenolic concentrations in pepper fruits in the hot summer spell, and enhanced the antioxidant activity, vitamin C and phenolic concentration in fruits. Carvalho *et al.* (2020) described that the detection and elucidation of plant resistance processes stimulated in heavy metal stress are crucial for crop yield, as well as quality and, ultimately, food security. They have reported that the extent of plant resistance during brief exposure of Cd might be linked with variable fluctuations in plant mineral composition, like Mg, Mn and B levels. These Cd-activated alterations modify physiological and developmental processes like root architecture, ionomic balance of Mg and B levels, biomass production of Mg and/or Mn level, and biomass distribution that is Mg/K ratio. Emamverdian *et al.* (2020) examined the function of three phytohormone groups, i.e. jasmonates, brassinosteroids and strigolactones, which can control networks of the stress signaling pathway in plants in heavy metal stress and enhance plant security systems, through the rise in antioxidant enzyme activity as well as provoke biosynthesis of phytochelatins and metallothioneins (Teklic *et al.*, 2021).

15.5 Conclusions

Plant development, just as resistance to abiotic stresses, have for quite some time been the mission of crop breeders. Techniques such as genetic engineering, conventional and current breeding methods like marker-assisted selection are used to help this research undertaking. Furthermore, agricultural management strategies such as increased irrigation and grafting are planned and implemented to assist plants in surviving harsh environmental conditions. However, these approaches and agricultural methods have limitations, and certain agricultural practices have greater environmental expenses. Therefore, a novel innovation like the application of biostimulants is certainly needed. Food and nutrition security worldwide require an imperatively additional and novel technique for managing the rising world population as well as the fluctuating environment. But there are still several gray areas that are required to be described to completely know the complexity of the relationship between the plant–biostimulant and abiotic stress. Metabolomic analysis help in the ongoing attempts of recognizing and defining the complex biochemical and molecular mechanisms stimulated by biostimulants in crop plants, increasing growth as well as plant immunity. The access to advanced research tools will certainly enhance our information on biostimulant composition, but this knowledge will not be complete. Thus, the biostimulant mechanism of action can be explained through plant responses at the physiological, biochemical, as well as molecular levels.

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16 Molecular Priming of Plants with Biostimulants: An Effective Strategy for Alleviation of Environmental Stresses in Crop Plants

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Abstract

The occurrences and frequencies of environmental extremities or fluctuations are the chief cause for crop failure worldwide. Innovative climate resilient crops and stress-reducing agro-chemicals for mitigation of undesirable impacts of environmental stresses are required. Application of natural compounds and synthetic derivatives, known as priming, has shown success for moderation of plants' responses to various environmental stresses of crop plants. Either seeds, plant parts, propagules or even entire seedlings are the common materials in practice of priming. The different biostimulants with their varied configurations can induce gene expression following integration in metabolic pathways to moderate not only the stress responses, but also the growth of plants. The complex web of reactions that communicate any signal, perception and the induction of gene cascade are still yet to be fully deciphered. The chemical residues influence the diminishing of the load of anti-nutrient metabolites and their turnover within the plant system. This chapter presents a concise review of different biostimulants, and their mode of functioning at cellular, metabolic and molecular levels have been described in pathways of stress remediation in crops species.

16.1 Introduction

Commonly, plant biostimulants have been referred to by many scientists as agricultural biostimulants with a wider category of chemical substances with complexities in residual structures and forms. They are applied through different pathways, resulting in optimization of plant responses with some stimulation or positive effects on plant growth and nutrition (Sible *et al.*, 2021). Biostimulants can minimize abiotic stress/biotic stress toxicities, resulting in improved crops growth under stressful conditions. They regulate the biological activity of plants and microflora in soil (Rouphael *et al.*, 2020). (Singh *et al.*, 2019). In most cases, biostimulants in the rhizospheric zone enhance nutrient uptake and mobilities, and also induce a protective role over diverse environmental extremities like salinity of soil, water deficits, suboptimal temperatures, abundance of heavy or toxic metals, etc. (Tekaya *et al.*, 2021). Typically, biostimulant residues are not like conventional plants nutrients: they precipitate the mobilization of nutrients in

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support of growth moderation even under stressful condition (Dubey *et al*., 2020). Microbiota like bacteria, fungi, actinomycetes, cyanophytes, microalgae and nematodes are often found within the rhizosphere; the majority of these organisms form mutually symbiotic relationships with plants (González-González *et al*., 2020). The coexistence of microorganism with plant include intracellular and extracellular relationships, which permits those plants to respond accordingly to stressful environment. Therefore, if this association is applied in optimal dose, there is ample opportunity to enhance and amplify their role in stress resistance for plants (SeutraKaba *et al*., 2021).

Classically, there are no absolute definition for such biostimulants. Still, in the recent past it has been defined as the specific compound or substance sourced directly from plants or its associating organisms (microorganisms) that are directly or indirectly released into the rhizosphere that stimulate the nutrient and water uptake mobilization in support of abiotic stresses and as a whole strengthened the crop productivity (Benidire *et al*., 2020). Biostimulants are therefore widely defined and are not restricted within specific groups or categories. Du Jardin (2015) redefined biostimulants as chemical residues obtained from higher plants or even microorganism as a whole, which on application, can modulate specific pathways of growth and development, stress remediation, and alter the qualitative and quantitative traits of crops irrespective of its inherent nutrient or residues content. This definition is still not ideal, but represents a transparent and concise form to classify and categorize the biostimulants (Fig. 16.1).

At present, the insight into biostimulants for their efficacy has been advancing at a significant rate. Biostimulants and their effects on plant growth promotion and nutrient delivery have been well addressed, where various categories of chemical residue have identified with both simples to complex formulations. They often include hydrolyzed products of proteins (Casadesús *et al*., 2019), extracts from sea weeds (EL Boukhari *et al*., 2020), Rhodophycean starch (Bello *et al*., 2021), elements like silicon (Hidalgo-Santiago *et al*., 2021), complex polymers of oligosaccharides like chitosan (Shahrajabian *et al*., 2021; Ghosh *et al*., 2021), heterogeneous mixtures or composites like humic acid (Gemin

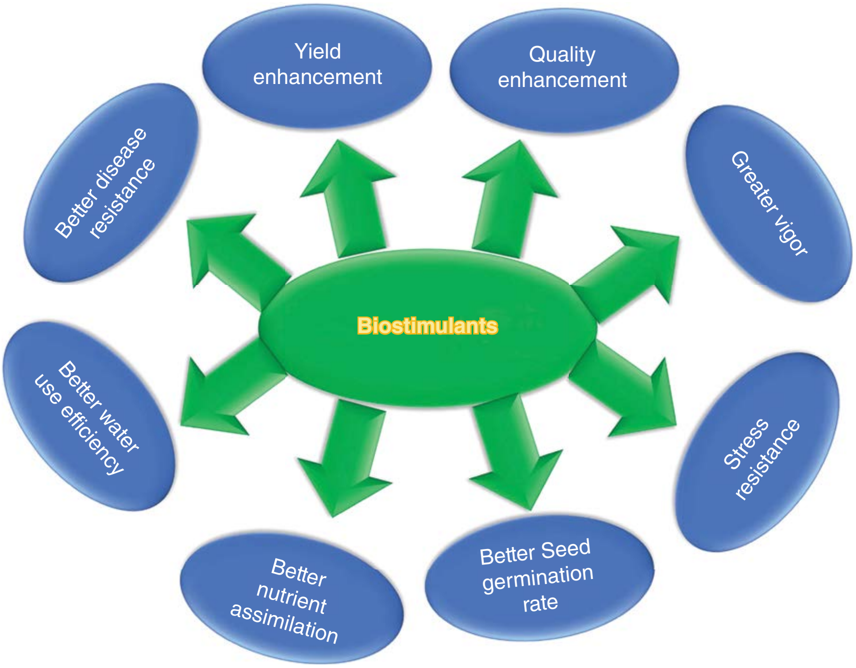

Fig. 16.1. Effects of biostimulants in plant growth and development.

et al., 2019), fulvic acid (do Rosário Rosa *et al.*, 2021), fungal organism from mycorrhizal partners of arbuscular mycorrhizal fungi (Saia *et al.*, 2021), *Trichoderma* (Silletti *et al.*, 2021), plant growth-promoting rhizobacteria (Adoko *et al.*, 2021). These different types of biostimulants can induce tolerance against abiotic stress and have plant growth-promoting properties. This chapter has consolidated the list of different plant biostimulants and describes their major roles in ameliorating abiotic stress factors. The data were obtained from *ex-situ* studies in artificial greenhouse conditions as well as from experiments in natural field conditions, where the specific effect of biostimulants either singly or in combination with other environmental facets were recorded. The chapter covers mainly agricultural crops, with an emphasis on cereal crops like rice, maize, wheat, barley etc. (Knapowski *et al.*, 2019; Schmidt *et al.*, 2020).

16.2 Abiotic stresses and biostimulants

Abiotic stresses in different forms reduce chances for sustainability by suppressing plant growth and other physiological functions, thus affecting the crop quantity and quality. Abiotic stresses include water deficit, salinity and heavy metal concentrations, fluctuation in temperature, high/low irradiance; xenobiotics like pesticides and herbicides have another common effect that is evidenced in by plants by an elevated oxidative redox reaction (Xie *et al.*, 2019). The accumulation of reactive oxidative species (ROS) or intermediaries characterizes plants with different levels of biomolecules degradation. The degenerative processes, through peroxidation and carbonylation of bioresidues, results in the deterioration of the plant's cellular metabolism and physiological activities (Ghosh *et al.*, 2020). Oxidative stress not only suppresses crop growth, but it can also switch on programmed cell death when the concentration of ROS is above the cellular threshold values. The ROS concentration and the effect on signaling molecules in the regulation of growth and development is a measure of the plants response to the environment. ROS are key to the plant's defense process through the upregulation or downregulation of stress-responsive genes. Many accumulated gene products in the form of enzymes are collectively required in stress tolerance process in plants. Similarly, common ROS (but not free radicals like H_2O_2) have emerged as prominent signaling residues that through various cascades can induce the stress response or programmed cell death. Like H_2O_2, other eco-friendly ROS are also regarded as effective transient agents in many biological processes even under low to high level of oxidative or other abiotic stresses. Therefore, ROS is a candidate for biopriming at molecular level.

Responses to abiotic stress in plants include well-defined signaling pathways that includes the interaction between ROS and reactive nitrogen species, plant growth substances, secondary messengers, etc. With regards to biopriming, plants hormone and related growth substances are also regarded as potential priming agents in either simple form or in combination with other residues (Makhaye *et al.*, 2021). As already mentioned, algae- and seaweed-based biostimulants (e.g. strobilurins) are also potential priming agents for different crop species. After application of the biostimulants, the plant's resistance system is induced for protection against subsequent related stresses by some common gene expressions at the nuclear level, a process called molecular priming. Once a treatment with such biostimulants (e.g. strobilurin, H_2O_2, trehalose, 1-methyl cyclopropane, brassinosteroids, and abscisic acid) has been applied to the plant biosystem, some simple compounds (e.g. acetate) stimulate the release of different methane residues that vary in duration. This collectively leads to subsequence stress resistance in the targeted tissues in three possible cascades: (i) elicitation of signaling residues like ROS/ROL, IP3, Ca^{2+}, jasmonic acid, different kinases and phosphatases, and hormones; (ii) activation of defense genes, a turnover of proteome, accumulation of secondary metabolites; and (iii) augmented defense (Sharma *et al.*, 2019) (Fig. 16.2).

Agro-chemicals that are applied through the broadcasting method may result in chemical wastage, excessive evaporation, corrosive damage to vegetation, spillage over the foliage, weathering, etc. In contrast, site directed activation reduces the longevity loss for individual plants. The uses of those are followed by technological devices like computer and machine, use

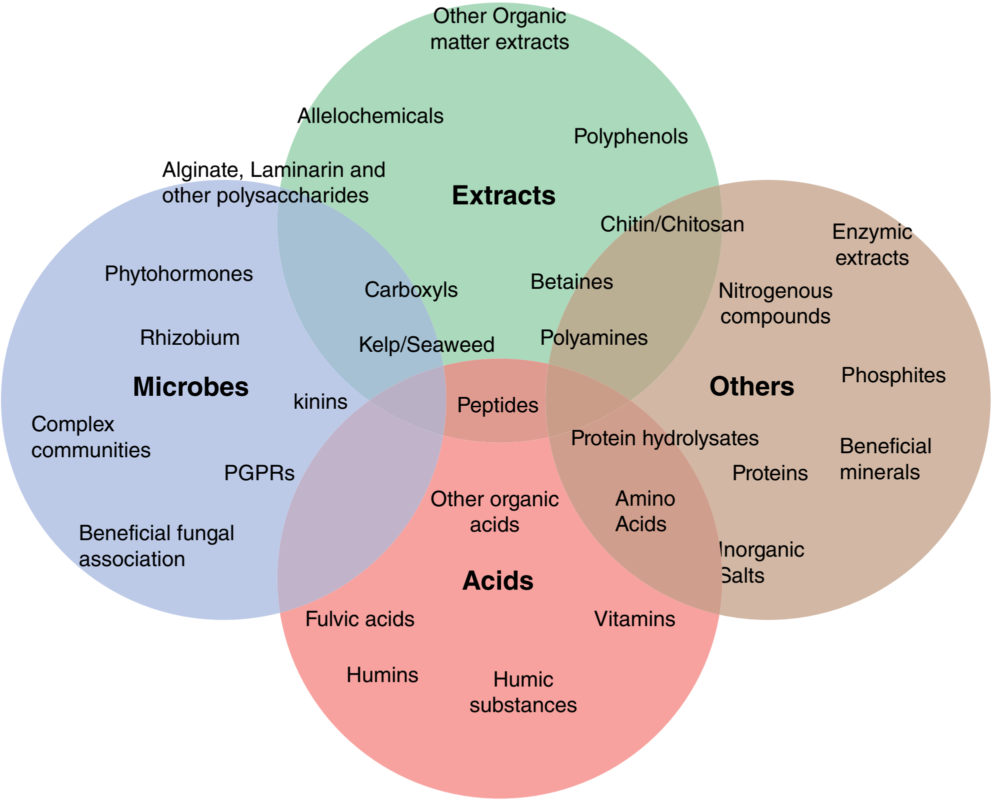

Fig. 16.2. Different forms of biostimulants used in plant growth and development.

of specific bio-adhesive or through systematic residues like fertilizers (Mora *et al.*, 2021). It is estimated that targeted application to individual plants can improve chemical wastage by over 90% which also reduces the level environmental toxicity. In shield crops, the combination of different agro-chemicals with nano-particle-based agents can specifically target the foliage and other plants parts, which increases the retention and stability of the biopriming agent over the crop as well as reducing volume of applied doses in natural field conditions. Many of the biopriming agents could be used to trigger the existing plant defense mechanism – they should not be toxic to plants and should have in sufficiently low concentration so that the crop is still suitable for human consumption. These biostimulants can be used in stress mitigation coupled with improving crop yield without compromising the plants and environment. The existing molecular biostimulants or biopriming agents and their mode in action and strategies for stress responses need to be investigated at the molecular level.

16.3 Chemical biostimulants used in agriculture

Over recent years, there has been an increase demand for eco-friendly agents to promote agricultural sustainability. With the advent of improved technology in agricultural science, the development of different residues like biostimulants are very important. Basically, biostimulants are the simple or complex fraction of organic extracts from plants that can stimulate the overall growth of plants (Bulgari *et al.*, 2019). Biostimulants estimated to have an economic value of US$3 billion by 2022, and they are expected to be used across and estimated 25 million hectares (Yakhin *et al.*, 2017). Commercial biostimulants are heterogeneous mixtures of multiple bioactive compounds that have a wider response for various crop species (da Silva *et al.*, 2017). Biostimulants can be categorized as broad spectrum in defense system in plants; roots and shoots enhancers; yield enhancers; moderators of fruit ripening at harvest and storage; and stress retardants in different crops. In general, biostimulants influence plant signaling cascades, thereby inducing different metabolic pathways. The main pathways affected include photosynthetic stimulation, senescence retardants, nutrient uptake and transport, enhancing phytohormones biosynthesis, alteration of C:N assimilation enhancing shelf-life, and inducing defense mechanism (Vitale *et al.*, 2021). Biostimulants are perhaps the most important treatment agents for stress tolerance. For example, the common fungus *Trichoderma* has been shown to control disease and pests along with alleviation of abiotic stresses. The different chemical agents produced by *Trichoderma* through root colonization elicit the plant defense mechanism and induces the production of antimicrobial compounds. The induced phenylpropanoide pathways can limit the pathogen in grass. Biostimulants from different non-pathogenic bacterial strains can also induce the stress tolerance at epigenetic level (Van Oosten *et al.*, 2017). Almost all plant growth-promoting rhizobacterium can activate the root to develop systemic acquired resistance against both biotic and abiotic factors. Complete plant extract and coating of hydrolyzed substances, particularly used in seed priming, have profound performance under environmental stress. Extracts from *Rosmarinus officinalis* improves the germination rate of maize seedlings and also improved their salt tolerance (Brito *et al.*, 2021). Priming agents like chitin and derivative such as deacetylase chitosan are good elicitors that can induce plant defense against pathogen (Zhao *et al.*, 2019). Chitosan is a hydrophilic compound that stimulates plant growth and abiotic stress tolerance. Chitosan not only includes water retention property of seeds, but is also effective for low temperature stress. Different protein fractions containing enzymatic hydrolyzes can improve the yield in cherry and tomato under low temperature conditions (Polo and Mata., 2018). The water retention capacity by activation of seaweed extracts induces stomatal regulation by ABA-signaling cascades. In *Arabidopsis* this extract has an antioxidation action. Other crops like tomato and chili are protected by extracts against herbicide-induced oxidative stress. At the nuclear level, the seaweed extract stimulates the gene expression along with some miRNA for regulation of genes that enhance tolerance through salt stress (Shukla *et al.*, 2018).

16.4 Organic residues and moieties as biostimulants

Organic residues have been more effective in priming function in plant growth and development particularly for abiotic and biotic stress. The simplest residue in the form of acetate have been found effective in GM crops against drought stress (Mirfattahi and Eshghi, 2020). Jasmonic acid biosynthesis and histone acetylation as reported as epigenetic modification by plants against drought (Banerjee and Roychoudhury, 2017; Wang *et al.*, 2021;). The osmolytes for compatible solutes like glycine betaine, proline, mannitol, etc. are crucial for osmotic adjustment as well as antioxidation. Glycine betaine has been shown to be effective in increasing the photosynthetic rate of maize, suggesting possible roles under water stress (Shemi *et al.*, 2021). Other osmolytes like trehalose and phosphorylated derivatives are effective in few solanaceous crop for both drought and water stress (Kosar *et al.*, 2019). Increasing permeability and membrane stability through specific activity and antioxidant level were responsible for drought tolerance in wheat (Aurangzaib *et al.*, 2021). Even small molecules like gamma amino butyric acid and its isomers used in the form of foliar spray or seed treatment increased stability against various abiotic stresses. The gamma-induced catalase (CAT) activity with lysis of H_2O_2 was the principal response for tolerance (Macovei *et al.*, 2014). The antioxidation activity of CAT and SOD was another control measure for effected plants to overcome the drought stress. Polyamines have undoubtedly been the most versatile biostimulants for abiotic stress tolerance. Polyamines have positively charged residues that can bind to the negatively charged domain of the membrane, thus functioning as a shield. Polyamines can also act as a messenger molecule which transmits the stress signal by the phosphorylation pattern of different proteins. This signaling has been interpreted by the stability of cellular organelles as well as sustaining the activities of the cellular functions (Podlešáková *et al.*, 2019). The overexpression of polyamines by transgenic modalities for different biosynthetic genes like polyamine catabolizing gene (PAO, DAO) are important. For example, in rice seedlings under salt stress conditions, the application of dipolyamines and tripolyamines reduces chlorophyll hydrolysis, electrolyte and solid loss, impairment of photosynthesis and gas exchange damage. Polyamines are effective in membrane sensitivity and regulating the decrease of quantum efficiency. Melatonin, however, is more effective in animal tissues and has also revealed multifaceted responses for growth and development as well as stress tolerance (El-Ghany *et al.*, 2020). The initial growth response shared by melatonin and auxin may be due to their common tryptophan precursor (Arnao and Hernández-Ruiz, 2021). However, although they also have a shared receptor, the auxin responsive gene induction is uncoupled from the melatonin treated seedlings. Melatonin mainly contributes to increasing the antioxidizing pathways as derived from different abiotic stresses. The responses to other stress signaling genes particularly for glutathione pathways are important for melatonin signaling. The protection of such genes has recorded a significant increase in some specific fractions with transcription control in *Arabidopsis* (Sharma *et al.*, 2020). Using seed pretreatment or priming the plant with different vitamin (mostly vitamin K3) has successfully mitigated salt stress in some plants. Physiologically, to upregulate osmotic turgidity at molecular level requires induction of transcription factors and ROS-dependent signaling, both being the domains for such activities (Rasheed *et al.*, 2018).

Other products from organic parts like humic and fulvic substances are important key organic constituents of soil and lignite. Principally, these two acids are the by-product of organic bio-degradation. They are a heterogenous mixture of phenolics and inorganic salts (phenolates). Fulvic acid and humic acid have a high proportion of O_2 content and low molecular mass (Elrys *et al.*, 2020). In fact, humic acid can increase the tissue hydration of roots and shoots of plants in under moisture-deficit soil (Kaya *et al.*, 2020). A combination of fulvic acids and cytokinin can induce cytokinin biosynthesis for drought tolerance (de Vasconcelos *et al.*, 2019). The application of humic acids also induces better absorption of macronutrients and micronutrients that support general nutrition. Humic acid acidification in *Phaseolus* can support osmotic adjustment and reduce the membrane leakage under high saline conditions. Highly composted fertilizer used to extract humic acid induces antioxidation stability by ROS scavenging enzymes (Aydin *et al.*, 2021). Another possibility for highly compost fertilizer is mediated by humic acid consumption based on plasma membrane-bound proton transfer. Therefore, humic and fulvic acid are beneficial for plants in two major domains: (i) nutrient availability and metal chelation when targeted at roots; and (ii) ROS scavenging osmotic adjustment, anionic balance, homeostasis, membrane flux and stability when targeted to whole plant response.

16.5 Exploration of molecular insights of biostimulants

As already discussed, there are several promising chemicals that can be used to mitigate multiple stress tolerance, but the molecular mechanism still to be determined. Several abiotic stresses, including water deficits, high salt concentrations, water exchange, temperature fluctuation, excess and low irradiance and heavy metal toxicity, have a significant effect on crop plants. The impact of an individual abiotic stress event is generally not as problematic compared to when several abiotic stressed combine to act in a synergistic or additive manner (Debbarma *et al.*, 2019). Alternatively, priming technologies are targeted to prepare the plants to recover or combat the stressful situations (Masondo *et al.*, 2018).

Priming is also known as sensitization or hardening, both of which can be initiated naturally or artificially on exposure of plants with those chemicals. At the cellular level, following challenge by chemicals the plants that are primed are able to switch on their defenses earlier and faster with more potential, thus reducing the impact of the stress (Fleming *et al.*, 2019). There was a significant variation in the physiological changes between primed plants and nonprimed plants when both were challenged with stresses. It is clear that chemical priming represents an effective control measure to alleviate the stress, and thereby improve the crop management. The introduction of a biostimulant in plants against combined abiotic stresses in plants means that there is no compromise in the total carbon allocation (Khalaki *et al.*, 2021). This change to carbon diversion in primed plants is not only reflected growth, but also in other plants responses and physiological behavior. In fact, pretreatment with priming residues results in both cellular tolerance and amelioration of stress-induced growth inhibition. At the cellular level, application of chemicals to roots initiates a signaling cascade that results in the systematic accumulation of dormant tolerance stress. Thereafter, plants introduced to stress are manifested with tolerance related responses. At the molecular level, the turnover of energy-yielding metabolism (Popko *et al.*, 2018), generation of ROS and its detoxification (Szparaga *et al.*, 2018), development of compatible solutes (Wu *et al.*, 2019), ion homeostasis (Awad-Allah *et al.*, 2020) are the predominant responses on priming. Chemical agents like donor NO, H_2O_2, H_2S and other sulfur donor polyamines initially accumulate in the tissues. Those may be transducer of systematic signals linked with general or specific metabolic paths. Endogenous development of different metabolites is required to combat the stresses. Accumulation of dormant tolerance signal may also be overexpressed for other chemicals to fight against abiotic stresses. Molecular adjustment to induce stress-related transcription factors are also important. These include, mostly, osmotic stress and DREB/CBF. In the gibberellic acid metabolic pathway, the most targeted transcription factor is MYB which also induces other gene expression related to abiotic stresses. In post transcriptional modification like carbonylation, nitrosylation is also included for protein homeostasis. The effective plant residue is grouped into heat-shock proteins and antioxidants (Haroun *et al.*, 2018). For the first, plants are corroborated with a number of cellular modifications like protein turn over, protein–protein dimer, redox changes of thiols, etc. For antioxidation, a number of chemicals like flavonoids and phenolics are important for quenching of excess energy or turnover of ROS directly. Therefore, physiological homeostasis photosynthesis and respiration, ion homeostasis (maintenance of Na^+/K^+), osmo-protection (proline, glycine betaine, mannitol biosynthesis), ROS detoxification (enzymatic or non-enzymatic pathways) cover a wider aspect of molecular insights when plants are subjected to chemical priming.

16.6 Revelation to oxidative stress: an improved stand in submission and achievement of priming

ROS with their varied chemical configurations are constantly formed inside plant cells through redox reactions. The ROS and their lysis is always in balance by a strongly regulated by fine tuning system (Gechev *et al.*, 2006). Transient or constant ROS bioaccumulation may trigger a signaling cascade of different reactions like peroxidation and nucleophilic reactions. Besides super oxide, it is the hydrogen peroxide that may also in antioxidation cascade to support chemical priming mechanism (Petrov *et al.*, 2012). H_2O_2-induced CAT activity has been used regardless of the crop species under salinity (Samaddar *et al.*, 2019), chilling (Yusefi-Tanha *et al.*, 2019), heavy metal (Corpas and Barroso, 2017) as well as biotic stresses exercised in crop improvement programs (Martínez-Gutierrez *et al.*, 2012). The precise roles or mechanisms of ROS-facilitated priming is not fully established. However, antioxidation cascade such as the glutathione-ascorbate system could be involved in priming, like H_2O_2 application. For cereal and vegetables crops, H_2O_2 treatment is sufficient to mitigate any shortage of peroxidase, catalases and other key enzymes in glutathione-ascorbate pathways (İseri *et al.*, 2013). Seed priming increases germination, seedling growth and vigor, and increase the potential in tolerance to abiotic stresses (Paul and Roychoudhury, 2017). Similar observations by Shatpathy *et al.* (2018) suggested PEG-primed seeds of rice seeds in reducing the time for seedling emergence in soil water deficit. In this case, antioxidants like glutathione and related enzyme activities are involved in establishing an effective defense (Bhattacharjee, 2012). Furthermore, glutathione was reported to play a role in the H_2O_2-induced tolerance through redox-sensing proteins that often undergo a reversible cysteine thiol oxidation process (Gill *et al.*, 2013). An inclusive transcriptional network rules the downstream responses prompted by exogenous H_2O_2. Crucial or key activators in the

H_2O_2 regulatory system include transcription factors like NAC017 and CRF6 (Zwack *et al.*, 2016). Involvement of aquaporins with their putative role in the kinetics of water exchange has been studied in *Arabidopsis*. DNA microarrays carrying aquaporin gene-specific tags and antibodies revealed higher and lower expression of tonoplast intrinsic proteins (TIP) isoforms (TIP3;1, TIP3;2, and TIP5;1) and plasma membrane aquaporins (PIPs), respectively.

Proteomic analysis of un-primed and primed seeds during germination *Arabidopsis* recorded 1300 seed proteins by 2-D gel analysis. Few of those were significantly changed during germination (prior to radical emergence and radical protrusion). Moreover, during the dehydration process used when priming the seeds, a few new proteins were generated including cytosolic glyceraldehyde 3-phosphate dehydrogenase (Gallardo *et al.*, 2001). During seed priming in wheat, 531 differentially expressed proteins (DIPs) were recognized in reference to non-primed seeds and several upregulated of those are involved in energy-yielding mechanisms in process of glycolysis, TCA cycle, and fatty acid oxidation and related anabolic processes.

16.7 Conclusion and future scopes

Molecular priming has been an efficient technology to counteract abiotic/biotic stresses through boosting the tolerance of plants. This is an eco-friendly approach available to a wider variety of species. The efficacy of priming agents depends on the critical concentration and duration of doses and exposure stage, for instance before the onset of stress. Even the appropriate time on crop developmental stages like seedling emergences, flowering, fruit setting could facilitate the better crop stand and productivity also. Despite contentment this often meets certain problems. Dependence on whether report and preparation of priming agents under threatened condition may often change the full potential of chemical agents. The spillage of chemicals into soil/water bodies is subjected to negative attitudes supported by environmental restrictive regulations. Besides, the possibility to predict the degrees of upcoming stress and therefore optimization of concern doses is likewise equally important. Documentation of synergistic/complementary possessions of biostimulants can be crucial to grow precise formulations to focus on environmental stress. A few such chemicals is discussed herein like peroxides, plant growth regulators which are easily bio-degradable and safe in use. In recent approach with advent of *omics* technologies, many new formulations have been in success as bio-agent to ameliorate the environmental stress. These residues are highly efficient for multiple stresses, economic and customized, eco and environmentally friendly and thus may opt a better choice for future. However, an in-depth understanding of underlined mechanisms once it has been reached may be released as next generation biostimulants. For example, biostimulants to support plant growth against water limiting environments with higher root shoot ratio and capacity to generate compatible solutes to re-establish plant growth are welcome. Similarly, a positive thrust would also be a factor for microbial biostimulants in order to absorption surfaces around rhizosphere.

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17 Biostimulants as Regulators of Stress Metabolites to Enhance Drought and Salinity Stress Tolerance in Plants

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Abstract

Plant biostimulants (BS), also termed bioeffectors, are viable microorganisms or active natural compounds applied to stimulate growth, nutrients uptake and stress tolerance in crop plants. The agricultural use of BS is discussed as a sustainable and resource-efficient approach to make optimal use of the biological potential that supports soil fertility, plant health and stress resilience of crops, contributing to profitable and sustained yield level. The use of BS-containing products (e.g. plant-beneficial microbes, seaweed, plant and compost extracts, protein hydrolysates, peptides, chitosan, and humic acids) as commercial formulations to enhance stress tolerance in plants provides a continuously increasing market potential with current annual growth rates of 12%. However, although the principal effectiveness of BS products is well documented, limited reproducibility of the expected effects, particularly under field conditions, still remains a major challenge. Therefore, it is essential to understand the physiological and functional basis of BS and their interactions in complex environments to get maximum benefit from these biological agents. This chapter provides a broad overview of the bio-protective effects of BS with the aim to make agriculture more sustainable and resilient to water limitations and salt stress. The positive effects of BS on physiological and metabolic events such as photosynthetic activity, phytohormonal balances, acquisition of nutrients and scavenging of reactive oxygen species to strengthen the defense mechanisms in water and salt-stressed plants are reviewed and discussed.

17.1 Introduction

Current trends in agricultural yields are considered as insufficient to meet the food demand of the global population expected to reach 9 billion by 2050 (Tilman *et al.*, 2011). The climate change scenario makes it more challenging due to high vulnerability of agricultural systems to sudden fluctuations in temperature and weather conditions resulting in heat waves, droughts, floods or heavy precipitation. The direct consequences of climate change on agricultural production have made it extremely difficult to reach the important goal of zero hunger by 2030 (Lobell *et al.*,

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2008; Szabo *et al.*, 2018). The continuous increase in human population has already put significant pressure on limited resources, suggesting an urgent implementation of sustainable measures to obtain a continuous enhanced food production while maintaining the robustness and resilience of cropping systems (Buono, 2021). The development of chemical fertilizers and plant protection agents in the 20th century, together with adapted breeding programs, brought a revolutionary change to boost crop yields (Nawaz *et al.*, 2020). On the other hand, the excessive use of fertilizers and synthetic pesticides in agricultural systems has resulted in massive problems related to the environmental quality and soil systems (Hillel *et al.*, 2008). To address these problems, recent approaches in modern agriculture focus on the development of more sustainable alternative strategies, trying to integrate and effectively exploit the natural biological potential of ecosystem services that support soil fertility and health, which have been largely replaced by industrial intensification of agriculture. The final goal is the achievement of profitable and sustainable yield levels without disturbing the resource efficiency (Glick, 2012).

In this context various alternative management strategies such as permaculture, regenerative and conservation agriculture, agroforestry and organic farming systems are increasingly emerging with the goal to reduce or even avoid the use of chemical fertilizers and pesticides (Mondelaers *et al.*, 2009; Hartmann *et al.*, 2015; Bender *et al.*, 2016; Hirschfeld and Van Acker, 2021). However, due to a reduced flexibility to adapt fertilizer application and plant protection to actual crop demands, many of these strategies are confronted with lower yield, and limited options for disease control and stress protection remain a major issue (Hartmann *et al.*, 2015). As a potential strategy to close these gaps, external application of active natural substances with protective potential, generally known as biostimulants (BS) or bioeffectors, has attracted increased attention in the last decade to protect the healthiness of crops as well as soils (Van Oosten *et al.*, 2017; Weinmann and Neumann, 2019). BS are based on plant-beneficial microorganisms or extracts and compounds obtained from different natural resources capable of improving plant productivity, health status and stress resilience of crops independent of direct fertilizer effects. As proposed modes of action, BS act by making nutrients available in the soil, improving plant efficiency in the use of nutrients, supporting the establishment of stress adaptations and stimulating the decomposition and humification of organic matter in the soil (Paradikovic *et al.*, 2011; Caradonia *et al.*, 2019). These bio-agents include microbes, plant and seaweed extracts, protein hydrolysates, and several other organic and inorganic substances that improve growth, nutrient acquisition and protection against various biotic and abiotic stresses (Römheld and Neumann, 2006; Brown and Saa, 2015; Dube *et al.*, 2018). A major benefit of using biostimulants is their ability to improve plant performance by stimulating the plant own adaptations and defense mechanisms even prior to the impact of stress events (Petrozza *et al.*, 2014; Buono, 2021), leading to a more rapid and efficient expression of adaptive responses upon challenging environmental conditions (stress priming). It is also well established that plants are host to numerous bacteria and fungi, and beneficial associations with soil microbiota allow them to strengthen their protection against biotic and abiotic stress factors (Toscano *et al.*, 2018; Pylak *et al.*, 2019; Rouphael and Colla, 2020). This chapter updates knowledge concerning the biostimulants as regulators of stress metabolites to enhance drought and salinity stress tolerance in plants.

17.2 Role of biostimulants in mitigation of drought and salinity

In recent years, studies related to the beneficial effects of BS have been extensively reported (Battacharyya *et al.*, 2015; Canellas *et al.*, 2020; Nephali *et al.*, 2020). This chapter reviews the different categories of BS and highlights the physiological and biochemical changes induced by the exogenous application of BS in plants under water limitations and salinity conditions.

17.2.1 Humic substances

Humic substances (HS) are believed to affect the growth and development of plants by two distinct mechanisms: (i) indirectly ameliorating the physiological, chemical and biological properties of soils (at higher application levels); and (ii) the direct regulation as signal compounds by affecting the nutrient transport systems, and synthesis of primary and secondary metabolites (Nardi *et al.*, 2017; Fig. 17.1). Interaction of HS with metabolic pathways such as glycolysis and tricarboxylic acid cycle affects physiological processes related to plant growth (Conselvan *et al.*, 2018). Application of HS, for instance humic acid (HA), has been unequivocally recognized as an effective approach to stimulate biological activity in plants (Pizzeghello *et al.*, 2020; Vujinović *et al.*, 2020). Humic acid can be applied directly to plants at low concentrations to affect plant growth, yield and nutrient uptake, and is therefore classified as one of the most important BS (Yakhin *et al.*, 2017). Humic acid-based BS regulate various biochemical processes in plant cells, including improvement of cell membrane permeability, photosynthetic rate, cell elongation and also improved water use efficiency of plants (Zhang *et al.*, 2013; Roomi *et al.*, 2018). HA enhances plant growth and development under abiotic stress by positively affecting primary and secondary metabolism, including osmotic adjustment and stimulating a variety of enzymatic antioxidants (Cordeiro *et al.*, 2011; Canellas *et al.*, 2015). Exogenous HA supply considerably enhanced the assimilation of nutrients in water-stressed safflower plants by stimulating plant cell metabolism and increasing the uptake of nutrient uptake resulting in high grain yield, mainly due to its chelating properties (Karimi and Tadayyon, 2018). Emerging evidence indicates the regulatory effects of HA on the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) to reduce malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) accumulation in plants (Panfili *et al.*, 2019; Elansary *et al.*, 2020). Rassam *et al.* (2015) reported significant improvement in emergence rate, shoot dry weight and growth of sugar beet plants pretreated with HA under drought stress. Similarly, foliar spray of HA was observed to alleviate drought stress in two melon genotypes by increasing biomass, accumulation of calcium (Ca) and potassium (K), and activation of antioxidant defense mechanism (Kıran *et al.*, 2019).

The potential role of HA to mitigate drought-induced oxidative damages in rice was reported by García *et al.* (2016). They observed a significant increase in the activity of peroxidase (POD) and proline levels as well as decreased H_2O_2 accumulation to restore cytosolic redox homeostasis in water-stressed plants. A recent study by Roomi *et al.* (2018) provides further evidence that HS stimulus plays a pivotal role in the regulation of ROS-related enzymes to sustain growth under oxidative stress conditions. They found that HS application considerably

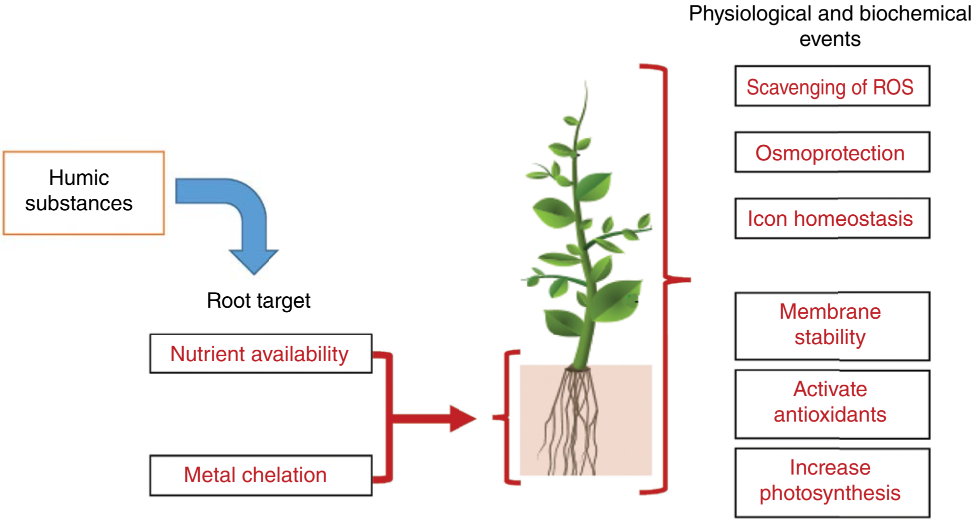

Fig. 17.1. Effects of humic substances on key mechanisms of plants subjected to drought and salinity stress.

increased the levels of 40 compounds associated with the stress response and upregulation of proteins responsible for redox homeostasis. Moreover, the mechanisms of action of HA to promote plant growth are related to processes involved in root cell elongation, lateral root development, and nitrate uptake and metabolism (Canellas and Olivares, 2014). Lotfi *et al.* (2018) reported a significant increase in gas exchange characteristics and electron transport flux by HA application in rapeseed plants. Similarly, Man-Hong *et al.* (2020) showed that foliar spray of water-soluble HA markedly enhanced potato tuber yield by increasing leaf-soil plant analysis (SPAD) and gas exchange attributes (photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration) under water-deficit conditions.

While the positive effects of HS to improve salinity tolerance have been documented in many plant species (Meganid *et al.*, 2015; Gao *et al.*, 2020), the mechanisms behind these effects still need to be explored. HA induces salt stress tolerance by improving the physical, chemical and microbiological properties of soil, and regulating physiological and biochemical processes as well as hormonal levels in plants (Canellas *et al.*, 2015; Fig. 17.1). Application of HA facilitates water uptake to maintain cell turgor and stimulates proline accumulation for osmotic adjustment, resulting in better adaptation to saline conditions (Van Oosten *et al.*, 2017). Common bean, *P. vulgaris* L., plants subjected to salinity stress and treated with HA exhibited higher proline content and lower membrane leakage than untreated control (Aydin *et al.*, 2012). Çimrin *et al.* (2010) reported that the application of HA to pepper plants significantly increased root growth and elevated the uptake of mineral nutrients in roots and shoots, to reduce membrane damage under salt stress conditions. In conditions with high salt concentrations, HA increases root surface area and facilitates nutrient uptake to minimize membrane damage and improve salt tolerance in plants (Daur and Bakhashwain, 2013; Saidimoradi *et al.*, 2019).

Under salt stress, HS limit the uptake of toxic minerals and increase the availability of essential nutrients (Kaya *et al.*, 2018). A greenhouse study showed that foliar spray of HA helped to maintain leaf water potential and increased the uptake of nitrogen (N), phosphorous (P) and potassium (K) with reduced sodium (Na) accumulation in maize plants exposed to salt stress (Kaya *et al.*, 2018). Latif and Mohamed (2016) suggested that HS substances decrease soil pH and increase the activity of soil-inhabiting microbes to promote the availability of nutrients such as iron (Fe). Moreover, they stimulate the activity of rubisco and photosynthetic pigments to enhance photosynthetic activity under salt stress (Ali *et al.*, 2020). Meganid *et al.* (2015) observed that soil amendment with HA helped to maintain the chlorophyll content of salt-stressed bean seedlings, reflecting better adaptability to saline conditions. A comparative study involving different methods of HA application (soil, foliar and soil + foliar) in beans showed that soil + foliar application is the most effective treatment to increase calcium (Ca) and K uptake and reduce damage induced by salt stress (Gulmezoglu and Ezgi, 2020).

17.2.2 Seaweed extracts

Seaweeds, found in coastal and marine ecosystems, are macroscopic multicellular red, green and brown algae that have been used as organic fertilizer for thousands of years (Craigie, 2011). They are a good source of polysaccharides, polyunsaturated fatty acids, enzymes and bioactive peptides, and are still used as natural fertilizers (Ahmadi *et al.*, 2015; Shukla *et al.*, 2016; Okolie *et al.*, 2018). Generally, seaweed extracts contain trace amounts of hormones such as cytokinins (CK) and auxins (IAA) (Hamza and Suggars, 2001), and several active mineral and organic compounds that may contribute to plant growth and development (Battacharyya *et al.*, 2015; Fig. 17.2). Application of seaweed extracts, either in soil or on leaves, has been reported to increase nutrient uptake, chlorophyll content, photosynthetic activity and water retention capacity of plant cells under biotic and abiotic stresses (Subramanian *et al.*, 2011; Sangha *et al.*, 2014).

The role of seaweed extracts to improve drought tolerance in plants is now emerging and has gained wide market acceptance (Jithesh *et al.*, 2012; Shukla *et al.*, 2018) with most formulations derived from the brown algae, *Ascophyllum nodosum* (Sharma *et al.*, 2014). The extracts of this intertidal seaweed have gained

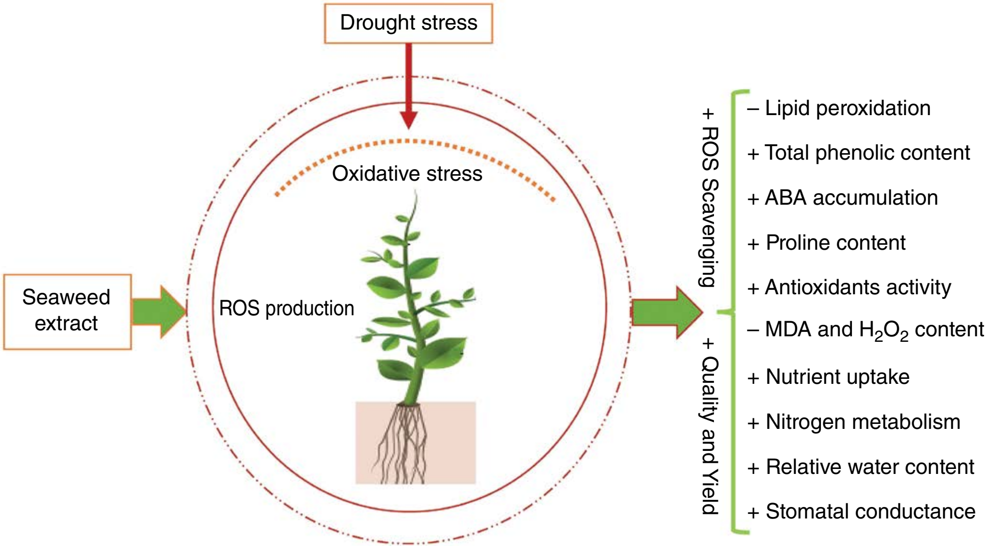

Fig. 17.2. Effects of seaweed extracts on key mechanisms of plants subjected to drought stress.

particular attention and popularity in agricultural crop production (Tandon and Dubey, 2015). Treatment of green bean (*Phaseolus vulgaris*) plants with *A. nodosum* extracts reduced lipid peroxidation, increased total phenolic content as well as stimulated the activity of antioxidant enzymes (SOD, CAT, APX), all of which would enhance tolerance to drought stress (Mansori *et al.*, 2015; Carvalho *et al.*, 2018). Similar effects were reported in *Paspalum vaginatum* plants foliar applied with *A. nodosum* extracts under drought stress (Elansary *et al.*, 2017). Recently, use of commercial extract of *A. nodosum* was reported to amplify the protective effects in drought stressed *glycine max* plants (Shukla *et al.*, 2018). Treated plants maintained higher relative water content (RWC) and stomatal conductance, accompanied by increased antioxidant activity under drought stress conditions. Also, the commercial extract of *A. nodosum* was observed to regulate leaf temperature, cell turgor, and many stress-responsive genes to overcome water-deficit stress in *G. max* plants (Martynenko *et al.*, 2016; Shukla *et al.*, 2018). Addition of *A. nodosum* extract in hydroponic culture of *Arabidopsis* induced partial stomatal closure through expression of genes involved in ABA-responsive pathways (Staykov *et al.*, 2020; Rasul *et al.*, 2021), and prevented drought mediated irreversible damages to photosynthetic apparatus by activation of antioxidant system pathways (Santaniello *et al.*, 2017; Omidbakhshfard *et al.*, 2020).

Extracts that are rich in *A. nodosum* regulate proline biosynthesis and stimulate enzymatic activities to induce drought tolerance in plants (Carvalho *et al.*, 2018). When *A. nodosum-based* extracts were applied to grapevine under water-deficit conditions, treated plants exhibited increased accumulation of proline, abscisic acid (ABA), total phenolics as well as enhanced activity of antioxidant system that may play a role in improved tolerance to drought stress (Irani *et al.*, 2021). Application of *A. nodosum* extracts in root medium could also be beneficial for soil-inhabiting animals, as root treatment of *Spinacia oleracea* plants with *A. nodosum* extracts considerably increased the survival rate of nematodes by 50% and stimulated flavonoid synthesis to improve nutritional quality under oxidative stress conditions (Fan *et al.*, 2011). Extracts from *A. nodosum* applied to broccoli plants elevated the uptake of nutrients such as nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg), copper (Cu) and manganese (Mn), which were associated with improved nutritional quality and general protective effect under drought stress (Kałużewicz *et al.*, 2018). One possible mode of action for *A. nodosum* extracts may be the differential regulation of genes in shoots and roots. Studies involving the treatment of *Brassica napus* plants with commercial seaweed extract AZAL5® showed increased expression of genes involved in nitrate and sulfate accumulation, and amino acid metabolism (Jannin *et al*., 2013).

Recent reports indicate that seaweed extracts derived from other algal species may also induce drought tolerance in plants. For instance, priming of wheat seeds with *Sargassum* and *Ulva* extracts resulted in higher ascorbic acid (AsA) content and increased activities of CAT and POD than untreated control plants, indicating the potential of these seaweed extracts to mitigate the adverse effects of drought (Kasim *et al*., 2015). In another study, treatment of wheat plants with *Gracilaria dura* extract resulted in higher wheat biomass (57%) and yield (70%) than untreated control under drought stress (Sharma *et al*., 2019). The protective effects of seaweed extract were found associated to higher accumulation of ABA in treated plants resulting in stomatal closure to prevent water loss, thereby facilitating mechanisms involved in water conservation strategies. A recent study involving the treatment of maize plants with K-sap (*Kappaphycus alvarezii* seaweed extract) showed significant upregulation of genes encoding root growth, gibberellic acid (GA) and auxin signaling, nitrate metabolism and transport as well as increased activities of glutathione *S*-transferase and POD under water-deficit conditions (Kumar *et al*., 2020). The results suggested that K-sap-mediated increased expression of N metabolism-related transcripts may lead to higher N content in all plant parts, including grains under limited soil moisture conditions. Further studies showed that the plants treated with seaweed extracts exhibited high concentration of bioactive molecules such as antioxidant enzymes as well as mineral nutrients (Aziz *et al*., 2011; Fan *et al*., 2011).

In salt-stressed plants, the application of seaweed extracts strengthens the plant defense system by osmoregulation and elevated uptake of mineral nutrients (Ronga *et al*., 2019; El Boukhari *et al*., 2020). Recent studies provide evidence that exogenous supply of algal based products regulate metabolic pathways to activate defense mechanisms involved in increasing tolerance to salt stress (Rouphael *et al*., 2018; Zou *et al*., 2019; Fig. 17.3). Chemical analysis of these seaweed extracts revealed the presence of a wide range of plant growth regulators such as IAA and CK, and compounds able to interact with plant hormonal stress signaling, which are helpful in maintaining plant growth and development under normal as well as stressful conditions (Zhang and Ervin, 2008; Di Stasio *et al*., 2018). However, apart from signaling functions, direct nutritional effects are also possible. Accordingly, Bradáková *et al.* (2016) showed cold stress protective effects by soil application of various seaweed extracts in maize. However, in this case only extracts rich in Zn and Mn exerted protective effects in terms of root growth promotion and oxidative stress mitigation with similar responses inducible also by supplementation with the respective micronutrients. By contrast, more highly purified preparations, containing only trace amounts of Zn and Mn remained inactive.

Among various seaweed extracts, the protective effects of *A. nodosum* extract to mitigate saline toxicity in plants have been well reported. For example, salt-stressed avocado plants treated with *A. nodosum* extract showed improved resource use efficiency and higher nutrient uptake, resulting in improved growth and yield than untreated control (Bonomelli *et al*., 2018). Similarly, *A. nodosum*-based extracts were used to counteract the negative effects of salt stress in turf grasses. Treated plants showed a higher K^+/Na^+ ratio as well as increased accumulation of proline content under saline conditions (Elansary *et al*., 2017). Reports of Al-Ghamdi and Elansary (2018) involving *Asparagus aethiopicus* showed that *A. nodosum*-based extracts significantly increased antioxidant activities as well as the concentration of pigments and stress metabolites (sugars, phenols and proline content) under salt stress. Moreover, a marked increment in photosynthetic rate and stomatal conductance was recorded, indicating the enhanced salt tolerance of treated plants. Similar results were reported by Orfanoudaki *et al.* (2019) in eggplant and Di Stasio *et al.* (2018) in tomato treated with *A. nodosum* under saline conditions. Addition of *A. nodosum* extract in growth medium with P deprivation was observed to regulate specific genes involved in increased P uptake and homeostasis under salt stress conditions (Shukla *et al.* 2018). The promise of *A. nodosum* to increase salinity tolerance holds great importance for salt-affected

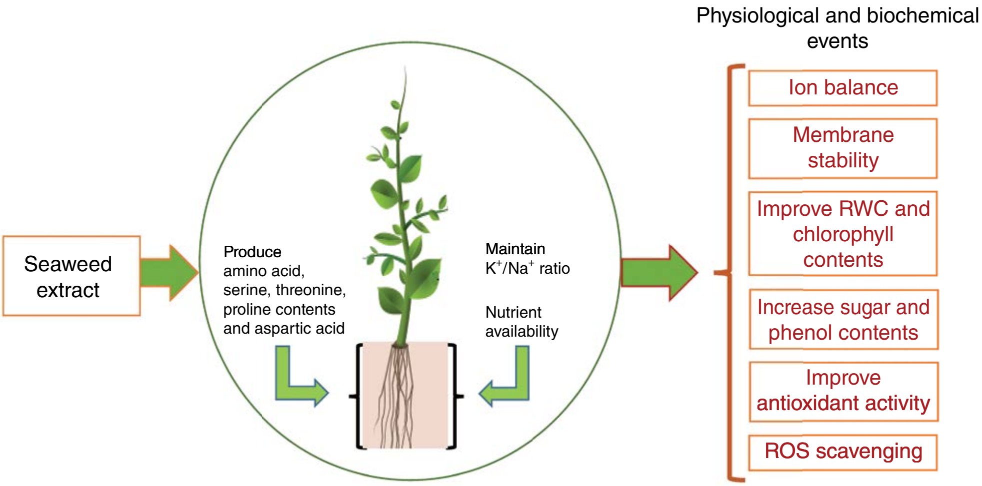

Fig. 17.3. Effects of seaweed extracts on key mechanisms of plants subjected to salinity stress.

regions where it is difficult to obtain optimum crop yields due to high concentration of salts in the growth medium. A similar application potential arises from declining quality (increased salinity) of irrigation water for vegetable production particularly in the Mediterranean area.

Seaweed extracts from other algal species have also been found to effective to mitigate salinity stress in plants. In chickpea, the application of *Sargassum muticum* and *Jania rubens* extracts significantly ameliorated the negative effects of salt stress by regulating amino acid metabolism, balancing ion content and improving antioxidant defense (Abdel Latef *et al.*, 2017). Moreover, a high concentration of serine, threonine, proline and aspartic acid was recorded in the roots of treated plants, all of which would enhance tolerance to salt stress. Use of seagrass *Zostera marina* extract as a foliar spray in salt-stressed tomato plants was observed to increase RWC and chlorophyll content (Vinoth *et al.*, 2017). Also, the changes in the activity of antioxidant enzymes (APX, CAT, SOD) were found to decrease salinity-induced ROS damage in treated plants. More recently, *Vigna sinensis* and *Zea mays* seeds pretreated with three Egyptian seaweeds, viz. *Ulva fasciata*, *Cystoseira compressa* and *Laurencia obtuse*, showed increased tolerance to salt stress upon germination. However, seed priming with *L. obtuse* extract resulted in the highest activity of amylase and protease along with maximum activity of guaiacol peroxidase (GPX) to promote seedling growth under salt stress conditions (Hussein *et al.*, 2021).

17.2.3 Plant growth-promoting bacteria

Microorganisms are widely used to produce BS formulations (Colla *et al.*, 2015a; Matyjaszczyk, 2015) and have the longest application history with the first patent for *Rhizobium* seed inoculants to improve N acquisition of leguminous plants by atmospheric N_2 fixation, dating back to 1896 (Hiltner, 1902). Although *Rhizobium* inoculants are still among the most widely used microbial BS products, particularly for industrial soybean production in South America, the total market share of microbial BS is comparatively small, and the market is clearly dominated (>75%) by non-microbial products. Microbial plant BS, especially plant growth-promoting (rhizo-)bacteria (PGPRs), have been discussed as a sustainable and efficient tool to ensure yield stability under low-input conditions (e.g. N and P deficiency) due to the ability to adapt and thrive under extreme environmental conditions (Rouphael and Colla, 2020). These microbe-based preparations contain living or non-living microorganisms and their metabolites (Matyjaszczyk, 2015). The most widespread PGPR strains are members of a limited number of genera (*Pseudomonas*, *Rhizobium*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Rhizobium*, *Bradyrhizobium* and *Burkholderia*) that have usually been isolated from different ecosystems, frequently also from stress-affected environments due to their ability to adapt and thrive under adverse conditions (Calvo *et al*., 2014). These adaptations include changes in cell wall composition and accumulation of high concentrations of soluble substances, which allow improved water retention and increase tolerance to osmotic and ionic stress (Van Oosten *et al*., 2017). Accordingly, a recent meta-analysis covering 166 publications on microbial BS revealed the largest yield responses (20%) in dry climate (Schütz *et al*., 2018).

For successful expression of beneficial effects on plant performance, efficient root colonization is a prerequisite (Berg *et al*., 2014). PGPRs are able to colonize roots and release exopolysaccharides (EPS) as well as polysaccharide lipids and lipopolysaccharide proteins, resulting in the formation of a protective biofilm on the root surface to maintain favorable water potential under severe arid conditions (Sandhya *et al*., 2009). However, endophytic colonization inside apoplastic spaces in plant roots or even intracellular incorporation of bacterial cells via endocytosis is usually considered to be more efficient than surface colonization due to less competition with native soil microbiota and a more direct access to root exudates acting as carbon source. Accordingly, this mode of root colonization is most widespread in real symbiotic interactions, such as *Rhizobium* symbiosis in leguminous plants or mycorrhizal associations characterized by a long co-evolutionary history (Marschner, 1995). On the other hand, for most PGPR inoculants, efficient root colonization reflecting a high rhizosphere competence remains a major challenge, which is easily achieved on artificial growth media without competitors and free accesses to the root surface. By contrast, under soil conditions, only very irregular and spotty colonization patterns by formation of micro-colonies are usually observed. Accordingly, quantitative assessments of root colonization by PGPR inoculants under field conditions frequently show only transient propagation patterns in the rhizosphere several weeks after the last inoculation and strongly declining population densities during the further culture period. Repeated application strategies could be a measure to counteract this problem, but the development of efficient and cost-effective inoculation techniques still remains a major issue particularly for field crops. Another problem arises from the impact of stress factors during the sensitive rhizosphere establishment phase of PGPR inoculants. Any stress factor severely affecting root growth and activity will also affect rhizosphere colonization by PGPRs due to limitation of carbohydrate supply to the inoculants as frequently shown, for example, for extreme nutrient limitations (Thonar *et al*., 2017; Mpanga *et al*., 2019), and also for the establishment of symbiotic interactions. However, after successful establishment of PGPR inoculants, beneficial effects on stress resilience of crops are well documented.

In this context, the secretion of high levels of phytohormones, phosphate solubilization, biological N_2 fixation and production of siderophores are discussed to alter root system architecture and improve nutrient uptake to increase survival under environmental extremes including drought and salinity (Barnawal *et al*., 2017; Kang *et al*., 2019). However, in terms of improved nutrient acquisition, limited reproducibility of PGPR effects under field conditions (Menzies *et al*., 2011) promoted a critical re-assessment of various, so far widely accepted, modes of action. This applies, for example, to the phosphate-solubilizing potential of PGPRs, which is a widespread feature in bacteria (Sharma, 2013) but a significant direct contribution of bacterial P solubilization to plant P acquisition by PGPR inoculants is not very likely based on the available experimental evidence (Menzies *et al*., 2011; Mpanga *et al*., 2019; Raymond *et al*., 2020). A similar situation applies for the direct contribution of biological N_2 fixation by associative diazotrophic bacteria belonging to the genera *Azospirillum*, *Herbaspirillum* or *Azotobacter* to N acquisition of crops, which can hardly be demonstrated in temperate climates, although numerous additional beneficial effects of the respective inoculants are clearly documented (Dobelaere *et al*., 2001).

Apart from contributions to nutrient acquisition, the growth enhancement by PGPR is discussed to be mediated by interactions with phytohormonal balances including IAA, GA, CK, ABA, salicylic acid (SA), jasmonic acid (JA) and ethylene (ETH), as well as the production of antioxidant enzymes and stress metabolites to increase tolerance to ionic and osmotic stress

(Govindasamy *et al.*, 2010; Kumar *et al.*, 2020). The inoculation of *B. subtilis* in *Platycladus orientalis* seedlings was observed to enhance root to shoot signaling of CK, thereby increasing plant tolerance to drought stress (Liu *et al.*, 2013). Similarly, the application of microbial consortia (*B. endophyticus* J13, *B. tequilensis* J12, *P. aeruginosa* PM389 and ZNP1) was reported to alleviate the damaging effects of drought by increasing the internal GA concentrations of inoculated *Arabidopsis* plants (Ghosh *et al.*, 2019). Also, certain PGPR possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme that can degrade ACC to α-ketobutyrate and ammonia to reduce ETH levels in plants under adverse conditions (Raghuwanshi and Prasad, 2018).

A plethora of pathways are influenced by PGPR to promote growth and development in plants under severe water-deficit conditions (Table 17.1). Maize plants inoculated with *Alcaligenes faecalis* (AF3) showed significant increase in growth, owing to the development of root system leading to increased uptake of water under drought stress (Naseem and Bano, 2014). In sorghum, inoculation with *Bacillus sp.* strain KB129 helped to maintain high RWC, which is an important drought tolerance strategy under water limitations (Grover *et al.*, 2014). Inoculation of tomato with *Azospirillum brasilense*, a bacterial strain originating from arid environment, has been shown to increase the area of xylem vessels that not only improves water uptake from soil but also reduces the symptoms of vascular diseases in plants under water-deficit conditions (Romero *et al.*, 2014). In *Arabidopsis*, the application of PGPR was observed to increase photosynthesis, ABA content and water use efficiency under limited water conditions (Bresson *et al.*, 2013; Cohen *et al.*, 2015). Similarly, several PGPR have been reported to increase flower size and the number and quality of ornamentals grown in greenhouse under water limitations (Leoni *et al.*, 2019). Nordstedt and Jones (2020) suggested that PGPR-mediated drought tolerance in greenhouse ornamentals is associated with decrease in electrolyte leakage accompanied by an increase in fluorescence attributes such as quantum yield, photosystem II (PSII) efficiency, and electron transport rate.

Stress-induced disturbance in the cellular metabolic processes of plants results in the formation of ROS that damage membrane proteins and interfere with the overall homeostasis of plants (Majeed *et al.*, 2020). Inoculation with PGPR triggers the antioxidant machinery to scavenge ROS and protect plants against oxidative stress (Bhat *et al.*, 2020). For instance, treatment of gladiolus with PGPR resulted in an enhancement in CAT and APX activities to increase tolerance against drought-induced oxidative stress (Damodaran *et al.*, 2014). Compared to uninoculated plants, inoculation of maize with *Bacillus licheniformis* (FMCH001) stimulated the antioxidant activity of CAT, consequently improving water use efficiency (WUE) as well as shoot and root dry weight under water-deficit conditions (Akhtar *et al.*, 2020). Similarly, basil plants inoculated with *Pseudomonas* sp. were reported to maintain photosynthetic pigments and exhibited the increased activity of CAT, while a marked increment in GPX and APX activity was observed by the application of microbial consortia (*Pseudomonas* spp., *Azospirillium brasilense* and *B. lentus*) (Heidari and Golpayeganib, 2012). In rice, the application of microbial consortia (*P. synxantha* R81, *P. jessenii* R62 and *A. nitroguajacolicus* strain YB3 and YB5) was also observed to ameliorate water stress by increased levels of CAT, GPX and APX (Gusain *et al.*, 2015). A few studies also report the combined application of PGPR with other BS, for example, the application of PGPR and seaweed extract (*Kelpak*) has been shown to improve growth, photosynthetic pigments and nutritional value of *Amaranthus hybridus* subjected to drought stress (Ngoroyemoto *et al.*, 2020).

Soil-inhabiting bacteria employ a wide range of mechanisms to mitigate the toxic effects of salt stress in plants (Table 17.1). Some of these strategies include solubilization of P and K to facilitate nutrients uptake (Etesami, 2018) alterations in root system architecture to increase the hydraulic conductance (*L*) of roots (Arora *et al.*, 2012), regulation of the expression of Na^+/H^+ and high affinity K^+ transporters (HKT), and secretion of EPS to trap Na^+ in roots (Bhat *et al.*, 2020). Moreover, they promote the accumulation of osmoprotectants and volatile organic compounds (Shabbir *et al.*, 2019), induce phytohormone signaling, and stimulate antioxidant machinery to increase plants' survival under saline conditions (Fazeli-Nasab *et al.*, 2019; Pan *et al.*, 2019). In plants, water uptake from saline soil is regulated by the expression of

Table 17.1. Summary of the effects of bacterial species to confer drought and salinity tolerance in plants.

Drought stress			
Bacterial species	Plant species	Response	Reference
Alcaligenes faecalis	*Zea mays*	Increase water uptake and root growth	Naseem and Bano (2014)
Burkholderia phytofirmans	*Z. mays*	Enhance RWC, chlorophyll and photosynthetic efficiency	Naveed *et al.* (2014)
Azospirillum brasilense	*Solanum lycopersicum*	Increase area of xylem vessel to facilitate water uptake	Romero *et al.* (2014)
Pseudomonas brassicacearum	*Arabidopsis thaliana*	Increase photosynthesis, ABA content and water use efficiency	Bresson *et al.* (2013)
A. brasilense	*A. thaliana*	Increase growth, photosynthesis, ABA content and water use efficiency	Cohen *et al.* (2015)
Bacillus cereus, Bacillus pasteurii	*Ocimum basilicum*	Increased antioxidant activity and photosynthetic pigments	Heidari and Golpayeganib (2012)
Bacillus licheniformis,Pseudomonas fluorescens	*Amaranthus hybridus*	Increased the nutritional value and mineral content of the leaves	Ngoroyemoto *et al.* (2020)
B. licheniformis	*Z. mays*	Increase root and shoot dry weight and antioxidants activity	Akhtar *et al.* (2020)
B. cereus, Bacillus subtilis Serratia spp.	*Camelina sativa*	Increase antioxidant enzymes, proline content and photosynthetic efficiency	Wang *et al.* (2012)
Achromobacter xylosoxidans, Bacillus pumilis	*Helianthus annuus*	Increase growth and phytohormones production	Castillo *et al.* (2013)
Pseudomonas spp.	*Pisum sativum*	Improve growth and ACC deaminase activity	Arshad *et al.* (2008)
B. pumilus, Bacillus firmus	*Solanum tuberosum*	Increase antioxidant enzymes, proline content and photosynthetic activity	Gururani *et al.* (2013)
Bacillus amyloliquefaciens A. brasilense	*Triticum aestivum*	Improve growth, fresh and dry weights and water content also show lower antioxidant enzymes activity.	Kasim *et al.* (2013)
Bacillus thuringiensis, Paenibacillus polymyxa	*T. aestivum*	Increase seedling germination, plant dry mass and production of antioxidant enzymes	Timmusk *et al.* (2014)
P. fluorescens, B. subtilis	*Vigna radiata*	Improve growth parameters, antioxidant activity and proline content and	Saravanakumar *et al.* (2011)
Pseudomonas aeruginosa	*V. radiata*	Increase germination rate, seedling vigor, plant growth, RWC, and the expression of drought specific genes	Sarma and Saikia (2014)

Burkholderia spp.	*Z. mays*	Increase production of antioxidant enzymes, water use efficiency and reduce production of reactive oxygen species	Fan *et al.* (2011)
Azospirillum lipoferum	*Z. mays*	Increase RWC, accumulation of free amino acids and proline	Bano *et al.* (2013)
Salinity stress			
Burkholderia cepacia, Promicromonospora sp., *Acinetobacter calcoaceticus*	*Crocus sativus*	Improve growth, elevate K^+ and P uptake, decrease Na^+ concentration	Kang *et al.* (2014)
Azotobacter spp.	*T. aestivum*	Increase plant biomass, nitrogen content and grain yield	Chaudhary *et al.* (2013)
Hartmannibacter diazotrophicus	*Hordeum vulgare*	Increase in root and shoot biomass and RWC	Suarez *et al.* (2015)
Enterobacter spp.	*Abelmoschus esculentus*	Increase in germination rate, growth parameters, chlorophyll content and antioxidant activity	Habib *et al.* (2016)
B. amyloliquefaciens	*Z. mays*	Increase total soluble sugar and antioxidants activity	Chen *et al.* (2016)
Sphingobacterium	*S. lycopersicum*	Increased enzymatic activities and expression of multiple isoforms like polyphenol oxidase (PPO), superoxide dismutase (SOD) and peroxidase (POD)	Vaishnav *et al.* (2020)
Pseudomonas putida, Novosphingobium spp.	*Citrus macrophylla*	Decrease abscisic acid and salicylic acid content, reduce maximum efficiency of photosystem, increase IAA accumulation in leaves	Vives-Peris *et al.* (2018)
Staphylococcus sciuri	*Z. mays*	Enhance plant growth and reduce ROS level by increasing antioxidant activities	Akram *et al.* (2016)
Bacillus aquimaris	*Z. mays*	Increase chlorophyll content, RWC, proline content, soluble sugar, total phenolic compound and enzymatic activities	Li and Jiang (2017)
A. brasilense, Rhizobium tropici	*Z. mays*	Enhance antioxidant enzymes (CAT, SOD, APX) in leaves proline content in roots and leaves and MDA content in leaves	Fukami *et al.* (2017)

intrinsic membrane proteins called aquaporins (Qin *et al*., 2016). The inoculation of maize roots with *Pantoea agglomeran* and *B. megaterium* has been shown to upregulate the expression of specific aquaporin proteins, thereby increasing *L*-values and contributing to salt tolerance in plants (Gond *et al*., 2015). Similarly, an expression analysis of *Arabidopsis thaliana* roots inoculated with *B. subtilis* GB03 revealed the downregulation of *HKT1* to restrict Na^+ uptake from saline medium (Zhang *et al*., 2008). The decreased uptake of Na^+ at the root surface stimulates the expression of *HKT1* in shoots, resulting in increased recirculation of Na^+ from shoots to roots to induce high K^+/Na^+ ratio in plants (Ali *et al*., 2019). The salt tolerance of *A. thaliana* inoculated with *Burkholderia phytofirmans* PsJN was found associated to changes in the expression of several genes including *HKT1* involved in ion homeostasis under salinity stress (Pinedo *et al*., 2015). Maize inoculated with *Azotobacter* strains C5 and C9 showed an enhanced exclusion of Na^+ and high uptake of K^+, which increased the accumulation of polyphenols and proline, all of which contributed to salt tolerance (Rojas-Tapias *et al*., 2012). Similarly, under high salt conditions, *Puccinella tenuiflora* seedlings inoculated with *B. subtilis* GB03 showed reduced Na^+ accumulation, which was found associated to upregulation of Pt*HKT1 and* Pt*SOS1* (Na^+/H^+ antiporter) genes (Niu *et al*., 2016). In another study, the inoculation of cucumber with *Acinetobacter calcoaceticus* SE370 and *Burkholdera cepacia* SE4, *Promicromonospora sp.* SE188 showed a decrease in Na^+ accumulation, and an increased uptake of P and K^+ to improve growth and enhance tolerance against salt stress (Kang *et al*., 2014). Chaudhary *et al.* (2013) reported that an exclusion of Na^+ in maize roots inoculated with *Azotobacter* was followed by simultaneous elevated uptake of K^+, P and N, resulting in higher grain yield than uninoculated control under saline conditions.

PGPR-mediated enhancement of antioxidant activities counteracts the generation of toxic metal-byproducts in plants exposed to oxidative stress (Halo *et al*., 2015). The inoculation of okra with ACC deaminase-containing PGPR *Enterobacter* spp. UPMR18 was observed to confer salt tolerance by increasing germination, chlorophyll content and upregulation of ROS scavenging antioxidant genes such as *CAT, APX* and *GR* (Habib *et al*., 2016). In hydroponically grown maize inoculated with *B. amyloliquefaciens* SQR9, an increase in sugars, chlorophyll content and activities of antioxidant enzymes was followed by a decrease in uptake of Na^+ to minimize salt-induced damages (Chen *et al*., 2016). Similarly, the treatment of tomato with salt tolerant *Sphingobacterium* BHU-AV3 was observed to induce the expression of multiple antioxidants such as polyphenol oxidase (PPO), SOD and POD in roots (Vaishnav *et al*., 2020). The inoculation of maize with *S. sciuri* SAT -17 showed that the inoculated plants maintained higher plant growth and had reduced levels of ROS due to an increase in proline and antioxidant enzyme activities, thereby exhibiting less salinity-induced oxidative damage than uninoculated control plants (Akram *et al*., 2016). Modulation in the plant defense mechanisms was also observed in maize plants treated with *B. aquimaris* DY-3 under salt stress (Li and Jiang, 2017). The inoculated plants demonstrated an increase in chlorophyll content, RWC, proline, soluble sugar, and total phenolic compounds with a significant decrease in lipid peroxidation and Na^+ content. Moreover, ROS generation was also decreased with upregulation of SOD, CAT, APX under saline conditions. Similar results were found by Fukami *et al.* (2017) when maize was singly or co-inoculated with *R. tropici* CIAT 899 and *A. brasilense* Ab-V5 and Ab-V6. The stimulated activity of ROS detoxifying antioxidant enzymes (CAT, SOD, APX) considerably increased the proline content in roots and leaves of plants inoculated with Ab-V6 or Ab-V6+CIAT 899, whereas *A. brasilense* Ab-V5 had no marked effect on salinity tolerance in maize. Inoculation of barley roots with *Hartmannibacter diazotrophicus* E19 (T) under saline conditions showed a significant increase in root and shoot biomass and RWC compared to control plants (Suarez *et al*., 2015). Use of a commercial BS TNC Bactorr^{S13} containing *Bacillus* spp. in lettuce, grown in a hydroponic system, showed positive effects under saline stress resulting in improved plant height, biomass accumulation, WUE, and nitrogen use efficiency (Moncada *et al*., 2020).

The regulation of phytohormones by PGPR is a key factor associated with increased salinity tolerance in plants (Shahzad *et al*., 2016). PGPR modulate the biosynthesis of ABA or regulate ABA-mediated signaling pathways to increase plant survival under saline conditions (Bhat *et al.*, 2020). In *Citrus macrophylla*, inoculation of *P. putida* KT2440 or *Novosphingobium* spp. HR1a significantly enhanced IAA and decreased ABA and SA content under saline conditions. Inoculated plants showed less cell injury by restricting chloride (Cl^-) and proline accumulation in roots under salt stress (Vives-Peris *et al.*, 2018). A decrease in ABA levels of salt-stressed *A. thaliana* inoculated with *Phyllobacterium brassicacearum* STM196 was found to reduce transpiration at the leaf surface, thereby increasing tolerance against salinity stress (Bresson *et al.*, 2013). The chickpea plants inoculated with *Pantoea dispera* PSB3 maintained high biomass, seed number and seed yield, owing to an enhancement in IAA and ACC deaminase production to restrict Na^+ uptake under salt stress (Panwar *et al.*, 2016). A similar effect on GA levels and antioxidant activities of *Cucumis sativus* inoculated with *Promicromospora* sp. SE188 and *B. cereus* MJ-1 was observed to alleviate salt-induced damage in seedlings (Kang *et al.*, 2014).

17.2.4 Plant growth-promoting fungi

Soil-borne fungi possess immense potential to improve plant growth and development (Table 17.2) and are widely used as commercial inocula in agriculture (Smith and Read, 2008). The plant growth-promoting fungi belonging to genera *Trichoderma*, *Fusarium*, *Penicillium*, *Phoma*, etc. have been reported as effective biocontrol agents against several plant diseases, owing to the destructive antibiotic properties of these microbes (Chandanie *et al.*, 2009). *Trichoderma* spp. (TR) comprises more than 200 cosmopolitan species, most of which parasitize other fungi worldwide (Atanasova *et al.*, 2013). TR usually acts by establishing chemical communication with plant roots, i.e. secretion of auxins, small peptides, volatiles and other active metabolites. TR colonization in plants has been reported to enhance plant resistance to several biotic and abiotic stresses by inducing intense root branching, increasing nutrient uptake, thereby improving plant growth and yield (López-Bucio *et al.*, 2015). The colonization of rice with *T. harzianum* T35 was observed to reduce ROS-induced oxidative damage by upregulation of SOD, CAT, and APX under drought stress (Gusain *et al.*, 2014). Similar effects of *T. harzianum* inoculation were observed in water-stressed tomato plants (Mona *et al.*, 2017). Inoculated plants showed increased regulation of phytohormones, photosynthetic efficiency and secondary metabolites, all of which would contribute to drought tolerance.

Among different fungal species, arbuscular mycorrhizal fungi (AMF) are undoubtedly the most widely studied species to explore the communication events between plants and soil-borne fungi (Begum *et al.*, 2019). They are asexual obligate biotrophs belonging to the subphylum *Glomeromycotina* and need a non-specific host to complete their life cycle. AMF develop a mutualistic symbiosis with the fine roots of most plant species (Avio *et al.*, 2018). This symbiotic relationship enables them to obtain photosynthetic products from the host plant, while in return they allow the plant to absorb more water and soil nutrients such as P, N, Cu and Zn through an improved root system (Ferrol *et al.*, 2019). AMF inoculation has been found helpful to overcome the deleterious effects of drought, salinity, and other abiotic stresses in plants (Abdel Latef and Chaoxing, 2014; Huang *et al.*, 2017; Begum *et al.*, 2019). AMF symbiosis regulates root hydraulic properties and stimulates many genes that help plants to overcome water stress (Petropoulos *et al.*, 2020). Application of AMF-based BS induce changes in root architecture, increases nutrient uptake and translocation of macronutrients and micronutrients, enhances photosynthetic activity and regulate plant hormones and antioxidant enzymes, all of which would stress tolerance in plants (López-Bucio *et al.*, 2015; Rouphael *et al.*, 2015; Zhang *et al.*, 2018). AMF symbiosis with chickpea was observed to increase drought tolerance (Habibzadeh and Abedi, 2014) as AMF hyphae can penetrate soil pores to access water and mineral resources that are not available to uninoculated plants (Baum *et al.*, 2015). Under water-deficit conditions, tomato plants inoculated with AMF showed 16–32% increase in fruit yield, owing to increased uptake of water and availability of nutrients (Wang *et al.*, 2014). In another study, inoculation of AMF (viz. *Funneliformis mosseae* and *R. irregularis*) considerably increased plant height, biomass, water use efficiency index (iWUE), proline content and stomatal density of tomato under drought stress

Table 17.2. Summary of the effects of fungal species to confer drought and salinity tolerance in plants.

Drought stress			
Fungal species	Plant species	Response	Reference
Trichoderma harzianum	*Oryza sativa*	Improve antioxidant activity and water uptake	Gusain *et al*. (2014)
T. harzianum	*Solanum lycopersicum*	Increase photosynthetic efficiency and secondary metabolite concentration	Mona *et al*. (2017)
Funneliformis mosseae, Rhizophagus irregularis	*S. lycopersicum*	Increase biomass, water use efficiency, proline content and stomatal density	Chitarra *et al*. (2016)
Glomus mosseae, Glomus versiforme	*S. lycopersicum*	Increase drought tolerance and yield	Wang *et al*. (2014)
F. mosseae, Rhizoglomus intraradices	*S. lycopersicum*	Increase water use efficiency and nutrient content	Volpe *et al*. (2018)
F. mosseae	*Poncirus trifoliata*	Improve water and nutrient uptake	Zhang *et al*. (2019)
G. mosseae, Glomus etunicatum	Juglans regia	Increase total phenolic content, proline content, peroxidase activity, total soluble sugar and starch content	Behrooz *et al*. (2019)
F. mosseae,Claroideoglomus etunicatum	Puccinellia tenuiflora	Increase P and K content and decrease Na^+ accumulation	Liu *et al*. (2018)
Salinity stress			
Arbuscular mycorrhizal fungi	*Triticum aestivum*	Increase gas exchange capacity, stomatal conductance and accumulation of sugars, free amino acids, proline and glycine betaine	Talaat and Shawky (2014)
Claroideoglomus claroideum	*Lactuca sativa*	Increase in nutrient uptake, total chlorophyll content, carbohydrates and proline accumulation	Santander *et al.* (2019)
F. mosseae, Diversispora versiformis	*Chrysanthemum morifolium*	Increase in root length, root and shoot dry weight, total dry weight and N content in roots	Wang *et al.* (2018)
C. etunicatum, R. intraradices, F. mosseae	*Crocus sativus*	Increase in biomass, pigment synthesis, higher enzymatic antioxidant activity, and ascorbic acid content	Hashem *et al.* (2018)
F. mosseae, R. intraradices, D. versiformis, Acaulospora scrobiculata, C. etunicatum	*Leptocanna chinensis*	Promote plant growth, increasing nutrient uptake, photosynthetic efficiency and maintain ionic balance	Cao *et al.* (2020)
Trichoderma longibrachiatum	*T. aestivum*	Increase RWC chlorophyll content and proline content while, decrease the MDA content	Zhang *et al.* (2016)
T. harzianum	*T. aestivum, O. sativa*	Increase plant RWC, performance index, dark-adapted quantum yield, photochemical quenching, stomatal conductance, pigment concentrations and antioxidant enzymes	Yasmeen and Siddiqui (2018)
Trichoderma asperellum	*Zea mays*	Increase in K^+ and Ca^{2+} content, accumulation of osmolytes, activities of antioxidant and decrease Na^+ content	Fu *et al.* (2017)
Trichoderma yunnanense, Trichoderma afroharzianum	*T. aestivum*	Increase in net photosynthesis, WUE and biomass in plants	Oljira *et al.* (2020)

(Chitarra *et al.*, 2016). Also, the use of *F. mosseae* in *Poncirus trifoliata* seedlings showed a significant improvement in plant growth, root hair growth and hormonal levels in roots, supporting the drought tolerance in plants (Zhang *et al.*, 2019). A recent study of commercial tomato cultivars by Volpe *et al.* (2018) highlighted the effects of *F. mosseae* and *R. intraradices* on physiological, biometric and gene expression of plants under drought stress. They found that inoculation of *R. intraradices* was more effective than *F. mosseae* to increase water use efficiency and nutrient content of plants under water limitations.

Microbial-based BS are widely used to overcome the deleterious effects of salt stress (Table 17.2), thereby help to maintain plant growth and productivity (Djighaly *et al.*, 2018; Li *et al.*, 2019; Wani *et al.*, 2020). They increase plant tolerance to salinity by enhancing nutrient uptake through increased root surface area (by AMF), and better solubilization of micronutrients as well as regulation of phytohormones (by *Trichoderma spp.*) (Abdel Latef and Chaoxing, 2011; Talaat and Shawky, 2014; Hashem *et al.*, 2016). A greenhouse experiment involving the use of fungal-based BS *T. atroviride* and *R. intraradices* in salt-stressed lettuce showed an increase in upregulation of antioxidant enzymes in plants inoculated with fungal strains. It was observed that such changes in antioxidant machinery helped to maintain photosynthetic activity, and improved the nutritional status of plants under salinity stress (Rouphael *et al.*, 2017). Treatment of wheat and rice with *T. harzianum* (Th-6) in a hydroponic system showed that the inoculated plants maintained higher RWC, chlorophyll, performance index, photochemical quenching (qP), dark-adapted quantum yield (Fv/Fm ratio), and stomatal conductance (*gs*) than uninoculated control plants under saline conditions (Yasmeen and Siddiqui, 2018). Besides, stimulated activity of antioxidant enzymes prevented salt-induced oxidative damage by reducing H_2O_2 content in the treated plants. Fu *et al.* (2017) reported the inhibition of salt stress by inoculation of *T. asperellum* in salt-sensitive (Xianyu 335) and tolerant (Jiangyu 417) maize cultivars. The treated plants showed an increase in K^+ and Ca^{2+} content along with a decrease in Na^+ content. Moreover, higher accumulation of osmolytes and upregulation of enzymatic and non-enzymatic antioxidants markedly reduced ROS levels to minimize the adverse effects of salinity. Inoculation of wheat with *T. longibrachiatum* T6 has been demonstrated to have positive effects under salinity stress by increasing RWC and proline concentration in leaves as well as stimulating antioxidant enzymes SOD, POD and CAT to reduce MDA content (Zhang *et al.*, 2016). In another study, the single and co-inoculation of bacterial (*B. licheniformis*) and fungal isolates (*T. yunnanense* Th4 and *T. afroharzianum* Th6) in wheat increased biomass, net photosynthesis and WUE; however, fungal isolates were found more effective than bacteria strain to induce salinity tolerance in wheat (Oljira *et al.*, 2020).

Mycorrhizal fungi are known to occur naturally in salt-affected soils (Yamato *et al.*, 2008; Beltrano *et al.*, 2013), and are well reported for their role to regulate different physiological and metabolic events to induce salinity tolerance in plants (Evelin *et al.*, 2009; Amanifar *et al.*, 2019). The colonization of AMF in wheat was reported to reduce salinity-induced oxidative damage by increasing photosynthetic rate, stomatal conductance, and accumulation of osmoregulators such as sugars, free amino acids, proline, and glycine betaine under saline conditions (Talaat and Shawky, 2014). Also, lettuce plants inoculated with microbial consortia (*Claroideoglomus claroideum* and AMF) showed increased tolerance to salt stress by elevated nutrient uptake, and higher total chlorophyll content, carbohydrates and proline than uninoculated control (Santander *et al.*, 2019). Similarly, the individual or combined application of mycorrhizal fungi *Diversispora versiformis* and *F. mosseae* in medicinal plant *Chrysanthemum morifolium* significantly increased root length, root and shoot dry weight, and total dry weight, which were associated to high N uptake in inoculated plants under saline conditions (Wang *et al.*, 2018). The possible modes of action for mycorrhizal fungi to reduce salinity-induced toxicity include the maintenance of pigments, accumulation of phenols, JA, AsA, and SA as well as upregulation of enzymatic antioxidants, also reported in AMF inoculated *C. sativus* by Hashem *et al.* (2018). Inoculated plants showed high uptake of K, Ca, Mg, Zn, Fe, Mn and Cu along with a decrease in Na^+ uptake, indicating that AMF-treated plants could better withstand harsh saline conditions. However, AMF may also differ in their ability to tolerate salt stress, hence the selection of most appropriate mycorrhizal species in essential get maximum benefits. For instance, Cao *et al.* (2020) performed a comparative evaluation of different AMF (*D. versiformis, R. intraradices, F. mosseae, C. etunicatum,* and *A. scrobiculata*) to increase salinity tolerance in *Leymus chinensis*. They found that among the five different mycorrhizal strains, *F. mosseae* and *R. intraradices* were the most effective strains to maintain ionic balance by facilitating the uptake of nutrients, and increasing photosynthetic activity under saline conditions.

17.2.5 Biopolymers

Biopolymers are naturally occurring polysaccharides or polypeptides found in the living organisms. These pure organic substances have gained wide prospects of application in agricultural systems due to their bioactivity, biocompatibility and biodegradability (García-García *et al.*, 2020). More recently, vegetal-based biopolymers like protein hydrolysates and lignosulphonates have been introduced as an efficient, environment friendly, and sustainable tool to increase tolerance in crops against various environmental stresses (Colla *et al.*, 2015b; Rouphael *et al.*, 2017; Hidangmayum *et al.*, 2019). Evidence indicates that the exogenous application of biopolymers triggers the accumulation of numerous stress related metabolites such as ABA, GA, CK, glucosinolate, carotenoids and flavonoids in plants (Lucini *et al.*, 2018). Here, we exemplarily overview the use of certain biopolymers as elicitors of plant resistance against drought and salinity stress.

Chitosan is a linear polymer synthesized from chitin (Kurita, 2006). It is formed by glycosidic linkage between *N*-acetyl-D-glucosamine and D-glucosamine, and is considered a safe and inexpensive organic substance capable of relieving the adverse effects of abiotic stresses in plants (Katiyar *et al.*, 2015). Chitosan application has been reported to mitigate drought stress by enhancing the capacity of photosynthetic apparatus, modulation of antioxidant machinery, and stimulating root growth to strengthen the ability of water and nutrient uptake in plants (Zeng and Luo, 2012; Shehzad *et al.*, 2020). Foliar spray of chitosan in *Phaseolus vulgaris* was reported to enhance growth and yield attributes of plants under water-deficit conditions (Farouk and Amany, 2012). Similar effects have been reported in *Hordeum vulgare*, suggesting the use of chitosan as an antitranspirant compound that can effectively reduce transpiration rate by stomatal closure under water limitations (Koers *et al.*, 2011). Also, treatment of *Thymus daenensis* Celak with chitosan was observed to growth and oil yield of plants under drought stress conditions (Bistgani *et al.*, 2017). Chitosan-mediated enhancement of drought tolerance involves the upregulation of tyrosine ammonia-lyase, α-amylase, and phenylalanine ammonia-lyase enzyme, which could improve the seedling vigor, leaf surface area, chlorophyll content and dry biomass (Tourian *et al.*, 2013). Investigations by Li *et al.* 2017 demonstrated that chitosan treatment stimulated the synthesis of amino acids and metabolites such as serine, valine, threonine, phenylalanine, proline, aspartic acid and γ-aminobutyric acid in white clover subjected to drought stress. On the contrary, no significant effects on proline concentration of chitosan-treated *Ricinus communis* seedlings were reported by Karimi *et al.* (2012). The contradictory results could be due to the application of different chitosan levels as low chitosan levels were observed to decrease proline content, which was significantly increased by the application of high dose in water-stressed *Carthamus tinctorius* seedlings (Mahdavi *et al.*, 2011). In chitosan-treated bean plants, a marked increment in photosynthesis levels was observed under water stress (Sheikha and Al-Malki, 2011), providing evidence that chitosan may contribute to the increased synthesis of chlorophyll by increasing the number of chloroplast per cell. A recent study by Shehzad *et al.* (2020) concluded that the combined application of K and chitosan significantly enhanced the total chlorophyll content, sugars, phenolics and activities of antioxidant enzymes, consequently improving the yield and oil quality attributes of *Helianthus annuus* under drought stress.

Several researchers have also reported the positive role of chitosan to mitigate salinity stress in plants (Jabeen and Ahmad, 2013; Krupa-Małkiewicz and Smolik, 2019; Ullah *et al.*, 2020). Pretreatment with chitosan has been shown to reduce salt-induced oxidative damage by increasing the activities of antioxidant enzymes in *Zea mays* (Younas *et al.*, 2021), *Oryza sativa* (Martínez *et al.*, 2015), *Vigna radiata* (Sen *et al.*, 2020) and *H. annuus* (Jabeen and Ahmad, 2013). Further, 2-year experiments done on *H. annuus* showed that chitosan seed treatment significantly improved growth, photosynthetic pigments, yield and IAA content to alleviate salinity-induced damages in plants (Bakhoum *et al.*, 2020). Similar results have been reported with soil application of chitosan in *Silybum marianum* (Safikhan *et al.*, 2018). The protective effects of chitosan supply were found to be associated to increase in chlorophyll *a*, *b* and proline concentrations, and a decrease in H_2O_2 by modulation of intercellular ion concentration and activities of antioxidant enzymes. In hydroponic systems, chitosan-treated wheat seeds showed high levels of SOD, CAT and POD in seedlings, thereby alleviating the damages of salt-induced oxidative stress (Ma *et al.*, 2012). More recently, Ullah *et al.* (2020) demonstrated that foliar spray of chitosan considerably improved the growth, chlorophyll, fruit firmness, fruits per plant, total soluble solids and fruit yield of tomato subjected to salinity stress. These studies provide evidence and highlight the importance of chitosan as a potential low-cost bio-fertilizer to confer salinity tolerance in plants.

Poly(γ-glutamic acid) (γ-PGA) is another inexpensive, water-soluble, biodegradable polypeptide, which is synthesized by microbial fermentation (Chen *et al.*, 2005). A few studies describe the role of γ-PGA as an anti-stress agent, for instance, the treatment of *B. napus* seedlings by this polymer was observed to increase the accumulation of ABA, proline and antioxidant enzymes, thereby enhancing the drought tolerance of plants (Xu *et al.*, 2020). In another study, it has been observed to promote the growth of microbial communities to increase the availability of soil moisture and mineral nutrients for maize seedlings under water-deficit conditions (Yin *et al.*, 2018). Foliar treatment of wheat seedlings with γ-PGA was reported to stimulate antioxidant activities and regulate ionic imbalance under saline conditions, particularly by increasing the accumulation of K^+ and decreasing Na^+ concentration in wheat leaves (Guo *et al.*, 2017). Xu *et al.* (2017) demonstrated that γ-PGA mediated activation of Ca^{2+} promoted the biosynthesis of brassinolide and JA, resulting in enhanced accumulation of proline and total antioxidant capacity to confer salt tolerance in canola seedlings.

17.3 Conclusion and future perspectives

The non-toxic and environment friendly nature of many BS offers numerous perspectives for the development of more sustainable agricultural practices. As a common feature, application of BS induces numerous stress defense mechanisms in plants, especially under water-deficit and saline conditions. The beneficial effects of BS in plants including changes in root system architecture and improvement in hydraulic conductivity of roots to increase uptake of water and nutrients, regulation of stress metabolites such as proline, phenolics, sugars, glycine betaine and phytohormones to maintain photosynthetic activity and upregulation of enzymatic and non-enzymatic antioxidants to scavenge ROS, all of which would result in overall increase in plant performance. This common mode of action of BS has been exemplarily demonstrated in a study by Moradtalab *et al.* (2017) on drought-protective effects of various seaweed and compost extracts in maize, which exerted very similar effects in reduction of oxidative damage, accumulation of osmoprotectants and improved enzymatic and non-enzymatic (total antioxidants, phenolics) ROS detoxification despite the completely different origin of the applied BS.

The physiological base of common patterns in plant responses to different BS and even different stress factors is most probably based on the general organization of stress defense mechanisms in higher plants and also on common physiological effects of different stress factors (e.g. impairment of root growth and function, excessive ROS accumulation). The plant immune system involves stress signal perception with receptors in the plasma membrane termed as pattern-recognition receptors (PRRs), which enable a recognition of invaders and stress factors via characteristic molecular patterns, for instance fungal chitin or bacterial flagellin, and siderophores (Teixeira *et al.*, 2019) or plant metabolites liberated during stress exposure.

Accordingly, these molecular signals have been classified as general microbe-associated molecular patterns (MAMPs), damage associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) (Barrett and Heil, 2012; Saijo *et al*., 2018). Activation of PRRs after recognition of MAMPs, originating for example from microbial inoculants, or DAMPS, liberated from damaged plant cells and consequently also included in extracts obtained by homogenization of plant material (seaweed, plant and compost extracts), leads to activation of adaptive plant defense responses. Accordingly, similar to the effects of moderate, sublethal stress stimuli, the priming of plant immunity by application of BS even prior to exposure to stress conditions, allows the plant to respond more quickly to environmental stress factors. The plant hormones salicylic acid, jasmonic acid, and ethylene and their signaling pathways are acting upstream the PRR-mediated signal perception and play a central role in shaping physiological defense responses (Kniskern *et al*., 2007; Lebeis *et al*., 2015; Liu *et al*., 2020). Root growth responses are frequently associated with BS-mediated supplementation of metabolites with hormonal activity or indirect interactions with auxin metabolism. A model for the different events involved in BS-induced stress priming is presented in Fig. 17.4.

The interactions of BS as signal compounds with plant hormonal balances can explain their effectiveness at very low application doses but also implicates the high variability of responses frequently observed during field applications. Depending on the environmental conditions and the plant developmental stage, the plant hormonal status is highly variable. Accordingly, the responses to external application of agents with hormonal activity, i.e. BS can also cause variable responses depending on the current internal status of the phyto-hormonal balances. Therefore, a major challenge for practical applications remains the correct timing and dosage of the

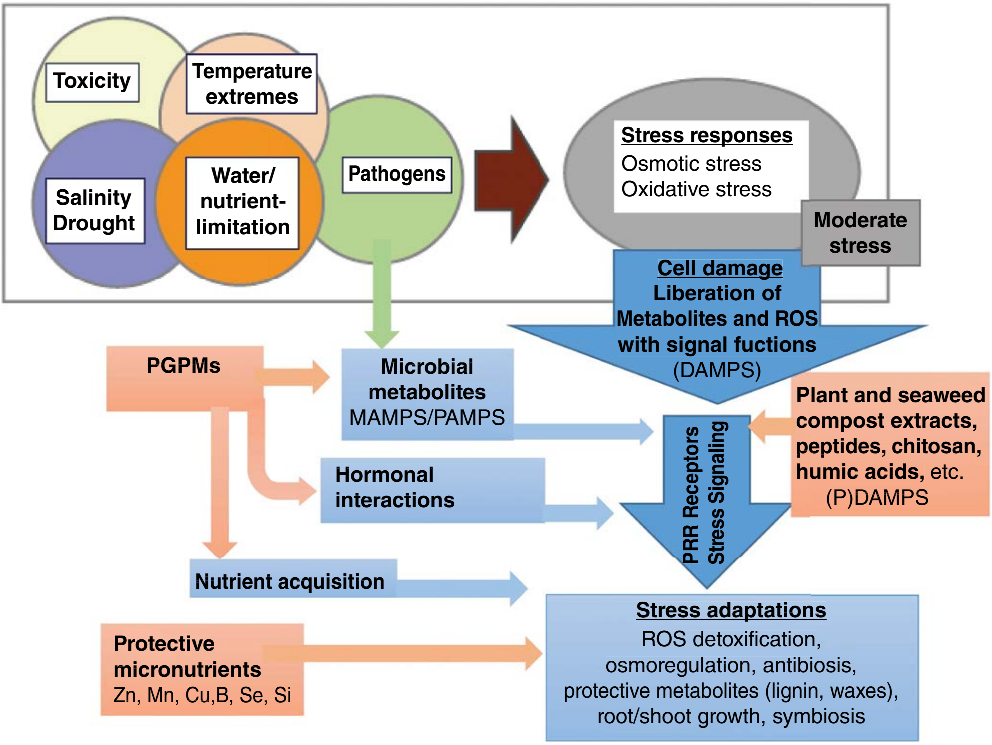

Fig. 17.4. Schematic representation of common modes of action of biostimulants in plants exposed to various biotic and abiotic stresses.

respective BS, adapted to the developmental and physiological stage of the receiver plants. However, this is only possible with highly standardized BS formulations as an additional challenge for product development.

Despite the work done to date, further studies based on metabolomics, proteomic and genomic approaches have been proposed to better understand the underlying mechanisms of stress tolerance in plants and the interactions with BS. Moreover, future research on identifying complementary and synergistic relationships among BS can be pivotal for many unanswered questions associated to plant resilience under adverse conditions.

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18 Seed Priming with Biostimulants for Better Crop Production Under Stresses

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Abstract

If biotic and abiotic stresses occur at the most sensitive phase of plant's life cycle, they can adversely affect the growth and development processes along with quality of production. The use of biostimulants is a promising strategy since they enhance the plant growth and resistance tolerance against abiotic stress factors, and decrease the use of synthetic fertilizers. Biostimulants, in a small amount, are more effective in improving the crop performance by stabilizing important plant functions that increase crop yield and quality, particularly under stress conditions. Biostimulants increase the plant nutrient uptake and their use efficiency, which triggers the plant natural defense system. Various studies have indicated that biostimulants are modulators of plant life processes that strengthen plants and enables them to perform better under abiotic and biotic stresses and also enhance the resource use efficiency. Diversity of raw materials, including plant growth-promoting bacteria (PGPB), humic acid, algae extract and hormones, are being used to produce biostimulants.

18.1 Introduction

Biotic (weeds and diseases) and abiotic stresses (drought, submergence, extremes temperature, salinity and nutrients deficiency) considerably hampers the growth and crop yield, globally (Bray *et al.*, 2000; Alexieva *et al.*, 2001; Munns and Tester, 2008; Fageria *et al.*, 2010; Farooq *et al.*, 2011, 2014, 2015). Different environmental factors (biotic and abiotic) determine the crop potential to produce yield, and thus good crop performance can be ensured when both of these factors are within in optimum range. However, abiotic stresses incur significant losses in grain yield such as 15% by chilling temperature, 40% by heat, 20% by salinity, 17% by drought and 8% by other factors (Ashraf and Harris, 2004). Fedoroff *et al.* (2010) stated that climatic variation-induced

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acceleration in the intensity of these stresses and crop production alteration are needed to fulfill the dietary requirements of an increasing population. Furthermore, low germination and poor seedling emergence can cause non-uniform establishment and lead to reduced grain yield (Singh *et al.*, 2015). Most of the farmers do not have enough resources to prepare good seed-bed and practice other agronomic practices. Thus, inadequate soil moisture and unfavorable temperature (at sowing) cause poor and erratic germination and seedling emergence resulted in huge yield losses (Angadi and Entz, 2002).

Optimum germination and good stand establishment are pre-requisite factors for high crop yield. In order to achieve better crop emergence, 'seed priming' is a well-known pre-sowing method where the seeds are partially hydrated so that pre-germination events occur but restrict radicle protrusion (Farooq *et al.*, 2019). The mechanism of primed seeds involves the initiation of epigenetic modifications and production of inactive signaling proteins and transcription factors. As plants experience stress, these mechanisms are modulated and turn into a highly efficient defense system to overcome stress-induced damage (Bruce *et al.*, 2007; Tanou *et al.*, 2012). Priming of seed helps to improve the germination rate and seedling emergence by protecting cellular proteins, repairing DNA damage during storage and improve functioning of protein synthesis machinery (Thornton *et al.*, 1993; Varier *et al.*, 2010), resulting in improved crop performance under biotic and abiotic stresses. Moreover, seed priming is an easy, less expensive and low-risk technique. and its effect becomes more visible under stressful conditions (Chen, 2011). A study by (Harris *et al.*, 2005) reported seed priming contributes to 20–70% increase in grain yield of field crops including wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), pearl millet (*Pennisetum glaucum* L.) and chickpea (*Cicer arietinum* L.).

Biostimulants – a product comprised of natural materials such as higher plants, bacteria, seaweeds and fungi – has the potential to promote crop growth and stress tolerance mechanisms (Ugena *et al.*, 2018). Biostimulants exhibit a wide array of diversity, ranging from one compound to different complex combination of bioactive compounds. Recently, its global market has expanded to satisfy crop requirement and improve resistance against stressful environment (Sharma *et al.*, 2014). Biostimulant application is an important innovative and promising approach to increase plant tolerance to many abiotic and biotic growth suppressing factors. Yakhin *et al.* (2017) stated that biostimulants help plants in improving nutrient use efficiency and nutrient uptake, and can induce resilience to the changing climate. Various researchers (Zeng *et al.*, 2012; Colla *et al.*, 2014; Pichyangkura and Chadchawan, 2015; Van Oosten *et al.*, 2017) have shown that priming seed with biostimulants enhances seed germination and also promotes tolerance against environmental stresses during imbibition and germination stages.

18.2 Seed priming – biotic and abiotic stress tolerance in plants

Seed priming is useful method of seed enhancement for augmenting the plant tolerance toward biotic and abiotic stresses. Moreover, it also guarantees faster germination and uniform establishment under adverse environment (Prasad *et al.*, 2016). This is primarily a hydration process that involves overnight soaking of seed (in aerated solution) or in low water potential solution and then dried back to original weight (Bradford, 1986; Farooq *et al.*, 2006). Uniform and synchronized seed germination due to the priming attribute to reduced duration of lag phase, activation of enzymes, build-up of germination metabolism, DNA repairing and osmotic adjustment (Lee and Kim, 2000; Brocklehurst and Dearman, 2008).

Jisha *et al.* (2013) reported, priming in field crops improves the tolerance against abiotic and biotic stresses through the activation of cellular defense responses and latent defense protein accumulation. It has been observed that priming of seed enhances tolerance against drought and saline stress by activating the antioxidant defense that protect the protein, lipids and membrane from damage. Furthermore, it helps in improving efficiency of photosynthetic system and carboxylation, resulting in better crop performance and higher crop yield (Farooq *et al.*, 2019). Plants grown from primed seed in stress environments (e.g. temperature extremes, drought, salinity) showed high tolerance response by stabilization of proteins, antioxidant system activation, molecular adjustment and scavenging reactive oxygen species (ROS).

Jalali and Salehi (2013) reported that priming of sugar beet seed with NaCl (1.5 N), HCl (0.1 N) and polyethylene glycol (PEG 8000) induces faster germination and also increases crop competitive ability against weeds. Use of phosphate fertilizer as seed priming reduces the infection of parasitic weed *Striga hermonthica* in cereals by decreasing the production of strigolactones in the rhizosphere compared with non-primed seeds (Jamil *et al.*, 2013). Another study found seed priming is the best option to manage weed–crop competition in early crop growth stages (Dhage and Anishetter, 2020). Primed rice seeds showed synchronized germination resulting in better crop establishment that suppress the weeds growth by giving preliminary advantage to rice. Anwar *et al.* (2012) found that osmoprimed rice seed produced healthier seedlings, which increases competition and lowers weed growth and dry matter (22–27%). Moreover, healthier seedling development through primed seeds intensifies crop competition and causes weed suppression, and also improve plant resistance against stressful environment (Clark *et al.*, 2001).

18.3 Seed priming with biostimulants

Biostimulants are the natural or synthetic products that, when applied to plants, enhance nutrients, plant quality and stress tolerance. Some of the natural biostimulants and their effect on crop performance and tolerance to biotic and abiotic stresses are described in this section.

18.3.1 Amino acids

Amino acids have been used as priming substances to promote growth and the ability of plants to perform better under stressful conditions (Worrall *et al.*, 2012; Ugolini *et al.*, 2015; Amirkhani *et al.*, 2017). For instance, β-amino butyric acid (BABA) application as a priming agent induces plant resistance to abiotic stresses and biotic stresses including insect, pathogens and nematodes (Oka *et al.*, 1999; Cohen, 2002; Hodge *et al.*, 2005; Zimmerli *et al.*, 2008). Amino acid-induced tolerance against different stresses is linked with their interactions with different hormones (Ton *et al.*, 2005). Zhong *et al.* (2014) explained that soybean seed priming with BABA enables the plant defense system and improves the synthesis of plant hormones including abscisic acid and salicylic acid, which therefore improve stress tolerance (Zhong *et al.*, 2014).

Several authors (Farooq *et al.*, 2013; Fercha *et al.*, 2014; Jisha and Puthur, 2016a) confirmed that rice and wheat seedlings established through amino acid priming showed high tolerance against salinity and drought stress contribute to membrane integrity and antioxidant activities. Seed priming with ascorbic acid lessens the salinity-induced adverse effects by changing the abundance of protein playing a role in the different physiological metabolism (Fercha *et al.*, 2014). Seed priming with BABA in mung bean increased the activities of enzymatic antioxidant such as peroxidase (POD), super peroxidase (SOD) and enhance the photosynthetic efficiency resulting in better crop growth and stress tolerance (Jisha and Puthur, 2016b). Likewise, Italian ryegrass primed with L-methionine showed higher POD activities compared to non-priming and showed more salt tolerance (Lee *et al.*, 2021). The priming with amino acids increased germination, fresh biomass and decreased H_2O_2 content under heat stress conditions. This reduction in H_2O_2 was linked with increased activities of glutathione S-transferase, SOD and catalase and accelerates the expression of heat shock proteins (Campobenedetto *et al.*, 2020). In general, application of amino acid in seed priming showed an excellent potential to strengthen plant defense and tolerance against different biotic and abiotic stresses. However, more research is needed to underpin the mechanism lying behind the increase in stress tolerance by application of amino acids.

18.3.2 Seaweed extract

Seaweed extract (SWE) is a biodegradable, non-polluted and non-toxic organic biostimulant to human's, plants, animals and the environment

(Craigie, 2011). The beneficial impacts of SWE on the nutrient uptake, grain quality, grain yield (Papenfus *et al.*, 2013; Halpern *et al.*, 2015) and alleviation of drought and heat stress (Xu and Leskovar, 2015; Elansary *et al.*, 2016) have been documented by different researchers. Application of SWE improves the plant antioxidant defense and detoxifies stress-induced ROS and physiological process during germination (Thirunavukkarasu *et al.*, 2020). Under heat stress (30°C), SWE (0.3%) application as priming showed higher germination, vigorous seedling and reduce ROS and malondialdehyde by enhancing the antioxidants function (Anjosneto *et al.*, 2020). Likewise, application of SWE as seed priming balance the water contents, improve photosynthetic components, soluble proteins and proline contents as indication of salt tolerance compared to control (Kasim *et al.*, 2016).

Seed treatment with SWE improves the nutrient uptake, photosynthesis, chlorophyll contents and ameliorates the effect of biotic stress (e.g. *Sclerotinia sclerotiorum* and *Pseudomonas syringae*) and water-deficit stress (Sangha *et al.*, 2010; Subramanian *et al.*, 2011; Shukla *et al.*, 2018). Likewise, SWE improved the antioxidant activities, gene expression, water retention and stomatal conductance and therefore leads to significant increase in drought tolerance (Shukla *et al.*, 2018). Seaweed extract application induced the physiological and morphological changes in plants that facilitate the tolerance against saline and drought stress throughout the growing season (Fleming *et al.*, 2019; Stasio *et al.*, 2020). Seed priming with SWE effectively improved stomatal conductance, physiological activities and gene expression and activities of antioxidants which favor the plant tolerance toward stresses (Rasul *et al.*, 2021). In conclusion, SWE priming can be used in wide range of conditions to decrease the negative impact of various biotic and abiotic factors.

18.3.3 Moringa leaf extract

Moringa (*Moringa oleifera*) possesses excellent potential to increase growth and grain yield of crop under both adverse and normal conditions (Semida and Rady, 2014). Moringa leaf extract (MLE) contains an appreciable amount of antioxidants, osmo-protectants and secondary metabolites (Rady *et al.*, 2013). Furthermore, moringa leaves have high mineral and vitamin content, and is also enriched with zeatin (a cytokinin – natural derivative) and thus has gained attention as natural biostimulant (Rehman *et al.*, 2015). Moringa leaf extract mitigates the adverse impacts of high temperature stress by improving plant water use efficiency, photosynthetic rate and chlorophyll contents (Rashid *et al.*, 2018). Likewise, applying extract of moringa leaves helps to increase the plant tolerance against drought and also improves crop performance. Afzal *et al.* (2012) found that priming maize seed with MLE increased the germination percentage and germination energy, decreased time to germination, and improved chlorophyll content, total sugars and amylase activities, resulting in high drought tolerance.

Bean seeds treated with MLE showed improved growth rates owing to an increase in antioxidant activities and osmolyte accumulation as compared to control (Rady *et al.*, 2013). Likewise, another study by Abdel-latef *et al.* (2017) noted that priming with MLE promotes growth in fenugreek under salt stress, which contributes to upregulation of salt stress-related genes, high uptake of minerals and metabolic activities. Another author noticed that priming with MLE improved fenugreek seedling emergence, nitrogen and carbon metabolism, ionic homeostasis and reduced the oxidative stress caused by lead stress (Al-Khazan, 2020). The exogenously applied MLE improved the performance of heat-stressed maize crop owing to improved chlorophyll content, stand establishments, and leaf and grain filling duration, which therefore increases the maize production (Bakhtavar *et al.*, 2015). Membrane leakage, along with the reduced net assimilation rate and photosynthetic pigments, has been observed in heat-stressed plants. However, maize seed primed with MLE improves the seedling development, membrane stability, water content, assimilation rate, membrane stability, photosynthetic pigments, biological and grain yield and protein content in high temperature environments (Iqbal *et al.*, 2020). Under salt stress, common bean (*Phaseolus vulgaris* L.) priming with MLE increased the plant weight, shoot length and physiological attributes including water content, membrane stability, chlorophyll content, total sugars, proline and caroteniod content (Zaki and Rady, 2015). Moreover, MLE also improves the uptake of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and antioxidants activities compared to control. Thus, all these improvements lead to significant enhancement to salt tolerance (Zaki and Rady, 2015).

18.3.4 Humic acid

Humic substances produced from decomposition of plant and animal residues have been widely used as organic amendments for their biostimulating effects on growth and nutrient acquisition within crops (Canellas *et al.,* 2015). Using humic acid (HA) in salt-stressed bean plants showed an increase in endogenous proline levels and a reduction in membrane leakage, both of which are indicators of better adaptation to saline environments (Aydin *et al.,* 2012). In rice, seed treatment with HA played a crucial role in activating the antioxidant enzymes which detoxify salt and drought stress-induced ROS (García *et al.,* 2012). Use of HA (1.5%) as a seed-priming agent promotes germination attributes (germination index, seedling emergence) and root growth under drought stress conditions (Nouriyani, 2019). Moreover, exogenously applied HA regulates non-enzymatic and enzymatic antioxidant activities and favors sugar accumulation, which increases the ability of plant to survive under water-deficit environment (Khodadad *et al.,* 2020).

Priming maize seed with HA (300 and 450 ppm) increased antioxidant activities and improved drought tolerance (Moghadam, 2013). Moreover, HA enhances the plant water use efficiency and lowers the root transpiration rate, which then increases drought tolerance (Asli and Neumann, 2010). The use of HA helps in strengthening the drought tolerance system by stimulating SOD and POD and scavenging the ROS (Sheteiwy *et al.,* 2017). In addition, HA also enhances drought tolerance by favoring ascorbate and glutathione metabolism and promoting flavonoids biosynthesis and increasing the antioxidant activities (Sun *et al.,* 2020).

Plant tolerance against salinity also improved significantly with HA application. Pretreatment with HA also improved the radical length, germination index, germination vigor and improved the salt tolerance in barley crop (Cavusoglu and Ergin, 2015). Seed priming with HA improved the shoot length, shoot weight, plant height, chlorophyll contents and antioxidant activities which lead to significant increase in salt tolerance (Kandil *et al.,* 2018). Mridha *et al.* (2021) noted that arsenic (As) stress significantly hampers rice germination, seedling development and important physiological processes. However, the application of HA enhanced the uptake of minerals, regulated antioxidant function and reduced the As entrance in plant, leading to better crop performance (Mridha *et al.*, 2021). Thus, applying HA can be a promising approach to increase tolerance in plant toward biotic and abiotic stresses and to ensure the better crop production.

18.4 Application of biostimulants on field crops

The European Biostimulants Industry (EBI) explained that biostimulants have the potential to improve the crop performance (germination to maturity), plant metabolism, nutrients assimilation and plant tolerance against stressful environment (European Biostimulants Industry Council, 2012). The mechanism of action of biostimulants is connected with higher tolerance to abiotic stress, thus improving quality and nutrients availability. This biological activity occurs due to the presence of peptides, hormones, saccharides, phenolic compounds and some other organic compounds. Use of SWE as biostimulants promotes germination and seedling emergence, which results in uniform crop stand, and ultimately crop yield and plant ability to withstand against stressful conditions (Craigie, 2011; Mattner *et al.,* 2013). Biostimulants are important element of agro-ecological practices due to their high potential in minimizing use of chemicals, saving energy and providing farmers with new opportunities for sustainable fertilization and disease control (Calvo *et al.,* 2014; Le Mire *et al.,* 2016). The effectiveness of biostimulants is determined by numerous factors including suitable choice of preparations, their concentration and dose, methods of application, environmental factors and cultivar of plants (Grabowska *et al.,* 2012; Kolomazník *et al.,* 2012).

Application of HA to common bean (*P. vulgaris* L.) increased the tolerance to salinity stress (120 mM NaCl) by increasing the endogenous proline concentration and decreasing membrane leakage (Aydin *et al.*, 2012). Studies have also found that extracts from HA are also beneficial for monocot crops. Vermicompost extract applied to rice plays an effective role in activating antioxidative enzyme functions and increased the ROS scavenging enzymes in water-deficit and saline environments (García *et al.*, 2012). In another study, inoculation of maize and wheat with *Azotobacter* strains has a positive effect under salinity by facilitating uptake of K^+ and exclusion of Na^+ as well as enhancing nitrogen and phosphorus availability results in higher yield (Rojas-Tapias *et al.*, 2012). Several researchers (Zhou *et al.*, 2007; Rodríguez-Lucena *et al.*, 2010; Yuan *et al.*, 2013) explained that the application of biostimulants to plant leaves and roots increased the uptake of nutrients and nutrients use efficiency for both macro and micronutrients in cereals and legumes.

18.5 Assessment of biostimulants priming on plant growth and development

Biopriming with seed biostimulant (strain: *Bacillus* spp. MGW9) improved the salt-stressed maize crop by increasing the germination (%), germination energy, seedling fresh weight and root length. Moreover, biopriming with *Bacillus* spp. (MGW9) also lowered the salt-induced oxidative stress by enhancing SOD, POD, CAT activities and also through stabilization of relative water contents, chlorophyll and proline contents (Li *et al.*, 2021). Biostimulants cause changes in vital and structural processes in order to influence plant growth through improved tolerance to biotic and abiotic stresses and also reduce the need of fertilizers (Du Jardin, 2015). However, in order to fine tune application rates, specificities of plant biostimulants and technique are identified that may bring the highest impact on stress protection.

Anjosneto *et al.* (2020) assessed the priming of spinach seed with SWE (*Ascophyllum nodosum*) and found that seed priming with SWE (0.3%) had the highest antioxidant activities and showed better germination (rate, speed) and seedling vigor under high temperature stress. Another interesting study was conducted by Chrysargyris *et al.* (2020), who evaluated the biostimulants effect of eco-product (EP) (containing essential oils of rosemary and eucalyptus) on tomato crop. Results showed that application of EP increased plant height, chlorophyll contents, stomatal conductance and CAT activity.

18.6 Conclusion

Plants are frequently subjected to unfavorable environments such as biotic and abiotic stresses, which play a vital role in determining crop yield. Biotic (weeds, diseases) and abiotic (drought, extremes temperature, salinization, heavy metals) restrict plant growth and development and cause yield losses. The use of biostimulants, including amino acid, humic acid, moringa leaf extract and seaweed extract, is an excellent and cost-effective method for counteracting the stress-induced growth instability. Seed priming with biostimulants helps plants in nutrient acquisition from the rhizosphere, protects cell membrane integrity by enhancing antioxidant system, and increases photosynthesis and carbohydrate metabolism under stress condition, resulting better crop growth and performance.

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19 Biological Trace Elements Confer Abiotic Stress Tolerance in Plants

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Abstract

Abiotic stresses have become a major concern now a days as they act as the limiting factors in plant growth and development. Due to the advancement of science, intensive research has been carried out globally to explore the underlying mechanisms of plant nutrient uptake, their metabolism, homeostasis and protection against abiotic stresses. Plants require trace elements at very low concentrations for growth and other physiological activities. Exogenous supplementation of trace elements in plant systems mitigates various stress conditions of plant species. Trace elements not only improve plant physiological processes and growth, but also play vital roles in improving plant tolerance toward varied abiotic stresses. The addition of trace elements ameliorates plant antioxidant response to counter oxidative stress. This chapter presents the plant responses to different abiotic stresses and the beneficial effects of different trace elements in conferring plant tolerance against various abiotic stresses.

19.1 Introduction

Rapid industrialization, globalization and increased human population have elevated the demand of plants as a source of food, oil, fiber, medicine, timber, etc. However, plants being immobile are bound to grow within their natural habitat and are exposed to a wide range of environmental stresses that hamper their morphology, physiology, biochemistry and molecular functions (Sharma *et al.*, 2019). Abiotic stresses can reduce the productivity and yield of crop plants by more than half (Dhankher and Foyer, 2018). A few plants have naturally adapted through various morphological changes against stress; however, some plants undergo physiological and molecular adaptations to avoid abiotic variations. The combined effect and longer duration of all these stresses and plant species drastically effect the plant growth and productivity (Pandey, 2015). The increased abiotic stress leads to overproduction of reactive oxygen species (ROS) in plants such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (•OH), and singlet oxygen ($^1O_2$), causing oxidative stress (Choudhury *et al.*, 2017). It adversely affects the synthesis of biomolecules such as DNA, proteins, carbohydrates and the antioxidant network, and may cause severe damage to cellular processes and other membrane systems. To cope with abiotic stress, plants have developed various mechanisms that help to mitigate stress-induced

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alterations in plants. Various antioxidants, hormones and metabolites have vital roles in providing tolerance to plants against abiotic stress. The exogenous application of micronutrients or trace elements is a novel approach to develop abiotic stress tolerance in plants.

Trace elements are the class of nutrients that are required in small proportions and help plants to perform a wide range of physiological functions. They play a vital role in providing protection against stressful conditions to plants (Hasanuzzaman *et al*., 2017). Trace elements perform multidimensional role in enhancing plant's tolerance to abiotic stresses. They may include boron (B), copper (Cu), cobalt (Co), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), zinc (Zn), etc. Trace elements are components of cell membranes and organelles and also play a key role in activating different ROS scavenging enzymes. Trace elements control important metabolic pathways involved in nitrogen assimilation, gene regulation, cell signaling, cell wall lignification and protection against environmental stresses. For instance, Tripathi *et al*. (2018) reported the role of Fe in alleviating drought, salinity and heavy metal stress in plants that might be attributed to activation of different antioxidants such as catalase (CAT), superoxide dismutase (SOD) and peroxidase that scavenges ROS in plants. Hawrylak-Nowak *et al*. (2018b) reviewed the role of selenium in enhancing abiotic stress tolerance in plants through stimulation of various antioxidative enzymes. However, the actual mechanisms involved in alleviating abiotic stress are still missing. This chapter outlines the roles of trace elements in induction of abiotic stress tolerance in plants.

19.2 Abiotic stress in plants

Rapid fluctuations in climate have led to generation of various abiotic stresses such as salinity, drought, extreme temperature, flooding, metal toxicity, air pollution, ozone and UV radiation in plants (Fig. 19.1; Pereira, 2016). Abiotic stress leads to inhibition of growth, photosynthesis, crop yield and impaired nutrient uptake in plants hampering the crop quality that deteriorates agricultural productivity (Hasanuzzaman *et al*., 2020). The primary sites for generation of

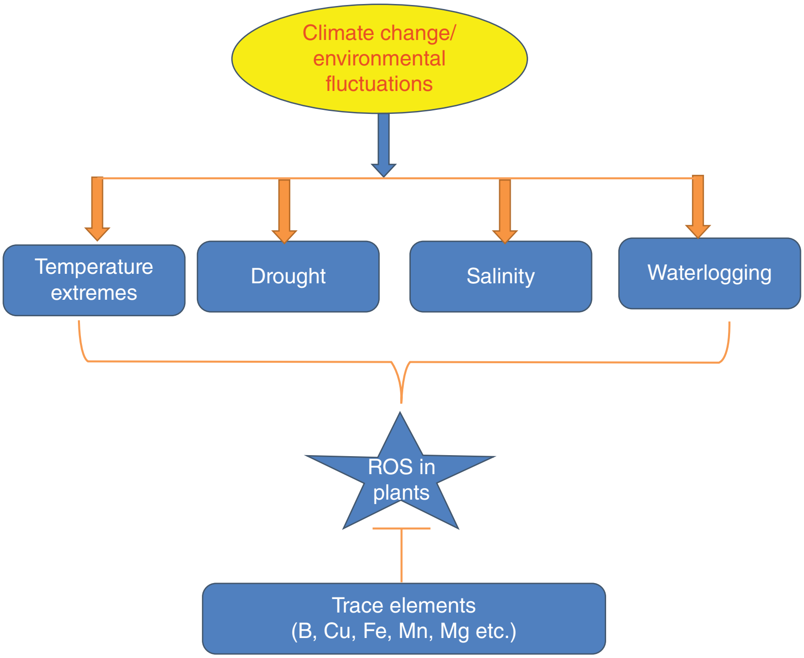

Fig. 19.1. Climate change induced abiotic stress in plants and their management by trace metals.

ROS in plants are chloroplasts, peroxisomes, mitochondria, etc. (Singh *et al.*, 2019). Abiotic stress disturbs the balance between ROS generation and antioxidant defense systems leading to excessive ROS accumulation that causes oxidative stress in plants (Hasanuzzaman *et al.*, 2020). The oxidative stress may severely damage the cellular machinery of plants, mainly the DNA, proteins and carbohydrates (Raja *et al.*, 2017).

Plants have a complete set of endogenous defense mechanisms in the form of enzymatic (SOD, CAT, APX, GPX, GR, MDHAR and DHAR) and non-enzymatic antioxidants (GSH, ascorbic acid, alkaloids, phenolics and flavonoids) to mitigate oxidative stress (Sidhu *et al.*, 2018). Besides antioxidants, some other mechanisms adopted by plants include root exudation, initiation of stress-related proteins and use of various trace elements that regulate plant defense mechanisms. The accumulation of ROS under stressful conditions leads to cell death in plants. Besides causing toxicity in plants, ROS play an important role in signaling pathways related to stress responses (You and Chan, 2015). The improved plant stress resistance is very important for both agricultural productivity and environmental sustainability.

19.3 Plant response to abiotic stress

Plants show various physiological, metabolic and genetic alterations in response to a range of environmental stresses. The signaling pathways in plants under abiotic stress occurs in four stages: (i) perception of the signal and (ii) its transduction; (iii) expression of stress responsive genes; (iv) and activation of physiological and metabolic pathways. The response of plants upon exposure to different abiotic stresses and the pathways involved in plants to alleviate stress are discussed in this section.

19.3.1 Temperature extremes

Temperature is a vital factor that affects plant growth and metabolism. Extreme variations in temperature have harmful effect on agricultural crops worldwide. According to the IPCC (2014) report, the annual daily temperature will rise by approximately 1–3°C by the end of the 21st century. Enhanced global temperature has caused severe loss in yield in tropical countries (Long and Ort, 2010). Heat stress severity is affected by the duration, stage and intensity of the stress (Fahad *et al.*, 2016b). The elevated temperature decreased the number of spikelets and florets in rice (Wang *et al.*, 2019) and seed set in sorghum plants (Singh *et al.*, 2017). Barlow *et al.* (2015) studied the extreme variation in temperature (i.e. frost and heat) in wheat plants and found that lower temperatures or frost caused sterility in grains and high temperatures or heat reduced grain number and grain filling period in wheat.

The high temperature, stress-induced negative effect on plants primarily depends upon the stage of plant development. Plant responses to temperature extremes are complex and have harmful effects on plant metabolism that disrupt cellular balance and damages physiological and biochemical processes of plants (Awasthi *et al.*, 2015). Photosynthesis, an important physiological process in plants, is temperature sensitive and high temperature can damage photosystem I and II (PSII) and enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in plants (Singh and Thakur, 2018). High temperature regulates important metabolic processes such as membrane stability, water relations and level of secondary metabolites in plants (Awasthi *et al.*, 2015).

In contrast, cold stress or low temperature stress is another factor that hampers plant growth and productivity and leads to huge crop losses worldwide (Yadav *et al.*, 2019). Cold stress includes both chilling and freezing stress that can affect the development and yield of agricultural plants (Awasthi *et al.*, 2015). Low temperature mainly affects growth, cell division, transport of water, photosynthesis and, ultimately, crop yield in plants (Yang *et al.*, 2018). The high- and low temperature stress-induced changes in cellular responses leads to excessive generation and accumulation of toxic compounds, mainly ROS that causes oxidative stress in plants (Schieber and Chandel, 2014). Increased ROS production damages proteins, lipids, carbohydrates and nucleic acids in plants.

19.3.2 Waterlogging stress

Waterlogging is the saturation of soil with water due to extreme rainfall, poor drainage and irrigation practices (ICID, 2019). It is a type of flooding that is superficial and covers only the root of the plant (Fukao *et al.*, 2019). Approximately 12% of the agricultural area globally is affected by waterlogging (Kaur *et al.*, 2020). It is an important factor that affects growth, development and survival of numerous plant species (Manik *et al.*, 2019). Waterlogging leads to soil compaction and accumulation of phytotoxic by-products, leading to hypoxia and further anoxia within a few hours near plant root systems (Isweiri *et al.*, 2021). Water logging also reduces leaf water potential, photosynthesis, root permeability, root hydraulic conductivity and stomatal conductance in plants (Rodríguez *et al.*, 2019). Increased exposure of plants to flooding stress results in restricted photosynthesis that alters the chlorophyll content in plants (Zhang *et al.*, 2019). The main reason for excessive waterlogging is the enhanced occurrence of extreme precipitation events (Kunkel, 2003).

Flooding or waterlogging also decreases nodulation and nitrogenase activity in plants. For example, El Msehli *et al.* (2016) studied the impact of hypoxia on nodulation and growth of legume plant *Medicago truncatula* living in symbiosis with *Sinorhizobium meliloti* and found an almost 45% decrease in nodulation under stressful conditions. Moreover, under waterlogged conditions plants showed reduced water and nutrient uptake, which might be attributed to decreased root conductance that causes poor growth in plants (Elzenga and van Veen, 2010). Waterlogging stress alters the sugar concentration, especially glucose and sucrose, that leads to change in gene expression in plants (Mutava *et al.*, 2015). The reproductive stage of the plant is a crucial stage that determines crop productivity and yield (Zhou *et al.*, 2020). Zhou *et al.* (2018) reported reduced grain weight and starch composition in winter wheat exposed to waterlogging stress after flowering stage.

19.3.3 Heavy metal stress

Heavy metals are those metals or metalloids having atomic density higher than 6 g cm^{-3}. It includes both essential elements that are required in very low amount (Co, Cr, Cu and Zn) and non-essential elements such as Cd, As, Hg and Pb (Pinto *et al.*, 2015). An increased amount of these elements is toxic for both the flora and fauna. Globally, metal contamination has become a serious environmental hazard. Injudicious industrialization, urbanization and exponential growth of the population have added a large amount of heavy metals in the soil or water ecosystem and cause serious harm to living organisms (Kumar *et al.*, 2019). The enhanced concentration of heavy metals in the soil leads to increased metal uptake by plants that cause decreased growth, biomass, photosynthesis and yield in plants (Ramzani *et al.*, 2016). Heavy metals disrupt the process of photosynthesis and destabilize enzymes involved in oxidation of PSII, thereby disturbing electron transport chain and mineral metabolism in plants (Seneviratne *et al.*, 2019).

The increased amount of metal accumulation affects the morphology, cellular metabolism and physiological pathways in plants. For example, Ni stress reduced root and shoot length, dry weight, chlorophyll and mineral content in plants causing oxidative stress in *Eleusine coracana* plants (Kotapati *et al.*, 2017). Sidhu *et al.* (2017) evaluated the effect of Cd on the growth and photosynthesis of *Coronopus didymus* plants and found that Cd toxicity reduced growth and increased generation of ROS species in plants. Furthermore, plant showed effect of chlorosis and necrosis at higher concentrations (Sidhu *et al.*, 2017). Moreover, Seneviratne *et al.* (2019) documented reduced proteolytic enzyme activity and increased amino acid content under metal stress. The major result of metal stress is enhanced production of ROS due to altered metabolic processes under metal toxicity. The accumulation of ROS leads to oxidative stress in plants that causes peroxidation of lipids, DNA damage, leakage of ions and disintegration of membranes (Sharma *et al.*, 2020).

19.3.4 Drought stress

Drought can be defined as a type of water deficit where soil water available for plants is insufficient for plants metabolic activity. It impairs growth and development of plants and is the major abiotic factor among various environmental stresses. Drought stress causes adverse effect on morphological, physiological, biochemical and molecular processes of plants (Table 19.1;

Table 19.1. Abiotic stress-induced morphological, physiological and biochemical changes in plants.

Stress type	Plant species	Effect	References
High temperature	*Lycopersicon esculantum*	Photoinhibition of PSII	Li *et al*. (2021)
	Oryza sativa	Reduced leaf area, photosynthesis, water-use efficiency	Fahad *et al*. (2016a)
	Zea mays	Decreased dry biomass and increased APX and GR activities	Khanna *et al*. (2016)
	Vigna radiata	Reduced leaf chlorophyll, leaf relative water content, enhanced oxidative stress	Nahar *et al*. (2015)
	Cucumis sativus	Inhibited plant growth and decreased chlorophyll concentration	Zhou *et al*. (2016)
Low temperature	*Solanum lycopersicum*	Reduced growth, relative water content, photosynthesis and proline content	Ghorbanpour *et al*. (2018)
	Fragaria × ananassa	Declined net photosynthetic rate, chlorophyll content, incremented MDA and H_2O_2 content	Huang *et al*. (2018)
	Cynodon dactylon	Limited growth, chlorophyll content, increased MDA, EL, H_2O_2 content	Liu *et al*. (2016)
	Kappaphycus alvarezii	Declined photosynthetic capacity and RuBisCo large subunit protein levels	Li *et al*. (2016)
	Stevia rebaudiana	Decreased quantum yield of photosystem II, net photosynthesis, chlorophyll a,b and carotenoid content	Hajihashemi *et al*. (2018)
Drought	*Oryza sativa*	Reduced growth, chlorophyll and carotenoid content and oxidative damage in plants	Gusain *et al*. (2015)
	Amaranthus tricolor	Declined total biomass, specific leaf area, relative water content, photosynthetic pigments and soluble proteins	Sarkar and Oba (2018)
	Achillea spp.	Increased MDA and H_2O_2 content	Gharibi *et al*. (2015)
	Eugenia uniflora	Reduced Fv/Fm, net photosynthetic assimilation, Increased MDA and proline content	Toscano *et al*. (2016)
	Photinia × fraseri	Decreased Fv/Fm ratio, net assimilation rate, lipid peroxidation and increased activity of SOD	Toscano *et al*. (2016)
Salt stress	*Phaseolus vulgaris*	Reduced shoot length, dry weight, chlorophyll, carotenoids and relative water content in plants	Rady and Mohamed (2015)
	Lemna gibba	Declined PSI and PSII activity, electron transport chain and increased ROS generation	Oukarroum *et al*. (2015)
	Cicer arietinum	Inhibited growth, biomass yield, leaf relative water content and chlorophyll content	Ahmad, P. *et al*. (2016)
	Calendula officinalis	Decreased stem length, fresh weight, photosynthetic pigments and increased proline and MDA content	Kozminska *et al*. (2017)
	Brassica napus	Reduced growth, leaf relative water content, osmotic potential and proline accumulation	Khalid *et al*. (2015)
Waterlogging	*Gossypium hirsutum*	Decreased leaf area, plant biomass, yield, photosynthetic rate	Zhang *et al*. (2015)

Continued

Table 19.1. Continued.

Stress type	Plant species	Effect	References
	Solanum lycopersicon	Reduced shoot length, leaf number, chlorophyll a, b, sugar and protein content	Singh *et al*. (2017)
	Zea mays	Declined leaf number, leaf area and dry weight	Jaiswal and Srivastava (2015)
	Cucumis sativus	Stunted growth, decreased leaf area, net photosynthesis and reduced leaf area	Barickman *et al*. (2019)
	Sesamum indicum	Decreased leaf water content, proline, photosynthetic pigments. Increased MDA and H_2O_2 in plants	Anee *et al*. (2019)

Hussain *et al.*, 2019). It reduces the crop yield, changes chlorophyll content, hampers photosynthetic processes and alters enzyme activity in plants (Devi *et al.*, 2012). Drought stress is affected by climatic, soil and agronomic factors. The effect of drought stress on plants depends upon amount of stress, plant species and stage of development in plants (Anjum *et al.*, 2011).

Drought stress leads to closure of stomata and reduced gaseous exchange in plants (Yadav and Sharma, 2016). It is a limiting factor that affects the germination of seeds in plants and also enhances mean germination time (Soleymani and Shahrajabian, 2018). Recently, Lotfi *et al.* (2019) evaluated the effect of drought stress on germination of walnut seeds and found decreased starch content and increased amount of proline and antioxidant enzymes in tested plant species. Further, Ahanger *et al.* (2016) reported reduced transport of nutrients from root to shoot due to the decreased rate of transpiration and alteration in functioning of membrane transporters. Earlier, Selvakumar *et al.* (2012) suggested decreased diffusion and mass flow of various macro- and micronutrients like magnesium, calcium and silicon upon exposure to drought stress.

19.3.5 Salt stress

Salinity stress is one of the important abiotic stresses worldwide. The adverse effect of increased salts such as Na^+ and Cl^- on morphological, physiological and biochemical processes of plants is defined as salinity or salt stress (Parihar *et al.*, 2015). According to Nawaz *et al.* (2010), areas with little precipitation, increased transpiration rate and high temperature are more likely to get affected by salt stress. The adverse effect of salinity on plants mainly depends upon climatic conditions, edaphic factors, intensity of light and species growing in that area (Tang *et al.*, 2015). High concentration of salt in plant cells induces ionic, osmotic and oxidative stress in plants (Yang and Guo, 2018). Roots are the primary organ of the plant that senses the salinity stress. Salt stress hampers germination, photosynthesis, biochemical pathways and yield of the plant (Kaleem *et al.*, 2018). For example, NaCl treatment reduced the growth, yield and physiological processes in maize plant (Baghel *et al.*, 2019). Moreover, salt stress affected the growth of different plant species such as *Amaranthus cruentus* (Gandonou *et al.*, 2018), *Calendula officinalis* (Baniasadi *et al.*, 2018) and *Oryza sativa* (Hussain *et al.*, 2017).

Salt stress-induced reduction in growth, photosynthesis, transpiration, stomatal conductance and leaf osmotic potential in *Amaranthus cruentus* was observed by Gandonou *et al.* (2018) when plants were exposed to 0–90 mM NaCl. Furthermore, the exogenous application of NaCl reduced the total chlorophyll content, Fv/Fm ratio and increased MDA and H_2O_2 content in *Calendula officinalis* (Baniasadi *et al.*, 2018). The reduced photosynthesis might be attributed to the decreased availability of CO_2 due to closure of stomata (Flexas *et al.*, 2004). Recently, Castanares and Bouzo (2019) evaluated different morphological and physiological parameters in *Cucumis melo* plants and observed reduced growth, relative electrolyte leakage, peroxidase activity and relative water content under salt stress.

19.4 Oxidative outburst in plants due to abiotic stress

Abiotic stresses are environmental challenges to which plants are exposed. It leads to reduced productivity resulting in large economic loss. These abiotic stresses include drought, temperature, salinity, heavy metals and waterlogging. The extreme environmental conditions increase the generation of ROS in plants. ROS accumulation leads to oxidation of biomolecules such as lipids, carbohydrates, enzymes and proteins, leading to molecular and cellular damage that ultimately causes plant death.

The extreme change in optimum temperature induces heat or freezing stress in plants that alters photosynthesis, reproduction and yield of plants (Bita and Gerats, 2013). Extreme temperature (cold or heat) affects the photosynthetic activity and yield in *Brassica oleracea capitata* and *B. oleracea acephala* that might be attributed to accumulation of ROS in chloroplasts that provoked photoinhibition in plants (Soengas *et al.*, 2018). The increased temperature enhanced O_3 (ozone) generation in the troposphere, which causes oxidative stress in plants (Rai *et al.*, 2018). Similarly, cold stress treatment induced oxidative damage in *Camellia sinensis* due to enhanced ROS accumulation that affected photosynthetic processes in plants (Li *et al.*, 2018).

Drought stress-induced water scarcity, reduced growth, water potential and yield in plants that hampers normal cellular functioning (Feng *et al.*, 2013). It reduces opening of stomata, increases photorespiration and causes ROS generation and oxidative damage in plants (Jalil *et al.*, 2017). Sarkar and Oba (2018) evaluated the mechanisms of growth, physiological and biochemical responses of *Amaranthus tricolor* against drought stress. Furthermore, excessive accumulation of water in soil is harmful for growth and development of plants. Waterlogging stress causes anoxic conditions that inhibits electron transport chain and results in ROS. Xiao *et al.* (2020) studied the effect of waterlogging stress on germination and growth of *Prunus persica* and found that stress-induced plants resulted in reduced stomatal opening, root activity and accumulation of ROS that leads to oxidative damage.

Salinity stress is a major environmental problem worldwide and accumulation of salts in higher concentration leads to decreased stomatal conductance, photosynthetic enzymes that causes ROS generation in plants (Hasanuzzaman *et al.*, 2018). Recently, Mushtaq *et al.* (2020) observed increased osmotic stress in plants due to excessive amount of salts that causes loss of water from plant leaves and hampers water absorption by roots. This might be attributed to the enhanced generation of ROS in salt-stressed plants (Mushtaq *et al.*, 2020).

19.5 Trace elements confer tolerance in plants toward different abiotic stresses

Trace elements play a vital role in combating abiotic stress in plants. They are an important part of all the metabolic activities of plants. Moreover, different key enzymes that confer tolerance in plants against stressful conditions contain trace elements that impart structural integrity to enzymes and help in providing protection to the plants. The following section discusses in detail the function of individual trace elements in providing tolerance toward varied abiotic stresses.

19.5.1 Boron

Boron is considered to play an important role in carbohydrate, protein and nucleic acid metabolism, cell wall structure and synthesis, phenolic metabolism and membrane integrity (Uluisik *et al.*, 2018). It helps in maintaining growth of meristematic tissues, enzyme activation, flowering and development of pollen grains in plants. Transport of sugars, hormone development and growth of pollen tube are also mediated by B in plants. The deficiency of B causes abnormal cell wall formation, altered plasma membrane permeability and reduced root elongation in plants. Thus, B deficiency leads to morphological, physiological and biochemical variations in plants that impede growth and development of plants (Hasanuzzaman *et al.*, 2017).

Boron's role in imparting stress tolerance in plants under different abiotic stressful conditions is multifaceted. Recently, Aydin *et al.* (2019) reported alleviation of drought stress by boron in tomato seedlings due to enhanced expression of stress-related genes and antioxidant enzymes activity in plants. Moreover, exogenous application of B improved wheat yield at different growth stages under drought stress (Abdel-Motagally and El-Zohri *et al.*, 2018). It might be attributed to the increased plant pigments content and reduced stress markers (proline and H_2O_2) in wheat upon foliar application of B (Abdel-Motagally and El-Zohri, 2018). Boron (5 mg kg^{-1} soil) treatment improved growth and performance of *Pistacia vera* under severe NaCl stress. The ameliorating effect of B might be due to the increased accumulation of glycine betaine that improved osmoregulation and water availability in plants (Karimi *et al.*, 2018). Extreme variation in temperature causes reduced crop productivity worldwide. B is essential element in alleviating injury caused by temperature stress. According to Shahid *et al.* (2018), B application decreased the harmful effect of high temperature at both vegetative and reproductive stage of rice plants suggesting role of B on cell membrane stability, pollen viability and mobilization of sugars in rice.

19.5.2 Copper

Copper (Cu) is an essential micronutrient for growth and development of plants. According to Wuana and Okieimen (2011), Cu in the range of 5–30 mg kg^{-1} is considered optimum for plant tissues. Cu plays a vital role in various physiological processes of plants such as chlorophyll formation, oxidation, respiration, photosynthesis and in metabolism of carbohydrate, protein and cell wall in plants (Rehman *et al.*, 2019). It is needed for the synthesis of enzymes responsible for chlorophyll synthesis in plants. Cu is main constituent of different enzymes e.g. plastocyanin, cytochrome *c* oxidase and Cu–Zn SODs in plant cells (Khan *et al.*, 2018). Plastocyanin is an important electron carrier in the process of photosynthesis between PSI and PSII. It is a vital component of plant metabolism. Similarly, Cu–Zn SOD helps in scavenging ROS in plants and provide resistance in plants to combat different environmental stresses.

Plants are subjected to numerous abiotic stresses during their growth that negatively affect their productivity. The role of Cu in conferring tolerance to abiotic stress has been highlighted by many studies. Under saline conditions, foliar application of Cu enhanced performance and growth of tomato plants (Pérez-Labrada *et al.*, 2019). The stress tolerance in plants upon treatment with Cu might be attributed to the increased antioxidant mechanisms (Pérez-Labrada *et al.*, 2019).

Drought stress negatively affects the yield of crop plants that results in food shortage and affects the agriculture productivity of the country. Van Nguyen *et al.* (2022) reported that application of Cu enhanced growth and yield of maize plants under drought stress conditions. The treatment of Cu nanoparticles increased leaf water content, anthocyanin, carotenoids and chlorophyll content, and biomass of maize plants, thereby regulating protective mechanisms of plant and providing drought tolerance to the crop (Van Nguyen *et al.*, 2022). Studies have reported that Cu/Zn SOD plays a key role in scavenging ROS under temperature stress. Che *et al.* (2020) studied that overexpression of Cu/Zn SOD in transgenic potato plants provided tolerance under low temperature. Earlier, Zhang *et al.* (2017) also reported upregulation of *SiCSD*, a novel Cu/Zn SOD gene in response to cold treatments in tobacco plants.

19.5.3 Iron

Iron (Fe) is the fourth most abundant microelement on the Earth's crust. It is required by plants in a very small amount for its growth and development. Fe regulates various processes of plants such as photosynthesis, respiration, chlorophyll synthesis and energy transfer and also acts as the main component of various proteins and enzymes. It also acts as a cofactor for different antioxidant enzymes such as CAT, POD and APX in plants (Tripathi *et al.*, 2018). A few studies have reported that Fe application significantly alleviates abiotic stress in plants (Sharma *et al.*, 2012). According to Mozafari *et al.* (2018), application of iron nanoparticles to grape softwood cuttings mitigates salt-induced damage in plants. The results revealed that iron treatment enhanced total protein content and reduced malondialdehyde content in grapes (Mozafari *et al.*, 2018). Similarly, Maswada *et al.* (2018) studied the role of nano-iron (III) oxide in providing salinity tolerance to sorghum. The treatment of n-Fe_2O_3 enhanced growth and decreased lipid peroxidation caused by salt stress providing protection to sorghum from stress (Maswada *et al.*, 2018).

Drought is one of the major threats to food security globally. It affects the growth, biomass accumulation, cell division and leaf size in plants (Tripathi *et al.*, 2018). Foliar application of Fe improves drought induced damage in plants. For example, Baghizadeh *et al.* (2013) reported reduced oxidative stress caused by enhanced H_2O_2 and lipid peroxidation upon treatment of Fe in cumin plants under drought stress.

Excess heavy metals in soils affect yield and productivity of crops worldwide. Many reports have suggested role of Fe in mitigating heavy metal stress in plants. Recently, Adrees *et al.* (2020) reported improved photosynthesis and yield, and reduced Cd concentration in wheat upon application of Fe nanoparticles. This might be attributed to the alleviation of oxidative stress by Fe in leaves of plants grown under Cd toxicity (Adrees *et al.*, 2020). Similarly, Zaheer *et al.* (2020) studied role of Fe-lysine on growth and development of rapeseed under Cr toxicity. Fe-Lys chelation significantly increased plant growth, biomass and chlorophyll, and reduced oxidative stress and Cr concentration in roots and shoots of rapeseed plants, thus mitigating negative effects of Cr (Zaheer *et al.*, 2020).

19.5.4 Manganese

Manganese (Mn) is required in the range of 20–40 mg kg^{-1} dry weight in plants for different metabolic functions. It is an essential micronutrient for growth, reproduction and development of plants. Mn is a component of many enzymes, such as MnSOD, oxalate oxidase and Mn-protein complex in PSII (Ye *et al.*, 2019). It also plays a key role in activation of some enzymes involved in physiological activities of plants. For example, enzymes involved in the shikimic acid pathway require Mn for their activation. It acts as a cofactor for the oxygen-evolving complex involved in photosynthesis and catalyze water-splitting reaction in PSII (Alejandro *et al.*, 2020). Furthermore, Mn is important for protein glycosylation, pectin biosynthesis, Ca^{2+} signaling and urea catabolism in plants (Alejandro *et al.*, 2020).

Abiotic stress (drought, temperature, salinity) in plants leads to production of ROS in plants. ROS generation damages proteins, lipids, carbohydrates and DNA in plants. Mn helps in mitigating stress caused by abiotic factors in plants. In a study conducted by Khan *et al.* (2016), foliar application of Mn ($MnSO_4$) promoted growth, fruit production, chlorophyll and carotenoids biosynthesis in drought-stressed *Brassica juncea* plants. Earlier, Karim *et al.* (2012) reported alleviation of harmful effect of drought stress in winter wheat plants upon foliar application of Mn. Salt stress is a major abiotic stress that affects agriculture production worldwide. Mn plays an important role in alleviating salt stress-induced toxicity in plants. Exogenous application of Mn mitigates salt-induced reduction in growth and chlorosis in rice seedlings (Rahman *et al.*, 2016).

19.5.5 Magnesium

Magnesium is an abundant and important nutrient involved in growth and development of plants (Guo *et al.*, 2015). Mg plays a vital role in the synthesis of chlorophyll and carbon metabolism in plants. It is a component of various enzymes involved in phosphorylation processes such as phosphatases and ATPases. Mg is helpful in providing conformational stability to proteins, nucleic acids and cell walls (Guo *et al.*, 2016). It balances the cation and anion ratio in the cell and maintains cell turgor in the plants. The impaired photosynthetic activity and carbon metabolism leads to generation of ROS in plants that causes oxidative damage.

The increased expression of MnSOD imparted stress tolerance under abiotic stressful conditions (Tounsi *et al.*, 2019). Earlier, Wang *et al.* (2010) reported increased salt stress tolerance in transgenic popular plants that might be attributed to expression of MnSOD from *Tamarix androssowii*. Sumesh *et al.* (2014) evaluated the drought tolerance in MnSOD-transgenic *Hevea brasiliensis* and found that MnSOD-transgenic *Hevea* lines showed better drought tolerant capacity and reduced inhibition of photosynthetic rates under stressful conditions.

19.5.6 Selenium

Selenium (Se) has both positive and negative effects on the environment. Previously, Se was regarded as a toxic element but now it is considered essential for both microbes and humans (Chauhan *et al*., 2019). The average concentration of Se in soil is 0.4 mg kg^{-1} (Hasanuzzaman *et al*., 2020). The natural and anthropogenic activities adds Se into the environment and also leads to its recycling in the atmosphere. The uptake of Se in plants is carried out by transporters present in root cell membranes.

Selenium has been reported to mitigate different abiotic stresses such as drought (Ahmad *et al*., 2016), temperature (Balal *et al*., 2016), salinity (Jiang *et al*., 2017) and heavy metals (Shekari *et al*., 2019) in plants. For example, Ashraf *et al*. (2018) investigated the effect of selenium on growth and physiological attributes of maize plants under salt stress. The results revealed mitigation of salt-induced oxidative damage in maize and improved growth in response to Se treatment (Ashraf *et al*., 2018). Similarly, Hawrylak-Nowak *et al*. (2018a) observed that exogenous application of Se enhances growth and thermo-tolerance in *Valerianella locusta* plants that might be due to the increased activity of antioxidant enzymes under Se exposure (Hawrylak-Nowak *et al*., 2018a).

19.5.7 Zinc

Zinc is an important microelement for all living organisms. Plants exhibiting zinc deficiency shows reduced growth, loss of chlorophyll, necrosis and impeded seed development (Sidhu, 2016). Several biological roles are assigned to Zn in plants such as activation of enzymes responsible for growth, production of chlorophyll and as a cofactor for SOD (Khan *et al*., 2018). Moreover, Zn metalloenzymes are involved in formation of DNA, RNA polymerases, transcription factors and reverse transcriptases in plants (Sidhu *et al*., 2016).

The role of Zn in conferring stress tolerance in plants is multidimensional. Wu *et al*. (2015) evaluated drought tolerance mediated by zinc in *Gossypium hirsutum*. The authors opined that Zn supplementation enhanced photosynthetic rate, chlorophyll *a* and *b* and dry matter content that might be attributed to the Zn induced increased antioxidative defense and osmotic adjustment in plants (Wu *et al*., 2015). Further, Jan *et al*. (2017) observed enhanced tolerance in *Triticum aestivum* to salt stress in response to Zn treatment. The results revealed that Zn application significantly increased photosynthetic pigment and antioxidant activities, and decreased MDA content in stressed plants (Jan *et al*., 2017). Furthermore, Ma *et al*. (2017) reported increased grain yield in *T. aestivum* in response to severe drought stress which might be due to the Zn regulated multiple defense systems at transcriptional level in response to drought.

19.6 Conclusions

Essential trace elements or micronutrients are important components of cellular machinery and play a vital role in the metabolism of plants. Extreme environmental conditions such as drought, heavy metals, salinity, high and low temperatures hamper the growth and metabolism of plants. The biological trace elements mitigate abiotic stress-induced alterations in plants by various mechanisms including activation of antioxidant enzymes, modulation of metabolic activities and maintaining ion balance.

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20 Role of Polyamines in Abiotic and Biotic Stress Tolerance in Plants

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Abstract

Polyamine (PA) metabolism and functions have started to gain the attention of plant scientists in the last few years. In plant organisms, the most common biogenic amines (spermine, spermidine and putrescine) are known primarily for their ability to mitigate the negative effects of biotic and abiotic stresses on plants and are involved in a number of various processes that ensure proper cell function. Plant PAs have been recognized for their roles as membrane, protein and nucleic acid stabilizers, as protectors of cellular integrity and photosynthetic machinery, as direct and indirect signaling agents, and as emerging members of the non-enzymatic antioxidant system. Currently, the knowledge of the role of PAs in many developmental and morphogenetic processes occurring in plants can be extended by interfering with PA biosynthesis pathways with appropriate inhibitors, the availability of PA mutants, and molecular biology methods. The accumulation of PAs in response to many abiotic and biotic stresses is one of the most remarkable plant metabolic responses. This chapter provides an overview of available data on PA functions in plants, synthesis, catabolism and involvement in plant cell responses to drought, salinity, low and high temperatures and biotic stressors.

20.1 Introduction

Polyamines (PAs) are relatively simple organic aliphatic substances that play a key role in many life processes in both animal and plant organisms. Investigations of this group of compounds began as early as in 1888, and the first reviews of their occurrence and physiological role were published in the second half of the 20th century (Cohen, 1998). The first reports on PAs described their presence and hypothetical functions in animal cells (Kubiś, 2006). Putrescine was identified in tissues of *Datura stramonium* L. plants in 1911 and in orange juice in 1948 (Bachrach, 2010). PAs are one of the basic components of living cells, participating in many physiological and biochemical processes (Flores, 1990). Due to their involvement in plant morphogenesis, they are classified as growth regulators (Galston and Kaur-Sawhney, 1995), and their concentrations necessary for exerting physiological effects are many times higher (at the millimolar level) than the concentrations of plant hormones. PAs are an important element in the regulation of many metabolic processes in plants and contribute to plant adaptation to unfavorable or stressful environmental conditions (Sobieszczuk-Nowicka and Legocka, 2007).

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PAs are common organic aliphatic polycationic compounds whose molecules contain two or more $-NH_2$ groups (Miller-Fleming *et al.*, 2015). Diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm) are the most common PAs found in all living cells. In addition to these PAs, cadaverine and agmatine are often present in biological material; the former is linear, likewise putrescine, spermidine, and spermine, and the latter is branched. Moreover, a spermidine isomer, i.e. thermospermidine, is synthesized in plant tissues (Takano *et al.*, 2012). Such PAs as norspermine, norspermidine, homospermidine, and homoagmatine are usually detected in lower amounts in biological material (Hamana *et al.*, 1995).

The chemical properties of PAs are determined by the presence of at least two positively charged amino groups in the molecule (Miller-Fleming *et al.*, 2015). These groups are responsible for the polar properties of PAs; hence, they strongly interact with polar water molecules, dissolving perfectly and showing the ability to form a hydration shell on the surface (Gupta *et al.*, 2012). In physiological pH conditions, they are nitrogen polycations, which determines their biological activity. Their unique feature is the distribution of the positive charge at regular intervals on a linear molecule, which is responsible for their kinetics and strong affinity for such polyanions as DNA or RNA (Basu *et al.*, 1988). PAs occur naturally in three forms: free bases, conjugated PAs and PAs bound to other macromolecules, such as phenols or proteins (Urano *et al.*, 2003). PAs in the form of free bases constitute the smallest part of the total PA pool (approximately 7–10%). They are mainly located in the cytosol and cell nucleus (Sempruch, 2008). The conjugated PA forms account for approx. 90% of the total PA content in cells and most often form amide bonds with phenylpropenoic acids, e.g. cinnamic acid. In plant cells, PAs are most often located in the cytoplasm, apoplast, vacuoles, chloroplasts, mitochondria and cell nucleus (Sempruch, 2008). They can also form complexes with such compounds as membrane phospholipids or various types of proteins, e.g. membrane, receptor, enzymatic, structural and other proteins (Martin-Tanguy, 2001). The PAs are present in all eukaryotic and most prokaryotic cells (Hamana *et al.*, 1985). Moreover, due to their affinity for many chemical compounds, they have the ability to bind to many cell structures, thus exerting an influence on many physiological processes in cells. This chapter reviews literature on the role of PAs in enhancement of stress tolerance in plant.

20.2 Polyamines in plants

In plants, the highest level and activity of PAs have been detected in meristematic tissues, chloroplasts, mitochondria and ribosomes (Sińska, 1997). Their concentration (from 10 μM to 1 mM) depends on the species, plant organ and environmental factors. Moreover, PAs are natural constituents of cell walls and easily bind to polygalacturonic acids (Lee, 1996). The activity of the conjugated form increases during flowering (Tanguy, 2001), while the activity of PAs increases during sprouting, seed germination, and formation of roots and shoots (Mengoli *et al.*, 1992). PAs are involved in many fundamental processes such as DNA replication, RNA modification, synthesis of protein, modulation of enzyme activities, and transcription (Takahashi and Kakehi, 2010). Many studies have reported an impact of PAs on the regulation of plant growth and development mechanisms (Ge *et al.*, 2006). Through interactions with phytohormones, mainly auxins, cytokinins and gibberellins, PAs participate in many physiological and biochemical processes in plant cells. They are involved in protein synthesis, cell division, stabilization of chromatin structures and cell membranes, inhibition of lipid peroxidation, ethylene synthesis, and other biochemical processes (Czerpak and Bajguz, 1999). Moreover, they can induce differentiation of organs and flower buds and exert an impact on plant flowering and fruiting (Singht and Singht 1995).

PAs have also been found to inhibit plant senescence (Baraniak and Kostecka, 1999) by delaying the process of degradation of chlorophylls and proteins, stimulating an increase in RNase and protease activity, and inhibiting the production of ethylene, which is referred to as a plant-senescence hormone (Baraniak and Kostecka, 1999). PAs, especially spermidine, are usually detected in young plant organs, and their concentration decreases with plant age (Galston and Kaur-Sawhney, 1987). Treatment of plants with exogenous PAs inhibits senescence symptoms, which proves that the activity of arginine decarboxylase, i.e. an enzyme involved in PA synthesis, decreases with the age of plant organs and so does the concentration of PAs. However, no clear results have been obtained in many studies conducted to confirm this thesis, and there are divergent views on this issue (Galston and Kaur-Sawhney, 1990).

It has also been shown that PA has an inhibitory effect on the activity of calcium channels in the tonoplast. Even a micromolar concentration of each of the three PAs was found to inhibit the activity of vacuolar ion channels: both the slow-activating vacuolar and fast-activating vacuolar types (Dobrovinskaya and Muniz, 1999). Detailed elucidation of the mechanism of action of PAs toward ion channels and changes caused by PAs in the cell ionic balance is currently one of the greatest challenges for researchers of these compounds (Kusano *et al.*, 2007).

In studies on *Arabidopsis thaliana* mutants with genes blocking the synthesis of PAs, putrescine and spermidine were proved indispensable for the proper course of the full development cycle in plants, whereas spermine was not (Imai *et al.*, 2004a, 2004b). In their experiment on *Arabidopsis thaliana* plants, Tun *et al.* (2006) ascribes the role of an inducer of nitric oxide production to spermine, which sounds very attractive but requires confirmation in further research (Yamasaki and Cohen, 2006). Another role attributed to spermine is the signaling function referred to as a 'spermine-dependent signal transduction pathway' (Takahashi *et al.*, 2003). There are two possible routes of spermine signaling. One involves an increase in the content of hydrogen peroxide produced via spermine oxidation by polyamine oxidase (PAO) (Cona *et al.*, 2006), as confirmed by the use of a PAO inhibitor blocking the signal transduction pathway (Takahashi *et al.*, 2004). The other route comprises the activation of calcium channels by spermine, which allows the influx of Ca^{2+} ions into the cell (Kim *et al.*, 2001). However, the exact determination of the potential involvement of all PAs, not only spermine, in stress signal transduction requires further investigations.

20.3 Biosynthesis of polyamines

The synthesis of the three basic polyamines in plants starts with decarboxylation of the two basic amino acids (i.e. arginine and ornithine) catalyzed by arginine decarboxylase (ADC, EC 4.1.1.19) or ornithine decarboxylase (ODC, EC 4.1.1.17) enzymes, respectively. The action of ADC results in conversion of arginine into agmatine. Arginine can also be converted into ornithine (through the activity of arginase EC 3.5.3.1) and then directly into putrescine by ODC. In turn, the formation of putrescine from agmatine is a two-stage process: the first product of the agmatine iminohydrolase-catalyzed reaction is N-carbamoylputrescine, which is subsequently converted into putrescine by N-carbamoylputrescine aminohydrolase. It is generally believed that the ODC-dependent putrescine synthesis pathway is dominant in animals and fungi. In some plants, putrescine synthesis may be associated primarily with ADC activity. For instance, the results of molecular analyses in *Arabidopsis thaliana* have demonstrated that the plant has no ODC-encoding genes, but has two genes whose expression product is ADC (Kusano *et al.*, 2007). In turn, the presence of an ODC-encoding gene and the activity of the ODC enzyme have been demonstrated in *Malus domestica* callus tissue and in *Solanum lycopersicum* seedlings (Hao *et al.*, 2005).

The synthesis of the other two PAs is associated with the presence of methionine, which donates propylamine groups indirectly via S-adenosylmethionine (SAM) (reaction catalyzed by S-adenosylmethionine decarboxylase - SAMDC, EC 4.1.1.50). Spermidine synthesis consists of the attachment of one propylamine group to putrescine via spermidine synthase (SPDS, EC 2.5.1.16), whereas spermine is formed by attachment of another propylamine group to spermidine via spermine synthase (SPMS, EC 2.5.1.22) (Slocum, 1991). Enzymes involved in polyamine biosynthesis, i.e. ADC and ODC, are found mainly in the nucleus, mitochondria, and chloroplasts, while SAM decarboxylase is a cytoplasmic enzyme. The synthesis of polyamines is directly linked with the synthesis of ethylene due to the competition for the common precursor SAM; therefore, one of the synthesis pathways may be inhibited while the other is stimulated (Bouchereau *et al.*, 1999). In *Lathyrus sativus* plants, an alternative spermidine synthesis pathway has been found with aspartic acid instead of S-adenosylmethionine as a donor of propylamine groups (Smith, 1985). PA biosynthesis is modulated by different factors and mechanisms. Transcriptional and/or translational regulation of PA biosynthetic enzymes by plant hormones, light, ozone, salt, chilling, wounding, drought or cold has been demonstrated in several plant species (Milhinhos *et al.*, 2013; Baima *et al.*, 2014). Moreover, the concentration of PAs with higher molecular weight is determined by the amount of putrescine in the plant cell. In turn, the level of putrescine depends on the activity of enzymes involved in its synthesis (ODA and ADC). It is also determined by such factors as the activity of ornithine (and arginine) synthesizing enzymes (Majumdar *et al.*, 2015), the activity of PA-catabolizing enzymes, the intensity of conversion of free putrescine into bound forms and the amount of putrescine utilized for the production of spermidine and spermine (Pérez-Amador and Carbonell, 1995).

20.4 Polyamine catabolism

The regulation of production of the three basic PAs is probably a result of a negative feedback, since exogenous application thereof inhibits the activity of enzymes involved in their biosynthesis. This is part of protection against the formation of excessive concentrations of these compounds, which may be harmful to plant cells. For this reason, precise regulation of the PA content in cells is essential (Kusano *et al.*, 2007). In plant cells, the control of the PA concentration is associated with the regulation of the expression of the SAMDC-encoding gene, which is necessary in their biosynthetic pathway. This gene has two elements coding for PA concentration-dependent translation regulators. One of the products represses the gene in conditions of a high PA concentration, whereas the other one stimulates translation at low PA content (Kusano *et al.*, 2007).

The level of PAs in plant cells depends on the biosynthesis, degradation and transport thereof (Groppa and Benavides, 2008). Putrescine, as well as spermidine and spermine, which have a larger number of amino groups, are degraded via oxidative deamination carried out by amine oxidases. The degradation of putrescine is catalyzed by diamine oxidase (DAO, EC 1.4.3.6), i.e. a copper-containing enzyme oxidizing the primary amino groups of diamine. In turn, another enzyme, i.e. flavin-containing PAO, EC 1.5.3.11), oxidizes the secondary amino groups of spermidine and spermine (Flores and Filner, 1985). Both DAO and PAO are located in the apoplast. PAO is also present in vacuoles, peroxisomes, and cytoplasm (Tavladoraki *et al.*, 2006; Angelini *et al.*, 2010). It has been shown in *Arabidopsis thaliana* that PAO can be involved in the conversion of spermine into spermidine and spermidine into putrescine (Tavladoraki *et al.*, 2006). The degradation products of putrescine include pyrroline, hydrogen peroxide and ammonia, whereas the other two polyamines are decomposed to pyrroline or aminopropyl pyrroline and then to diazobicyclononan. Diaminopropane and hydrogen peroxide can be formed in parallel. Subsequently, diaminopropane can be converted into β-alanine, and pyrroline is converted into γ-aminobutyric acid (GABA) and further into succinate, which can become part of the Krebs cycle (Flores and Filner, 1985). The enzymes involved in the degradation of PAs are associated with the cell wall, and their activity is related to the processes of lignification, suberinization and stiffening of the cell wall (Slocum and Furey, 1991), while GABA is classified as an immunostimulator (Bouchereau *et al.*, 1999).

Plant cells usually produce sufficient amounts of PAs to cover their demand for these compounds (Kakkar *et al.*, 1997). However, in the case of lower availability, mechanisms of PA uptake from the extracellular environment are triggered (Fujita *et al.*, 2012). PAs are readily taken up by plant roots and transported via the xylem along with the transpiration current (DiTomaso *et al.*, 1992). PA transport into the cell is an active process. It is strongly stimulated by auxins and occurs with the involvement of a specialized transmembrane protein (RMV1) located in the cell membrane (Fujita *et al.*, 2012).

20.5 Polyamines and abiotic stresses

Plants are a specific group of organisms characterized by a sedentary lifestyle and autotrophy. Since plants cannot move to find more optimal living conditions, they had to evolve effective stress-resistance mechanisms. The impact of stress factors on the plant organism is reflected in changes in the structure of cell membranes and physiological processes that determine cell metabolism. These disturbances consequently result in changes in gene expression and synthesis of proteins and other metabolites. The increase in PA synthesis in response to abiotic stress is one of the adaptations to adverse environmental conditions (Takahashi and Kakehi, 2010). The very first insight into the relationship between PAs and stress responses in plants dates back to the 1950s, when Richards and Coleman (1952) observed increased levels of putrescine in barley plants grown in potassium-deficient medium. Since then, growing evidence has linked PAs with enhanced plant tolerance to different kinds of adverse conditions. PAs play a protective and stabilizing role in various cell organelles, especially cytoplasmic membrane lipids, proteins and nucleic acids, which is associated with their hydrophilicity (Kuznetsov and Shevyakova, 2007). An increase in the PA concentration has been observed as a response to various types of abiotic stresses, e.g. salt stress, osmotic stress, light excess or deficiency stress, low and high temperatures, UV-C radiation, heavy metal stress, mechanical damage, and oxygen or potassium deficiency (Bouchereau *et al.*, 1999; Rangan *et al.*, 2014; Todorova *et al.*, 2014). Additionally, all types of stresses produce reactive oxygen species in the biological system, which are highly toxic and lead to oxidative stress (Alexieva *et al.*, 2003). The extent of the oxidative stress consequences depends on the duration of exposure of the plant species to the stress conditions. Several researchers have observed the role of PAs in mitigation of the consequences of various stresses, as these compounds are regarded as radical scavengers (Kim and Jin, 2006). On the contrary, several researchers have reported that the cell counteraction to the stresses is related to the increase in the antioxidant defense systems by PA application (Velicova *et al.*, 2000).

20.5.1 Drought and salinity stress

One of the most common stresses accompanying growing plants is related to drought. Due to their polycationic nature, PAs can prevent excessive water loss by increasing the amount of ionic compounds in the cell sap, which increases kinetic energy required for the transition of water to the gas phase. Numerous studies have reported an increase in the PA concentration in cells of plants exposed to drought stress; however, the plant response has been found to vary depending on the species, cultivar, plant organ, or persistence of stress conditions (Maiale *et al.*, 2004). Montesinons-Pereira *et al.* (2014) demonstrated an increase in the endogenous spermine concentration in cherry tomato cells exposed to drought stress, which in turn increased resistance to this stressor. In turn, foliar spermidine treatment of plants growing in water deficit conditions contributed to enhancement of the photosynthesis efficiency of chloroplasts and a substantial increase in the positive effects of restitution (Murkowski, 2002). An increasing problem in horticultural production is posed by salinity stress, which disturbs water metabolism in plants, likewise the drought stress. Generally, PA-rich plants show strong tolerance to salt stress. It has been indicated that the spermidine level in plants is an important indicator of salt tolerance (Li and He, 2012). A positive effect of exogenous PA application in salinity stress on various physiological parameters of plants has been observed in soybean (Wang and Bo, 2014), pea and bean (Shevyakova *et al.*, 1981), and broad bean (Priebe and Jager, 1978). In experiments on abiotic stresses and the possibility of mitigation of their effects by PAs, the efficiency of the photosynthetic apparatus is frequently analyzed, as it is directly associated with crop productivity. An experiment conducted by Velikova *et al.* (1998) showed a protective role of spermine on the photosynthetic apparatus activity. In turn, Murkowski (2001) found that a high concentration of PAs inhibits electron transport in PSII and causes a decline in PSI activity.

20.5.2 Temperature stress

As reported by Tiburcio *et al.* (1997), various abiotic and biotic stress factors influencing plants contribute to intensification of the metabolism and accumulation of polyamines in tissues. An increase in PA synthesis has been observed under high-temperature stress (Cheng *et al.*, 2009). PAs in plant cells exposed to this stressor stimulate the synthesis of heat-shock proteins (Konigshofer and Lechner, 2002) and enhance the photosynthetic efficiency, antioxidant capacity, and osmotic adjustment capacity in plants (Tian, 2012; Tian *et al.*, 2012). Moreover, exogenous application of PAs was found to increase the resistance to high-temperature stress in cucumber seedlings (Tian, 2012; Tian *et al.*, 2012). In turn, in an experiment on wheat plants, Racz *et al.* (1996) reported substantial accumulation of putrescine induced by low temperatures, which was also accompanied by increased frost resistance. In their studies, Kramer and Wang (1989, 1990) also observed an increase in SAM decarboxylase activity accompanying increasing PA levels. The protective effect of PAs on plant cells exposed to temperature stress is attributed to, for example, their stabilizing effect on cytoplasmic membrane lipids through interactions of positively charged amino groups with negatively charged phospholipid groups. In stress conditions, PAs can reduce damage to cell membrane phospholipids caused by enhanced lipoxygenase activity (Besford *et al.*, 1993; Lester, 2000).

In an experiment carried out on wheat and rice plants in low temperature conditions, Lee *et al.* (1997) reported an increase in the content of abscisic acid (ABA), which stimulated arginine decarboxylase activity and, consequently, produced an increase in the putrescine content. The use of an ABA synthesis inhibitor reduced the hormone content, enzyme activity and PA content successively. In turn, the effect of the inhibitor was eliminated by application of exogenous ABA. These results suggest that one of the functions of ABA synthesized during cold stress is the increase in the putrescine content resulting from increased ADC activity (Bouchereau *et al.*, 1999), which suggests indirect involvement of PAs in the action of phytohormones or a partial response to their signal (Rastogi and Davies, 1991).

In their experiment on cold-tolerant and cold-sensitive cucumber cultivars exposed to low temperatures, Shen *et al.* (2000) detected different PA contents in the leaves of the cultivars. Under the cold stress, a substantial increase in the spermidine content was found in the cold-tolerant plants, which can be explained by the increase in ODC activity. No increase in the content of any of the tested PAs was observed in the cold-sensitive plants, but the exogenous application of putrescine, spermine, and spermidine clearly mitigated the cold-induced plant damage. Other studies conducted on mangoes showed that administration of exogenous putrescine prior to cold exposure did not prevent cold-induced damage. In turn, exogenous administration of the other two polyamines (i.e. spermidine and spermine) largely protected the mango fruit against the destructive effects of cold, which allows a conclusion that the progression of cold damage is correlated with the biosynthesis of high molecular weight PAs (Nair and Singh, 2004). In poplar seedlings growing at 4°C, considerable accumulation of putrescine was observed immediately after the exposure to the stress, while the spermidine and spermine content increased after 4 and 7 days, respectively (Renaut *et al.*, 2005). Therefore, it may be suggested that the putrescine concentration increases rapidly in response to changes in environmental conditions. However, it should be added that such a high increase in the PA content is transient and only persists when the stress is relatively mild, which suggests that the putrescine content may reflect suboptimal growth conditions, whereas spermine and spermidine play a significant role as antioxidants or stabilizers of macromolecules or cell membranes (Larher *et al.*, 2003). Although the stress-induced levels of spermidine and spermine are lower than that of putrescine and these compounds exhibit much slower activity, they seem to be better markers in assessment of the level of stress in plants (Hausman *et al.*, 2000).

Although the higher levels of PAs in cells are correlated with higher stress tolerance in some cases (Tajti *et al.*, 2018; Hasanuzzaman *et al.*, 2019), this relationship should not be generalized in such a simplistic way. Putrescine, spermine and spermidine appear to have different functions under abiotic stress. In stress-sensitive plant species, the putrescine content usually increases quickly in response to changes in the environment, which is reflected in a decrease in the (spermine + spermidine)/putrescine ratio, and these changes are usually accompanied by the generation of reactive oxygen species, which is regarded as a stress signal (Groppa and Benavides, 2008; Paul *et al.*, 2018). In contrast, stress-tolerant species and cultivars are usually able to maintain higher levels of spermine and spermidine under stress, while putrescine levels remain relatively low, which may imply their higher resilience (Sánchez-Rodríguez *et al.*, 2016). Excessive putrescine accumulation in cells under stress can cause serious negative effects, such as the depolarization of membranes leading to potassium leakage, tissue necrosis, and protein loss, especially in leaf tissues. On the other hand, spermine and spermidine have anti-senescence effects under stress, being crucial for preserving the integrity of thylakoid membranes (Zhao *et al.*, 2008).

20.6 Polyamines and biotic stresses

Plants struggle with many kinds of biotic stresses caused by different living organisms, e.g. fungi, virus, bacteria, nematodes and insects. These biotic stress agents cause various types of diseases, infections and damage to crop plants and ultimately affect crop productivity. However, different mechanisms have been developed through research approaches to overcome biotic stresses (Gull *et al.*, 2019). One of the first reports describing the alterations in PA levels during pathogenic infections was carried out by Greenland and Lewis, who reported an increment in spermidine levels in barley leaves infected with the biotrophic fungus *Puccinia hordei* (Walters, 2000). A comparable phenomenon was observed in the same plant species after the inoculation with *Blumeria graminis* f. sp. *hordei* and in the interaction between wheat and *Puccinia graminis* f.sp. *tritici* (Walters, 2000).

Biotic stress results in considerable changes in the expression of genes involved in the synthesis of PAs in plant cells and PA catabolism, which in turn lead to changes in the content of these substances in plant tissues (Walters, 2003). Since both the plant and the pathogen are capable of synthesizing PAs, it is difficult to determine clearly whether the increase in the PA concentration is associated with the metabolism of the plant or the pathogen. Moreover, many studies indicate a possibility of plant infection by pathogenic microorganisms through partial control of the metabolism of the host. Therefore, investigations of PA metabolism in terms of plant–microorganism interactions should take into account changes in gene expression, which may constitute part of plant defense responses to biotic stress or may be a consequence of pathogen virulence mechanisms. The available research results do not provide an unambiguous answer to the question of the mechanisms involved in PA biosynthesis during pathogen attack. Changes in the PA content are probably a consequence of both mechanisms described. This hypothesis is supported by results of studies on PA homeostasis in plant tissues inhabited by beneficial microorganisms. The level of PA in the cells of these plants changed as in the case of infection by pathogenic microorganisms (Jiménez-Bremont *et al.*, 2004; Romero *et al.*, 2018).

Changes in the PA content in plant cells are one of the earliest plant responses to pathogen attack. Hence, the alterations in the levels of free and conjugated forms of PAs in response to pathogens with different lifestyles have been well documented (Walters, 2003; Jiménez-Bremont *et al.*, 2014; Pal and Janda, 2017; Singh and Farsodia, 2018; Seifi and Shelp, 2019). Various studies have shown that both free and conjugated putrescine levels rise considerably in plant tissue infected by fungi (Rodríguez-Kessler *et al.*, 2008; Wojtasik *et al.*, 2015) and bacteria (Vilas *et al.*, 2018). As mentioned earlier, changes in the putrescine content may be a result of the *de novo* synthesis thereof in infected plant tissues and/or putrescine is excreted by the pathogen during plant tissue colonization (Vilas *et al.*, 2018). Although a complete picture of the roles of PAs in plant defense is still difficult to determine, it has been found that free PAs can restrict the growth of some fungal pathogens *in vitro* (Mo *et al.*, 2015; Wojtasik *et al.*, 2015) and that exogenous application of putrescine can suppress nematode development in infected plants (Khajuria and Ori, 2018). Application of PAs or their precursors to leaves increased the activity of peroxidase and polyphenol oxidase in bean plants (Haggag, 2005), which is related to the increased resistance against the attack of pathogens (Kumar and Balasubramanian, 2000).

The concentrations of spermine and spermidine change in the cells of plants exposed to biotic stress, especially viral infection-associated stress (Jabłońska-Trypuć and Czerpak, 2007). Particularly interesting is spermine and its potential involvement in the activation of plant defense mechanisms against pathogens. Currently, two hypotheses regarding its regulatory impact have been proposed in the literature. One of them assumes a possibility of an impact of a signaling molecule, i.e. hydrogen peroxide, which is a by-product of PA oxidation processes, on the regulation of the expression of genes involved in defense reactions (Angelini *et al.*, 2010). The other hypothesis about the involvement of spermine in the mechanisms of activation of defense pathways suggests its ability to regulate the closure of ion channels, thereby influencing the transport of Ca^{2+} ions, which play a signaling role in plant cells (Sempruch, 2008; Takahashi and Kakehi, 2010). Furthermore, enhancement of PAO activity and, consequently, increased H_2O_2 synthesis indirectly contribute to strengthening and stiffening of the cell wall through lignification and suberinization, which facilitates formation of a mechanical barrier and impedes the spread of pathogens (Angelini *et al.*, 2010). It has also been shown that oxidized PA derivatives have strong antibacterial properties and are able to inactivate viruses (Bachrach, 2007). Besides the activation of the H_2O_2 signaling molecule, analyses of the role of PAs in the activation of the cellular signaling system should consider the activation of NO, i.e. a molecule triggering the signaling cascade in plant response to biotic stresses (Yamasaki and Cohen, 2006; Wimalasekera *et al.*, 2011). However, the questions of the impact of PAs and NO on specific reactions and the exact mechanisms of their interactions are still to be elucidated (Wimalasekera *et al.*, 2011; Ciącka and Krasuska, 2014).

20.7 Conclusion

In recent years, PAs have aroused considerable interest in the scientific milieu, which seems obvious given the wide spectrum of activity of these growth regulators. PAs are responsible for the regulation of many physiological processes in plant organisms. Moreover, their positive effect on mitigation of damage caused by biotic and abiotic stresses and plant adaptation to unfavorable environmental conditions has been proven. It seems that the PA biosynthesis and catabolism pathways have been well explored at the present stage of research. Nevertheless, the relationships between the PA and NO synthesis pathways have not been elucidated. Furthermore, the mechanisms responsible for increased NO production in plant tissues exposed to exogenous PAs have not been clarified. The role of NO in the regulation of plant metabolism is a highly interesting issue arousing growing interest.

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21 Role of Osmolytes in Enhancement of Plant Abiotic Stress Tolerance

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Abstract

Plants are subjected to various external abiotic stress factors like heat, drought, cold, salinity and heavy metal pollutants in soil. These have a deleterious effect on their growth and yield parameters. It depends on the extent of exposure to a particular abiotic stress factor. However, plants have adapted to these environments gradually by way of some kind of tolerance or by an innate resistance caused by changes in its morphological, biochemical and physiological characteristics. One such biochemical change is the synthesis of osmolytes in plants to protect themselves from oxidative damage caused by stress. Osmolytes are also called osmoprotectants, cytoprotectants or compatible solutes. There are different classes of osmolytes produced by plants. This chapter deals with the biochemical and molecular basis for the production of osmolytes in plants, and their types, significance and role in the abiotic stress tolerance in plants.

21.1 Introduction

Plants are sessile and this has made them prone to various environmental stressors like temperature (heat and cold), drought, flooding, salinity and heavy metal pollutants. However, over the ages, plants have learned to adapt to these stressors by way of morphological, physiological and biochemical changes. These changes vary depending on the type of abiotic stress. Morphological changes involve the change or adaptations that the plant has undergone with its external characters estimated in the form of leaf area, plant height, fresh and dry weight, seed weight, etc. The physiological parameters are relative water content which has a direct impact on transpiration, respiration, photosynthesis, seed germination, stomatal performance, etc. Finally, the biochemical parameters are the chlorophyll content, antioxidant, polyamines, amino acids, osmolytes, lectins, secondary metabolites and enzymes like peroxidase, catalase, polyphenol oxidase and superoxide dismutase.

These parameters vary between the type of stress involved. Interestingly, the stressors like drought, heat, salinity and cold stress lead to a common condition of water deficit. Many of these stress responses by plants seem to overlap or are similar between the stress factors involved. A very common response is the generation of reactive oxygen species (ROS) in the form of free radicals when plants are subjected to stress. Antioxidants are a group of phytochemicals that help in the scavenging of free radicals which have a deleterious effect on the physiology of the plant. Osmoprotectants or osmolytes are another

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class of phytochemicals which play a major role in protecting the plants from osmotic stress which is a major side effect in most of the stressors which result in water deficit.

The osmolytes are accumulated during abiotic stress to maintain the homeostasis (Yancey, 2005; Sharma and Dietz, 2006; Abhimanyu, 2019). They are overproduced and accumulated during osmotic stress and are inert and hence called compatible solutes (Burg and Ferraris, 2008). The term osmolyte is now referred to all molecules that are compatible and aid in protecting the cells during the abiotic stress (Roychoudhury and Chakraborty, 2013; Roychoudhury and Banerjee, 2016; Roychoudhury and Das, 2014). Moreover, since they protect the cells in times of osmotic stress, they are also called cytoprotectants (Groppa and Benavides, 2008; Yancey, 2005; Khan *et al.*, 2010).

The osmolytes belong to various classes of phytochemicals like sugars, amino acids, polyamines, polyols, methylamines and secondary metabolites (Hayat *et al.*, 2012; Roychoudhury *et al.*, 2015). Its biosynthesis is governed by mitogen activated protein (MAP) kinase and phytohormones along with calcium signaling. Their accumulation is necessary to protect the plants from reduction in photosynthesis and growth of plants during abiotic stress. Their biosynthesis is extensively studied in different plants (Giri, 2011). The genes responsible for the synthesis of osmolytes are transferred from stress tolerant plants to susceptible plants to develop transgenic plants for stress tolerance (Yang *et al.*, 2009). They are accumulated in plants in the cytoplasm, chloroplast and in few cell organelles (Yancey, 2005)

There are three phases in abiotic stress in plants namely, stress sensing, signaling and exhaustion. The plants first sense the abiotic stress stimuli by way of sensors (Roychoudhury and Banerjee, 2017) and then it leads to a signaling cascade that leads to various changes in the plants biochemically, physiologically and morphologically (Rosa *et al.*, 2009; Duque *et al.*, 2013), the extent of which leads to exhaustion. There might be yet another phase which is regeneration when a plant has overcome the stress and has started growing normally (Bhattacharya and Kundu, 2020). Various types of osmolytes and their role in abiotic stress tolerance in plants are discussed in this chapter.

21.2 Osmolytes

Osmolytes are low-molecular-weight compounds that affect the properties of the cell sap (Yancey, 2005). It alters the viscosity and ionic strength of the biological fluid. They help in maintaining the cell intact free from the damage due to osmotic stress and also has an impact on protein folding (Bolen and Baskakov, 2001). Osmoprotectants act as osmolytes and protect the plants in times of stress (Lang, 2007). The term osmolyte came into existence in the 1970s when it was first mentioned in the *American Journal of Physiology*. Osmolytes may be classified into betaines and associated compounds, sugars and polyols and amino acids. Osmolytes are present in most of the living organisms (Yancey, 2001; Burg and Ferraris 2008). Bacterial cells produce glutamate, glycine betaine, trehalose and proline in times of stress (Csonka 1989). Glycerol is accumulated in yeast cells during stress. Marine organisms accumulate dimethylsulphoniopropionate (DMSP) to protect themselves from sea water which has more salt. The application of exogenous osmolytes has been useful to protect the plants from abiotic stress (Jeyanthi Rebecca *et al.*, 2010). It has been observed in tobacco, barley, wheat, green gram, soybean, etc. (Borojevic *et al.*, 1980; Agboma *et al.*,1997). Moreover, it has also been reported that in Arabidopsis exogenous application of proline and glycine betaine triggered the expression of genes responsible for the synthesis of antioxidants (Oono *et al.*, 2003; Einset *et al.*, 2007).

Osmolytes are highly soluble, electrically charged, low-molecular-weight molecules. They rarely interfere with cellular metabolism and is non-toxic even at high concentrations. They protect the plant cells from dehydration by maintaining membrane fluidity and by stabilizing the cellular proteins (Wani *et al.*, 2013; Roychoudhury and Paul, 2012).

21.3 Types of Osmolytes

The osmolytes can be classified into sugar alcohols or polyols, amino acids and their derivatives, quaternary ammonium compounds, tertiary sulphonium compounds and polysaccharides (Table 21.1). Sorbitol which is soluble in water is

Table 21.1. Types of osmolytes (Slama, 2015).

Sl. No.	Group	Osmolyte
1	Sugar alcohol (polyol)	Sorbitol, mannitol, glycerol, pinitol and inositols
2	Amino acid and their derivatives	Polyamines, proline
3	Quaternary ammonium compounds	Betaines and choline-O-sulfate
4	Tertiary sulphonium compounds	Dimethylsulphoniopropionate (DMSP)
5	Polysaccharides	Trehalose, sucrose, fructose and glucose

present in significant amounts in salt marshes in *Plantago crassifolia* while it was subjected to high temperature and evapotranspiration. Similarly, sucrose, fructose and glucose were found in *Juncus acutus* and *J. maritimus* also in salt marshes (Gil-Ortiz*et al.*, 2011).

21.3.1 Sugar Alcohols or Polyols

Sugar alcohols are aldose or ketose sugars reduced to their respective hydroxyl residues. They are also called poly alcohols or polyhydric alcohols. They are water soluble and are classified into cyclic or acyclic polyols. The cyclic polyols are myo-inositol, pinitol, ononitol, etc., and the acyclic polyols are mannitol, sorbitol and inositols. They play a major role in reducing osmotic stress as compatible solutes in many organisms where they accumulate in times of stress (Sengupta *et al.*, 2008).

21.3.1.1 Mannitol

Mannitol is structurally similar to the aldohexose sugar mannose (Stoop *et al.*, 1996). It has been found in plants, fungi, bacteria, algae etc. (Ruijter *et al.*, 2003). It is sometimes synthesized as a photosynthate translocated via the phloem when sucrose is exhausted in certain plants. However, it functions as an osmolyte and scavenges free radicals during stress, as a storage of reducing power and as a storage for carbon (Stoop and Pharr, 1992). It is synthesized in higher plants from mannose-6-phosphate catalyzed by NADPH-dependent mannose-6-phosphate reductase yielding mannitol-1-phosphate, which is then dephosphorylated by mannitol-1-phosphate phosphatase to mannitol (Rumpho *et al.*, 1983; Loescher *et al.*, 1992). Mannitol is converted to mannose by mannitol dehydrogenase.

21.3.1.2 Sorbitol

Sorbitol is present in plants, fungi and bacteria as a 6C polyol and is also translocated as a photosynthate via phloem when sucrose is scarce like mannitol in mature leaves (Noiraud *et al.*, 2001; Jain *et al.*, 2010). It is synthesized in the source tissues from glucose-6-phosphate by the action of sorbitol-6-phosphate dehydrogenase to form sorbitol-6-phosphate, which is then converted to sorbitol and later converted to fructose in the sink tissues by sorbitol-6-phosphatase and sorbitol dehydrogenase, respectively (Jain *et al.*, 2010).

21.3.1.3 Inositols

Inositols are present in many plants and are required for their normal growth and metabolism. It belongs to a family of cyclohexitols and the most prominent of them is myo-inositol. It is synthesized from glucose-6-phosphate by the action of myo-inositol-1-phosphate synthase to produce myo-inositol-1-phosphate, which is then dephosphorylated to myo-inositol by myo-inositol mono phosphatase. It plays a major role in stress as an osmolyte and also regulates metabolic responses. Moreover, other osmoprotectants like D-oninitol and D-pinitol are myo-inositol derivatives (Bhattacharya and Kundu, 2020).

21.3.1.4 Glycerol

Glycerol is the first osmolyte identified referred to as compatible solute in yeast. Yeast cells accumulate it when they are at high salt concentrations (Albertyn *et al.*, 1994). It is also observed in *Chlamydomonas* that are grown at elevated salt concentrations. Glycerol, like other osmolytes, is soluble, chemically inert and non-toxic. There are reports of its accumulation in microalgae like *Chlorella* spp., Dunaliella spp., Scenedesmus spp. and Micrasterias spp. (Ahmad and Hallebust, 1984). Triacylglycerol is a major storage lipid in plants (Lung and Weselake, 2006). However, the biosynthesis of glycerol in higher plants has not been investigated much comparison to that in animals. It is synthesized from glucose via the glycolytic pathway from the reduction of dihydroxyacetone-P by glycerol dehydrogenase to yield glycerol-3-phosphate, which is then converted to triacylglycerol in plastids (Banas *et al.*, 2000).

21.3.2 Amino acid and its derivatives

21.3.2.1 Polyamines

Polyamines are nitrogenous compounds of low-molecular weight that are positively charged at physiological pH and are found ubiquitously in all living organisms. The most common polyamines in higher plants are the diamines, 1,4-diaminobutane (putrescine) and 1,5-diaminopentane (cadavarine), the triamine 1,8-diamino-4-azaoctane (spermidine) and the tetraamine 1,12-diamino-4,9-azadodecane (spermine). Apart from these, a large number of other linear and branched chain polyamines like norspermidine, homospermidine, norspermine and canavalmine have also been detected in plants (Livingstone *et al.*, 2002; Chen *et al.*, 2014; Chen *et al.*, 2019). Polyamines occur either in the free form as molecular bases or conjugated with small molecules or macromolecules like phenolic acids and proteins, respectively. Polyamines are present within the vacuole and in mitochondria and chloroplasts (Cai *et al.*, 2006).

Polyamines play a major role in cell division and also in other developmental processes of the plant tissue (Chen *et al.*, 2019). They are involved in senescence and stress responses of the plant (Jones *et al.*, 2012). They help in the differentiation of vascular strands similar to that of auxins and possess an antisenescence effect similar to that of cytokinins. Polyamines behave as cations at cellular pH levels and they can interact with other anionic molecules like DNA, RNA, phospholipids and certain proteins (Pál *et al.*, 2015; Sun *et al.*, 2018). The level of the diamine putrescine was found to increase during potassium deficiency (Mohammadi *et al.*, 2018) and also due to an exposure to a variety of osmotica (Bouchereau *et al.*, 1999). The polyamines serve as precursors for secondary metabolites like nicotine and also produce defense-related compounds by conjugating with phenolic acids (Sun *et al.*, 2018).

The biosynthesis of polyamines begins with the synthesis of the diamine precursor putrescine (Hanfrey *et al.*, 2001; Yanagisawa, 2001). Putrescine is synthesized either directly from the decarboxylation of ornithine or indirectly from arginine via agmatine and N-carbamoyl putrescine. The reactions are catalyzed by ornithine decarboxylase and arginine decarboxylase, respectively. Spermidine and spermine are formed from putrescine by the addition of an aminopropyl moiety in reactions catalyzed by spermidine synthase and spermine synthase, respectively. The aminopropyl moiety is derived from the decarboxylation of S-adenosylmethionine (SAM) in the reaction catalyzed by the enzyme S-adenosylmethionine decarboxylase (SAMDC). SAM is the common precursor for both ethylene and polyamine biosynthesis.

21.3.2.2 Proline

Proline is accumulated in plants when plants are subjected to drought, salinity, heavy metal stress and high temperature (Hayat *et al.*, 2012). It is synthesized by glutamate and ornithine pathway. During stress it is synthesized from the glutamate pathway. Glutamic acid is converted to proline via the intermediate Δ'-pyrroline-5-carboxylate catalyzed by Δ'-pyrroline-5-carboxylate synthase and Δ'-pyrroline-5-carboxylate reductase (Sekhar *et al.*, 2007). Application of proline exogenously in plants under abiotic stress has shown better growth and yield in soybean plants (Hayat *et al.*, 2012).

21.3.3 Quaternary ammonium compounds

21.3.3.1 Betaines

Betaines are amino acid derivatives with a fully methylated nitrogen atom. They are non-toxic at high concentrations and they do not carry net charge even at physiological pH. Betaines are in different forms namely, glycine betaine, proline betaine, β-alanine betaine, hydroxyproline betaine, pipecolate betaine and other related compounds like choline-O-sulfate (McNeil *et al.*, 1999; Ashraf and Harris, 2004). Among them, glycine betaine is the most widely available form.

Glycine betaine is accumulated in animals, fungi, cyanobacteria, bacteria and algae. They are also found in many higher plant families like Amaranthaceae, Chenopodiaceae, Compositeae, Gramineae and Malvaceae (Türkan and Demiral, 2009). It is accumulated in plants in response to salt stress, chilling stress and water stress (Chen *et al.*, 2000; Xing and Rajashekar, 2001; Guo *et al.*, 2009). Holmström *et al.* (2000) had suggested that the increased stress tolerance in *Nicotiana tabacum* transgenic lines created to accumulate glycine betaine was probably due to an improved protection of the photosynthetic apparatus. Various inorganic salts like KCl, $MgCl_2$, H_2O_2 and Ca^{2+} ions are also found to be inducers of glycine betaine (Jagendorf *et al.*, 2001).

It is synthesized from choline by a two-step oxidation (Fig. 21.1). There was an increase in the accumulation of glycine betaine due to choline supplementation (Velasco-García *et al.*, 1999; McNeil *et al.*, 2000; Huang *et al.*, 2000). The two-step oxidation is carried out in darkness and is promoted by light in *Spinacia oleracea* (Weigel *et al.*, 1988). In Chenopodiaceae and Amaranthaceae, choline is first oxidized to betaine aldehyde. The reaction is catalyzed by a ferredoxin-dependent choline monooxygenase. The betaine aldehyde is then oxidized to glycine betaine. This step is mediated by an NAD-dependent betaine aldehyde dehydrogenase (Burnet *et al.*, 1995; Rathinasabapathi *et al.*, 2001).

β-Alanine betaine is synthesized from β-alanine by S-adenosylmethionine-dependent N-methylation (Rathinasabapathi *et al.*, 2001; Duhaze *et al.*, 2003). Proline betaine (stachydrine) is a dimethyl proline present in non-halophytes namely, citrus and *Medicago* species and is an effective osmoprotectant than proline itself (Trinchant *et al.*, 2004).

21.3.4 Tertiary sulphonium compounds

Dimethylsulphoniopropionate (DMSP) is a tertiary sulphonium compound has a methyl substituted sulfur atom. It is present in marine organisms like algae, crabs and prawns. It is seldom present in plants, though it is found in saltmarsh grasses of the genus *Spartina*, sugarcane and in *Wollastonia biflora* (Otte *et al.*, 2004). It is synthesized from methionine in *S. alterniflora* where S-methyl methionine is converted to DMSP-aldehyde (Kocsis and Hanson, 2000)

21.3.5 Polysaccharides

Trehalose is accumulated in plants due to water stress but has not been much found in halophytes (Lunn *et al.*, 2014). It is a non-reducing disaccharide which is soluble and is inert even at high concentrations. Sucrose, glucose and fructose are also accumulated in plants in response to abiotic stress where they play a role in membrane stability and in osmotic adjustment (Lokhande and Suprasanna, 2012). In temperate grasses, and in some higher plants, soluble polymers of fructose called fructans are accumulated in stress conditions (French and Waterhouse,1993; Hendry, 1993). It is distributed in different tissues in plants and is either in pre-vacuolar or vacuolar vesicles synthesized from sucrose. Fructans are of five types: inulin, levan, graminan, neo-levan and neo-inulin (Lewis, 1993). It is synthesized from sucrose by the enzyme fructosyltransferase, which transfers fructose units from sucrose molecules leading to the formation of different chain length of fructans.

Another class of polysaccharides are the raffinose family oligosaccharides (RFOs). They are also derived from sucrose by the transfer of galactosyl moieties from galactinol. Some of the prominent RFOs are stachyose, raffinose and verbascose. They are detected from plants belonging to Lamiaceae under cold stress. Other plants like legumes, grapes, mint and cereals also accumulate RFOs under abiotic stress conditions (Bachmann *et al.*, 1994; Sheveleva *et al.*, 1997).

21.4 Diversity of osmolytes in plants

The type of osmolyte is diverse and depends on the type of abiotic stress factor and the genus of the plant (Slama*et al.*, 2015; Sanchez *et al.*, 2008; Lugan *et al.*, 2010; Szabados *et al.*, 2011). Moreover, some plants produce more of the osmolyte while others in less amounts (Ashraf and Foolad, 2007). It is present in more quantities in plants subjected to an abiotic stress factor continuously, like plants in marshy areas where the plants have adapted to the type of habitat.

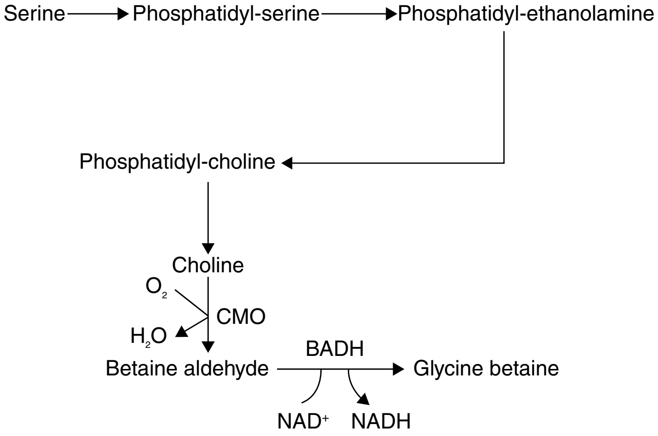

Fig. 21.1. Biosynthesis of Glycine betaine.

Halophytes are known to accumulate large amounts of proline, sorbitol, glycine betaine, β-alanine betaine, choline-O-sulfate and sugar (Tipirdamaz *et al.*, 2006; Gagneul *et al.*, 2007; Arbona *et al.*, 2010; Lugan *et al.*, 2010). In *Limonium* sucrose, fructose and proline is accumulated during drought stress (González-Orenga *et al.*, 2019).

The plants belonging to the family Amaranthaceae are mostly salt tolerant and they predominantly accumulate glycine betaine and very low levels of proline and sucrose (Tipirdamaz *et al.*, 2006; Yang *et al.*, 2008; Hameed *et al.*, 2012). In plants belonging to family Plumbaginaceae, the predominant osmolytes are choline-O-sulfate and β-alanine betaine (Gagneul *et al.*, 2007). Members belonging to the family Plantaginaceae and Aizoaceae accumulate polyols to a greater extent (Messedi *et al.*, 2004; Koyro, 2006; Pommerrenig *et al.*, 2007; Ghnaya *et al.*, 2007; Slama *et al.*, 2008). The grasses belonging to Poaceae accumulate glycine betaine in times of abiotic stress and those coming under Brassicaceae seemed to accumulate proline (Hutterer and Albert, 1993; Fan *et al.*, 1993; Marcum, 1999; Inan *et al.*, 2004; Ghars *et al.*, 2008; Koch and German, 2013; Saxena *et al.*, 2013).

21.5 Role of osmolytes in abiotic stress

The osmolytes, or compatible solutes as they are also called, are accumulated in plants during the time of abiotic stress. However, the accumulation does not seem to have any detrimental effect on the plant as it does not react with any macromolecule present in the plants (Yancey, 2005). The compatibility hypothesis proposed by Yancey (2005) states that osmolytes are neutral or zwitterionic at physiological pH. They are paired with K^+ to achieve neutrality (Martin *et al.*, 1999). It also suggests that osmolytes are interchangeable or a cell can produce a mixture of osmolytes whether it will use it or not. There may be compatibility among different classes of osmolytes in an organism. The type of osmolyte produced by an organism varies depending on the nutrition. Thus, green plants produce more carbon and sulfonium based osmolytes due to reduced nitrogen in the medium (Yancey, 2005). The mechanism behind the stabilization of proteins and membranes in the presence of osmolytes is still not known. However, it has been concluded that the most common water-solute-macromolecule interactions are involved (Yancey, 2005).

Certain protein destabilizers like salt and urea tend to unfold the proteins for efficient interaction as a greater number of groups are exposed. However, the osmolytes do not bind to the proteins but are excluded from the hydration layer or water shell of the protein (Timasheff, 1992). Moreover, Bolen and Baskakov (2001) introduced a term called ‘osmophobic effect’, which envisages that the expulsion of osmolytes arises due to the repulsion between the peptide back bone and the osmolyte. Hence this leads to the compact folding of proteins. Osmolytes are synthesized immediately after abiotic stress through various stress signaling pathways like calcium signaling, MAP kinase signaling, ABA signaling and ROS signaling pathways (Abhimanyu, 2019). The genes for the biosynthesis of osmolytes can be transferred to susceptible plants through selective breeding methods or by recombinant DNA technology to develop transgenic plants for abiotic stress tolerance (Serraj and Sinclair 2002). The metabolic engineering of the pathway for the biosynthesis of osmolytes also leads to the development of plants shows increased accumulation of osmolytes to protect them from abiotic stress (Chen and Murata 2002; Rontein *et al.*, 2002).

21.5.1 Role as an osmoregulatory

The osmolytes help in reducing the osmotic potential of water in cells during abiotic stress aiding in osmotic adjustment. This ability has resulted in an increase in the solute concentration in a cell resulting in the increase in osmolytes (Yancey, 2005). This is a positive approach as the external water potential is reduced under high salinity (Niu *et al.*, 1995). In halophytes, as there is high ion accumulation, the osmolytes and the ions are partitioned in such a way that the ions are accumulated in the vacuole whereas the osmolytes are accumulated in the cytoplasm (Glenn *et al.*, 1999). Betaines are found to be accumulated in the cytoplasm of many salt tolerant plants (Gagneul *et al.*, 2007). Moreover, there is an osmotic balance between the cytosol and the vacuole and the cytosol and the apoplast owing to the distribution of osmolytes in cells (Glenn *et al.*, 1999).

21.5.2 Role as a molecular chaperone

Osmolytes help in the stabilization of proteins and membranes under abiotic stress conditions that may lead to protein denaturation by increasing the thermodynamic stability of proteins by hydrogen bonding. This role helps in protecting the protein integrity, enhancing its activity, preventing protein aggregation and stabilizing the enzymes during extreme environmental conditions (Rajendrakumar *et al.*, 1994). Proline is found to form hydrophilic colloids in aqueous solutions and interacts with the protein with a hydrophobic backbone. There are reports of proline protecting the enzymes like lactate dehydrogenase, nitrate reductase, ribonucleases and proteases in this manner (Rajendrakumar *et al.*, 1994). Glycine betaine stabilizes the chloroplast and the photosystem II complex proteins to alter its thermodynamic properties (Subbarao *et al.*, 2001). Trehalose also helps in stabilizing the proteins and membranes during drought stress (Crowe, 2007).

21.5.3 Role as an antioxidant

In plants subjected to drought and cold stress leading to water deficit, the polyols like mannitol help in water retention as well as in scavenging the ROS (Shen *et al.*, 1999; Ozgur *et al.*, 2013). Glycine betaine has been implicated with the lipid peroxidation in stressed plants (Cushman, 2001). Mannitol has been reported to protect the enzyme phosphoribulokinase, ferredoxin, thioredoxinand glutathione from ROS (Shen *et al.*, 1997). Proline preferentially scavenges singlet oxygen and hydroxyl radicals (Alia *et al.*, 1997). Proline increases the enzyme activity of catalase, peroxidase and polyphenoloxidase and also stabilizes the thylakoid membranes (Paleg *et al.*, 1984). Osmolytes help the cells during hypoxia especially in salt marshes (Hanson *et al.*, 1994).

21.5.4 Role as a sulfate detoxifier

Plants growing in mangroves accumulate choline-O-sulfate as an osmolyte. This osmolyte helps to detoxify the sulfate which is a major anion in seawater which has inhibitory effects on the growth of the plants at high concentrations (Hanson *et al.*, 1994). In marsh grass Spartina the osmolyte DMSP is found to aid in sulfate detoxification (Slama *et al.*, 2015).

21.6 Conclusions

Osmolytes play a pivotal role in stress response in plants. Their accumulation is considered to be an immediate response by the plants subjected to abiotic stress. The type of osmolyte depends on the type of abiotic stress and the genus of the plant. The amount of osmolyte produced varies depending on the type of plant, tolerant plants accumulating more than the susceptible ones. Their accumulation does not affect any physiological process in the plant as they only maintain the osmotic balance of the cells. They act as molecular chaperones in protecting proteins and membranes apart from being an osmoprotectant. They also act as antioxidants in scavenging the free radicals which accumulate during stress. The accumulation of osmolyte can be enhanced in plants susceptible to abiotic stress by selective plant breeding methods and through genetic engineering.

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22 Enhancement of Plant Productivity and Stress Tolerance by the Application of an Exogenous Supply of Vitamins

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Abstract

Plants growing in nature often fail to exhibit their full genetic potential for productivity and reproduction due to frequent exposure to diverse stressful phenomenon from seed emergence to throughout the life cycle. On the basis of triggering factors, the stresses are classified into biotic and abiotic stresses. Biotic stress occurs by various organisms living in nature, for instance, pests, pathogens, parasitic weeds, etc. Abiotic stress is associated with climate, earth physiographic and geographic elements, for instance drought, waterlogging, salinity, heat, cold, ultraviolet radiation, limiting nutrients and environmental toxicities, etc. Both biotic and abiotic stresses limit growth, development, productivity and survival of plants. Increasing the biotic and abiotic stresses tolerance in a crop through genetic engineering requires time-consuming breeding programs and distinct cultivation circumstances for verification of crop performance. Vitamins are organic molecules essential for both plant and animal metabolism with strong antioxidant potential, and have a role as enzymatic cofactors that can accelerate plant stress tolerance, minimize crop loss and improve crop quality. In this chapter, we critically review and summarize the protective role of some vitamins, including water-soluble vitamins B and C, and fat-soluble vitamins A, E, D and K, in plants subjected to abiotic stress as well as their contribution in crop production.

22.1 Introduction

Plants are sessile organisms and continuously face various biotic and abiotic stresses attributable to climate change (Jamil *et al.*, 2010; Osakabe *et al.*, 2011). The most deleterious abiotic stresses limiting agricultural productivity through inhibiting plant growth globally are drought, salinity, waterlogging, extreme temperatures and infertile soil. Among these, drought, salinity and nutritional deficiencies are the most important problems in developing countries where agriculture is the main income source of the rural people. According to the report by the Food and Agricultural Organization on the United Nations (FAO, 2009), only 3.5% of the worldwide terrestrial area is safe from several environmental constrictions (Van Velthuizen, 2007). Since 1982, yield losses due to unfavorable conditions have been as high as 70%, according to Boyer (1982).

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Although the effects of abiotic stresses on crop output are difficult to predict with certainty, it is thought that abiotic stresses have a significant negative impact on crop productivity depending on the level of damage to the entire area under cultivation. Since then, Cramer *et al.* (2011) have estimated that 64%, 13%, 57%, 6%, 15% and 9% of global land area are badly affected by drought, flooding, cold, salinity, acidic soil and low fertility, respectively, which reduces crop production, considering the 2000 and 2007 FAO reports. Salinity affects more than 20% of total arable land globally (approximately 45 million hectares), and the area is growing every day, potentially resulting in a 50% loss of cultivable land by the middle of the twenty-first century (Hasanuzzaman *et al.*, 2013a, 2013b). According to Farooq *et al.* (2009), yield decline might range from 13% to 94% of different crops, depending on the severity and length of drought stress. Drought, salt and other abiotic stressors all cause substantial crop damage, affecting around half of the world's total crop (Acquaah, 2007). Furthermore, by 2050, the total population of the world will be 2.3 billion people, and a major challenge for world agriculture would be to produce 70% more food crops to feed this expanding population (FAO, 2009).

In the field, crops are frequently subjected to multiple abiotic stressors at the same time. Several abiotic stimuli can cause oxidative stress, which happens due to disruption of balance between the formation and quenching of reactive oxygen species (ROS) (Kusvuran *et al.*, 2016). Growth and development of plants mainly depends on the lower production of ROS from various biological reactions during physiological metabolisms such as photosynthesis and respiration. Under abiotic stress, ROS levels rise dramatically, causing toxicity to proteins, nucleic acids and lipids, which ultimately leads to cellular damage and death. On the other hand, during stress conditions, a high volume of ROS build-up in plant cells plays an important part in the nexus-signaling network of plant stress responses. Plant cells collect a variety of antioxidant molecules, both enzymatic and non-enzymatic, to quench ROS produced by stress and retain it at a safe level (Mittler *et al.*, 2011; Suzuki *et al.*, 2012; You and Chan, 2015). For osmotic equilibrium and to reduce cell damage induced by abiotic stressors, plants enhance the manufacture and storage of osmoprotectants such as sugars and proline. In response to unfavorable growing conditions, plants use both short- and long-term adaptive mechanism by activating and regulating the expression of certain stress-related genes (Xu *et al.*, 2015). Recent research aims to transfer one or more genes involved in cell signaling and regulatory pathways, as well as genes coding molecules such as antioxidants and osmolytes to give tolerance to a certain abiotic stress. (Wang *et al.*, 2003). Numerous functioning and administrative genes conferring abiotic stress tolerance have been recognized and analyzed, and they can be used to improve cultivated crops by adding tolerance traits. It does, however, necessitate several years of effort and a variety of growing settings.

Another strategy is *in vitro* selection, which is founded on inducing genetic variety among cells, tissues or organs by exposing them to stress, and then regenerating the entire organism from the surviving cells (Rai *et al.*, 2011). Such culture-based method to develop salt and drought tolerant genotypes in a variety of different plant species, including vegetables was used by Pérez-Clemente and Gomez-Cadenas (2012). However, *in vitro* selection is a less expensive and time-consuming method than traditional molecular engineering, but the stability of the selected features is unknown.

To improve plant resistance to abiotic stresses, agronomic management techniques such as selecting the right cultivar, the ideal growing season, sowing density, and particular irrigation or fertilizer doses are used (Mariani and Ferrante, 2017). In addition to these measures, biostimulants or particular bioactive substances are used on crops that are grown in less-than-ideal conditions to help them avoid damage (Colla and Rouphael, 2015; Van Oosten *et al.*, 2017; Yamauchi, 2018; Bulgari *et al.*, 2019). External use of electrically neutral non-toxic molecules like also minimize antagonistic consequence of stress on plants (Rohman *et al.*, 2020).

New roles of vitamins in plants related to induced resistance and immunological modulation have recently been discovered, with an emphasis on distinct hormonal signaling governing vitamin-triggered resistance responses (Boubakri *et al.*, 2016). The significance of exogenous vitamins in alleviating the damaging effects of abiotic stressors and increasing agricultural output is summarized in this chapter.

22.2 Vitamins

Vitamins are organic molecules or a group of related molecules crucial for both plant and animal metabolism and differ from other food nutrients having their specific organic nature, and their classification depends on their chemical nature and function (Ibrahim and El-Sayed, 2015). Sometimes, vitamins act as antioxidants to sequestrate ROS in plant cells or enzymatic cofactors that enhance both biotic and abiotic stress tolerance and improve crop production (Boubakri *et al.*, 2016). Vitamins are classified into two groups:

22.2.1 Water-soluble vitamins

- Vitamin B complex: Vitamin B_1 (thiamin), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxal/pyridoxine/pyridoxal 5′-phosphate/pyridoxamine/pyridoxine-5′-phosphate/pyridoxamine 5′-phosphate), vitamin B_7 (biotin), vitamin B_9 (folate: dihydrofolates, methyl folate, poly glutamyl folates and mono glutamyl folates), vitamin B_{12} (cobalamin).
- Vitamin C (ascorbic acid).

22.2.2 Fat-soluble vitamins

- Vitamin A: Vitamin A derived from animal sources is known as retinoid and from the plant source is called provitamin A carotenoids (Cho, 2016).
- Vitamin D (calcitriol).
- Vitamin E: Vitamin E contains four types of tocopherols and tocotrienols, consisting of eight naturally occurring forms of vitamin E, which comprises alpha, beta, gamma, and delta classes of tocopherol and tocotrienol (Rizvi *et al.*, 2014).
- Vitamin K: K_1 (phylloquinone), K_2 (menaquinone) and K_3 (menadione).

22.3 Significance of vitamins in plant biosynthesis

Vitamins benefit plants in the same way that they benefit animals. Carotenoids or vitamin A derived from plants are typically C_{40} terpenoids, a group of hydrocarbons that play a role in various biological activities in plants, including photosynthesis, photomorphogenesis, photoprotection and development, as well as serving as precursors for two plant hormones and a variety of apocarotenoids (Nisar *et al.*, 2015). B vitamins are precursors of a number of metabolic cofactors that all serve as plant hormones (Hanson *et al.*, 2016). Vitamin C (ascorbic acid) is a very important and powerful non-enzymatic antioxidant required for plant growth. In the tissue culture technique, for example, it is desirable or even essential to add some of the vitamins A, C, D, B_7, B_1, B_2, B_3, B_5, B_6, B_8, B_9, and B_{12} to the plant growing mix. Seed germination can also be enhanced by including a tiny amount of B vitamins (niacin, thiamin and folic acid) as well as vitamin C. Many plant tonics that combine vitamin B_1 with other helpful vitamins, hormones and botanical components help plants grow faster, produce stronger roots, and fight disease. The amount of vitamins in plants is regulated by their growing conditions as well as the type of plant. Apples and roses cultivated in cold climates have four to ten times more vitamin C than kinds grown in hot climates. During photosynthesis, vitamin C protects plants from UV damage (Paciolla *et al.*, 2019). Vitamin B_6 has been reported to be an antioxidant with effectiveness equivalent to vitamins C and E, and it plays an important role in many stress responses (Vanderschuren *et al.*, 2013).

22.4 Role of water-soluble vitamins in growth and development of different crops and abiotic stress tolerance

Thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate and cobalamin are eight water-soluble cofactors of enzymes and their derivatives. Plants need the active form of thiamine (vitamin B_1), known as thiamine pyrophosphate (TPP), which is involved various metabolic processes including acetyl-CoA biosynthesis, amino acid biosynthesis, Krebs cycle and Calvin cycle (Du *et al.*, 2011). Thiamine acts a potent antioxidant in plants. It is found that thiamine application protected paraquat (herbicide)-driven oxidative stress in *Arabidopsis* plants and also induced an accumulation of thiamine and its derivatives (TPP and thiamine monophosphate, TMP) compared with controls under exposure of various abiotic constraints, including paraquat, extreme temperatures, high light, drought and salt stress (Tunc-Ozdemir *et al.*, 2009). Thiamin application induces salinity tolerance in coriander and fenugreek, duranta, maize and sunflower (Hamada and Al-Hakimi, 2009; Kaya *et al.*, 2015; EL Sayed *et al.*, 2017; Aminifard *et al.*, 2018), as well as drought tolerance in white clover and turnip (Ghaffar *et al.*, 2019; Jabeen *et al.*, 2021) by modifying antioxidant enzymatic activity and osmotic balance and enhance plant growth and development.

Riboflavin (B_2), a precursor of the cofactors flavin mononucleotide (FMN) and flavin adenine (FAD), is used by a variety of oxidoreductases in the cytosol, plastids, mitochondria, and peroxisomes (Roje, 2007). It is also required for optimal plant growth and development. Riboflavin supplementation has been reported to improve disease resistance in plants in previous research (Dong and Beer, 2000; Zhang *et al.*, 2009; Boubakri *et al.* 2013). Salinity and drought inducing oxidative stresses are minimized by foliar application of riboflavin (B_2) in seedling stage of plants modulating the antioxidant enzyme activity (Azooz, 2009; Deng *et al.*, 2014).

The active forms of niacin (vitamin B_3) are nicotinamide adenine dinucleotide and its 2′-phosphate ester (NADP), acts as a coenzyme and plays a role in the metabolic hydrogen transfer to $NADP^+$ via ATP that participates in cellular growth and antioxidant defense in plants (Gerdes *et al.*, 2012). Exogenous application of various forms of vitamin B_3 influence photosynthetic pigments, amino acid and glucose content and lessen salinity effects in plants (Magdi *et al.*, 2013; Hussein *et al.*, 2014).

Pantothenate (Vitamin B_5) is a precursor of coenzyme A (CoA) and an acyl-carrier protein that is essential for all living cells' metabolic and energy-yielding pathways (Webb and Smith, 2011; Kleinkauf, 2000). Pantothenate 4′-O-b-D-glucoside was discovered in tomato fruit (Amachi *et al.*, 1971) and is reported to present in many different species and tissues (Yoshizumi and Amachi, 1969).

Pyridoxal 5′-phosphate (PLP), the active form of vitamin B_6, plays an important cellular role as a coenzyme for various of metabolic enzymes and possible antioxidant molecule. In the *Arabidopsis* plant model, functional and regulatory genes related with vitamin B_6 production are described, revealing the vitamin's importance in plant development, photosynthesis, and stress response (Mooney and Hellmann, 2010). Plants can also employ exogenously provided non-phosphorylated B_6 vitamers and can reduce singlet oxygen accumulation produced by excessive light exposure in wild-type and pdx1.3 mutant *Arabidopsis* plants (Havaux *et al.*, 2009; Huang *et al.*, 2011). Exogenic application of vitamin B_6 or its vitamers promote salinity and drought tolerance in plants attenuating antioxidant enzymatic activity (Emam, 2012; Orabi *et al.*, 2016; Liu *et al.*, 2019).

Biotin (vitamin B_7) is an important cofactor for some enzymes involving in the transfer of CO_2 during carboxylation, decarboxylation and transcarboxylation reactions (Dakshinamurti and Cauhan, 1989; Knowles, 1989) and helps in the synthesis of vitamins B_9 and B_{12}.

Folates are a collection of vitamin B_9 water-soluble vitamins involved as cofactors in ‘carbon one’ transfer reactions occur in two vital cycles in mammalian and plant cells namely, the DNA biosynthesis cycle (*de novo* biosynthesis of the purines and pyrimidines) and the methylation cycle (Scott and Weir, 1994). Recently, Wittek *et al.* (2015) proposed that folic acid-induced systemic resistance in *Arabidopsis* depends on the salicylic acid (SA)-signaling pathway. Kilic and Aca (2016) and Emam and Helal (2008) also reported enhanced seed germination and physiological growth in barley and flax under salinity, whereas there was an increased in yield parameters in potato under drought (Youssif, 2017).

Vitamin B_{12} (cobalamin) is unavailable in vascular plants, but is abundant in algae that acquire it from bacteria (Smith *et al.*, 2007). Active biological forms of cobalamin are adenosylcobalamin and methylcobalamin, which take part in radical and methyl transfer reaction. Exogenous application of cobalamin influences the activities of antioxidant enzymes and content of chlorophyll in common bean and enhance tolerance toward salinity (Keshavarz and Moghadam, 2017).

Vitamin C is not only a very important non-enzymatic antioxidant but also a cofactor for a number of enzymes in cellular metabolism of plant and human (Smirnoff, 2000). Therefore, it takes part in regulation of several key physiological and biochemical processes like the germination of seeds, photosynthesis, hormone biosynthesis, floral initiation, fruit expansion, ROS regulation and leaf senescence. In these regards, a good number of reports have studied the role of external application of water-soluble vitamins in the improvement of plant growth, productivity and different metabolic functions in plants both in normal and stress environments. The roles of vitamins in salt and drought tolerance have been reported extensively; however, very few studies are available for extreme temperature, waterlogging and heavy metal toxicity. The findings of the recent studies on role of water-soluble vitamins in plant metabolic fictions in normal and abiotic stress environment are summarized in Table 22.1 and Table 22.2, respectively.

22.5 Role of fat-soluble vitamins in the growth and development of different crops and abiotic stress tolerance

Plant vitamin A or carotenoids are structurally diverse pigments, which harvest light and play multiple roles as antioxidants and attractants for pollinators and seed dispersers. Photosynthesis produces significant number of extremely reactive intermediates as by-products that induce oxidative injury to the photosynthetic equipment, lowering photosynthesis efficiency (Niyogi, 1999). Under stress, singlet oxygen (ROS) is formed in plant chloroplasts, which can oxidize carotenoids and yield a wide range of oxidized products such as aldehydes, ketones, endoperoxides, and lactones (Ramel *et al.*, 2012a, 2012b). To minimize this damaging effect of light during photosynthesis, plants utilize carotenoids as antioxidant to mitigate those reactive molecules (ROS) resulting cope with the damaging effects of light (Niyogi, 1999). Some carotenoid derivatives, such as b-ionone and b-cyclocitral, are reactive electrophile species that are biologically active, and can regulate gene expression, resulting in stress adaptation (Havaux, 2014). The importance of carotenoids as antioxidants have been demonstrated in algae and plants (Dong *et al.*, 2007; Qin *et al.*, 2007). Carotenoids provide a photoprotective role in mutants of *Arabidopsis* with altered xanthophyll composition (e.g. szl1, chy1chy2, lut5, etc.) (Kim *et al.*, 2009; Cazzaniga *et al.*, 2012). Besides the essential function as photoprotectants and antioxidants of carotenoids, a new function has been identified associated with plant response to environmental stresses. Incorporation of exogenous carotenoids in wheat plants minimize the detrimental effect of salinity (Hemida *et al.*, 2015).

As vitamin D precursors, sterols play a critical function. Vitamin D is divided into five classes, numbered 2–6, with vitamin D_2 being created in fungi and yeast after ergosterol is exposed to UVB (provitamin D_2). In the *Arabidopsis* plant model, enzymes involved in 24-demethylsterol and 24-ethylsterol production, which is the precursor of vitamin D_3, have been found (Jäpelt and Jakobsen, 2013). Vitamin D_3 compounds increase root growth and differentiation by regulating the Ca^{2+} messenger system, which is regulated by vitamin D_3 and $1\alpha 25(OH)_2D_3$ binding proteins in plants (Boland *et al.*, 2003).

In plastid membranes, the interaction of isopentyl diphosphate with homogentisic acid produces lipid soluble vitamin E, also known as alpha-tocopherol (αToc). This alpha-tocopherol has biological in a number of plant metabolic processes, including quenching oxidative cations to neutralize and block lipid peroxy radicals, maintaining the integrity and fluidity of photosynthetic membranes, cellular signaling, and promoting growth and development of plants under stress and non-stress conditions (Sadiq *et al.*, 2019). Exogenous administration of αToc has recently been broadly reported as an important way to promote tolerance in plants under environmental conditions such water shortage and excessive salinity, noxious metals, high or low temperatures, and radiations.

Phylloquinone (vitamin K_1) is ubiquitous in the plant kingdom and is structurally distinguished having the same phytyl (hexahydrotetraprenyl) side chain as found in the plant pigment chlorophyll (Langemann and Isler, 1965) and directly plays role in photosynthesis. Menaquinone (vitamin K_2) is an isoprenoid quinone found in a limited number of obligate and facultative anaerobic bacterial respiratory (Collins and Jones, 1981; Fernandez and Collins, 1987; Nowicka and Kruk, 2010). Menadione sodium bisulphite (MSB) is a water-soluble precursor of menadione (vitamin K_3) extracted from fungus and

Table 22.1. Improvement in plant growth, productivity and different metabolic functions by external application of water-soluble vitamins.

Vitamins	Plant	Activities	References
Vitamin B_1 and vitamin B_3	Rice (*Oryza sativa* L.)	Increased physical growth, relative chlorophyll and fertility	Vendruscolo *et al.* (2019)
Vitamin B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}	Quinoa (*Chenopodium quinoa* Wild.)	Enhanced APX, NADPH converting activity Reduced CAT activity Increased Pro content	Pitzschke *et al.* (2015)
Vitamin B_3	Kiwifruit (*Actinidia deliciosa* L.)	Increase in chlorophyll content, O_2^- production, and H_2O_2 content, and promoted NADPH content	Zhu *et al.* (2018)
Vitamin C, Vitamin B_3	Bitter lupine (*Lupinus termis* L.)	Increased in protein, oil, omega fatty acid group content Enhanced microbiological activity	Mahmud *et al.* (2014)
Vitamin C	Sugarcane (*Saccharum officinarum* L.)	Enhanced fresh weight, number of shoots/roots, shoot/root length, soluble protein contents Enhanced enzymatic antioxidants activities	Munir *et al.* (2013)
Vitamin C	Olive (*Olea europea* L.)	Increased plant height, leaf number, leaf area and lateral shoot number	Mayi *et al.* (2014)
Vitamin C	Pearl millet (*Pennisetum glaucum* L.)	Increased leaf area and number of leaves	Hussein and Alva (2014)
Vitamin C	Chickpea (*Cicer arietinum* L.)	Increased plant height, seed yield and harvest index	Zarghamnejad *et al.* (2014)
Vitamin C	Wheat (*Triticum aestivum* L.)	Increased growth, grain yield and yield components	Mohamed (2013)
Vitamin C	Tea (*Camellia* spp.)	Increased chlorophyll a and b Enhanced PPO and PAL activity Improved brewed tea liquor characteristics	Murugan *et al.* (2012)
Vitamin C	Shoe flower (*Hibiscus rosasinesis* L.)	Increased fresh and dry weights, number of flowers/plant, carotenoids, chlorophyll a and b, soluble sugars, N, P and K contents	Fatma *et al.* (2009)
Vitamin C	Wheat (*T. aestivum* L.)	Enhanced CAT, K, Ca^{2+}, photosynthetic pigments, AsA contents and plant growth	Athar *et al.* (2008)

APX, Ascorbate peroxidase; CAT, Catalase; PAL, Phenylalanine ammonia lyase; PPO, Polyphenoloxidase.

phanerogams (Binder *et al.*, 1989). MSB, a plant growth promoter, has been utilized in a variety of commercial nutritional formulations. It acts as a plant defense elicitor in a variety of plant species against a variety of diseases (Liu *et al.*, 2006; Borges *et al.*, 2009; Ashraf *et al.*, 2019; Askari *et al.*, 2021). The gene expression pattern is changed by exogenous MSB, including upregulation of biotic and abiotic stress response genes (Borges *et al.*, 2009). Therefore, like water-soluble vitamins, fat-soluble vitamins play important biological role in plant cells both in non-stress and stress conditions. Their functions in both conditions are summarized in Table 22.3 and Table 22.4.

Table 22.2. Improvement in plant growth, productivity and different metabolic functions by external application of water-soluble vitamins under environmental stresses.

Vitamins	Abiotic stress	Plant	Activities	References
Vitamin B_1	Salinity	Coriander (*Coriandrum* L.), Fenugreek (*Trigonella foenum-graecum* L.)	Increased in TGW, contents of N, P, carotenoid, chlorophyll b, phenolic compounds Enhanced total antioxidant activity and vegetative growth	Aminifard *et al.* (2018)
Vitamin B_1	Salinity	Duranta (*Duranta plumieri* L.)	Increased in growth, physiological processes and Pro content	EL Sayed *et al.* (2017)
Vitamin B_1	Salinity	Maize (*Zea mays* L.)	Increased in N, P, Ca^{2+}, K^+, photosynthetic pigment concentration Decreased in Na^+ concentration, MDA content, H_2O_2 levels Reduced CAT, SOD and POD enzyme activity	Kaya *et al.* (2015)
Vitamin B_1	Drought	White clover (*Trifolium repens* L.)	Increased in plant growth, chlorophyll pigments, and total phenolic compounds No considerable change was observed in accumulation of AsA, total soluble proteins, and activities of antioxidants (SOD, CAT, and POD)	Ghaffar *et al.* (2019)
Vitamin B_1	Drought	Turnip (*Brassica rapa* L.)	Increased in photosynthetic pigments, Pro and GB contents Enhanced SOD, POD and CAT activities Decreased in H_2O_2 and MDA content	Jabeen *et al.* (2021)
Vitamin B_2	Salinity	Roselle (*Hibiscus sabdariffa* L.)	Enhanced MSI and CAT, POD, APX and GR antioxidant enzyme activity Reduced MDA content	Azooz (2009)
Vitamin B_2	Drought	Tobacco (*Nicotiana benthamiana* L.)	Enhanced SOD, CAT, APX and GR enzymatic activities	Deng *et al.* (2014)
Vitamin B_3	Salinity	Onion (*Allium cepa* L.)	Increased in height and fresh weight, bulb dry weight and plant biomass	Hussein *et al.* (2014)
Vitamin B_3	Salinity	Faba bean (*Vicia faba* L.)	Increased in photosynthetic pigments, polysaccharides, total carbohydrates, total N concentration, sucrose, total soluble sugars, total free amino acids and Pro Decreased in MDA Reduced oxidative enzymes (PPO and POD enzymes).	Magdi *et al.* (2013)
Vitamin B_3	Drought	Sunflower (*Helianthus annuus* L.)	Enhanced growth parameters, yield components, stomatal opening, levels of IAA, GA, photosynthetic pigments, total carbohydrates, total nitrogen, minerals (K, Ca, Mg, P) Decreased in ABA content	Abdallah *et al.* (2013)

Vitamin B_6	Salinity	Canola (*Brassica napus* L.)	Increased in the AsA content in the seeds, reduced GSH and total phenols in seeds Enhanced PAL, PPO, APX enzyme activity and DPPH free radical scavenging activity (%) in seeds	Orabi *et al.* (2016)
Vitamin B_6	Salinity	Wheat (*T. aestivum* L.)	Increased in RWC, biomass, photosynthetic pigments, Pro content Decreased in MDA and H_2O_2 content Enhanced SOD, CAT and POD activities	Liu *et al.* (2019)
Vitamin B_6 and Vitamin B_3	Drought	Rice (*O. sativa* L.)	Reduced ion leakage, membrane peroxidation and high ratio of chlorophylls/carotenoids Enhanced AsA accumulation, GSH and total phenol contents as well as GR, SOD, and POX activities	Emam (2012)
Vitamin B_9	Salinity	Barley (*Hordeum vulgare* L.)	Induced germination, seedling growth and leaf parameters	Kilic and Aca (2016)
Vitamin B_9 Vitamin C and Vitamin B_{12}	Salinity	Flax (*Linum usitatissimum* L.)	Enhanced seed germination, accumulation of polyphenols, free amino acids and Pro content Increased in AsA and GSH content	Emam and Helal (2008)
Vitamin B_{12}	Salinity	Common bean (*Phaseolus vulgaris* L.)	Enhanced antioxident enzyme activity Increased in lipid peroxidation, carotenoid and Pro accumulation	Keshavarz and Moghadam (2017)
Vitamin B_{12}, Vitamin B_9 and Vitamin C	Drought	Potato (*Solanum tuberosum* L.)	Increased in potato plant growth, tuber yield and its components as well as chemical composition and total chlorophyll	Youssif (2017)
Vitamin C	Salinity	Rice (*O. sativa* L.)	Increased in chlorophyll content, fresh weight, dry weight and the lengths of shoot and root and AsA content	Arshad *et al*. (2015)
Vitamin C and Vitamin B_1	Salinity	Maize (*Z. mays* L.) And Sunflower (*H. annuus* L.)	Increased in net photosynthetic rate, pigments biosynthesis and membrane integrity Decreased in K^+ leakage	Hamada and Al-Hakimi (2009)
Ascobin (Compound composed of vitamin C acid and citric acid with ratio of 2:1)	Salinity	Cowpea (*Vigna sinensis* L.)	Increased in photosynthetic pigments accumulation, yield and yield components, total soluble sugars, Pro, GB as well as N, P and K	Abdelgawad (2014)
Vitamin C	Salinity	Tomato (*Solanum lycopersicum* L.)	Enhanced CAT enzyme activityIncreased in the contents of macro and micronutrient mineral elements (N, P, K, Ca, Mg, Mn, Fe, Cu, Zn, B, Na and Cl)	Ahmed *et al.* (2016)

Continued

Table 22.2. Continued.

Vitamins	Abiotic stress	Plant	Activities	References
Vitamin C	Salinity	Wheat (*T. aestivum* L.)	Increased endogenous AsA, root and shoot growth as well as chlorophyll content Enhanced CAT, POD, and SOD enzymatic activities	Athar *et al*. (2009)
Vitamin C	Salinity	Milk thistle (*Silybum marianum* L.)	Enhanced seed germination and increased in plant growth, water status, carotenoids, endogenous AsA content Enhanced CAT and GR antioxidant enzyme activities	Ekmekçi and Karaman (2012)
Vitamin C	Salinity	Chickpea (*Cicer. arietinum* L.)	Increased in roots, shoots and leaves fresh and dry weight and chlorophyll content	Beltagi (2008)
Vitamin C	Salinity	Sugarcane (*Saccharum* spp.)	Increased in vegetative growth and Pro contents Enhanced antioxidant enzyme activities (POD and SOD)	Ejaz *et al*. (2012)
Vitamin C	Salinity	Tomato (*Lycopersicon esculentum* Mill. var. *Cerasiforme*)	Increased in growth, total available carbohydrates, protein, amino acids, Pro and inorganic minerals Enhanced CAT, POD and SOD enzymatic activities	El-Sayed and El-Sayed (2013)
Vitamin C	Salinity	Wheat (*T. aestivum* L.)	Increased in chlorophyll ‘a’ and Na^+ content in leaves	Athar and Ashraf (2006)
Vitamin C	Salinity	Flax (*L. usitatissimum* L.)	Increased total soluble carbohydrates, free amino acids, Pro contents and SOD activity Reduced lipid peroxidation, PPO, POD activities	El-Bassiouny and Sadak (2015)
Vitamin C	Salinity	Wheat (*T. aestivum* L.)	Increased in plant growth, endogenous AsA level, photosynthetic capacity, K^+ and Ca^{2+} accumulation in the leaves Enhanced CAT activity	Khan and Ashraf (2008)
Vitamin C andVitamin B_1	Salinity	Maize (*Z. mays* L.)	Increased in shoot and root dry weights, macro-element, AsA contents and SOD activity POD activity increased considerably with beta-carotene application AsA was more effective than the others	Tuna *et al.* (2013)
Vitamin C	Salinity	Tomato (*S. lycopersicum* L.)	Enhanced seedling germination Decreased in lipid peroxidation products in roots, stems and leaves and active oxygen species	Shalata and Neumann (2001)
Vitamin C (Ascorbic acid)	Salinity	Lentil (*Lens culinaris* Medik.)	Increased yield and yield components	Alami-Milani and Aghaei-Gharachorlou (2015)
Vitamin C and Vitamin B_3	Salinity	Broad bean (*V. faba* L.)	Increased in the photosynthetic pigments, soluble carbohydrates and proteins, Pro, free amino acids content and K^+/Na^+ ratio Decreased in transpiration, ion leakage, Na^+ and Cl^- ion content	Azooz *et al.* (2013)

Vitamin C	Salinity	Barley (*H. vulgare* L.)	Increased Pro, RWC and chlorophyll content Enhanced enzymatic antioxidants activities Improved leaf anatomy	Agami (2014)
Vitamin C	Salinity	Safflower (*Carthamus tinctorius* L.)	Increased germination percentage, seedling fresh and dry weights, shoot and root lengths and vigor index	Razaji *et al.* (2012)
Vitamin C	Salinity	Squash (*Cucurbita maxima* D.)	Increased seedling growth, fresh and dry matter, protease activity and chlorophyll contents	Rafique *et al.* (2011)
Vitamin C	Salinity	Wheat (*Triticum durum* L.)	Increase leaf area, chlorophyll and carotenoid and Pro contents Decreased H_2O_2 content	Azzedine *et al.* (2011)
Vitamin C	Salinity	Wheat (*T. durum* L.)	Enhanced antioxidant enzyme activities, ascorbate, phenol, carotenoids, K, Ca and Mg content. Reduced adverse effects on leaf senescence	Farouk (2011)
Vitamin C	Salinity	Canola (*B. napus* L.)	Increased protein conten Decreased activities of antioxidant enzymes and MDA in leaf	Dolatabadian *et al.* (2008)
Vitamin C	Salinity	African mahogany (*Khaya senegalensis*)	Increased chlorophyll a, b and carotenoid content, total sugars and uptake of P, K and N content.	Aziz *et al.* (2006)
Vitamin C	Salinity	Common bean (*P. vulgaris* L.)	Increased chlorophyll content. Decreased ABA	Dolatabadian and Jouneghani (2009)
Vitamin C	Salinity	Sorghum (*Sorghum bicolor* L.)	Increased germination percentage, thickness of xylem and phloem tissues and leaf blades	Arafa *et al.* (2009)
Vitamin C	Drought	Sunflower (*H. annuus* L.)	Enhanced germination rate, germination percentage, plumule length and seedling fresh biomass	Ahmed *et al.* (2014)
Vitamin C	Drought	Soybean (*Glycine max* L.)	Increased total soluble protein Enhanced SOD and POD activities	Hasanah *et al.* (2017)
Vitamin C	Drought	Common bean (*P. vulgaris* L.)	Increased in photosynthetic pigments, growth and seed yield, secondary metabolites (phenolic, flavonoids, and tannins) and MDA content Enhanced carbonic anhydrase, POD activity and antioxidant activities	Gaafar *et al.* (2020)
Vitamin C	Drought	Broccoli (*Brassica oleracea* L.)	Increased in yield, RWC, MSI, and leaf photosynthetic pigments, endogenous AsA, soluble sugars, Pro, DPPH activity and WUE	Osman *et al*. (2018)
Vitamin C	Drought	Wheat (*T. aestivum* L.)	Increased in chlorophyll contents, net photosynthesis and plant growth	Malik and Ashraf (2012)
Vitamin C	Drought	Maize (*Z. mays* L.)	Increased in plant growth, biomass, and photosynthetic pigments Enhanced the activities of SOD and POD	Noman *et al*. (2015)
Vitamin C	Drought	Sunflower (*H. annuus* L.)	Enhanced germination rate, germination percentage, seed stamina index and fresh and dry weights	Fatemi (2014)

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Table 22.2. Continued.

Vitamins	Abiotic stress	Plant	Activities	References
Vitamin C	Drought	Wheat (*T. aestivum* L.)	Increased chlorophyll a and b, total soluble proteins, carbohydrates and carotenoids	Hussein *et al.* (2014)
Vitamin C	Drought	Canola (*B. napus* L.)	Increased shoot and root fresh weights, root dry weight, qN, NPQ, shoot and root P and AsA content	Shafiq *et al.* (2014)
Vitamin C	Drought	Sunflower (*H. annuus* L.)	Increased stearic acid, linoleic acid and palmitic acid percentage and oil yield	Ahmed *et al.* (2013)
Vitamin C	Drought	Maize (*Z. mays* L.)	Increased RWC, seed yield and chlorophyll content	Darvishan *et al.* (2013)
Vitamin C	Drought	Sunflower (*H. annuus* L.)	Decreased flavonoids, anthocyanins and total soluble sugars	Ebrahimian and Bybordi (2012)
Vitamin C	Drought	Savory (*Satureja hortensis* L.)	Increased growth, Pro and soluble proteins content	Yazdanpanah *et al.* (2011)
Vitamin C	Drought	Basil (*Ocimum basilicum* L.)	Increased fresh and dry weights, RWC, photosynthetic pigments, growth and oil percentage	Khalil *et al.* (2010)
Vitamin C	Drought	Maize (*Z. mays* L.)	Increased stem and leaf dry weights and leaf fresh weight as well as grain weight	Dolatabadian *et al.* (2010)
Vitamin C	Drought	Okra (*Hibiscus esculentus* L.)	Increased fresh and dry weights, sugar content, Pro, chlorophyll a and b, carotenoids and leaf area	Amin *et al.* (2009)
Vitamin C	Drought	Wheat (*T. aestivum* L.)	Increased chlorophyll a content and Na+ accumulation	Khan *et al.* (2006)
Vitamin C	sandy soil	Wheat (*T. aestivum* L.)	Increased number of tillers and spikes per plant, spike length, spikelets/spike, grain and straw yield	Bakry *et al.* (2013)
Vitamin C	Waterlog	Adzuki beans (*Vigna angularis* L.)	Decreased in MDA Reduced CAT, SOD, PPO, POD and APX activities	Ullah *et al*. (2017)
Vitamin C	low temperature	Maize (*Z. mays* L.)	Enhanced seedling growth, chlorophyll b, leaf relative water content, membrane stability and activities of enzymatic antioxidants	Ahmad *et al.* (2014)
Vitamin C	Heavy metal toxicity (Led)	Wheat (*T. aestivum* L.)	Enhanced SOD, CAT and GR. Increased in the content of essential nutrients (N, P, K, Ca and Mg) and RWC Enhanced the activity of rubisco Decreased the MDA, H_2O_2 and chlorophyll degradation.	Alamri *et al*. (2018)
Vitamin C	Pb stress	Faba bean (*V. faba* L.)	Decreased micronucleus frequency and chromosomal aberration. Improved mitotic index.	Yu *et al.* (2014)

ABA, Abscisic acid; APX, Ascorbate peroxidase; AsA, reduced ascorbic acid; CAT, Catalase; CK, Cytokinin; GA_3, Gibberellic acid; GB, Glycine betaine; GR, Glutathione reductase; GSH, Reduced glutathione; IAA, Indole-3-acetic acid; MDA, Melondialdehyde; POD, Peroxidase; PPO, Polyphenoloxidase; Pro, Proline; RWC, Relative water content; SOD, Superoxide dismutase.

Table 22.3. Improvement in plant growth, productivity and different metabolic functions by external application of fat-soluble vitamins.

Vitamins	Plant	Activities	References
Vitamin D_3 (sterols and stigmasterol)	Common bean (*P. vulgaris* L.)	Enhanced the growth of roots in culture	Talmon *et al.* (1989)
Vitamin D_3	Aspen (*Populus tremula* L.)	Enhanced adventitious rooting and synergy	Pythoud *et al.* (2006)
Vitamin E	Common bean (*P. vulgaris* L.), Pea (*Pisum sativum* L.)	Increased the number of pods per plant, TGW	Abd El-Hakim (2014)
Vitamin E	Garden snapdragon (*Antirrhinum majus* L.)	Increased plant height, number of leaves, shoot and root biomass and floral characters	Badawy *et al.* (2015)
Vitamin E	Faba bean (*V. faba* L.)	Increased plant height, biomass, number of branches, and total number of pods	Marzauk *et al.* (2014)
Vitamin E	Common bean (*P. vulgaris* L.)	Increased plant height, number of leaves, leaf dry weight, and seed quality	Shafeek *et al.* (2014)
Vitamin E	Capcicum (*Capsicum annum* L.)	Increased plant height, leaf area, plant dry weight and fruit yield	Abeer *et al.* (2015)
Vitamin E	Onion (*A. cepa* L.)	Enhanced length and diameter of bulb, protein level and total soluble sugars	Hassan *et al.* (2013)
Vitamin E	Calendula (*Calendula officinalis* L.)	Increased shoot growth and seed yield	Soltani *et al.* (2012)
Vitamin E	Sunflower (*H. annuus* L.)	Increased plant height, number of leaves, floral head diameter and yield characters	Al-Qubaie (2012)
Vitamin E	Rose geranium (*Pelargonium graveolens* L.)	Increased plant biomass, length and crude protein contentsDecrease lipid peroxidation	Ayad *et al.* (2009)
Vitamin E	Jasmine (*Jasminum grandiflorum* L.)	Increased soluble sugars, non-soluble sugars, weight of flower and yield	Eid *et al.* (2010)
Vitamin E	China rose (*Hibiscus rosa-sinenses* L.)	Increased plant height, number of leaves and branches per plant and plant biomass	El-Quesni *et al.* (2009)
Vitamin E	Linseed (*L. usitatissimum* L.)	Increased proteins, phenol and PPO	El-Lethy *et al.* (2010)
Vitamin E	Faba bean (*V. faba* L.)	Enhanced antioxidant enzymes, Pro, and carotenoidsDecreased MDA levels	Orabi and Abdelhamid (2016)

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Table 22.3. Continued.

Vitamins	Plant	Activities	References
Vitamin E	Sunflower (*H. annus* L.), Arabidopsis (*Arabidopsis thaliana* L.)	Increased in endogenous vitamin E level	Gala *et al.* (2005)
Vitamin E	Tomato (*Lycopersicon esculentum* Mill.)	Increased in photosynthetic pigments, N, P, K, Fe, Zn, Mn, total carbohydrates and crude protein content in leaves and fruits Increased in GAs and CKs level in shoots Decreased in IAA and ABA	Mady (2009)
Vitamin K soluble form (Menadione sodium bisulphite)	Tomato (*S. lycopersicum* L.), Alfalfa (*Medicago sativa* L.), Mung bean (*Vigna radiata* L.), Cucumber (*Cucumis sativus* L.), Corn (*Z. mays* L.) and Capsicum (*Capsicum annuum* L.)	Increased the tomato plants growth Increased alfalfa callus. Stimulated rooting of mung bean cuttings Decreased activities of the enzymes involved in IAA oxidation in tomato plants IAA level were increased by about threefold to fourfold following application of MSB to tomato, cucumber, corn and capsicum plants	Rao *et al.* (1985)

ABA, Abscisic acid; CK, Cytokinin; GA, Gibberellic acid; IAA, Indole-3-acetic acid; MDA, Melondialdehyde; MSB, Menadione sodium bisulphite.

Table 22.4. Improvement in plant growth, productivity and different metabolic functions by external application of fat-soluble vitamins under environmental stresses.

Vitamins	Abiotic stress	Plant	Activities	References
Vitamin A (β-carotene)	Salinity	Wheat (*T. aestivum* L.)	Enhanced the level of free amino acids, Pro, GB, choline, GSH, AsA, phenolic contents and total antioxidants activities	Hemida *et al.* (2015)
Vitamin A	Salinity	Maize (*Z. mays* L.)	Increased in shoot and root dry weights, macro-element, AsA content Enhanced SOD and POD activity	Tuna *et al.* (2013)
Vitamin E	Salinity	Onion (*A. cepa* L.)	Increased the levels of total phenolics and flavonoids	Mohammad and Aly (2008)
Vitamin E	Salinity	Wheat (*T. aestivum* L.)	Increased AsA, phenolics, ions such as Mg, K and Ca	Farouk (2011)
Vitamin E	Salinity	Orange (*Citrus aurantium* L.)	Increased membrane permeability, chlorophyll a and b content Reduced lipid peroxidation and H_2O_2 content	Kostopoulou *et al.* (2014)
Vitamin E	Salinity	Soybean (*G. max* L.)	Increased photosynthetic pigments, RWC and concentration of K^+	Mostafa *et al.* (2015)
Vitamin E	Salinity	Sunflower (*H. annuus* L.)	Increased total soluble sugars, antioxidant enzymes and levels of N, P, K, and Mg	Rady *et al.* (2011)
Vitamin E	Salinity	Broad bean (*V. faba* L.)	Increased biomass, length, number of leaves and pods, 100 seed weight	Semida *et al.* (2014)
Vitamin E	Salinity	Wheat (*T. aestivum* L.)	Increased levels of AsA, total phenols, GSH and activities of antioxidants.	Sakr and El-Metwally (2009)
Vitamin E	Salinity	*Vigna sinns*	Increased plant biomass and plant height	Hussein *et al.* (2007)
Vitamin E	Salinity	Asian short-stem sedge (*Carex leucochlora* L.)	Decreased in H_2O_2 content, the rate of O_2^- generation Increased chlorophyll *b*, carotenoids, Pro and soluble protein content	Ye *et al.* (2017)
Vitamin E	Salinity	Flax (*L. usitatissimum* L.)	Increased total soluble carbohydrates, free amino acids, Pro contents and SOD activity Reduced lipid peroxidation, PPO, POD activities	El-Bassiouny and Sadak *et al*. (2015)
Vitamin E	Salinity, heavy metal, and osmoticstress	Mustard (*Brassica juncea* L.)	Increased in RWCDecreased in electrolyte leakage, MDA content and H_2O_2 accumulation, APX and GSH content Enhanced activities and transcript levels SOD, CAT, APX, and GR	Kumar *et al.* (2013)
Vitamin E	Drought	Tobacco (*Nicotiana tabacum* L.), Arabidopsis (*A. thaliana* L.)	Increased in vitamin E content of plants Enhanced tolerance to environmental stresses	Liu *et al.* (2008)

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Table 22.4. Continued.

Vitamins	Abiotic stress	Plant	Activities	References
Vitamin E	Drought	Sunflower (*H. annuus* L.)	Enhanced growth parameters, yield components, stomatal opening, levels of IAA, GA, photosynthetic pigments, total carbohydrates, total N, minerals (K, Ca, Mg, P) Decreased in ABA content	Abdallah *et al.* (2013)
Vitamin E	Drought	Cotton (*Gossypium barbadense* L.)	Increased chlorophyll a, b, total soluble sugars and total free amino acids	Mekki *et al.* (2015)
Vitamin E	Drought	Chinese rye grass (*Leymus chinensis* L.)	Enhanced antioxidants (SOD and POD) and free Pro Decreased MDA content	Jie *et al.* (2008)
Vitamin E	Drought	Sunflower (*H. annuus* L.)	Increased photosynthetic pigments, total carbohydrates, growth hormones and some mineral ions	Badawy *et al.* (2015)
Vitamin E	Sandy soil	Common bean (*P. vulgaris* L.)	Increased vegetative growth, yield, CKs, IAA and GA3 content, total chlorophyll and carbohydrates content Reduced fibers	El-Tohamy (2007)
Vitamin E	Chilling	Alfalfa (*Medicago sativa* L.)	Enhanced CAT, APX and GR activities	Bafeel and Ibrahim (2008)
Vitamin E	Low temperature	Cucumber (*Cucumis sativus* L.)	Increased in Pro, soluble solid content and enhanced POX activity	Orabi *et al.* (2017)
Vitamin K	Salinity	Okra (*Abelmoschus esculentus Moench*)	Decreased in H_2O_2 and MDA accumulation Increased in antioxidant compounds (anthocyanins, AsA, favonoids, and phenolics), total free amino acids and Pro content Enhanced activities of CAT, POD, and SOD	Ashraf *et al.* (2019)
Vitamin K	Chromium (Cr) toxicity	Okra (*A. esculentus* Moench)	Increased plant growth Enhanced activities CAT, POD and SOD Decreased H_2O_2 and MDA	Ashraf *et al.* (2021)
Vitamin K	Chromium (Cr) toxicity	Okra (*A. esculentus* Moench)	Reduced lipid peroxidation, ROS production, Mg levels, total Cr contents Enhanced antioxidant enzyme (SOD, POD, CAT) activities	Askari *et al.* (2021)

ABA, Abscisic acid; APX, Ascorbate peroxidase; AsA, reduced ascorbic acid; CAT, Catalase; CK, Cytokinin; GA_3, Gibberellic acid; GB, Glycine betaine; GR, Glutathione reductase; GSH, Reduced glutathione; IAA, Indole-3-acetic acid; MDA, Melondialdehyde; POD, Peroxidase; POX, Guaiacol peroxidase; PPO, Polyphenoloxidase; Pro, Proline; RWC, Relative water content; SOD, Superoxide dismutase.

22.6 Conclusion and future prospective

All vitamins, as well as their precursors and derivatives, play a vital role in plant growth and development, as well as in protecting plants against a wide range of abiotic and biotic stress conditions. These are also essential metabolites in regulating of key physio-biochemical roles in plants, such as seed germination, membrane stability, photosynthesis, respiration, precursors of enzymatic cofactors, nucleic acid biosynthesis, energy-generating pathways, senescence, cellular ROS regulation, and the oxidative defense system, among others. Exogenous vitamins A, B_1, B_2, B_3, B_6, B_9, B_{12}, C, D, E, and K minimize various abiotic stresses (salinity, waterlogging, drought, heavy metal and temperature stress) and result in reduction of oxidative damage, osmolytic and electrolytic balance in plant cells, whereas vitamins B_1, B_2, B_9 and K induce disease resistance. The effectiveness of vitamin application depends on time, dose and mode of application. They could be applied on seeds, early stages of growth, on soil or foliar spray depending on the desired results. Exogenous vitamin usage has the potential to be a beneficial strategy for good crop production in both biotic and abiotic harsh conditions. Vitamins help improve the crop's nutritional quality, which is very essential for customers. Vitamins must be explored more thoroughly with multidisciplinary approaches for a better understanding of crop responses and adaptation to practical agronomic strategies for minimizing stressful impacts and preserving agricultural productivity.

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23 Sugar Signaling in Plants under Physiological and Stress Conditions

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Abstract

Sugars, including glucose (Glc), fructose (Fru), sucrose (Suc) and trehalose (Tre), are not only energy, nutrient and structural materials, but are also signaling molecules in plants. Sugars, as signaling molecules, regulate seed germination, seedling establishing, plant growth and development, maturation and senescence, and response to unfavorable environmental stress. Sugars can be sensed by different sensors or receptors in a concentration-dependent manner or energy-dependent manner in plant cells, which in turn regulate gene expression, enzyme activity and cellular metabolism. In plants, sugar signaling pathways mainly include hexose kinase (HXK)-dependent and metabolism-independent signaling pathway, HXK-dependent and metabolism-dependent signaling pathway (also known as glycolysis-dependent signaling pathway), and regulator of G-protein signaling (RGS)-dependent G-protein signaling pathway (also known as HXK-independent signaling pathway). In the HXK-dependent and metabolism-independent signaling pathway, HXK is responsible for the function of glucose sensor, while HXK performs the catalytic activation in the HXK-dependent and metabolism-dependent signaling pathway. In addition, the RGS-dependent G-protein signaling pathway negatively regulates G-protein-coupled receptor (GPCR) by activating GTPase to degrade GTP, which accelerates the cycle of G-proteins, followed by generating second messengers such as Ca^{2+}, cyclic adenosine mononucleotide phosphate (cAMP) and diacetylglycerol (DAG). Thus, the different sugar signaling pathways form an intricate signaling network to regulate plant growth and development as well as response to environmental stress. In this chapter, the HXK-dependent and metabolism-independent signaling pathway, HXK-dependent and metabolism-dependent signaling pathway, and RGS-dependent sugar signaling pathways are highlighted under physiological and stress conditions, which aims to further understanding the action mechanisms of sugar signaling, and accelerate the rapid progress on sugar signaling research in plants.

23.1 Introduction

Sugars, also known as carbohydrates, are derived from the photosynthesis of green plants. Sugars can be divided into structural and non-structural sugars based on their physiological functions. The structural sugars mainly include cellulose, hemicellulose and lignin, which contribute to plant biomass and structures. Non-structural sugars are composed of monosaccharides

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(including glucose, fructose and galactose), disaccharides (mainly sucrose, trehalose and maltose), oligosaccharides (like raffinose and stachyose) and polysaccharides (such as starch and fructan) (Amist and Singh, 2020). Because the non-structural sugars can dissolve in water, they have multiple physiological functions. They are a fuel for plant growth and development; a precursor for the biosynthesis of biomolecules such as lipids, proteins and nucleic acids; an osmotic adjustment substance to maintain cellular osmotic balance; and a scavenger of reactive oxygen species (ROS) (Bhattacharya and Kundu, 2020). Interestingly, the non-structural sugars (mainly glucose, sucrose and trehalose) are short- and long-distance signaling molecules in plants, which in turn regulate seed germination, seedling establishing, plant growth and development, as well as the response and adaptation to environmental stress by cooperation of complex sugar signaling pathways (Gangola and Ramadoss, 2018; Sakr *et al.*, 2018; Amist and Singh, 2020).

As mentioned above, sugars are not only energy, nutrient and structural materials, but are also signaling molecules in plants. The concentration, energy and nutrient status of sugars can be sensed by the different sugar sensors or receptors, which in turn trigger different signaling pathways (Li and Sheen, 2016; Dröge-Laser and Weiste, 2018). In plants, there are many sugar signaling pathways in a nutrient-dependent and/or energy/concentration-dependent manner. In general, the sugar signaling pathways mainly include hexose kinase (HXK)-dependent and metabolism-independent signaling pathway, HXK-dependent and metabolism-dependent signaling pathway (also known as glycolysis-dependent signaling pathway), and regulator of G-protein signaling (RGS)-dependent G-protein signaling pathway (also known as HXK-independent signaling pathway) (Chen *et al.*, 2006; Coello *et al.*, 2011; Burkart and Brandizzi, 2021). In the HXK-dependent and metabolism-independent signaling pathway, HXK is responsible for the function of glucose sensor (not enzymatic function), while in the HXK-dependent and metabolism-dependent signaling pathway, HXK performs the function of enzyme (catalytic activation, not sugar sensor) (Xiao *et al.*, 2000). In addition, the RGS-dependent GPCR by activating GTPase to degrade GTP, which accelerates the cycle of G-proteins, followed by generating second messengers such as Ca^{2+}, cyclic adenosine mononucleotide phosphate (cAMP) and diacetylglycerol (DAG) (Rolland *et al.*, 2000; Pandey, 2016). In many plant physiological processes, the interactions among sugar signaling pathways can be observed, which in turn form the complex sugar signaling network to regulate plant growth, development and response to environmental stress (Smeekens, 2000; Rolland *et al.*, 2002; Sheen, 2014; Zhao and Wang, 2021).

Sugars play a very important role in the life cycle of plants in a nutrient and/or signaling manner. The nutrient role of sugars has been extensively studied in many physiological processes in various plant species, but the signaling role of sugars has emerged in recent years (Siddiqui *et al.*, 2020; Saddhe *et al.*, 2021; Zhao and Wang, 2021). The accumulating evidence also shows that the sugar signaling exerts a key role in the plant growth and development as well as response to environmental stress (Shah *et al.*, 2019; Amist and Singh, 2020; Sami *et al.*, 2021). Sugars can regulate the expression of hundreds of genes in plants. In the *Arabidopsis* plant model, 290 sugar-responsive genes have been identified using a functional genomics approach. These genes can rapidly and specifically respond to a low concentration (1 mM) of glucose, fructose and/or sucrose within 1 hour (Kunz *et al.*, 2014). Therefore, in this chapter, the well-studied sugar signaling pathways in plants, that is, HXK-dependent and metabolism-independent signaling pathway, HXK-dependent and metabolism-dependent signaling pathway, and RGS-dependent sugar signaling pathways, are discussed under physiological and stress conditions. The aim of which is to further understand the action mechanisms of sugar signaling, and accelerates the rapid progress on sugar signaling research in plant biology.

23.2 The homeostasis of sugar in plants

Though sugars can act as an energy source and nutrient in plants, their excessive accumulation in plant cells can generate carbonyl stress, mainly methylglyoxal stress. Reactive sugars (such as glucose, fructose, glucose 6-phosphate and fructose 6- phosphate) and their derivatives (like methylglyoxal) can rapidly react with biomolecules such as proteins, nucleic acids and lipids, which in turn produce advanced glycation end products and advanced lipid-peroxidation end products, followed by damage to cells by changing the structure and function of biomolecules (Shumilina *et al.*, 2019; Liu and Gendron, 2020). On the contrary, when the level of sugars in plant cells declines, the plant growth and development will be severely constrained, and may even lead to plant death (Depaepe *et al.*, 2021; Yoon *et al.*, 2021). Therefore, the level of sugars in plant cells must be maintained in homeostasis. Generally, the homeostasis of sugars, especially glucose, in plant cells (similar to that of in animal cells) is tightly regulated by many pathways (Fig. 23.1). The homeostasis pathways are involved in the conversion of glucose into polysaccharides (such as starch), disaccharides (including sucrose and trehalose) and other non-sugar substances (like lipids, proteins and nucleic acids); adversely, when the sugar is insufficient in plant cells, the non-sugar substances (such as lipids and amino acids) can convert into glucose to maintain the homeostasis of glucose, which is known as gluconeogenesis (Li and Sheen, 2016; Saddhe *et al.*, 2021).

In addition, glucose in plant cells can be catabolized by glycolysis (also known as the Embden–Meyerhof–Parnas pathway: EMP)-tricarboxylic acid (TCA) cycle to synthesize nicotinamide adenine dinucleotide (NADH) and adenosine triphosphate (ATP), which in turn maintains redox balance and energy equilibrium in plant cells (Dröge-Laser and Weiste, 2018). Also, glucose can be decomposed by glucose oxidase (mainly occurring in animals and microbes) to produce hydrogen peroxide (H_2O_2), which further converts to water and oxygen by catalase (CAT), glutathione-S-transferase or ascorbate peroxidase (APX) in the different subcellular structures. In addition to these, to maintain the homeostasis of glucose in the plant cells, glucose can be transported into or out of the cells, this process is also known as partitioning (Saddhe *et al.*, 2021; Zhao and Wang, 2021). Therefore, the different levels of sugars (especially glucose) can be sensed by the different sugar sensors/receptors, which in turn trigger the different sugar signaling pathways, forming a complex sugar signaling network, followed by regulating gene expression, enzyme activity and sugar metabolism, thus modulating plant growth and development as well as response to environmental stress.

23.3 Inter-conversion of sugar as signaling molecules in plants

To maintain homeostasis, the sugars acting as signaling molecules, such as monosaccharides (including glucose and fructose) and disaccharides

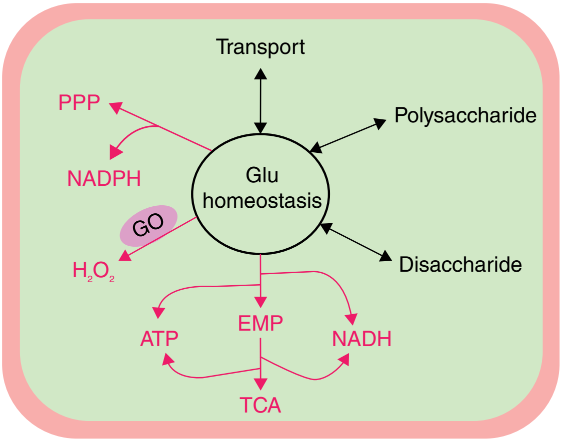

Fig. 23.1. Homeostasis of glucose in plant cells.

(like sucrose and trehalose), can be mutually transformed in plant cells. In general, the sucrose can be degraded to glucose and fructose by invertases (INVs), which include cytosol invertase, cell wall invertase and vacuole invertase (VIN) located in the different subcellular structures; similarly, the sucrose also can be converted into uridine diphosphate-glucose (UDP-G) and fructose by sucrose synthase (SUS) (Li and Sheen, 2016; Poonam *et al.*, 2016) (Fig. 23.2). Also, the sucrose can be transported into or out of the plant cells by sucrose transporter (SUT) (Fig. 23.2). In addition, the glucose and fructose derived from the sucrose can be phosphated by hexose kinase (HXK) to generate glucose-6-phosphate (G-6-P) and fructose 6-phosphate (F-6-P), respectively. Analogously, G-6-P also can be isomerized into F-6-P by phosphoglucose isomerase (ISM) (Lejay *et al.*, 2008) (Fig. 23.2).

Otherwise, the glucose and UDP-glucose can be converted into trehalose-6-phosphate (Tre-6-P) in the catalysis of Tre-6-P synthase (TPS), which in turn produce an important osmotic adjustment substance trehalose by the catalytic role of Tre-6-P phosphatase (TPP) (Li and Sheen, 2016; Morgutti *et al.*, 2019) (Fig. 23.2). Contrary to the synthesis of the sucrose, the F-6-P can combine with UDP-G and produce sucrose-6-phosphate (Suc-6-P) by the catalysis of Suc-6-P synthase (SPS). Subsequently, the Suc-6-P is converted into sucrose by the dephosphorylation of Suc-6-P phosphatase (SPP) (Rolland *et al.*, 2006) (Fig. 23.2). As shown in Fig. 23.2, the UDP-G is an important intermediate to synthesize trehalose and sucrose, which plays a key role in the homeostasis of trehalose and sucrose. In addition, the sugar inter-conversion pathway also implies the interaction of the different sugar signaling pathways in plant growth and development, as well as response to environmental stress.

23.4 Sugar signaling pathways in plants

As above-mentioned, to maintain homeostasis, the types and levels of different sugars can be sensed by the different sensors or receptors in plant cells, which in turn trigger the different sugar signaling pathways. These signaling pathways include the HXK-dependent and metabolism-independent signaling pathway, HXK-dependent and metabolism-dependent signaling pathway (also known as glycolysis-dependent signaling pathway), HXK-independent signaling pathway (also known as RGS-dependent signaling pathway), energy-dependent signaling pathways including sucrose non-fermenting 1-related protein kinase (SnRK)

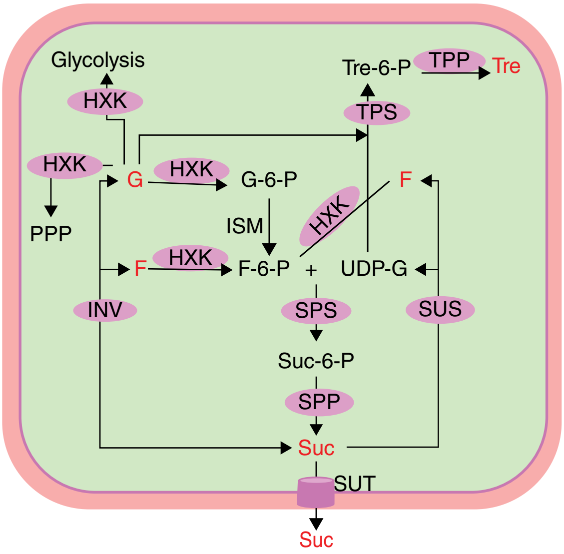

Fig. 23.2. Inter-conversion of sugars as signaling molecules in plants.

signaling pathway and target of rapamycin (TOR) kinase-dependent signaling pathway and other sugar signaling pathways (Fig. 23.3). These pathways are stated in detail in the following sections.

23.4.1 HXK-dependent and metabolism-independent signaling pathway

As mentioned above, HXK is a protein with double functions (a glucose sensor triggering sugar signaling and an enzyme converting glucose to G-6-P in glycolysis). In HXK-dependent and metabolism-independent signaling pathway, the HXK acts as glucose sensor (not an enzyme to phosphorylate glucose), which in turn triggers sugar signaling pathways, followed by regulation of corresponding gene expression, enzyme activity and cellular metabolism, thus modulating seed germination, seedling emergence, plant growth and development, as well as response to environmental stress including abiotic and biotic stress (Rolland *et al*., 2006). The most significant feature of HXK-dependent and metabolism-independent signaling pathway is that it is able to inhibit the expression of genes related to photosynthesis, such as chlorophyll a/b-binding protein1(CAB1), plastocyanin (PC), ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (rbcS), etc. (Martínez-Noël and Tognetti, 2018). In the *Arabidopsis* plant model, the glucose insensitive2 (*gin2*) mutants have been clearly confirmed that AtHXK1 is a core component in sugar sensing and signaling in plants. Subsequently, the independence of glucose metabolism and signaling can be identified by the analysis of two catalytically inactive AtHXK1 alleles, one deficient in ATP binding site and the other deficient in phosphoryl transfer. The results show that the alleles have been found to sustain wild-type growth, repression of the expression of genes related to photosynthesis, and response to plant hormones (auxin and cytokinin) when expressed in a *gin2* background (Xiao *et al*., 2000; Rolland *et al*., 2006). These imply that *Arabidopsis* should have other HXK (AtHXK2) and HXK-like proteins (AtHXL), which further identified six HXK and HXK-like genes in the *Arabidopsis* genome.

In addition, the HXK as a glucose sensor can be revealed by the glucose analogs, such as 2-deoxyglucose (2-DOG), mannose, 6-deoxyglucose (6-DOG) and 3-O-methyl glucose (3-OMG). The

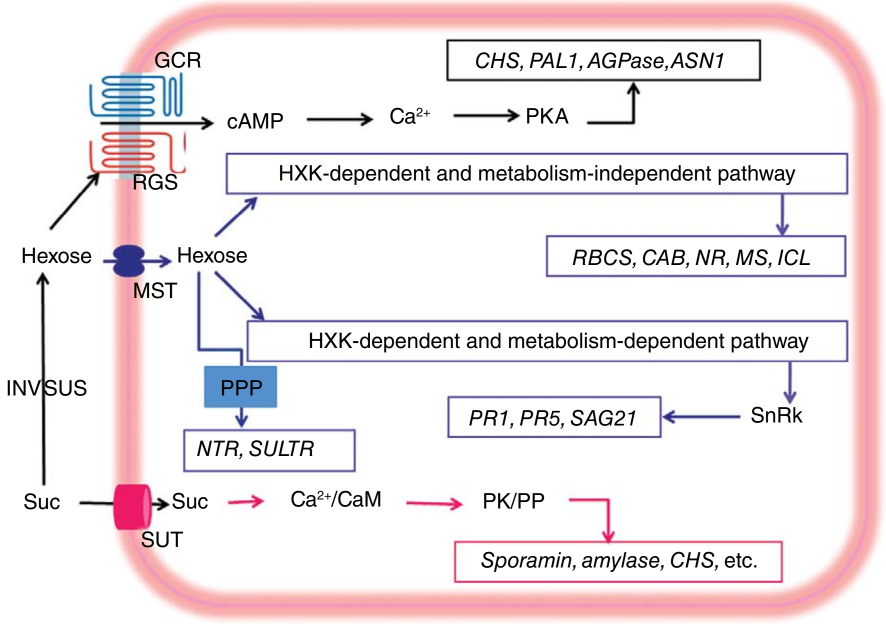

Fig. 23.3. Potential sugar signaling pathways in plants.

2-DOG and mannose can be phosphorylated by HXK, simulating glucose signaling in the regulation of photosynthetic and glyoxylate genes; whereas 6-DOG and 3-OMG can not be phosphorylated by HXK, that is, they are non-metabolizable, being accumulated in plant cells, thus triggering other sugar signaling response in the regulation of INV and patatin genes, no significant effect on the expression of photosynthetic and glyoxylate genes (Xiao *et al.*, 2000; Shah *et al.*, 2019). These indicate that there are both HXK and non-HXK sugar sensors in plants. Also, the HXK1 can form an HXK1-nuclear complex that directly binds to promoters of glucose-regulated genes by interacting with the vacuolar H^+-ATPase B1 (VHA-B1) and the 19S regulatory particle of proteasome subunit (RPT5B) in a glucose-dependent manner (Ahmad, 2019; Ahmad *et al.*, 2020). Moreover, glucosinolate, a secondary metabolite, takes part in regulating the acquirement of abiotic and biotic stress tolerance in plants. The glucose can upregulate the expression of genes (*CYP79F1* and *CYP79F2*) related to glucosinolate biosynthesis in *Arabidopsis*, while the gene expression and the total aliphatic glucosinolate content substantially reduce in *gin2-1* mutants, abscisic acid (ABA)-insensitive 5 (*abi5-7*) mutants and sugar-insensitive RGS1 mutant (*rgs1-2*) (Amist and Singh, 2020; Bhattacharya and Kundu, 2020). These indicate that the HXK-dependent signaling pathways and/or RGS-dependent G-protein signaling pathways are involved in the biosynthesis of glucosinolate, which in turn improve the resistance of plants to abiotic and biotic stress.

In general, the low concentrations (29–60 mM) of sugar can promote seed germination by a different signaling pathway, augmenting the energy supplies of seeds. On the contrary, the different concentrations (1%, 3% and 6%) of glucose can delay the germination of seeds, and this delay is closely associated with the accumulation of abscisic acid by suppressing catabolism and enhancing anabolism, similar to the osmotic effect induced by mannitol (Finkelstein and Lynch, 2000). Similarly, the low concentrations of both mannose at 3 mM and 3-OMG at 35–60 mM can inhibit the germination of seeds in the absence of ABA, which is enhanced in the presence of ABA. The further study has been shown to the mannose-inhibited seed germination is mediated by HXK via depleting energy and carbon sources, similar to the effect of glucose on the activities of glyoxylate cycle enzymes (Garciarrubio *et al.*, 1997). In addition to inhibition of seed germination, the sugar at high concentration (greater than 300 mM) can inhibit seedling growth. This inhibition might be largely osmotic effect, which can be mediated by abscisic acid (Finkelstein and Lynch, 2000).

23.4.2 HXK- and metabolism-dependent signaling pathway

The HXK-dependent and metabolism-dependent signaling pathway is also known as glycolysis-dependent signaling pathway. In this pathway, HXK acts as enzymatic function, converting glucose to G-6-P, which in turn degrades to pyruvate via the next nine steps in glycolysis, finally enters into tricarboxylic acid cycle to regulate energy and redox balance in plant cells (Xiao *et al.*, 2000; Ahmad *et al.*, 2020). Therefore, in this pathway, many intermediates (not clear) derived from glycolysis, and even from tricarboxylic acid cycle, might act as the role of signaling molecules (Xiao *et al.*, 2000), but their sensors need to be further illustrated in the future. In this signaling pathway, the glycolysis can be sustained by the heterologous yeast HXK2 activity, and the glucose can induce the expression of specific genes related to pathogen resistance (such as pathogen-related protein *PR1* and *PR5*) and senescence-associated gene *SAG21* (Xiao *et al.*, 2000; Rolland *et al.*, 2006), indicating the glycolysis-dependent signaling pathway is closely associated with disease resistance. In *Arabidopsis*, the glucose signaling can regulate root growth deviation, root waving and coiling, lateral root emergence and hypocotyl elongation by interacting with auxin, cytokinin and brassinosteroid signaling in an HXK-dependent and independent signaling pathway manner (Saksena *et al.*, 2020). Similarly, the tobacco transgenic plants over-expressing a yeast invertase in the vacuole or cell wall increased the accumulation of sugars and transcripts (*PAR-1*, *PR-1b*, *SAR8.2* and *PR-Q*), which in turn enhanced resistance to potato virus Y. These effects also could be induced by exogenous glucose supply (Xiao *et al.*, 2000). In this case, the sugar signaling molecule is not glucose itself, but rather some unknown intermediates downstream in the glycolysis pathway. These indicate that the induction of pathogen-related proteins by glucose may depend on the catalytic activity of HXK, but not the sensor function of HXK1.

23.4.3 RGS-dependent G-protein signaling pathway

In addition to the HXK-dependent and metabolism-independent signaling pathway and the HXK-dependent and metabolism-dependent signaling pathway, RGS-dependent G-protein signaling pathways belong to HXK-independent signaling pathway in plants. In this pathway, glucose can regulate the expression of genes related to the biosynthesis of chalcone (chalcone synthetase, *CHS*), lignin (phenylalanine ammonia lyase, *PAL1*), aspartic acid (aspartic synthase, *ASN1*), ADP-glucose pyrophosphorylase (AGPase) and carotenoids (Ryu *et al.*, 2004; Rolland *et al.*, 2006; Ahmad, 2019). Briefly, the glucose or sucrose can be sensed by the G-protein-coupled receptor system (mainly RGS1), which in turn stimulates guanosine triphosphatase (GTPase), thus promoting the cycle of G-protein-coupled receptor system. The activated G-protein-coupled receptor system by sugar can further stimulates the activity of adenylate cyclase, which catalyzes the generation of the second messenger (cyclic adenosine monophosphate, cAMP). The cAMP can trigger calcium signaling in cytosol by binding to its receptor located in endoplasmic reticulum (calcium storage) (Chen *et al.*, 2006; Pandey, 2016). Thus, the sugar signaling is converted into cAMP and calcium signaling, finally regulating plant growth and development as well as response to abiotic and biotic stress.

Also, the calcium-mediated sugar signaling pathway can be further identified by pharmacological experiments. The sugar-triggered physiological processes, such as fructan synthesis, can be inhibited by Ca^{2+} channel blockers ($LaCl_3$), EGTA and calmodulin antagonists (trifluoperazine and chlorpromazine) (Vitrac *et al.*, 2000; Martínez-Noël *et al.*, 2006). Similarly, the gene expression of sporamin and α-amylase in sweet potato and of anthocyanin biosynthesis in cell suspension cultures of *Vitis vinifera* by sugar also have been confirmed using pharmacological experiments related to calcium signaling (Ahmad, 2019; Ahmad *et al.*, 2020).

23.4.4 Energy-dependent signaling pathway

In plants, in addition to HXK-dependent and metabolism-independent signaling pathways, there are energy-dependent signaling pathways to regulate gene expression, enzyme activity and cellular metabolism, finally regulating seed germination, seedling emergence, plant growth and development, as well as response to changing environments. The energy-dependent signaling pathways include the TOR-dependent signaling pathway and SnRK-dependent signaling pathways. They regulate plant growth and development as well as response to environmental stress by sensing the different status of energy and nutrient in plant cells (Fig. 23.4). The two sugar signaling pathways are stated in this section.

23.4.4.1 TOR-dependent signaling pathway

Under normal physiological conditions, plants contain a high sugar concentration (and even a high nutrient status) to construct cellular structure and accumulate biomass. A high sugar concentration can be sensed by TOR kinase, which triggers a specific signaling pathway, regulates gene expression, enzyme activity and cellular metabolism, followed by the regulation of transcription, translation, cellular autophagy and the metabolism of starches, lipids and proteins, eventually regulating plant growth, development, flowering, senescence and response to environmental stress (Dong *et al.*, 2017; Burkart and Brandizzi, 2021). In yeast and animal systems, the two TOR kinases, TOR complex1 (TORC1) and TORC2, have been found, which have the different structure and function. In *Arabidopsis*, only a gene coding TORC1 has been found. To investigate the TOR-dependent signaling pathway, in pharmacological experiments, the chemicals AZD-8055, torin2 and PP242 are commonly used as the inhibitors of TOR kinase (Smeekens, 2000; Zhao and Wang, 2021).

The numerous studies show that the TOR-dependent signaling pathway is involved in the

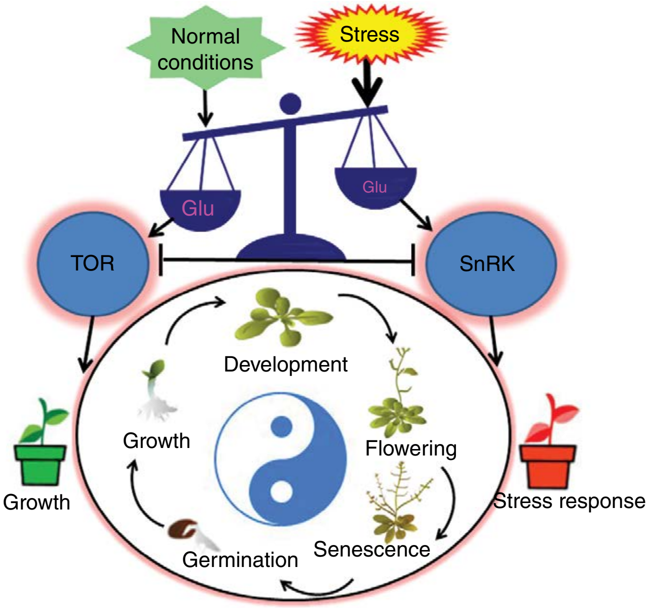

Fig. 23.4. Crosstalk between TOR- and SnRK-dependent sugar signaling pathways in plants.

regulation of a number of physiological processes from seed germination to organ senescence, and even plant death under physiological and stress conditions. Under physiological conditions, the TOR-dependent signaling pathways can modulate auxin and brassinosteroid signaling by regulating the activities of hormone corresponding receptors, protein translation and cell cycle by phosphorylating transcription factors, and nitrogen assimilation and stress response by inhibiting protein phosphatase2A (PP2A) (Rodriguez *et al*., 2019; Shah *et al*., 2019). Similarly, under stress condition, the TOR-dependent signaling pathway also can regulate the plant stress response by modulating hormone signaling (abscisic acid, ethylene, salicylic acid and jasmonic acid), redox balance, cellular autophagy, meristem activity and starch biosynthesis, and even blocking the SnRK-dependent signaling pathway (Rodriguez *et al*., 2019; Yoon *et al*., 2021). At the same time, besides sugar and nutrient status, the TOR-dependent signaling pathway also can be controlled by light, bioclock and abiotic/biotic signaling at the upstream of TOR kinase (Rodriguez *et al*., 2019). In addition, the TOR-dependent signaling pathway is involved in the tolerance of plants to heat, cold, drought, salt, osmotic and hypoxia stress (Rodriguez *et al*., 2019; Sharma *et al*., 2020). In *Arabidopsis* seedlings, the glucose treatment can significantly induce the thermotolerance; while the glucose-induced thermotolerance was arrested by the inhibitor of TOR kinase (AZD-8055) (Sharma *et al*., 2019), indicating the thermotolerance induced by glucose was involved in the TOR-dependent signaling pathway. Similarly, both glucose and sulfur treatment can promote the growth of Arabidopsis, while the sulfur-induced growth of Arabidopsis was regulated by the glucose-TOR signaling pathway (Dong *et al*., 2017).

23.4.4.2 SnRK-dependent signaling pathway

In general, under stress conditions, the content of sugar in plant cells can be reduced due to limiting of photosynthesis and the acceleration of sugar catabolism. Similar to TOR-dependent signaling pathway, a low sugar and nutrient status can be sensed by SnRK, which in turn triggers the downstream signaling pathway, finally modulating plant growth and development as well as response to stress conditions (Coello *et al*., 2011; Rodriguez *et al*., 2019; Takahashi *et al*., 2020). In plants, the SnRK is a large family, which includes three subfamilies, SnRK1, SnRK2 and

SnRK3, whereas the SnRK1 is composed of ten subunits, of which can sense sugar status (Rodriguez *et al.*, 2019). Accumulating data show that SnRK can regulate the expression of thousands of genes, which in turn promote the accumulation of osmolytes (such as proline and total soluble sugar) and heat shock proteins, as well as enhancement of antioxidant system composed of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, peroxidase, monodehydroascorbate reductase and dehydroascorbate reductase) and non-enzyme antioxidants (glutathione, ascorbic acid, flavonoids, total phenols and carotenoids), thus improving the resistance of plants to stress conditions (Rodriguez *et al.*, 2019; Saksena *et al.*, 2020). To study SnRK-dependent signaling pathway, in pharmacological experiments, the chemicals glucose-6-phosphate, glucose-1-phosphate and trehalose-6-phosphate are usually used as the inhibitors of SnRK (Amist and Singh, 2020).

The growing studies have been found that the SnRK-dependent signaling pathway takes part in the regulation of many physiological processes under normal and stress conditions. Under normal physiological conditions, the SnRK-dependent signaling pathway represses plant hormone signaling (auxin, gibberellin, jasmonic acid and ethylene) and the TOR-dependent signaling pathway, as well as activating the energy-producing pathway, which in turn modulates plant growth (Smeekens, 2000; Rodriguez *et al.*, 2019). Under stress conditions, the SnRK-dependent signaling pathway can trigger abscisic acid-dependent and metabolism-independent signaling pathways, cellular autophagy signaling and energy-producing signaling pathways, which in turn primes the resistance of plants to abiotic/biotic stress (Smeekens, 2000; Rodriguez *et al.*, 2019; Ahmad *et al.*, 2020). Similar to the TOR-dependent signaling pathway, besides a low energy status, the trehalose-6-phosphate, glucose-6-phosphate, glucose-1-phosphate, calcium signaling and abiotic/biotic signaling all can modulate the SnRK-dependent signaling pathway at the upstream of SnRK in plants (Rodriguez *et al.*, 2019). Also, the acquirement of abiotic stress tolerance of plants to cold, drought, salt, osmotic, flooding, hypoxia and nutrient deficiency stress is closely associated with the SnRK-dependent signaling pathway (Rodriguez *et al.*, 2019; Ahmad, 2019). These further indicate the interaction between TOR-dependent and SnRK-dependent signaling pathways in plant growth and development as well as response and adaptation to environmental stress, but the detailed interactive mechanisms should be further dissected using omics and multiple omics in the future.

23.4.5 Other signaling pathways

23.4.5.1 Trehalose-6-phosphate (T6P)-dependent signaling pathway

As mentioned above, the Tre-6-P is an intermediate during the trehalose biosynthesis, which can be generated from UDP-glucose and G-6-P by the catalysis of TPS. Subsequently, Tre-6-P can be converted into trehalose by TPP (Smeekens, 2000; Morgutti *et al.*, 2019). In *Arabidopsis*, the 11 HXKs, that is, AtTPS1-11 and 10 TPPs (namely AtTPPA–J) have been identified. The trehalose is a disaccharide with two glucose residues, which is not only an osmoprotectant, but also a signaling molecule in plants under stress conditions such as drought, salt, heat and cold. The trehalose can induce the accumulation of anthocyanins (an important antioxidant to maintain cellular redox) during the development of leaf under high carbon supply (Shahri *et al.*, 2014). Similarly, the Tre-6-P can regulate the physiological processes from embryo development to leaf senescence by interacting with phytohormones, such as auxin (ÓHara *et al.*, 2013). In addition, the Tr-6-P regulate starch synthesis via redox activation of adenosine diphosphate (ADP)-glucose phosphorylase, which catalyzes the first step in starch biosynthesis, and inhibits the activity of the KIN10/11 regulatory kinase. Also, in yeast, the Tre-6-P, as a signaling molecule, allows HXK to perceive carbon status, while the *Arabidopsis* plants have no direct link between HXK inhibition and Tre-6-P (Paul *et al.*, 2001; Eastmond *et al.*, 2002), but a link between protein kinase (PK) and sucrose non-fermenting-related kinase-1 (SnRK1) might exist in plants (Schluepmann *et al.*, 2004). Our previous study also showed that the treatment with hydrogen sulfide could increase the content of endogenous trehalose in maize seedlings, which in turn improved the heat tolerance of seedlings. The hydrogen sulfide-induced heat tolerance was enhanced by trehalose treatment, while weakened by the inhibitors of trehalose biosynthesis (Li *et al.*, 2014). These indicate that the interaction between hydrogen sulfide and trehalose signaling might exist in the formation of plant heat tolerance.

23.4.5.2 Sucrose (Suc)-dependent signaling pathway

In addition to glucose, fructose and trehalose, sucrose has a signaling role in plant growth and development as well as response to environmental stress. Relatively, the sucrose signaling pathway is more complex as compared to HXK-dependent signaling pathways. The sucrose is easy to be hydrolyzed to the glucose and fructose, which in turn exert their signaling role via HXK-dependent signaling pathways (Yoon *et al.*, 2021). Therefore, it is difficult to separate the sucrose-dependent and HXK-dependent signaling pathways in plants. However, in sugar beet, the sucrose-specific gene, proton-sucrose symporter, has been found in a HXK-independent manner (Vaughn *et al.*, 2002). Similarly, the post-transcriptional control of a leucine zipper and translational inhibition of the ATB2/bZIP11 in *Arabidopsis* are mediated by sucrose (Rook *et al.*, 1998). Also, the bZIP transcription factors (i.e. bZIP1, bZIP2, bZIP11, bZIP44 and bZIP53) can be translationally repressed by sucrose. Moreover, starch synthesis in potato tubers and seed development in transgenic *Vicia narbonensis* have been found to be mediated by sucrose (Shahri *et al.*, 2014).

In addition to these, the sucrose transporter SUT2/SUC3 is considered to be a sucrose sensor in yeast, but CitSUT1 and CitSUT2 in citrus. The CitSUT2 can be expressed in young leaves, but that of CitSUC1 in the mature leaves in citrus (Eckardt, 2003). Interestingly, the SUS1 can be regulated by glucose and mannose in an HXK-dependent (at low concentration) and osmoticum-dependent (at high concentration) manner, which is identified by HXK inhibitor (N-acetyl glucosamine) and mutational approach (plants with impaired HXK1 expression). These further imply the interaction between HXK-dependent and sucrose-dependent signaling pathways in plants.

23.4.5.3 Oxidative pentose phosphate pathway-dependent signaling pathway

In plants, oxidative pentose phosphate pathway-dependent signaling pathway can be observed in recent years. This pathway can induce the expression of specific sugar response genes, such as nitrate (NO_3^-) transporter 1.1, nitrate (NO_3^-) transporter 2.1, sulfate (SO_4^{2-}) transporter 1.1 and sulfate (SO_4^{2-}) transporter 3.5, which in turn integrates the nitrogen, sulfur and carbon metabolism, followed by promotive biosynthesis of amino acids, thus responding environmental stress (Lejay *et al.*, 2008; Ahmad, 2019; Ahmad *et al.*, 2020). Similar to other sugar signaling pathways, to explore oxidative pentose phosphate pathway-dependent signaling pathway, in pharmacological experiments, the chemical 6-aminonicotinamide is usually used as the inhibitor of phosphogluconate dehydrogenase (Lejay *et al.*, 2008; Ahmad, 2019; Ahmad *et al.*, 2020). Nowadays, how oxidative pentose phosphate pathway-dependent signaling pathway regulates plant growth and development as well as response to environmental stress is not clear, which needs to be further illustrated in the future.

23.4.5.4 MicroRNA-dependent signaling pathways

Recently, microRNA 156 (miRNA 156)-dependent sugar signaling pathway has been found to regulate the phase transition from juvenile to adult in plants, thus mediating floral transition. In general, in juvenile stage, plants have a low sugar content, which largely up-regulates the expression of microRNA156 gene, repressing the expression of transcription factor *SQUAMOSA PROMOTER BINDING PROTEIN-LIKES* (*SPLs*), thus blocking plant flowering (Wang, 2014). On the contrary, with the growth and development of plants, they accumulate a high sugar and accelerate *SPLs* expression, promoting the transition from juvenile to adult, therefore accelerating plant flowering (Wang, 2014). However, the detailed mechanism by which miRNA 156 regulates plant flowering needs to be further expounded in the future. Recently, it was found that the acquirement of plant heat tolerance is involved in the regulation of miRNA 156, while the sugar priming could improve the heat tolerance of plants (Szaker *et al.*, 2020), further implying the crosstalk between sugar signaling and miRNA 156 in the formation of plant heat tolerance.

23.5 Conclusion and prospects

Until now, the different sugar sensors and the corresponding signaling pathways have been identified in various model plants and other plant species. These signaling pathways at least include HXK-dependent and metabolism-independent signaling pathway, HXK-dependent and metabolism-dependent signaling pathway, and RGS-dependent G-protein signaling pathway (also known as HXK-independent signaling pathway). The sugar signaling pathways form a complex signaling network by interacting with each other to regulate seed germination, seedling emergence, plant growth and development, as well as response and adaptation to environmental stress. There are many key components in the sugar signaling network in plants. These components include sugar sensors (like HXK, RGS, SUT, TOR, SnRK, TPS and TPP), PKs, protein phosphatases, cyclic nucleotides (cAMP), Ca^{2+}/calmodulin-centered calcium signaling and the downstream transcription factors (such as ATB2, bZIP1, bZIP2, bZIP11, bZIP44 and bZIP53), which in turn regulate the gene expression and cellular metabolism. For example, the high energy and nutrient status (under normal conditions) can be sensed by TOR, which inhibits the SnRK-dependent sugar signaling pathway, thus promoting cell division, plant growth and development; on the contrary, the low energy and nutrient status (under stress conditions) can be sensed by SnRK, which blocks the TOR-dependent sugar signaling pathway, finally improving the response and resistance of plants to stress conditions by reducing cell division, plant growth and development (Fig. 23.4). Therefore, the TOR-dependent and SnRK-dependent sugar signaling pathways can critically regulate the plant growth and development as well as response to changing environments by sensing the status of sugar and nutrient.

It is clear that sugar metabolism is the center of cellular metabolism, while sugar signaling is the hub of cellular signaling network in plants. The sugar signaling has been preliminarily found to interact with plant hormones (such as auxin, gibberellin, cytokinin, abscisic acid and ethylene), calcium, nutrient (like nitrogen and sulfur), light, development and abiotic/biotic signaling to regulate plant growth and development as well as response to adverse environments, but their detailed mechanisms should be further investigated in the future. In addition, sugar (glucose, sucrose and trehalose) priming can improve abiotic/biotic stress tolerance (including heat, cold, drought, salt and heavy metal tolerance) in plants, while the physiological-biochemical and molecular mechanisms remain unclear. Interestingly, the novel sugar sensors and their trigger signaling pathways are all the time waiting for finding. Also, the crosstalk between sugar signaling and other signaling (such as hydrogen sulfide, methylglyoxal, ROS, nitric oxide and carbon monoxide) in plants under physiological and stress conditions should be further uncovered in the future.

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24 Role of Nanoparticles in Improving Stress Tolerance in Crop Plants

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Abstract

Agriculture is facing various challenges due to the negative consequences of global climate change. In this scenario, designed nanoparticles are considered to be a useful tool for improving stress tolerance in crop plants for assuring sustainability of crop production in order to achieve food security. Various kinds of nanomaterials have shown great promise in promoting sustainable agriculture as they help to improve agricultural production by increasing the efficiency of inputs and minimizing yield losses. Nanomaterials offer a wider specific surface area to fertilizers and pesticides. In addition, nanomaterials are unique carriers of agrochemicals that facilitate the site-targeted controlled delivery of nutrients and pesticides for increased crop protection. Due to their direct and intended applications in the precise management and control of inputs (fertilizers, pesticides, herbicides), nano-tools, such as nano-biosensors, support the development of high-tech agricultural farms. The integration of biology and nanotechnology into nano-sensors has greatly increased their potential to sense and identify the environmental conditions or impairments.

24.1 Introduction

The constant growing human population is the main reason for the recent problems in the world. Whether it is an issue of food insecurity, water scarcity or shortage of energy resources, every country in the world is affected by it or will be. McCalla (2001) stated that the human population in the world is increasing on a daily basis and it will reach 9.1 billion in 2050, but resources are not going up at a comparable pace. Most of the population of the world is directly or indirectly dependent on agro-biased resources. 'Zero hunger', which is among the 17 sustainable development goals of the United Nations, is one of the highest priorities of the scientific community. Better crop production is one of the best remedies to elevate hunger, but due to the

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increase in conversion of agricultural land for non-agricultural use it is a big challenge to combat food shortage from the area under farming, which sustains a constant or even a decline in production. There are many factors (shrinking amount of arable land, shortage of water resources, climate change, and low use efficiency agrochemicals) that affect the crop yield, but reduced yield due to abiotic and biotic stresses is one of the most important (Shabala *et al.*, 2014; Suzuki *et al.*, 2014). According to Acquaah (2007), an approximate 70% reduction in crop yield was noted by the influence of abiotic stresses on crops directly or indirectly. To cope with this issue, the scientific community has developed stress tolerant crop varieties, but due to slow progress yet we have yet to develop a robust salt-tolerant wheat variety that is available on a commercial level (Genc *et al.*, 2019). De Lange *et al.* (2018) flagged the issue of safety in crop varieties that have been bred through transgenic approaches. Thus, it is important to develop new techniques that protect crop plants from various stresses and enhance efficacy of agrochemicals to reach the food requirements of the constant increasing world population with a safe and sustainable approach.

Among the recent approaches, nanotechnology is a promising approach in the era of sustainability related to plant biotechnology and agricultural techniques (Scrinis and Lyons, 2007). According to Gogos *et al.* (2012) and Giraldo *et al.* (2019), nanotechnology is defined as an operating material, system or process at a scale of 100 nanometers or less, that is, 'nano' means a small size scale between 1 nm and 100 nm. A composition of nanomaterials is made up of very small components but they show an impact at macrolevel with the properties of given material. Nanoparticles or nano devices affect the various developmental stages of crop plants which showed both positive and negative impacts on crop growth. The novel properties of nanomaterials make it an easy and safe way for agriculture researchers to improve crop growth as well as alleviate the various stresses of crop plants (Carmen *et al.*, 2003). The reason for better crop production is that nanomaterials have a relatively large external area when matched to the similar mass of material produced in a larger form. It can make more responsive and chemically effective materials, which affect the electrical properties as well as strength. Due to the high surface to volume ratio of particles, it causes an increase in the reaction and biochemical activity (Dubchak *et al.*, 2010).

In agriculture, the different forms of stresses like drought, salinity, alkalinity, flooding and mineral poisoning or deficiency are suggested to be the main causes of reduced crop growth and production (Boyer, 1982). During their whole life cycle, plants face various types of environmental stresses, which is the main reason to develop a defense system to counter these environmental stresses at different levels by modifying the various pathways (molecular, biochemical and physiological) by plants. With the help of appropriate alteration of gene expression, plants can adopt a molecular route that is another way to cope with these stresses. Many recent studies have shown that that the effect of nanoparticles on crop plant growth and development is dependent on its concentration. Various researchers (Kalteh *et al.*, 2014; Ashkavand *et al.*, 2015) have stated that nanoparticles and silicon nano-fertilizer had significant effects on various traits (physiological and morphological) and the vegetative features of basil under salinity and drought stress conditions. In another study it is reported that nanoparticles are responsible in up-regulating the antioxidant enzyme (superoxide dismutase, SOD; catalase, CAT and peroxidase, POD) activities (Jiang *et al.*, 2012; Laware and Raskar, 2014). This chapter summarizes the types of nanomaterials and their application in agriculture, which may provide unique reference for researchers and outline the importance of nanomaterials in agriculture and crop stress tolerance.

24.2 Types of nanomaterials used

Nanomaterials can be grouped into various categories on the biases of the size, morphology, and physical and chemical properties of the material. The most promising types of nanomaterials are carbon-based nanomaterials, ceramic nanomaterials, metal nanomaterials, semiconductor nanomaterials, polymeric nanomaterials and lipid-based nanomaterials. Carbon-based nanomaterial includes two main types: (i) carbon nanotubes (single-walled carbon nanotubes [SWCNTs] and multi-walled carbon nanotubes [MWCNTs]); and (ii) fullerene (Aqel *et al.*, 2012;

Ibrahim, 2013). Fullerene is an allotrope of carbon comprising a hollow cage of sixty (60 C) or more carbon atoms (Astefanei *et al.*, 2015). Ceramic nanomaterials are inorganic solids made up of oxide, carbide, carbonate and phosphate (Sigmund *et al.*, 2006; Thomas *et al.*, 2015). Examples of metal-based inorganic nanomaterial are silver (Ag), gold (Au), aluminum (Al), cadmium (Cd), copper (Cu), iron (Fe), zinc (Zn) and lead (Pb). The examples of metal oxide-based inorganic nanomaterial are zinc (Zn), copper (Cu), magnesium (Mg), Al, titanium (Ti), cerium (Ce), iron (Fe), silica (SiO_2) and iron oxide (Fe_2O_3). These nanomaterials have resistance against heat and unresponsiveness to chemicals. The organic-based nanomaterials are made up of organic materials without carbon materials, for example dendrimers, cyclodextrins, liposomes and micelles (Tiwari *et al.*, 2008; Rao and Geckeler, 2011; Mansha *et al.*, 2017). Metal nanomaterials are made up from metal predecessors. These nanomaterials can be manufactured by various methods (chemical, electrochemical and photochemical). Dreaden *et al.* (2012), Salavati-Niasari *et al.* (2008) and Tai *et al.* (2007) reported that semiconductor nanomaterials have properties like those of metal and non-metal elements. They are in groups II–VI of the periodic table (ZnO, ZnS, CdS, CdSe and CdTe), III–V (GaN, GaP, InP and InAs) or IV (silicon and germanium). Polymeric nanomaterials are organic-based nanomaterials and characterization depends upon the preparation method. They have a nano-capsular or nano-sphere structure. Lipid nanomaterials are spherical-shaped materials with a diameter of 10–100 nm. They contains a solid core which is made up of lipids and a soluble lipophilic-molecule matrix. The outer core of these nanomaterials becomes stable with surfactant and emulsifier.

24.3 Uptake, translocation and accumulation of nanoparticles (NPs) into the plants

Nanomaterials have presented an encouraging potential tool and strategy at the nanometer scale to increase the production of food and meet the future needs of agricultural and food security. Although this new approach has shown potential benefits to date, their use in field conditions is limited. Concerns about uptake, accumulation, bio-availability and toxicity limits the implementation of this approach in the agricultural community. Absorption, translocation and accretion of nanomaterials depend on the species of crop plants and the size, type, chemical configuration, performance and constancy of nanomaterials (Das and Das, 2019). Generally, nanomaterials enter in the root system of crop plants through the junctions of lateral roots and reach to the xylem tissue through the cortex and the pericycle (Dietz and Herth, 2011). The mechanism of interaction of nanomaterials with the system of crop plants is mainly based on chemical processes that generate reactive oxygen species (ROS), transport activity of ion cell membrane, oxidative damage and peroxidation of lipids. When nanomaterials enter into the plant cells it reacts with two groups (sulfhydryl and carboxyl) and eventually changes the activity of protein. Nanomaterials may form complexes with the transporters of membrane or exudates of plant roots and can be successively shifted into crop plants (Watanabe *et al.*, 2008; Kurepa *et al.*, 2009). Nanomaterials transfer from leaf to root, stem and developing grains, and from one root to another root of crop plants. The main passage of uptake and transportation into the shoot and leaf of crop plants is xylem (Birbaum *et al.*, 2010; Miralles *et al.*, 2012). Nanomaterials are able to penetrate into the leaf cuticle and the cell cytoplasm (Sharif *et al.*, 2013). In the cell cytoplasm, nanomaterials may bind with various cytoplasm organelles and interfere with the process of metabolism (Zhang and Monteiro-Riviere, 2009). Plasmodesmata is another pathway by which nanomaterials, including Ag (20 nm in particle size), can be shifted inside the cell (Unrine *et al.*, 2012). Another study, which determined generational transmission of C70-NOM in rice plants, determined the presence of black aggregate C70 in the leaf of second-generation rice plants treated with fullerene only in their first generation (Lin *et al.*, 2009).

24.4 Nanoparticles as a tool to alleviate abiotic stresses

Abiotic stresses, whether in the form of drought and/or salinity, are the prime reason for crop loss worldwide by affecting the morphological and physiological components of plant growth. In recent years, nanotechnology is gaining momentum as a key strategy to alleviate the restraints related with abiotic stress and secure crop production.

24.4.1 Role of nanoparticles in mitigating drought

Drought is an environmental issue that affects crop yield, nutrition of the crop products and crop potential; it can even affect human nutrition. Nanotechnology plays a key role in mitigation of drought stress. Therefore, nanomaterials can be used for sustainable crop production in reducing nutrient loss, suppressing diseases and then enhancing the yields (Nair, 2016; Khan *et al.,* 2017). In past years, nano biotechnology occupied a significant position by gaining secure and sustainable agricultural future. This activity copes with biotic and abiotic stresses worldwide. Nanoparticles are usually called nano-scale particles, being very small molecules with a size dimension between 1 nm and 100 nm (Roco *et al.,* 2003). Nanoparticles can regulate the activity of antioxidant enzymes (superoxide dismutase SOD, catalase CAT and peroxide POD) (Laware and Raskar, 2014). The mechanism of interaction between plant system and nanoparticles based on various chemical processes like ROS creation, lipid peroxidation, oxidative damage and ion transport activity. Once nanoparticles have entered into the plants, they react with sulfhydryl and carboxyl groups and alter the protein activity of plant. These particles form complexes with root exudates or membrane transporters so can easily transported in plants (Kurepa *et al.,* 2010).

Recent studies show that utilization of various amounts of silica nanoparticles (SNPs) enhances the plant endurance toward scarcity stress. Hawthorns (*Crataegus* spp.) demonstrated enhanced drought resistance; the physiological and biochemical response in hawthorn seedling to various concentrations of SNPs at various levels of drought from average to intense stress. The outcomes recommended the significant effect on physiological parameters (photosynthesis parameters, relative water content-RWC, malondialdehyde (MDA), membrane electrolyte leakage as well as chlorophyll, carotenoid, carbohydrate and proline content) by pre-treatment of SNPs (Ashkavand *et al.,* 2015). Another study showed the positive effect on plants of iron nanoparticles applied to plant stress under drought stress, for example boll numbers per branch, seed numbers per boll, weight of thousand seeds and production at 1% probability level. The application of iron nanoparticles also increased the yield and yield components at two growth stages (flowering and granulation), although it was healthier at the stage of flowering than seed formation in comparison to scarcity stress condition without iron nanoparticle application (Davar *et al.,* 2014).

The reduction of the negative impact due to resource limitation stress by using foliar application of titanium nanoparticle on wheat plants has also revealed positive outcomes on certain agronomical parameters (e.g. gluten and starch content in seeds of wheat plants). The study determined that foliar spray of titanium dioxide (0.02%) nanoparticle displayed improvement in different parameters related to plant agronomy (height of plants, weight of ear, number of ears, seed count, thousand weight of seeds, final productivity, biomass, index of yield including contents of gluten and starch under drought stress) (Jaberzadeh *et al.,* 2013). Ag-NPs applications also reduced the adverse effects of water stress in lentil crops. Studies showed the effect of various doses of PEG and Ag-NPs on percent (%) sprouting, length of roots, and fresh and dry weight of lentil plants. Furthermore, utilization of Ag-NPs decreased the drought stress mediated loss of crop plant development and production (Hojjat, 2016). Various studies related to metal-based or oxides of metal-based nanoparticle are being conducted to check their progress related to growth, development and protection of plants from biotic as well as abiotic stress. It also provides data regarding crop production and its role in moderating the numerous procedures in plants. To achieve sustainability in agriculture, there is still a long way to go and develop a technology.

24.4.2 Role of nanoparticles in mitigating salinity

Sustainability of crop production grown in high saline conditions is a major concern in the scientific community. Across the world, it is estimated that more than 20% of land that is cultivated is affected by salinity problems; the affected land area is increasing on a daily basis. Silicon NPs and fertilizers have shown encouraging results in basil (*Ocimum basilicum*) physiology and morphology grown under salinity stress conditions. The results showed a statistically significant improvement in the levels of chlorophyll *a* and proline level and the indices related to plant growth and development when silicon NPs and silicon fertilizer was applied to the basil plants (Kalteh *et al.*, 2014). Many other scientists have also demonstrated the ability of nano-SiO_2 in the alleviation of stresses related to salinity. A significant improvement was recorded in the level of chlorophyll *a* and proline, the fresh weight of leaf, dry weight of leaf, and the up regulated antioxidant enzyme activity under stress of salinity by treating plants with nano-SiO_2 particles. This increase in various attributes may confirm the improvement in stress tolerance of plants related to abiotic stress (Haghighi *et al.*, 2012; Siddiqui *et al.*, 2014).

In another study, the application of silicon to various genotypes of lentil crop under the stress of salt showed a statistically significant rise in germination percentage and seedling growth when compared to control plants where no treatment of nanoparticles resulted in a significant decrease in germination percentage and seedling growth due to the stress of salts. The addition of SiO_2-NPs not only improved the germination and seedling growth, but also increased the other parameters related to lentil genotypes under the stress of salt. So, SiO_2-NPs enhance various mechanisms of plants related to defense against salinity (Sabaghnia and Janmohammad, 2015). SiO_2-NPs tolerate the salinity in plants and decrease sodium ion ratio. Salinity directly affects plant growth due to the decline in osmotic potential and toxicity of sodium ions. Si-NPs increase the plant growth under the stress of salts due to sodium ion toxicity (Raven *et al.*, 1983).

24.4.3 Role of nanoparticles in improving the antioxidant mechanism of plants

Nanoparticles have the potential to interact with plant biological systems chemically or mechanically. This interaction largely originates from their intrinsic catalytic activity, large surface area and small size. The treatment of Ag and Au nanoparticles in *Brassica juncea* improved the actions of CAT, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase along with higher concentration of proline (Sharma *et al.*, 2012; Gunjan *et al.*, 2014). ROS are prepared in cell organelles in which enzymatic machinery activates the ROS-induced oxidative stress under different abiotic stresses. Nanoparticles support the reduction of such stress by triggering specific genes to accumulate osmolyte and supply free nutrient and AA (Mittal *et al.*, 2020).

Ceria oxide (CeO) nanoparticles can be used as antioxidants, and they have been shown to have an intense effect on plant growth. These types of nanoparticles increase the resistance against biotic and abiotic stresses. Nano-Ce can lessen the stress impact due to a unique redox property that depend upon the superficial passage between Ce^{3+} and Ce^{4+} oxidation state and therefore act as ROS collector (Collin *et al.*, 2014). Furthermore, CeO NPs with their high Ce^{3+}/Ce^{4+} ratios imitate SOD and make hydrogen-peroxide. It can also imitate CAT activity at low Ce^{3+}/Ce^{4+} ratios, and shows a scavenging effect (Wang *et al.*, 2012; Pulido-Reyes *et al.*, 2015).

Silicon NPs have an anti-stress effect at different concentration toward water deficit stress and saline stress by enhancing antioxidant system (Ashkavand *et al.*, 2015; Soleymanzadeh *et al.*, 2020). In faba bean, application of titanium dioxide (TiO_2) NPs improve the hydration status by improving the action of nitrate-reductase (NR) enzyme, resulting in enhanced osmolyte accumulation. The higher NR activity causes nitric oxide (NO) synthesis that eventually induce glycine betaine and proline synthesis (Khan *et al.*, 2020). Ahmed *et al.* (2018) noted that TiO_2 NPs tend to show both enzyme and non-enzyme defense systems against adverse conditions. TiO_2 NPs have a vital role in regulation of other enzymes, including glutamine synthase and glutamate hydrogenase. Another study (Song *et al.*, 2012) found TiO_2 NPs applied at low concentration (200 mg mL^{-1}) increased the enzymatic antioxidant (CAT, POD, SOD) through the elimination of ROS. However, at high concentration (500 mg mL^{-1}) TiO_2 NPs caused disruption of cell membrane. Use of zinc oxide NPs in tomato crop improved the development by enhancing the photosynthetic functions and antioxidant system (Faizan *et al.*, 2018).

24.4.4 Role of nanoparticles on the molecular mechanism of plants

Silver NPs modify the metabolic pathway and physiochemical properties of plants. It has been reported that Ag-NPs are genotoxic in plant cells. For example, Ag NP applied to onion crop plants resulted in chromosomal abnormality and cell wall interruption in onion crop plants (Kumari *et al.*, 2009). Moreover, Ag-NPs cause damage to DNA and numerous breaks in chromosomes, create chromatin bridges and affects metaphase (Panda *et al.*, 2011), resulting in NP-induced gene expression changes in plants. MicroRNAs (miRNAs) have also shown a statistically significant role in plant reaction to NPs by adjustable gene expression (Zhang *et al.*, 2006). Another study, which was reported by Garcia-Sanchez *et al.* (2015), showed that NPs introduced a significantly downregulated number of genes that are involved in the response to microbial pathogens, thus resulting in improved bacterial survival and colonization.. The use of TiO_2 NPs resulted in an enhancement in the expression of light harvesting complex II (LHC-II), resulting in photosynthesis improvement which transfer energy from chlorophyll *b* and carotenoids to chlorophyll, and spreading of light energy between PS-I and PS-II also enhanced the amount of light production (Ze *et al.*, 2011).

In wheat, proteomic and genomic alteration induced by Ag-NPs has also been analyzed with the help of DNA finger-printing technique and 2-DE coupled with LC-ESI-MS/MS (Vannini *et al.*, 2014). No significant DNA polymorphism was witnessed with Ag-NPs (10 mg L^{-1}) treatment; however, 2-DE profiling of plant parts (root, shoot) showed changed expression of various proteins mainly engaged in protein metabolic processes, protein preparation and metabolic adaptation of plant that are essential in alleviating unfavorable changes (Vannini *et al.*, 2014). In other study, Ag-NPs treatment in soybean grown under flooding stress affected the abundance of 107 root protein mainly linked with stress signals and cell metabolic process. A decrease was observed in the fermentative pathway toward normal cellular process and generation of moderately low cytotoxic byproduct resulted in better performance of soybean (Mustafa *et al.*, 2015).

24.5 Conclusion and future perspectives

Abiotic stress, including drought and salinity, is the major constraints that adversely affects agricultural crop productivity. The response of crop plants to these abiotic stresses is complex and involves changes in their morphological and physiological mechanisms. Nanomaterials can improve germination and seedling growth of crop plants by stabilizing physiological activities, including photosynthesis, nitrogen metabolism and activities of CAT, POD, SOD, ascorbate peroxidase and chlorophyll content. Nanoparticles enhance stress tolerance through root hydraulic conductance, increasing water uptake and the abundance of proteins involved in ROS detoxification and stress signaling. However, further investigation should be focused on characterization, standardization, biodegradability and the environmentally friendly nature of nanoparticles and their translocation in plants.

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25 Role of Organic Amendments in the Stress Tolerance of Plants

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Abstract

The defense mechanisms of plants to different types of environmental stresses becomes lower down due to the use of uneven artificial fertilizers. The synthetic fertilizers have detrimental impacts on soil, for example changing the soil pH and polluting the soil and underground water. On the other hand, the organic amendments or natural fertilizers improve the water saturation within the soil profile and makes water available to the plants under drought stress condition. Farmyard manure not only provides large amount of NPK, but also many other macronutrients and micronutrients. Cover crops and green manuring crops secure the plant from heat stress and chilling stress. Biofertilizers provide microorganisms, which helps in the degradation of large molecules and prevents the plant from nutrient deficiency. Compost maintains the soil temperature and soil pH. Compost containing chemicals such as phenols or tannins may have an antagonistic effect on soil-borne pathogens and protect composted trees and plants from different types of soil-borne diseases. Soil acidity and salinity can be controlled by the application of organic or natural fertilizers. In this chapter, we discuss different types of organic amendments, their comparison with synthetic fertilizers, and how they mitigate the effect of environmental stresses. This chapter also updates the understanding about sustainable agriculture farming by using organic amendments under climate change scenario.

25.1 Introduction

The activity of agriculture production systems has increased as a result of organic waste usage – the use of organic waste has not only increased, but it is also a beneficial approach in economic development (Hofmann, 2008). The impact of climate change and intensive horticultural practices would be a great practice for managing the organic waste product intending to improve the soil fertility and its health (Azim *et al*, 2017). Growth of plants could be retarded due to many environmental factors or abiotic stresses such cold, heat, drought, salinity, etc. which results in inhibited plant growth and development that ultimately causes low crop production and yield.

Stress conditions for any crop production could be negative as it adversely affects the yield and production. Among all the stresses, drought stress is the major cause of low production in those areas where water scarcity is the big issue. To overcome these issues, different types

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of organic amendments such as compost, biochar and plant growth-promoting rhizobacteria (PGPR) are very helpful in maintaining the soil health and fertility. Many experiments are carried out to check the individual and combined impacts of biochar and PGPR on productivity under stressful environments (Ullah *et al*, 2021). Under salinity stress, organic amendments (e.g. biochar, humic acid, PGPR and compost) play an important role by mitigating the stress affects. When the organic amendments are applied in abiotic stress conditions, they promote the nutrient uptake and availability to plants, which results in improving not only the physical and chemical soil health, but also results in increased yield in the plant. Therefore, organic amendments have an ability to maintain the soil fertility even in stressed environment (Rekaby *et al*, 2020).

Soil fertility is increased usually by the use of organic agriculture (OA); it is also helpful in alleviating the effects of climate change (Lehmann *et al*., 2011). Short- and long-term changes occur in the soil when it is provided with biotic or abiotic amendments that produce fluctuations in soil structure and also in microbial community. Microbiota present within the soil are important for plant growth and health – they are either beneficial or pathogenic in nature. Fluctuations in plant health and functioning is the result of external agricultural inputs, either organic or inorganic, and they should be avoided to induce unpredictable changes and effects for cultivated crops (Lehmann *et al*., 2011).

Soil structure and its physical properties such as availability of different nutrients, pH, nitrate content and organic matter content could be changed by the addition of OA products to the soil(Ansari and Mahmood, 2017). Also, OA products will help in inducing the changes in the microbial community of the soil. Residence and activities of soil microbiota are affected by the application of organic amendments to the soil. Due to OA, an improvement is found in soil quality as a result soil pH increase and a reduction in trace elements availability (Montiel-Rozas *et al*. 2016). In this chapter, we compare the use of organic fertilizers with inorganic fertilizers to minimize nutrient stress conditions for improved plant growth, as well as the role of organic amendments in protecting the plants from biotic and abiotic stresses.

25.2 Organic amendments and their comparison with synthetic fertilizers for nutrient availability

Environmental pollution is the result of excessive use of chemical fertilizers. Water bodies are polluted through eutrophication via the exposure of chemical fertilizers, which also causes air pollution (Youssef and Eissa, 2014). Presently, the demand for better quality produce under environmentally safe and sound practices by agricultural producers is very high. Use of organic fertilizers has advantages not only in crop production, but it also fulfills all the requirements necessary for sustainable agriculture as indicated by previous studies. Organic fertilizers show more benefits when compared to synthetic fertilizers. Soil biological and microbial activities could improve efficiently by using organic amendments as they have a high amount of organic matter content, which not only improves the physical and chemical health of the soil, but also has an ability to decrease the soil bulk density by improving aggregate stability (Zhang *et al*., 2009; Diacono and Montemurro, 2010). However, the excessive use of organic fertilizers does not provide the plants with sufficient nutrients, as the organic nutrients are released slowly into the soil. After application of organic amendments, in the first year only a single fraction of nitrogen and other nutrient elements becomes available for plant growth (Hartl *et al*., 2003).

Excessive use of artificial fertilizers as agricultural practice for crop production results in reduced ability of the ecosystem to provide better services and goods. Ground and surface water become polluted by the excessive use of synthetic fertilizers which may increase the contamination risk that produces toxic chemicals causing eutrophication and soil degradation (Tilman *et al*., 2002). All the sources necessary for crop production such as fertilizers and pesticides application for better yield are needed in intensive agriculture farming and for this use of capital and inputs is ensured on a large scale (Eurostat, 2015). If the fertilizers and pesticides are used excessively, then there is a chance to increase the risk of contamination of groundwater through leaching (Eurostat, 2015).

25.2.1 Farmyard manure (FYM)

Organic fertilizers applied as a source of mineral nutrients in the soil are present in different forms having different percentage compositions. For example, cattle slurry (49%) is the largest source of organic wastes in the form of farmyard manure (FYM 38%). Biosolids (sewage and sludge in treated form) and waste from industries (such as compost, brewery effluents and paper waste etc.) each account for ~2% of organic fertilizer (DEFRA, 2018). Around 5.4% of farms carry anaerobic digestion on farm processing of organic wastes (DEFRA, 2019).

Risk of CH_4 and N_2O emission to the atmosphere is also a result of using these organic wastes, therefore, the productive use of such manures should be in consideration by applying manures to grassland which are essential for maintaining productive swards and ensuring the positive or beneficial use of animal wastes for nutrient supplying (Chadwick *et al*., 2000; Rodhe *et al*., 2006). Animal wastes are applied to the soil for making it more fertile and enables it to get all the essential nutrients such as N, P and K, as they are applied from the synthetic sources of fertilizers (Ohara *et al*., 2004; Rees *et al*., 2013) (Table 25.1).

25.2.2 Press mud

Better crop production is also obtained through the application of press mud, which is the waste product of sugar industries as organic amendment (Diaz, 2016).The negative impact of chemical pesticides applied in large amount on soil could be controlled by the application of filtercake. So organic fertilizers are a rich source for making the soil more fertile and healthier. It not only helps in plant nourishment, but it also plays a role in plant's growth and development; it is also a rich source of sugar and minerals. Improved root proliferation and better aeration are also the result of different plant growth regulators, hormones, enzymes, auxins and vitamins that are present in sugar cane press mud. It is also a good source of retaining water in the soil and it is non-toxic in nature (Sardar *et al*., 2012).

Alterations in physical and chemical properties of soil are the result of secondary and micronutrient deficiencies due to excessive use of NPK fertilizers, which ultimately results in poor crop yield and production. Use and recycling of by-products of industries serve as the source of macronutrients, micronutrients and secondary nutrients (Dotaniya *et al*., 2016). Sugar filter cake is a rich source of mineral nutrients and when applied to soil as a manure improves its health and as well as crop yield. Press mud application also increases the organic carbon content in the soil (Fantaye *et al*., 2016).

A constant increment is noticed through analysis of copper and nickel concentrations when filter press mud is used (Khan *et al*., 2013). Biofuel and fertilizer production are the result of different sources such as nitrogen, cellulose, lignin, protein and sugar which are present in press mud (Table 25.2).

25.2.3 Poultry manure

Agricultural land is also benefited through the manure or litter produced by poultry wastes.

Table 25.1. Chemical composition of farmyard manure (Punjab State Council for Science and Technology, 2010).

Nutrients	Farmyard manure
N (%)	0.5
PO (%)	0.2
KO (%)	0.5
Ca (%)	0.9
Mg (%)	0.2
Fe (ppm)	146.5
Cu (ppm)	2.8
Zn (ppm)	14.5
Mn (ppm)	69.0
C:N Ratio	31.3

Table 25.2. Chemical composition of press mud (Diaz, 2016).

Nutrients	Ave amount/100 g of press mud (%)
OM	50
N	1
P	2–3
K	1–2
Ca	11
Mg	1
S	0.3
Fe	0.05
Cu	Trace
Zn	Trace
Mn	Trace

Nutrients such as nitrogen, phosphorus and potassium are recycled through the proper application of poultry manure in the soil if it is managed properly (Table 25.3). Nutrients that are available when manures are applied to the soil are not so much favored as they produce pollution and nuisance issues which are unsuitable for environment (Casey *et al*., 2006; Kaiser *et al*., 2009).

Soil biological and chemical properties are enhanced through the application of poultry manure and it also helps in overcoming the disposal issues of poultry manure (Friend *et al*., 2006; McGrath *et al*., 2009). Crop yield is reduced as a result of excessive manipulation of soil as the arable land is cultivated continuously, which ultimately results in soil degradation. Adding the poultry manure to the soil results in not only increasing the soil fertility, but it is also very helpful in improving soil organic matter content, water-holding capacity, soil bulk density and oxygen diffusion (Adeli *et al*., 2009).

25.2.4 Green manure

In situ plowing of any crop plant is commonly known as 'green manure'. In previous years, when use of inorganic fertilizers was not as familiar, green manuring was done as organic amendments for soil and crop like rice, wheat and sugarcane etc. (Chanda *et al*., 2011). Foliage of green manure crops is used as organic amendment for the soil and such crops may be leguminous and non-leguminous in nature that are usually grown on wastelands or on bunds.

Table 25.3. Chemical composition of chicken manure (Zayed *et al*., 2013).

Nutrients	Chicken manure
N (%)	3.52
P (%)	0.86
K (%)	1.83
Ca (%)	0.45
Mg (%)	051
Fe (ppm)	1050
Cu (ppm)	60.4
Zn (ppm)	230
Mn (ppm)	240

Valuable nutrient sources are gained from cereal residues which are retained in the soil having different proportions, i.e. 25% N and P, 50% S and 75% K. However, they could be used as organic sources to improve the soil fertility (Takkar, 2009). Soil health is improved by using green manure crops as they increase the nitrogen level in the soil, nitrate leaching is reduced and there is less nitrogen fertilization. Green manure crops not only enhance the grain yield of cereal crops, but they can also entirely or partially fulfill the need of their nitrogen fertilization (Jani *et al*., 2015).

Different soil properties (i.e. organic matter and other nutrients such as N, P, K, Ca and Mg) are improved by adding green manure crops in the soil. Crops like pawpaw, neem, moringa and mesquite are helpful in enhancing soil bulk density. While NPK individually do not increase the soil bulk density and soil organic matter content (Table 25.4), the addition of mesquite is more useful in enhancing soil health and the growth and yield of crops compared with NPK and use of other green manure crops (Adekiya *et al*., 2017).

25.2.5 Sewage sludge

Sewage sludge is comprised from different resources such as municipal wastewater plants and human excretory wastes that are mixed together. It can contain different types of heavy metals, organic wastes, pathogens and pharmaceuticals (Ostman *et al*., 2014). As waste material

Table 25.4. Nutrient content of important green manure and green leaf manure crops (Ramanjaneyulu *et al*., 2017).

	Nutrient content (% on dry weight basis)		
Crops	N	P	K
Green manure			
Sesbania aculeate	3.3	0.7	1.3
Crotolarea juncea	2.6	0.6	0.2
Tephrosia purpurea	2.4	0.3	0.8
Phaseolus trilobus	2.1	0.5	–
Green leaf manure			
Glyearicidia maculeata	2.9	0.8	2.8
Pongamia glabra	3.2	0.3	1.3
Azadirachta indica	2.8	0.3	1.3
Calotropis gigantean	2.1	0.7	3.6

from sewage and sludge contains high number of pollutants, it ultimately limits its use in agriculture. Different harmful results are obtained through the use of sewage and sludges as organic amendments because it contains a large number of heavy metals which are added to arable lands which ultimately causes toxicity in the soil that badly affects the soil microbial activities and biological processes (Giller *et al.*, 1998; Kirchmann *et al.*, 2017). Sequential fraction of sewage sludge reveals that only 10% of water soluble and extractables phosphorus is present in sewage sludge; all other forms of phosphorus are strongly bonded with iron (Fe) or aluminum (Al) (Xu *et al.*, 2012). Pot and field trials were also conducted to check nutrient availability by using sewage sludge as organic amendments, which is lower than that of mineral fertilizers (Delin, 2015; Krogstad *et al.*, 2015). Utilization of mineral nutrients from sewage and sludge by the plants is very low due to its negative impacts on crops (Hamner and Kirchmann, 2015).

Due to the following reasons the large amount of sewage sludge that accumulates in cities and towns are not equitably dispensed back into arable land: (i) the high moisture content and minimum fertility makes sewage sludge transportation to the disposal areas impassible; and (ii) therefore reduced availability of macronutrients and micronutrients to the plants in sewage sludge results in low yield even after heavy use of sewage sludge in low fertile soils for many years (Table 25.5). Nutrient availability extracted from city wastes rather than direct recycling of organic waste is a possible way forward (Kirchmann *et al.*, 2017).

Table 25.5. Chemical composition of sewage sludge (Ozdemir *et al.*, 2004).

Nutrients	Sewage sludge
Dry matter (%)	75
Organic matter (%)	35
Ph	6.03
EC (dS m^{-1})	2.14
Total N (%)	2.7
P (%)	1.25
K (%)	0.41
Ca (%)	3.76
Mg (%)	1.76
Na (%)	0.27
Fe (ppm)	41
Cu (ppm)	83
Zn (ppm)	1200
Mn (ppm)	402

25.2.6 Compost

Compost as natural amendments are critical in maintaining farming through furnished flora with vitamins and enhance the fertility and physical characteristics of land (Konsaeth et al., 2002; Mohamed *et al.*, 2020). Use of activator in the process of making compost might be play an important role in accelerating the process by decreasing the carbon nitrogen ratio (C:N). Microorganisms within the compost can degrade the unprocessed substances within the material (Iewkittayakorn *et al.*, 2018). Examples are sewage, fowl droppings, cow dung, goat dung, pig dung, etc. (Huang *et al*, 2017).

Nitrogen content should not be less than 1% and the phosphorus and potassium content should not be less than 1.5% according to the agricultural standards. In addition, the fertilizer must also contain calcium, zinc, copper and other important micronutrients in the quantity that range from 0.01% to 0.05% (Ayilara *et al.*, 2020; Table 25.6).

The mixing of compost into the soil should: (i) enhance soil physicochemical characteristics to improve seed germination and early plant maturity; (ii) increase fertility and accelerate activity of soil microorganisms, which may result in

Table 25.6. Chemical composition of garden compost (George and Duke, 1994).

Nutrients	Garden compost
pH	7.80
EC (mmhos/cm)	3.60
Total nitrate nitrogen (ppm)	156.5
P (%)	0.35
K (%)	0.48
Ca (%)	2.27
Na (%)	0.01
Mg (%)	0.57
Fe (ppm)	11690
Cu (ppm)	17
Zn (ppm)	128
Mn (ppm)	414
B (ppm)	25
Al (ppm)	7380

improving the crop nutritional status and an enhancement of the plant vegetative growth of plants; and (iii) have a low effect on the composition of the microorganisms population, with an improvement in bacterial dominance due to mixing of nitrogen-rich compost (Domínguez *et al*, 2020).

25.2.7 Biochar

Biochar is a brand-new sort of carbon (C) composite with excessive carbon, natural amendment and inorganic fertilizer content that has obtained massive interest because of its easy availability in soil remediation, chemical engineering and wastewater treatment (Ahmad *et al.*, 2013; Qambrani *et al.*, 2017). It can properly hold fertility in the soil, enhance the hydraulic traits of the soil, and decrease nutrient loss (leaching) from the soil through adsorption. These traits make biochar a helpful soil organic product for accomplishing efficient water and mineral utilization.Recent research has shown that the mixing of biochar in soil enhances the fertility of soil by providing nutrients (e.g., K, Ca, Mg and P) and improving physicochemical and organic traits of the soil, therefore also increasing plant growth (Liang *et al.*, 2009; Hasan, 2018). The percentage of nutrients is given in Table 25.7.

Biochar addition stepped forward the nutrients concentration along with phosphorus (P) and potassium (K) in loamy soil (Minhas *et al.*, 2020). Biochar is characterized by excessive natural carbon content, huge surface area, excessive porosity, and its fertility when applied in the field (Wang *et al.*, 2017). In addition, the pore shape of biochar gives more surface area for the attachment of microorganisms, resulting in anincrease of microbial activities in the soil (Ding *et al.*, 2017), improving soil fertility, enhancing crop growth and promoting plant resistance. The capacity of biochar to neutralize contaminated soils sourced from mining practices has been described by El-Naggar *et al.* (2020).

Table 25.7. Chemical composition of biochar. All analyses are expressed on a percent or concentration of oven dried biochar (Altland and Locke, 2012).

Nutrients	Biochar
C (%)	59.5
N (%)	0.2
P (%)	0.07
K (%)	0.50
Ca (%)	1.15
Mg (%)	0.27
S (%)	0.02
Si (%)	3.01
B (mg kg^{-1})	17.01
Cu (mg kg^{-1})	10.87
Fe (mg kg^{-1})	1609.9
Mn (mg kg^{-1})	323.3
Mo (mg kg^{-1})	4.13
Zn (mg kg^{-1})	9.26

25.3 Organic amendments and stress tolerance

25.3.1 Abiotic stress

At the time of their emergence land plant life 25.3.1 dwell a in genetically harsh surroundings. A huge range of physicochemical factors are aggressive to them, including cold and heat, drought or waterlogging conditions, heavy metals, excessive salinity and ultraviolet radiation (UV), among others. These abnormal conditions are collectively called abiotic stresses. These abiotic stresses are posing a serious threat to agricultural farming and the ecosystem, accounting for a huge reduction in crop yield (Wang *et al.*, 2003; Wania *et al.*, 2016).

25.3.2 Salt stress

The modern agriculture sector is facing a major environmental problem in the form of salt stress, which is related to drought. It causes a continuous loss of yields and the erosion of the surface of arable land, which damage the balance of global food (Munns, 2002; Rafat and Rafiq, 2009).

25.3.2.1 Effect of biofertilizers

Application of organic amendments is one of the best solutions to agricultural loss (Walker and Bernal, 2008; Ould Ahmed *et al.*, 2010). Biofertilizers can help in improving organic matter quantity and recovering degraded soils through the improvement of physical structure and enhancing essential nutrient quantity and thus crop production (Al-Moshileh and Motawei, 2007; El-Tantawy *et al.*, 2009). PGPR and actinobacteria improve plant production by producing siderophores, phytohormones, antifungal and antibiotic compounds, solubilizing phosphate and protection against phyto-pathogenic microorganisms. Water stress and salt stress resistance *Actinobacteria* have many plant growth-promoting characteristics that can potentially enhance plant production in salty conditions (Saidi *et al.*, 2021). Providing major nutrients, essentially N, P and K, the biofertilizers increase the nutrient concentration and growth of plants in high saline soils (Qadir *et al.*, 2008). Organic amendments can also increase the microbiological action of soil that has its main part in carbon mineralization and cycling of nutrients. Thus, biofertilizers indirectly affect the alteration and accessibility of important nutrients in plants (Wichern *et al.*, 2006).

25.3.2.2 Effect of organic manure

The mechanisms of salt stress tolerance mainly considers the balanced selectivity maintenance of Na and K uptake, which represents one of the necessary situations for the enhancement of crop growth. In this case, the transposition of ions, the main cause of salt stress (mainly Na) by mixing natural manure is a feasible plan in recovering salty soils (Garcia *et al.*, 2000). Such improvements in organic products will be effective only with a given quantity of biofertilizers in the presence of salt stress situations. Different organic amendments such as dung, compost and mulches have been utilized for soil application in order to minimize alternate effects of soil salt stress and enhancing plant output (Walker and Bernal, 2008; Mahdy, 2011). Due to the short growth period of potato crops, the use of a fast mineralization bio-fertilizer (e.g. poultry manure)as the main source of nutrients is important. Poultry manure has all the essential plant nutrients and without the use of inorganic fertilizers supply it is applied with the required nutritional quantity.

25.3.2.3 Effect of biochar

Biochar is taken into consideration as an activated soil carbon conditioner and as an organic amendment. It is one of the key organic products. Commonly, biochar is known to have a huge cation exchange capacity and alkaline in nature. It has many beneficial effects on soil characteristics as it increases the action of biological factors in soil (Lehmann *et al.*, 2011; Paz-Ferreiro *et al.*, 2014), Other physical and chemical characteristics of soil such as the pH of soil, porosity, electrical conductivity (EC) and soil accumulations are also changed due to the application of organic amendment in the form of biochar (Amonette and Joseph, 2009). As a soil organic product, biochar can be used for solving the problem of high salinity soil (Lehmann and Joseph, 2009). There are many research reports on the recovery of saline crop land after the use of biochar (Lashari *et al.*, 2013). Biochar has many beneficial actions toward resistance to salt stress in wheat crop and increasing crop stand, and growth improvement and performance. Biochar as an organic amendment, when applied in different levels, resulted in best germination, vegetative production, physiological, biological and chemical processes in both unaffected and salt-stressed conditions (Kanwal *et al*, 2017).

25.3.2.4 Effect of bagasse

Organic amendments such as poultry and farmyard manure, compost and organic waste products such as bagasse are known to have potential to recover saline condition and their effects on crop by changing the physical, chemical and biological characteristics of soil (Rady *et al.*, 2016; Malik *et al.*, 2018). The by-product of the sugarcane industry known as bagasse is the chief source of fiber content and essential nutrients and can also be used in enhancing agricultural output (Muhieldeen *et al.*, 2014; Oo *et al.*, 2015). Additionally, the mixing of bagasse in soil enhances the microorganism activities in soil, enhances soil water-holding capacity, maintains pH of the soil and the nutrient availability and sodium uptake ratio, and defends the plant from drought stress and toxicity situations (Oo *et al.*, 2015; Seleiman and Kheir, 2018). Some research reports on the use of bagasse or bagasse ash for crop output enhancement in normal soil conditions (Muhieldeen *et al.*, 2014) or in salt-affected soils (Seleiman and Kheir, 2018) are available. Research work on the use of bagasse compost for wheat production under saline conditions is clearly missing.

25.3.3 Drought stress

Water stress is the primary abiotic stress that affects the plant growth and yield components as well as decrease crop growth (Aslam *et al.*, 2020). Water stress is the main threat for the dry land and semi-dry land zones of the world. More severe drought situations are expected in the future, due to increase in average global temperature by 1.5°C and also due to variations in climate (IPCC, 2018) which will significantly change the agricultural output and farming system (World Bank, 2018). At the same time, the Pakistani population is rising (2.1%), a rate that is much higher when compared to the average world population rate (1.1%) (World Bank, 2018).

25.3.3.1 Effect of biochar

As an organic fertilizer, biochar is used to recover soil carbon, organic products, enhance soil fertility, improve soil water-holding capacity and hold desired soil structure. Soil organic amendment as (biochar) is helpful in increasing soil nutrient status and maintaining soil moisture contents. Application of biochar significantly enhance the crop production (Haider *et el.*, 2020).

Biochar as pyrolyzed organic material can be utilized as a soil organic fertilizer (Khalili *et el.*, 2020) for the uptake of carbon and simultaneously increasing the physicochemical characteristics and functions of soils. Amendment of biochar has no negative impact in both the short-term and in long-term (Lehmann and Joseph., 2009). An enhancement in the water-holding capacity of the soil has been found by mixing the soil with biochar under various situations (Lehmann *et el.*, 2011). Biochar application not only improves the transfer ability of ions and as well as the physicochemical structure and fertility of the soil (KeshavarzAfshar *et al.*, 2016), but it also decreases the effect of heavy metal toxicity on different crops (Chan *et al.*, 2007). It also enhances the mode of action of microorganisms, nutrient holding and soil exchange capacity (KeshavarzAfshar *et al.*, 2016). Biochar has a high concentration of pores and surface area, which is useful in minimizing the effect of drought stress (Downie *et el.*, 2009).

25.3.2.2 Effect of compost

Different plant materials and animal wastes, which are used in compost production, have been found to enhance the output of different crops cultivated on low nutrients or nutrient-deficient soil (Adejumo *et al.*, 2010). Other than increasing the soil fertility status, it has also been used in enhancing crop production on heavy metal affected soils by minimizing the effect of heavy metals on crops (Adejumo *et al.*, 2013). On the other hand, the physiological performance for tolerance to water-deficient conditions in crops at various growth stages and the use of natural manure in removing the damage caused by water-deficient conditions in crops still needs further research. In another report, it was narrated that use of organic fertilizer significantly improved the drought tolerance in okra when induced at the reproductive stage (Adejumo *et al.*, 2018).

25.3.3.3 Effect of sewage sludge

Biosolids can be used with inorganic fertilizers for maintaining the fertility of soil. Sewage sludge is considered to be the origin of nutrients in various agronomic crops and is the secondary product of the process of wastewater treatment (Samara *et al.*, 2017). Soil application of sewage sludge can enhance the soil profile by sustaining or enhancing the content of organic matter in the soil and enhancing different characteristics of the soil (Bueno *et al.*, 2011). In addition, using sewage sludge in the agriculture sector is considered to be an alternate, cheap waste disposal method of this material compared to landfill or burning (Antonkiewicz *et al.*, 2019). Sewage sludge used as a fertilizer can be the most prominent way to minimizing the adverse effects of drought stress on crop output in dry land and semi-dry land areas. Application of various levels of sewage sludge-based fertilizer improved the morphological, physiological and production performances of wheat under water-deficient conditions (Boudjabi *et al.*, 2019).

Use of sewage sludge under extreme water stress (Zhang *et al.*, 2014) improved all the variables which were studied, and in addition positive correlation was found in attributes and with increased doses of sewage sludge (Debiase *et al.*, 2016). Water shortage and warmth pressure can adversely affect achene yield and oil best, depending at the timing of pressure with respect to crop phenological processes and the degree of compensation among yield additives (Hussain *et al.*, 2018). Specifically, drought pressure from early flowering to seed filling, and excessive temperatures at flowering stages adversely affect leaf growth and transpiration ratesin addition to flower pollination, resulting in a decrease in achene yield (Garcia-Lopez *et al.*, 2014).

25.3.3.4 Effect of farmyard manure

Animal manure can provide the necessary nutrients required by the plant during the growth season and also improved the organic matter and physiochemical properties of soil, and reduced the influence of water scarcity through improving water-retention capacity of the soil (Jalilian *et al.*, 2012). As a good way to promote sustainable agriculture, the blended chemical-manure-biological fertilizer mixture can alleviate the negative effects of water deficiency in the vegetative plant; it can also enhance the secondary medicinal compounds, particularly omega-three unsaturated fatty acids (Hosseinzadeh *et al.*, 2021). Farmyard manure can reduce the prices and the risks of soil, water and plant infection with nitrogen fertilizer residues as well produce as boom crop yields (Heidarian *et al.*, 2018). The addition of organic fertilizers, such as cow manure to soil results in increasing the combination balance, the water infiltration rate, and the water-holding capability and decreases soil bulk density (Barzegar *et al.*, 2002).

25.3.4. Temperature stress

Recent climate alternate research has tested that international intensity of storms, flooding and different extreme climate activities with increasing temperatures may also ultimately influence food production. Worldwide movement fashions are expecting that floor air temperatures may additionally boom 4–5.8°C over the next few years (IPCC, 2007). This temperature increase will mean that most plants will be exposed to heat stress conditions during some stage of their life cycle. The increase in average temperature will also affect night-time temperatures, which may show a decrease in radiant warmth loss due to increased cloud cover (Peng *et al.*, 2004; Welch *et al.*, 2010). Both long- and short-term exposure are predicted to occur more frequently over the coming years, affecting many factors of crop growth and development and reducing crop yield and lowering crop quality. Most studies assessing how heat stress influences crop production have assumed there is no difference between day or night temperature effects (Peng *et al.*, 2004).

25.3.4.1 Effect of biochar

Major nutrients such as nitrogen, phosphorus, potassium are supplied by the application of biochar. During the recent years damage induced by the heat stress in plants is mitigated by exogenous application of nutrients like phosphorus and potassium (Hasanuzzaman *et al.*, 2013). Biochar applied along with phosphorus in heat stress was found to be more effective than individual application of biochar and phosphorus (Fahad *et al.*, 2016).

The impact of biochar and phosphorus on heat stress and its effect on pollen properties, physiological variation and pollen germination are still unknown. Novak *et al.* (2009) reported that the availability and uptake of phosphorus is enhanced by the application of biochar, and availability of aluminum and iron is reduced by biochar in the soil. Under extreme heat stress, rice crops field treated with biochar and phosphorus enhances the growth and yield of rice even in stressful conditions (Fahad *et al.*, 2016).

25.3.4.2 Effect of farmyard manure

Excessive application of nitrogen fertilizers such as urea becomes the reason for weak resistance and elasticity of soil while. However, fertilizers that are applied in balanced way along with organic manures like farmyard manure results in high soil resistance and elasticity, which improves the physical and chemical health of the soil (Kumar *et al.*, 2013). It is hypothesized that the resistance and resilience of soil functions against abiotic stresses like high temperature are affected by the use of long-term fertilizer application (Rakshit *et al.,* 2012; Chakraborty *et al.*, 2014).

Soil organic matter content is enhanced by the application of farmyard manure, and as a result soil resistance against abiotic stresses is enhanced and its fertility improved. The soil also shows sustainable microbial activity (Kumar *et al.,* 2014).

25.3.4.3 Effect of press mud

Press mud is a lightweight, light-colored, amorphous, spongy material (Mohamad *et al.* 2018). The application of compacted press mud can significantly improve soil structure and crop production (Dotaniya *et al.*, 2016; Chattha *et al.,* 2019). Press mud creates a problem of dumping and extreme heat (65°C) and a bad smell in the area in its application (Bhat *et al.*, 2014). The interest in heat tolerant plants has never been greater, driven mainly by concerns about how the environment will be affected by climate change (IPCC, 2014). Reused material such as press mud prevents their dumping in the environment, thereby maintaining a balance between economic development and environmental protection (Kumar *et al.,* 2016).

25.3.5 Waterlogging

Water logging is one of the most pressing abiotic pressures affecting plant growth (Setter and Waters, 2003; Lone *et al.,* 2018). In the summer, it is common to see large agricultural areas flooded with water. When the soil is immersed, air is not added and the soil quickly becomes anoxic and reduced, changing the biological and chemical properties of soil (Kirk, 2004). Soil pH is probably the soil's most important chemical parameter (Bloom, 1999). Levels of biological degradation are much faster under oxidizing conditions, prior to free O_2 (Macias and Camps Arbestain, 2010).

25.3.5.1 Effect of compost

Waterlogging of sandy soil for up to 3 weeks and clay loam soil for as long as 4 weeks decreased their pH values and thereafter those values tended to barelygrow and the addition of compost further decreased soil pH. In both soil types, barely growhardly and the addition of compost further decreased soil pH. In both soil types, decline in pH results in low cost and productivity if prolonged in the firstweek of water logging (Amel and El-Latif., 2015). Waterlogging soil increases the availability of P, Fe and Mn, whereas the availability of Zn and Cu decreases. The addition of compost decreased the availability of the investigated factors. This trend can be due to the formation of insoluble organic complexes (Amel and El-Latif., 2015).

25.3.5.2 Effect of green manure

The incorporation of herbaceous perennial legumes such as lucerne, clovers and Messina (*Melilotus siculus*) adapted to waterlogging, and including the material into cropping systems has been suggested to reduce waterlogging (Cocks, 2001; Nichols, 2018). Another way of reducing waterlogging is through a similar practice where large volume of organic matter with high N levels are placed within and above the heavy clay layers. This practice is referred to as sub-soil manuring (Gill *et al.*, 2009; Celestina *et al.*, 2018).

25.3.5.3 Effect of farmyard manure

According to Xu (2001), paddy soil organic matter is reduced while soil bulk density is increased by extensive chemical fertilization. Tian *et al.* (2021) stated that in several parts of the world, crop yield is reduced the firstweek of water due to abiotic stresses, especially salinity. Clark (2007) reported that salt-affected soils can be reclaimed by the addition of the organic matter in the soil that improved the growth and development of the plants. Addition of organic matter accelerates the process of cation exchange and leaching of toxic salts. However, according to Ghafoor (2008), the rate of dissolution of $CaCO_3$ liberating Ca for the exchange of Na is increased by the addition of the organic matter.

25.4 Biotic stress

25.4.1 Disease and insect pest attack

Across the world, the annual loss due to a particular disease is relatively small, but when combined with loss due to quality and aesthetic values of plants, the accumulative loss is a substantial loss to the farmer. Maloy (2005) stated that reduction in the economic and aesthetic losses caused by plant diseases is the major goal of the plant disease management.

25.4.2 Effect of organic manure

To stay protected, the plants must grow in a balanced environment. Over lush plants also result in economic loss as they are more prone to plant diseases. Visual clues appear on the surface of the over fertilized plants, and these plants become an easy target to the diseases and insects. Surekha and Rao (2000) stated that the addition of the organic manures to the plant results in the production of the defense compounds within plant that prevent the plant from pest attack. They also noted that the effect of fruit borers was much reduced in plants where organic materials (e.g. farmyard manure and vermicompost) were applied when compared to those plants treated with straight fertilizers (e.g. NPK inorganic fertilizers).

25.4.3 Effect of neem cake

Balasubramanian and Muralibaskaran (2000) stated that as compared to a full dose of nitrogen, if a 75% dose of nitrogen along with organic amendments (e.g. neem cake, castor cake, poultry manure, sewage sludge and sheep manure) was applied to the crops, then it will show better results in controlling the sucking pests of cotton. Rajaram and Siddeswaran (2006) showed that 45 and 60 days after sowing, the population of leafhopper was reduced by 0.62 and 1.27/leaf when neem cake was applied at the rate of 250 kg/ha. Balakrishnan *et al.* (2005) stated that damage caused by cotton stem weevil was significantly reduced when basal application of neem cake and farmyard manure was done at the rate of 250 kg/ha and 12.5 ton/ha followed by earthing up 25 DAS.

The lowest (14.3%) infestation by the shoot borer and fruit borer of brinjal was observed when neem cake was applied at 1.7 ton/ha (Godase and Patel, 2003). When nitrogenous fertilizers were applied, this increased the level of incidence further. However, in mango orchards it was observed that farmyard manure remained effective for 3 months in controlling the termites while the neem cake remained effective for 4 months (Singh and Singh, 2003).

25.4.4 Effect of compost

According to Ramesh (2005), organically grown crops show more resistance to the attack of insects. Altieri (2012) stated that organic crops are less susceptible to insects, pests and diseases because these have balanced nutrition. Natural predators of plant pests are also affected by the type of fertilizer used (Banfield-Zanin, 2012). Up to 51–72% inhibition in the mycelial growth of pathogens was observed when vermicompost and vermi-wash was applied to plants (Sinha *et al.*, 2010a). In managing the insect, pest and diseases, the most crucial factor is planning and execution of the soil fertility program.

The most vital thing about vermicompost and earthworms is that they provide a double benefit to the crop. It controls the insects, pest and diseases in addition to the successful crop production as stated by Sinha *et al.* (2010b).

25.4.5 Effect of biochar

There is limited information on the impact of biochar as a soil amendment on plant resistance to diseases. An experiment was conducted by Matsubara (2002) on the effect of arbuscular mycorrhiza (AMF) fungal inoculation on asparagus tolerance to fusarium root rot. He incidentally revealed that soil-borne fungus were significantly controlled by the amendment of soil with charcoal. He further revealed that AM colonization of asparagus seedlings was enhanced by the addition of charcoal produced by the coconut fiber, which also reduced the impact of various fungi. Another study showed that as compared to non-amended control, root lesion caused by *Fusarium oxysporum* f. sp. asparagi and *F. proliferatum* was significantly reduced by the addition of biochar made from ground hardwood (Elmer and Pignatello, 2011).

The mechanisms of disease suppression by biochar can be summarized as follows: (i) induction of systemic resistance in host plants through bioactive translocated metabolites; (ii) enhanced biodiversity of beneficial microbes such as mycorrhizal fungi in soils; (iii) modulation of soil fertility in terms of bioavailability of nutrient and abiotic factors like liming effect; (iv) direct fungi-toxic effect; and (v) indirect effects through allelopathic and phytotoxic compounds sorption, which can directly harm plant rhizosphera and therefore stimulate pathogen occurrences.

However, according to Ippolito (2016), a side effect of the biochar which reduces its efficacy is that it absorbs the agrochemicals such as insecticides, herbicides and pesticides.

25.5 Conclusion and outlook

It can be stated that not only is plant growth and soil fertility improved by organic fertilizers, but it also helps in the eradication of environmental stress. There is no doubt that plants show faster growth when inorganic fertilizers have been applied, thus helping with the mitigation of food shortage. However, inorganic fertilizers also have drastic effects such as the production of several harmful compounds and gases like methane, carbon dioxide and ammonia. Our environment and water bodies are affected by these chemicals and it also causes both biotic and abiotic stress. In comparison, organic fertilizers like compost, green manures, biochar, press mud, poultry manures and farmyard manures relieve various biotic stress (insects, diseases, pests and microbes) and abiotic stress (gases, nutrients, water, chilling, heat, temperature and light).

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26 Endophytes as Potential Biostimulants to Enhance Plant Growth for Promoting Sustainable Agriculture

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Abstract

Plant growth-promoting endophyte (PGPE) is a prerequisite for plant productivity, health, ecosystem functions and community organization. The endophytic relationship of microbes with the host plants is important for potential plant growth enhancement. These endophytes originate from interior tissues of roots and aerial plant parts of various host plants. The endophytic plant–microbe relationship is initiated with the colonization of root surfaces and invasion of the interior root structure, followed by the movement of endophytes into aerial tissues of the host plants. The ability of some endophytic diazotrophs in fixing N_2 provides a promising source of N input which can substitute chemical nitrogen fertilizers for the host plants. Other than N, endophytes are also capable of solubilizing inorganic phosphate, potassium and promoting P and K uptake. Additionally, endophytes are also known as producers for indole-3-acetic acid (IAA) which is among the vital phytohormones for plants. They are also capable of expressing 1-aminocyclopropane-1-carboxylate (ACC) deaminase to lower the levels of plant ethylene produced when they are exposed to stress and subsequently enhance plant growth. Most endophytes can also synthesize iron-chelating siderophore that solubilizes insoluble iron in soil and facilitate in reducing heavy metals stress. In addition, with the current development of the next-generation sequencing technology, fundamental knowledge on genomic information of endophytes has improved the understanding of gene functions of selected plant-associated PGPE. This chapter discusses the importance of the application of selected beneficial endophytes as biostimulants for the promotion of sustainable crop production.

26.1 Introduction

Endophytes are endosymbionts that persist in interior tissues of healthy plants during their lifecycle, either throughout or part of their life span, without harming the host plants or its exterior structure (Kuklinsky-Sobral *et al.*, 2004; Hardoim *et al.*, 2008; Pedraza *et al.*, 2009; Taghavi *et al.*, 2010; Jasim *et al.*, 2013; Nair and Padmavathy, 2014; Trujillo *et al.*, 2014; Miliute *et al.*, 2015). The endophytes (‘endo’, inside; ‘phyte’, plant) exists *in planta* or in plants for

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beyond 120 years. Endophytes that endure in leaves have been recognized as efficient plant invaders (Dublan *et al.*, 2014; Berg *et al.*, 2013) and growth enhancers (Taghavi *et al.*, 2010; Tharek *et al.*, 2021). However, its presence *in planta* has brought up numerous questions on how it could ascendingly move from the rhizosphere into the phyllosphere. According to previous studies, strategies of ascending migration *in planta* involves preliminary root adhesion, invasion and colonization of the plant tissues followed by its establishment (Böhm *et al.*, 2007; Hofkin, 2010; Dublan *et al.*, 2014). The ascending actions are also believed to occur once endophytes that exist in the soil enters plant roots (Chi *et al.*, 2005).

The endophytes are categorized either as obligate or facultative, depending on their lifestyle. Obligate endophytes stringently rely on the host plant for their survival and growth, whereas facultative endophytes can survive in both the interior and exterior tissues of the host plant. Facultative endophytes can be classified into passenger, opportunistic and competent endophytes (Hardoim *et al.*, 2008). Passenger endophytes are endophytic bacteria that unintentionally enter the host plant via root invasion by nematodes or through natural wounds. Opportunistic endophytes occasionally penetrate the host plants in response to chemotactic stimuli toward root exudates, followed by rhizoplane colonization and invasion of the internal root tissues via cracks at the root tips and lateral root emergence. However, colonization and spreading of both passenger and opportunistic endophytes are limited only in the root cortex. In contrast, competent endophytes have the capability to actively invade and spread into the entire plant and sustain *in planta* even though in the presence of high densities of bacteria (Hardoim *et al.*, 2008). These ubiquitous endophytes colonize both locally and systemically, besides influencing plant health via disease suppression, contaminant degradation and plant growth promotion (Pedraza *et al.*, 2009; Kumara *et al.*, 2014).

Isolation of endophytes *in planta* can be obtained from surface sterilized healthy plant tissues (Hallmann *et al.*, 1997; Zinniel *et al.*, 2002; Chi *et al.*, 2005). Various strains of endophytes which includes both Gram-negative and Gram-positive bacteria have been previously isolated from the interior plant tissues of root, stem and leaf of miscellaneous species of plants (Table 26.1). Interestingly, several different bacterial species have been discovered from a single host plant (Zinniel *et al.*, 2002; Tharek *et al.*, 2011; Khairuddin, 2012). The endophytes include culturable and unculturable bacteria (Suzuki *et al.*, 2005; Taghavi *et al.*, 2010; Pedrosa *et al.*, 2011). Research performed on culturable and unculturable microorganisms of the rice roots cv. APO determined diversities of prokaryotic endophytes originating from 16 classes or phyla (Hardoim, 2015). In this study, Gammaproteobacteria was found to be the most frequently isolated group, followed by Alphaproteobacteria (Hardoim, 2015). The presence of broad diversities of bacteria *in planta* is probably due to their capability to invade and sustain in interior plant parts (Hardoim *et al.*, 2008). Sustainability of the indigenous endophytes within the plant tissues varies between 2.0 and 6.0 $\log_{10}$ CFU per g for sweet corn, potato, alfalfa, squash, sugar beet and cotton. Inoculation of endophytic bacteria on root or via seed drenching also exhibited similar population concentrations (Zinniel *et al.*, 2002). However, concentration of endophytes inoculated to the host plants may exceed 8.0 $\log_{10}$ CFU per g or higher (Zinniel *et al.*, 2002; Nautiyal *et al.*, 2010).

The population of both introduced and indigenous endophytes varies depending on the plant tissue type, plant age, plant source, environment and time of sampling (Lamb *et al.*, 1996). These endophytic bacteria can colonize both the intracellular and intercellular spaces of different plant parts (Compant *et al.*, 2011; Nair and Padmavathy, 2014; Miliute *et al.*, 2015). Populations of endophytes were found higher in the root and decreases in the stem and leaf of the host plants (Lamb *et al.*, 1996; Tharek *et al.*, 2021). However, the limitation of their ascending actions after initial root invasion, *in planta* population dynamics and the potential benefits to the host plants remains a vital concern. This chapter discusses on endophytes as potential biostimulants to enhance plant growth for the promotion of sustainable agriculture.

26.2 Plant growth-promoting endophyte

Plant growth-promoting endophytes (PGPE) are endophytes that have the ability to promote

Table 26.1. Endophytic bacteria isolated from different host plants and different plant parts/tissues.

Endophytic bacteria	Host plant	Plant tissues	References
Pseudomonas spp., *Sphingomonas* spp., *Stenotrophomonas* spp.	Cucumber	Roots	De Hita *et al*. (2020)
Burkholderia spp.,	Poplar	Roots	Kandel *et al*. (2017)
Pseudomonas spp.	*Phalaris arundinacea*	Roots	Fan *et al*. (2020)
Bacillus spp.	*Solanum dulcamara*	Roots	Fan *et al*. (2020)
Mucilaginibacter spp.	*Scorzoneroides autumnali*	Roots	Fan *et al*. (2020)
Rhizobium spp.	*Glycine max*	Roots	Fan *et al*. (2020)
Rhizobium spp., *Rhodococcus* spp., *Agrobacterium* spp.	Tomato	Roots	Abbamondi *et al*. (2016)
Pseudomonas guinea, *Rhizobium giardinii*, *Sphingomonas insulae*	*Stellera chamaejasme* L.	Roots	Jin *et al*. (2014)
Bacillus megaterium, *Burkholderia diazotrophica*, *Rhizobium tropici*	Sugar cane	Roots	Paungfoo-Lonhienne *et al*. (2014)
Burkholderia phytofirmans PsJN	Onion	Roots	Sessitsch *et al*. (2005)
Burkholderia sp., *Curtobacterium* sp., *Rahnella aquatilis*, *Pseudomonas* sp.	Poplar	Stem	Kandel *et al*. (2017)
Curtobacterium sp.	Willow	Stem	Kandel *et al*. (2017)
Achromobacter piechaudii	*Sedum plumbizincicola*	Stem	Ma *et al*. (2016)
Pseudomonas cremoricolorota, *Sphingomonas insulae*, *Bacillus safensis*	*Stellera chamaejasme* L.	Stem	Jin *et al*. (2014)
Pseudomonas spp., *Microbacterium oleivorans*, *Sphingomonas* spp.	*Salix purpurea*	Stem	Gan *et al*. (2014)
Herbaspirillum rubrisubalbicans,*Burkholderia brasilensis*	Banana	Stem	Cruz *et al*. (2001)
Curtobacterium spp.	Poplar	Leaf	Kandel *et al*. (2017)
Pseudomonas resinovorans, *Pantoea dipersa*, *Mesorhizobium chacoense*	*Stellera chamaejasme* L.	Leaf	Jin *et al*. (2014)
Escherichia coli	Spinach, rocket salad, oil palm	Leaf	Meric *et al*. (2013); Tharek *et al*. (2017)
Bacillus subtilis, *Pseudomonas fluorescens*, *Sphingomonas parapaucimobilis*	Switchgrass	Leaf	Gagne-Bourgue *et al*. (2013)
Gordonia terrae	Mangrove	Propagule	Soldan *et al*. (2019)

growth of its host plant. The PGPE have been found in interior tissues of plant roots and shoots (Lindow and Brandl, 2003; Tharek *et al.*, 2021). Its presence in leaf tissues has been hypothesized to be caused by the migration of endophytes from roots into leaf tissues via xylem vessels. Occurrence of the migration is probably due to the abundance of nutrient and iron resources in the phyllosphere of the host plants (Bodenhausen *et al.*, 2013; Tharek *et al.*, 2021). Furthermore, the ability of PGPEs in the establishment of mutualistic relationships with the host plants contributes toward beneficial effects to the host plant (Hardoim *et al.*, 2015). Recently, the numbers of PGPEs have increased significantly, which comprise numerous bacteria species such as *Azospirillum, Burkholderia, Enterobacter, Bacillus, Pseudomonas, Arthrobacter, Alcaligenes, Azotobacter, Serratia, Klebsiella* and *E. coli*. These PGPEs have been described as plant growth enhancers (Kloepper *et al.*, 1989; Okon and Labandera-Gonzalez, 1994; Glick, 1995; Gururani *et al.*, 2012; Tharek *et al.*, 2021). The process could be accelerated via establishment of symbiosis between tissue culture plant materials and PGPEs (Lim *et al.*, 2018).

PGPEs such as *Azomonas*, *Klebsiella*, *Azospirillum*, *Pseudomonas*, *Azotobacter* and *Bacillus* have been commonly used as bioinoculants to enhance growth and development of crucial agricultural crops. The application of PGPEs as bioinoculants has become an attractive approach to replace and reduce pesticides, chemical fertilizers and supplements (Stefan *et al.*, 2008; Ashrafuzzaman *et al.*, 2009), thus resulting in decreased health risks and environmental pollution due to use of excessive agrochemical fertilizers (Tambalo *et al.*, 2015). Besides that, PGPE inoculation has also increased plant growth, photosynthetic activity, harvest index, N fertilizer use efficiency, N content of shoots and grains, and grain yield of rice (Chi *et al.*, 2005). *Pisum sativum* inoculated with *Enterobacter cloacae* MSR1 which was isolated from internal tissues of *Medicago sativa* also exhibited significantly enhanced dry weight and length of its host plant compared to the control plants (Khalifa *et al.*, 2016). Alternatively, a conventional micropropagation method via biotization of an endophytic diazotroph, *Herbaspirillum seropedicae* strain Z78 (ATCC 35893) introduced to *in vitro* plant materials of oil palm (*Elaeis guineensis* Jacq.) revealed the success of pelleted and sonicated Z78 on growth promotion of *in vitro* embryogenic calli and calli of oil palm under symbiotic conditions (Lim *et al.*, 2016).

According to Glick *et al.* (1999), there is more than one mechanism of action that affects plant growth directly at different times through the plant lifecycle. It could occur *via* direct supply of nutrients through solubilizing and mineralizing of phosphate (Kiers *et al.*, 2003; Berg, 2009; Richardson *et al.*, 2009) and potassium (Meena *et al.*, 2014). Additionally, many PGPEs can supply vitamins and iron to the host plant (Richardson *et al.*, 2009). Endophytes in tomatoes also harbored several plant growth-promoting traits such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, indole-3-acetic acid (IAA) and siderophore production (Abbamondi *et al.*, 2016). The presence of plant growth-promoting traits in PGPEs plays a crucial role in the enhancement of plant growth (Ahemad and Kibret, 2014; El-Sayed *et al.*, 2014; Khalifa *et al.*, 2016; Tharek *et al.*, 2017). Recently, Tharek *et al.* (2021) demonstrated *in planta* invasion, colonization and plant growth enhancement by *Escherichia coli* USML2 in association with rice seedlings. The study also revealed the potential of *E. coli* USML2 as a genetically amenable endophyte that can be employed in a sustainable agriculture practice especially for rice planting. Earlier findings by Lim *et al.* (2018) had successfully shown modification on the biochemical profiles of oil palm calli and embryogenic calli associated with endophytic diazotroph *H. seropedicae* (Z78). Thus, this exhibits that selection of the most appropriate endophyte which consists essential traits for plant growth enhancement is essential (Etesami *et al.*, 2015).

26.3 Plant growth-promoting traits

26.3.1 Nitrogen fixation and metabolism

Beneficial bacteria that are equipped with the ability to fix atmospheric N_2 and convert it into ammonium provides a promising source of N input in agriculture. In fact, biological nitrogen fixation (BNF) acts as a substitute to chemical nitrogen fertilizers (Carvalho *et al.*, 2014). BNF bacteria such as *Azospirillum*, *Gluconoacetobacter diazotrophicus*, *Azotobacter* and *Azocarus* are known as diazotrophs (Ahemad and Kibret, 2014). According to Tan *et al.* (2015), free-living diazothrophs capable of producing nitrogenase enzyme can have a major impact on the growth of its host plant. Significant effects of different N concentrations supplied to endophytic diazotroph *H. seropedicae* (Z78) was observed on nitrogenase enzyme activity. However, nitrogenase enzyme activity significantly decreased in the presence of higher N levels, resulting in increased auxin production, which are important factors influencing growth of associated host plants (Tan *et al.*, 2015).

Unfortunately, not all endophytes have the capability to fix atmospheric N_2. Endophytes lacking nitrogen fixation properties would assimilate nitrate as its nitrogen source. The assimilation of nitrate is initiated by active nitrate uptake by ABC-type transporters. In the cytoplasm, the assimilatory nitrate reductase (encoded by the *nas* genes) will thus convert nitrate to nitrite. Finally, nitrite is reduced to ammonium by the assimilatory nitrite reductase which is readily available for the plant uptake (Rediers *et al*., 2009). Non-diazotrophic endophyte such as *Enterobacter* sp. 638 consists necessary genes for the assimilatory nitrate reduction (Taghavi *et al*., 2010). Genes for nitrate assimilation are expressed when nitrate is available but other nitrogen fixing forms such as ammonia is limited (Shapleigh, 2009). However, once the endophytic bacteria are *in planta*, it is uncertain whether the host plants acquire nitrogen from these endophytes. Beltran-Garcia *et al.* (2014) revealed that the presence of endophytes *in planta* stimulated higher plant biomass than heat-killed bacteria due to transfer of more nitrogen to the host plants. In addition, under nitrogen restraint, some plants might degrade the endophytes as nitrogen source (Beltran-Garcia *et al*., 2014). Induction of nitrogenase enzyme activity by an endophytic diazotroph *H. seropedicae* (Z78) was also reported to successfully promote the proliferation and differentiation of oil palm embryogenic calli and calli under *in vitro* conditions (Lim *et al.*, 2016).

26.3.2 Phosphorus solubilization

Phosphorus (P) is among the most limited macronutrients after nitrogen (N). However, P is particularly crucial for almost all major metabolic processes in plants, including photosynthesis, macromolecular biosynthesis and respiration (Sharma *et al*., 2013). Plants deficient in P usually show inhibited stem and root development, poor flowering and lack of seed and fruit formation (Ji *et al*., 2014). It is applied to soil in the form of phosphatic fertilizers. However, only 0.1% of the P content in soil (~0.05% w/w) is available to the plants due to poor solubility (Sharma *et al*., 2013). While a large portion of soluble inorganic phosphate applied is rapidly immobilized, this makes it unavailable to plants. Selected endophytic bacteria capable in solubilizing phosphate are considered to be important soil inhabitants that are also known as phosphate solubilizing bacteria (PSB) (Zhu *et al*., 2011). Abundant species of endophytic PSBs have been isolated, including *Pseudomonas, Arthrobacter, Azotobacter, Bacillus, Agrobacterium, Flavobacterium, Erwinia, Micrococcus, Enterobacter, Salmonella, Bradyrhizobium, Chromobacterium, Alcaligenes, Streptomyces, Thiobacillus Serratia* and *Escherichia*.

These PSBs are involved in a range of processes that affect the transformation of soil P which are an integral part of the soil P cycle. The PSBs contribute to the release of P from organic and inorganic pools of total soil P via mineralization and solubilization (Hilda and Fraga, 1999; Chen *et al*., 2006). The ability of PSBs in conversion of insoluble P into accessible form of P is an important trait for a PGPE to increase plant growth and yields (Chen *et al*., 2006; Sharma *et al*., 2013). Additionally, increase in P uptake by plants was observed due to the application of endophytic PSBs as inoculants. Subsequently, *Rhizobium* sp. MR-54 mutants which are deficient in mineral phosphate solubilization demonstrated reduced plant growth compared to the wild-type strain under greenhouse conditions. Thus, this indicated the importance of phosphate solubilization in growth and development of the host plants (Dahale *et al*., 2016).

26.3.3 Potassium solubilization

Plants require potassium (K) in large quantities. In fact, K is the third most critical macronutrient required for plant growth and development (Prajapati *et al*., 2013). The necessity of K in plant growth promotion is due to its important role in the enhancement of root growth, development of stronger stems, and capability in increasing cold and water stress resistance. The enhancement of crop resistance contributes to improved crop quality and reduced pest and disease incidence (Bagyalakshmi *et al.*, 2012). Moreover, K is also crucial for the activation of numerous metabolic processes which includes enzyme activation, protein synthesis and photosynthesis (Prajapati *et al.*, 2013). Thus, K deficiency in host plants cause slow growth, burned leaf edge and incomplete root development (Zhang and Kong, 2014). Even though the K supply in soil is abundant, only 1–2% of K is available to the plants (Setiawati and Mutmainnah, 2016). The unavailable K can be solubilized into available K by endophytic potassium solubilizing bacteria (KSB) such as *Bacillus edaphicus, B. mucilaginosus*, *Acidothiobacillus ferrooxidans, Paenibacillus* spp. and *B. circulans* (Meena *et al.*, 2014). The K solubilization by KSBs involves acidolysis, chelation, exchange reactions, complexolysis and production of organic acids (Meena *et al.*, 2014). The application of KSB is a promising approach to increase K availability in the soil for enhancing plant growth and development (Ahmad *et al.*, 2016). Sheng (2005) reported that inoculation with a KSB strain *B. edaphicus* NBT not only resulted in efficient K mobilization in cotton, but also increased root and shoot of the host plant (Sheng, 2005). Inoculation of efficient KSB also contributed to enhanced K uptake in plants, thus contributing to plant growth promotion under plant house and field conditions (Sindhu *et al.*, 2016).

26.3.4 Phytohormone production

Phytohormones play vital roles in plant growth and development. Among the most important phytohormone is auxin (Idris *et al.*, 2007; Glick, 2012). Although many naturally occurring auxins have been reported, IAA is the most frequently studied (Glick, 2012). IAA is a common product of L-tryptophan metabolism produced by several microorganisms including PGPEs (Mohite, 2013; Tan *et al.*, 2015). According to Tan *et al.* (2015), auxin production by endophytic diazotrophs *H. seropedicae* (Z78) requires a minimum of 0.25 g L^{-1} NH_4Cl in the growth medium as a starter N. The IAA aids in the development of longer roots with an increased number of root hairs and lateral roots which are prerequisites for nutrient uptake in plants (Datta and Basu, 2000). Additionally, IAA stimulates cell elongation by modifying certain conditions such as by increasing osmotic content of the cell, increasing permeability of water into cells, decreasing wall pressure and increasing cell wall synthesis and protein synthesis (Mohite, 2013). It also delays or inhibits abscission of leaves and induces flowering and fruiting (Zhao, 2010; Mohite, 2013). Regulations of these plant physiological processes are caused by IAA production by most PGPEs. Seed bacterization of chickpea cultivar C235 with *Pseudomonas* isolates that are capable in IAA production also exhibited growth stimulation of the host plant (Malik and Sindhu, 2011). Similarly, canola seeds inoculated with wild-type *Pseudomonas putida* GR12-2 exhibited induced root formation that were 35–50% longer than those inoculated with mutant deficient in IAA production. In contrast, overproduction of IAA by mutant strains showed higher numbers of short roots compared to the control. Overproduction of IAA stimulated the activity of ACC synthase and triggered the rise of ethylene due to stress, thus suppressed root elongation. This showed that plant hormone balance and stress response are significantly affected by different levels of phytohormone (Glick, 2012). Proliferation and differentiation of embryogenic calli and calli of oil palm was also successfully induced by the production of IAA by endophytic diazotroph *H. seropedicae* (Z78) (Lim *et al.*, 2016). This was shown by the formation of friable embryogenic calli by Z78 pellet cells as early as 60 days. However, treatment with Z78 sonicated cells exhibited faster development of embryoids at 30 days after inoculation (Lim *et al.*, 2016).

26.3.5 ACC deaminase production

Plant enhancement by PGPEs that are capable of producing ACC deaminase occurs due to lowering of the plant ethylene levels (Onofre-Lemus *et al.*, 2009). Naturally, plants produce ethylene under stress condition such as flooding, extreme temperature, radiation drought, insect predation and high salt. The production of ethylene is a form of protective response, hence increases the survival of plants. However, the overproduction of ethylene will thereafter induce deleterious effects such as senescence, chlorosis and abscission that may possibly cause major plant growth inhibition during plant growth and development. Fortunately, in PGPEs with the ability to produce ACC deaminase, ACC, which is the ethylene precursor, will be converted into α-ketobutyrate and ammonia (Glick, 2012). Both the α-ketobutyrate and ammonia produced can thus be exploited by the plants as nitrogen and carbon sources for their growth and development (Klee *et al*., 1991). ACC can also be used as a sole source of nitrogen for plant growth promotion in various ACC deaminase producing bacteria such as *Herbaspirillum* spp., *Azospirillum* spp., *Azoarcus*, *Pseudomonas* spp. and *Gluconoacetobacter diazotrophicus* (Ahmad *et al*., 2008).

Bacteria producing ACC deaminase also have positive correlation between root elongation and ACC deaminase activity per unit time (Shaharoona *et al*., 2006), thus showing significantly increased tolerance to high salt conditions and the ability to promote plant growth (Akhgar *et al*., 2014). The importance of ACC deaminase producing bacteria in the enhancement of root elongation was agreed by Glick (2014). *Burkholderia unamae* MTl-641^T inoculated in to tomato plants also showed significantly higher root length and higher dry weight of the shoot and root compared to the *acds* knockout mutants deficient in ACC deaminase production. This suggested that ACC deaminase production could play an important role in plant growth enhancement (Onofre-Lemus *et al*., 2009).

26.3.6 Siderophore production

Iron is among the essential constituents required by bacteria and plants. In fact, it is the fourth most abundant element on earth. Unfortunately, in nature, its bioavailability is often diminished due to its low solubility (Glick, 2012). To overcome this problem, most PGPEs can synthesize iron-chelating siderophore that solubilizes insoluble iron in soil and subsequently take up the iron complex using efficient transport systems. These siderophore-producing PGPEs also affects plant uptake of various metals including Cu, Zn and Fe (Ji *et al*., 2014). Siderophore-producing bacteria also aids in stress reduction in plants that is usually caused by high heavy metal concentrations in the soil (Ahemad and Kibret, 2014). The efficiency of siderophore-producing bacteria in Fe uptake contributes toward promotion of plant growth. For instance, an increase of chlorophyll *a* and *b* was observed in plant inoculated with siderophore-producing *Pseudomonas* sp. GRP3 (Ahemad and Kibret, 2014). Growth of *Arabidopsis thaliana* inoculated with a siderophore-producing bacteria *P. fluorescens* C7 was also enhanced due to increased iron levels inside the plant. In contrast, a siderophore-deficient endophyte (*Streptomyces* sp. GMKU 3100 mutant) showed significantly lower biomass of root and shoot and lower height of mung bean and rice compared to the wild type, hence, demonstrating that siderophore production is an essential trait in plant–microbe interaction toward plant growth enhancement (Rungin *et al*., 2012).

26.4 Genome sequence of plant growth-promoting endophytes

Application of omics tools in comparative genome and gene analysis provides novel information of sequenced bacteria genomes (Gupta *et al*., 2014). Genome analysis is essential to understand the genetic information of endophytes. Recently, numerous genomes of PGPE isolated from various plant tissues have been documented. Megías *et al*., 2016 announced a draft genome sequence of *Pantoea ananatis* AMG521 which is a rice endophyte. This strain promoted rice yield and consists of plant growth-promoting genes that encode fusaric acid resistance, N-acylhomoserine lactone (AHL) hydrolases, AHL synthases, lignin oxidation and hyperadherence (yidQ, yidP, and yidR) (Megías *et al*., 2016). In addition, endophytic *Enterobacter* sp. 638 harbored genes encoding for survival in the rhizosphere, colonization, establishment *in planta* and improved poplar growth and development *via* production of phytohormones, e.g. IAA, acetoin and 2, 3-butanediol (Taghavi *et al*., 2010). Genes contributing to plant-beneficial functions might be an intrinsic feature for PGPEs. Furthermore, findings also showed associations between genes that contributed to phytobeneficial traits, thus providing new insights into the emergence of plant growth-promoting bacteria (Bruto *et al.*, 2014). Moreover, sequencing of PGPE genomes aids in better understanding of the synergistic interactions between PGPEs and its host plant (Taghavi *et al.*, 2010).

Chaudhry and Patil (2016) reported that *Staphylococcus epidermidis* isolated from surface sterilized rice seeds consists of gene clusters required for stress tolerance, survival and adaptation to the plant habitat, which is indeed unique to this strain. Since *S. epidermidis* originates from humans, this study demonstrated the first evolutionary insight into adaptation of a rice seed endophyte in plants. Besides this, pathogenic and commensal *E. coli* strains were discovered from the phyllosphere of salads grown in agricultural fields across England. This plant-associated *E. coli* was found to harbor traits for plant colonization (Méric *et al.*, 2013). Similarly, *E. coli* isolated from the soil also successfully colonized the internal tissues of inoculated maize seeds. This study revealed the potential of inoculated *E. coli* in promoting growth of its plant host (Nautiyal *et al.*, 2010). Similarly, genes involved in plant growth promotion of endophytic *E. coli* strain USML2 were also recognized which include genes for solubilization of phosphate and potassium, production of ACC deaminase and biosynthesis of auxinic phytohormone IAA. The presence of these genes highlighted the potential of *E. coli* strain USML2 as a plant growth-promoting endophyte (Tharek *et al.*, 2017).

26.5 *In planta* ascending migration and plant growth enhancement of *E. coli* USML2 in association with rice seedlings

Non-pathogenic PGPE *E. coli* USML2, which harbor traits involved in plant growth enhancement (e.g. phytohormone IAA, siderophore and ACC deaminase production) was successfully isolated from aerial tissues of oil palm (Tharek *et al.*, 2017). In addition, the isolate has the ability to solubilize potassium and phosphate (Table 26.2). The isolate was tested on 7-day-old rice seedlings for 42 days under plant house conditions. Based on the results, rice seedlings inoculated with *E. coli* USML2 and *A. brasilense* Sp7 exhibited similar good growth responses compared to the control (-N) (Fig. 26.1a-d). Interestingly, seedlings inoculated with *E. coli* USML2 and *A. brasilense* Sp7 also showed significantly higher root and shoot dry weight of the host plants (Fig. 26.1a). It is believed that presence of plant growth-promoting traits such as siderophore, phytohormones, and phosphate solubilization promotes plant growth (Liu *et al.*, 1992; Glick, 1995; Bowen and Rovira, 1999; Ashrafuzzaman *et al.*, 2009). Similarly, inoculation of soil-originated *E. coli* NBRIAR3 into maize seeds under microplot conditions also showed significant plant growth enhancement compared to the uninoculated control (Nautiyal *et al.*, 2010). Since the strain *E. coli* USML2 was discovered from interior tissues of oil palm leaf, its ability to invade into the rhizoplane of its rice host plant and ascendingly migrate into the leaf tissues was investigated. The results have successfully shown the presence of viable cells in the tissues of root, stem and leaf of the inoculated rice seedlings. Bacteria enumerated from the internal tissues of root was the highest (19.2×10^4 CFU/g [fresh wt]$^{-1}$) compared to viable cells present in the internal stem and leaf tissues 12 hours after inoculation (Table 26.3). The extent of colonization was highest in roots compared to stems and leaves. A similar trend was observed 24 hours after inoculation and no presence of bacteria was observed from uninoculated plants. The presence of *E. coli* USML2 in internal tissues of surface sterilized root, stem and leaf were confirmed via PCR amplification of three selected essential genes of *E. coli* (*groEL*, *ftsZ*, *marB*) (Table 26.3). The presence of endophytic bacteria *in planta* without impairing its host plant is considered to

Table 26.2. Presence of traits involved in plant growth promotion of endophytic *E. coli* USML2 (Tharek *et al.*, 2017).[a]

Traits	Observation
IAA production (μg ml^{-1})	41.63 ± 0.83
Siderophore production (μg ml^{-1})	1.81 ± 0.18
ACC Deaminase production (μmol mg^{-1}h^{-1})	2.31 ± 0.07
Phosphate solubilization (μg ml^{-1})	0.32 ± 0.04
Potassium solubilization (μg ml^{-1})	0.37 ± 0.05

[a] Non-pathogenic plant-associated endophytic *E. coli* USML2 (UPMC432) CC BY 4.0.

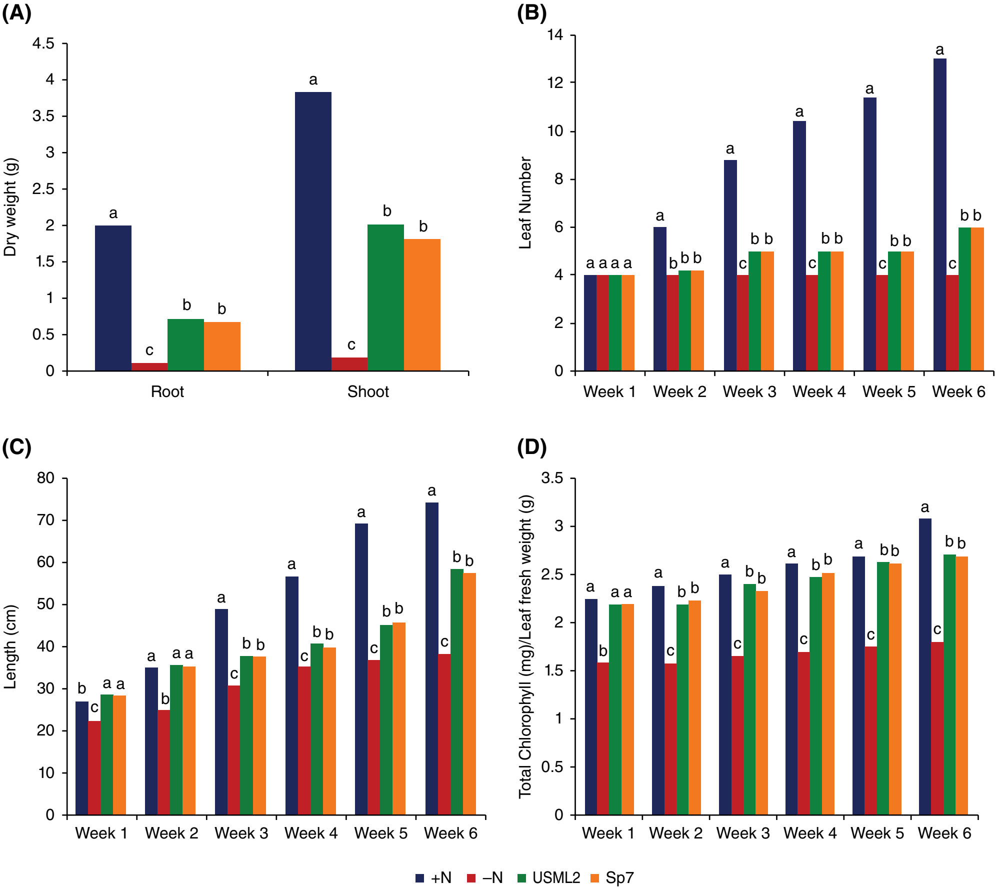

Fig. 26.1. Influence of rhizosphere inoculation on growth of 42 days old rice seedlings. **(A)** Plant dry weight; **(B)** leaf number; **(C)** shoot length; and **(D)** chlorophyll content. Mean values with the same letters in each root, shoot and weekly observations of the host plants is not statistically different (Tukey HSD, $P < 0.05$).

be a sign of a healthy plant system. These endophytes will promote health, growth and development of their host plant and provide protection against harmful conditions outside the plant (Taghavi *et al.*, 2010; Méric *et al.*, 2013).

26.6 Conclusions and future perspectives

Interactions between the endophytes and host plants have been regarded as the major key to plant productivity, health, ecosystem function and community organization. Its occurrence *in planta* is also important in shaping intense sustainable agricultural practices. Our findings revealed that the plant-originated *E. coli* USML2 could migrate from the rhizoplane into the interior leaf tissues of rice seedlings. Its ability in *in planta* ascending migration was also found to affect growth promotion of the host plant. Successful *in planta* ascending migration and plant growth promotion occurred probably because *E. coli* USML2 harbor traits involved in movement toward the roots, attachment, colonization, invasion and plant growth enhancement. Application of PGPE as biostimulants for improved plant growth and yield offers an attractive approach to replace and reduce pesticides, chemical fertilizers and supplements. Additionally, interactions between *in vitro* plant cells and endophytic diazotrophs would elucidate acceleration of embryo regeneration and maturation. As a result, application of

Table 26.3. Microbial enumeration of *E. coli* USML2 on the rhizoplane and internal tissues of different plant parts of rice seedlings after 12 and 24 hours of inoculation.

	Viable cell count (10^4 CFU g [fresh wt]$^{-1}$)			Verification of *E. coli* USML2 *in planta* via PCR								
				groEL			ftsZ			marB		
Plant part	0 h	12 h	24 h	0 h	12 h	24 h	0 h	12 h	24 h	0 h	12 h	24 h
Rhizoplane	0^{c}	4.0^{b}	722.3^{a}	–	+	+	–	+	+	–	+	+
Internal root tissues	0^{c}	19.2^{b}	125.5^{a}	–	+	+	–	+	+	–	+	+
Internal stem tissues	0^{c}	0.7^{b}	23.6^{a}	–	+	+	–	+	+	–	+	+
Internal stem tissues	0^{b}	0.02^{a}	0.4^{a}	–	+	+	–	+	+	–	+	+

Note: Mean values of viable cell count with the same superscript letters for each plant part is not statistically different (Tukey HSD, $P < 0.05$). No bacteria cells were observed in all plant parts of uninoculated rice seedlings (control).

excessive agrochemical fertilizers and plant hormones could be reduced. Thus, decreasing health risks and environmental pollution for a more sustainable agriculture.

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27 Plant Endophytes in Growth Promotion and Sustainable Management of Crop Pests

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Abstract

Plant endophytes can be defined as the microorganisms that reside inside the plant tissues but do not harm the host plants. Diverse endophytic microbes in the seeds deliver nutrients to plants while simultaneously suppressing pathogenic microbial virulence, preventing insect feeding, and reducing competing weed growth, which can lead to less environmental pollution and environmental degradation. Thus, plant endophytic microbes could be a novel alternative for the management of insect pests and plant diseases through various mechanisms including production of antimicrobial substances, phytohormones and induction of systemic resistance in the host plants. Plant endophytes can even pass from generation to generation. A plethora of endophytes such as bacteria, fungi, viruses, archaea, etc. are present in seeds. Mechanisms of their co-evolution with hosts and their beneficial roles to growth stimulation and protection from pests are poorly understood. A better understanding of the taxonomic and functional diversity of endophytic microorganisms are needed for their practical application in sustainable agriculture and bioprospecting. This chapter reviews and discusses the effects of plant endophytes on the promotion of growth and protection of crop plants from pests.

27.1 Introduction

The seed and other plant organs harbor taxonomically diverse endophytic microorganisms that generally promote growth and fitness of the host plants (Table 27.1) (Mano *et al.*, 2006). The ability to reproduce and adapt to harsh environmental conditions are adaptable features of seed endophytes. Seed endophytes can form endospores, thus protecting them from mutations within the seed (Mano *et al.*, 2006; Compant *et al.*, 2011; Kane, 2011). They retain some features, such as cell flow and phase activity, so that microbiomes can move freely inside the plant and insert the seeds before they harden. However, there have been relatively few studies examining the biological diversity of seed-bearing endophytes. To date, endophyte research has focused on fungal or bacterial structures separately. Indeed, few researchers have considered the combined effect of the endo-microbiome. Endophytes in seeds are particularly interesting as they are passed from one generation to the next. By carrying seeds, these endophytes ensure their availability in new plants. This vertical transmission, defined as a direct transmission from parent to offspring, should be selective against pathogenicity and favored consensus as

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Table 27.1. Taxonomic diversity in seed endophytes.

Genus	Bacteria endophyte	Host plant	References
Bradyrhizobium	*Bradyrhizobium* sp. SUTNa-2	*Oryza sativa*	Greetatorn *et al*. (2019)
Bacillus	*Bacillus tequilensis* PBE1	*Solanum lycopersicum*	Bhattacharya *et al*. (2019)
	Bacillus tequilensis PO80	*Citrus* spp.	Daungfu *et al*. (2019)
	Bacillus amyloliquefaciens EPP90	*Pennicetum glaucum*	Kushwaha *et al*. (2020)
	Bacillus subtilis	*Pennicetum glaucum*	Kushwaha *et al*. (2020)
	Bacillus pumilus	*Pennicetum glaucum*	Kushwaha *et al*. (2020)
Pantoea	*Pantoea dispersa* IAC-BECa-132	*Saccharum officinarum*	De Silva *et al*. (2019)
Pantoea	*Pantoea*,	*Eleusine coracana*	Misganaw *et al*. (2019)
Pseudomonas	*Pseudomonas*	*Eleusine coracana*	Misganaw *et al*. (2019)
Pseudomonas	*Pseudomonas* sp.	*Saccharum officinarum*	De Silva *et al*., 2019
	Pseudomonas granadensis T6	*Oryza sativa*	Shen *et al*. (2019)
Enterobacter	*Enterobacter* sp.	*Saccharum officinarum*	De Silva *et al*. (2019)
Enterobacter	*Enterobacter cloacae* RCA25	*Oryza sativa*	Andreozzi *et al*. (2019)
	Enterobacter	*Eleusine coracana*	Misganaw *et al*. (2019)
Herbaspirillum	*Herbaspirillum huttiense* RCA24	*Oryza sativa*	Andreozzi *et al*. (2019)
	Rhizobium larrymoorei E2	*Oryza sativa*	Shen *et al*. (2019)
Gordonea	*Gordonea terrae*	*Avicena marina*	Soldan *et al*. (2019)
Curtobacterium	*Curtobacterium* sp. SAK 1	*Glycine max*	Khan *et al*. (2019)

these endosymbionts rely entirely on their host to survive and reproduce (Ewald, 1989; Rudgers *et al.*, 2009).

Seeds play a vital role in the life cycle of spermatophytes. These can be in a torpid state for a long time until favorable conditions are available to a new plant (Geisen *et al.*, 2017). Seeds may have benefited from seed-bearing endophytes, which promote seed conservation and facilitate seed germination in the soil (Rodríguez *et al.*, 2018; Shearin *et al.*, 2017). Seed-bearing endophytes are particularly important because they are passed on to the next generation of plants by direct transfer (Cope-Selby *et al.*, 2017; Shade *et al.*, 2017). This interaction supports and promotes plant survival and bacterial growth (Rudgers *et al.*, 2009). Plants grow successfully in their natural niche and form cooperative relationships that help biodiversity in the ecosystem. Some tissue-binding bacteria form a close relationship with the host plant and provide plant benefits under favorable conditions and challenges. Endophytic bacteria mitigate the plants to reduce growth that reduces biotic and abiotic impacts (Miliute *et al.*, 2015). Fungal entomopathogens can be used as environment-friendly alternatives to chemical control. Unfortunately, their effectiveness continues to be limited by their susceptibility to ultraviolet light and low moisture. The fungal endophytes might overcome the traditional obstacles impeding the widespread adoption of fungal entomopathogens. These also provide a novel alternative to synthetic chemicals for the management of insect pests and plant pathogens. Besides, some fungal entomopathogens could also function as biofertilizers through various mechanisms including fixation of atmospheric nitrogen (Vega, 2018).

Endophytic *Bacillus* spp. produce a variety of secondary metabolites and lytic enzymes that are useful in the field of biotechnology and agriculture (Dame *et al.* 2021). They are good sources of bioactive chemicals, enzymes, vitamins, pigments and many other secondary metabolites that could be used for industrial use. Members of the genus are also known for their contribution to the growth and development of plants. They improve crop yield by increasing plant tolerance, disease prevention, and facilitating access to minerals and water. In addition, the current trend of global population growth and climate uncertainty calls for faster and more efficient strategies to increase crop production. Endophytic bacteria or plant probiotics may help to reduce synthetic chemicals and fertilizers that can make the whole production process less susceptible to unwanted environmental effects (Dame *et al.* 2021; Chakraborty *et al.* 2021). The application of biocontrol agents remains limited despite their high prospects in industrial application as biopesticides. Many scientific reports have focused on bacterial selection, basic chemical studies such as mass production, biocontrol agents, toxic and environmental safety, and integration into some control strategies.

A plethora of endophytes is present in the plant seeds and other organs. The mechanisms of their beneficial effects on host plants are poorly understood. However, these endophytes can pass from generation to generation to their offspring's. We need more study for commercial application of seed endophytes to get benefit in agriculture and bioprospecting. This chapter reviews recent updates of seed endophytes and find out the research gaps for future researchers.

27.2 The entry and formation of endophytes within plant tissues

Endophytes follow the same pattern as pathogenic microorganisms entering the plants. The exposure of protective molecules to the host is found to be lower in endophytes. In addition to other endophytes transmitted by seeds or vegetable distribution material, an effective endophytic colony goes through several key stages including capture acquisition, recognition and colonization of the plant area, and intrusion of internal plant tissue. Endophytic bacteria enter plants through natural openings such as hydathodes, stomata and lenticel cells, lesions, abrasions of soil particles, pathogen damage, lateral root formation, micropores, and abiotic mechanical damage (Fig. 27.1). Bacteria that produce enzymes that break down cells such as cutinase, pectinase, cellulase, hemicellulase, protease and lignin-peroxidase gain entry into the host plants via cuticle and cell walls (de Vries and Visser 2001). Proteinase was examined during endophytic *Acremonium typhinum* infection in the *Poa ampla* grass (Lindstrom and Belanger 1994). This proteinase was abundant inside the fungal membrane vesicles and on the walls of the plant and/or the fungus at the time of infection. In the case of root endophytes, *Piriformospora indica* disrupts the host cellular regulation process to establish coexistence with plants (Deshmukh *et al.* 2006). Already established plant endophytes can be passed from one generation to the next using plant seeds. Rhizospheric bacteria can enter and form as endophyte roots through the emergence of lateral roots or hair follicles, primary and posterior cracks, and various tissue lesions that occur due to plant growth (Sørensen and Sessitsch 2007). The genotype of host and fungus is an important factor in determining the establishment of a harmonious relationship. The PCR-based genetic analysis developed in *Venturia ditricha*, a fungal endophyte of birch trees, has shown that the frequency of infection with a particular endophyte genotype is influenced by the host genotype (Ahlholm *et al.* 2002).

27.3 Mechanisms of disease resistance by endophytes

Several lines of evidence suggest that endophytes enhance host defense against various diseases and reduce the damages attributed by pathogenic microorganisms (Ganley *et al.*, 2008; Mejía *et al.*, 2008). The most common strategy is *in vitro* direct plate antagonistic reaction against pathogens or by comparing the rate of survival of plants inoculated with control. Some studies present mechanisms of disease suppression by plant endophytes (Table 27.2). However, the precise mechanisms of plant protection by diverse classes of endophytic microorganisms are still not fully known (Ganley *et al.*, 2008). In the direct mechanism, endophytes directly produce antibiotics that help in suppressing pathogens (Matsumoto *et al.*, 2021). Direct and indirect endophyte-pathogen interactions are responsive to species-specific antagonism (Table 27.3) (Arnold *et al.*, 2003). Some examples of direct and indirect mechanisms use by endophytes are discussed in the following sections.

27.4 Direct mechanisms

27.4.1 Antibiotics are produced by endophytes

Endophytes produce various secondary metabolites with antibacterial and antifungal properties that help inhibit the growth of phytopathogenic microorganisms (Gunatilaka, 2006). Research

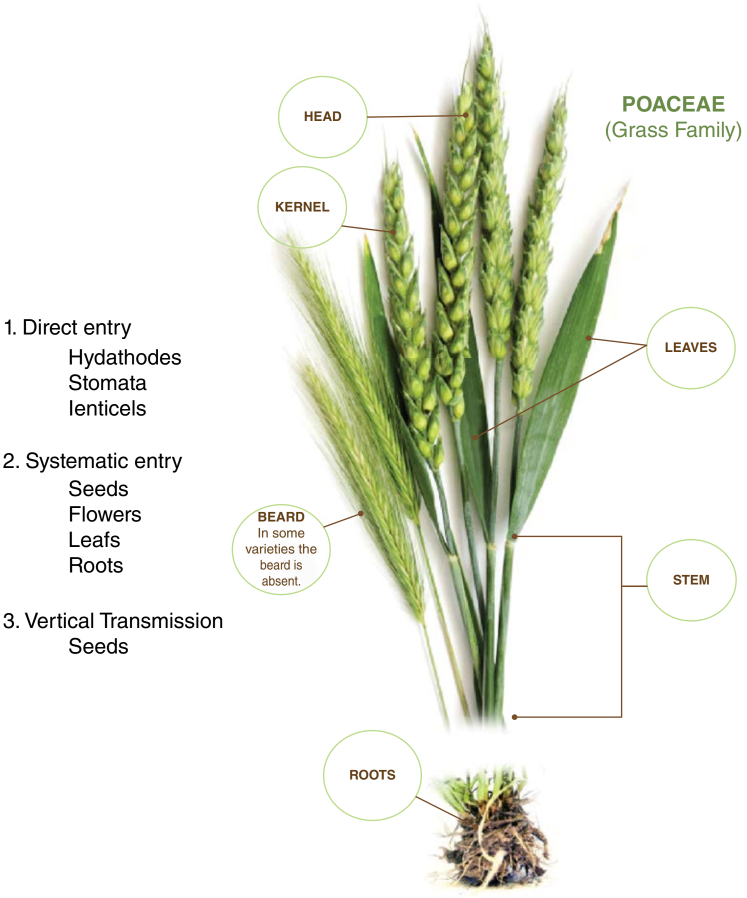

Fig. 27.1. Entry, establishment and transmission of endophytes in plants.

is ongoing in the process of identifying metabolites from plant endophytes for commercialization as agrochemicals or human drugs. Various bioactive compounds discovered from endophytic microorganisms inhibit various phytopathogens (Suryanarayanan, 2013; Daguerre *et al.*, 2016). Also, many metabolites with antimicrobial properties are found in endophytes, some of which have recently been characterized as flavonoids, peptides, quinones, alkaloids, phenols, steroids, terpenoids and polyketides (Mousa and Raizada, 2013; Lugtenberg *et al.*, 2016). When multiple bacterial species are present in a single plant, the organization promotes the secretion of metabolites by endophytes or a host that inhibits the growth of harmful bacteria (Kusari *et al.*, 2012). In some cases, endophytes and the host plant use several different mechanisms to increase the production of metabolites, others use a dynamic metabolism that assists the production of another (Kusari *et al.*, 2012; Ludwig-Müller, 2015). However, many endophytic species are unable to produce compounds independently (Heinig *et al.*, 2013). The endophytes isolated from *Cassia spectabilis*, named *Phomopis cassia*, were able to combine five substances such as 3, 11, 12–trihydroxycadalene and cadinane sesquiterpenes where one of the five ingredients produced the most effective antifungal metabolite against *Cladosporium* spp. Alkaloids are reported to have great potential in inhibiting the growth of pathogenic microbes. For example, altersetin, a novel alkaloid produced by the endophyte *Alternaria* spp., has shown antibacterial power against many Gram-positive pathogenic bacteria (Hellwig *et al.*, 2002). Another metabolite

Table 27.2. Functional diversity of seed endophytes on host plant.

Endophytic microbes	Host	Function	References
Bacteria			
Paenibacillus polymyxa	*Oryza sativa*	Glucanase production, anti-phytopathogenic microbe	Liu *et al*. (2017)
Proteobacteria, *Frimicutes*, *Actinobacteria*	*Cucumis melo*	NA	Glassner *et al*. (2017)
Micrococcus yunnanensis, *Micrococcus luteus*, *Enterobacter soli*, *Leclercia adecarboxylata*, *Pantoea dispersa*, *Staphylococcus epidermidis*	*Oryza sativa*	IAA production, plant growth promotion	Shahzad *et al*. (2017c)
Enterobacter asburiae, *Pantoea dispersa*, *Pseudomonas putida*	*Oryza sativa*	IAA production, phosphate-solubilizing, antifungal, plant growth promotion	Verma *et al*. (2017)
P. fluorescens, *Psedomonas* spp., *Pantoea* spp., *Enterobacter* spp.	*Phragimates australis*	Phosphorus-solubilizing, protease production, antifungal, plant growth promotion	White *et al*. (2017)
Panibacillus spp., *Pantoea* spp., *Bacillus* spp.	*Triticum aestivum*	IAA production, antifungal, siderophore production, phosphate-solubilizing, plant growth promotion	Díaz Herrera *et al*. (2016)
Massilia, *Kosakonia*, *Pseudorhodoferax*, *Caulobacter*, *Pantoea*, *Sphingomonas*, *Burkholderia*, *Methylobacterium*, *Bacillus* spp., *Curtobacterium*, *Microbacterium*, *Mucilaginibacter*, *Chitinophaga*	*Tylosema esculentum*	Plant growth promotion, phytohormone and metabolite production	Chimwamurombe *et al*. (2016)
Bacillus amyloliquefaciens	*Oryza sativa*	Phytohormone production, growth promotion	Shahzad *et al*. (2016)
Bacillus subtilis	*Lycopersicum esculentum*	Plant growth promotion, phytohormone and metabolite production	Xu *et al*. (2014)
Undibacterium, *Sphingomonas*, *Acinetobacter*, *Burkholderia*, *Pantoea*, *Limnobacter*, *Burkholderia*, *Pantoea*, *Staphylococcus*, *Serratia*, *Cronobacter*, *Enterobacter*, *Escherichia*, *Acinetobacter*	*Zea mays*	NA	Liu *et al*. (2013)
B. thuringiensis, *B. cereus*, *B. amyloliquefaciens*, *B. megaterium*, *B. subtilis*, *Bacillus* spp., *Paenibacillus,* spp., *Pseudomonas* spp., *B. thioparans*, *Cyanobacterium*	*Arachis hypogaea*	Antifungal	Sobolev *et al*. (2013)
Bacillus massilensis, *Bacillus* spp. *Bacillus pumilus*, *Bacillus flexus*, *Bacillus korlensis*, *Bacillus silvestris*, *Paenibacillus*, *Enterococcus*, *Staphylococcus*, *Arthrobacter*, *Kocuria*, *Micrococcus*, *Brachybacterium*, *Methylobacterium*, *Paracoccus*, *Acinetobacter*	*Phaseolus vulgaris*	NA	Rosenblueth *et al*. (2012)

Continued

Table 27.2. Continued.

Endophytic microbes	Host	Function	References
Actinobacteria, *Firmicutes*, *Gammaproteobacteria*	*Triticum aestivum; Elymus trachycaulus; Agropyron fragile*	NA	Ringelberg *et al*. (2012)
Pseudomonas protegens, *Pseudomonas* spp., *Stenotrophomonas maltophilia*, Uncultured*Stenotrophomonas clone*, *Ochrobactrum tritici*, *Ochrobactrum* spp., *Ochrobactrum grignonense*, *Sphingomonas yanoikuyae*, *Flavobacterium johnsoniae*, *Flavobacterium* spp., *Paenibacillus humicus*, *Paenibacillus* spp. *Agromyces mediolanus*, *Curtobacterium citreum*, *Curtobacterium* spp., *Curtobacterium herbarum*, *Frigoribacterium faeni*, *Microbacterium oleivorans*, *Microbacterium* spp., *Mycobacterium abscessus*	*Oryza sativa*	Plant growth promotion, mitigating biotic and abiotic stress	Hardoim *et al*. (2012)
Plantibacter flavus			
Bacillus spp., *Methylobacterium*, *Tukamurella*, *Alcaligenes*, *Erwinia*, *Microbacterium*, *Rhodococcus*	*Zea mays*		Rosenblueth *et al*. (2012)
Bacillus spp., *Pseudomonas chlororaphis*, *Lysobacter gummosus*, *P. chlororaphis*, *Paenibacillus polymyxa*, *Serratia plymuthica*	*Cucurbita pepo*	Antifungal	Fürnkranz *et al*. (2012)
Bacillus altitudinis, *Bacillus simplex*, *Bacillus thuringiensis*, *Paenibacillus amylolyticus*, *Staphylococcus aureus sub*spp. *aureus*	*Vitis vinifera*	Tissue colonization	Compant *et al*. (2011)
Pantoea agglomerans, *Staphylococcus succinus*, *Aerococcus viridans*	*Fraxinus*	Antibiotic production	Donnarumma *et al*. (2011)
Pantoea agglomerans, *Acinetobacter* spp., *Curtobacterium citreum*, *Microbacterium* spp., *Pantoea ananatis*, *Pseudomonas* spp., *Paenibacillus* spp., *Pantoea* spp., *Staphylococcus cohnii*, *Curtobacterium citreum*, *Microbacterium* spp., *Sphingomonas* spp., *Rhizobium larrymoorei*, *Curtobacterium* spp., *Sphingomonas* spp.	*Oryza sativa*	Phytohormone and metabolite production,phosphate-solubilizing, antifungal, plant growth promotion	Ruiza *et al*. (2011)
Acinetobacter, *Bacillus*, *Enterococcus*, *Nocardioides*, *Paracoccus*, *Phyllobacterium*, *Sphingomonas*	*Glycine max*	Phytate-solubilizing	López-López *et al*. (2010)
Enterobacter spp., *Xanthomonadaceae*, *Pseudomonas* spp., *Enterobacter* spp., *Pseudomonas fulva*, *Sanguibacter*spp., *Stenotrophomonas* spp., *Clostridium aminovalericum*, *Stenotrophomonas* spp., *Sanguibacter* spp.	*Nicotiana tabacum*	Mitigating metal toxicity, promote plant growth	Mastretta *et al*. (2009)

Bacillus pumilus, *Kocuria palustris*, *Pantoea ananatis*, *Methylobacterium radiotolerans*, *Methylobacteriumfujisawaense*	*Oryza sativa*	Enzyme production, osmotic stress tolerance	Kaga *et al*. (2009)
Bacillus spp., *Enterococcus* spp., *Paenibacillus* spp., *Methylobacterium* spp.	*Eucalyptus*	Growth promotion	Ferreira *et al*. (2008)
Pantoea spp., *Microbacterium* spp., *Frigoribacterium* spp., *Bacillus* spp., *Paenibacillus* spp., *Sphingomonas* spp.	*Zea mays*	Antifungal	Rijavec *et al*. (2007)
Xanthomonas translucens, *Pantoea ananatis*, *Methylobacterium aquaticum*, *Sphingomonas melonis*,*Sphingomonas yabuuchiae*, *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus*, *Acidovorax* spp., *Curtobacterium flaccumfaciens*, *Paenibacillus amylolyticus*, *Xanthomonas translucens*	*Oryza sativa*	Enzyme production, osmotic stress tolerance	Mano *et al*. (2006)
Bacillus spp., *Burkholderia cepacia—GC subgroup B*, *Burkholderia gladioli GC subgroup A*, *Burkholderia gladioli—GC subgroup B*, *Clavibacter michiganense insidiosum*, *Curtobacterium flaccumfaciens-flaccumfaciens*, *Curtobacterium flaccumfaciens-poinsettiae*, *Escherichia vulneris*, *Micrococcus* spp., *Pantoea agglomerans*, *Pseudomonas putida biotype A*, *Pseudomonas putida biotype B*, *Stenotrophomonas* spp., *Stenotrophomonas maltophilia*, *Yersinia frederiksenii*	*Coffea Arabica*	NA	Vega *et al*. (2005)
Pseudomonas fluorescens, *Pseudomonas* spp.	*Fragaria*	NA	Kukkurainen *et al*. (2005)
Agrobacterium radiobacter, *Aeromonas* spp., *Bacillus* spp., *Chryseomonas luteola*, *Flavimonas oryzihabitans*, *Sphingomonas paucimobilis*	*Glycine max*	Seedling growth, root colonization	Oehrle *et al*. (2000)
Fungi			
Alternaria spp., *Phoma* spp., *Penicillium corylophilum*	*Invasive Phragmites*	Improved seed germination and seedling growth	Shearin *et al*. (2017)
Fusarium spp., *Beauveria* spp., *Tulasnella violea*, *T. violea*, *Epulorhiza* spp., *Trichosporiella multisporum*	*Dendrobium friedericksianum*	Growth promotion	Khamchatra *et al*. (2016)
Diaporthe spp.	*Cinchona ledgeriana*	Alkaloid production	Maehara *et al*. (2016)
Cladosporium spp.	*Toona sinensis Roem*	Antioxidant potential	Rahmawati *et al*. (2016)
Neotyphodium spp.	*Lolium perenne*	NA	Wiewióra *et al*. (2015)
Epicholë ceonophiala	*Schedonorus phoenix*	Improved resistance against herbivores and environmental stresses	Young *et al*. (2013)

Continued

Table 27.2. Continued.

Endophytic microbes	Host	Function	References
Epichloë typhina	*Dactylis glomerata*	Improved host plant growth and photosynthesis	Rozpadek *et al*. (2015)
Acremonium strictum, *Alternaria alternate*, *Aspergillus niger*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Chaetomium cochliodes*, *Clodosporium cladospriodes*, *Cladosporium oxysporum*, *Cladosporium sphaerospermum*, *Colletotrichum dematium*, *Epicoccum nigrum*, *Fusarium avenaceum*, *Fusarium equiseti*, *Fusarium merismoides*, *Fusarium tricinctum*, *Fusarium* spp. *A*, *Geotrichum candidum*, *Mucor hiemalis*, *Penicillium* spp. *A*, *Penicillium* spp. *B*, *Phialophora verrucosa*,*Rhabdospora coricea*, *Sterile* spp. *A*, *Sterile* spp. *B*	*Centaurea cyanus;* *Papaver rhoeas;* *Senecio vulgaris;* *Centaurea nigra;* *Plantago lanceolata;* *Rumex acestosa*	NA	Hodgson *et al*. (2014)
Helotiales spp.	*Laelia speciosa*	NA	Ávila-Díaz *et al*. (2013)
Collelotrichum spp., *Fusarium* spp.	*Ipomoea carnea*	Antimicrobial	Tayung *et al*. (2012)
NA	*Swietenia macrophylla* King	a-Glucosidase inhibition	Ramdanis *et al*. (2012)
Neotyphodium oenophialum	*Festuca arundinacea*	Ergovaline and loline alkaloid production and improved protection against herbivores	Pennell *et al*. (2010)
Epichloë festucae var. *lolii*	*Lolium perenne*	Improved drought tolerance	Kane (2011)

Table 27.3. Mechanisms of disease resistance by endophytes.

Endophyte	Host	Broad mode of action	Mechanism involved	References
Pseudomonas fluorescens	Wheat	Competitive root colonization	Differential phase of growth, ability to stick onto the roots, ability to move, effective use of the organic acids present in root exudates and the synthesis of various components including amino acids, type III secretion system	Whipps (1997), Lugtenberg and Kamilova (2009), Turnbull *et al*. (2001)
Bacillus and *Pseudomonas*	–	Antibiosis and antibiotics suppressing pathogens	Production of antibiotics like phenazines, pyoluteorin, pyrrolnitrin and the volatile HCN Production of antibiotics, namely, D-gluconic acid, 2–hexyl-5–propyl resorcinol and the volatiles 2,3– butanediol, 6–pentyl-α-pyrone and DMDS Among lipopeptides, surfactin, fengycin, polymyxin, bacitracin and the group of iturin can elicit relevant properties of disease control Production of phloroglucinol, pyrrolnitrin, phenols and volatile organic compounds like pyrazine (2,5–dimethyl), benzothiazole, phenolic derivatives	Gupta *et al*. (2001), Fravel (1988), Haas and Défago (2005), Dandurishvili *et al*. (2011), Tabbene *et al*. (2009), Zhang *et al*. (2013), Ongena and Jacques (2008), Ramkumar *et al*. (2013), Zeriouh *et al*. (2011), Touré *et al*. (2004), Yánez- Mendizábal *et al*. (2011), Ongena *et al*. (2007); Henry *et al*. (2011), Torres *et al*. (2016), Dwivedi and Johri (2003), Pierson and Pierson (2010), Shanmugaiah *et al*. (2010), Pierson and Thomashow (1992), Perneel *et al*. (2008), Ligon *et al*. (2000), Wildermuth *et al*. (2001), Liechti and Farmer (2002), Díaz *et al*. (2003) and Gao Zhenbeng *et al*. (2017)
Bacillus thuringiensis	–	Signal interference	Inactivation of AHL molecule required for exo-enzyme production	Dong *et al*. (2004) and Dandurishvili *et al*. (2011)
Pseudomonas fluorescens	–	Competition for ferric iron ions	Production of siderophores to catch hold of ferric ion and to deprive the pathogens for iron	Loper and Henkels (1997) and Whipps (2001)
Azoarcus sp. strain BH72 *Pantoea* sp. *Lysinibacillus* sp. *Burkholderia* sp. strain PsJN	Rice Maize Banana grapevines	Competition for nutrients and niches (CNN)	The mechanism involved in competitive root colonization applies for CNN also	Pliego *et al*. (2008) and Malfanova (2013)
Xanthomonas albilineans	–	Detoxification and degradation of virulence factors	Fusaric acid detoxifies the toxins produced by pathogens. Quorum-sensing ability by degrading autoinducer signals, thereby inhibiting expression of numerous virulence genes	Toyoda and Utsumi (1991), Uroz *et al*. (2003), Von *et al*. (2003) and Compant *et al*. (2005)

Continued

Table 27.3. Continued.

Endophyte	Host	Broad mode of action	Mechanism involved	References
F. solani *Sclerotium rolfsii* *Bacillus amyloliquefaciens* CM-2 and T-5 *Bacillus subtilis* strain E1R-j *P. fluorescens* strain EP1	Cotton Beans Tomato Wheat Sugarcane	Induced systemic resistance (ISR)	Resistance induced by the production of salicylic acid, c-LPs, pyocyanins, siderophores, etc. Combined application of endophytic bacteria and chemical elicitors such as chitosan, a chitin derivative, will enhance ISR Increased production of peroxidases, PPO and PAL enhances ISR The action of lipoxygenase products which contributes to induction of phytoalexins	Li *et al*. (1991), Wei *et al*. (1991), Van Peer *et al*. (1991), Daniel and Purkayastha (1995), Young *et al*. (1995), Hallmann *et al*. (1995), Liu *et al*. (1995), Van Wees *et al*. (1997), Benhamou *et al*. (1998), Pieterse *et al*. (1998), Romeiro (2000), Chen *et al*. (2000a, 2000b), van Wees *et al*. (2000), Dekkers *et al*. (2000), Audenaert *et al*. (2002), Iavicoli *et al*. (2003), Ryu *et al*. (2003), Van Loon and Bakker (2003), Raj *et al*. (2003), Silva *et al*. (2004), Kloepper *et al*. (2004), Saikia *et al*. (2004), Kamilova *et al*. (2005), Saikia *et al*. (2006), Kloepper and Ryu (2006), Schuhegger *et al*. (2006), Nakkeeran *et al*. (2006), Ongena *et al*. (2007), Van Wees *et al*. (2008), Pliego *et al*. (2011) and Pérez-García *et al*. (2011)

indicating that antibiotic principle is a flexible oil. Endophytic fungi from tropical fungi known as *Muscodor albus* have produced a wide range of biological variants, including aciphyllene, 2–butanone and 2–methyl furan, that have reportedly produced antibiotic properties (Atmosukarto *et al.*, 2005). Also, *in vitro*-separated fungal endophytes from *Artemisia annua* can suppress the growth of many phytopathogenic microorganisms by the production of antifungal chemicals (Liu *et al.*, 2001). Tian *et al.* (2017) examined the role of antifungal protein produced by *Epichloë festucae* in the control of *Sclerotinia homoeocarpa* in *Festuca rubra*. The plant protection method used by the *Paraconiothyrium* strain SSM001 is linked to the production of a taxol from the yew tree (*Taxus* spp.) Fights of endophytes against harmful wood rot pathogen of yew tree were investigated by Rafiqi *et al.* (2013) and Soliman *et al.* (2015). The antibiotics and many other human health benefiting compounds produced by plant seed endophytes are high potential sources for bioprospecting. It has recently been reported that bacterial seed endophyte shapes resistance in rice (Matsumoto *et al.* 2021). Matsumoto *et al.* (2021) demonstrated that *Sphingomonas melonis* that is accumulated and transmitted across generations in disease-resistant rice seeds confers resistance to disease-susceptible phenotypes by producing anthranilic acid. Without affecting cell growth, anthranilic acid interferes with the sigma factor RpoS of the seed-borne pathogen *Burkholderia plantarii*, probably leading to impairment of upstream cascades that are required for virulence factor biosynthesis. These findings highlight the hidden role of seed endophytes in the phytopathology paradigm of ‘disease triangles’, which encompass the plant, pathogens and environmental conditions (Matsumoto *et al.* 2021).

27.4.2 Lytic enzymes

Most endophytic microorganisms release lytic enzymes for the hydrolysis of polymers (Gao *et al.*, 2010). For the endophytes to colonize the surface of plants, they produce several enzymes that help to sequence the hydrolysis of the plant cell wall. These enzymes help to reduce phytopathogens indirectly and also contribute to suppress cell wall damaging fungal phytopathogens. There are many types of enzymes some of which are chitinase, hemicellulases, and 1,3–glucanases. The use of mutagenesis in genes of 1,3–glucanase present in the genus *Lysobacter enzymogenes*. *L. enzymogenes* reduces the activity of *Pythium hypertension*, a pathogen causing the chronic fescue leafspot disease (Gao *et al.*, 2010). Lytic enzymes produced by *Streptomyces* have a strong effect on the resistance of cocoa witches broom (Macagnan *et al.*, 2008). Although enzymes are not as effective as antagonizing agents, they can enhance antagonistic functions when combined with other antibiotics. Pectinase has also been reported to help reduce pathogenesis in plants (Babalola, 2007).

27.4.3 Phytohormone production

Endophytes produce various phytohormones that promote plant growth and alter plant structure. As a result, endophytes have found a place in the agricultural environment (Sturz *et al.*, 2000). The mechanism adopted by endophytes in the production of phytohormones in the host plant is related to the method used by rhizobacteria in promoting plant growth. Endophytes can help to grow and promote non-corn plants by secreting gibberellic acid (Khan *et al.*, 2014), auxins (Dutta *et al.*, 2014), indole-3–acetic acid (IAA, Khan *et al.*, 2014; Patel and Patel, 2014) and ethylene (Babalola, 2010; Kang *et al.*, 2012). IAA stimulates plant cell division, differentiation and proliferation; stimulates seed germination and tuber grown; increases the rate at which roots and xylem develop, improves early onset and regulates the growth rate of vegetables, and the formation of new root and biosynthesis of metabolites; controls responses to gravity, light and fluorescence; and affect photosynthesis and resistance to extreme conditions (Gao *et al.*, 2010). The IAA secreted by endophytes that promote plant growth sometimes slows the body's processes listed above by affecting the plant's immune system. Also, IAA produced by endophytic bacteria can increase root length and surface area, thus providing the plant with better access to nutrients from the soil.

Besides, IAA production increases fluid exudates in addition to providing additional nutrients to boost the growth of other beneficial bacteria present in the rhizosphere. Therefore, IAA produced by endophytic bacteria is accepted as a major effect molecule on phytostimulation, pathogenesis and plant-bacterial interactions (Gao and Tao, 2012). Several studies have shown that endophytic actinomycetes also produce plant growth stimulants. Compounds such as the IAA have been reported to promote the formation and elasticity of plant roots in plants (de Oliveira *et al.*, 2010; Shimizu, 2011).

27.4.4 Solubilization of essential nutrient elements

The second and third most important nutrient for plant growth are phosphorus (P) and potassium (K). Some endophytes can also colonize rhizoplane of plants and solubilize insoluble nutrients such as P and K. Many endophytic and epiphytic microorganisms can dissolve insoluble phosphate to improve the production of ionic phosphates that make it available for plant use (Alori *et al.*, 2017). The most common mechanisms of nutrient element solubilization by endophytes include production and secretion of organic acid, protons, siderophores, carbon dioxide (CO_2) and hydroxyl ions (Olanrewaju *et al.*, 2017). The presence of small amounts of potassium-soluble bacteria makes potassium available for the plant uptake (Rogers *et al.*, 1998). Some endophytes that colonize roots can also decompose soil organic matter to release nutrients for the host plants. Many species of bacteria such as *Bacillus mucilaginosus*, *B. circulans*, *Pseudomonas* spp., *Burkholderia* spp., *Paenibacillus* spp., *Acidothiobacillus ferrooxidans* and *B. edaphicus* have been identified as potassium solubilizers (Yadav, 2018). Phosphorus is abundant in the soil. Unfortunately, most of its residues are not soluble (Miller *et al.*, 2010). Similarly, endophytic actinomycetes reportedly play an important role in phosphate solubilization and also improves its availability to plants through chelation, acidification, and mineralization, and redox changes of organic phosphorus (Singh and Dubey, 2018). Resolution of phosphate beside phytase fluid as indicated by an *endophytic actinomycete*, *Streptomyce* spp., which is the most important for improving plant growth by solubilizing nutrient for plant (Jog *et al.*, 2014).

27.4.5 Siderophore production

Siderophores are small iron-binding compounds that can be produced by endophytes and can make iron available to plants (Yadav, 2018). Some siderophores are known to be produced by endophytes that can provide biocontrol functions such as hydroxymate, phenolate and/or types of catecholate (Rajkumar *et al.*, 2010). Siderophores assist nitrogen fixation since diazotrophic organisms require Fe^{2+} and Mo for nitrogenase activity and function (Kraepiel *et al.*, 2009). A large body of literature supports the theory that endophytes protect plants from various pests in addition to siderophore production (Azevedo *et al.*, 2000). Some endophytes reduce the entry of stone insects by strengthening the endodermal cell wall (Gao *et al.*, 2010). Although some of the toxic metabolites are remediated by endophytes. Other metabolites produced by endophytes are pyrrolizidine, alkaloids, pyrrolopyrazine alkaloid, peramine ergot alkaloid and ergovaline (Wilkinson *et al.*, 2000). In the case of bacterial growth-promoting bacteria, Fe^{2+} is linked to the Fe^{3+}-siderophore complex in the bacterial membrane, which is later introduced into the cell by endophytes (Gao *et al.*, 2010). The melting of the molten metal increases when siderophores bind to the surface of the metal (Rajkumar *et al.*, 2010). Once the level of heavy metal contamination has been removed, a variety of methods are used by plants to add iron to viral siderophores. For example, iron chelates help directly absorb the properties of siderophore-Fe, or ligand exchange (Schmidt, 1999). The endophyte *Pseudomonas* GRP3 that produces siderophore was tested in *Vigna radiate* for iron intake. The result showed that after 45 days, the plants showed a decrease in iron and chlorotic signals (Sharma *et al.*, 2003). Other endophytic actinomyces such as *Streptomyces* sp. GMKU 3100, *Streptomyces* sp. mhcr0816, *Streptomyces* sp. UKCW/B and *Nocardia* spp. reportedly produced siderophores (Singh and Dubey, 2018). Similarly, *S. acidiscabies* E13 has also been reported as an excellent siderophore producer that promotes the growth of *Vigna unguiculata* under nickel stress conditions (Sessitsch *et al.*, 2013).

27.4.6 Competition with pathogens

Competitiveness is a strong mechanism used by endophytes that prevent viruses from binding to gripping tissue (Martinuz *et al.*, 2012). Endophytes have the ability to form large amounts of plant tissue locally (Latz *et al.*, 2018). For example, they work by colonialism and by concentrating on available nutrients and by staying in place is found in bacteria to perform their functions (Rodríguez *et al.*, 2009). Mohandoss and Suryanarayanan (2009) found that the destruction of endophytes in mango leaves by the use of fungicides allows other fungi to remain in this niche, especially pathogenic fungi. The method used to compete with multiple endophytes usually occurs in combination with other methods, rather than working independently (Lahlali *et al.*, 2014). It indicates the potential limitations of competition as a biocontrol method, as it may not work in the presence of high levels of pathogens. Symptoms of *Phytophthora* spp. are effectively reduced when treated with compounds produced by endophytes in the leaves of the cork tree. Competition of endophytes with pathogenic microorganisms through nutrient, space and chemical weapons are important means to protect the host plants from their enemies. However, other factors have also been identified in the production of other active metabolites which is an indication that competition may not be the only method used to control the disease (Arnold *et al.*, 2003).

27.4.7 Seed germination

Seeds are genetically and physically perfect to grow into new plants under ideal conditions. Seeds contain nutrients, usually in the endosperm. Some groups of plants (e.g. orchids) do not have food storage for the seedlings. Previous results have shown that endo-mycorhizal fungi increase seed germination and promote development in orchids; indeed this partnership is needed for successful seed germination (Masuhara and Katsuya 1989; Stewart and Zettler 2002; Rasmussen *et al.* 2015). Endo-mycorhizal fungi help to uptake carbohydrates, essential nutrients and, in particular, phosphate in orchid seeds through the mycotrophy process. *In vitro* studies have shown that the symbiotic germination of orchid seeds requires a specific mycobiont organization (Taylor and Bruns 1997; Batty *et al.* 2006; McCormick *et al.* 2006; Stewart and Kane 2006). Hubbard *et al.* (2012) found that wheat seed germinated better under heat and drought stress when colonized by fungal endophytes. Furthermore, seedlings infected with endophytes demonstrated fitness parameters even under drought condition which is similar to unstressed seedlings.

27.5 Indirect methods of plant protection against diseases

In long-term evolution, indirect (normal) resistance and specific resistance are examples of natural resistance designed to pathogen resistance (Király *et al.*, 2007). Those with a specific resistance can resist infection from one or more pathogens while unspecified resistance works against many viruses. Endophytes amplify plant protection through the production of secondary metabolites and improved systemic resistance.

27.5.1 Plant prevention performance

For more than 20 years now, many studies have focused on how plants respond to parasites and pathogens using various weapons. Induced systemic resistance (ISR) and systemic acquired resistance (SAR) are two resistance methods that have attracted the attention of researchers. The ISR, induced by other pathogenic rhizobacteria, is moderated by ethylene or jasmonic acid which can be linked to the formation of pathogenesis-related proteins (PR). The SAR, caused by viral infections, is regulated by salicylic acid and is linked to the formation of PR proteins (Tripathi *et al.*, 2008). These PR proteins are rich in enzymes, such as 1,3–glucanases and chitinases, that help in the direct activation of invading cells, and to strengthen cell wall barriers to build resistance to infection and cell death (Gao *et al.*, 2010). The ISR produced by endophytes can also be linked to genetic development expressed in pathogenesis. The root of tomato pools contains important endophytes called *Fusarium solani* that stimulate the ISR to counteract *Septoria lycopersici*, the causative agent of tomato foliar bacteria, and utilize PR, PR7 and PR5 gene functions in the roots (Kavroulakis *et al.*, 2007). Redman *et al.* (1999) reported that the incorporation of the pathogen *Colletotrichum magna* into *Cucumis sativus*, and *Citrullus lanatus* produced high levels of peroxidase, lignin deposition and phenylalanine ammonialyase which help protect the plant from infections caused by *Fusarium oxysporum*. Disease reduction in the leaves was observed when *Neotyphodium lolii* was involved in four different bacteria, which could be caused by enhanced peroxidase activity and superoxide dismutase activity in the host plant. (Tian *et al.*, 2008).

27.5.2 Stimulation of plant secondary metabolites biosynthesis

The secondary metabolites are compounds that are not directly involved in plant growth and reproduction but involved in fitness of plants to stressful environment (Bourgaud *et al.*, 2001). There is huge structural diversity in secondary metabolites produced by plants and their endophytes. Among them, terpenoids, flavonoids and alkaloids are the major classes of these bioactive compounds. *Orchis morio* and *Loroglossum hircinum* produce phytoalexins in response to a fungal attack developed by a French botanist named Noel Bernard. Phytoalexins production in plants can be induced by certain abiotic stress factors such as heavy-ion metals, salt pressure and UV light (Gao *et al.*, 2010). Some studies focus on the production of phytoalexins when plants are infected by bacteria (Pedras *et al.*, 2008). Production of secondary metabolites induced by endophytes is still a new area of research. The findings revealed that *Fusarium* E5 elicitors can induce triterpene and dipertene cellular production of *E. pekinensis*. Li and Tao (2009) reported that endophytes induce paclitaxel production in *Taxus* cell culture. Endophytic colonization induces plant production of hydrolase to reduce growth fungi (Gao *et al.*, 2010).

27.5.3 Promoting the growth of flowers and physiology

Endophytes sometimes support a plant's immune system against pathogenic microorganisms (Gimenez *et al.*, 2007). As plant growth increases, it increases the strength and resistance to abiotic and biotic pressures. This is one of the methods plants use to protect themselves from disease (Kuldau and Bacon, 2008). Numerous studies have shown that plants injected with endophytes had increased growth and drought resistance (Gao *et al.*, 2010), and tolerance to any type of soil pollutants (Malinowski *et al.*, 2004). Plant growth can be enhanced endophytic microorganisms by the production of IAA (Lu *et al.*, 2000). Dai *et al.* (2008) reported that extracts of endophytic *Fusarium* sp. E5 contains auxin. Production of various phytohormones by endophytes is an important strategy to modulate plant physiology for higher growth and fitness (Dai *et al.*, 2008).

27.5.4 Hyperparasites and predation

Hyperparasitism is another mechanism endophytes use to protect their host ecologically. In this mechanism, endophytes directly attack identified pathogens or their propagules (Tripathi *et al.*, 2008). Endophytic fungi capture the pathogens penetrating their hyphae to destroy the cell wall of the pathogen. For instance, *Trichoderma* spp. are able to capture and penetrate the hyphae of *Rhizoctonia solani* and show the biocontrol activities (Grosch *et al.*, 2006). Another mechanism is microbial predation. This entails a general way of reducing pathogens of plants. Most endophytes exhibit their predatory characteristics in nutrient-deficient conditions. For example, enzymes produced by *Trichoderma* spp. digest the cell wall of fungal pathogens directly to get nutrition (Gao *et al.*, 2010).

27.6 Methodical advancement, challenges, and prospects for industrial application

The endophytic population is known to vary from plant to plant and also from species to species. The same species of plant may also show different endophytic populations occurring from region to region. Hence temporal and climatic changes affect the occurrence of entophytes (Nair and Padmavathy, 2014). Bacteria and archaeal endophytes are metagenomically identified by 16S ribosomal RNA gene sequencing (Caporaso *et al.*, 2012). However, eukaryotic 18S rRNA (Stoeck *et al.*, 2010) and fungal intergenic transracial spacer (Menkis *et al.*, 2012) are used to assess the diversity of eukaryotic endophytes in the plant tissues. Without a clear prediction, results are simply descriptive, which is especially dangerous when phenotypes are naturally complex, such as successive clusters in operational taxonomic units and by scattering their relatives. To avoid these pitfalls, the experimental design should develop basic studies that previously described the critical characteristics of plant microbiomes, such as biological variability, density and strength (Lebeis, 2015). Endophyte–host relationships are believed to be complex and possibly vary depending on the handling to the catch and on the small-to-small animals (Boursnell, 1950). Many studies have compared plants infected with endophyte and uninfected plants concerning environmental stress, as well as invasion by insects and animals (Owen and Hundley, 2004). A better understanding of the complex cross-talks between and among plants and their endophytes and environmental microorganisms could help their practical application in sustainable crop production and bioprospecting. The bacterial world, in particular endophytes, shows genetic and metabolic variability, which is very high at a level that has not been fully tested. In conclusion, there are still many barriers to be passed to allow commercial use of antimicrobial agents to reduce resistance, and even more studies are needed to allow the use of endophytes.

Although first described in the 19th century, the details of the function of plant endophytes have been known since 1980s. They are regarded as important tools for promoting plant growth and plant protection to control pests and diseases. The endophytic microbial community may also be related to plant adaptation to biotic and abiotic pressure. Some endophytic microorganisms can produce compounds of biotechnological value such as antibiotics and antimicrobials. A small number of plant species have been investigated for their endophytes. Therefore, knowledge concerning the plant endophytes and their functions to host plants are still only partly understood. Endophytic microbiomes play an important role in protecting plants from disease, environmental stressors and helping them accelerate growth and development by providing better nutrition. Therefore, it is speculated that new mechanisms of interaction and interaction between endophytes and their host will be established for simplicity. More work is needed to understand the physiology, biochemical mechanisms, the role of the immune system, and the production of secondary metabolites, related to endophytes and the host. There is an urgent need to study the role of endophytes in the production of secondary metabolites both in vivo and *in vitro* (Rai *et al.*, 2014). Recent advancement in the methods of genomics, metagenomics, transcriptomics, proteomics, metabolomics and bioinformatics would accelerate our understanding of plants and their interactions with the endophytic microbiome in the changing climate that help utilize the beneficial endophytes for sustainable agriculture and bioprospecting.

27.7 Possible use of endophytic pesticides in agriculture

In the face of many barriers to the production of food, there is an urgent need to look beyond these challenges in a modern, sustainable agricultural way to maintain an environmental balance and achieve sustainable production from enhanced crop production. The potential use of endophytic fungi and bacteria to improve agricultural production is another ongoing approach considered in recent years. Endophytic bacteria and fungi have shown great potential in promoting plant growth in the biocontrol of phytopathogens, pests and insects, in reducing tolerance to abiotic stress and in reducing plant resistance against weeds (Chhipa and Deshmukh, 2019; Khare *et al.*, 2018).

The ability of endophytic microorganisms to invade, establish and tighten plant tissues causes them to produce multifunctional factors that have a positive effect on plant production. The question then arises: how do endophytes succeed in delivering these beneficial properties to plants? Endophytes are a group of ubiquitous and diverse organisms found in a wide variety of organisms in plant tissues. They live in all plants indiscriminately and act as a repository of biologically important metabolites that can be used to promote plant growth, a powerful biocontrol to fight germs and germs, to promote the immune system, and to provide functional properties to the plant to allow it to withstand or withstand external stresses. Biological control of destructive wheat blast disease by seed endophytic *Bacillus* species has been demonstrated (Surovy *et al.*, 2017; Dutta *et al.*, 2018). Genomic analysis reveals that these endophytic Bacilli protect wheat plants by secretion of antibiotics and induction of systemic resistance in the host plants (Dutta *et al.*, 2018).

27.8 Conclusion

Microbial endophytes are used to improve plant health and productivity in commercial production of crop plants. Benefits also obtain when endophytes reduce bacteria and insect damage and compete with weed plants. Increasing crop production without compromising the health of agricultural soils and endangering the quality of food with agrochemicals is a challenge through current agricultural practices. Modern efforts to obtain small-scale plant incentives are a start that could lead to a significant reduction in the use of chemicals in crop production. Endophytes can help reduce the requirements for chemical mineral fertilizers, fungicides, insecticides or herbicides for crop production. In the future, we are considering changes in performance to focus more on the functioning of plant and insect relationships between soil and endophytic microorganisms. Increasing microbial diversity through the application of microbial supplements to the plants that work to deliver nutrients to plants while simultaneously suppressing microbial virulence, preventing insect feeding, and reducing competing weed growth, can lead to less environmental pollution and environmental degradation. To develop sustainable agriculture in the future, we must develop a better understanding of how microbiomes work in soils and plants. We should also learn how we can use microbial functions to improve plant production and protection. Recent advances in genomics and post-genomics techniques would facilitate our better understand of the complex interactions between plants and their endophytes would facilitate their efficient use in eco-friendly sustainable crop production with improvement of soil health.

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28 Plant Growth-Promoting Rhizobacteria as Biostimulants in Sustainable Crop Production

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are regarded as the biostimulants for plant growth and development. These bioagents are also used as the bioprotectants of plants to combat the biotic and abiotic stresses in plants. The diverse mechanisms of the PGPR for regulating plant nutrition and defending the stressful conditions of plant growth are the major triggering indicators for their growth-promoting feature. Bacteria from diverse taxonomic genera such as *Pseudomonas*, *Bacillus*, *Paraburkholderia*, *Serratia*, *Enterobacter*, *Azospirillum*, *Rhizobium*, *Delftia* and *Azotobacter* belong to the group of PGPR that show high potential as biofertilizers and biopesticides. Therefore, the PGPR have been applied for plant growth enhancement and combating plant diseases and abiotic stresses, simultaneously. Cycling, solubilization and enhancement of use efficiency of essential plant nutrient elements, and production of phytohormones (indole acetic acid, gibberellins, cytokinins, etc.), siderophores, and ACC deaminase are predominant mechanisms of plant growth promotion by the applied PGPR. However, the key challenges for the universal application of the PGPR include storage incompatibility, poor shelf-life, inconsistent field performance and delicate application techniques required in field conditions. A consortium application of the multifunctional and compatible PGPR is the formulation that shows better performance than the formulation of a single bacterium. A variety of multi-omic approaches are applied to overcome these shortcomings for sustainable plant nutrition, moderation of phytohormone chemistry, induction of gene expression in host plants and screening of the target genes for overall genetic advancement of the PGPR in agriculture. A large body of literature is available on the beneficial effects of the PGPR and their mode of action on crop plants. This chapter focuses on the contemporary research and knowledge gap concerning the widespread application of the PGPR for promoting low-input eco-friendly agriculture. This systematic and meta-analysis of the PGPR should offer a new opportunity for further research and practical application of the PGPR-based formulations as sustainable biostimulants in crop production under the changing climate.

28.1 Introduction

In modern agriculture, extensive application of numerous agrochemicals, including chemical fertilizers, pesticides and growth hormones, are evident to keep pace with the enhanced growth and higher yields of cultivated crops (Galloway *et al.*, 2008; Gupta *et al.*, 2015). This heavy use

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of synthetic chemical inputs gradually deteriorated the quality of soil health and fertility of soils needed for sustainable crop production (Carsten and Mathis, 2014; Rai *et al.*, 2020). The non-judicious and several-fold higher doses of chemical fertilizers are being applied in many Asian and African countries to meet the food security requirements of the ever-increasing population (Alavaisha *et al.*, 2019; Singh *et al.*, 2020). The application of unplanned chemical fertilizers also deteriorates the physical and chemical properties of soils (Slepetiene *et al.*, 2020). These improper handlings and incidental overuses of agrochemicals are also regarded as the major barriers to sustainable agriculture in the future.

The conventional agrochemicals could largely be replaced by the potential beneficial soil microbes such as PGPR to sustain future agriculture in an eco-friendly manner (Bhattacharyya and Jha, 2012; Ahemad and Kibret, 2014). The PGPR are the group of soil-inhabiting bacteria that are useful for the enhancement of plant growth and development by a variety of modes of action (Kang *et al.*, 2014; Sarker *et al.*, 2014; Saha *et al.*, 2016; Etesami and Maheshwari, 2018; Majeed *et al.*, 2018; Khan *et al.*, 2019; Kour *et al.*, 2019; Khoshru *et al.*, 2020). Thus, PGPR can be treated as plant biostimulants due to their multifunctional features, together with enhancement of plant growth, replenishment of soil nutrition, biocontrol and stress management toward sustainable plant growth (Singh, M., *et al.*, 2019a; Khatoon *et al.*, 2020; Gupta *et al.*, 2020). A countable number of bacterial strains of diverse genera comprising of *Bacillus*, *Pseudomonas*, *Burkholderia*, *Paraburkholderia*, *Klebsiella*, *Serratia* and *Enterobacter* are documented for their plant growth-promoting traits (Islam and Hossain, 2012; Khan *et al.*, 2017; Mukta *et al.* 2017; Rahman *et al.*, 2018; Dutta *et al.*, 2020). They can directly or indirectly affect plant growth for the establishment of green and sustainable agriculture. Sustainability is a broad terminology for holistic agriculture, but PGPR can enhance eco-friendly plant development to sustain one of the sustainability components of holistic agriculture (Syed and Prasad Tollamadugu, 2018). Although PGPR is regarded as a sustainable replacement for chemical fertilizers, the single strain has not exhibited reproducible performance in the real field conditions (Bashan *et al.*, 2014). The loss of potentiality of the PGPR may be rendered by several factors such as storage conditions, carrier materials for PGPR formulation, mutation of stored strains and poor shelf-life.

Considering the current circumstances, a mixed microbial consortium including PGPR with other potential microbes can be used as an alternative to a single PGPR strain to boost the plant growth and sustainable practices of contemporary agricultural research (Kumar *et al.*, 2017; Santoyo *et al.*, 2021). However, PGPR was a decade-long concept for sustainable agriculture without hampering the surrounding ecosystems, but the mechanistic insights and the inconsistent performance of potential PGPR strains in field conditions are still under exploration. The growth stimulation of plant and biocontrol of diseases and other pests by the effects of applied PGPR have been the subject matter of many original research reports and reviews (Islam *et al.*, 2005, 2007; Deora *et al.*, 2005; Dutta and Podile, 2010; Islam 2010, 2011; Islam and Hossain 2012; Sarkar *et al.*, 2012; Paterson *et al.*, 2016; Kumar *et al.*, 2017; Mukta *et al.* 2017; Rahman *et al.*, 2018; Dutta *et al.*, 2020; Sarker *et al.*, 2021a). This chapter reviews current knowledge on the effects and mode of action of PGPR on the stimulation of plant growth and productivity. The bottlenecks of the field efficacy of the PGPR and application of advanced biotechnological and genetic approaches for overcoming the prevailing research pitfalls toward the commercial application of the PGPR for crop growth enhancement are also discussed.

28.2 The concept of PGPR and rhizosphere

Plant growth-promoting rhizobacteria (PGPR) are the beneficial bacteria that colonize the plant roots and augment plant growth through various direct and indirect mechanisms, including fixation of atmospheric nitrogen, solubilization of essential nutrient elements (e.g. phosphorus, potassium, zinc, etc.), production of phytohormones and siderophores, suppression of diseases by the production of antimicrobial substances (e.g. antibiotics), induction of systemic resistance in plants to biotic and abiotic stresses, increasing beneficial plant–microbe symbioses, interference with biofilm and pathogen toxin production. (Islam *et al.*, 2005; Ongena *et al.*, 2007; Islam 2010, 2011; Islam and von Tiedmann, 2011; Bhattacharya and Jha, 2012; Khan *et al.*, 2017; Verma *et al.*, 2018; Chakraborty *et al.*, 2021; Dame *et al.*, 2021). The demand for PGPR for sustainable crop production is increasing day by day (Bhattacharya and Jha, 2012).

The term 'rhizosphere' was first coined by the famous scientist Hiltner (Hiltner, 1904). The rhizosphere is a hotspot of plant–microbe interplay. It is the zone of soil surrounding a plant root. Intensive interactions among the plant, soil and microfauna take place in the rhizosphere (Pinton *et al.*, 2007). The physical, chemical and biological properties of soil are modulated by the plant processes (Huang *et al.*, 2014). The complex interactions and relationship between the rhizosphere and the PGPR are shown in Fig 28.1. There are mutualistic dealings and interdependence between microorganisms and their host plants (Suman *et al.*, 2016; Sharaff *et al.*, 2020). The rhizosphere-inhabiting microorganisms play an important role in the growth and ecological fitness of the specific host plant. Moreover, plant roots release a large number of photosynthates (various metabolites), mostly water-soluble compounds such as amino acids, sugars, secondary metabolites, signaling compounds and organic acids, which supply mainly food and signals for the inhabiting microorganisms (Backer *et al.*, 2018). On the other hand, the PGPR produces diverse metabolites and supports plant nutrition in various ways to establish a mutualistic relationship with the host plant. However, some of the microorganisms in the plant rhizosphere are antagonistic to plants and they can even cause various plant diseases (Islam and Tahara, 2001).

Higher root colonization is critical for the efficacy of the applied PGPR to plant growth and development (Islam *et al.*, 2005; Islam 2010). The colonization of microbial communities and their activity is mostly influenced by the plant species and development stages, composition and pattern of root exudates, and also the type and texture of soil (Somers *et al.*, 2004; Broeckling *et al.*, 2008; Yurgel *et al.*, 2019). Plant–microbe interactions may thus be considered beneficial, neutral or harmful to the plant depending on the particular plants and microorganisms involved, and on the prevailing environmental conditions (Islam *et al.*, 2003, 2005; Bais *et al.*, 2006).

The PGPR are widely used as biofertilizers, biopesticides, biostimulators, plant stress alleviators and maintaining the soil health conditions (von der Weid *et al.*, 2000; Orhan *et al.*, 2006; Rana *et al.*, 2011; Zhang *et al.*, 2012; Sharma *et al.*, 2014). They are an integral part of soil organic matter and their activities are mostly plant species-specific. Most of the representative root-associated bacterial community includes

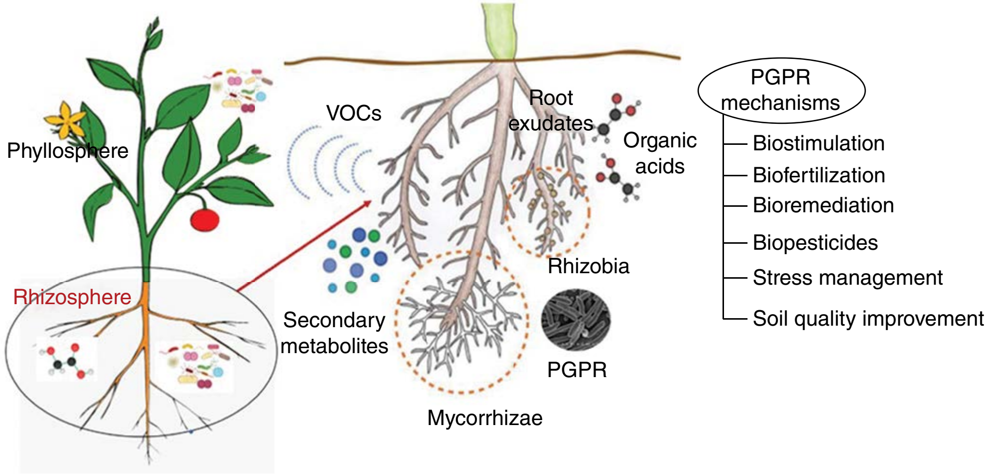

Fig. 28.1. Interactions of PGPR and other beneficial microbes in the rhizosphere of a plant.

Alphaproteobacteria (e.g. *Rhizobia*), Betaproteobacteria (e.g. *Burkholderia*), Bacteroidetes, Gammaproteobacteria (e.g. *Pseudomonas*), and Firmicutes (e.g. *Bacillus*) (Nuccio *et al*., 2020). Furthermore, Martinez-Viveros *et al.* (2010) reported two forms of PGPR: extracellular and intracellular. The extracellular PGPR that reside in the rhizosphere include *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Caulobacter*, *Chromobacterium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* spp. (Ahemad and Kibret, 2014). Conversely, Rhizobiaceae family members are intracellular PGPR like *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and rhizobium endophytes with *Frankia* spp. Interestingly, in association with the higher plants, all the intracellular PGPR can fix atmospheric nitrogen (Bhattacharya and Jha, 2012; Wani *et al.*, 2013). In addition to Rhizobiaceae, some other groups of bacteria could also be endophytic and/or epiphytic.

Soil fertility is the key factor affecting world food security, livelihood, energy renewability and agricultural sustainability. However, the anthropogenic activities cause cropland degradation which led to 24 billion tons of loss of fertile soil that has received great attention worldwide (FAO, 2011). Besides, climate change due to global warming and the growing population of the earth will create a crisis for food security and nutrient availability in developing countries (Çakmakçi *et al.*, 2007; De *et al.*, 2015; Reeves, 2017). Therefore, without restoring soil fertility, it is impossible to satisfy the basic demand of an ever-increasing population by 2030 (IFPRI, 2012). Additionally, excessive use of chemical fertilizers and synthetic pesticides possess a major threat to the environment and other non-targeted organisms including humans, which led to the search for alternative sources to hazardous synthetic chemical fertilizers and pesticides (Glick *et al.*, 2007; Ansary *et al.*, 2018). The diversified species of PGPR represent an economically and ecologically sustainable strategy for future agriculture that can replace harmful and environmentally unhygienic chemical fertilizers, and pesticides (Gupta *et al.*, 2021). It is estimated that the global population will rise to 9.7 billion by the year 2050. Using the modern biotechnological tools, rhizosphere and PGPR together can make green earth to meet the demand for the growing population (Haymer, 2015; Thijs *et al.*, 2016; Ahmadi *et al.*, 2017; Reeves, 2017; Wallenstein, 2017).

28.3 PGPR interactions in the rhizosphere

PGPR offers an attractive way to replace chemical fertilizers, pesticides or other supplements, therefore, their potentiality in the agricultural sector has steadily increased (Kloepper *et al.*, 1980; Pii *et al.*, 2015; Timmusk *et al.*, 2017). The complexity of PGPR genera, their functions and the interactions in the rhizosphere is still an interesting subject for exploration. However, rhizodeposits, root exudates and root border cells are vital components of the rhizosphere that have a significant impact on the capacity of root colonization and rhizosphere microbe multiplication, as well as on the secretion of organic bioactive compounds (Ahkami *et al.*, 2017). The rhizosphere is an ecological niche in which beneficial bacteria compete for organic carbon compounds with other microbiota and communicate with plants through the root colonization operation of soils (Islam *et al.*, 2005; Hassan *et al.*, 2019). Some of these beneficial root-colonizing rhizobacteria also endophytically colonize and multiply inside plant roots (Khan *et al.*, 2017). These components contribute to complex physiological processes in the rhizosphere, including cell growth, cell differentiation, and plant pathogenic microbe suppression (Hassan *et al.*, 2019). Beneficial free-living soil bacteria are typically referred to as rhizobacteria that can promote plant growth (Ahmadi *et al.*, 2017). The PGPR can colonize the rhizosphere, the rhizoplane (root surface), or the root itself (within radicular tissues) independently of the mechanisms of vegetable growth promotion (Gray and Smith, 2005). It is well established that only 1–2% of bacteria in the rhizosphere has the capability to promote plant growth (Glick *et al.*, 2007; Backer *et al.*, 2018). The PGPR affects plant growth in two different ways: direct growth promotion and indirect growth promotion. The direct promotion of plant growth by PGPR involves either supplying the plant with a bacterium-synthesized compound (e.g. phytohormones) or facilitating the absorption from the environment of certain nutrients

(Glick, 1995; Martinez-Viveros *et al.*, 2010). Indirect plant growth promotions occur when PGPR decreases or prevents one or more phytopathological organisms from having deleterious effects by producing antagonistic substances or inducing resistance to pathogens (Kumari *et al.*, 2019). The use of one or more such mechanisms can influence the growth and development of plants. The PGPR, therefore, can act as the biocontrol agent (Patten and Glick, 2002), plant ethylene reduction, or nitrogen fixation associated with roots by different mechanisms irrespective of its role in supporting direct growth (Kloepper *et al.*, 1980; Glick *et al.*, 2007).

28.4 Potentiality of PGPR as biostimulants

28.4.1 Plant growth promoting attributes of PGPR

The soil near the plant root, called the plant rhizosphere, is where raw material is produced from large quantities of metabolites of living root hair or fibrous root systems. These metabolites serve as the signaling chemicals for the root surface to attract/move the bacteria, but also provides the main nutrient sources to support rhizosphere growth and persistence (Çakmakçi *et al.*, 2007; De *et al.*, 2015). The bacteria that colonize the root or rhizosphere soil of the plant can effectively enhance the growth of the plant without hampering the environment. These bacteria are known as PGPR (Gupta *et al.*, 2021). This PGPR performs important functions in different ways for plant growth and health. The promotion of direct plant growth can be either through the improved acquisition of nutrients or hormonal stimulation. Different mechanisms are involved in eliminating the pathogens of plants, which are often indirectly linked to plant growth. In recent times, scientists have attempted, by directly providing the plant with the necessary compound or indirectly preventing the deleterious effect of one or more plant pathogens via the production of antagonistic substances, to categorize these actions into the form of exciting growth or benefits that this gives to the plant (Huang *et al.*, 2014; Kumari *et al.*, 2019; Gupta *et al.*, 2021). It can be described as a direct way of its mechanism as the positive interactions of PGPR in the form of biofertilization, root growth stimulation, rhizo-remediation and production of phytohormones, plant stress control, and the efficient use of certain nutrients in the environment. Although the impact of antibiotics, antifungal metabolites, systemic resistance induction and competition for nutrients and niches are reduced, PGPR exhibits indirect action (Kawalekar, 2013; Ahemad and Kibret, 2014; Pii *et al.*, 2015). In general, the PGPR work by synthesizing special plant-oriented components and phytohormones (collectively called 'biostimulants'), facilitating the uptake of certain environmental nutrients (called 'biofertilization') and avoiding diseases of plants (called 'bioprotectants' or 'biocontrol') (Bais *et al.*, 2006; Glick *et al.*, 2007; Kumari *et al.*, 2019). Both direct and indirect plant growth promoting attributes of PGPR for sustainable plant growth are illustrated in Fig 28.2.

28.4.2 Hormonal crosstalk between plant and PGPR

Hormones are organic chemical compounds that influence plant growth. They are, in other words, called plant growth regulators or phytostimulants. Examples of phytohormones are auxin (indole-3-acetic acid [IAA], gibberellic acid [GA], cytokinins, and ethylene). Over the years, these chemical molecules are known to be the four major plant hormones for biochemical and physiological development (Odoh, 2017). The PGPR species belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, *Alcaligenes*, *Bradyrhizobium*, *Rhizobium*, *Enterobacter*, *Acetobacter* and *Klebsiella*, and also the species of *Bacillus pumilus*, *Paenibacillus polymyxa*, *B. licheniformis*, *Glucanoacetobacter* spp., *Phosphobacteria* spp., *Aspergillus* spp. and *Penicillium niger* possess the ability to produce phytohormones (Ahemad and Kibret, 2014; Akram *et al.*, 2017). Auxin is a key molecule that controls most plant processes directly or indirectly. Being the first phytohormone found by Darwin in the year 1880 in *Phalaris canariensis* seeds, the plant has since been the most active and renowned plant hormone of the auxin group, leading to further discovery of IAA. Regardless of whether the plant can synthesize

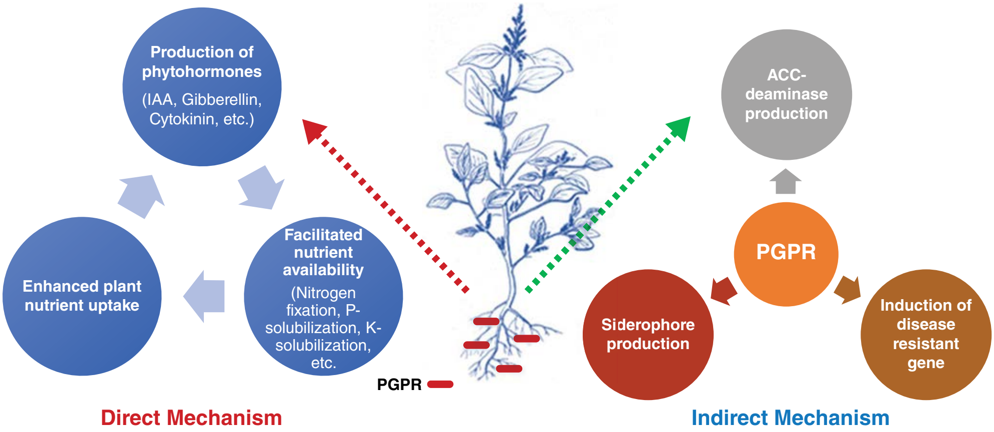

Fig. 28.2. Mechanisms of PGPR for growth enhancement of sustainable agriculture.

this chemical molecule (endogenous supply), they still largely depend on external (exogenous) supply for optimal performance. This external meeting is mainly controlled by PGPR and associated soil bacteria (Patten and Glick, 2002; Kumar *et al.*, 2015). Auxin triggers several cellular functions, ranging from vascular tissue differentiation, lateral and adventitious root initiations, cell division stimulation, the elongation of stems and roots, the orientation of root and root growth in light, and seriousness response (Glick, 1995). To make IAA more efficient, the developmental stages of the availability of nutrients in the rhizosphere, given the type and strain cultivable, are important (Kumar *et al.*, 2015). Although plants were identified with additional auxins such as indole-3-butyric acid (IBA) and phenylacetic acid (PAA), scientists still need to understand their complexity and mode of action.

There is still no comprehensive understanding of the precise mechanism through which PGPR promotes plant growth. It was generally thought that GA promotes stem tissue development, root elongation, and the side extension of the root (Kumar *et al.*, 2015). The GA is a group of tetracyclic diterpenes, which greatly affect seed germination, leaf expansion, stem elongation, fruit growth, flora and trichome initiation (Yamaguchi, 2008). Gibberellins and their generations remain the primary objective during environmental stress conditions due to their vital role in the improvement of efficient photosynthetic processes in plants, making them an important biosynthetic factor that may increase the stress tolerance of many crops. Gibberellin involves modification of plant morphology and stimulates the aerial part's development (Van Loon, 2007). During cell division, cytokinin plays a key role in the mobilization of vascular differentiation nutrients, chloroplastic biology, shoot distinction, leaf senescence, apical domination, anthocyanin production and the development of photomorphosis (Backer *et al.*, 2018). It also involves vascular change sensitivity, root hair proliferation and inhibition of root lateral formation and the primary radical lengthening (Aloni *et al.*, 2006). Either the plant or the PGPR can acquire this molecule in an endogenous and exogenous way. This phytohormone is unique and has a wide variety of biological activities. The useful role is best documented at low concentrations of this biomolecule. It hinders some key developmental properties such as root elongation, defoliation and other high-concentration cellular processes, resulting in a reduction in crop outcomes (Bhattacharyya and Jha, 2012; Odoh, 2017). The 1-aminocyclopropane-1 carboxylic acid (ACC) deaminase enzyme is required in plants to overcome stressful situations. The function of this biocatalyst is to degrade the plant ACC, a direct precursor to α-ketobutyrate and ammonium for ethylene synthesis in plants (Glick *et al.*, 2007). Degradation results in reduced production of plant ethylene by several potential mechanisms, while the PGPR-produced ACC deaminase controls the plant's level of ethylene and prevents high levels of ethylene to prevent growth inhibitions (Kumari *et al.*, 2019). The PGPR is capable of inducing exogenous production of ethylene via degradation of the endogenous product using enzymes include *Bacillus*, *Azospirillum*, *Burkholderia*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Achromobacter*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium* (Odoh, 2017). The deaminase activities of the PGPR ACC are vital to the growth of various vegetables (Glick *et al.*, 2007). Pierik *et al.*, (2004) suggested that plant yield, growth and germination properties of tobacco accelerates at low concentrations of PGPR-mediated ethylene and GA. However, it also governs the root induction, fruit maturation, germination of the seeds, abscission of the leaf and wilting (Kour *et al.*, 2019).

28.4.3 Bio-priming and advancement of PGPR application

The term organic priming was used if the seed is immersed during a predetermined period into a microbial suspension. The seed can then be dried to prevent the start of sprouts. For several decades, seeds have been primed using osmotic solutions. In recent decades, the PGPR seed inoculation has proved to be an efficient and eco-friendly agrotechnology practice that enhances the long-lasting life and reduces its ecological impact. The application process mainly contributes to the survival efficiency of the soil and seed bacteria. Seed treatment, soil modification and root dipping in bacterial suspensions before transplanting in crops are the most common methods being developed and examined.

The demand for agricultural output also increases with the world population increasing rapidly. The agricultural systems face a major challenge. People also want more and better food as nations evolve and large-scale agricultural chemicals and pesticides are used (Carvalho, 2017; Pivoto *et al.*, 2018). However, traditional farm equipment and methods reach their efficiency limits to increase farm productivity (Pivoto *et al.*, 2018). Conventional fertilizers are used to increase productivity in high doses for high yield varieties. However, chemical fertilizers cause deterioration of soil health, including pollution of soil water, and can also be stored in plants and seeds (Timsina, 2019). Consequently, eco-friendly techniques and tools are demanded by environmentalists, scientists, researchers' consumers and progressive farmers (Kawalekar, 2013; Bahadur *et al.*, 2014). A solution for chemical fertilizer and pesticide can be different types of bioactive compounds (Hassan *et al.*, 2019), enzymes (Backer *et al.*, 2018) or antimicrobial substantial activity/biocontrol activities of the PGPR (Backer *et al.*, 2018; Liu *et al.*, 2018). For agriculture, efficient seed germination is important. Indeed, successful early planting requires a fast and consistent appearance and root growth. Even if farmers and seed companies use priming to improve the quality of seeds for two to three decades, it may also occur under natural plant conditions. This is especially true for serotinous plants that grow and retain their seeds for a long time in deserts. These seeds are subject to several cycles of hydration–dehydration, improving their sprouts after the final dispersal of seed due to heavy rain (Santini and Martorell, 2013).

In general terms, the process of priming concerns not only seeds, but the whole plant system itself. The process can be defined as an induced state in which the plant reacts to stress more quickly and efficiently (Balmer *et al.*, 2015). This acceptance leads to a temporary metabolism adaptation to a stress memory, which enables crops exposed to main stress to adapt more effectively to subsequent stresses (Tanou *et al.*, 2012; Gamir *et al.*, 2014). Although there is much evidence of interest in the priming of seed, the basis of this intriguing method is not well understood. Physiologically and biochemically holistic approaches can offer new possibilities to elucidate the molecular components of priming. However, to examine kinetic imbibition in the change in the seed ultrastructure, non-destructive and non-invasive technologies like digital image technology is more accurately used. Intensive research is needed to examine and validate the effects with some elicitors or microbes of various priming on seed germination (physiological), changes in the antioxidant system, induction of polyphenol intakes and photosynthetic pigments, effects on plant vigor by simulating PEG 6000 seed drought and metabolic changes (Muscolo *et al.*, 2014).

28.5 PGPR for abatement of plant stress conditions

The PGPR are beneficial microbes that can induce tolerance in plants to biotic and abiotic stresses. Several environmental stresses often challenge plants. The term stress is defined as any adverse condition or substance or factor that affects and impairs the metabolism, growth or development of plants. The stress factors are divided into biotic stress (living) and abiotic stress (non-living). Biotic stress comprises a variety of pathogens, including human interference, insects, pathogenic microbes and higher animals. Abiotic stress includes tactors such as waterlogging, drought, heat, cold, wind, intense light, soil salinity, and an unsatisfactory or excess of mineral nutrients also (Wahid *et al.*, 2007; Khoshru *et al.*, 2020). Plant growth-promoting bacteria (PGPB) have the potential to relieve environmental stress and induce tolerance in plants for growth and development. Various plant defense changes in the form of systemically induced resistance (ISR) in biotic stress result from physical and/or chemical changes. Researchers have highlighted the absence of plant disease by the PGPB-related ISR in greenhouse and fields, caused by a variety of pathogens. The PGPB-induced physical and chemical plant modifications result in increased drought tolerance, salt, and other factors described as a form of systemic abiotic tolerance induced (Etesami and Maheshwari, 2018; Gupta *et al.*, 2020).

According to the early observations, the major mechanisms of the PGPR and other related potential microbes for stress alleviation include high root colonization, production of phytohormones, higher uptake of nutrients, fixation of atmospheric nitrogen, induce expression of stress-tolerant genes, induce systemic resistance, increase ACC deaminase activity, produce siderophores, produce antioxidants, enhance water and nutrient use efficiency in plants and production of various signaling compounds (Gupta *et al.*, 2020; Mitra *et al.*, 2021). A plant's ability to respond to a specific type of stress is inherently equipped. Plants have retained specific mechanisms to mitigate salt stress, including hormonal stimulation, ionic exchange, antioxidant enzymes and signal activation cascades on their metabolic and genetic boundaries that ease stress (Numan *et al.*, 2018). However, to boost up stress tolerance and plant growth, the PGPR also has a specialized mechanism that is essential to the plant's inherent mechanisms. This triggers plants to produce various growth hormones such as auxin, cytokinin and gibberellins, and organic volatile compounds. These bacteria also produce growth regulators such as siderophores, fix atmospheric nitrogen, solubilize nutrient elements from and inorganic reserves (Tariq *et al.*, 2017).

28.6 Application of PGPR for bioremediation of pollutants

Pesticides, heavy metal ions and various toxic chemical substances in soils limit plant growth and crop yield. Higher concentrations of these chemical substances are also hazardous for food safety as some of them are taken up by the plants and contaminate food products. PGPR have the innate potentiality for the bioremediation of various pollutants including pesticides and heavy metals (Khatoon *et al.*, 2020; Sarker *et al.*, 2021b). A diverse group of PGPR may remediate pesticides, persistent organic pollutants (POPs), toxic heavy metals, hazardous phenolic products and emerging pollutants through varied potential mechanisms (Khatoon *et al.*, 2020; Manoj *et al.*, 2020; Sarker *et al.*, 2021b). Effective sequestration of trace metals including Fe, Mn, Zn and Cd by a potential PGPR strain (*Bacillus cereus*) was reported in *Trifolium repens* plant species (Azcón *et al.*, 2010). Similarly, other *Bacillus* species simultaneously exhibited the potential for both plant growth promotion and remediation of heavy metals such as, Zn, Cu, Pb, Cr and Cd through biosorption and oxidation of trace metals (Ma *et al.*, 2015; Naureen and Rehman, 2016). Additionally, a comprehensive review documented the effective and enhanced bioremediation of heavy metals using various PGPR strains (Manoj *et al.*, 2020). The complex molecular mechanism toward the heavy metal removal or transformation comprises siderophore production, ACC deaminase production and growth hormone modulation. These mechanisms of PGPR can be merged with phytoremediation properties (phytoextraction, phytostabilization, phytovolatilization, etc.) of hyperaccumulating plants for the enhancement of the bioremediation approaches.

The PGPR is considered a popular option due to encouraging remediation capacity for various pesticides. Therefore, PGPR is treated as the leading candidate for bioremediation of pesticide polluted samples (Pant *et al.*, 2013). Several PGPR genera (*Bacillus*, *Enterobacter*, *Staphylococcus*, etc.) indicated 80–90% degradation of studied endosulfan and chlorpyrifos (Bhattacharjee *et al.*, 2014; Duraisamy *et al.*, 2018). However, the complete degradation of studied pesticides such as diazinon and atrazine by various novel PGPR strains have been reported (Wang and Liu, 2016; Jiang *et al.*, 2019). Thus, the multivariate mechanism of PGPR strains should be adopted for sustainable remediation of pesticide polluted sites. The multifunctional PGPR are now getting the researchers' attention for global sustainable agriculture. The application of PGPR for the bioremediation approach is also beneficial for plant growth and biostimulation, concurrently. Early research findings noticed the use of numerous PGPR traits for the effective bioremediation of chloronitrophenols (Arora *et al.*, 2018). A bacterial isolate (*Bacillus megaterium*) was documented for a 55.93% removal of hydrolyzed polyacrylamide (HPAM) within a 7-day incubation period (Song *et al.*, 2019). Similarly, azo dye (methyl orange) was remediated and decolorized by *Aeromonas veronii* in a 24-hour static incubation experiment (Mnif *et al.*, 2016). Thus, a wide range of pollutants, including pesticides, POPs, heavy metals, phenolics, and emerging environmental pollutants, were effectively managed through the sustainable application of PGPR. The selected studies exhibited the effective remediation potentialities of PGPR strains for pollutants from contaminated sites is listed in Table 28.1.

28.7 PGPR in plant protection

The PGPR can be used as biopesticides based on their metabolic activity (Bhattacharya and Jha, 2012). Biological pesticides or biopesticides are eco-friendly biological agents, used to control the growth of the pest population by secreting various secondary metabolites or extracellular enzymes (Islam *et al.*, 2005; El-Sayed *et al.*, 2014; Akram *et al.*, 2017; Barnawal *et al.*, 2017; Rajamani and Negi, 2021). They have exclusive mechanisms against viruses, bacteria, fungi and nematodes, including the production of antibiotics, siderophore and enzymes, and also improve plant defense mechanisms by releasing secondary metabolites like flavonoids (Pathak and Kumar, 2016). Therefore, PGPR-mediated biopesticides play a crucial role in managing plant diseases worldwide (Table 28.2).

Flavonoids and phenolic compounds exudate by plant roots significantly stimulate plant symbiosis with beneficial rhizobacteria (Palaniyandi *et al.*, 2013). Other root exudates including organic acids, vitamins and sugars serve as necessary nutrients for rhizobacteria (Dakora and Phillips, 2002). Microorganisms compete with each other for available nutrients. Antagonism through competition is one of the indirect plant disease suppression and biocontrol mechanisms of PGPR (Palaniyandi *et al.*, 2013). Production of antibiotics such as amphisin, phenazine-1-carboxylic acid, 2,4-diacetyl phloroglucinol (DAPG), oomycin, pyoluteorin, pyrrolnitrin, kanosamine, zwittermycin-A, pantocin, and kanosamine is the most common direct biocontrol mechanism of PGPR against pests (Milner *et al.*, 1996; Koch *et al.*, 2002; Fernando *et al.*, 2005: Islam and von Tiedemann, 2011). In this way, they either kill the pathogenic microbes or induce systemic resistance in the plant. They also produce lytic enzymes and volatile compounds to protect the plant from the undesirable effect of devastating pathogens (Islam 2010, 2011; Tariq *et al.*, 2017). Plant growth is improved by some specific proteins and chemicals produced by PGPR also helps in plant defense mechanisms (Akhtar and Siddiqui, 2010).

The PGPRs are capable of producing low-molecular-weight compound siderophores in an iron-limiting situation, which is a vital metal for the growth of all living organisms (Kloepper *et al.*, 1980; Whipps, 2001) and deprive the pathogenic microorganisms of iron (Gupta and Gopal, 2008). Thus, PGPR indirectly protects the plant from the notorious phytopathogens (Islam *et al.*, 2017, 2019). Cell wall degrading enzymes such as peroxidase, chitinase, glucanase and protease is produced by PGPR (Gupta *et al.*, 1995; Chater *et al.*, 2010; Islam, 2011). Using this mechanism, the PGPR disrupts the cell wall of insects and pathogenic fungi. The resistant capacity of crop plants is the best strategy to manage the pathogenic attack.

Table 28.1. Application of PGPR in bioremediation of pesticides, persistent organic pollutants (POPs), heavy metals, and emerging pollutants.

Studied chemicals	PGPR	Mechanism	Result	Reference
Heavy metals (Fe, Mn, Zn, and Cd) in *Trifolium repens*	*Bacillus cereus*	Heavy metal sequestration by the bacterium	Effective alleviation of studied heavy metals	Azcón *et al.* (2010)
Heavy metal (chromium Cr-III)	*Bacillus subtilis*	Biosorption of heavy metal	Above 90% reduction of Cr-III	Al-Gheethi *et al.* *(*2015)
Heavy metals (Cd, Pb, and Zn) in *Brassica napus*	*Bacillus* spp.	Trace metal biosorption	Exhibited high tolerance of metal (300 mg/L for Cd, 730 mg/L for Zn, and 1400 mg/L for Pb)	Ma *et al.* (2015)
Heavy metal (Mercury-Hg)	*Pseudomonas aeruginosa*	Biodegradation	Removal of 99.7% of Hg^{2+} in saline solution	Imron *et al.* (2019)
Heavy metals (As, Cu, Pb, Cr, Cd)	*Bacillus cereus* (1.1S) and *Acinetobacter junii* (1.3S)	Heavy metal oxidation	Exhibited oxidation tolerance to 40 mM As, 10 mM Pb, Cu, 8 mM Cd, and 6 mM Cr	Naureen and Rehman (2016)
Pesticide (Endosulfan)	*Staphylococcus equorum*, *Enterobacter* spp.*Bacillus subtilis*	Biodegradation	81–84% degradation through various PGPR strains	Bhattacharjee *et al.* (2014)
Pesticide (DDT)	*Bacillus* spp.	Biodegradation	89.3% degradation during incubation	Pant *et al.* (2013)
Pesticide (Chlorpyrifos)	*Bacillus cereus* Chlorpyrifos Gordoniasp.JAASI*Bacillus subtilis*	Biodegradation	Maximum 89% degradation of CP was recorded	Duraisamy *et al.* (2018)
Pesticide (Diazinon)	*Serratia marcescens* Ralstoniasp. DI-3	Biodegradation	100% degradation of diazinon was achieved within 60 h reaction time	Wang and Liu (2016)
Pesticide (Atrazine)	*Arthrobacter* sp. DNS10 co-culture with *Enterobacter* sp. P1	Biodegradation	More than 99% degradation of atrazine and metabolites	Jiang *et al.* (2019)
Emerging pollutant (4-chloro-2-nitrophenol)	*Pseudomonas* sp. JHN	Chemotaxis and biotransformation	Decolorization of 4C-2-nitrophenol by effective transformation to metabolite	Arora and Bae (2014)
Chlorotoluene,Chlorobenzene	*Comamonas testosteroni* strain KT5 and *Bacillus subtilis* strain DKT	Biodegradation by biofilm formation	Effective dual degradation of studied CT and CB within 48 h	Nguyen and Ha (2019)
Azo dye (Methyl orange)	*Aeromonas veronii*	Decolorization of dye through bioremediation	>80% decolorization within 24 h reaction time in the static situation	Mnif *et al.* (2016)
Hydrolyzed polyacrylamide(HPAM)	*Bacillus megaterium*	Biodegradation	55.93% removal of HPAM within 7 days incubation	Song *et al.* (2019)

Table 28.2. PGPR strains to combat against certain plant diseases.

Targeted diseases	PGPR strain	References
Black root rot of tobacco	*Pseudomonas fluorescens*	Laville *et al.* (1992)
Blue mold of tobacco	*Streptomyces marcescens*	Zhang *et al.* (2002)
Fungal disease of sesame	*Paenibacillus polymyxa*	Ryu *et al.* (2006)
Powdery mildew	*Pseudozyma focculosa*	Hynes and Boyetchko (2006)
Crown rot of sorghum	*Bacillus cereus* *B. subtilis*, *B. circulans*, *B. licheniformis and* *B. stearothermophilus.*	Idris *et al.* (2007)
Fungal diseases of pea and chickpea	*Enterobacter* sp.	Hynes *et al.* (2008)
Root rot of sorghum	*B. cereus* *P. fluorescens* *S. marcescens* *Brevibacterium laterosporus*	Idris *et al.* (2008)
Bacterial wet rot of potato	*B. subtilis*	Aliye *et al.* (2008)
Rice blast of rice	*A. strains*	Naureen *et al.* (2009)
Maize rot of maize	*Burkholderia* sp.	Gijon-Hernandez *et al.* (2010)
Downy mildew of pearl millet	*B. pumilus*	Chandrashekhara *et al.* (2010)
Banana bunchy top virus	*P. fluorescens*	Kavino *et al.* (2010)
Anthracnose of pepper	*Paenibacillus polymyxa*, *B. subtilis*	Lamsal *et al.* (2012)
Damping-off of cucumber	*B. pumilis*	Huang *et al.* (2012)
Damping-off of chickpea	*Streptomyces* sp.	Loliam *et al.* (2013)
	S. rubrolavendulae	Ashokvardhan *et al.* (2014)
Lupin root rot	*S. vinaceusdrappus*	Yandigeri *et al.* (2015)
Sclerotium rot of Melon	*B. subtilis*	Darma *et al.* (2016)
Black rot of Chinese cabbage	*B. velezensis*	Liu *et al.* (2016)
Bacterial spot of tomato, bacterial speck of tomato	*B. altitudinis* *B. velezensis*	Liu *et al.* (2017)
Red rot of sugarcane	*B. xiamenensis*	Xia *et al.* (2020)
Late blight of potato	*Pseudomonas* sp. *Bacillus* sp.	Islam *et al.* (2021)
Blue mold of citrus	*P. fluorescence*	Wang *et al.* (2021)
Fusarium wilt and tuber dry rot of potato	*B. subtilis*	Khedher *et al.* (2021)
Southern blight of chili	*B. megaterium* *P. fluorescence*	Sharf *et al.* (2021)

Interestingly, PGPR help to elicit induced systemic resistance in host plant to resist plant pathogens and insect pests (Alstrom, 1991; Walters *et al.*, 2013; Dame *et al.*, 2021).

Bacillus and *Pseudomonas* are two broadly discussed PGPR genera due to their availability and importance in biological control (Malfanova *et al.*, 2012, Ali *et al.*, 2014; Dutta *et al.*, 2018; Prasad *et al.*, 2019; Dame *et al.*, 2021). They mostly used antibiotic mechanisms to manage pests and insects (Jayaprakashvel and Mathivanan, 2011; Dominguez-Nuñez *et al.*, 2016). However, the efficiency varies due to different abiotic and biotic factors like environmental conditions, soil physical and chemical properties, competency of the bacterial rhizosphere and pathogen pressure (Benizri *et al.*, 2001; Ortíz-Castro *et al.*, 2009; Dutta and Podile, 2010). The genus *Bacillus* produces different antifungal and antibacterial compounds such as polymyxin B, cerexin A, colistin, sublancin, TasA, subtilosin A, subtilin, bacilysin, mycobacillin, chlorotetain, iturin, surfactin and fengycin (Babasaki *et al.*, 1985; Zuber *et al.*, 1993; Stöver and Driks, 1999; Paik *et al.*, 1998; Matar *et al.*, 2020; Das *et al.*, 2021; Dame *et al.*, 2021; Yue *et al.*, 2021). Different strains of *Pseudomonas* frequently produce phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (Phl), lipopeptide amphisin, tolaasiin, syringopeptin and syringomycin to suppress diseases in crop plants (Brodney *et al.*, 1991; Raaijmakers *et al.*, 1997; Koch *et al.*, 2002; Islam and von Tiedemann, 2011).

Synthetic pesticides are costly and undergo a lot of processes during formulation, which is considered a potential environmental threat to the environment of developing countries (Sarker *et al*., 2021c). In contrast, biopesticides are natural, living and cost effective. They are species specific and target very closely related species. Therefore, they are not harmful to non-targeted organisms such as mammals, birds and beneficial insects (Marrone, 2009). The efficacy is much higher than the synthetic pesticides and is being increasingly applied in the field (Cawoy *et al*., 2011; Kannojia *et al*., 2019). As a result, PGPRs are now available in the international market (Table 28.3) and the information regarding a better understanding of commercialization is available (Guthrie, 1896; Perret *et al*., 2000). However, the quality and efficacy of the PGPR-based formulations are still not satisfactory. Therefore, effective commercialization of the PGPR in many developing countries is a distant dream and needs to develop the technologies and skills.

The PGPR are essential not only to protect the plants from pests, but also to increase the growth and efficiency of plants. This multi-site action of PGPR is the most attractive part for the plant growers. Food security might be guaranteed after the effective implementation of PGPR (Gusain and Bhandari, 2019). Therefore, large-scale production and successful commercialization of biopesticides are highly time demanding to feed the growing population and make a green environment.

28.8 Challenges of PGPR application from laboratory to field

There is a desperate need to eliminate chemical fertilizers and pesticides in the current climate change scenario, and replace them with environmentally healthy, more efficient natural resources. Since the significance of microorganisms has been well documented with the advent of new technology and development, many areas are still unknown toward sustainable field applications. Therefore, there is a gap between the lab and field applications of the PGPR as biofertilizers or biopesticides.

During the last few decades, researchers have been focused on PGPR-based formulation preparation and commercialization. Consequently, some of the commercial products, especially biofertilizers, have become available in the international market (Zhang *et al*., 2004; Haden *et al*., 2007; Gupta and Dikshit, 2010). Later on, researchers introduced nanotechnology; however, quality and cost-effective nano products are time-demanding issues (Vejan *et al*., 2016; Delshadi *et al*., 2017; Reeves, 2017). At the same time, a promising research area rhizo-engineering has been created to expose the interactions between microbes and the rhizosphere, but most of the interactions are still not clearly described (Ahmadi *et al*., 2017). The successful application of biofertilizers or biopesticides largely depends on proper screening, laboratory formulation processes and the method of application in the field. The majority of the PGPR-based bioformulations have a limited success rate in the field level due to the lack of well-developed technology, quality carrier material, quality control legislation, training programs and on-farm demonstrations (Gagné *et al*., 1993; Murphy *et al*., 2000; von der Weid *et al*., 2000). Furthermore, inoculated PGPR may interact with native soil microflora that could influence crop production, and the diversified mechanisms of PGPR strain limit the widespread application of PGPR-based bioformulations (Gusain and Bhandari, 2019).

Besides, lack of understanding, diverse expectations, and the climate change effect make a huge gap between the laboratory and field application of the PGPR-based products. For a better understanding of PGPR commercialization, there is an urgent need for a better understanding of the signaling system and rhizobiocomplex in the plant rhizosphere through visualization and molecular bioengineering, biotechnology and multi-omic studies (Kumari *et al*., 2019; Riaz *et al*., 2021). Furthermore, the response of PGPR in diversified soil, and gradual changes of PGPR after inoculation need to be explained in detail (Ruiu, 2018; Gusain and Bhandari, 2019). The global biopesticides market is predicted to reach nearly $7.7 billion in 2021 from $4.0 billion in 2016 (BCC Research, 2016). However, we should carefully handle the pre-market authorization, legislative frameworks and health safety issues (Ruiu, 2018). Further investigations on well understanding the crosstalk between plants and microbes, selection of highly compatible PGPR-plant combinations, the dynamics of PGPR effects (short, mid or long-term) concerning the variable sites and host crop are highly needed.

Table 28.3. List of existing commercial biopesticides and their potential application. Adapted from Karnwal and Kapoor, (2021) and respective company's websites.

Active substances	Commercial names	Main targets	Application and other information
B. thuringiensis var. kurstaki (Bt)	Bactur®, Bactospeine®, bioworm®, Caterpillar killer®, Dipel®, Futura®, javelin®, SOK-Bt®, Thuricide®, topside®, Tribactur®, worthy attack®, Biobit, Cordalene®, Costar-WG®, Crymax-WDG®, Deliver®, Foray®, Javelin-WG®, Lepinox Plus®, Lipel®, Rapax®	Caterpillars (larvae of moths and butterflies)	Sunlight responsive should be sprayed directly on the lower surface of leaves and does not cycle in the environment. Effective against Indian meal moth in stored grain and foliage feeding caterpillars
B. thuringiensis var. israelensis (Bt)	Aquabee®, Bactimos®, Gnatrol®, LarvX®, mosquito attack®, Skeetal®, Teknar®, Vectobac®, Vectobar®	Larvae of Aedes and Psorophora mosquitoes, black flies, and fungus gnats	Only when ingested is it effective against larvae. In the case of Culex and Anopheles mosquitoes, the standard application rate is ineffective. In the environment, do not cycle extensively
B. thuringiensis var. tenebrinos	Foil®, M-one®, Mtrack®, Novardo®, trident®	Larvae of Colorado potato beetle, elm leaf beetle adults	Elm leaf beetle larvae and Colorado potato beetle larvae are both affected. It must be ingested. Breakdown in the presence of UV light. In the environment, do not cycle extensively
B. thuringiensis var. aizawai	Certan®, Able-WG, Agree-WP®, Florbac, XenTari®	Wax moth caterpillars	Effective only for control of wax moth infestations in honeybee hives
B. popilliae and *B. lentimorbus*	Doom, Japidemic®	Larvae (grubs) of Japanese beetle	The annual white grub, *Cyclocephala* sp.) is not susceptible to milky spore disease
Lysinibacillus sphaericus (formerly *B. sphaericus*)	Vectolex CG®, Vectolex WDG, VectoMax	Mosquitoes and blackflies	Effective only if ingested, for use against *Culex*, *Psorophora*, *Culiseta*, and *Aedes vexans*. Remains effective in stagnant or turbid water
Burkholderia spp.	Majestene®	Nematodes	It works on eggs, juveniles, and adults and is species-specific. Nematodes that cause lesion, root-knot, dagger, stunt, reniform, and soybean cysts are all susceptible to this treatment

Continued

Table 28.3. Continued.

Active substances	Commercial names	Main targets	Application and other information
Saccharopolyspora spinosa	Tracer™ 120, Conserve	Insects	Disturb the central nervous system of insect
Chromobacterium subtsugae	Grandevo®	Chewing and sucking insects and mites	Work as a deterrent. Sucking and chewing insects, flies, and mites inflict damage to a wide range of organic and conventional crops, including fruits, vegetables, and nuts, by stopping feeding, reducing reproduction, and inducing mortality
B. firmus	Bionemagon™	Nematodes	Infects and kills both larvae and adult stage of many plant pathogenic nematodes such as *Meloidogyne* spp, *Hetrodera* spp, *Helicotylenchus* spp, *Hoplolaimus* spp.

28.9 Advancement of microbial biotechnology through multi-omic techniques to improve PGPR genetic makeup

The PGPR-based formulations are experiencing significant growth as an alternative to chemical fertilizers, growth regulators and pesticides in the global market. For sustainable agriculture and to feed the world's increasing population, there are no alternatives rather than a green, environmentally friendly and cost-effective technology. Since PGPR seems an ideal tool as an efficient biostimulator, biofertilizer, biopesticides and stress mitigator, further improvement is needed to overcome the large-scale utilization of these renewable bioresources using multi-omic techniques including metagenomics (Kumari *et al.*, 2019; Riaz *et al.*, 2021). Brenner *et al.* (2015) reported that <1% of microbial species have been recognized due to a lack of advanced techniques and facilities. Therefore, to identify the novel activity and their variations in different environments, the study of modern biotechnological tools such as metagenomics is highly necessary (Krishna *et al.*, 2019; Jha and Kumar, 2021).

With the advent of next-generation sequencing, including pyrosequencing and high-throughput sequencing, it is possible to screen new microbes with PGPR activity in complex soil ecosystems which is not clear through the conventional screening methods (Palazzotto and Weber, 2018). Next-generation sequencing permits higher sequencing capacities at a lower cost (Klindworth *et al.*, 2013). Significant development of sequencing technologies exhibited the intact metabolic potential of PGPR prompting a revolution in the discovery of PGPR-based formulations over the past decade. The silent gene clusters within the genome of extensively studied species have been identified through modern genomic analyses (Ziemert *et al.*, 2016). Genomics coupled with bioinformatics has created a new revolution that combined life science with mathematical, electrical, and engineering and thus plays an important role in sustainable agriculture.

With the advent of science, our knowledge regarding the relationship between microbes and plants has been increased through transcriptome techniques (Wang *et al.*, 2005). During the DNA sequencing, the majority of the biosynthetic gene clusters of PGPR remain silent due to the complex regulation at transcriptional, translational and post-translational levels. To reveal the actual mechanisms, transcriptional changes in gene expression levels have been extensively studied (Abdelmohsen *et al.*, 2015). Additionally, proteomics is a powerful approach to disclose the morphological variations, the association among metabolic pathways and biosynthesis of secondary metabolites (Palazzotto and Weber, 2018).

Similarly, metabolomics is a promising tool to understand the underlying reality of plant–microbe interactions. The nuclear magnetic resonance-based metabolomic analyses and advanced mass spectrometry could be used to identify novel secondary metabolites produced by the PGPR while interacting with plant and rhizosphere microorganisms (Crüsemann *et al.*, 2017). All these genomic-based methods could widely be used as a means for discovering the biosynthetic ability of both cultured and uncultured microorganisms including 'silent' gene clusters (Liu *et al.*, 2015).

An integrated meta-omic approach, including metagenomics, metatranscriptomics and metaproteomics, allowed us to explore the hidden chemistry of culture-independent PGPR and their physiological properties (Krishna *et al.*, 2019). Metagenomics (functional and structural) allowed us to identify the microbial heterogeneity in plants and the rhizosphere (Unno and Shinano, 2013; Mendes *et al.*, 2014; Yadav *et al.*, 2015; Bramhachari *et al.*, 2017). In addition, the metagenomics approach has a significant contribution in decoding the interaction of PGPR with the rhizospheric zone and other microbes, and better understanding the genetic features and functions, presence of novel microbes, and their biochemical and metabolic pathways. Furthermore, the role of PGPR in the production of antibiotics can be described through metagenomics (Fernández-Arrojo *et al.*, 2010; Jha and Kumar, 2021). Multi-omic approaches like metabologenomics (genomic analyses integrated with metabolomics) have the potential to accelerate the discovery of novel metabolites. Using combined omics technologies, the limitation of the application of the PGPR-based formulation at the field level can be minimized and a new avenue for the commercialization of PGPR-based biofertilizers, biostimulators, and biopesticides can be launched.

28.10 PGPR in single-use versus consortium for sustainable plant growth

In general, PGPR is used as a single potential strain for sustainable crop production. However, several constraints such as inconsistent performance, loss of viability due to storage conditions, etc., could limit the single strain effectiveness for plant growth concerns (Sarker *et al.*, 2021b; Santoyo *et al.*, 2021). The alternative to single strain PGPR is the application of a consortium of several multifunctional and compatible PGPR for ensuring better performance in field conditions (Santoyo *et al.*, 2021). There are several benefits of using a microbial consortium over a single strain for plant growth promotion because of the complex biochemical mechanisms of diverse microbes. The compatible microbes can coexist for mutual benefit and consequently enhance the growth and development of cultivated crops both for usual agriculture and stress conditions (Bashan *et al.*, 2020). However, PGPR may act as one of the best candidates for combating plant stress conditions while a mixed consortium of the microbiome was merged with PGPR. The microbial consortia may be bacteria–bacteria, bacteria–AMF (mycorrhizae), fungi–bacteria, etc., combinations based on their mutual compatibility in coexisting culture (Panwar *et al.*, 2014). The soil–plant–microbe interaction is facilitated by the mixed microbial consortia under abiotic and biotic stress conditions of plants (Vimal *et al.*, 2017). The non-stress agricultural practices can be mediated by the PGPR consortium for the improvement of the micronutrient profile of soils to enhance the growth and development of crop plants. The vascular uptake of micronutrients and higher yield of wheat was achieved without compromising the soil quality through bacterial consortia using PGPR strains (Rana *et al.*, 2012).

Early observations noted a positive correlation of plant growth and inoculation of microbial consortia for the growth of vegetable seedlings in a nursery experiment managed by farmers (Jayashree and Jagadeesh, 2017). Likewise, the co-inoculation of a rhizobial strain with a PGPR stain for the enhanced growth of common bean under deficit phosphorus condition was documented (Korir *et al.*, 2017). Thus, microbial consortia can act as an effective biological tool for the sustainable management of soil fertility and plant nutrition. Interestingly, microbial consortia along with PGPR may perform better in low fertile soil. The mutual co-culture of methanotrophs, diazotrophs and PGPR can boost the combined performance of a mixed microbial consortium as compared to single use of respective strains. Therefore, the microbial consortium was regarded as the driving tool for future soil microbiology (Korir *et al.*, 2017; Singh, *et al.*, 2019b). The microbial consortium performs more aggressively in stressful conditions such as salinity, drought, etc. of plants. Consequently, the effective and mutually compatible consortium of microbes is vitally applied to combat the abiotic stress of soil–plant interactions (Vimal *et al.*, 2017; Bashan *et al.*, 2020).

Furthermore, an effective microbial consortium is reported for the biocontrol approach to fighting against phytopathogens. Thus, a plant defense mechanism is also rendered by redirecting cumulative exploitation of direct growth promotion and indirect disease suppression of effective microbial consortium (Sarma *et al.*, 2015). An early study revealed the modulation of phenolic profile by an immediate transformation of the studied chickpea plant to alleviate the biotic stress affected by *Sclerotium rolfsii* (Singh *et al.*, 2014). Significant changes in the biochemistry of plant enzymes including peroxidases, polyphenols and other related proteins for the induction of systemic resistance by a rhizobacterial consortium to fight against infestation of pathogenic *Macrophomina phaseolina* in *Vigna radiate* plant species have been demonstrated (Sharma *et al.*, 2018). Thus, the multifunctional feature of an effective microbial consortium including PGPR and other compatible microbes may open a mesmeric gateway instead of single strain PGPR toward sustainable agriculture.

28.11 Prospects of PGPR as biostimulants for commercial application

Plant growth-promoting rhizobacteria have the direct potentiality for plant growth promotion and indirect potentiality for alleviation of plant stresses (Yadav *et al.*, 2020). Although PGPR has sustainable and eco-friendly benefits for future agriculture, the various limitations may hinder their extensive application in real field conditions. The PGPR is a decade-long soil microbiological research for advanced and sustainable agriculture. Many commercial products of PGPR under various trade names such as Azo-Green (*A. brasilense*), Epic (*B. subtilis*), Blue circle (*B. cepacia*), Quantum 4000 (*B. amyloliquefaciens* GB99), Conquer (*P. fluorescens*), etc. have been registered for farming applications (Chet and Chernin, 2002). These commercial formulations are biofortified through various plant-growth regulators to enhance the potentiality in real field conditions. They also attempt to mend by tinkering with the genetic makeup for the sustained application of PGPR as prospective biostimulants (Bhattacharyya and Jha, 2012). Genetic engineering of some PGPR has improved PGPR activity to combat heavy metal and water stress conditions in combination with transgenic plant species (Wu *et al.*, 2006; Farwell *et al.*, 2007). Similarly, the phytoremediation approaches of heavy metals are enhanced by various advanced engineering of PGPR with growth regulators (Ali and Hj, 2010).

The prospect of PGPR as biostimulants is somehow curtailed due to the lack of proper formulations, carrier materials and storage conditions (Bashan *et al.*, 2014). Therefore, several liquid formulations are gaining popularity instead of solid, carrier-based formulations (Goljanian-Tabrizi *et al.*, 2017). The stability of growth and shelf-life of PGPR in the liquid formulation is enhanced by the addition of different additives (Yadav *et al.*, 2017). Thus, various additives including polyvinylpyrrolidone (PVP), sodium alginate, gum *Arabica* and related potential polymers can improve the stability of the liquid formulation of PGPR biostimulants for an enhanced shelf-life of living organisms with innate potentiality (Arora and Mishra, 2016; Brahmaprakash *et al.*, 2020). A number of biostimulating PGPR comprised of the species *Pseudomonas*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Azobacter*, *Variovorax*, *Azosprillum* and *Serratia* are commercially available from different reputable manufacturers (Novozymes, Monsanto, Lallemand, IIsa sPA, etc) in the USA, Canada, Europe, Asia–Pacific regions (Brown and Saa, 2015; Kumari *et al.*, 2019). The commercialization of PGPR biostimulants is still under development, but the promising PGPR (either single strains or mixed consortium) should be considered as the microbiological pillar to eco-friendly, green, and sustainable agriculture. A recent study depicted the current trend of PGPR application as biostimulants in sustainable agriculture and the possible prospect through advanced biotechnological tools (Gupta *et al.*, 2021). The prospective application of PGPR as commercial biostimulants is dependent on the merger of industry–laboratory research collaboration and the optimization of formulations for better shelf-life (Arora *et al.*, 2016). A decade-long screening of various potential PGPR for sustainable agriculture and enhanced plant growth has been reported as an eco-friendly approach toward prospective application in the field of microbiological biotechnology (Verma *et al.*, 2019). The basic steps, including isolation, screening, laboratory culture, mass production via bioreactors, effective formulation, confirmation of better shelf-life and advanced biotechnological tools, should be optimized toward commercialization of prospective PGPR biostimulants.

28.12 Conclusion and future perspectives

Plant growth promoting rhizobacteria are regarded as a promising and green approach for stimulation of plant growth and yield for ensuring food and nutritional security under the changing climate. These microorganisms exert beneficial effects to the applied plants in various ways such as enhancement of growth, improvement of nutrition, and increase tolerance to biotic and abiotic stresses. Despite numerous advantages, the commercial application of the PGPR is limited due to unpredictable performance in the real field and loss of viability during storage conditions. The optimization of formulation, application of advanced genomic and biotechnological tools, and development of an effective protocol for commercial application of the PGPR are encouraged to develop widely applicable commercial formulations in real field conditions. Although various biostimulants such as bioactive materials, secondary metabolites, volatile organic compounds, growth regulators, etc. are found to be effective for sustainable plant growth, the PGPR as a live biostimulant could occupy special attention for the multipurpose, green, and cheap tactics for eco-friendly crop production. The PGPR biostimulants are treated as the potential microbiological pillar for the future agricultural revolution. Further meticulous and advanced studies should be designed to overcome the prevailing research drawbacks for the establishment of prospective PGPR formulations as effective plant biostimulants for sustainable agriculture under the changing climate.

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29 Endophytes: The Immune System Modulators of Rice Plants Under Abiotic Stresses

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Abstract

Endophytes are the microorganisms, including bacteria and fungi, that reside within healthy plant tissues and promote plant growth under favorable as well as unfavorable conditions. Endophytes are present in all the plant species examined to date. They can enhance growth of a plant, stimulate defense responses against different pathogens and can act as immune system modulators for plants in abiotic stresses. Rice plants (*Oryza glaberrima* [or African rice] and *O. sativa* [or Asian rice]) are one of the major cereal grains that is the most widely consumed staple food for a large human population throughout the world. Therefore, it is very important to increase the production by controlling diseases, increasing growth in abiotic stresses, etc. of rice in order to feed the growing population worldwide, especially Asian and African people. This chapter focuses on the potential of endophytic microbes that induce abiotic stress tolerance in rice crops by improving the immune system of the plant. This chapter also discusses how endophytic microbes can be used for biotechnological applications in rice crop growth promotion and improvement under abiotic stress conditions.

29.1 Introduction

Rice is the seed of a plant species Oryza glaberrima (African rice) or Oryza sativa (Asian rice) belonging to the family Poaceae. It is the highest consumed staple cereal grain for a huge portion of the world's human population, especially in Asia and Africa. It is the agriculture product with the fourth-highest worldwide production after sugarcane, maize and wheat (FAO, 2019). There is a need to increase the production of rice to fulfill the requirements of the ever-growing population worldwide. Different approaches have been implemented to overcome the challenges in agricultural sectors for its successful production. Different types of chemical fertilizers and pesticides are being utilized (Wang *et al.*, 2019). However, the use of chemical growth stimulants leads to a hazardous impact on human health as well as the environment (Deka *et al.*, 2017). Rigorous cultivation of high-yielding crop varieties like rice and uneven use of chemical fertilizers are the principal factors that develop inconsistency in soil nutrient, reduced yield, decrease in soil fertility and poor quality of food (Verma *et al.*, 2018). Therefore, it results in a severe difficulty to develop sustainable strategies to mitigate the adverse effect of intensive

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practices used by farmers worldwide (Verma *et al.*, 2018). The primary production of rice may be enhanced in a sustainable way with the use of biostimulants. Agricultural scientists are working on novel options to enhance agricultural productivity and sustainability, which is undeniably an immense challenge for them (Verma *et al.*, 2018). The use of beneficial microbial symbionts like endophytes associated with plants for the improvement of rice productivity is one of the most significant sustainable practices under any adverse conditions (Khare *et al.*, 2018). Endophytes are the microbes that inhabit the healthy plant tissues without any overt symptoms (Bills, 1996). Plants have been bestowed with a diverse population of endophytic microorganisms, including bacteria, fungi, actinomycetes, etc. (Bills, 1996). Endophytes can be considered to be the plants' immune system modulators, which help the host plants to survive by nutrients supply, environment acclimatization, biotic and abiotic stress protection, growth promotion and enhancing community biodiversity of host plants (Gond *et al.*, 2010; Pandey *et al.*, 2011; Li *et al.*, 2012). There is a lot of evidence that endophytes help the host plants to withstand abiotic stresses in an eco-friendly manner (Gond *et al.*, 2010; Pandey *et al.*, 2011; Li *et al.*, 2012). Rice plants have a large number of endophytic microorganisms that help them to tolerate abiotic stresses like drought stress, nutrient stress, salinity and alkalinity tolerance, temperature stress, heavy metal stress, etc. (Kharwar *et al.*, 2008; Berg, 2009; Gond *et al.*, 2010; Pandey *et al.*, 2011; Li *et al.*, 2012). This chapter highlights how endophytic microorganisms induce abiotic stress tolerance in rice, which can also be called the immune system modulators in rice plants.

29.2 Impact of abiotic stresses on rice plant

Due to abiotic stresses, plants lose their normal physiological pattern of growth and development as a result of changing intensities of water content, soil pH, salinity, ion toxicity, drought etc., which lead to decrease in the germination rates, low rate of photosynthesis, membrane integrity loss, increase in reactive oxygen species (ROS) etc. (Munns and Tester, 2008; Greenberg *et al.*, 2008). Protracted water stress results in a decline in leaf water potential as well as stomatal opening, reduced leaf size, suppressed root and shoot growth, and reduced seed number, size and viability. Flowering and fruiting are suspended, which results in limited growth and productivity of rice (Xu *et al.*, 2016). High soil salinity and drought are the major causes of osmotic stress to rice plants. Also, higher temperatures can have cause extensive denaturation and aggregation of cellular proteins, which if unimpeded, leads to cell death in the plant (Theocharis *et al.*, 2012). On the other hand, low temperatures weaken metabolic processes by altering the plant membrane properties, changing the structure of proteins and the interactions between macromolecules as well as by restricting the enzymatic reactions (Theocharis *et al.*, 2012). Heavy metals Pb, Hg, Cd, As, etc. also obstruct numerous biochemical as well as physiological processes, including photosynthesis, respiration, nitrogen and protein metabolism and nutrient uptake as well (Zhang *et al.*, 2009).

29.3 Endophytes as an immune system modulator for rice plants under abiotic stresses

The symbiotic association between rice plant and endophyte may result in several beneficial consequences for the host (Lewis, 1985). The outer environment has a deep impact on the plant body, but in spite of the changing environment, endophytes help to maintain a homeostasis in the plant body metabolism, which occurs through some molecular interactions between the plant and the endophytic microorganisms (Gill and Tuteja, 2010). Endophytic microbes assist the rice plant health under abiotic stresses by inhibiting different environmental stresses through nutrient uptake (modifying of root morphology, altering nitrogen accumulation and metabolism), enhancing water uptake (osmotic adjustment, stomatal regulation etc.) (Ripa *et al.*, 2019).

Abiotic stress tolerance in rice plants with the help of endophytes, symbiotically, involves at least two mechanisms: (i) activation of the host plant system to evade or alleviate the impacts of the abiotic stress (Redman *et al.*, 1999); and

(ii) synthesis of stress-reducing biomolecules by endophytes (Schulz *et al.,* 2002). Verma *et al.* (2018) observed that ten species of endophytic bacteria *Pantoea* promoted growth in rice seedlings through restoration of root geotropic response, increased root and shoot growth, stimulation of root hair formation, etc. These endophytic bacteria were able to produce plant growth-promoting phytohormones like indole-3-acetic acid (IAA) in rice plants and also could solubilize phosphate under stress conditions too (Verma *et al.*, 2018). Among all the species *Pantoea hericii* produced the highest amount of IAA (Verma *et al.*, 2018). Endophytic bacteria *Arthrobacter* spp. and *Bacillus* spp. associated with rice plants showed significant decrease in upregulation and also downregulation of some stress-inducible genes when compared with gene expression in uninoculated plants (Ripa *et al.*, 2019). Endophytic fungi *Phoma glomerata* and *Penicillium* spp. significantly increased rice plant biomass and related growth parameters (Waqas *et al.*, 2012). Essential nutrients like potassium, calcium, magnesium were assimilated in the host body and sodium toxicity was reduced under sodium chloride, polyethylene glycol-induced salinity and drought stress in the plant associated with endophytic *P. glomerata* and *Penicillium* spp. On the other hand, these activities were not observed in the control plants (Waqas *et al.,* 2012).

29.4 Mitigation of abiotic stresses in rice plants via microbial endophytes

29.4.1 Augmentation of water-use efficacy during drought conditions

Rice plants associated with endophytes have been reported to use considerably less water and have an increased biomass compared to non-endophytic plants. The process of drought tolerance may be described by the higher accumulation of solutes in the tissues of endophyte-inhabiting plants as compared to non-endophytic plants, or by reduction of leaf conductance and weakened transpiration process in the host, or due to the formation of thicker cuticle in the leaf (Malinowski and Beleskey, 2000). There are some reports revealing that endophytic microbes emphasize some changes in gene expression patterns in the host plant undergoing drought stress, resulting into drought tolerance in the plant (Bailey *et al.*, 2006).

29.4.2 Extenuation of osmotic stress by plant physiological responses

A varied range of responses have been observed in rice plants against osmotic stress at the molecular, cellular and whole plant system level, such as controlling shoot (inhibition) and root (enhancement) growth, regulation of ion transport (uptake, extrusion, compartmentalization of ions) and alterations of metabolic reactions, for example, carbon metabolism, synthesis of compatible solutes, etc. (Eid *et al.*, 2019). Some of these responses are the result of the primary osmotic stress signals (OSS), others may be the outcome of secondary stresses/signals triggered by the primary OSS (Ram *et al.*, 2019). These secondary signals are the phytohormones (e.g. abscisic acid, ethylene), ROS, intracellular secondary messengers (e.g. phospholipids), etc. (Ram *et al.*, 2019). However, all the secondary signal molecules may not be confined within the primary stress sites such as the root. Root-induced ABA can move upward due to transpiration pressure to influence the closing and opening of stomatal aperture in leaves under drought stress (Zhang and Davies, 1991).

29.4.3 Enhancement of salinity or alkalinity tolerance

Enhancement of salt stress tolerance in rice plants is associated with the enhancement of antioxidant enzymes as proposed by earlier studies (Sekmen *et al.*, 2007). The ROS scavengers (e.g. glutathione, ascorbate and tocopherol) and enzymes like catalases (CAT), superoxide dismutases (SOD), glutathione reductases, ascorbate or thiol-dependent peroxidases (APX), dehydroascorbate reductases (DHAR), monodehydroascorbate reductases (MDHAR), etc., are involved in this process (Rouhier *et al.*, 2008). They are involved in the removal of ROS either directly (SOD, CAT, APX, etc.) or indirectly through the regeneration of ascorbate and glutathione in the host cell (Rouhier *et al.*, 2008). Endophytic *Piriformospora indica* induces salt tolerance in barley and rice by increasing the levels of antioxidants (Baltruschat *et al.*, 2008). In an experiment of constant exposure to 500 mmol L^{-1} NaCl solution, *Leymus mollis* (dune grass) plant having endophytic infection by *Fusarium culmorum*, could survive easily without showing any wilting symptoms until 14 days post exposure (Rodriguez *et al.*, 2008). In contrast, the non-symbiotic plants of the same species became brutally wilted and shriveled within 7 days and died after 14 days under the same exposure (Rodriguez *et al.*, 2008).

29.4.4 Encompassing alleviation of nutrient stress

Endophytes facilitate the supply of macro- and micro-nutrients to their host body. Root exudates of the rice plant are processed by nitrogen-fixing as well as phosphate-solubilizing bacteria and fungi, which in turn provides nitrogen to the plant for amino acid synthesis (Lata *et al.*, 2018). The endophytic microbes enhance plant growth by phosphate solubilization, inducing the synthesis of IAA, cytokinins, gibberellic acids (GAs), siderophores, etc., and by providing essential vitamins (Jha *et al.*, 2011). *Pseudomonas* spp. isolated from rice and wheat was found to mediate phosphate solubilization by producing gibberrellic acid (Choi *et al.*, 2008). Endophytic *Piriformospora indica* and *Azotobacter chroococcum* also amended the uptake of different mineral nutrients (especially Zn) in rice and wheat (Abadi and Sepehri, 2015). Different studies have established the role of endophytes in biodegradation of the litter of its host plants (Rodriguez *et al.*, 2008). Endophytic microbes primarily colonize the plants and expedite the saprophytic microbes to act on increasing the litter decomposition in the soil (Terekhova and Semenova, 2005). Another study revealed that all endophytes have the capability to decay organic components, including lignin, cellulose and hemicelluloses, which in turn enable in nutrient cycling (He *et al.*, 2012).

29.4.5 Heavy metal stress tolerance

Under increased Cd concentration in soil, *Exophiala pisciphila*, a root-associated dark septate endophyte (DSE), isolated from maize and some bacterial endophytes isolated from rice showed enhanced synthesis of some enzymes having antioxidant activity, inhibiting Cd stress in the host plants (Wang *et al.*, 2016; Verma *et al.*, 2018). Three vital genes were identified that are involved in the uptake, detoxification and transport of Cd as downregulation of ZIP (Zrt- and Irt-related protein family), upregulation of phytochelatins (PCs) and metal tolerance protein in the host, upon inoculation with DSE and consequent treatment with high Cd and other heavy metal (Zn, Mn, Co, Ni, etc.) concentrations (Wang *et al.*, 2016; Ram *et al.*, 2019). Endophytic *Pseudomonas* spp. and *Gigaspora* spp. can alter the levels of 1-aminocyclopropane-1-carboxylate (ACC) enzyme, resulting in the induction of tolerance to different heavy metals (Cd, Mn, Pb, etc.) in rice directly through the manipulation of plant ethylene levels (Friesen *et al.*, 2011).

29.4.6 Coping with soil temperature stress

There is some compelling evidence indicating that the interactions between microbes and plants influencing abiotic stresses is impacted at the tri-trophic level (Eid *et al.*, 2019). The incidence of viruses in fungi (mycoviruses) and their impact on mutualistic relationships with the host plants has attracted recent attention, implying a remarkable example of tri-trophic level interaction (Lata *et al.*, 2018). The capability of a grass species *Dichanthelium lanuginosum* to withstand in soil temperatures ranging from 38°C to 65°C in Yellowstone National Park was directly influenced by the association of one of its endophytic fungi *Curvularia protuberata* and its mycovirus *Curvularia thermaltolerance* (CThTV) (Redman *et al.*, 2002). The endophytic fungi associated with wheat and rice improved heat tolerance ability in the hosts, increasing their height, weight of the grains as well as germination of the second-generation seeds (Hubbard *et al.*, 2014). There exists a multidimensional interaction between endophytes and their host plants, resulting in the maintenance of the health of the host plants under different environmental stresses. Figure 29.1 illustrates such multidimensional interactions of endophytes with their host plants (Khare *et al.*, 2018).

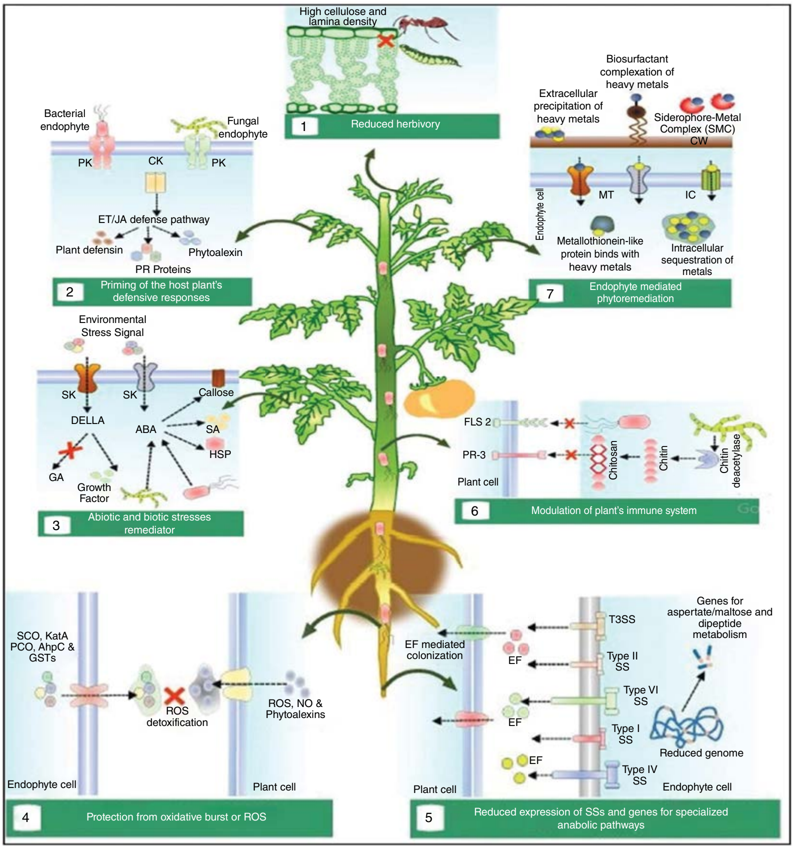

Fig. 29.1. The multifaceted interaction of endophytes with host plants. (1) Fungal endophytes change chemical and physical characteristics of the leaf such as high-cellulose content and lamina density, which provide toughness resulting in reduced herbivory rates, specifically by leaf-cutting ants. (2) Endophytes prime the host plant's defensive responses against phytopathogens. Early detection of the phytopathogen by cell surface receptor kinases and subsequent cytoplasmic kinases mediate intracellular responses and trigger ethylene/jasmonic acid transduction pathway. (3) Abiotic and biotic stress signals positively induce expression of the stress-responsive genes, preinvasion defense and enhanced callose deposition. However, ABA affects negatively signals that trigger systemic acquired resistance. The endophyte significantly modulate stress through the downregulation of ABA. Gibberellins synthesized by plants or endophytes hamper the inhibitory effects of DELLA proteins over the plant-growing signals. (4) Reactive oxygen species, generated by the plant, are neutralized by the production of enzymes such as superoxide dismutases, catalases, peroxidases, alkyl hydroperoxide reductases, and glutathione -*S*-transferases in endophytes. (5) Protein secretion systems, which deliver effector proteins into the plant are either absent or present in low abundance in mutualistic endophytic bacteria. Endophytes also encode specific genes for utilizing aspartate/maltose and dipeptides metabolism. (6) Fungal endophytes modulate the plant's immune system by the production of chitin deacetylases, which deacetylate chitosan oligomers and, hence, prevent themselves from being recognized by chitin-specific receptors of the plants that recognize chitin oligomers. Perception of flagellin from endophytes also differs from phytopathogens. (7) Endophytic microbes alleviate metal phytotoxicity via extracellular precipitation, intracellular accumulation, sequestration, or biotransformation of toxic metal ions to less toxic or non-toxic forms. RK, receptor kinase; CK, cytoplasmic kinase; ET, ethylene; JA, jasmonic acid; SK, sensor kinase; GA, gibberellic acid; DELLA, DELLA protein; ABA, abscisic acid; SA, salicylic acid; HSP, heat shock protein; ROS, reactive oxygen species; SOD, superoxide dismutases; CatA, catalases; POD, peroxidases; AhpC, alkyl hydroperoxide reductases; GSTs, glutathione-s-transferase s; EF, effector protein; PR-3, chitin-specific receptors; FLS 2, flagellin; TTSS, type III secretion system; SS, secretion system; MT, metal transporters; IC, ion channels; CW, bacterial cell wall. (From Khare *et al.,* 2018 CC-BY-4.0).

29.5 Conclusion and future prospects

The endophytes, boosters of plant immune system, represent an eco-friendly approach for the promotion of plant growth and productivity. Endophytes are very important unique biological resources, which need to be explored for sustainable development of agricultural needs. Rice plant health is obstructed by different abiotic stresses resulting in poor growth and development, less productivity, etc. This problem can be mitigated by microbe-induced stress tolerant abilities in plants rather than chemical-induced stress tolerance, which is an eco-friendly approach for better crop yield. The hidden friends of the rice plant, i.e. the endophytes, help the plant to exist under drought conditions, high concentration of salt, heavy metals, temperature, etc., by synthesizing and releasing different phytohormones and other growth-promoting molecules under such stress conditions, thereby increasing global rice production. Thus, with increasing population worldwide, growing interest in environmental protection, food security, sustainable agriculture, etc., exploration of beneficial endophytes is urgent. However, there is a need to know about the lifestyle, biochemistry, physiology and molecular pathways of the endophytes at least up to the genomic and metabolomics levels in the rice plants so that they can be implemented in a proper way in the agricultural field. The signal mechanisms to produce different plant growth regulators under stress conditions by the host plants induced by the endophytes are yet to be understood properly. As endophytes can reduce the abiotic stresses in rice plants, therefore, they should be properly investigated for future applications in agriculture worldwide.

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30 Plant–Microorganism Interactions Remediate Heavy Metal-contaminated Ecosystems

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Abstract

Technogenic advances have led to the exploitation and contamination of soil/water ecosystems. The alleviation of heavy metal contaminants from polluted soils through non-conventional methods is a cost-effective and eco-friendly approach. Phytoremediation is a prominent green technology employed to remediate contaminated soil/water ecosystems. Rhizospheric microorganisms are omnipresent and can tolerate a wide range of contaminants, hence they can facilitate the removal of noxious contaminants. Microorganism-assisted phytoremediation is a safe and innovative method for remediation of toxic substances. Microorganisms like bacteria and fungi in the rhizosphere can sense signals and enable the plants to tolerate metal-induced toxicity along with growth promotion. These rhizosphere microbes can accumulate, transform or detoxify the toxic substances. In this chapter, we discuss heavy metal-induced toxicity, microorganism–plant interactions and microorganism-assisted phytoremediation of contaminated ecosystems. This chapter is also intended to give an overview of the recent findings, challenges and opportunities in microorganism-assisted plant-based reclamation of contaminated soils.

30.1 Introduction

Environmental contamination by heavy metals is a serious environmental concern worldwide. Heavy metals have been reported to cause severe health concerns in humans (Bali *et al.*, 2020; Sidhu *et al.*, 2020). Heavy metals are non-biodegradable and persistent in nature (Kumar *et al.*, 2019a, 2019b). They are known to cause soil/water pollution, inducing toxicity, genotoxicity and mutagenic effects on the living organisms. Heavy metals have an atomic number greater than 20 and have an elemental density greater than 5 g cm^{-3}. Heavy metals such as Pb, Cd, As, Cr and Hg do not have any biological function and are known as non-essential elements (Ali and Khan, 2018). A number of environmental protection agencies have listed heavy metals as priority pollutants that are liable to cause serious health hazard to humans (Sarwar *et al.*, 2017). Therefore, there is an urgent need to employ environmentally sound strategies to alleviate the heavy metals from the contaminated ecosystems.

Conventional approaches are costlier and environmentally destructive in nature (Sidhu

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et al., 2018a). They are highly expensive, viable for relatively small areas, cause secondary pollution and hamper the soil fertility and affect the agroecosystems. Phytoremediation is a sustainable alternative to the conventional remediation techniques (Sidhu *et al.*, 2018b). It is a cost-effective and eco-friendly approach for the prevention and control of heavy metal contamination. According to Mahar *et al.* (2016), this aesthetically pleasant technique can be commercially utilized by extracting the usable forms of economically viable metals during the process of phytomining. In addition, burning of plant biomass can generate energy and land restoration can be achieved for sustainable agriculture. Nevertheless, physiologic damage to the remediating plant and long time frame are the major limitations of phytoremediation.

Bioremediation is another effective non-conventional remediation technique in which microorganisms in association with the plants are used to mitigate the soil/water ecosystems contaminated with organic and inorganic contaminants (Sidhu *et al.*, 2019). Microorganism-assisted phytoremediation is the remediation of contaminated ecosystems via degradation and detoxification of heavy metals from polluted sites. It acts as a better substitute to promote plant growth and phytoremediation of metal contaminated sites (Calvo *et al.*, 2014). Therefore, the integration of the two different processes needs better understanding. Hence, the integrated processes should be carried out in such a manner that they should be experimentally feasible in pilot-scale experiments, relatively less expensive and economically viable. Owing to the encouraging outcomes, integrated approaches are gaining in importance for heavy metal alleviation from various ecosystems. This chapter outlines the microorganism-assisted phytoremediation of heavy metal-contaminated matrices, metal acclimatization and detoxification, and discusses the challenges with recommendations for the future research.

30.2 Heavy metals as environmental contaminants

Heavy metals are the naturally occurring compounds found in the earth's crust. Their contamination in the environment is caused by both anthropogenic and natural sources. Anthropogenic sources, including mining, smelting and excessive use of the fertilizers, are the main culprits for heavy metal pollution of soil/water ecosystems. According to Nagajyoti *et al.* (2010), agricultural fertilizers especially phosphate fertilizers contain excessive concentration of Pb, Cr, Ni, Zn and Cd. In agricultural soils, use of untreated sewage sludge, pesticides, liming and untreated water used for irrigation contribute toward heavy metal contamination. Heavy metal contamination at wastewater irrigation sites in Zambia have resulted in their acclimatization in the soils and crops above the permissible limits (Kapungwe, 2013). Moreover, discharge of heavy metals into the water deteriorates the quality of water and makes it unfit for drinking and agricultural practices (Nazeer *et al.*, 2016). The release of effluents from various domestic activities is also responsible for heavy metal contamination of the environment and thus is of serious concern worldwide (Bhardwaj *et al.*, 2017).

In aquatic environments, metal stress negatively affects the production of gametes, quality of sperm and embryo development, and leads to physical abnormalities in fishes (Fatima *et al.*, 2014). Heavy metal exposure induces synaptic damage, oxidative stress, and neurotoxicity and genotoxicity in fishes (Lee *et al.*, 2019). It causes a decline in glycogen reserve and causes weakness in fishes (Javed and Usmani, 2015).

Soil is the major sink for heavy metals. The metal contamination of agricultural soil affects its physiochemical and biological properties leading to decreased land area for farming thus causing food insecurity (Wuana and Okieimen, 2011). The existence of metals in the soil leads to entry of metals into the food chain causing severe health hazards in humans (Sidhu *et al.*, 2018a). The irrigation of agriculturally edible crops with contaminated water causes its movement from soil to animals/humans, thus disrupting vital metabolic processes. Heavy metals affect different enzymes such as nitrogenase, ribonuclease, nitrate reductase, phosphatase, RuBisCo, etc. (Yadav, 2011). It disrupts the biochemical processes in plants like nitrogen metabolism, transpiration, photosynthesis, water balance and ionic balance that ultimately cause death of plants (Sidhu *et al.*, 2017). Metal exposure harms the different cytological processes in plants such as cellular permeability, cell elongation and mitotic activity, leading to chromosomal aberrations in plants (Gupta *et al*., 2018a).

Heavy metals may cause oxidative stress due to the formation of reactive oxygen species (ROS) that harm the antioxidant defense system in living organism, resulting in cellular damage. Oxidative stress can be caused by high concentrations of metals and leads to inhibition of growth (Fryzova *et al*., 2017). ROS can induce oxidation of proteins and lipids, DNA malfunction and inhibition of enzyme activity, leading to cell death (Sharma *et al*., 2012). However, Schutzendubel and Polle (2002) suggested that ROS play a major role in plant defense and so it cannot be completely removed from plants.

30.3 Microbe-assisted phytoremediation of contaminated ecosystems

Rapid urbanization and industrialization has led to contamination of the environment. The discharge of wastewater from different industries is a major source of soil and water pollution. A number of physical and chemical methods are employed for treatment of industrial waste; however, they are costly and use large amounts of chemicals for the treatment process (Deb *et al*., 2020). On the other hand, the non-conventional approach like phytoremediation utilizes plants for alleviating heavy metal contaminants while bioremediation utilizes specific microbial strains for mitigating inorganic contaminants from polluted ecosystems (Conesa *et al*., 2012). These biological techniques for remediation are environmentally safe and economical (Sarma and Prasad, 2019).

Phytoremediation is the most effective remediation technique for contaminated sites. It is the use of green plants and associated soil microbes to remediate toxic compounds from contaminated soil and water using degradation and detoxification mechanisms (Ali *et al*., 2013). It is an eco-friendly technology that extracts heavy metals from contaminated areas (Chirakkara *et al*., 2016). Phytoremediation employs a range of plant-based remediation techniques that decreases the chance of metal dispersion. The efficacy of phytoremediation depends upon the physiochemical properties of soil or water, species of plant, class of pollutant and its bioavailability (Sreelal and Jayanthi, 2017). However, plants with small biomass and slow growth cycle pose a hindrance to the phytoremediation technology. Therefore, exploitation of plant–microbe interactions to augment growth and phytoremediation potential of plants could be employed on metal-contaminated sites. Table 30.1 shows recent studies that use microbes (plant growth promoting rhizobacteria, endophytes and arbuscular mycorrhizal fungi [AMF]) in the remediation of metal-contaminated soils.

Plant growth promoting rhizobacteria (PGPR) are free-living, soil-borne, root colonizing bacteria that enhance growth of plants by modulating different plant growth mechanisms under metal-contaminated areas (Manoj *et al*., 2020). PGPR produces various growth promoting substances such as phytohormones, ammonia, osmolytes, organic acids and siderophores that promote growth in plants (Ma *et al*., 2015). The pathways or procedure for promotion of plant growth varies among different bacterial strains and mostly depend upon different secondary metabolites/compounds produced by plants (Backer *et al*., 2018). PGPR-induced improved plant growth mainly depends upon two methods: specific and non-specific mechanisms. Under specific mechanisms, PGPR promote root growth, cell division and elongation and help in availability of soil nutrients to the plants. However, non-specific mechanisms alleviate metal-induced toxicity and promote initiation of systemic resistance thereby enhancing growth (Manoj *et al*., 2020) (Table 30.1). Therefore, PGPR usage in remediation of contaminated environments could be an important approach for survival of plants in stressful conditions.

Endophytes (bacteria and fungi) are microorganisms that live in intercellular and intracellular spaces in plant tissues. They do not cause harm to the host plant and they promote plant growth under stressful environment. Heavy metal stress causes ROS generation that leads to membrane disintegration, protein malfunction and DNA damage in plants (Sidhu *et al*., 2018a). Various reports have suggested that endophytes activate ROS-detoxification mechanisms in host plants and thus mitigate metal toxicity in plants (Wang *et al*., 2020b) (Table 30.1). The removal of ROS causes enhanced biomass of plant that

Table 30.1. Role of plant growth promoting rhizobacteria, endophytes and arbuscular mycorrhizal fungi in mitigation of metal stress in plants.

Bacteria strain	Host plant	Metal	Medium	Positive effects	References
Plant growth promoting rhizobacteria					
Pseudomonas spp. (PsA4) and *Bacillus* spp. (Ba32)	*Brassica juncea*	Cr	Soil	Siderophores production and solubilization of phosphate	Rajkumar *et al*. (2006)
Pseudomonas spp. (Ps29C) and *Bacillus megaterium* (Bm4C)	*Brassica juncea*	Ni	Soil	Promoted plant growth and alleviate Ni toxicity	Rajkumar and Freitas (2008a)
Pseudomonas spp. (PsM6) and *Pseudomonas jessenii* (PjM15)	*Ricinus communis*	Ni, Cu and Zn	Soil	Increased shoot and root biomass	Rajkumar and Freitas (2008b)
Microbacterium oxydans (JYC17), *Pseudomonas thivervalensis* (Y1-3-9), *Burkholderia cepacia* (J62)	*Brassica napus*	Cu	Soil	Increased Cu remediation efficiency	Ren *et al*. (2019)
Zinc tolerant bacteria (ZTB15, ZTB24,ZTB28,ZTB29)	*Zea mays*	Zn	Soil	Enhanced SOD, POD, PAL, CAT and ppo	Jain *et al*. (2020)
Pseudomonas putida (ATCC 39213)	*Eruca sativa*	Cr	Soil	Increased plant growth andCr phytoextraction	Kamran *et al*. (2017)
Kocuria flava and *Bacillus vietnamensis*	*Oryza sativa*	As	Soil	Promoted growth and decreased As uptake and accumulation	Mallick *et al*. (2018)
Bacillus and *Halobacillus*	*Arachis hypogea*	Zn, Al and Pb	Soil	Decreased lignification, intact protoxylem and cortical parenchyma	Banik *et al*. (2018)
T2Cr and CrP450	*Zea mays*	Cr	Soil	Increased plant growth, declined MDA and H_2O_2 level	Islam *et al*. (2016)
Serratia marcescens S2I7	*Oryza sativa*	Cd	Soil	Incremented root and shoot length	Kotoky *et al*. (2019)
Burkholderia spp.D54 and *Burkholderia* spp. D416	*Sedum alfredii*	Cd, Pb and Zn	Soil	Enhanced soil respiration and growth	Guo *et al*. (2020)
Bacillus licheniformis, *Micrococcus luteus*, *Pseudomonas florescens*	*Grapevine*	As	Soil	Peaked antioxidant activity, protein and biomass	Pinter *et al*. (2017)
Pseudomonas aeruginosa KUJM	*Lens culinaris*	Cd, As, Co, Cu, Cr, Ni andZn	Wastewater	Increased IAA production	Biswas *et al*. (2017)
Pseudomonas and *Azotobacter*	*Eichhornia crassipes*	As	Wastewater	Enhanced As removal	Kaur *et al*. (2018)
Pseudomonas spp. (CPSB21)	*Helianthus annuus*	Cr	Soil	Increased uptake of Cr	Gupta *et al*. (2018b)
Bacillus spp. KUJM2	*Lens culinaris*	As, Cd, Cu, Ni	Wastewater	Enhanced germination rate, index and seed production	Mondal *et al*. (2019)
Paenibacillus mucilaginosus and *Sinorhizobium meliloti*	*Medicago sativa*	Cu	Soil	Improved soil fertility and biological activity	Ju *et al*. (2019)

Continued

Table 30.1. Continued.

Bacteria strain	Host plant	Metal	Medium	Positive effects	References
Bacterial endophytes					
Pantoea stewartii ASI11, *Enterobacter* spp. HU38 and *Microbacterium arborescens* HU33	*Leptochloa fusca*	U and Pb	Soil	Enhanced plant growth and phytoremediation capacity	Ahsan *et al*. (2017)
Paneibacillus RM	*Tridax procumbens*	Cu, Zn, Pb and As	Soil	Produced secondary etabolites, IAA, siderophores	Govarthanan *et al*. (2016)
Acinetobacter spp. BRSI56, *Bacillus cereus* BRSI57, *Bacillus licheniformis* BRSI58	*Brachiaria mutica*	Cd, Cr, Fe and Ni	Wastewater	Enhanced removal efficiency of *B. mutica*	Ijaz *et al*. (2015)
Bacillus, *Staphylococcus* and *Aerococcus*	*Lolium multiflorum*	Cr	Soil	Improved plant growth and metal removal capacity	Khan *et al*. (2015)
Microbacterium arborescens HU33, *Pantoea stewartii* ASI11 and *Enterobacter* spp. HU38	*Brachiaria mutica*	Cr, Cu, Fe, Mn, Ni, Cd and Pb	Wastewater	Increased growth and reduced toxicity of pollutants	Ashraf *et al*. (2018b)
Pseudomonas azotoformans ASS1	*Trifolium arvense*	Cu, Zn and Ni	Soil	Enhanced chlorophyll content, growth and metal uptake	Ma *et al*. (2017)
Acidovorax spp. U3, *Ralstonia* spp. U36, *Pseudomonas* spp. R16 and *Ochrobactrum* spp. R24	*Juncus acutus*	Cr	Groundwater	Mitigated Cr toxicity	Dimitroula *et al*. (2015)
Proteus vulgaris H7, *Pseudomonas* spp. H15, *Pseudomonas helmanticensis* H16	*Sinapsis alba*	Zn and Cd	Soil	Promoted plant growth and resistance to heavy metals	Płociniczak *et al*. (2019)
Pantoea stewartii ASI11, *Microbacterium arborescens* HU33 and *Enterobacter* spp. HU38	*Leptochloa fusca*	Cr	Wastewater	Increased bioremediation and detoxification potential	Ashraf *et al*. (2018a)
Paenibacillus spp. SB12, *Bacillus* spp. SB31, *Bacillus* spp. LB51 and *Alcaligenes* spp. RB54	*Helianthus annuus*	Cd	Soil	Enhanced Cd accumulation and growth	Siripan *et al*. (2018)
Bacillus megaterium BM18-2	*Pennisetum americanum* × *P. purpureum*	Cd	Soil	Peaked growth and tolerance to Cd toxicity	Wu *et al*. (2019)
Fungal endophytes					
Phialocephala fortinii, *Rhizodermea veluwensis* and *Rhizoscyphus* spp.	*Clethra barbinervis*	Cu, Zn, Ni, Cd	Soil	Tolerated high heavy metal concentration	Yamaji *et al*. (2016)

Penicillium ruqueforti	*Triticum aestivum*	Ni, Cd, Cu, Zn and Pb	Soil	Higher plant growth, nutrient uptake and decreased concentrations of heavy metals	Ikram *et al*. (2018)
Fusarium spp. CBRF44, *Alternaria* spp. CBSF68, *Penicillium* spp. CBRF65	*Brassica napus*	Pb and Cd	Soil	Increased biomass and metal extraction efficiency	Shi *et al*. (2017)
Mucor spp. MHR-7	*Brassica campestris*	Cr, Mn, Co, Cu and Zn	Soil	Improved bioremediation potential	Zahoor *et al*. (2017)
Trametes hirsuta	*Triticum aestivum*	Pb	Soil	Increased uptake of metal	Malik *et al*. (2020)
Penicillium funiculosum LHL06	*Glycine max*	Ni, Cu, Pb, Cr and Al	Soil	Higher antioxidant activity, expression of stress-related proteins	Bilal *et al*. (2019)
Aspergillus fumigatus, *Rhizopus* spp., *Penicillium radicum* and *Fusarium proliferatum*	*Lactuca sativa*	Cr	Soil and culture media	Restored normal growth of the plant	Hussain *et al*. (2018)
Arbuscular mycorrhizal fungi					
Claroideoglomus etunicatum	*Zea mays*	La and Cd	Soil	Increased shoot and root Cd and La concentrations	Chang *et al*. (2018)
Glomus versiforme	*Zea mays*	As	Soil	Enhanced root colonization, dry matter accumulation and antioxidant enzyme activity	Wang *et al*. (2018)
Rhizophagus irregularis	*Solanum nigrum*	Cd	Soil	Alleviation of Cd stress	Wang *et al*. (2020a)
Glomus mossae	*Solanum lycopersicum*	Cd and Pb	Soil	Improved growth, chlorophyll content, enzymatic defense mechanism	Chaturvedi *et al*. (2018)
Funneliformis mosseae and *Rhizophagus intraradices*	*Capsicum annuum*	Cu	Soil	Higher total dry weight and leaf area	Ruscitti *et al*. (2017)
Glomus mosseae	*Vetiveria zizanioides*	Zn, Fe, Cu, Cd and Pb	Wastewater	Increased biomass production, chlorophyll content and photosynthetic plants	Kafil *et al*. (2019)
Rhizophagus irregularis, *Acaulospora longula*, *Funneliformis mosseae*, *Scutellospora calospora*	*Plantago lanceolata*	U, Th, As and Pb	Soil	Enhanced accumulation of metals in roots and shoots	Rosas-Moreno *et al*. (2021)
Rhizophagus irregularis	*Phragmites australis*	Cu	Soil	Promoted growth	Wu *et al*. (2020)

CAT, catalase; IAA, indole-3-acetic acid; MDA, malondialdehyde; PAL, phenylalanine lyase POD, peroxidase PPO, polyphenol oxidase; SOD, superoxide dismutase may be useful in phytoremediation process (Durand *et al*., 2021).

Arbuscular mycorrhizal fungi form symbiotic association with roots of vascular plants that help plant to absorb nutrients and perform better in natural environment and in turn take food from host plants (Riaz *et al*., 2020). Mycorrhiza improves physiochemical properties of soil and can cause immobilization of metals in roots of plants. There are many studies related to AMF-assisted remediation of metals from contaminated soils, and recently this technique is receiving major attention since it is environmentally safe and cost effective (Miransari, 2017; Krishnamoorthy *et al*., 2019; Ma *et al*., 2019; Dhalaria *et al*., 2020) (Table 30.1).

30.4 Mechanism of plant–microbe interaction for remediation of metal-contaminated ecosystems

Plants growing in contaminated soils often encounter stressful conditions that degrade growth and development of the plant. However, the pessimistic effect of contaminants can be mitigated by using microorganisms. Soil environment is rich in diverse microbes that maintain physiochemical properties and nutrient cycling in soil (Ahemad and Khan, 2013). Many reports have confirmed plant–microbe interaction to alleviate metals from contaminated soils (Ma *et al*., 2017; Wang *et al*., 2018). The microbe-assisted remediation can also enhance the growth and development of plants under stressful conditions. The association between plants and microbes in metal-contaminated ecosystems largely depend upon both the host plant and microorganisms used (Egamberdieva *et al*., 2016). The plant-associated microbes improve the soil texture, modulate defense responses in plants, detoxify pollutants and, in turn, get nutrients from the host plant. The microbe-aided phytoremediation occurs by both direct and indirect mechanisms. The direct mechanism includes enhanced uptake, accumulation and translocation of toxic metals. The indirect mechanisms enhance plant tolerance and promote plant growth under stressed conditions.

30.4.1 Direct mechanisms

In contaminated ecosystems, metals get adsorbed into the soil particles and are not available for the plant roots for uptake (Gamalero and Glick, 2012). Microbes secrete biosurfactants and organic acids, release siderophores and solubilize phosphates that enhance the solubility and availability of metals to plants. Soils are a major source of phosphorus; however, since it is present in insoluble form, it is not available to the plants (Ahemad, 2015). Recently, many reports have opined the role of microbes in phosphate solubilization by producing organic acids (Bi *et al*., 2019; Sowmya *et al*., 2020). For example, Teng *et al*. (2019) studied the role of phosphate-solubilizing bacteria for Pb immobilization in soil. The results revealed a strong Pb-resistant capability of bacterial strains that could efficiently solubilize 200 mg L^{-1} of P due to enhanced concentrations of organic acids, pH and acid phosphatase. In another study, Jeong *et al*. (2012) reported enhanced bioavailability and phytoextractability of Cd in *Brassica juncea* and *Abutilon theophrasti* by phosphate-solubilizing bacteria, *Bacillus megaterium*.

Another important class of secondary compounds are siderophores, that are low-molecular weight substances capable of binding Fe and functioning as iron solubilizing agents in soil (Bhandari and Bhatt, 2021). Siderophores can form strong complexes with other metal ions, like Cd, Cu, Pb and Zn. Several genetically diverse group of microbes have been reported to secrete siderophores and assist in providing metal tolerance to plants (Ullah *et al*., 2015). For example, Yu *et al*. (2017) investigated the siderophore-producing characteristics of *Bacillus* spp. and also studied its role in uptake and translocation of Pb in *Brassica juncea*. The results revealed siderophore induced enhanced phytoextraction of Pb from soil in *B. juncea*. Recently, Sepehri and Khatabi (2021) concluded role of siderophore-producing bacteria in minimizing Cd toxicity and enhancing growth rate in alfalfa plants. Further, numerous PGPRs have reported production of low-molecular weight organic acids in increasing solubility and mobility of harmful metals (Ullah *et al*., 2015). In this context, Arwidsson *et al*. (2010) reported the role of oxalic acid and citric acid secreted by fungi in remediation of Ni-, Zn- and Cu-contaminated soils.

30.4.2 Indirect mechanisms

The indirect mechanism includes production of phytohormones and enzymes, enhanced nutrient uptake and tolerance to stressful conditions (Deb *et al.*, 2020). A variety of microbes are reported to produce phytohormones like IAA (indole-3-acetic acid), gibberellins and cytokinins under adverse environmental conditions. IAA regulates various morphological and physiological mechanisms in plants (Glick, 2012). It is mainly involved in stimulating growth of lateral roots. For example, Khiangte and Lalfakzuala (2021) reported enhanced production of IAA by phosphate-solubilizing bacteria under metal stressed conditions. Ethylene is a crucial phytohormone that regulates different functions in plants; however, its overproduction causes a negative effect on the growth of plants. Microbial ACC (1-aminocyclopropane-1-carboxylic acid) deaminase enzyme converts ACC into ketobutyrate and ammonia that is used as a nitrogen source by microorganisms. Thus, plants inoculated with bacterial strains that contain ACC deaminase will indirectly hamper the root growth and enhance the biomass and heavy metal remediation efficiency (Hassan *et al.*, 2016).

30.5 Challenges to the application of microbial mediated phytoremediation

The progress of phytoremediation process depends upon plant–soil–metal interaction. Some plants show reduced biomass and limited growth under increased concentration of toxic compounds or pollutants. Therefore, selection of a plant species is very important for remediation of contaminated soils. Microbes in soil positively affect the growth of the plant by growing in strong association with them. However, as microbes are highly susceptible to metal pollution, they show decreased biomass and number when grown in contaminated sites. Stressful environment alters the physical, chemical and biological properties of soils and affect the interaction among plant and microbe. Moreover, isolation of microbes for the remediation process is a very tedious task and requires analysis of large number of strains for an effective phytoremediation process (Rajkumar *et al.*, 2012). Further, extensive research is needed to explore some novel microbial strains to alleviate metal toxicity in plants.

30.6 Conclusions

The contamination of environment with heavy metals is a serious threat to the ecosystem. The use of microbes (PGPRs, endophytes and AMFs) is an efficient strategy to increase metal detoxification in the soil. Therefore, a complete understanding of plant–microbe interaction is must for remediation of metal-contaminated sites. The identification of some important genes that are responsible for peaked growth and metal sequestration is crucial for the remediation process. The production of genetically engineered metal accumulators could be a beneficial method for increased phytoremediation in plants. Thus, microbe-aided phytoremediation technique is promising option for sustainability of environment.

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31 Alleviation of Salinity Stress in Non-legumes by Beneficial Salt-tolerant Bacteria

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Abstract

Salinity is a chronic agricultural problem, hindering the crop productivity in arid and semi-arid regions of the world where a vast area of lands remains fallow due to various degrees of salt accumulation. The severity of salinity is higher in the dry season owing to low rainfall where glycophytic crop plants cannot be grown because of the deleterious effects of salt. The harmful effects of high salinity include creation of osmotic stress, ionic imbalance due to excessive uptake of Na^+ and Cl^- ions and leakage of nutrients caused by membrane damage. Various approaches have been taken to overcome the toxic effects of harmful stress; however, no economically viable and sustainable technologies have yet to be developed for boosting the crop productivity. Recently, plant growth-promoting rhizobacteria bacteria (PGPR) have been shown to exert beneficial effects on various crop plants, especially the non-legumes, and the effects are carried out in multidimensional approaches in addition to biological N_2 fixation (BNF) process. They create a conducive environment in the rhizosphere as well as in the shoots for providing benefits to the host plants, consequently increased the crop productivity. PGPR also alleviate the abiotic stresses like drought and salinity. The dominant candidates of PGPR include *Azospirillum, Herbaspirillum, Pseudomonas*, *Burkholderia, Azotobacter* and *Bacillus*. This chapter discusses the beneficial salt-tolerant plant growth-promoting bacteria, their mechanisms of beneficial effects and future projections for developing a sustainable technology for salt-affected crop plants.

31.1 Introduction

There is a need to produce 87% more food than the present figure to meet the demand of the ever-increasing population in the world (Fróna and Szenderák, 2019). However, the productivity of crops is currently not increasing to that extent. In addition, crops are also affected by various biotic and abiotic stresses, with salinity being one of the devastating, hindering the yield drastically. Soil salinity is an ever-increasing global problem that stress hampers crop growth, development and, finally, the yield. The term salinity includes the accumulation of NaCl and other salt ions (e.g. K^+ and Ca^{2+}) in the soil. It is reported that nearly 20% of irrigated lands in the world are affected by various degrees of salinities (Egamberdieva *et al.*, 2019). In the saline soils, the key soluble cations are Na^+, K^+, Mg^{2+}, Ca^{2+} and anions Cl^-, HCO_3^-, and NO_3^-, where

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Na^+ and Cl^- have been found to be the most dominating ion species (Rengasamy, 2006). The concentration of soluble ions increased by gradual increment of saline water in the soil. Thus, a salt level increase at the surface of soil results in the appearance of a salty soil. Higher concentration of salts in the soil also disturbs soil processes, and the level of Na^+ at the interchange complex of the soil (sodicity) affects the mechanical strength of soil. This is visible in the morphological, physiological, biochemical and molecular levels of the plant. Based on the reaction to salinity, plants can be classified as either glycophytic (i.e. susceptible) or halophytic (i.e. tolerant). Unlike many toxins and herbicides, excess NaCl has no single cellular target but injures the cells by multidimensional approaches. The harmful effects on plant growth due to salinity are accomplished by various processes, namely low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion toxicity or a combination of those (Ashraf, 1994; Khaware *et al.*, 1995). Although the response of plants to salt stress is a complex phenomenon involving processes from the cellular to the whole-plant level, the exclusion of cytotoxic ions such as Na^+ from the cytosol is an absolute requirement (Kalampanayil and Wimmers, 2001). Organic solutes like sugar, proline and other ammonium compounds are also produced in response to salt stress. These are localized into cytoplasm. The inorganic ions such as Na^+ and Cl^- are preferentially sequestered into the vacuole, thus leading to turgor maintenance for the cell under osmotic stress.

The composition of the soluble salts in these saline soils can indicate possible management strategies for crop production. The use of saline water for irrigation without proper management may render the irrigated soils as affected and, consequently, crop production may be seriously depleted. Currently, this problem receives a lot of attention because of its widespread occurrence as a result of climate change. To mitigate the salinity problem affecting crop productivity, the development of saline-tolerant, high-yielding genotypes should be a top priority. However, crop tolerance to salt stress is a complex field that can be addressed in various ways, including ion accumulation, tissue-specific growth rates, production of biomass and reproductive capacity. It would be judicious to optimize various salt responses in order to get higher yield depending upon the nature of crop.

Dilution of excessive amount of Na^+ and Cl^- ions accumulated in the plant body is one of the important mechanisms of salinity tolerance in a crop (Razzaque *et al.*, 2011). In this process excessive amounts of ions are distributed throughout the plant body, thereby reducing the deleterious effect of salt ions. It is important to mitigate the salinity problem to achieve a sustainable crop production system in arid and semi-arid regions where salinity limits the crop productivity. Various means have been used to alleviate the deleterious effects of salinity in crop plants, for example the development of salt-tolerant crop varieties, improved agronomic practices and the use of bio-stimulating microbial inoculants for sustainable crop production systems in the problem areas. Among the microbial inoculants, application of plant growth-promoting bacterial technology for alleviating abiotic stress in different crop plants is gaining prominence for creating a sustainable environmentally friendly crop production system (Bacilio *et al.*, 2004; Grover *et al.*, 2011). Remarkable progress has been achieved in mitigating the salinity problems by the use of plant growth-promoting/stimulating bacteria (PGPB), which can be improved both the crop productivity and the soil fertility. PGPB are root-associated bacteria that, being both rhizospheric and endophytic, can exert beneficial effects in a multidimensional approach. Their adaptive response to halotolerance creates a new avenue for research.

31.2 Occurrence of salt-tolerant, plant growth-promoting bacteria

Soil is the main reservoir for the vast array of microbes that can withstand salt stress. The bacteria also inhabitant the plant roots in both a rhizospheric and endophytic manner. The salt-tolerant, plant growth-promoting bacteria (STPGPB) can be classified as rhizospheric, endophytic, symbiotic and phyllospheric. The plant growth-promoting rhizobacteria inhabit the rhizosphere region of the plant, where the root exudates provide the main source of their food materials. The endophytic bacteria reside in the internal structure of the plant roots, stem and in the leaves. They are mainly found in the apoplastic area of cortex, in the xylem and the mesophyll tissue.

They obtain carbohydrate from the host plants and provide beneficial activities to the plant, such as secretion of phytohormone and fixation of N_2 (Mia *et al.*, 2007). The symbiotic bacteria are also regarded as endophytic bacteria that make a strong association with the host plant. The phyllospheric bacteria are located on the leaf and stem surface and exert beneficial effects. It is reported that the genera *Pseudomonas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Streptomyces*, *Klebsiella* and *Ochromobacter* are best recognized for enhancing the productivity of various crops under diverse saline conditions (Sharma *et al.*, 2016; Singh *et al.*, 2016; Sarkar *et al.*, 2018). The various types of plant growth-promoting bacteria are shown in Fig. 31.1.

The beneficial effects that rhizospheric, endophytic and phyllospheric PGB exert on a large number of crop plants have been documented by various researchers (Mia *et al.*, 2016). Many of PGB are isolated from different types of halophytic plants ranging from 4–8% NaCl salinity levels (Sharma *et al.*, 2016) (Table 31.1).

As reported by Sharma *et al.* (2016), the productivity and yield of peanut (*Arachis hypogea* L.) can be increased by 4–8% through the application of STPGB diazotrophic *Klebsiella*, *Agrobacterium*, *Pseudomonas* and *Ochrobactrum* species that have isolated from the halophytic plant *Arthrocnemum indicum*. In addition, the plant growth of *Chrysanthemum* was found to be increased under saline conditions by the application of *Bacillus licheniformis* (Zhou *et al.*, 2017). The genome sequence of several salt-tolerant plant growth-promoting bacteria has shown to predict the effectiveness of salt tolerance (Kothari *et al.*, 2013). Liu *et al.* (2016) reported a whole genomic study of halotolerant PGPR having beneficial traits such as phosphate solubilization, indole-3-acetic acid (IAA) and siderophore-producing and N_2-fixing capacity. Albdaiwi *et al.* (2019) isolated a good number of salt-tolerant PGPB belonging to the genera *Bacillus*, *Halomonas* and *Oceanobacillus* that possess growth-promoting characteristics such as N_2 fixation, ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity, auxin production, P solubilization and siderophore production for absorbing Fe under saline condition.

31.3 Isolation and identification of STPGPB from halophytes

Various types of STPGPB have been isolated from diverse halophytic crop plants, and easy standard techniques have been established. Generally, they are isolated through dilution plate or streak techniques on selective saline media. The capacity of beneficial effects is tested *in vitro*, pot culture and, finally, in the salt-affected fields. The sequential steps of isolation, identification and proof of their beneficial effects shown in flow chart, as modified from Mia and Shamsuddin (2013) (Fig. 31.2).

31.4 Mechanisms of alleviation of salt stress by STPGPB

The PGPB exert beneficial effects on a diverse arena of plant attributes through increased root

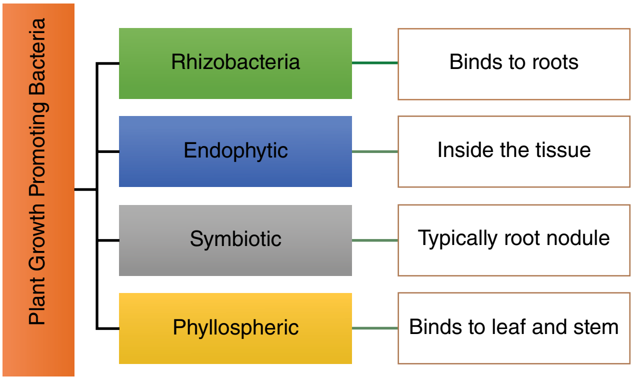

Fig. 31.1. Various types of plant growth-promoting/stimulating bacteria with their occurrence in the plant body.

Table 31.1. List of salt-tolerant growth-promoting bacteria, their effect on the crop plants and mode of beneficial effects.

Name of plant growth-growth-promoting bacteria	Name of crop	Mode of beneficial effects	References
Achromobacter spp.	*Solanum lycopersicum*	Decreased the level of ethylene	Mayak *et al*. (2004)
Acinetobacter spp.	*Cucumis sativus*	Decreased the ethylene content	Kang *et al*. (2014)
Aeromonas spp.	*Triticum aestivum*	Production of exopolysaccharide	Ashraf *et al*. (2004)
Arthrobacter spp.	*Pisum sativum, Triticum, aestivum*	Enhanced nutrient accumulation thereby improves plant growth	Barnawal *et al*. (2014), Upadhyay *et al*. (2011)
Acinetobacter spp.	*Cucumis sativus*	Decreased ethylene content	Kang *et al*. (2014)
Azospirillum spp.	*Helianthus annuus*, *Zea mays*	Improved chlorophyll content	Naz *et al.* (2009), Hamdia *et al*. (2004)
Azotobacter spp.	*Zea mays* L.	Enhanced nutrient accumulation	Rojas-Tapias *et al*. (2012)
Brachybacterium spp.	*Arachis hypogaea*	Higher accumulation of K^+	Shukla *et al*. (2012)
Burkholdera spp.	*Cucumis sativus*	Enhanced water and chlorophyll content	Kang *et al*. (2014)
Curtobacterium spp.	*Hordeum vulgare* L.	Production of proline	Cardinale *et al*. (2015)
Enterobacter spp.	*Oryza sativa, Brassica napus, Triticum aestivum, Solanum lycopersicum, Zea mays, Arabidopsis thaliana*	Decreased the production of ethylene	Nadeem *et al*. (2006, 2013), Kim *et al*. (2014), Sarkar *et al*. (2017)
Enterococcus spp.	*Vigna radiate* L.	Lower uptake of Na^+ and resulting the salinity tolerance	Panwar *et al*. (2016)
Geobacillus spp.	*Zea mays*	Increased proline content and enhance photosynthetic activity	Abdelkader and Esawy (2011)
Haererohalobacter spp.	*Arachis hypogaea* L.	Higher accumulation of K^+	Shukla *et al*. (2012)
Klebsiella spp.	*Triticum aestivum, Avena sativa*	Enhanced accumulation of K and greater synthesis of proline	Singh *et al*. (2015), Sapre *et al*. (2018)
Microbacterium spp.	*Triticum aestivum*	Higher accumulation of K^+	Ashraf *et al*. (2004)

formation, production of phytohormone and enhanced uptake of plant nutrient elements. The mode of beneficial effects are described in the following sections.

31.4.1 Production of phytohormone

Plant growth regulators are endogenous biochemical substances that regulate the growth and development of plants. Recently, plant growth-promoting bacteria were introduced as biostimulants for the growth and development of both legumes and non-legumes. They improve plant growth under a large range of salinity stress conditions by producing (and regulating) various phytohormones, including IAA, gibberellic acid, zeatin, abscise acid (ABA) and ethylene, in addition to enhancing P solubilization. The plant growth-promoting bacteria can withstand the increased concentration of salt by the

Fig. 31.2. Flowchart of isolation, identification and development of commercial of STPGPB products.

production of IAA, ethylene, ACC deaminase, volatile organic compounds and antioxidant as demonstrated by several research groups (Gupta and Pandey, 2019). The enhanced growth and alleviating the deleterious effect of salinity stress by the application of *Bacillus pumilus* could be attributed to the production of ACC deaminase as well as siderophore production (Barnawal *et al.*, 2012). The PGPR can produce various types of growth-promoting hormones (e.g. auxins, gibberellin, cytokinin) under salt stress condition (Hashem *et al.*, 2018). Species that use this mechanism include *Azotobacter*, *Arthrobacter*, *Azospirillum*, *Pseudomonas*, *Stenotrophomonas* and *Rahnella* (Egamberdieva *et al.*, 2008, 2018; Piccoli *et al.*, 2011; Abd Allah *et al.*, 2017).

31.4.2 Regulation of ion homeostasis

The ability of an organism to maintain a steady internal environment regardless of external variation is very important under biotic and abiotic stresses, as is the regulation of vital internal variables in a state of relative constancy. Various hormones control the homeostasis, for example ethylene. STPGB can degrade plant ACC to acquire ATP and N. They also have the capacity to alleviate the deleterious effect of ethylene by enhancing stress tolerance (Tewari and Arora, 2014). They are able to maintain the ion homeostasis and enhanced K^+/Na^+ ratio in shoots through reducing Na^+ and Cl^- accumulation by decreased uptake of those ions. They can also exclude the Na^+ ion and increase the activity of K^+ transporter in the plasma membrane for better influx of K^+ into the cytoplasm. This is supported by the findings by Ilangumaran and Smith (2017) where higher uptake of K^+ and exclusion of Na^+ by the inoculation of *Azotobacter* strains C5 and C9 in *Zea mays* L. under salt stress conditions were reported.

31.4.3 Alleviation of reactive oxygen species (ROS)

Various types of reactive oxygen species are produced as a result of salt stress in crop plants. The ROS are highly active oxygen molecules that contain an additional electron; examples include peroxides, hydrogen peroxide (H_2O_2), singlet oxygen, superoxide and alpha oxygen. They are produced as a natural by-product of normal aerobic metabolism and have significant effects on cell signaling and homeostasis. The ROS are produced in mitochondria, peroxisomes and chloroplasts. They are harmful and responsible for aging and senescence through damaging various biomolecules, including lipids, proteins and nucleic acids in crop plants (Apel and Hirt, 2004). It has been reported that application of STPGPB modulated the levels of peroxidase (POX), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase, etc. in saline-affected plants, especially in tomato, where Wang *et al.* (2012) documented the enhanced synthesis of SOD, POX and CAT. The inoculation of STPGPB had beneficial effects by enhancing the activity and level of proline, protein and sugar content in tomato (Kousar *et al.*, 2020).

Recently, researchers reported the beneficial effects of STPGPB in tomato crops by the alleviation of ROS due to the production of antioxidant and proline (Egamberdieva *et al.*, 2017). In addition, the STPGPB help plants grow under biotic and abiotic stress by decreasing the level of stress that can inhibit plant growth (Fig. 31.3). STPGPB produce ACC deaminase (ACCD) that degrades ACC with nitrogen and energy as by-products, which consequently mitigate the stress. *Achromobacter piechaudii* ARV8 ameliorates salt stress in tomato by producing ACCD as reported by Singh *et al.* (2015), although this is an inducible enzyme that is secreted in the presence of substrate ACC. Ethylene-induced stress can also be mitigated by ACCD (Glick, 2007). The STPGPB also help to reduce lipid peroxidation and superoxide dismutase activity in salt-sensitive rice. ROS are highly reactive, and to minimize the effect of oxidative stress, plants have developed efficient antioxidant systems to protect themselves from their effect (Azooz *et al.*, 2011; Parvaiz *et al.*, 2012). The ROS-scavenging enzymes, which include peroxidase (POX), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (Apel and Hirt, 2004; Koyro, *et al.*, 2012), are present in different cellular compartments in the form of isoenzymes, in particular chloroplasts and mitochondria (Apel and Hirt, 2004).

31.4.4 Enhanced uptake of nutrient ions

Balanced nutrients can enhance the repair of deleterious effect caused by salinity in both glycophytes and halophytes, as described by Mia (2015). It is suggested that PGPB inoculation enhances the uptake of nutrients despite the presence of nitrogen, which could be due to the enhanced root formation by hormone production (Mia *et al.*, 2010a). Increased uptake of K, Ca and Mg decreases the uptake of Na, and consequently maintains the chlorophyll content in plants. Application of arbuscular mycorrhiza in

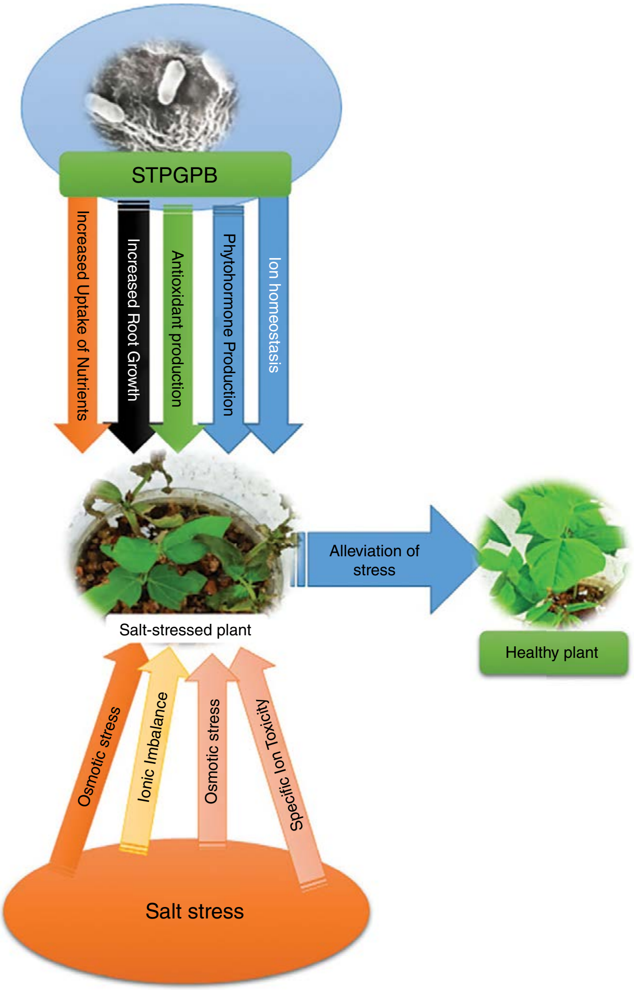

Fig. 31.3. Schematic showing the harmful effects on crop plants and mode of beneficial actions after the inoculation of STPGPB.

Cucurbita pepo L. showed higher chlorophyll content and greater relative water content, as well as more K absorption and reduced Na uptake (Colla *et al*., 2008). The salinization process enhances the uptake of Na and reduces the uptake of K, which reduces the growth and yield of crop plants. Application of STPGPB reduces the uptake of Na^+ and decreases the ratio of Na^+/K^+ in cytosol, thus easing the deleterious effect of salt. Similarly, the monoculture of inoculant and co-inoculation also resulted in an increased K^+/Na^+ ratio in cytosol (Mia and Shamsuddin, 2010). The reduced uptake of Na^+ by roots and subsequent translocation to shoot tissue could be mediated by co-inoculation, which triggers the enhanced uptake of Ca (Giri *et al*., 2003). Ashraf (2002) and Wieneke *et al.* (1987) suggested that the high K^+/Na^+ selectivity is an important selection criterion for salt tolerance.

31.4.5 Remediation of salt stress by enhanced roots activity induced by STPGPB

Roots of higher plants are complex organs encompassing several descriptive categories, such as hairy, elongation and cap/tip possessing distinct roles (Mia, 2015). The spatial distribution of primary, secondary and tertiary roots, along with their number and length, are integrated by root system architecture (Vacheron *et al.*, 2013). The activity of roots, especially the absorption and uptake of nutrients, is influenced by rhizospheric ecology. The soil microbiome around the roots plays a significant role in the beneficial interactions in diverse ways. Enhanced root stimulation is one of the most important effects, stimulating nutrient and water uptake. The root stimulation also plays an important role in alleviating the abiotic stresses like drought and salinity. Application of rhizobacteria enhances the root growth in monocotyledonous plants, for example in *Musa* spp. where both primary and secondary roots are enhanced by the application of *Azospirllum brasilense* and *Bacillus sphaericus* and subsequently increased the uptake of Ca (Mia *et al.*, 2009, 2010a, 2010b). The structural and functional integrity of cell membrane is improved by Ca ions, which also stabilizes the cell wall structure. The Ca molecule also influences the signal transduction in the membrane of the plant cell. PGPR can stimulate the root membrane by secreting a signal that triggers the membrane-bound ATPase, consequently increasing the proton efflux and cation influx by antiporter (Bashan, 1990; Mia *et al.*, 2013; Mia, 2015).

31.4.6 Osmotic adjustment in a salinized plant cell by STPGPB

It is reported that under salt stress the STPGPB produce compatibility osmolytes for adjusting the osmotic stress in the cell of the host plant. They can influence the stomatal conductance, hydraulic conductivity and transpiration rate in different crop plants (Sarig *et al.*, 1988; Saad *et al.*, 1999; Amir *et al.*, 2001). Enhanced root hydraulic conductivity and expression of ZmPIP (plasma membrane aquaporin protein) isoforms were observed in maize under salt stress inoculated with *Bacillus megaterium* (Marulanda *et al.*, 2010). The initial osmotic shock can be overcome by STPGPB-induced osmolyte accumulation and phytohormone signaling in the host cell. It is reported that proline and glycine betaine are accumulated during salt stress, where proline is the key amino acid that is produced by the breakdown of protein (Krasensky and Jonak, 2012). Glycine betaine protects the plants against stress without necessarily contributing significantly to cellular osmotic potential. Niu *et al.* (2016) reported that application of *Bacillus subtilis* in rice under salt stress resulted in a downregulation of OsHKT2;1, reflecting the reduced uptake of Na^+ and an associated upregulation of SOS1 and HKT1;5, transcripts for Na^+ efflux and xylem Na^+ unloading, respectively. The productivity of rice on saline soils can be achieved by altering the OsHKT2;1 function.

31.5 Conclusions and future perspectives

Several morphological, physiological, biochemical and molecular alterations in plants occur as a result of salt injury. These injuries can be mitigated by various means. It is necessary to explore the suitable measures for getting remedies from the deleterious effects of salt stress, and also search for alternative strategies for improving the stress-tolerant variety in non-legumes for sustainable crop production system. STPGPB play a significant role in alleviating the salt stress of different crop plants, both legumes and non-legumes in various biomes. The beneficial interaction between the microbes and host plants resulting in morphological, anatomical and biochemical aspects have been proved and reported by various researchers. They can change the root morphology by increasing the number of root hairs, resulting in absorption of more nutrients and water. Absorption of more K^+, Ca^{2+} ions can alleviate and suppress the harmful effects of Na^+ ions. Ion homeostasis could result in the induction of plant growth-promoting bacteria. They are involved in physiological aid of plants through the production of antioxidants, volatile organic compounds and extracellular polysaccharides. STPGPB are natural microbes that improve the plant attributes and productivity. They can be used as biofertilizer for sustainable crop production in high saline areas, therefore potentially bringing economic benefits to farmers. To achieve precise and sustainable beneficial effects, the whole genomic sequence of STPGPB should be studied and their beneficial activities should be optimized. Our understanding regarding the molecular mechanisms of beneficial effects of STPGPB have been improved; however, it should further investigated to gain a comprehensive understanding.

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Biostimulants for Crop Production and Sustainable Agriculture

Edited by **Mirza Hasanuzzaman**,
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and **Masayuki Fujita**

Agricultural biostimulants are a group of substances or microorganisms, based on natural resources, that are applied to plants or soils to improve nutrient uptake and plant growth, and provide better tolerance to various stresses. Their function is to stimulate the natural processes of plants, or to enrich the soil microbiome to improve plant growth, nutrition, abiotic and/or biotic stress tolerance, yield and quality of crop plants. Interest in plant biostimulants has been on the rise over the past 10 years, driven by the growing interest of researchers and farmers in environmentally-friendly tools for improved crop performance.

Focusing on recent progress on biostimulants and their role in crop production and agricultural sustainability, this book includes:

- 31 chapters on a wide range of biostimulants and their role in plant growth stimulation and stress tolerance.
- Mechanism of actions of diverse groups of biostimulants, such as trace elements, plant and seaweed extracts, humic substances, polyamines, osmolytes, vitamins, nanoparticles and microorganisms.
- New promising biostimulants with novel modes of action.

Improved crop production technologies are urgently needed to meet the growing demand for food for the ever-increasing global population by addressing the impacts of changing climate on agriculture. This book is of interest to researchers in agriculture, agronomy, crop and plant science, soil science and environmental science.