Tariq Aftab
Khalid Rehman Hakeem *Editors*

Plant Growth Regulators

Signalling under Stress Conditions

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(1908–1999)

Hakim Abdul Hameed *was a great philanthropist, thinker, visionary, an Indian physician of the traditional medicine system of Unani, founder-chancellor of* ***Jamia Hamdard****, and a former chancellor of Aligarh Muslim University, India. He was honored by the Government of India in 1965, with the award of Padma Shri, the fourth highest Indian civilian award, and in 1992, the government awarded him the third highest Indian honor of Padma Bhushan.*

Preface

Agriculture faces many challenges to fulfil the growing demand for sustainable food production and ensure high-quality nutrition for a rapidly growing population. To guarantee adequate food production, it is necessary to increase the yield per area of arable land. A method for achieving this goal has been the application of growth regulators to modulate plant growth. Plant growth regulators (PGRs) are substances in specific formulations which, when applied to plants or seeds, have the capacity to promote, inhibit, or modify physiological traits, development, and/or stress responses. They maintain proper balance between source and sink for enhancing crop yield. PGRs are used to maximize productivity and quality, improve consistency in production, and overcome genetic and abiotic limitations to plant productivity. The use of PGRs in mainstream agriculture has steadily increased within the last 20 years as their benefits have become better understood by growers. Unfortunately, the growth of the PGR market may be constrained by lack of innovation at times when increase in demand for new products requires steady innovation and discovery of novel, cost-competitive, specific, and effective PGRs. It is expected that the need to raise agricultural production will lead to the increased use of plant growth regulators. They may make it possible to grow crops, changing the crop's time pattern so that it can mature and be harvested before adverse conditions. The effects of PGRs on plant functions such as root induction, control of flowering, sex expression, maturation, and aging have been documented, with many horticultural examples.

Apart from well-known PGRs, which are mostly plant hormones, there are a number of substances/molecules such as nitric oxide, hydrogen sulfide, seaweed extracts, melatonin, plant growth promoting rhizobacteria, etc., which act as PGRs. These novel PGRs, or biostimulants, have been reported to play important roles in stress responses and adaptation. They can protect plants against various stresses, including water deficit, chilling and high temperatures, salinity, and flooding.

The present book covers a wide range of topics, discussing the role and signalling of traditional as well as novel PGRs in challenging environments. Moreover, this will be a unique reference book on topics discussing the use of PGRs with latest biotechnological and omics approaches. In this volume, we highlight the working solutions as well as open problems and future challenges in PGR research. We believe that this book will initiate and introduce readers to state-of-the-art developments and trends in this field of study.

The book comprises 21 chapters, most of them being review articles written by experts, highlighting a wide range of topics, discussing the role and regulation of PGRs in plants under stressful conditions. We are hopeful that this volume furnishes the need of all researchers working or are interested in this particular field. Undoubtedly, this book will be a helpful general source of reference for research students, teachers, and those who have interest in PGRs.

We are immensely grateful to all our contributors for accepting our invitation for not only sharing their knowledge and research, but for venerably integrating their expertise in dispersed information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. We also thank the Springer Nature team for their generous cooperation at every stage of the book production.

Lastly, thanks to well-wishers, research students, and authors' families for their moral support, blessings, and inspiration in the compilation of this book.

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He has edited six books with with international publishers, including Elsevier Inc., Springer Nature and CRC Press (Taylor & Francis Group), co-authored several book chapters, and published over 50 research papers in peer-reviewed international journals. His research interests include physiological, proteomic, and molecular studies on medicinal and aromatic plants.

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So far, Dr. Hakeem has authored and edited more than 50 books with international publishers, including Springer Nature, Academic Press (Elsevier), and CRC Press. He also has to his credit more than 110 research publications in peer-reviewed international journals and 60 book chapters in edited volumes with international publishers.

At present, Dr. Hakeem serves as an editorial board member and reviewer of several high-impact international scientific journals from Elsevier, Springer Nature, Taylor & Francis Group, Cambridge, and John Wiley Publishers. He is included on the advisory board of Cambridge Scholars Publishing, UK. He is also a fellow of Plantae group of the American Society of Plant Biologists, member of the World Academy of Sciences, member of the International Society for Development and Sustainability, Japan, and member of Asian Federation of Biotechnology, Korea. Dr. Hakeem has been listed in Marquis *Who's Who in the World* since 2014. Currently, Dr. Hakeem is engaged in studying the plant processes at eco-physiological as well as molecular levels.

Chapter 1

Prospective Role of Plant Growth Regulators for Tolerance to Abiotic Stresses

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Abbreviations

ABA	Abscisic acid
ACC	Aminocyclopropane carboxylic acid
ARFs	Auxin response factors
AXs	Auxins
BRs	Brassinosteroids
CGR	Crop growth rate
CKs	Cytokinins
EPS	Exopolysaccharides

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ET	Ethylene
GAs	Gibberellins
ISR	Induced systematic resistance
JAs	Jasmonates
LAI	Leaf area index
NAR	Net assimilation rate
PGRs	Plant growth regulators
RNA	Ribonucleic Acid
ROS	Reactive oxygen species
SA	Salicylic acid
SLs	Strigolactones
VOCs	Volatile organic compounds

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1.1 Introduction

Several climate models predict a sharp decline in crop productivity owing to changing climate across the globe which may jeopardize the food security of increasing population (Battisti and Naylor 2009; IPCC 2014). The arid and semi-arid regions are expected to confront increasing desertification due to global warming and fluctuation of rainfall distribution leading to a decrease in the area under plow (Agbola and Ojeleye 2007). Another manifestation of climate change is a severe intensity and more frequent incidence of abiotic stresses especially drought, salinity, heat, and heavy metals toxicities, which are projected to drastically plummet staple crops' productivity. Heat stress alters the constancy of numerous proteins, RNA species, and construction of cytoskeleton along with reducing the effectiveness of several enzymatic activities which results in unbalancing of metabolic activity. The net result of heat stress is cell death especially at the reproductive growth stage which leads to complete crop failure. Similar to heat stress, drought negatively affects and even halts vital molecular, biochemical, physiological, and morphological processes in plants. Moreover, salinity is equally harmful to crops as it leads to osmotic stress and salt-induced ion toxicity (Munns 2005). These types of stresses are linked to unwarranted Cl^- and Na^+ toxicity, leading to the deficiency of Ca^{2+} and K^+ along with other macro- and micronutrients (Marschner 1995). The ROS-mediated ion toxicity leads to chlorosis and necrosis which hamper photosynthesis by accumulating Na^+ and ultimately inhibits numerous physio-biochemical activities in plants (Munns 2002; Kundu et al. 2018).

Plant growth regulators (PGRs) have become commercialized in some countries to improve the productivity of crops (Jahan et al. 2019; Iqbal et al. 2015). These PGRs play essential functions in modulating growth and development of plant by inducing morphological, physiological, and molecular adaptations under stressful environment (Iqbal et al. 2018; Afzal et al. 2015). Previous studies showed that PGRs bolster plant defense system (Iqbal 2015; EL Sabagh et al. 2019a, b, c) against plant pathogenic bacteria, which need alive cells to finish their life cycle (Bari and Jones 2009). Wani et al. (2016) studied that plant growth hormone is a signaling molecule of natural origin, recognized to play a vital and complex role in regulating boom, physiology, development, morphology, and response to abiotic stress. Plant growth regulator acts as a sign of transduction agent in a very complicated way (Iqbal 2014), keeping plant life sustainably optimal during growth and development, and is consequently considered to be the principal thing in plant reaction to abiotic and biotic stresses (Bücker-Neto et al. 2017; Pál et al. 2018a, b). Therefore, several hormonal signaling pathways are integrated to modulate different stress responses, in turn triggering stress tolerance mechanisms under changing climate; it will play a key role in environmental security.

This chapter synthesizes and critically evaluates the drastic impact of abiotic stresses especially heat, drought, and salinity on plant growth and development. In addition, PGRs' role in ameliorating the negative effects of abiotic stresses by imparting stress tolerance through morphological, physiological, and biochemical alterations has been objectively analyzed. An attempt through the synthesis of available literature has been made to develop PGRs as an effective and biologically viable tool to cope with abiotic stresses under changing climate leading to bolstering food and nutritional security of populace.

1.2 Abiotic Stress Effects on Crops

1.2.1 Temperature Fluctuations

Heat stress can have a hostile effect on almost all aspects of growth and development of plants (Mittler and Blumwald 2010; Lobell et al. 2011). For example, in the last three decades (1980–2008), wheat and maize yield were decreased by approximately 5.5 and 3.8% due to heat stress (Lobell et al. 2011). Generally, stage-specific, most of the plant is vulnerable to the temperature above or below its optimum levels; particularly the reproductive stage is more sensitive (Zinn et al. 2010). Heat stress alters the constancy of numerous proteins, RNA species, and construction of cytoskeleton, reduces the effectiveness of several enzymatic activities, and also unbalances metabolic activity (Ruelland and Zachowski 2010; Suzuki et al. 2011); ultimately cell death occurs as a result of excessive production of reactive oxygen species (ROS) (Mittler et al. 2012; Fig. 1.1). Although, a direct association exists between heat-induced ROS and plant survive to heat stress (Ruelland and Zachowski 2010; Suzuki et al. 2011).

Rising temperature results in cell death owing to water scarcity to plants (Radin et al. 1994; Grill and Zeigler 1998; Hetherington and Woodward 2003). For

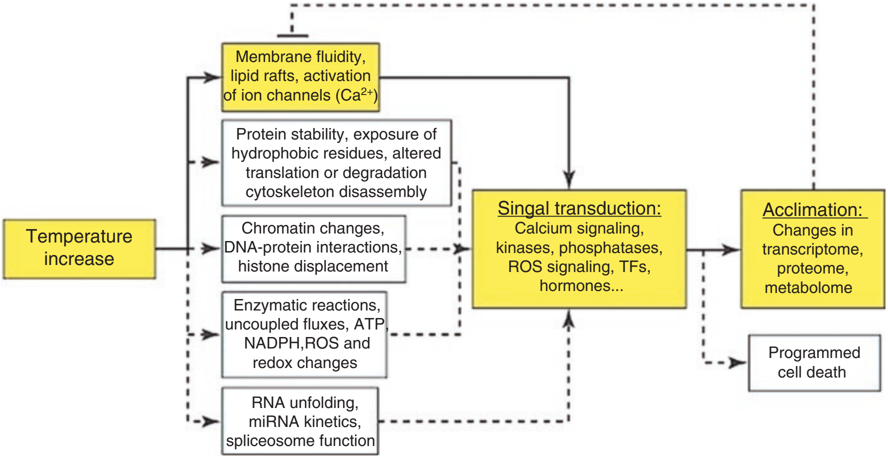

Fig. 1.1 A schematic model for temperature sensing in plants. Increases in ambient temperature affect many different processes (Modified, Mittler et al. 2012)

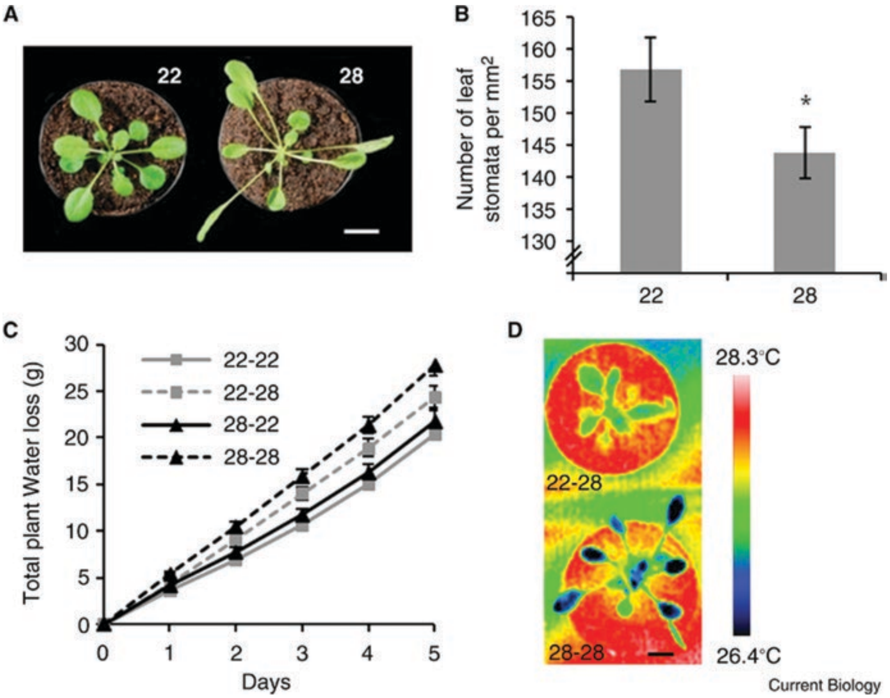

Fig. 1.2 The increasing temperature increased the transpiration and also enhanced the leaf cooling capacity of *Arabidopsis thaliana*. (Adapted from Crawford et al. 2012 with permission)

example, Crawford et al. (2012) observed the effect of heat stress-induced expansion on plant water usage approach in Fig. 1.2 (a–c). It has been observed that the growth of *Arabidopsis*, at heat stress (28 °C), results in improved water loss, conspicuous elongation of stems, improved leaf cooling capacity in these conditions, and enlarged leaf advancement from the soil surface, regardless of producing fewer leaf surface pores (stomata) (Crawford et al. 2012).

1.2.2 Drought Stress

Similar to heat stress, drought is also a big threat in agriculture, particularly in arid or semi-arid regions where rainfall is scarce and available water is limited (Silva et al. 2011; Barutcular et al. 2016, 2017). It is the greatest devastating stress and could cause a reduction in crop productivity (Molla et al. 2019). Since drought has an unfavorable consequence on agricultural ecosystems, it leads to a thoughtful threat to sustainable crop production systems all over the world (Kogan et al. 2019).

Plants need available water and nutrition throughout the life cycle (Taiz and Zeiger 2006; Ajum et al. 2011), and their reduction consequently affects all molecular, biochemical, physiological, and morphological processes in plants (Sarker et al. 2005; Sircelj et al. 2005; Silva et al. 2009). It is documented that drought could cause nutrient deficiency, even in the fertile soil, due to the reduction of nutrient movement and absorbance from the soil solution to the root systems (Silva et al. 2011).

Drought induces the secondary stress known as oxidative stress (Da Silva et al. 2013), leading to the excess generation of ROS, lipid peroxidation, and membrane injury, ultimately causing cell death (Abid et al. 2018). The plants survive in drought-induced oxidative stress in various ways such as altering growth pattern, phenology, phenotype, and morphology, and also follow several physiological mechanisms (Zandalinas et al. 2018), such as increasing enzymatic and non-enzymatic antioxidant activities (Da Silva et al. 2013; Abid et al. 2018). Lipid peroxidation increased in growth phases of maize, while antioxidant enzyme activities especially superoxide dismutase reduced in the last phases (Li-Ping et al. 2006; Abdelaal et al. 2017).

1.2.3 Salinity Stress

Among the abiotic stresses, soil salinity is another abiotic limitation that has threatened the agricultural productivity worldwide, predominantly in arid and semi-arid regions (Hussain et al. 2009; Huang et al. 2019; Yassin et al. 2019). It is estimated that approximately 800 million ha of arable lands across the globe are affected by soil salinity (Munns and Tester 2008). Under soil salinity, generally, plants face two types of stress such as osmotic stress and salt-induced ion toxicity (Munns 2005) which are linked with unwarranted Cl^- and Na^+ toxicity, leading to the deficiency of Ca^{2+} and K^+ and also other nutrients (Marschner 1995). ROS-mediated ion toxicity leads to chlorosis and necrosis (hampered the photosynthesis), as a result of the higher accumulation of Na^+ which inhibits numerous physio-biochemical activity in plants (Munns 2002; Kundu et al. 2018).

The damaging effect varies depending on growth environment, climatic conditions and genotypes (Islam et al. 2011; Tang et al. 2015). Based on salt-tolerant ability, generally, plants can be classified into three categories: glycophytes, euhalophytes, and halophytes (Munns 2005; Koyro 2006; Stepien and Johnson 2009; Tang et al. 2015). Glycophytes cannot grow in the presence of high salt levels (Munns and Termaat 1986); euhalophytes can grow in a medium level of salinity through developing different types of mechanisms (Larcher 2003); halophytes can be grown under high concentrations of NaCl (300–500 mM) as they could establish better salt resistance mechanisms (Parida and Das 2005; Flowers and Colmer 2015).

1.3 Overview of PGRs Under Stressful Environment

The plant hormones commonly related to stress responses are abscisic acid (ABA) (Suzuki et al. 2016), ethylene (ET) (Dubois et al. 2018; Debbarma et al. 2019), salicylic acid (SA) (van Butselaar and Van den Ackerveken (2020), etc. While ABA has been mainly involved in the regulation of abiotic stress responses, ET, SA, and JA have been associated with responses of defense against pathogens, insects, or wounding. Auxins (AXs), cytokinins (CKs), gibberellins (GAs), brassinosteroids (BRs), and strigolactones (SLs) are also required in stress-triggered responding networks (Korver et al. 2018; Kanwar et al. 2017).

ABA is a molecule classified as sesquiterpene, and it is accumulated in plants growing under abiotic stressful conditions, which activates several responses. When the environment is optimal, ABA levels are reduced to promote plant growth. Regulations of ABA levels are essential for balancing defense and growth processes when plants are exposed to stressful environments (Vishwakarma et al. 2017). ABA levels are finely regulated by their biosynthesis, degradation or conjugation reactions, and the control of conjugate or free ABA transport to hormone action sites. The gene-encoding enzymes involved in ABA biosynthesis are upregulated under adverse environmental conditions, and it leads to an increase of ABA synthesis and/or a decrease of ABA catabolism and regulates numerous ABA-mediated stress responses (Finkelstein 2013; Waadt et al. 2014; Tan et al. 2018). ABA-mediated responses involve a change in several proteins and anion channels for stomatal closure reducing the transpiration and water loss and limiting the carbon dioxide availability, which leads to reduced photosynthetic performance and increased ROS production (Mittler and Blumwald 2015).

ABA signaling begins with ABA receptors identified as RCAR/PYR1/PYL (regulatory components of ABA receptor/pyrabactin resistance 1, and protein/PYR-like proteins) receptors and protein phosphatases of type 2C (PP2C) as ABA receptors (Tischer et al. 2017). Binding of ABA to the receptor allows blocking of the active site of phosphatases and the release of a protein kinase from the PP2C complex. This protein kinase is part of sucrose non-fermenting-1 (SNF1)-related protein kinase 2 s (SnRK2s) subfamily which functions as link between guard cell movement and ABA signaling (Mittler and Blumwald 2015). ABA promotes the stomatal closure through a ROS wave, which allows the regulation of ROS-regulated Ca^{2+} channels and the generation of synthesis of new ROS (Drerup et al. 2013). PP2C complex can be inhibited by the action of ROS; thus, ROS and ABA interact in a positive regulation triggering the stomatal closure and inducing the specific gene expression during stressful conditions (Mittler and Blumwald 2015). Furthermore, it has been reported the essential role of ABA signaling mediated by the SnRK2s modulating the tricarboxylic acid cycle and metabolism of organic acids involved in osmotic stress tolerance mechanisms (Thalmann et al. 2016). In addition, ABA homeostasis and signaling are modified by the presence of other PGRs. For example, ABA accumulation and upregulation of ABA signaling are affected by the synthesis of JAs in plants under dehydration treatments; both hormones trigger several drought plant responses (de Ollas and Dodd 2016).

Ethylene (ET) is a gaseous hormone with the simple C_2H_4 structure; it increased the response to multiple stressful environmental conditions, such as salinity, drought, exposure to heavy metals, and high temperatures, among others (Llanes et al. 2016; Savada et al. 2017; Dubois et al. 2018). ET biosynthesis consists in the conversion of methionine into *S*-adenosyl methionine, which is turned to an amino-cyclopropane carboxylic acid (ACC) by the action of ACC-synthase (ACS) enzyme. The induction and activation of ACS are regulated by environmental factors that trigger ET accumulation. For example, ACS genes are transcriptionally upregulated by drought (Dubois et al. 2017). In destination organs, ET triggers a signaling cascade through ethylene receptors, ERS1 and ERS2 (ethylene response sensor), ETR1 and ETR2 (ethylene resistance), and EIN4 (ET insensitive). In the absence of ET, these active receptors subsequently bind to kinase protein (CTR1), but in the presence of ET, it relieves CTR1 inhibition, and these receptors induce the expression of numerous transcription factors. Ethylene response factors (ERFs) have been reported as the last downstream components of ET signaling pathways (Meng et al. 2013). ERFs play essential functions in the abiotic stress responses through the regulation of several stress tolerance genes (Klay et al. 2018). Therefore, this hormone regulates leaf development and some senescence mechanisms and processes such as fruit ripening and germination in some species under stressful conditions, being ACC a long-distance root-sourced signal under stress.

SA is involved in numerous plant growth responses, such as photosynthesis, ionic homeostasis, and seed germination. In recent years, there have been increasing reports about SA new roles in plants under different environmental stresses (Ghassemi-Golezani et al. 2016; Singh et al. 2017). Foliar application of SA in soybean plants exposed to different levels of salinity enhanced the quantity and quality of several proteins (Farhangi-Abriz and Ghassemi-Golezani 2016). Exogenous SA increases the physiological, yield, and growth parameters in wheat plants under drought treatments (Kareem et al. 2017), while JAs are involved in diverse developmental processes regulating several responses to biotic and abiotic stress conditions, mediating defense responses against pathogens and herbivores, and controlling growth and reproductive development (Pérez-Salamó et al. 2018). Farhangi-Abriz and Ghassemi-Golezani (2018) reported that foliar sprays of SA and JAs reduce the sodium influx to cells of leaves and mitigate the oxidative and osmotic damages by the enhancing antioxidant enzyme activities and increasing glycine betaines, carbohydrates, and proteins in soybean plants under salinity. The JAs and SA signaling pathways were characterized in *Arabidopsis* plants in response to stressful environments (Ahmad et al. 2016; Zhou et al. 2019; Fu et al. 2020). It has been suggested that both PGRs induce antioxidant system genes that help to eliminate ROS. Indeed, studies on JAs signaling in wheat plants under different stress conditions demonstrated that JA is involved in the induction of some antioxidant systems to cope with the stress (Kang et al. 2013; Qiu et al. 2014; Shan et al. 2015). There are several evidence that JAs and SA may have convergence points with ABA generating a complex and integral network for abiotic stress responses (Zhao et al. 2014; Prodhan et al. 2018). Future researches in hormonal signaling may be explored to elucidate the links between SA, JAs, and ABA and the multiple stresses abiotic responses.

Modifications in plant architecture and growth pattern of plants under stressful environment are regulated by AX and CK pathway. The action of AXs has been found to be antagonistic to CKs (Bielach et al. 2017). Different plant tissues during stressful environment decrease the synthesis and signaling of AXs and CKs to increase the ABA sensitivity for the regulation of abiotic stress responses (Rowe et al. 2016; Abid et al. 2017). Thus, stress-induced growth inhibition involves the repression of AX- and CK-responsive genes. For example, Pospíšilová et al. (2016) described that the transgenic barley plants overexpressing a CK dehydrogenase gene showed greater tolerance to drought conditions. These transgenic plants showed altered root morphology resulting in better dehydration avoidance. In addition, some studies demonstrated that components of cytokinin signaling, such as cytokinin oxidase/dehydrogenase genes, are regulated by ABA (Huang et al. 2018). Recently, Bouzroud et al. (2018) reported that auxin response factors (ARFs) are involved in auxin signaling in tomato plants responses under stressful environments.

Brassinosteroids are a group of PGRs classified as polyhydroxy steroidal compounds with strong growth and development promoting potential. More than 70 compounds have been identified from plants, being 28-homo-brassinolide, 24-epibrassinolide, and brassinolide the most bioactive compounds isolated (Vardhini and Anjum 2015). BRs play an essential role in several developmental processes such as shoot and root growth, floral initiation, and fruit development. Recent studies have shown the potential of BRs in the induction of components of antioxidant defense mechanisms decreasing ROS accumulation and increasing the protection of photosystems in plant abiotic stress responses (Vardhini and Anjum 2015; Sharma et al. 2017). Moreover, BRs could modify the architecture of cellular walls and the adjustment of cellular membranes to preserve the functionality of membranes during adverse environmental conditions. Indeed, BRs signaling have been reported to be critical for inducing expression of several enzymes involved in the loosening and extension of cellular walls (Guo et al. 2009) and increasing of pectin methylesterases enzymes (Yang et al. 2014), which are associated with the stress tolerance mechanisms. BRs also regulate several stress-responsive transcription factors by a negative regulator of BRs signaling called BIN2, which can regulate some transcription factors that activate numerous stress tolerance genes (Xu et al. 2015; Eremina et al. 2016; Chen et al. 2017).

Strigolactones (SLs) constitutes a small group of PGRs represented by carotenoid-derived compounds. SLs are germination-stimulating compounds in root parasitic plants such as *Striga*, *Orobanche*, and *Phelipanche* species (López-Ráez et al. 2017). Application of SLs in some plants suggests that SLs could play essential roles in the tolerance of abiotic stress factors, by regulating growth and development responses, source/sink transitions, and nutrient distribution (Saeed et al. 2017). SLs signaling and their action on the architecture of plants implicate that SLs interact with other PGRs, particularly AXs and ABA (Cheng et al. 2013) although there is emerging evidence for cross-talk between the signaling and function of SLs.

1.4 PGR-Induced Changes in the Agronomic Traits of Crop

Exogenous application of plant growth hormone like SA produces resistance against several abiotic stresses like moisture deficiency, salt stress, high-temperature stress, and heavy metal stress (Singh and Usha 2003). Earlier studies also showed that exogenous application of salicylic acid increased the resistance in *Cicer arietinum* against moisture stress (Khan et al. 2017) as well as also mitigated the drastic effects of lead (Pb) and mercury (Hg) (Kim et al. 2014). Putrescine (Put) also played a positive role in minimizing the drastic effects of abiotic pressures on crops by its acidic neutralization and stabilized capability of the cell membrane (Zhao and Running 2010). Application of plant growth regulator Put enhanced resistance in plants against moisture stress, ROS production, salt stress, and low-temperature stress in various plants (Duan et al. 2008). In spite of producing resistance against abiotic stresses, plant growth regulators also play an imperial role in various growth phases of plants (Pal et al. 2015).

1.4.1 Interactive Effect of PGRs on Growth and Yield Traits

In legumes, leaf area index and other growth-related traits were affected by various PGRs (Ullah 2006). Plant growth-related parameters like leaf area index (LAI), leaf area duration, crop growth rate (CGR), net assimilation rate, and total dry matter production were significantly affected by plant growth regulators. Grain yield is directly proportional to LAI, CGR, and net assimilation rate (NAR). Yield and quality traits of *Cicer arietinum* were significantly influenced by plant growth regulators, and also PGRs increased protein synthesis, seed yield, and straw production (Kumar et al. 2003). PGR application with twice rate increased bud's retention percentage (Gupta et al. 2007). Neelima et al. (2006) revealed that triacontanol alone or with KNO_3 improved pod development, hundred-grain weight, biomass, HI, as well as grain yield at harvest as well as reduced leaves senescence in *Cicer arietinum*. Rao et al. (2005) studied that yield-related traits including floral sustainability, pod formation percentage, pod number, and grain weight were increased by application of gibberellic acid and cystocele levels.

1.4.2 Role of PGRs in Plant Physiology

1.4.2.1 Role of PGRs on Chlorophyll Contents

Chaloupkova and Smart (1994) revealed that the application of plant growth regulators and other inputs like pesticide application usually declined chlorophyll pigments. Additionally, chlorophyll pigments also were suppressed by the AA application. Samet and Sinclair (1980) studied that application of abscisic acid causes falling of older leaves, while newborn leaves have little effect of abscisic acid. Xanthophyll intermediate produces abscisic acid. Taiz and Zeiger (2010) studied that chloroplasts and other plastids are the hosts for producing early phases of abscisic acid.

1.4.2.2 Role of PGRs in Photosynthesis

Giordano et al. (2000) observed that in legume crops due to sulfur (a growth hormone) deficiency exhibited minimum growth and photosynthetic efficiency. Li et al. (2007a, b) revealed that sulfur in the liquid condition of apoplast induces ROS that decreases photosynthetic efficiency of the plant and ultimately reduces plant yield. Root/shoot ratio is increased by kinetin application that may be accredited to cytokinins regulating photosynthesizing activity due to kinetin treatment that is associated with improving total biological yield with the rise in photosynthesizing activity. Application of cytokinins promoted photosynthesis efficiency mainly by means of improvement in leaf chlorophyll content (Caers and Vendrig 1986).

1.4.2.3 Role of PGRs on Protein Contents

Gupta et al. (2007) observed positive effect of plant growth regulators on the protein content in *Cicer arietinum*. Cumulative effects of plant growth regulators and sulfur on maximizing content of protein in *Cicer arietinum* also as reported by Yadav and Bharud (2006) through combined foliar application of gibberellic acid, NAA and cycoel, benzyladenine, biforce, and biopower. Mandavia et al. (2006) reported that foliar-applied SA and brassinolide improved protein contents in chickpea seeds. Ali and Bano (2008) documented that at flower initiation and grain formation stage, maximizing protein concentration and sugars contents of leaves are directly proportional to nodule activity; therefore, reduction in chlorophyll contents and protein contents become more vulnerable in older leaves than younger leaves.

1.4.3 Role of PGRs in Nodule Formation

Effects of PGRs (indoleacetic acid, gibberellic acid, and kinetin) on hemoglobin synthesis in *Cicer arietinum* nodules were noticed that almost all growth regulators showed an overall positive influence on the synthesis of hemoglobin (Jain et al. 2008). Scherer and Lange (1996) studied that kinetin is a plant growth regulator which improved the efficiency of nitrogenase enzyme of root nodules of *Cicer arietinum* as well as improved the biomass of pink bacterial tissues; it also enhanced leg hemoglobin percentage and nodule bacterial treatments over control. Kinetin causes increase in leg hemoglobin content and nodule bacteroid region over the control. The nitrogen-fixing capacity of sulfur-deficient plants is significantly reduced, which is an important factor for the low level of nodule development and low production of nitrogenase and hemoglobin (Ereifej et al. 2001). Ali and Bano (2008) revealed that nodule formation is positively affected by exogenous application of ABA and kinetin in *Cicer arietinum*.

1.5 Impact of PGRs on Abiotic Stress Tolerance

The prospective roles of PGRs for abiotic stress tolerance and their earlier evidence are discussed in Table 1.1.

1.5.1 Impact of PGRs on Drought Stress Tolerance

Various abiotic stresses like moisture deficiency are ameliorated by the application of ABA (Keskin et al. 2010). High cellular ABA promotes changes in stomatal conductance, root hydro-conductivity, photosynthesis, distribution of biomass between roots and shoots, water family members of plants, the formation of osmolytes, and the synthesis of stress-touchy proteins and genes to ensure resistance to moisture stress (Finkelstein et al. 2008; Kim et al. 2010). Water stress produces abscisic acid accretion in the leaves of many plants (Upreti and Murti 2005; Satisha et al. 2005). The increase in ABA allows flora to restrict water loss via transpiration after the stomata are closed and improves the plant's water reputation after the underpinning hydraulic conductivity increases (Thompson et al. 2007). Zhang et al. (2006) observed that abscisic acid also plays an important role in the transfer of materials from roots to stem by regulating stomatal conductance, allele exhibition, and catabolic variations under moisture deficit conditions.

1.5.2 Role of PGRs on Salt Stress Tolerance

The role of PGRs in regulating various physiological and biochemical processes and physiological characteristics of numerous crops underneath salt strain has been broadly elucidated (Fatma et al. 2013). The concentration of these plant genetic resources in plants may be a determinant of increased tolerance/sensitivity because the expression of stress-related proteins in crops is increased under stress conditions (Hamayun et al. 2010).

Due to the different plant growth regulators, plant growth may change under a persistent salt environment (Iqbal et al. 2012). Jackson (1997) proposed that changes and reductions in plant growth under stress are attributed to low endogenous levels of plant growth regulators. The exogenous application of plant growth regulators

Table 1.1 Plant hormones and abiotic stresses: recent advancement

Types of PGRs	Stress condition	Studied plants	Key findings and impact on plant growth and development	References
Thiourea	Salinity stress	Maize	Better K^+/Na^+ ratio, uptake of Ca^{2+}, K+ and N, and water relations	Waqas et al. (2019), Kaya et al. (2016)
		Mung bean	Improved contents of glycine betaine	Perveen et al. (2016)
		Wheat	Improvement in nutrient uptake and photosynthetic machinery	Seleiman and Kheir (2018)
		Indian mustard	Regulated various signaling and effector mechanisms	Srivastava et al. (2010)
	Heat stress	Sunflower	Configuration of plant protein modified	Akladious (2014)
		Wheat	Minimized damage to the cell membrane	Asthir et al. (2013)
		Wheat	Improved water use efficiency and Rubisco action	Freeha et al. (2011)
		Maize	The extra activity of the ascorbate-glutathione cycle	Khanna et al. (2017)
		Wheat	Alterations in carbon metabolism	Asthir et al. (2015)
	Cold stress	Jatropha	Reduced leaf senescence and injury to the membrane	Yadav et al. (2011)
		Maize	Better photosynthesis	Waqas et al. (2017)
		Capsicum	Improved the appearance of defense-associated genes	Patade et al. (2012)
	Drought stress	Mung bean	Improved plant metabolic working	Mathur et al. (2006)
		Wheat	Moderated opening and closing of stomata	Wakchaure et al. (2016)
		Chickpea	Enhanced expression of RbcL and glycolate oxidase to continue photosynthesis	Vineeth et al. (2016)
		Onion	Enhanced water efficiency	Wakchaure et al. (2018)
Auxins	Salinity	Arabidopsis	A membrane-bound NAC transcription factor NTM2 facilitates the signaling cross-talk between salt stress and auxin through the IAA30 gene at the time of seed germination	Park et al. (2011)
		Tomato	Cytokinin biosynthesis due to IPT gene enhanced growth and yield	Ghanem et al. (2011)

(continued)

Table 1.1 (continued)

Types of PGRs	Stress condition	Studied plants	Key findings and impact on plant growth and development	References
	Drought	Rice	A putative auxin efflux carrier gene, OsPIN3t, acts in auxin polar transport as well as involved in the response of drought stress in rice	Zhang et al. (2012)
		Potato	Increased content of auxin because of YUCCA6 gene (vital gene in auxin/ IPA biosynthesis) and improved drought tolerance	Kim et al. (2013)
Gibberellins	Salinity		Hormonal homeostasis	Fahad et al. (2015)
		Arabidopsis thaliana	Response to osmotic stress	Wani et al. (2016), Skirycz et al. (2011)
Cytokinins	Salinity	Tomato	Transgenic tomato presented higher salinity stress tolerance	Qiu et al. (2015)
	Drought	Tobacco	Transgenic tobacco showed greater drought tolerance	Rivero et al. (2007)
		Arabidopsis	Overexpressing cytokinin oxidase/dehydrogenase gene	Werner et al. (2010)
		Barley	Transgenic barley through improved avoidance to dehydration	Pospíšilová et al. (2016)
		Rice	ERF-1 (JERF1) response factors for ethylene and jasmonates	Zhang et al. (2010)
Abscisic acid	Salinity	Maize	Improved salinity tolerance due to increased Na + efflux and H+ influx	Zhang et al. (2016)
	Drought	Date palm	Phosphatases in the PP2C family, ATP-binding cassette (ABC) Transporters, late embryogenesis abundant proteins (LEAs), and MYB74 (a guard cell transcription factor)	Müller et al. (2017), Hazzouri et al. (2020)
		Soybean	Transgenic soybean exhibited improved biomass and yield	Li et al. (2013)
		Rice	Improved tolerance to drought	Zhang et al. (2012)

(continued)

Table 1.1 (continued)

Types of PGRs	Stress condition	Studied plants	Key findings and impact on plant growth and development	References
	Temperature	Arabidopsis	Transgenic *Arabidopsis* showed improved tolerance to salt, drought, and freezing stress related to reduced water loss, better osmotic potential and photosynthesis	Mao et al. (2010)
Jasmonic acid	Salinity	Soybean	The MeJA exogenous application successfully minimized symptoms of salinity stress	Yoon et al. (2009)
	Drought	Rice	Rice plants presented improved tolerance to drought	Zhang et al. (2010)
		Maize	Transgenic maize showed improved tolerance to drought with minimum ethylene levels	Habben et al. (2014)
Brassinosteroids	Salinity	Soybean	Overproduction of BR, improved growth rate, and grain yield	Li et al. (2007a, b)
	Drought		Enhanced tolerance to drought with dwarf phenotypes having purple false brome	Feng et al. (2015)

may be an effective and practical strategy for conferring crop salt resistance (Amjad et al. 2014). Without any delay in regulating gene expression or its interactions with specific DNA sequences, transcription factors, and other practical proteins, PGR supplementation can regulate many developmental factors of the flora cycle (Chapman and Estelle 2009).

1.5.3 Role of PGRs in Heat Stress Tolerance

Salicylic acid (SA) is a universal plant hormone that controls various physiological approaches of flora in response to abiotic stress (Hayat et al. 2010). Mohammed and Tarpley (2009) stated that the application of SA to rice could increase the distribution of dry count as much as 16%, thereby lowering the adverse effects of high temperatures at 32 °C at night. Foliar spray of salicylic acid (0.1 mM) prompted rice resistance to heat stress and progressed its effect (Chandrakala et al. 2013). Foliar application of SA (0–50 mmol L^{-1}) mitigated negative effect of high-temperature stress by increasing the proline; sugar contents; antioxidant enzymes, like CAT, POD, and APX; as well as phytohormone concentrations, like ABA, IAA, and GA_3 contents of rice spikelet and increased spikelet numbers, seed setting rate, and ultimately increased crop yield (Zhang et al. 2017).

Some growth regulators, which include methyl jasmonate, can increase the flowering time until the early hours of the morning and may be used to relieve heat stress (Kobayasi et al. 2009). Kobayasi and Atsuta (2010) reported that methyl jasmonate application resulted in the early opening of flowers and also improved flower numbers. The application of methyl jasmonate resulted in early flower opening, which was started just 80 min after its application (Zeng et al. 1999).

ABA plays a variety of vital roles in plants, for example, inside the biosynthesis of natural acids, phytohormones, and flavonoids, which regulate mobile department and elongation and act as an antioxidant enzyme to remove reactive oxygen species (Debolt et al. 2007). Exogenous ABA can protect flowers from oxidative strain and preserve the steadiness of photosynthesis function. Zhang et al. (2018) studied the results of endogenous ascorbic acid content material in transgenic rice that overexpressed at high temperature and inhibited GLDH (L-galactono-1,4,-lactone dehydrogenase) enzyme activity. Maximum reactive oxygen species were found in transgenic rice with suppressed GLDH. Foliar application of PGR such as ascorbic acid, methyl jasmonate, alpha-tocopherol, and brassinosteroid mitigated heat stress, by enhancing photosynthetic activity, water use efficiency (WUE), and enhanced crop yield (Fahad et al. 2016).

1.5.4 Effect of PGRs on Heavy Metal Stress

Heavy metal phytoextraction refers to the growth of crop plants to extract heavy metal from the soil through their roots along with nutrient and moisture absorption and accumulate them in their leaves and seed which are harvestable parts (McGrath and Zhao 2003). The PGRs are used to mitigate the negative effects of metal stress in crop plants to boost their biomass production (Ouzounidou and Ilias 2005). Plant PGRs particularly ABA, GAs, AXs, CKs, ET, JAs, BRs, and SA are also most important that can assist plants under heavy metal toxicity (Peleg and Blumwald 2011; Vriet et al. 2012). SA application to crops induced adaptive effect and then increased the resistance against heavy metal stress because, basically, it adjusts the metabolic pathways like antioxidative activities. Early research revealed that SA application produced defensive mechanisms as a result of copper toxicity in tobacco and cucumber (Strobel and Kuc 1995). While later on, it was suggested that foliar-applied SA facilitated protection against heavy metal stress. SA application improved the efficiency of APX, POD, and NADPH and diminished Hg toxicity in alfalfa. It specifies the role of reactive oxygen species signaling in such an improving procedure (Zhou et al. 2009). BRs are one of the important PGRs that can regulate ionic uptake and are effective to reduce heavy metal toxicity in plants. BRs may also induce tolerance against most of the abiotic and abiotic stresses like drought, heat, cold, heavy metal, salinity, and pathogen attack (Bajguz and Hayat 2009; Hao et al. 2013). Cadmium (Cd) is a very toxic heavy metal even where it is present in very low concentration, it stops chlorophyll synthesis, disturbs efficiency of various enzymes, and decreases photosynthetic efficiency by minimizing energy resources

(Vassilev and Yordanov 1997). Although BRs application decreased cadmium toxicity. Drastic effects caused by cadmium toxicity were minimized by the application of epibrassinolide (Janeckzo et al. 2005). Many scientists suggested that GA application minimizes abiotic stresses, especially heavy metal stress tolerance. Zhu et al. (2012) studied that GAs application with a rate of 5μM reduced the cadmium uptake and lipid peroxidation. Moreover, scientists confirmed that gibberellic acids decrease nitrogen oxide level that, in turn, down controls expression of IRT1 gene, that is, a Fe transporter involved in Cd uptake as indicated by no influence of gibberellic acid in reduction of cadmium uptake in an IRT1 knockout mutant irt1. The exogenous application of GAs regulates the growth of soybean by improving daidzein and genistein contents under stress conditions and mitigates its adverse effect (Hamayun et al. 2010). Siddiqui et al. (2011) studied that nickel has been shown to reduce growth, chlorophyll contents, and carbonic anhydrase activity by enhancing ROS production but the application of GAs ameliorates nickel-induced toxic effects. Exogenous application of GAs reduces the toxicity of chromium on growth and ammonium assimilation of pea seeds by regulating ROS production and an antioxidant defensive system (Gangwar et al. 2011). Meng et al. (2009) reported that exogenous application of gibberellic acid ameliorates the adverse effects of cadmium on seedling establishment and oxidative damage. Atici et al. (2005) observed that Pb and Zinc toxicity disturbs seed emergence in chickpea by varying hormonal balance, and foliar application of GAs augmented the heavy metals toxicity. Furthermore, GAs reduce the damaging effects of cadmium and molybdenum on broad bean and lupin plants by regulating activities of proteases, CAT, and POD (Sharaf et al. 2009).

1.6 PGRs-Induced Antioxidant Defense Under Abiotic Stress

In response to abiotic stresses especially heat stress, plants produce ROS which negatively affects plant growth by altering physio-chemical processes. It deserves mentioning that ROS are small molecules that are produced by plants under various abiotic stresses and tend to disrupt numerous vital metabolic as well as biological processes taking place within plants (Wahid et al. 2017). Few instances of ROS include singlet oxygen ($1O_2$), superoxide (O^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-), while mitochondria and chloroplasts are their production sites in the cells. The damages caused by ROS are generally of severe level irrespective of the plant growth stage. As ROS damages proteins and other related substances like DNA, lipids, etc., these become a prime reason for disrupting cellular functioning in all plant species (Halliwell 2006). To offset the drastic effects of abiotic stresses, detoxification of ROS is of the utmost pertinence to keep various biological processes of plants to work normally.

Detoxification of these molecules constitutes a vital strategy to avoid losses caused by oxidative stress as well as redox imbalances. Application of different PGRs, primarily through foliage means, can be a promising approach to neutralize

ROS in a biologically viable manner (Waqas et al. 2017). Exogenous application of PGRs substantially reduces malondialdehyde concentration through significantly increasing superoxide dismutase (SOD) and catalase (CAT) which are stress-ameliorating enzymes. The application of PGRs can be equally effective in reducing heavy metals stress for a variety of plant species. The scanning electron microscope-energy dispersive spectrometer has recorded significant evidence that IBA or DA-6 application significantly lowered the concentration of Cd in lower and upper epidermal cells of leaves. It has been concluded that lower concentration of Cd in epidermis cells suggests that more Cd migrates from shoots to roots of *Amaranthus*. These research findings suggested that IBA or DA-6 foliar application may be developed as an effective abiotic stress-ameliorating strategy that appears to be optimal for boosting *Amaranthus* phytoextraction efficiency in Cd-contaminated soils.

Heat stress which has recently emerged as the most threatening challenge and one of the pronounced impacts of climate change phenomenon and global warming may be tackled with PGR application under various agro-ecological conditions. Thiourea (TU) supplementation has been suggested for ameliorating and mitigating the drastic impacts of heat stress in crop plants (Asthir et al. 2013; Khanna et al. 2017). Both seed priming and foliage application of synthetic PGRs have been effective in boosting plant tolerance against terminal heat stress. Seed priming (6.6 mM TU) and exogenous foliage applied of TU (6.6 mM) at the anthesis stage have remained instrumental in imparting terminal heat tolerance by lowering oxidative damage caused by excessive production of ROS. It also lowered heat stress caused by cell membrane injury along with reducing the concentration of thiobarbituric acid reactive substances as well as H_2O_2. It has also been observed that at the grain-filling phase of the wheat crop, synthetic PGRs tend to increase antioxidant potential under heat stress (29 °C) (Asthir et al. 2013). The synthetic PGRs used as a seed treatment (10 mM) significantly improve the achene yield and oil content of sunflower (*Helianthus annuus*) under both mild (35 °C) and extreme heat stress (45 °C) through induction of antioxidant defense (SOD, CAT, and APX). They also assist sunflower plants under heat stress to maintain leaf water status as well as availability and uptake of primary nutrients (N, P, K). Another underlying mechanism of heat stress amelioration by PGRs is their role in improving nitrate reductase activity and phenylalanine ammonia-lyase activity, which tend to detoxify ROS and regulate photosynthesis rate even under extreme heat stress (Akladious 2014).

Water stress is another challenge confronted to modern farming systems, and shifting of rainfall patterns along with disruption in rainfall intensity and distribution throughout the year has aggravated water-related issues. Water-limited conditions hamper vital process such as photosynthesis, glycolysis, etc. which lead to stunted growth and even plants wilt under prolonged drought spell. PGR-treated plants show competitively better performance regarding vegetative growth through detoxification of ROS despite the fact that these experiences slightly severe water deficit owing to lipoprotein structure stabilization and considerably lesser production of malondialdehyde in comparison to non-treated control plants (Mohammadi and Karr 2001). When wheat plants were watered at 40% field capacity, these expressed drastic detrimental impacts on numerous physiological processes as well as biochemical reactions, such as translocation of synthases, uptake of nutrients ions, respiration, photosynthesis, metabolism of nutrients, carbohydrates, and hormones (Abdelkader et al. 2012). However, seed priming with salicylic acid (~1 mM) combined with two foliar applications of TU (5 mM) just before the onset of anthesis phase significantly multiplied grain yield and its nutritional quality (Abdelkader et al. 2012). Moreover, numerous studies have concluded that a significant increase in MDA and H_2O_2 concentration can take place in plants under water stress. This leads to extensive oxidative damage due to excessive production of ROS, while TU application as seed priming agent and foliar spray can potentially scavenge ROS by substantially improving the antioxidant defense of drought-affected plants (Hassanein et al. 2015). In addition, lipid peroxidation (LPO) induction has been reported as an effective mechanism caused by oxidative damage under drought stress. Previously, it has been inferred that seed priming with synthetic PGRs and their foliar application (1000 mg L^{-1} of TU) at vegetative growth stages significantly lowered LPO and improved photosystem II's quantum efficiency in chickpea plants prior to the onset of drought stress and later on reduced the concentration of hydrogen peroxide as well as hydroxyl ions suggesting that it can be effectively employed as a detoxification agent for ROS under water-limited conditions (Vineeth et al. 2016).

To ameliorate the drastic impacts of abiotic stresses, PGRs not only neutralize the ROS but also trigger extensive and vigorous root growth owing to rapid leaf expansion, improved photosynthesis rate, effective sucrose movement, and distribution from leaves to roots leading to balancing of source-sink relationship and essential nutrient uptake and availability. Moreover, it has been noted that PGRs reduce ROS concentration and improve plants' salt tolerance potential by enhancing chlorophyll content, which increases shoot growth and maintains water relations along with reducing H_2O_2 content. It has also been inferred that synthetic PGR application as seed treatment and foliar application increase the K^+/Na^+ ratio, which multiplies the uptake of K^+, Ca^{2+}, and N (Kaya et al. 2015). Moreover, PGR application, especially ABA and synthetic TU (20 mM), significantly hampered the negative effects of salt stress in mung bean by improving chlorophyll b and phenolic contents, the concentrations of soluble sugars and proteins, and enhanced POD activity. They were also effective in decreasing permeability of the membrane, free amino acids, as well as glycine betaine (Perveen et al. 2016).

Among PGRs, salicylic acid has been extensively reported for its role in ROS signaling and detoxification and antioxidant metabolism (Hernández et al. 2017). It alleviated the negative impact of abiotic stresses (heat and drought) and enhanced oil content and photosynthesis rate and efficiency of sunflower by inducing antioxidant defense (SOD, CAT, and APX). The antioxidant action was further bolstered by boosting the biosynthesis of salicylic acid (Akladious 2014). Recently, the role of synthetic PGRs has been reported in plant's defense-related transcription reprogramming under water-limited conditions.

In temperate regions, chilling stress is one of the leading causes of reduced germination and stunted growth caused by the toxicity of ROS. It is interesting to note that PGR-mediated gene expression pertaining to antioxidant defense (Cu/Zn SOD) and biosynthesis of metabolites (CAH) has been observed in the seedling of *Capsicum* which was exposed to moderate level of chilling stress (Patade et al. 2012). Thus, it may be inferred that PGR application as seed priming agent or exogenous foliage application may potentially detoxify ROS by strengthening the antioxidant defense in plants subjected to different abiotic stresses (heat, drought, chilling, heavy metals, wounds, etc.).

1.7 Relation Between Nutrient and PGPR Under Abiotic Stress

PGPR promote growth and development in their direct mechanism of action. In this mechanism, they act via accelerating the macronutrient's uptake, modulating the levels of phytohormones, and playing the roles of biocontrol agents through diminishing the adverse impacts of plant pathogens (Altaf et al. 2019).

1.7.1 Nitrogen Fixation

Nitrogen (N_2) plays numerous functions in plant metabolism, thereby essential for plant growth and development. Although it is abundantly present in the atmosphere (78%) due to its bonding properties ($N \equiv N$) and lack of nitrogen fixation machinery in plants, it is not available directly to the plants. Thus, plants rely on bacteria and cyanobacteria for the N_2 requirement as these microbes possess a nitrogenase enzyme (Kim and Rees 1994). Regarding PGPR, it was reported that both types PGPR, i.e., symbiotic and non-symbiotic, fix nitrogen. For example, *Azoarcus*, *Beijerinckia*, *Bradyrhizobium*, *Klebsiella*, *Mesorhizobium*, *Pantoea*, *Rhizobium*, and *Sinorhizobium* are the main symbiotic PGPR, while non-symbiotic PGPR are *Azotobacter*, *Burkholderia*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas*, cyanobacteria, etc. (Bhattacharyya and Jha 2012; Singh et al. 2019). The inoculation of PGPR has emerged as an integrated strategy for growth promotion, disease suppression in crops, and maintenance of nitrogen levels in the agricultural soils (Damam et al. 2016).

1.7.2 Phosphate Solubilization

Phosphorus (P) is another important macronutrient after N_2 (available in both organic and inorganic form) and required for optimal plant growth and development. It plays a significant role in energy transfer, respiration, photosynthesis, signal propagation, and macromolecular synthesis (Anand et al. 2016). Plants can take up P as mono- and dibasic phosphates. Therefore, organic and insoluble inorganic P forms need to be solubilized present in agricultural soils (Dipta et al. 2019). PGPR is reported as an efficient P-solubilizing agent. No doubt, there are other alternatives for the mitigation of P deficiency like the applications of phosphatic fertilizers. However, the major issue after applying the synthetic fertilizers is the marginal availability of P to the crops as they are applied in the precipitated form (Altaf et al. 2019). Thus, the use of synthetic fertilizers leads to the depletion of soil fertility and also modulates the surrounding microflora. Thereby, the use of phosphate-solubilizing PGPR as inoculants in P-deficient soils can provide new horizons to sustainable agriculture, and this strategy of P solubilization is economic and eco-friendly (Alori and Babalola 2018). The main PGPR known for P solubilization are *Arthrobacter*, *Burkholderia*, *Bacillus*, *Beijerinckia*, *Enterobacter*, *Erwinia*, *Mesorhizobium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, *Serratia*, etc. (Bhattacharyya and Jha 2012).

1.7.3 Potassium Solubilization

Potassium is a vital macronutrient that also plays an essential role in the growth and development of plants. Adequate potassium supply is especially observed to be associated with root development and seed yield. Whereas, potassium in soluble form is always low as a major part of naturally occurring potassium is present in the form of insoluble rocks and minerals (approximately 90%) (Parmar and Sindhu 2013; Singh et al. 2019). Henceforth, it is the need of the hour to explore for additional potential sources of potassium capable of sustaining plant growth by maintaining the necessary soil potassium concentration without causing adverse environmental effects (Kumar and Dubey 2012). In this context, PGPR has reported as the most effective and productive applicants because of their ability to solubilize potassium-containing rocks and minerals. The reported P solubilizes PGPR that are *Bacillus mucilaginosus*, *Bacillus edaphicus*, *Acidithiobacillus ferrooxidans*, *Paenibacillus* sp., *Burkholderia* sp., *Pseudomonas* sp., etc. (Liu et al. 2012). The application of PGPR is intended not only to reduce reliance on dangerous and expensive chemical fertilizers but also to promote sustainable crop production via using potassium-solubilizing PGPR as a biofertilizer (Altaf et al. 2019).

1.7.4 Plant Growth-Promoting Rhizobium (PGPR) and Phytohormone Production

Besides the roles of plant growth-promoting rhizobium (PGPR) as N_2 fixatives and solubilizers of phosphorous and potassium, they are also reported to be involved in various growth-promoting phytohormone production (indole-3-acetic acid,

Table 1.2 Plant growth-promoting rhizobium (PGPR) and phytohormone production

Phytohormones	Production and PGPR examples	References
Auxins	The number of studies has revealed that rhizospheric microorganisms form and secrete auxins as part of their secondary metabolites (~80%) Examples: *Agrobacterium, Azospirillum, Bradyrhizobium, Enterobacter, Klebsiella, Pseudomonas, Rhizobium,* etc.	Altaf et al. (2019)
Cytokinins	Different cytokinins are secreted by PGPR, but among all kinetin and zeatin are the most observed cytokinins in the rhizosphere Examples: *Rhizobium* sp., *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bradyrhizobium* sp., *Rhodospirillum* sp., *Paenibacillus* sp., etc.	Glick (2012), Singh et al. (2019)
Gibberellins	PGPR producing gibberellins are reported to enhance crops' growth and development via playing important roles during germination, flowering, stem lengthening, etc. Examples: *Bacillus sp., Sphingnomonas* sp., etc.	Khan et al. (2014), Kang et al. (2017)
Ethylene	Ethylene is an important plant hormone as it is involved in fruit-ripening. It also mediates abscission of the leaves, wilting the flowers, and activation of certain other phytohormones Examples: *Agrobacterium*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, *Ralstonia*, *Serratia*, etc.	Singh et al. (2019)

gibberellins, cytokinins, and ethylene). The details of phytohormone-producing plant growth-promoting rhizobium (PGPR) are given in Table 1.2:

1.7.5 Siderophore Production

Some of the PGPR are reported to produce iron-chelating compounds with a low molecular weight that facilitates the absorption of iron and referred to as siderophore. Siderophores with high iron specificity form complexes with Fe^{3+} and eventually bound to the membrane of bacterial cells. Furthermore, inside the cell, Fe^{3+} enzymatically reduced to Fe^{2+} and siderophore possess less affinity for Fe^{2+}, thereby it releases from siderophore (Deori et al. 2018; Tonziello et al. 2019). Thus, siderophores assist the growth and metabolism of microbes as well as assist plants in the uptake of iron. In addition, siderophores were reported to assist plants directly by absorbing iron from the bacterial siderophores through the direct absorption of iron from siderophore-Fe complexes, chelation, and release of iron and a ligand reaction exchange (Schmidt 1999). The indirect benefits of siderophores to plants are also reported by several workers as they improve plant growth due to the iron sequestration capacity that controls the iron nutrition of pathogens. Thus, they serve as a biocontrol agent and enhance crop outcomes and also are reported to form complexes with certain heavy metals. Thus, they increase soluble metal amount that is absorbed after the process of complex formation with heavy metals, which helps in the alleviation of heavy metal stress-mediated consequences in crop plants (Rajkumar et al. 2010). The examples of siderophore-forming PGPR are *Aeromonas*, *Bacillus*, *Rhizobium*, *Pseudomonas*, *Serratia*, and *Streptomyces* sp. (Sujatha and Ammani 2013).

1.8 The Indirect Mechanism to Abiotic Stress

In the indirect mechanism, the growth and development augmentation in plants is observed to be mediated by PGPR via the production of volatile organic compounds (VOCs), lytic and protective enzymes, exopolysaccharides (EPSs), and antibiotics (Fig. 1.3). Furthermore, the induction of systematic resistance is also an important part of the indirect mechanisms.

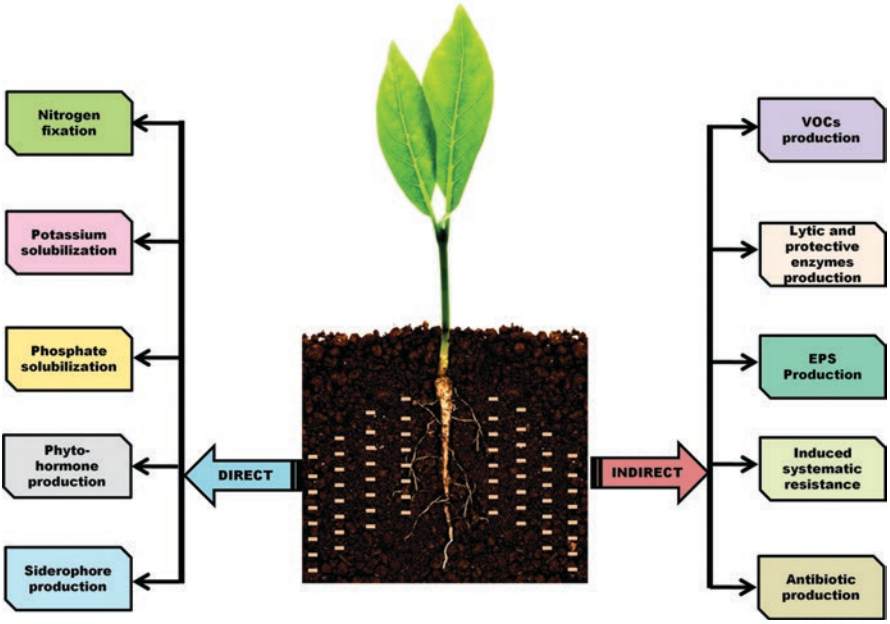

Fig. 1.3 Direct and indirect growth-promoting mechanisms of plant growth-promoting rhizobacteria

1.8.1 VOC Production

VOCs are low molecular weight compounds (<300 Da) that are produced by several PGPR. These compounds have high vapor pressure and are reported to inhibit the growth of bacterial and fungal phytopathogens and induce ISR due to their diffusible nature (Insam and Seewald 2010; Fincheira and Quiroz 2018). *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Serratia*, *Stenotrophomonas,* etc. are the main VOC-producing PGPR (Santoro et al. 2016).

1.8.2 Lytic and Protective Enzymes Production

Several PGPR are known for producing such enzymes that can hydrolyze cellulose, hemicellulase, chitinase, protease and glucanase, hemicellulose, chitin, protein, and glucans. Thus, they can cause degradation of cell walls via using these enzymes (Singh et al. 2019). This property of PGPR indirectly plays important roles in the promotion of plant growth by suppressing the growth of phytopathogenic fungi due to the secretion of such lytic enzymes. Furthermore, it was also observed that PGPR could also produce protective enzymes, i.e., antioxidative enzymes (catalase, superoxide dismutase, glutathione reductase, and ascorbate peroxidase). These enzymes are reported to scavenge the elevated levels of reactive oxygen species that are formed in plants during stressed conditions (Hahm et al. 2017; Singh et al. 2020).

1.8.3 EPS Production

Exopolysaccharides (EPSs) are the high molecular weight biopolymer and are synthesized by a wide range of PGPR and well-avowed for enhancing soil fertility and crop yield. They function as a potential signaling molecule that triggers plant defense responses during infection (Parada et al. 2006). Furthermore, they are reported to participate in the mitigation of salinity stress via their binding with Na^+ (Ilangumaran and Smith 2017). Similar to siderophores, EPSs are also observed to possess the ability of heavy metals sequestration via the formation of biofilms. Biofilm is a protective sheath of bacteria that converts toxic metal ions into nontoxic forms as they get adsorb on it (Gupta and Diwan 2017). *Azotobacter* sp., *Agrobacterium* sp., *Bacillus drentensis*, *Enterobacter* sp., and *Xanthomonas* sp. are some EPS-producing PGPR (Mahmood et al. 2016).

1.8.4 *Induced Systematic Resistance*

Induced systematic resistance (ISR) is characterized as a physiological condition of enhanced defense capability produced against certain environmental stimuli. For many plants, PGPR activates the protection mechanism via ISR to several biotic stresses involving phytopathogens (Prathap and Ranjitha Kumari 2015). In ISR, jasmonic acid and ethylene phytohormones are reported to provide immunity to plants (Tsukanova et al. 2017). Some of PGPR are also observed to induce systemic response via the salicylic acid pathway (van de Mortel et al. 2012), while other PGPR activates ISR via signaling pathways (Niu et al. 2011). However, the immunity provided via ISR is not specific to any pathogen, but it can enable crop plants to control several diseases (Kamal et al. 2014). Besides, several PGPR components have been identified as evoking the mediated systems reaction, like siderophores, cyclic lipopeptides, homoserine lactones, volatile agents, etc. (Berendsen et al. 2015).

1.8.5 *Antibiotic Production*

The antibiotic formation by PGPR is a well-studied and useful biocontrol mechanism against specific phytopathogens. Of all PGPR, *Bacillus* and *Pseudomonas* are the most effective against phytopathogen growth and proliferation via antibiotic production (Singh et al. 2019). Antibiotics are classified according to their ability to control root diseases in six groups. These are phloroglucinols, phenazines, pyoluteorin, cyclic lipopeptides, pyrrolnitrin (diffusible antibiotics), and hydrogen cyanide (volatile antibiotic) (Haas and Défago 2005).

1.9 Conclusion and Future Outlooks

This work has highlighted the role of plant growth regulators in imparting plant stress tolerance and developing them as potential mediators to bolster plant defense responses against abiotic stresses. Such an understanding of plant growth regulators and their role in enabling plants to cope with stresses could be an important step toward enhancing plant tolerance under stressful environmental conditions. Furthermore, PGR application tends to reduce drastic impact of stresses by integrating and optimizing the plant's defense circuits such as stress signaling, enzymatic and non-enzymatic antioxidants, metabolic processes, genetic expression, photosynthesis, and secondary metabolites biosynthesis. Lastly, it is concluded that plants need to enhance the concentration and activity of the antioxidant enzyme to ensure their survival in an environment where abiotic stress exists. The preceding researches established and suggested that exogenous supply of PGRs improves antioxidant defense along with reducing the associated metabolic cost.

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Chapter 2
Accumulation, Partitioning, and Bioavailability of Micronutrients in Plants and Their Crosstalk with Phytohormones

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2.1 Introduction

Micronutrients are essential for plants and animals, but unfortunately their deficiency has increased on global scale (Gupta et al. 2016). As a result of this, defected roots and shoots; problem in growth, development, and performance; and many times a sharp drop in the crop yield is observed in case of many edible plant species (Matthes et al. 2020). Moreover, almost about two billion people are suffering from their deficiency worldwide because of the low bioavailability and accumulation of these nutrients in cereals (Gupta et al. 2016). This problem is more pronounced in low-income countries where death rate has increased due to higher vulnerability to different diseases in adults and children (Detterbeck et al. 2016). Due to this malnutrition problem, scientific research focus has been diverted toward

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micronutrient availability, accumulation, and further partitioning within plant (Detterbeck et al. 2016; Naveed et al. 2020). Also, during the 2nd International Conference on Nutrition conducted in the year 2014, a commitment was made by more than 150 countries that they will take cautions to tackle micronutrient deficiencies, such as iodine, vitamin A, zinc, and iron (Shahzad et al. 2014). According to research done in the recent years, soil properties (Li et al. 2019), salt stress (Liu et al. 2020), agronomic practices like fertilization management (Dubberstein et al. 2019), and presence of other nutrients like high concentration of plant nitrogen (Hamnér et al. 2017) are considered to be the possible factors that are affecting the bioavailability and accumulation of micronutrients within plants (Greger et al. 2018). Presently, this growing problem is being countered in different ways, and for that purpose, different approaches, such as biofortification (Gupta et al. 2016), agronomic strategies (Detterbeck et al. 2016), and breeding options (Shahzad et al. 2014), have been adopted by scientific community to understand the mechanics of micronutrients within plants (Baxter et al. 2014; Riaz et al. 2020).

Phytohormones, indoleacetic acid (IAA), cytokinins (CKs), abscisic acid (ABA), gibberellic acid (GAs), salicylic acid (SA), jasmonic acids (JA), ethylene, and 1-aminocyclopropane-1-carboxylic acid (ACC), are basically chemical compounds with multiple functions to offer within plant body, such as control on growth and development along with serious responses to stress conditions like lack of micronutrients that can cause serious loss in plants (Navarro-León et al. 2016). Being the complex chemical compounds, these hormones have the ability to handle stress within plants, both biotic and abiotic in nature (Sytar et al. 2019). Phytohormones and micronutrients have a very close relationship because both assist each other in so many ways (Marschner 2011). The plant hormones, their production, and their affects are controlled by nutrient supply, and they themselves are the major regulators of plant micronutrients (Piotrowska-Niczyporuk et al. 2012). In return, the phytohormones assist in their availability and accumulation within plants along with development of homeostasis (Navarro-León et al. 2016).

Studies have shown that above or below a certain level, the micronutrients pose different threats to plants which can be effectively countered by exogenous application of phytohormones because their application can regulate all major functions within plants (Sytar et al. 2019; Bücker-Neto et al. 2017). Basically, there is cross-talk which is initiated by the phytohormones when they are introduced into the plant body, and the priming effect of these hormones allows increased performance of plant body to help in better adaption and tolerance toward stressful external and internal environment (Sytar et al. 2019). They are regulators and have the ability to provide plants with signals and provide pathway for plant relief against stress; therefore, they are of global interest right now (Bücker-Neto et al. 2017). They do this by modifying the molecular and physiological response or reaction of plants (Fahad et al. 2015a, b). For the management of crops, along with availability, accumulation, and partitioning of micronutrients, future research has been planned out by scientist to enhance management of crops with these chemical ingredients (Sytar et al. 2019).

2.2 Role of Micronutrients in Plant Growth and Physiology, Metabolic, and Biochemical Processes

Micronutrients, present in 0.1–100 mg per kg concentration in plants, are considered as essential source based on their significant roles in plant growth, cell metabolism, enzyme-catalyzed reactions, physiological response, metabolic activities, and biochemical processes (Moura et al. 2013; Pandey 2018). They have the ability to provide structural integrity to plants, play a role in fruit and flower formation, regulate respiration, and allow seed development, cell division, phloem formation, etc. (Bubarai et al. 2017). These micronutrients are essential for the growth and development as are actively involved in biological, physiological, chemical, and all type of processes occurring within the agriculture crop bodies (Pandey 2018; Umar et al. 2020). Therefore, farmers must be encouraged to use micronutrient fertilizers in order to promote their functionality and ultimately economic yield from their fields.

The extent to which a plant can lose its production depends on many factors; however, availability of micronutrients and their interaction with the soil and environment of the plant are the main factors that control it. In order to improve the overall yield, research should be conducted to understand the mechanism of uptake, translocation, and transfer of nutrients so that required homeostasis could be maintained within plant bodies. Various studies have been conducted on micronutrients, such as iron (Fe), zinc (Zn), copper (Cu), etc., but there is a long way to go. Research and development related to the exploration of molecular genetics, agronomic innovations, and biotechnology must be continued and further developed so that it could be made clear that how plants or crops would be able to better deal with situations where the nutrient's supply is limiting. This will be especially crucial for the food security worldwide and for its increasing demand with the passage of time. Techniques must be developed that not only increase the bioavailability, accumulation, and portioning of micronutrients but also increase overall plant produce yield quantity and quality.

2.3 Uptake Mechanism and Transportation of Micronutrients in Plants

2.3.1 Sources of Micronutrients

Environmental matrices contain micronutrients which are required by plant bodies. These micronutrients are crucial for normal growth and development, but surplus of these metals is also toxic for plants. Ingress of micronutrients in environmental matrices is from both natural and anthropogenic origins (Lajayer et al. 2018). Natural sources of micronutrients in environment are volcanic eruptions, erosion and mineral weathering, etc. (Antoniadis et al. 2017; Lajayer et al. 2017). In addition, anthropogenic activities involved in the contamination of environment with micronutrients are mining, industrial effluents, urbanization, fossil fuels burning, agricultural materials (fertilizers, pesticides, sludges, etc.), smelting of ores, technogenic materials, etc. (Tauqeer et al. 2016; Feng et al. 2017; Lajayer et al. 2017; Mahar et al. 2016).

2.3.2 Bioavailability from Soil

Bioavailability of micronutrients from soil depends on their interaction with the soil-water system. Micronutrient elements include metals, such as zinc (Zn), iron (Fe), copper (Cu), molybdenum (Mo), manganeese (Mn), nickel (Ni) and non metals like chlorine (Cl), and boron (B). Most of the times, these micronutrient elements are supplied to the soil in the form of chemical or organic fertilizer. In many pharmacies, these elements are made as a part of fungicides, insecticides, and even bactericides. As far as the bioavailability of these micronutrients is concerned, it mainly depends on the soil properties in which they exist. These properties mainly include the soil pH level, percentage of organic matter, forms and concentration of clay and carbonates in soil matrix, etc. Along with this, the presence of opposite ions within the soil system affect their bioavailability from soil (Feng et al. 2017). In many studies, it is shown that they interact with the phosphate contents in soil; thus they can bind to them or change into forms which might be unavailable to plants. Also, they can be converted into forms that are more available compared to when they were present in isolated form. There are also chances that these micronutrients get adsorbed by clay minerals present in soil, or the metal containing oxides, and organic materials. This interaction, and absorption, includes two different type of mechanisms: (1) the electrostatic force might have resulted in an ion exchange within the diffuse layer, and (2) there could be a development of covalent bond between the micronutrient ion and surface of mineral (Selim 2016).

2.3.3 Absorption by Roots

For the proper growth, development, and reproduction of plants, micronutrients such as Fe, Cu, Mn, Zn, etc. hold great importance. Within plant bodies, these mineral elements are responsible for many cellular functions and molecular activities. These include production of chlorophyll pigment and processing of photosynthesis by elements like Cu, Fe, and Mn and respiratory activities carried out by copper, and iron, and zinc which are used for stabilization of DNA and allow genes expression to be perfect. This is why any fluctuation above or below a threshold level of these nutrients results in stunted plant growth, toxicity, and problems in the overall development. When these nutrient elements are present in oxidized form in the soil system, their uptake becomes extremely difficult for the plants; therefore the growth is directly affected. In such situation, plants use various mechanisms in order to uptake these elements through rhizosphere into the roots and then transfer it to the plant edible parts and the developing tissues that require their supply. Therefore, uptake of these metals is not only programmed in a certain way but also is very regulated to make sure that they fulfill the needs of plants (Bashir et al. 2019).

2.4 Factors Affecting Micronutrient Uptake

There are several factors which affect the uptake of micronutrients by plants including soil factors and environmental and plant-related factors. It is difficult to choose which factors are more importantly affecting the micronutrient uptake because every factor plays an important role in nutrient acquisition.

2.4.1 Soil Texture

Texture of any soil plays an important role in nutrient supply to plants (Bada and Raji 2010). Sandy soils (coarse textured) is mostly deficient in nutrients as compared to clayey soils (fine textured) because nutrient depletion in fine-textured soils is lower than the coarse-textured soils and the clay and organic matter contents in fine-textured soils have the ability to store more nutrient and water contents (Choudhary and Suri 2014; Gil et al. 2012). Coarse-textured soils have high drainage of water and the drained water take dissolved nutrients with it below the root zone, and this phenomenon is known as nutrient leaching. The leached nutrients are not available for plant uptake (Chaudhari et al. 2013). Furthermore, the mineral soils which have low organic matter contents are most probably deficient in micronutrients. Strongly acidic soils are also deficient in most of the micronutrients because most of the originally present micronutrients are removed by acidic leaching (Kumar et al. 2014).

2.4.2 Organic Matter

Soil organic matter is considered as a pool of essential plant nutrients because it can continually supply nutrients to plants by the process of decomposition over the time. Organic matter is important to the nutrients which are in anionic form like boron which cannot bind to the soil matrices and are subjected to leaching. Soil organic matter can affect micronutrient availability differently like it can increase or decrease or it can also have no effect on micronutrient availability depending on the conditions (Mortvedt 2000). When organic matter forms complexes with high molecular weight organic molecules like lignin, humic acid, and others, it results in the formation of insoluble precipitates and ultimately reduce the availability of micronutrients (Choudhary and Suri 2009). On the other hand, solubilization of low molecular weight or short-chain compounds like amino acids increase the availability of micronutrients (Mortvedt 2000). Regular addition of organic manures in the soils reduce the incidence of micronutrient deficiencies. Micronutrient deficiencies can also occur due to imbalanced application of other macronutrients like Zn deficiency is a common phenomenon when phosphorus is applied in heavily manured soils (Kumar et al. 2014; Das 2011). Higher accumulation and slow release of heavy metals was reported in the soil rich in organic matter, which shows higher attraction of organic matter toward heavy metals (Kumar et al. 2016a, b). Some of the micronutrients like Mn, Zn, and Cu become unavailable in *peat* and *muck* soils due to strong chelation because of extremely high organic matter contents (Deb et al. 2009).

2.4.3 Clay Contents

Clay particles have high affinity for cations due to negatively charged surfaces. Soil clay contents contribute in soil cation exchange capacity (CEC) due to the presence of protonated functional groups (-COOH, -OH). Dissociation of -COOH and -OH (*especially those on organic matter*) depends on soil pH, so the increase in pH increases the CEC of the soil. Increase in the negative charge on colloids surfaces due to increased CEC attracts metal cations and reduces the availability of metal cations to plants (Kumar et al. 2016a, b).

2.4.4 Soil pH

One of the most important factors which affects micronutrient uptake is soil pH. Measurement of soil alkalinity or acidity is known as soil reaction (pH). pH of the soil plays an important role in the plant nutrient uptake because it regulates the solubility and mobility of the nutrients (Bradl 2004; Fageria et al. 1997). Availability of most of the micronutrients at low pH is at highest level. Protonated anions and metal cations are favored at low pH, while hydroxyl complexes and carbonates are available at higher pH. Thus, the availability of metal cations and other toxic ions is increased with the decrease in pH or increase in acidity, while the availability of the nutrients which are in anionic form (SeO_3^-, CrO_4^-, MoO_4^{-2}) is increased with the increase in pH or with reduction in acidity. The availability of micronutrients like Fe, Cu, B, Mn, Zn, etc. is increased, and the availability of Mo is reduced at low pH. Under highly acidic conditions, the availability of most of the micronutrients reached to their toxic level (Brady, 2002). When the pH increases from 5 to 7, most of the micronutrient cations remain strongly attached to the exchange sites and become less available for plant uptake. In acidic conditions, B is present in the form of boric acid, while the availability of B is high at alkaline pH as compared to acidic pH (Deb et al. 2009). At lower pH, deficiency of Mo is a major problem because at low pH, fixation of Mo takes place with Al, Fe, and silicates.

2.4.5 Nutrient Interaction

Nutrient interactions can take place in soil as well as in plant body. Micronutrient uptake is not only dependent on the soil's ability to supply nutrients, but several other factors also affect micronutrient nutrient acquisition by crop plants (Das 2011). Balance between micronutrients and macronutrients influence the availability of micronutrients for plant uptake (Deb et al. 2009) as this interaction is very critical in soil media (Das 2011). Availability of Zn is reduced in the soils with high phosphorus (P) contents or by excessive application of phosphate fertilizers (Grant 2011; Das 2011). Similarly, potassium (K) application has antagonistic effect on Mn and Fe avalibility in rice crop (Rietra et al. 2017). Use of $MgCO_3$ and $CaCO_3$ for the amelioration of acidic soils reduces the availability of Zn due to increase in soil pH (Singh et al. 2016). Increase in the soil pH reduced the heavy metal uptake in plants due to physiological antagonism between heavy metals and Ca^{+2} (Kumar et al. 2016a, b). It is reported that plant growth response and the concentration of various nutrients have been changed due to nutrient interactions (Fageria et al. 2002). Concentration of one micronutrient can affect the concentration of others due to antagonistic and synergistic effects. Higher concentration of Fe can induce Zn and Mn deficiencies (Rengel 2015). Similarly, in cereal crops, deficiency of Cu has been observed due to excessive Zn application. It is also observed that under submerged conditions, Zn application can increase the Mn concentration while reduce the extractable Fe (Singh et al. 2016). Deficiency symptoms of Fe have been noted on citrus plants due to higher Cu concentration, while on the other hand Fe application can reduce the Cu toxicity in plants (http://edis.ifas.ufl.edu/ss617). Competition between Fe^{+2} and Zn^{+2} for plant uptake was observed under waterlogged conditions.

2.4.6 Oxidation State

Redox potential measurement indicates the condition of soils (oxidized or reduced). Oxidized conditions represent the well-drained soils, while reduced soil condition is the indicator of waterlogging or poor drainage (Pezeshki and DeLaune 2012; Dass et al. 2016). At normal soil pH range, Mn, Cu, and Fe are more soluble in their reduced states. Reduced soil conditions are favored by lower soil pH, while alkaline pH favors oxidized conditions (Brady 2002; Deb et al. 2009). Reduced forms of micronutrients are more available at neutral soil conditions as compared to oxidized forms (Rengel 2015). Dass et al. (2016) reported that micronutrients availability is increased under waterlogged or reduced conditions which indicate that micronutrient availability is influenced by poor aeration and low pH conditions. Deficiency symptoms of Mn, Zn, and Fe were reported on plants under well-drained calcareous soils at higher pH range, because these nutrients exist in oxidation states (Singh et al. 2016; Deb et al. 2009; Brady 2002).

2.4.7 Soil Moisture and Temperature

Low moisture and temperature conditions reduce the availability of micronutrients to plants because of reduced growth and activity of plant roots (Morgan and Connolly 2013). A case study in north India showed that during winter season, deficiency symptoms of Zn appeared on wheat crop (Sadeghzadeh 2013). This deficiency of Zn might be due to the reduced solubility of Zn at lower temperature conditions (Deb et al. 2009). Wet and cold soil conditions may show more severe deficiency symptoms on maize crop as compared to wheat. Furthermore, moist soil conditions favor the mineralization of micronutrients from organic matter (Choudhary and Suri 2014; Almendros et al. 2013). Submergence of soils for a longer period of time reduced the redox potential (Eh) and increased the pH which in turn reduced the availability of Zn, B, Cu, and S in acid soils (Karan et al. 2014). Concentration of Mn reduced with the increasing temperature, while Zn and Cu concentration increased with the increase in temperature (Albrecht et al. 2011).

2.4.8 Rhizosphere Conditions

Plant nutrient availability significantly depends on rhizosphere condition. Interaction of soil microbes with plant roots and the properties of soil have combine effects on micronutrient availability in rhizosphere (Kumar et al. 2016a, b). Chemistry of rhizospheric soil is much different than bulk soil. Chelating agents produced during the decomposition of residual material by microbes make complexes with micronutrients and convert them to more soluble and plant available forms (Deb et al. 2009). Secretion of chelating agents or organic acids by arbuscular mycorrhizal fungi (AMF) significantly changes the conditions in rhizosphere (Kumar et al. 2014), which results in the release of several micronutrients from inorganic and organic complexes (Suri and Choudhary 2013). Hyphal network of AMF significantly increases the nutrient absorption by increasing the root surface area (Begum et al. 2019; Harrier and Watson 2003). Several other microbes also play important role in micronutrient acquisition by plants.

2.4.9 Other Factors

Nutrient uptake is a phenomenon in which nutrients from soil solid phase moved to the soil solution phase and then taken up by plant through root system (Suri and Choudhary 2013). Processes like diffusion, mass flow, and root interception are responsible for the uptake of nutrients by plant roots (McMurtrie and Näsholm 2018; Barber 1995). Uptake of Zn, B, and Cu (More than 90–95%) and about 65% of Fe is carried out through mass flow. About more than 20% of Fe, Mn, and B are transferred to plant roots by diffusion. On the other hand, root interception also plays an important role in nutrient uptake by plants. Nowadays, anthropogenic activities like fossil fuel burning, industrial waste, application of manures, increased pesticide application, and particulate matter significantly adding some toxic heavy metals in the soil-plant system (Alloway 2012).

2.5 Compartmentalization and Absorption in Plant Body

Long time ago, researchers started to study the mechanisms of nutrient uptake from soil and translocation within the plant body (Marschner 1995; Miller et al. 2009; Miwa et al. 2009). Plants take micronutrients from soil through plant roots. These nutrients cross the root system via symplastic or apoplastic pathways to the stele followed by transport to the shoot by xylem loading (Duan et al. 2018). All the cations which may cause toxicity to plant cells are transported in the form of chelates through symplastic or apoplastic ways (Sablok 2019). Elements like Al, Cd, and Mo remain in the plant roots of several species (Sajwan et al. 2005; Marschner 1995). The micronutrients which are loaded to the xylem are transported to transpiring vegetative parts of the plants, i.e., leaves, where they are taken up by specific type of tissues (Waters and Sankaran 2011). The transport of micronutrients to the non-transpiring plant parts takes place via phloem transport systems. Few of the micronutrients like Mo, Zn, Fe, I, and Cu have less mobility in phloem, while Mn is almost immobile in several plant species (Etienne et al. 2018; Marschner 1995). Mobility of B varies according to the plant species (Brown et al. 2002). It is reported that micronutrients which showed less mobility in phloem transport systems accumulate in high transpiring or xylem containing tissues which ultimately lead to the deficiency or lower concentration of those nutrients in edible plants parts, i.e., seed or tuber (White et al. 2009; White and Broadley 2009). The remobilization of micronutrients which are stored in different vegetative plant tissues to the seed is unclear up till now. Crop plants are considered as basic source of these nutrients for animals and humans (Alloway 2008). The concentration of most of the micronutrients in soil is seldomly increased and mostly remained constant during past 60 years but the concentration in plant edible parts is reduced continuously (Fan et al. 2008). In a work conducted by Granett and Graham it was reported that a significant drop in the remobilization of Cu and Fe (40–62 and 77%, respectively) to the seed has been recorded over the period of time (Garnett and Graham 2005). Zinc remobilization from leaves to the grains is significant in barley (Hegelund et al. 2012) and wheat (Kutman et al. 2012). It was reported that mobilization of Fe from leaves to grains in wheat was higher (80%) as compared to Zn (50%) (Kutman et al. 2011). It was also reported that rice genotypes which were Zn sufficient have greater ability to mobilize Zn from vegetative parts to reproductive parts as compared to the genotypes which are sensitive to Zn deficiency (Impa and Johnson-Beebout 2012). Pottier et al. (2014) reported that process of autophagy also plays an important role in micronutrient remobilization.

2.6 Strategies to Improve Bioavailability and Biofortification of Micronutrients

Micronutrients are the mineral elements required in much smaller quantity for plants. Soils and crops often lack in these elements (Dimkpa and Bindraban 2016). Micronutrient deficiency not only leads to the disturbance of plant physiological and biochemical processes causing stunted growth, reduced grain and biological yield, as well as poor nutritional food quality which ultimately leads to the malnutrition or hidden hunger in animals and humans (de Valença and Bake 2016). It's not only a concern related to crop growth and production but also related to the human health and nutrition.

Although required in much smaller quantities, these are essential for plant health nourishment, and plants are unable to complete their life cycle and development without their proper avalibility. Micronutrient status changes to a greater extent with different nutrient management practices (Moharana et al. 2017). Micronutrient deficiency extent in plants can be divided in four stages, i.e.:

1) Stored body micronutrients depletion and reduction in enzymes and carriers saturation.
2) Impairment of biochemical functions dependent on micronutrients.
3) Changes in cellular as well as physiological processes of plants.
4) Structural and functional lesions appearance, i.e., deficiency symptoms.

The first three stages are termed as hidden hunger and can only be diagnosed by tissue analysis causing impairment of plant physiological and biochemical processes, but the fourth stage is characterized by visual clinical deficiency symptoms causing significant yield loss (Shukla et al. 2018). Deficiency of micronutrients in soils and plants leads to the deficiency of specific minerals in humans.

Soils are source of most of the food of world. Healthy soil is linked to good human health because of the good quality and nutritious food produced by it (Shukla et al. 2018). Healthy soils having optimum supply of nutrient provide nutritious food which provides humans and animals with complete nutrition. Bioavailability as well as phytoavailability of micronutrients and biofortification of food can improve dietary intake of micronutrients. Bioavailability of the micronutrients refers to availability of micronutrients for normal functioning, reproduction, physiological and biochemical processes of human body, and phytoavailability in the same way for plants' growth and development. Biofortification of micronutrients refers to enrichment of seed contents or edible portion content of the plants with specific micronutrients for dietary intake of human being to accomplish the hidden hunger or malnutrition. Phytoavailability of micronutrients from soil, rhizosphere, or other source is dependent on various factors and can be increased by various strategies. In the same way, bioavailability and biofortification of micronutrients can be increased or improved with the following strategies for human dietary intake.

2.6.1 Genetic Manipulation

Genetic manipulation of crops, i.e., plant breeding and genetic engineering is a known practice for increasing the bioavailability of nutrients in staple food by improvement of micronutrients in edible portion of crops, lowering the concentration of antinutrients compounds, and increasing the compounds which are responsible for increasing bioavailability of these nutrients (Bouis 2003). Improvement in nutrient uptake by plant roots from soil, transport of these nutrients from root to shoot and aerial parts of plants, improved mineral sequestration to endodermis, and decrease in antinutrients concentration are main factors associated with biofortification and increased bioavailability of micronutrients in food crops. Genetic biofortification is a technique which can cover all these aspects. Conventionally fortified crops contribute significantly for reduction of micronutrient malnutrition (Finkelstein et al. 2015). Development of new genotypes and varieties which are efficient in achieving the different aspects of biofortification is the main factor of genetic biofortification of crops. These genotypes may increase the bioavailability of micronutrients to animals and humans as well as increase the crop productivity by efficient utilization of fertilizers and resources.

2.6.2 Agronomic Biofortification

Use of any other means for increasing the concentration of mineral nutrients other than genetic biofortification in edible portion of crops is termed as agronomic biofortification. Other means for improving micronutrients concentration and bioavailability in crops are pre- to post-harvest cultural operations, i.e., good agricultural practices, use of fertilizers for target nutrient fortification, improved soil fertility status for nutrient availability in soil, and enhanced uptake by roots and adaptation of precision agricultural techniques (Wakeel et al. 2018). These agronomic interventions not only increase the mineral concentration and bioavailability in crops but also increase the crop yields hence increasing the economic benefit (De Valença et al. 2017). Fertilization of target nutrient is the most common and effective method for biofortification of mineral nutrients such as zinc, iron, iodine, etc. in staple crops worldwide. It is an easy and cost-effective approach for amelioration of mineral nutrients malnutrition through dietary intake of staple food.

2.6.3 Good Agricultural Practices

Good agricultural practices (GAPs) is a set of principles, techniques, and methodologies from pre- to post-harvest of any food crop maintaining social, environmental, and economic sustainability to get safe and healthy food (FAO/WHO 2003). Use of fertilizers especially micronutrient fertilizers in excess can cause toxicity to plants. Agricultural sustainability is the basis of GAPs. Soil fertility management for maximum bioavailability of micronutrients through integrated nutrients management, site-specific application of nutrients, slow release fertilizers, and application of organic matter has more environmental, social, and economic sustainability than blind and conventional methods of fertilizers application. It can reduce volatilization loses and leaching loses of nutrients in the environment to reduce environmental deterioration. Judicious use of fertilizers can reduce cost of food production resulting uplift of social status of farmers. Improvement in social status and economic uplift of farmer community can encourage the use of fertilizers containing micronutrients and ultimately enrichment of micronutrients in plant-based foods (FAO/WHO 2003).

2.6.4 Nutrients Synergism

Crop yields are dependent on several factors, and nutrient interaction is definitely one of the most important factors. Nutrient interaction refers to the phenomenon when supply of one nutrient changes the absorption and utilization of another nutrient. It only occurs and affects plant growth and development when supply of a specific nutrient is too low as compared to applied ones (Fageria and Oliveira 2014). Although antagonistic affect is reported in some macronutrients with some of the micronutrients at specific pH and specific soil conditions, in case of agricultural crops, interaction between macronutrients and micronutrients is synergistic most of the time which means application of some specific macronutrients can enhance uptake and utilization of some micronutrients (Rietra et al. 2017). Application of primary and secondary nutrients, i.e., NPK and Ca can significantly increase the plant growth and development, and some of these nutrients are also associated with augmented uptake of micronutrients which are in lesser quantity in soil and without them can undergo severe deficiency system leading to the reduced food quality and yield of crops. Reduced food quality or lack of these mineral micronutrients in staple food crops are the main reasons for malnutrition and micronutrient deficiency in humans and animals. Balanced application of fertilizers keeping in view the synergistic and antagonistic effects of nutrients can reduce the use of excess fertilizers doses as well as cost of crop production leading to the highly nutritious food production without wasting the natural resources and deteriorating environment.

2.6.5 Precision Agriculture

A set of advanced methods and techniques involving the use of information technology for crop production and farm management to ensure precise application of inputs, i.e., water, nutrients, seeds, etc., is termed as precision agriculture. It involves the sustainable development of food production system with wiser use of inputs and lesser damage to natural resources and environment (Carolan 2017). Integrated soil fertility management (ISFM) or integrated nutrients management (INM) is one of the practices of precision agriculture with the advantages of improved soil health and plant nourishment. Agricultural systems with INM have less weeds and improved nutrient use efficiency leading to the production of quality food (Singh and Prasad 2016). Site-specific input application in precision agriculture is proved to be resource saving and enhance efficiency of inputs (Chivenge and Sharma 2019). Nutrients mapping of soil with the help of decision support systems and models can reduce the application of excessive fertilizers and site-specific application of required nutrients which leads to the efficient land and resource management for quality food production and elimination of malnutrition (Dong et al. 2018). Efficient management of nutrients and site-specific application increase the uptake of all the nutrients in turn providing balanced nutrition to plants produce good quality food.

2.7 Phytohormones: Domain, Principality, and Functionality in Plants

2.7.1 Phytohormones

The population of the world is being increased very rapidly, and according to a report of the united nations (UN), it will cross the figure of 7.8 billion in the starting few months of 2020 (Worldometer). Today’s food security is a controversial issue of the world, but the solution of this problem is only possible by increasing the production of food especially by increasing the agricultural yield per acre. The different types of stresses affect crop production and reduce total production. So, it is necessary to increase the productivity of agriculture up to 70% to feed more population expected in year 2050 (Wani and Sah 2014; Tilman et al. 2011). The mechanism against abiotic stresses in the plants is different from animals (Qin et al. 2011). The different plant species behave differently against abiotic stress, but the selection of any tolerant variety against these stresses is based on phytohormones. This eliminates the negative effects of abiotic stress such as drought, salinity, and some other climatic factors. The different abiotic stresses including salinity, drought, temperature, and some other environmental factors cause a negative impact on plant growth and reduce the yield under stress conditions (Ayub et al. 2020). The response of plants against different abiotic stress is different because it depends upon the period and types of stress, plant growth stages, and correlation with other biotic and abiotic factors (Feller and Vaseva 2014). The different crops have different resistance level against stresses at different stages. Mostly, crops were affected at an early stage of growth although they also depend upon genotype and variety of crops. The salinity and drought stresses are major stress conditions around the globe which affect the osmotic potential, photosynthetic rate, and cell membrane of the plant cell (Sanghera et al. 2011; Pathak et al. 2014; Ayub et al. 2020; Farooqi et al. 2020).

The phytohormones regulate the different metabolic activities in plants which are very essential for the growth and development of plants. They also act as a messenger and transfer cellular activities in plants (Voß et al. 2014). They also play a vital role in the signal transduction pathway and regulates the different metabolic processes under stress conditions (Kazan 2015).

2.8 Classification and Functions of Phytohormones in Plants

2.8.1 Abscisic Acid (ABA)

The abscisic acid (ABA) is involved in many metabolic processes and enhances the development of plant at different growth stages. It is involved in the dormancy of seed, embryo morphogenesis, and control the opening and closing of stomata (Wilkinson et al. 2012). It also plays a role in the synthesis of lipids and proteins (Sreenivasulu et al. 2010). It plays an important role in plants under stress conditions. Under stress conditions, the ABA increases rapidly and reduce the negative effect of stress. It also modifies gene expression and activates the signaling pathway (O’Brien and Benková 2013). The ABA improves the root growth under drought stress and deficiency of nitrogen (Giuliani et al. 2005; Zhang et al. 2007). It synthesizes some protective proteins and maintains the turgor pressure under abiotic stress conditions (Sreenivasulu et al. 2012).

2.8.2 Auxins (IAA)

The auxins (IAA) are important phytohormones which are involved in plant development at different growth stages under normal as well as stress conditions (Kazan 2013). The presence of auxins significantly enhances plant growth under the diverse impact of abiotic stresses (De Smet et al. 2011). It also improves the growth of root and shoot under salinity and heavy metal stress conditions (Iqbal et al. 2014; Sheng and Xia 2006). Fahad and Bano (2012) reported that the IAA correlates with salicylic acid as salinity decreases the quantity of IAA in maize, but the application of salicylic acid enhances the level of IAA. It stimulates many genes known as primary auxin response genes. It also stimulates the release of ethylene and controlled cell elongation (Fahad et al. 2015a, b).

2.8.3 Cytokinins (CKs)

The cytokinins (CKs) effectively enhance the different metabolic processes and increase plant growth. It plays a role in the mitigation of abiotic stresses such as drought and salinity (Nishiyama et al. 2011; Zalabák et al. 2013). The external application of CKs under stress conditions increases the level of cytokinins in plants and improves plant growth. It has an antagonistic mechanism with ABA; under drought condition, the concentration level of CKs is decreased and level of ABA is increased, as a result of which ABA/CKs ratio is increased. The decreased level of cytokinins improves the apical dormancy which helps the ABA to close the stomata and give compensation to the plant under stress. The CKs also release the seed from dormancy, while ABA is involved in inhibition of seed germination (Pospíšilová 2003).

2.8.4 Ethylene (ET)

Ethylene (ET) has an important role in the ripening of fruit, senescence, and abscission process of the plant. It also plays a role in alleviating the harsh effect of abiotic stress and boosting plant growth (Gamalero and Glick 2012; Groen and Whiteman 2014). The different abiotic stress such as salt stress and some climatic factors decreases the endogenous level of ethylene in plants (Shi et al. 2012). The ET integrates with other phytohormones which enhance the signaling pathway and boost the defense mechanism of the plant. The ET has a synergistic and antagonistic correlation with ABA, which help in controlling the growth and development of plants (Yin et al. 2015).

2.8.5 Gibberellins (GAs)

The gibberellins (GAs) are involved in the germination of the seed, leaf expansion, and growth of plant stem and flowers. It has a vital role in improving the quality of flowers and fruits (Yamaguchi 2008). It is an essential phytohormone because it takes part in many metabolic processes of plants from seed to its harvesting stage. It also decreases the harmful effect of abiotic stress and increases the adaptation process (Colebrook et al. 2014). The gibberellins showed significant results in the experiments conducted under osmotic stress conditions (Skirycz et al. 2011; Claeys et al. 2012). It also has a positive or negative interaction with ethylene and some other phytohormones in the different processes involved in plant growth (Munteanu et al. 2014).

2.8.6 Brassinosteroids (BRs)

The brassinosteroids (BRs) were first identified in the rape plant and has great potential to improve plant growth and development (Vardhini et al. 2006). It has contribution in the development of root and shoot as well as takes part in the initiation of the floral part and promote the quality of fruits (Bajguz and Hayat 2009). The different experimental results showed that the BRs mitigate the negative effect of abiotic stresses. It also enhances the antioxidant defense mechanism thus improves plant growth. It increases the tolerance level in plant against abiotic stresses such as salinity, temperature, drought, light, heavy metal, waterlogging, and organic pollutants (Janeczko et al. 2011; Wang et al. 2014a, b; Abbas et al. 2013; Kurepin et al. 2012; Vardhini and Anjum 2015).

2.8.7 Jasmonates (JAs)

The jasmonates (JAs) have a significant impact on flowering, reproductive process, and senescence (Fahad et al. 2015a, b). It enhances the defense mechanism of plants against pathogen and environmental stresses. The different experiments showed that the exogenous application of JAs remarkably improved the plant defense mechanism and enhanced the plant growth (Seo et al. 2011; Du et al. 2013). The JAs have tremendous potential against abiotic stresses. The result of the experiments indicated that the exogenous application of JAs enhances the crop yield and mitigate the salinity stress. Some other experiments manifested that the application of JAs increased the endogenous level of JAs under stress condition which diminished the injurious effect of salinity and heavy metals on rice crop (Dar et al. 2015).

2.8.8 Salicylic Acid (SA)

Salicylic acid (SA) plays an essential role in fruit ripening and plant growth under abiotic stress (Rivas-San Vicente and Plasencia 2011). The high concentration of SA causes cell death and reduces plant growth although the low level increases the defense system of plants under stress conditions (Jumali et al. 2011). It induces plant growth and significantly reduces the negative effects of abiotic stresses particularly drought, temperature, and salinity (Yang et al. 2012).

2.8.9 Strigolactones (SL)

The strigolactones (SLs) are growth promoter and stimulate seed germination (Ruyter-Spira et al. 2013). They are present in a different form in single plant specie and their quantity also changes from one variety to another one, but they are produced primarily in small amounts in roots as compared to other parts of the plant (Yoneyama et al. 2013; Koltai et al. 2013). The SLs take part in the adaptability of plant against environmental conditions and in higher plants; it participates in root and shoot architecture (Kapulnik et al. 2011). It improved the plant interaction with microbes by acting as a signaling molecule, and as a result of this, it increases the nodulation process in leguminous crops (Foo et al. 2013). It has great potential to enhance the adaptability of plants against adverse environmental conditions (Vurro and Yoneyama 2012).

2.9 Micronutrients and Phytohormones Homeostasis and Crosstalk

Plants need nutrients throughout their life in order to survive and to produce food. Plants need both macro- and micronutrients for proper growth and metabolism. Macronutrients, i.e., N, P, K, Ca, Mg, and S are required in concentration > 1–150 g per kg of total plant's dry weight. However, micronutrients Cu, Zn, Co, Fe, Mo, B, Cl, and Mn are required by plants in minor concentrations usually 0.1–100 mg per kg of total plant's dry weight (Pandey 2018). Micronutrients are as vital as macronutrients are, since they work as cofactor for hormonal biosynthesis (Zn), as enzyme activator (Mn), as catalyst in nitrogen fixation (MO), as sugar translocator (B), in breakdown of urea (Ni), and for nitrogen metabolism (Cu) and provide resistance from heat and drought. However, there is a very minute difference in sufficiency and toxicity ranges of micronutrients, for example, toxicity range for B is 50 to 200 ppm and sufficiency range is 10–200 ppm (Noreen et al. 2018). Hence, often the term "heavy metal toxicity" is used to reveal their excessive levels in plant's growing environment.

Plant existence and survival remain at stake throughout their life cycle. As the plants have to withstand several kinds of abiotic stresses including drought, waterlogging, salt stress, nutrient deficiency, elevated temperatures, chilling injuries, and heavy metal toxicity, leading to substantial downfall in plant's growth, development and quality (Hasanuzzaman et al. 2017a, b, 2018). In order to tackle the fluctuating external environment, plants have developed various mechanisms to protect themselves from the external shocks. There are several mechanisms that plants follow, for instance, in case of salt stress, excessive salts accumulate in the rhizosphere and plants respond by absorbing more osmolytes like proline and glycine betaine to maintain their osmotic balance and avoid osmotic shock (Hussain et al. 2018). Moreover, both micronutrients and phytohormones have synergistic and antagonistic roles in plants. For instance, both micronutrients and phytohormones (auxin, gibberellic acid, and ABA) work synergistically for protecting plants from the abiotic stresses and maintaining plant's homeostasis (Fahad et al. 2015a, b). In addition, when micronutrients are in excess, phytohormones serve as remediator to shield plant from heavy metal toxicity (Barbafieri 2016).

2.9.1 Role of Micronutrients in Hormonal Biosynthesis

Several micronutrients are involved in hormonal biosynthesis either directly or indirectly. For example, an iron enzyme 1-aminocyclopropane-1-carboxylic acid oxidase is involved in the biosynthesis of ethylene hormone. The Fe^{3+}/Fe^{2+} redox potential bestows iron with a unique dual behavior. On one hand, it acts as prooxidant through generating toxic radicals like OH, but on the other hand, it is constituent of several antioxidant enzymes like SOD, CAT, and POD (Chapple 1998; Becana et al. 1998; Pandey 2018). Moreover, among micronutrients Cu has a distinguished role of protecting plants from biotic and abiotic stresses. Copper as Cu-Zn cofactor in superoxide dismutase catalyzes the detoxification reaction of superoxide ions (O_2^-). Hence, it plays an imperative role in antioxidant defense system. Moreover, copper is eminent for its role in ethylene enzyme biosynthesis and as a constituent in ethylene receptor. Copper has structural role in molybdenum cofactor synthesis which in turn yields ABA and IAA (Peñarrubia et al. 2015; Kuper et al. 2004).

Moreover, as a cofactor Zn plays an important role in the structure and functioning of more than 300 enzymes. Hence, Zn has a vital role in various plant processes including protein synthesis, plant photosynthesis, and phytohormones regulation (Andreini and Bertini 2012). Further Zn has a direct role in the biosynthesis of growth hormones like IAA and gibberellin (GA3). For instance, recently a study revealed that spraying $ZnSO_4$ on apple trees resulted in a significant increase in the sucrose phosphate synthase (SPS) activity, zinc contents indoleacetic acid (IAA), gibberellin (GA3), and ABA contents in fruits. Enhanced Zn contents triggered higher activity of enzymes involved in carbohydrates metabolism leading to higher indoleacetic acid, gibberellin, and abscisic acid contents in plants (Zhang et al. 2016).

2.9.2 Role of Micronutrients in Alleviating Abiotic Stress

Mineral elements aid plants in their defense against pathogens, pests, and several types of stresses that plants have to endure throughout their life cycle. They play a role directly by generating metabolites like glucosinolates, lignin, phenols, and phytoalexins that help plants to survive under unfavorable conditions and indirectly by modifying root exudates and rhizosphere pH (Cabot et al. 2019; Latef et al. 2017). For instance, ZnO nanoparticles unveiled substantial potential to reverse the oxidative stress effects caused by heavy metal stress by elevating photosynthetic pigment, soluble protein contents, antioxidants enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) while decreasing malondialdehyde contents in leaf tissues of *L. leucocephala* seedlings (Venkatachalam et al. 2017). Besides that, silicon, although believed as non-essential nutrient, is an important micronutrient especially under stress condition. A bunch of studies have reported recently about its role in enhancing growth, crop quality with its special role against infectious diseases, heavy metal toxicity, elevated temperature, salinity, and drought stress (Coskun et al. 2016). Further exogenous application of silicon can ameliorate and recover the damage caused by reactive oxygen species (ROS) which are responsible for severe damage to cells, organelles, and their structure and function. Silicon works by regulating the production of ROS and malondialdehyde (Kim et al. 2017; Pascual et al. 2016; Etesami and Jeong 2018; Helaly et al. 2017). Apart from the above mentioned, foliar application of Fe and Mn displayed significant positive results on the grain yield when applied on grain filling stage under water stress condition (Babaeian et al. 2011). Proper application of boron under abiotic stress conditions helpful in enhancing the production of grain and transportation of the sugars within the plants (Waraich et al. 2011). Under abiotic stress conditions the B also improve the number of flower and fruits by protecting the loss of fruiting bodies and also prevents leaves chlorosis (Waraich et al. 2011). Plant growth also improved under abiotic stress conditions by the application of copper (Cu) because under abiotic stress conditions, Cu is one of the essential nutrients which enhanced the metabolism of N and CHO in the plant which are responsible for increasing the growth and development of plants under abiotic stress conditions (Waraich et al. 2011).

2.9.3 Interaction of Micronutrients and Phytohormones

Plants parade a variety of physiological and metabolic behaviors in order to adopt and survive in the continuously varying environments, one among those is the changes in the nutritional status of the rhizosphere (Bücker-Neto et al. 2017). Plants have developed certain sensing and signaling mechanism to monitor the changes in the internal and external nutritional status in order to survive under stress situations. Not only nutrients, hormones are also involved in the control of these regulatory mechanisms. Both nutrients and hormones work either independently or in corelation with each other, or sometimes they have been reported for being induced by increase and decrease in the concentration of one or the other (Rubio et al. 2009). For instance, increase in the concentration of abscisic acid was monitored in the presence of high concentrations of Cu^{2+} and Zn^{2+} suggesting its role in plant defense mechanism (Wang et al. 2014a, b). Likewise, auxin distribution was reported to be affected by boron deficiency resulting inhibition in the root elongation. This inhibition in the root elongation could be a result of crosstalk between auxin and ethylene because ethylene participate in the auxin biosynthesis and its transport toward the elongation zone

(Růžička et al. 2007; Eggert and von Wirén 2017). Further iron has been reported to be involved in the activation of brassinosteroids signaling by accelerating root growth of *Arabidopsis thaliana* (Singh et al. 2018a, b). The application of brassinosteroids was helpful to improve the antioxidant enzymatic activities of the plants suffering from Zn stress (Ramakrishna and Rao 2013). Wild et al. (2016) reported that the Fe has beneficial interaction with the GA and the deficiency of Fe leads to the inhibition of the synthesis of GA in their primary root due to poor availability of Fe in the plants. The auxin and ethylene also play an important role under Fe deficiency. Under such conditions, the growth and development of root hairs improve by the synthesis of auxin and ethylene and fulfill the requirements of Fe in the plants (Romera and Alcántara 2004). Similarly, plant hormones such as auxin and ethylene also regulate the effect of Fe deficiency on the plant physiology system by increasing the ferric reductase activity, and acidification (Curie and Briat 2003).

2.10 Role of Various Micronutrients in Uplifting Phytohormones in Plants Under Stress Conditions

2.10.1 Role of Hormones in Assuaging Heavy Metal Stress

Some of the heavy metals especially Cu and Zn are considered as essential micronutrients especially at lower concentration and have considerably important role in plant's metabolism. However, the same heavy metals at elevated concentrations are hazardous for plants and pose negative effects on plant's biomass, physiology, biochemistry, and metabolic processes, i.e., photosynthesis (Mathur et al. 2016; Ebbs et al. 2016; Ivanov et al. 2016). Phytohormones have exhibited substantial potential to mitigate heavy metal stress. For instance, exogenous application of tryptophan (auxin precursor) enhanced the growth and yield of rice grown under cadmium-treated soils, suggesting its role in improving plant's growth under Cd stress (Farooq et al. 2015). Similarly, another study revealed the role of abscisic acid in mitigating Cd stress in *Populus euphratica* by accelerating the activity of antioxidant enzyme (catalase, superoxide dismutase, and ascorbate peroxidase) which played a role in scavenging Cd^{2+} induced H_2O_2, also by limiting the uptake of Cd^{2+} (Han et al. 2016). Supplication of abscisic acid to the grapevine seedlings significantly reduced the uptake and accumulation of zinc by inducing detoxification-related genes and thus assisting in stress alleviation (Song et al. 2019). Further, apart from auxin and abscisic acid, brassinosteroids also revealed substantial potential to improve the activity of antioxidant enzyme against boron-induced oxidative stress in *Vigna radiata* plants (Yusuf et al. 2011). Likewise, in 2016 a study exhibited that application of 24-epibrassinolide to the chromium-stressed rice seedlings resulted in strengthening rice defense system by upregulating the activity of antioxidative enzymes and by restricting the uptake of chromium (Sharma et al. 2016). For comprehensive study about phytohormones and their potential to alleviate heavy metal stress, refer to Table 2.1. Schematic mechanism of phytohormone activity on HM stress management is given in Fig. 2.1.

Table 2.1 Phytohormone and their ability to ameliorate heavy metal stress (last 10 years)

Plant growth regulator	Metal	Effect	Crop	References
Auxin	Cd	Enhanced paddy yield, height, number of tillers, panicles, and 1000 grain weight	Rice	Farooq et al. (2015)
Auxin + Salicylic acid	Cd	Enhanced antioxidant defense system and improved leaf anatomy	Wheat (*Triticum* sp.)	Agami and Mohamed (2013)
Auxin	Pb	Total Pb extraction was improved by applying in combination with PGR and soil fungicide	Switchgrass (*Panicum virgatum L.*)	Aderholt et al. (2017)
Auxin	Cd	Increase in hemicellulose 1 contents and fixation of Cd^{2+} and eventually reduced translocation from root to shoot	Arabidopsis (*Arabidopsis thaliana L.*)	Zhu et al. (2013)
Auxin	Cd	Reduced Cd accumulation resulted in increased photosynthetic activity and antioxidant potential	Fenugreek (*Trigonella foenum-graecum L.*)	Bashri and Prasad (2015)
Auxins + cytokinins	Pb	Enhanced redox status of algal cells, contents of antioxidants, and activity of antioxidant enzymes	Green alga (*Acutodesmus obliquus*)	Piotrowska-Niczyporuk et al. (2018)
Abscisic acid	Cd	Enhanced activity of antioxidant enzyme and reduced Cd uptake	*Populus euphratica*	Han et al. (2016)
Abscisic acid	Cd	ABA inhibited the growth of the plant; however its higher concentration (40µmol L^{-1}) was helpful to extract Cd	*Bidens pilosa*	Liu et al. (2017)
Abscisic acid	Zn	Reduced uptake and accumulation of Zn and more expression of detoxification-related genes in the roots	Grapevine (*Vitis vinifera L.*)	Song et al. (2019)
Abscisic acid	Cd	Reduction in the levels of reactive oxygen species (ROS) by increased activity of antioxidant enzyme system	Purple flowering stalk (*Brassica campestris L. ssp. chinensis var. purpurea Hort.*)	Shen et al. (2017)
Abscisic acid	Cd	Augmented Biomass, chlorophyll contents, as well as Cd extraction	*Solanum photeinocarpum*	Wang et al. (2016)

(continued)

Table 2.1 (continued)

Plant growth regulator	Metal	Effect	Crop	References
Abscisic acid	Pb	Stimulation in Pb uptake and its vascular loading as well as alleviation in ROS production	*Populus × canescens*	Shi et al. (2019)
Abscisic acid	Cd	Upregulated antioxidant enzyme activity and reduction in Cd buildup	*Populus euphratica Cells*	Han et al. (2016)
Brassinosteroids	B	Elevated antioxidant activity and proline contents	*Vigna radiata*	Yusuf et al. (2011)
Brassinosteroids	Cr	Reduction in Cr accumulation, improved growth, increased antioxidant enzyme activity	*Oryza sativa L.*	Sharma et al. (2016)
Brassinosteroids	Cd	Elevated levels of antioxidant enzymes and proline contents	Bean (*Phaseolus vulgaris L.*)	Rady (2011)
Brassinosteroids	Cr	Significant increase in IAA, abscisic acid concentration, phenols, and soluble sugars	Radish (*Raphanus raphanistrum* subsp. *sativus*)	Choudhary et al. (2011)
Brassinosteroids	Cd	Reduced Cd accumulation and enhancement in the uptake of other micronutrients	*Pisum sativum L.*	Jan et al. (2018)
Brassinosteroids + salicylic acid	Pb	Improved root, shoot length, elevated chlorophyll, carotenoid, glutathione, ascorbic acid, and tocopherol contents	Mustard (*Brassica juncea L.*)	Kohli et al. (2018a, b, c)
Brassinosteroids + salicylic acid	Pb	Improved growth, phenolic, and pigment contents	Mustard (*Brassica juncea L.*)	Kohli et al. (2017)
Brassinosteroids	Cu	Reduced Cu uptake and enhancement in antioxidant defense system	Mustard (*Brassica juncea L.*)	Yadav et al. (2018)
Brassinosteroids	Cd	Strengthening of antioxidant defense system and enhanced stimulation of flavonoids, anthocyanins, and proline	Mustard (*Brassica juncea L.*)	Kaur et al. (2017)

(continued)

Table 2.1 (continued)

Plant growth regulator	Metal	Effect	Crop	References
Cytokinins	Ni	Significant increase in plant biomass and transpiration rate	*Alyssum murale* Waldst. & Kit	Cassina et al. (2011)
Cytokinins	Cd, Cu, Pb	Improvement in algal adaptation ability and response to abiotic stressor	*Chlorella vulgaris L.*	Piotrowska-Niczyporuk et al. (2012)
Cytokinins	Cd	Alleviation in the negative effects caused by Cd on fresh mass and decrease in the ROS levels was observed	Tomato	Singh et al. (2018a, b)
Cytokinins	Cd, Zn	Improvement in plant growth, stomatal conductance, net photosynthesis, total ascorbate and a decrease in oxidative stress was recorded	*Kosteletzkya pentacarpos*	Zhou et al. (2019)
Salicylic acid	Cd	Improved growth, chlorophyll contents, photosynthesis, mineral nutrition, and decrease in oxidative stress	Peanut (*Arachis hypogaea*)	Xu et al. (2015)
Salicylic acid	Zn, Cu, Mn, Cd, Hg and Pb	Enhancement in the activity of catalase and superoxide dismutase enzymes	Malting Barley (*Hordeum uhulgare L.*)	Song et al. (2014)
Salicylic acid + nitric oxide	Cd	Reduced root to shoot translocation of Cd and increased activities of antioxidant enzymes	Perennial ryegrass	Wang et al. (2013)
Salicylic acid	Pb	Enhancement of heavy metal tolerance index, water contents, relative water contents and lipid-soluble antioxidants	Mustard (*Brassica juncea L.*)	Kohli et al. (2018a)
Salicylic acid	Pb	Increased dry matter content, heavy metal tolerance index and activities of guaiacol peroxidase, catalase, glutathione reductase and glutathione-s-transferase	Mustard (*Brassica juncea L.*)	Kohli et al. (2018b)

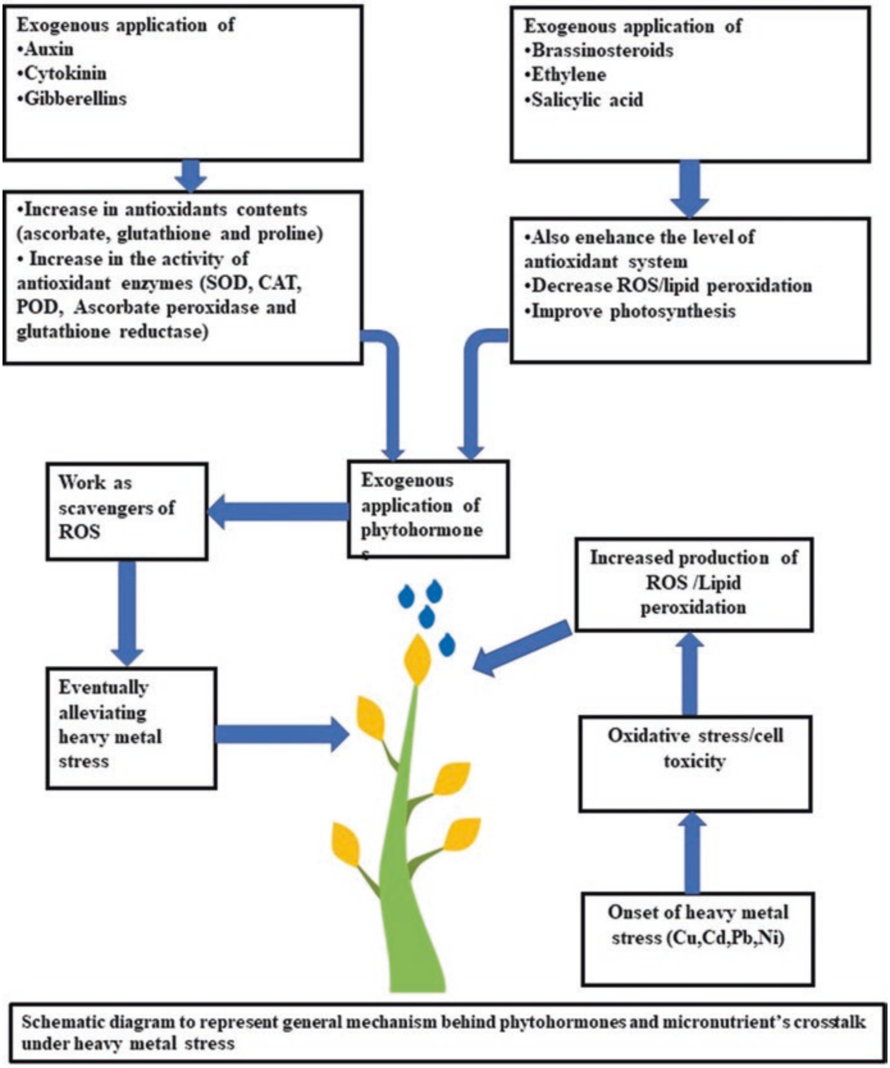

Fig. 2.1 Schematic diagram of mechanism behind effect of exogenous applied phytohormones in HMs stress management

2.11 Summary and Future Prospects

Agriculture sector has an economical importance for many countries, but different abiotic stresses are the enormous challenges for this sector. The different phytohormones are produced in response to stress conditions which reduce the harmful effects of these stresses. The micronutrients play a vital role in plant growth and development and alleviate the abiotic stress conditions by the production of phytohormones which play an important role as a plant defense system. These phytohormones manage the production of ROS in plant cells and controlling the plant growth under stress conditions. In this chapter, we overviewed the role of micronutrients in production of phytohormones and presented crosstalks between micronutrients and different hormones such as abscisic acid (ABA), auxins (IAA), cytokinins (CKs), ethylene (ET), gibberellins (GAs), brassinosteroids (BRs), jasmonates (JAs), salicylic acid (SA), and strigolactones (SL). These hormones are involved directly or indirectly in plant growth under stress conditions and their production in plant is in direct control of micronutrients. Exogenious application of phytohormones and/or micronutrients is an intresting approach to check their cross talk in effective management of abiotic stresses in plants and must be studied in future.

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Chapter 3

An Insight into the Role of Plant Growth Regulators in Stimulating Abiotic Stress Tolerance in Some Medicinally Important Plants

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3.1 Introduction

The most devastating threat to the environment, now gaining considerable attention from researchers, farmers, and policy makers, is the global climate change, because of its major influence on agriculture (Hasanuzzaman et al. 2013). Abiotic stresses such as drought, chilling, salinity, extreme temperatures, flooding, and toxicity of heavy metals and metalloids frequently reduce crop productivity and limit plant growth (Lata et al. 2018). More than 10% of arable land is affected by drought and salinity resulting in 50% decrease in the yield of crops worldwide. Several stresses can affect plants simultaneously, and tolerance to these stresses is a complicated process as stress can affect multiple stages of plant development (Lata et al. 2011).

Heavy metals (HMs) are considered as environmental pollutants and potentially hazardous to plants, human, and animals. It has been reported that HMs adversely affected plant growth and development; however in little amount some metals are essential for the growth of plants and play as key component of many vital compounds (Azevedo and Azevedo 2006). The specific responses of plants found under HMs stress decreased chlorophyll content and stomatal density (Da Silva et al. 2012). Decrease in chlorophyll content directly affects photosynthetic machinery resulting in disturbed carbohydrate metabolism and initiates the secondary stresses such as oxidative stress and nutrition stress, which leads to reduced plant growth and productivity (Yang and Chu 2011). Soil salinity is a major restraint to agriculture because it reduces plant growth and development adversely. About half of the world's irrigated land and 20% of the world's cultivated area are affected by salinity.

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High salt concentration adversely affects the various processes in plants such as flowering and fruit set, seedling growth and vigor, and seed germination that ultimately decrease the yield and quality of the plant (Sairam and Tyagi 2004).

Drought is commonly defined as a period without significant rainfall. Scarcity of water in soils and continuous loss of water through transpiration and evaporation causes drought stress (Jaleel et al. 2009). Drought stress has found to decrease photosynthetic carbon reduction cycle enzymes, mainly ribulose 1,5-bisphosphate oxygenase/carboxylase, and also reduce CO_2 assimilation due to decreased stomatal conductance (Reddy et al. 2004). It also decreases stem and root growth, reduces water use efficiency, and disturbs plant water relation (Anjum et al. 2011).

Soil waterlogging and submergence, collectively called as flooding, is a complex stress that directly affects species composition and productivity in several plant communities (Jackson and Colmer 2005). It has been found that a large number of crops have been affected by prolonged seasonal rainfall in tropical and subtropical regions (Liao and Lin 2001). Soil inundation causes disruption in many physiological processes that limits plant growth and survival. Flooding causes reduction in water and nutrient uptake and lower the metabolism (Dat et al. 2004).

It is predicted that temperature will rise to 2–3 °C by the end of the twenty-first century, which is a major threat to crop plants (Shah et al. 2011). Temperature is found to be the primary factor affecting the rate of plant growth. In all species, pollination is the most sensitive phenological stage to high temperature, and production would be greatly affected during the developmental stage (Hatfield and Prueger 2015). Extreme temperatures usually interrupt photosynthesis and limit the supply of carbohydrate resulting in abortion of kernels during reproductive phase, and starch synthesis in endosperm of kernels is diminished by reduction in the activity of several enzymes resulting in chalky and opaque kernels (Shah and Paulsen 2003).

Since ancient times, aromatic plants have been used in many ways such as in perfumery and drugs. Aromatic plants are usually characterized by pleasant fragrant smell that is carried by the essential oil fraction. It has long been known that aromatic plants have medicinal properties as they contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids, and other chemical compounds. In the recent years, scientist investigated the medicinal properties of these plants enormously due to their potent antioxidant activity (Okigbo et al. 2009; Ekren et al. 2013). It has been reported by the World Health Organization that around 35,000 to 70,000 aromatic plant species have been used in medication which is a total of 14–28% of 250,000 plant species occurring worldwide (Padulosi et al. 2002). Aromatic plants that bear essential oils (EOs) are known to be used in various human activities (odoriferous and volatile oily liquids), from religious ceremonies to embellishments and to herbal remedies and personal use, even before the recorded history of mankind (Sangwan et al. 2001). The EOs have many biological properties such as antibacterial, anti-fungal, and antioxidants (Złotek et al. 2016). These oils have been used in medical microbiology, phytopathology, and food preservation as naturally occurring antimicrobial agents (Pandey et al. 2010).

Plant growth regulators (PGRs) often regarded as “phytohormones” are known to act as signal molecule, produced within the plants. They are derived from plant biosynthetic pathways which usually mediate growth and developmental processes that either act locally (at the site of synthesis) or are transported to the other site in plant body under climatic or stressful conditions. Several phytohormones like auxins (IAA), abscisic acid (ABA), cytokinins (CKs), gibberellins (GA), ethylene, and brassinosteroids regulate growth and development in a coordinated manner and control many physiological and bio-chemical processes. However, in recent years, some other compounds like nitric oxide, polyamines, and strigolactone have also been included to this list (Fahad et al. 2015). However, a lot of work has been done and widely studied on the morphology and physiology of agricultural crop plants under different abiotic stresses (Fig. 3.1). The aim of this chapter is to highlight the morphological, physiological, and metabolic responses of MAPs under various abiotic stress conditions and provide the insights of PGRs in abiotic stress conditions.

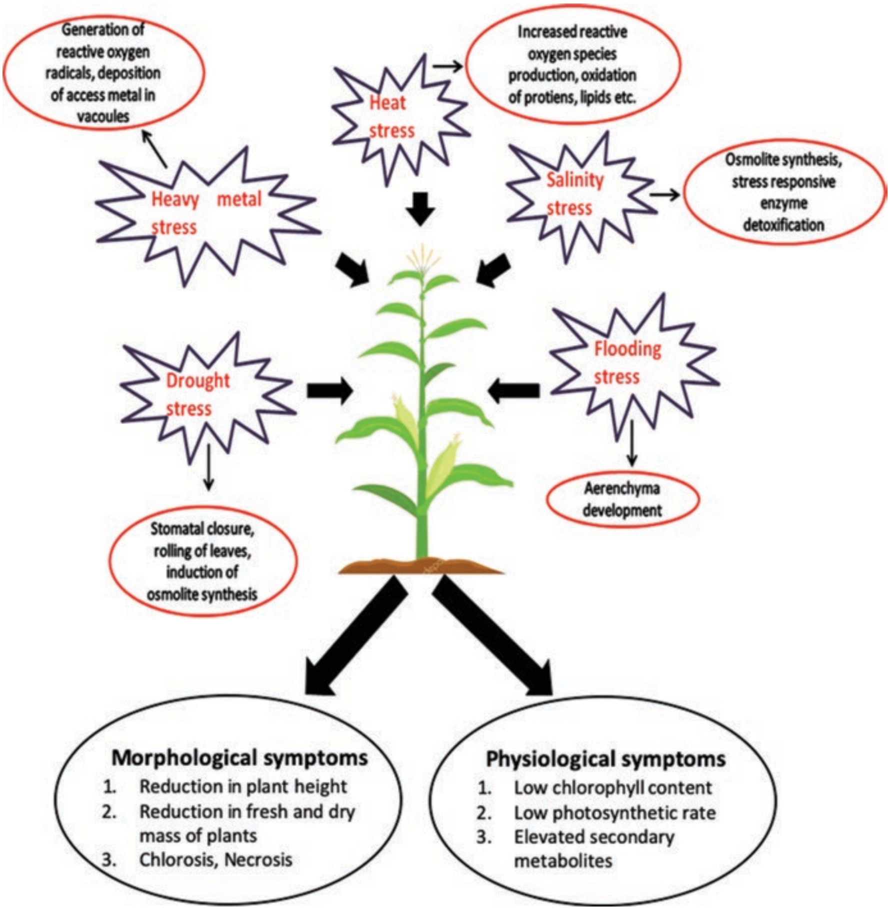

Fig. 3.1 Common morphological and physiological symptoms under different abiotic stress

3.2 Effect of Different Abiotic Stresses on MAPs

3.2.1 Heavy Metals and Metalloid Stresses

Heavy metals are a group of elements that are specific in weights higher than about 5 g/cm^3. Some of them (Co, Fe, Mn, Cu, Mo, Ni, Zn) are necessary for the normal development and growth of plants and take part in redox reaction, electron transport, and many metabolic processes, considered as essential micronutrient (Michalak 2006). The relation of HMs toxicity and use of medicinal plants have been reported worldwide. Contaminated agricultural resources and poor production practices may be the reason for accumulation of HMs in MAPs. The proposed key mechanism for the contamination of HMs into medicinal plants products are inadvertent cross-contamination during processing, contamination during cultivation, and/or deliberate addition of heavy metals for alleged medicinal purposes (Denholm 2010; Street 2012).

Among all the highly toxic HMs, cadmium (Cd) and lead (Pb) have great importance as their presence in environment is concerned (Pandey et al. 2007). It has been reported that small amount of Pb can affect plants by reducing metabolic processes such as photosynthesis, nitrogen assimilation, cell division, and germination (Gupta et al. 2011). Furthermore, accumulation of Pb was observed in many medicinal plants including *Acalypha indica* (Venkatachalam et al. 2017) and aromatic rice (Ashraf and Tang 2017). Anti-inflammatory activity of *COX1* and *COX2* in *Eucomis autumnalis* and *Eucomis humilis* was reduced by the different concentrations of Cd (Street et al. 2009).

Copper (Cu) is considered as an essential micronutrient for plants; it takes part in various metabolic processes being a component of several protein and enzymes (Ali et al. 2006). It has been reported that Cu toxicity significantly reduced leaf size followed by chlorosis in *Oregano* (Panou-Filotheou et al. 2001). Similar results were observed in *Withania somnifera* (Khatun et al. 2008), *Jatropha curcas* (Gao et al. 2008), and *Matricaria chamomilla* (Kováčik et al. 2009) with increased antioxidant activity.

Arsenic (As) is a ubiquitous, non-essential, and toxic element to plants (Zhao et al. 2009). Recently, some medicinal plants of West Bengal were found to contain higher concentration of As than the permissible limits of 1 mg Kg^{-1} dw (Siddiqui et al. 2013). *Withania somnifera* was treated with different concentrations of As, showed tolerance up to lower concentrations for long periods and high concentrations for short periods, and increased the synthesis of thiols. The authors suggested that *W. somnifera* treated with As may be used in herbal remedies under proper guidance of experts, but it will cause threat to As entry into humans due to medicinal uses (Siddiqui et al. 2015). Similar results were found in *Artemisia annua*, (an antimalarial drug) plant, which was treated with As that exhibited high artemisinin content and showed tolerance to As. The common As toxicity symptoms reported are wilting and necrosis of leaf blades, reduced fruit and grain yield, and lowered chlorophyll content and photosynthesis (Rai et al. 2011).

Mercury (Hg) is a highly toxic non-essential HMs. Mercury usually reduced photosynthesis, transpiration, and chlorophyll content and, by interrupting the function of antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), and nonenzymatic antioxidants glutathione (GSH) and non-protein thiols (NPSH), affects the antioxidant defense system (Azevedo and Rodriguez 2012). Effect of different concentrations of Hg (0.5, 1 and 2 mg L^{-1}) on germination and seedling growth was reported in *Bowiea volubilis*, *Eucomis autumnalis*, and *Merwilla natalensis*, medicinal plants belonging to the family Hyacinthaceae. Mercury (0.5, 1, and 2 mg L^{-1}) significantly reduced the percentage germination of *E. autumnalis* and *B. volubilis*; however no effect was observed in percentage germination of *M. natalensis*, and seedling growth in *E. autumnalis* was not affected by Hg (Street et al. 2007).

Selenium (Se) is an essential micronutrient and causes toxicity at higher concentrations than the safe limit. There are two ways through which Se causes toxicity in plants, (a) malformed selenoproteins and (b) inducing oxidative stress, both are found to be harmful for plants (Gupta and Gupta 2017). Stunting, chlorosis, fading of leaves, reduced shoot, and root biomass are the common symptoms of Se toxicity. It has been observed that Se causes toxicity by interrupting the process of nitrate accumulation as well as chlorophyll combination in plants. Selenium toxicity reduces the plant defense system against oxidative stress and hydroxyl radicals by interfering with the production of glutathione (Garousi 2017). Leaves of *Portulaca oleracea* were treated with combination of Se and Hg and individually with both of the chemicals that exhibited reduction in shoot and root development and enhanced antioxidant system; therefore Hg was to be highly toxic individually in comparison to Se (Thangavel et al. 1999).

Cadmium, a non-essential heavy metal, affects plant growth and development negatively. Several authors have reported the toxic effects of Cd on plants. Cadmium affects photosynthesis, transpiration, and stomatal opening. Chlorosis, leaf rolls, and stunting are the common symptoms of Cd toxicity in plants. Cadmium interferes with the functions of membrane through lipid peroxidation and interrupts the chloroplast metabolism by reducing chloroplast biosynthesis and decreases the activity of photosynthetic enzymes (Benavides et al. 2005). Plants of *Mentha aquatica* treated with Cd showed stunted growth, smaller leaves, curled and chlorotic leaf margins, veins exhibiting red brown coloration. Total chlorophyll content and protein content were also reduced under Cd stress. Research showed that physiochemical absorption on the cell surface enhanced the uptake of Cd. It has been found that sulfhydryl and phosphate group of several compounds showed greater affinity to cadmium ions and might be the reason for Cd toxicity (Aslan et al. 2003). Different concentrations of Cd reduced the fresh mass of leaves, bulbs, and roots of *Merwilla plumbea*. Cd accumulated in the bulb of *M. plumbea*, 24-fold higher than the WHO guideline of 0.3 mg Cd/kg, when irrigated with 2 mg/L, and bulb treated with Cd showed greater antimicrobial activity. These findings raise concern for consumer safety, and the quality of medicinal plants sold might be in question as bulb of *M. plumbea* accumulated high Cd (Street et al. 2009). Decrease in fresh mass of shoot and root, plant height, and chlorophyll and carotenoid content were observed in *Phyllanthus amarus* under high concentration of Cd, and an increase in phyllanthin and hypophyllanthin, therapeutically active compounds, was enhanced at certain levels of Cd (Rai et al. 2005). Similar results were observed in *Bacopa monnieri* L.; however, the plant was able to tolerate metal-induced oxidative stress through enzymatic and non-enzymatic antioxidant activities (Singh et al. 2006). Cadmium was found to reduce menthol content significantly in peppermint oil (Zheljazkov et al. 2006). Reduction in plant height, fresh weight of seedlings, and seed germination were reported in *Silybum marianum* under high Cd levels (Khatamipour et al. 2011). Cadmium toxicity caused browning and rot root in *Catharanthus roseus*. Concentration of vinblastine, vindoline, catharanthine, and loganin was increased with the increasing concentration of Cd. *Catharanthus roseus* can be used in phytoremediation of Cd, due to substantial accumulation of biomass and secondary metabolite, used in pharmaceuticals when facing Cd toxicity (Chen et al. 2018).

3.2.2 Salinity Stress

The presence of high concentration of soluble salts in the soil moisture of root zone is referred to as soil salinity. In the arid regions, salinity stress is the most hazardous factor for the normal plant growth and development. By reducing the osmotic potential of soil solutes, salt stress causes water deficit condition even in the well-watered soils and causes unavailability of water to roots (Jaleel et al. 2007a; b). Common symptoms of salinity stress are reduced growth, accelerated development, and senescence and death during longer durations (Jouyban 2012). Different physiological processes were found to be affected by salt stress in medicinal plants. With the increasing concentration of salt, the most sensitive growth stage which is severely affected is seed germination. It has been reported that salt stress reduced the seed germination of *Thymus maroccanus*, sweet marjoram, *Petroselinum hortense*, and *Ocimum basilicum* (Aghaei and Komatsu 2013). Reduction in fresh and dry masses of both root and shoot was observed in *Trachyspermum ammi* L. Oil concentration of seeds of *T. ammi* was not affected by the high salt concentrations. It has been suggested that *T. ammi* is a moderately salt-tolerant plant (Ashraf and Orooj 2006). The seed germination in *Catharanthus roseus* was delayed at low salt level and completely inhibited at high concentrations. Glycine betaine and proline content was accumulated in the seedlings under high concentrations of salt. Moreover, a significant reduction in chlorophyll *a* and *b* and total chlorophyll content was observed in *Catharanthus roseus* (Jaleel et al. 2007a, b, 2008a, b). In another study, a reduction in fresh and dry flower weight, number of branches per plant, peduncle length and head diameter, and essential oil content were observed in *Matricaria chamomilla*, an important essential oil-bearing medicinal plant (Razmjoo et al. 2008). Effect of NaCl salinity was reported in *Cymbopogon nardus*. Plants were treated with 25, 50, 100, 200, and 300 mM NaCl, respectively. An increase in total chlorophyll content was observed in mature leaves with the increasing concentration of salt up to 200 mM; however drastic changes were observed at

300 mM. Decrease in chl a, chl b, and carotenoids was observed at high salt levels. The concentration of polyphenols increased with the increasing concentration of salt, and maximum increase was observed at 300 mM. These findings suggest that elevated levels of polyphenol at increased level of salinity enhance accumulation of secondary metabolite in *C. nardus* in order to tolerate high level of salt stress (Mane et al. 2010). Similar results were reported in *Vetiveria zizanioides* (Mane et al. 2011).

Several previous researches showed that medicinal plants (*Lepidium sativum*, *Linum usitatissimum*, *Plantago ovata*, and *Trigonella foenum-graceum*) might be grown under saline stress. It has been reported that these plants have the ability to tolerate moderate concentrations of salt which might be tried for cultivation under salt-stressed soils. However, reduction in fresh and dry weights of seedling was observed in all the tested plants (Muhammad and Hussain 2010). Seed germination, growth rate, and length of radicle and plumule were decreased in *Lepidium sativum* L. under salt stress (El-Darier and Youssef 2000). In *Withania somnifera*, germination percentage, early seedling growth, and chlorophyll content were significantly reduced under salt conditions (Jaleel et al. 2007a, b). Salinity stress decreased nodules, nodule weight and nitrogenase activity, reduction in nitrogen assimilation, and fixation potential in *Sesbania sesban* L. Enzymatic antioxidant (SOD, APX, and GR) and non- enzymatic antioxidant (ascorbic acid and glutathione) showed great variation under salinity stress in *Sesbania sesban* (Abd_Allah et al. 2015).

Rhizomes of *Mentha pulegium* were treated with different concentration of salt (25, 50, 75, and 100 mM), and growth of the plant was prevented even at the lowest dose of salt, and reduction was more pronounced in shoots than roots (Oueslati et al. 2010). Salt stress significantly reduced the fresh and dry masses of shoot and root and seed yield in *Foeniculum vulgare*. Increasing salt stress was found to decrease the oil content of *F. vulgare* (Ashraf et al. 2004). Also effect of salt stress on micro- and macronutrient uptake and biomass of *Andrographis paniculata* (king of bitters) was observed. Decrease in the content of nitrogen (N), phosphorus (P), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and manganese (Mn) and increase in sodium (Na^+), iron (Fe^+), zinc (Zn^+), and copper (Cu^+) were reported. Changes in the uptake of micro- and macronutrient under salinity stress caused low photosynthetic activity and respiration (Talei et al. 2012). Different concentrations of salt (0, 50, 100, and 150 mmol LG^1) were applied on *Mentha* plants, and it has been found that *Mentha* plants died and the synthesis of essential oil was stopped at the concentration 150 mmol LG^1 (Khorasaninejad et al. 2011). Plants of *Artemisia annua* were subjected to different concentrations of salt, and growth was completely diminished at 160 mM NaCl. Moreover, salinity induced oxidative stress that damaged the photosynthetic machinery leading to decrease in chlorophyll content, photosynthetic rate, and artemisinin content. However, an increase in artemisinin content was observed in the early growth stage of plant, possibly due to the conversion of artemisinic acid/dihydroartemisinic acid into artemisinin by oxygen species under stress conditions (Qureshi et al. 2005). The effects of different stresses on plants have been summarized in Table 3.1.

Table 3.1 Morphological, physiological, and metabolic changes in MAPs toward various abiotic stresses

Type of stress	Plant name	Morphological symptoms	Physiological changes and antioxidant response	Tolerant or intolerant	References
Heavy metal stress (Pb)	*Mentha crispa* L.	Significant resistance showed by leaf number, budding, and green mass	Increased essential oil yield and carvone content	Highly tolerant	Sá et al. (2015)
Heavy metal stress (Hg)	*Mentha arvensis*	Decreased seedling growth and biomass	Increased antioxidant enzyme activity, reduced chlorophyll pigments	Highly tolerant	Manikandan et al. (2015)
Heavy metal stress (Cr)	*Cymbopogon flexuosus*	Reduced fresh and dry weights of root and shoot, enhanced lengths of root and shoot	Decreased chlorophyll content, increased proline accumulation and antioxidant enzyme activity	Moderately tolerant	Patra et al. (2018)
Heavy metal stress (Cr)	*Catharanthus roseus*	Chlorosis, senescence occurred in the beginning but later plants grew well	Reduced total chlorophyll, chl a and chl b content, increased total carotenoid content, increased level of cysteine and proline, increased antioxidant enzyme activity, increased vincristine and vinblastine content	Less tolerant	Rai et al. (2014)
Heavy metal stress (Cd)	*Artemisia annua*	–	Decreased photosynthetic pigments content, increased artemisinin content	Tolerant	Li et al. (2012)

(continued)

Table 3.1 (continued)

Type of stress	Plant name	Morphological symptoms	Physiological changes and antioxidant response	Tolerant or intolerant	References
Heavy metal stress (Pb)	*Matricaria chamomilla*	Decreased biomass, shorter lateral roots and reduction of root hair, increased shoot/ root ratio	Reduced total chlorophyll content, increased proline content	Tolerant	Grejtovský et al. (2008)
Heavy metal stress (cu)	*Withania somnifera*	Reduced shoot and root lengths and fresh weights	Decreased total chlorophyll and carotenoid content, induced MDA content, reduced antioxidant activities, increased total phenolic contents	Less tolerant	Khatun et al. (2008)
Heavy metal stress (Cr)	*Ocimum tenuiflorum*	–	Reduced photosynthetic pigments, protein, cysteine, ascorbic acid and non-thiol contents, decreased nitrate reductase activity and increased proline content and antioxidant enzymes activity, enhanced eugenol content	Tolerant	Rai et al. (2004)
Salinity stress	*Mentha canadensis*	Biomass and water content were reduced, decreased root and branch lengths, plant height, number of branches and nodes	Increased Na+/k + ratio, reduced essential oil content and menthol content, increased pulegone and menthone content	Less tolerant	Yu et al. (2015)

(continued)

Table 3.1 (continued)

Type of stress	Plant name	Morphological symptoms	Physiological changes and antioxidant response	Tolerant or intolerant	References
	Cymbopogon flexuosus	Decreased plant height, fresh and dry weights of shoot and root	Reduced photosynthetic pigments and activity of carbonic anhydrase and nitrate reductase, increased proline content, decreased essential oil yield and content, citral content	Less tolerant	Idrees et al. (2012)
	Catharanthus roseus	Reduced fresh and dry weights of plant, plant height and root length	Decreased total chlorophyll content, chl a, and chl b, increased total indole alkaloid content	Less tolerant	Jaleel et al. (2008a, b)
	Artemisia annua	Decreased shoot and root lengths, fresh and dry weights of shoot and root	Decreased total chlorophyll content, reduced activity of carbonic anhydrase and nitrate reductase, increased proline content and antioxidant enzyme activity, enhanced artemisinin content at some salt concentrations	Tolerant	Aftab et al. (2010)
	Matricaria chamomilla	Decreased shoot fresh weight and increased root fresh weight	Increased proline content and Na + and Fe concentration in plant	Tolerant	Heidari and Sarani (2012)
	Foeniculum vulgare	Reduced fresh and dry mass of both root and shoot	Increased concentration of Na^+ and Cl^- in both shoot and root, decreased essential oil content	Moderately sensitive	Ashraf et al. (2004)

(continued)

Table 3.1 (continued)

Type of stress	Plant name	Morphological symptoms	Physiological changes and antioxidant response	Tolerant or intolerant	References
Drought	*Matricaria chamomile*	Reduction in plant height, number of branches and flower, peduncle length, and head diameter	Reduced essential oil content	Moderately tolerant	Razmjoo et al. (2008)
	Cymbopogon nardus and Cymbopogon pendulus	Plant growth (plant height, leaf length, no. of tillers) was reduced	Increased activity of PEP carboxylase, and essential oil production, increased geraniol and citral content	Moderately tolerant	Sangwan et al. (2001)
	Catharanthus roseus	–	Increased H_2O_2 content and lipid peroxidation, glycine betaine and proline content, decreased proline oxidase activity and increased γ-glutamyl kinase	Moderately tolerant	Jaleel et al. (2007a, b)
	Mentha piperita L.	Reduction in internodes length, shoot and root lengths, shoot fresh and dry weights and stolon length	Reduction in essential oil yield and content, menthone and menthafuran content	Moderately tolerant	Khorasaninejad et al. (2010)
	Matricaria recutita L.	Reduced plant height, flower yield, shoot weight, and root weight	Decrease in epigenin content but no effect on oil content and yield	Tolerant	Baghalian et al. (2011)
	Bunium persicum	Decrease no. of umbels, reduced the umbellet, no. per umbel, reduced plant height	Increased essential oil percentage with the increasing level of drought, decreased seed and oil yield. Elevated antioxidants	Highly tolerant	Saeidnejad et al. (2013)

(continued)

Table 3.1 (continued)

Type of stress	Plant name	Morphological symptoms	Physiological changes and antioxidant response	Tolerant or intolerant	References
	Salvia officinalis	Thinner stems, dry and small leaves, reduced plant height, fresh and dry weights	Slightly increased essential oil content, yield, and camphor, α-thujone, 1,8-cineole	Moderately tolerant	Bettaieb et al. (2009)
	Ocimum basilicum	Decreased plant height, internode no., and dry weight per plant	Increased essential oil percentage	Tolerant	Forouzandeh et al. (2012)
	Cuminum cyminum	Decreased no. of umbels per plant, no. of umbellet per umbel, and seed yield under severe water stress	Increased activity of antioxidant enzymes, decreased essential oil yield under severe water deficit	Tolerant in moderate water stress, however moderately tolerant under severe water deficit	Rebey et al. (2012)
	Withania somnifera	Reduction in root and shoot lengths and leaf area	Increased withaferin content, total carotenoid content, anthocyanins and flavonoids, starch, and proline content, decreased chlorophyll content	Tolerant	Kannan and Kulandaivelu (2011)
	Cymbopogon martini	Decreased plant height and herbage yield	Increased PEP carboxylase activity, decreased oil content and yield, reduced geraniol content	Moderately tolerant	Fatima et al. (1999)
	Tagetus minuta	Reduced leaf dry weight	Reduced essential oil yield and content	Moderately tolerant	Mohamed et al. (2002)

(continued)

Table 3.1 (continued)

Type of stress	Plant name	Morphological symptoms	Physiological changes and antioxidant response	Tolerant or intolerant	References
	Plantago ovata and *Plantago psyllium*	–	Decreased relative water content, leaf water potential, severely damaged photosynthetic organs in *P. psyllium* than in *P. ovata*	Moderately tolerant	Rahimi et al. (2010)
	Silybum marianum	Reduced plantlet height, root growth, fresh and dry weights of plant, decreased growth rate of leaves	Increased concentration of total phenolics and flavonoids, high antioxidant activity observed	Less tolerant	Zahir et al. (2014)
Flooding	*Matricaria chamomilla L.*	–	Reduced chlorophyll content, reduction of osmolytes, high concentration of proline	Moderately tolerant	Pirzad et al. (2011)
	Ocimum sanctum	Reduction in plant height, fresh weight, and number of leaves	Decreased leaf chlorophyll content, increased proline level, enhanced ethylene production	Moderately tolerant	Barnawal et al. (2012)
	Ocimum sp.	Reduction in total fresh and dry weights of plants	Reduced chlorophyll content and photosynthetic efficiency, increased essential oil percentage and proline content	Moderately tolerant	Khalid (2006)
	Cichorium intybus	Reduced root length, shoot/root ratio	Higher stomatal conductance, decrease in chlorophyll content, lower PS II efficiency, decreased inulin concentration	Tolerant	Vandoorne et al. (2014)

(continued)

Table 3.1 (continued)

Type of stress	Plant name	Morphological symptoms	Physiological changes and antioxidant response	Tolerant or intolerant	References
	Mentha piperita	Premature senescence, chlorosis and leaf fall, decrease in the no. of internodes	Reduction in chlorophyll content	Less tolerant	Phukan et al. (2014)

3.2.3 *Drought and Flooding Stress*

Among the major environmental stress, drought causes alternation in morphology, physiology, and biochemistry of plants as well as a significant loss in agricultural production. It is estimated that 25% of the total world's land is severely affected by drought stress (Moinuddin et al. 2012). An increase in solute concentration inside cell under drought stress causes osmotic flow of water out of the plant cell, thus decreasing water potential of the cell and disrupting membranes along with essential metabolic processes like photosynthesis (Farahani et al. 2009). In semi-arid regions, it has been found that drought stress causes metabolic changes, responsible for the increasing concentration of active substances in plant cells. Plants suffer from drought stress due to less water supply and much higher light intensities, causing water shortage, closure of stomata and decrease uptake of CO_2 leading to lower the CO_2 fixation via Calvin cycle. This decrease of Calvin cycle lowers the consumption of reduction equivalents (NADPH + H^+), producing in turn a massive oversupply of NADPH+ H^+, resulting in the synthesis of highly reduced compounds such as alkaloids, phenols, or isoprenoids (Kleinwächter and Selmar 2015). Drought stress significantly affects the yield of medicinal and aromatic plants; the three possible mechanisms are:

1. Reduction in the whole canopy absorption of incident photosynthetically active radiation either by temporary leaf wilting or rolling during severe stress, by stress-induced limitation of leaf area expansion, or by early senescence
2. Reduction in the efficiency through which photosynthetically active radiation might be used by the crop to produce new dry matter
3. Limitation of grain yield of medicinal and aromatic plant by reducing the Harvest Index (HI) (Sharafzadeh and Zare 2011)

It has been reported that moderate drought stress reduced the biomass production and enhanced the content of rosmarinic acid, ursolic acid, and oleanolic acid in *Prunella vulgaris* (Chen 2011). Plants of *Trachyspermum ammi* treated with different drought levels exhibited decrease in transpiration rate and stomatal conductance and growth parameters such as plant height; fresh and dry weights of plants were also reduced with the increasing level of drought, while total chlorophyll contents, phenolic contents, and internal CO_2 concentration increased under drought stress. These findings suggested that the production of secondary metabolite of *T. ammi* could enhance under drought stress (Azhar et al. 2011).

Severe crop losses are caused by prolonged seasonal rainfall in tropical and subtropical regions. Within few hours, flooding or water submergence produces anoxic soil conditions. The characteristic change occurs in soil during soil inundation and flooding stress causes alteration in the availability of different nutrients. According to Sepehr et al. (2012), nitrogen fixation decreased more than vegetative growth under flooding stress in cowpea (*Vigna unguiculata*). The common symptoms of flooding are lower leaf expansion, chlorophyll breakdown, decrease membrane permeability, protein degradation, peroxidase, stomatal closure, and petiole epinasty. Internal CO_2 concentration lowers due to stomatal closure; subsequently, photosynthetic activity declines due to diminished availability of CO_2 for carbon fixation. Decrease in CO_2 concentration enhanced the amount of reactive oxygen species in the leaf due to ongoing light reaction, leading to senescence and death of the plant (Lin et al. 2006).

3.2.4 Heat, Light, and Temperature Stresses

Increased occurrence of heat in the changing environment and its metabolic effects have become major concern of plant researchers to study on crop and food plants. An increase in the levels of antioxidants and phenolics under heat stress has been found in some fruits such as strawberries, raspberries, and blueberries. These elevated antioxidant levels might protect plant tissues against reactive oxygen and nitrogen species generated by the increased levels of temperature. The inflated levels of temperature have been known to be critical to maintain the antioxidant enzymes system and chemical composition of medicinal plants. It has been observed that drying temperature up to 80° showed significant decrease in total antioxidant capacity, and rosmarinic acid was completely absent in *Mentha spicata* (Fletcher et al. 2005). An increase in chlorophyll *a* and *b* and carotenoid content was observed at 30° but decline at 40°; osmoprotectants such as soluble protein, soluble sugar, amino nitrogen, and proline were also increased in fenugreek (*Trigonella foenum-graceum*). Heat stress enhanced the thermotolerance of fenugreek by two ways: (i) increasing the catalase activity and glutathione content and (ii) decreasing the rate of lipid peroxidation, the activities of super oxide dismutase, peroxidase, and ascorbic acid peroxidase (Hassanein et al. 2012). Additionally, previous researches have shown that UV-B spectrum between 290 nm and 380 nm enhanced the synthesis of dimeric alkaloids in *Catharanthus roseus* shoots. UV-B light has also been found to enhance the expression of several genes involved in early stages of terpenoid indole alkaloids (Binder et al. 2009).

3.3 Role of Plant Growth Regulators in Alleviating Different Abiotic Stresses

Plant responses to abiotic stress are highly regulated by endogenous plant growth regulators through sensitizing growth and developmental processes. The role of ABA and cytokinins and their physiological and molecular mechanisms associated with stress tolerance are well explained, and there is a growing interest in understanding the association of auxins, ethylene, gibberellins, brassinosteroids, and polyamines in stress tolerance mechanism and elucidating their possible cross-talk mechanisms during stress tolerance acquisition. Recent progresses on hormonal signaling, mutant research, and physiological actions, and the identification and characterization of the gene regulating synthesis of different endogenous growth regulators come up with the scope for utilizing their biosynthetic pathways for developing transgenic crop plants with enhanced abiotic stress tolerance (Upreti and Sharma 2016).

3.3.1 Abscisic Acid

Abscisic acid (ABA) is an isoprenoid phytohormone, critical for plant growth and development, which regulates various physiological processes ranging from stomatal opening to protein storage and allows alterations to several stresses like salt, drought, and cold stresses. Abscisic acid is considered as the central regulator of abiotic stress tolerance in plants and coordinates an array of functions (Sah et al. 2016). Several ABA transporter genes have been reported in vascular tissues in *A. thaliana* belonging to the ATP-binding cassette (ABC) family. One such gene which encodes ABA transporting protein is *AtABCG25*. It exports ABA from the inside to the outside of the cell. On the other hand, another transporter *AtABCG40* has been reported, expressed in guard cells importing ABA from the outside to the inside of the cells. Both these genes are found to be involved in ABA signaling.

Several studies have attempted to understand the mechanisms and core components of ABA signaling under several stress conditions. ABA signaling involved three major components: pyrabactin resistance (PYR)/PYR1-like (PYL) regulatory component of ABA receptor (RCAR), protein phosphatase 2C (PP2C; a negative regulator), and (sucrose non-fermenting) SNF1-related protein kinase 2 (SnRK2, a positive regulator). These three components form a signaling complex known as “ABA signalosome” which acts as a double negative regulatory system (Mehrotra et al. 2014).

3.3.2 Cytokinins

Besides ABA, further hormones including cytokinins (CKs), which regulate an extensive range of processes crucial to plant development, are activated when a plant is exposed to stress. Several stresses (drought and high salinity) are found to alter the endogenous CK levels elucidating their role in stress response (O'Brien and Benková 2013; Zwack and Rashotte 2015). These severe environmental conditions hamper the production and transport of CKs from roots. Exogenously applied CKs can improve stomatal apertures and transpiration in many plants and can influence photosynthetic activity positively, suggesting their role in the regulation of plant adaptation to environmental stresses. It is necessary to take several physiological functions into consideration, when evaluating the role of CKs in stress responses; predominantly, cell division, which is a high energy consuming process; efficiency of main energy source, i.e., photosynthesis, and stimulation of the sink strength (Ha et al. 2012).

Cytokinins release seeds from dormancy and are often considered as ABA antagonists, as ABA inhibits seed germination. An increased ABA/CK ratio due to accumulation of ABA and decreased CK was observed in water deficit plants. Under low concentrations, CKs promote apical dominance which along with the increased level of ABA controls opening of stomatal aperture and helps plants to adapt to drought stress (Rasool et al. 2018).

Cytokinins usually travel from roots to shoots and are considered as an important signal class. The CK content of xylem sap has been reported in many plants providing information of its changed content under drought conditions. The main CK biosynthesis gene *isopentenyl transferase (IPT)*, which is driven by senescence-inducible promoter *SAG12* positively correlated with ample flooding tolerance, is over-expressed under increased CK levels. A drought/maturation-induced promoter, *senescence-associated receptor kinase (SARK)* was found to control the expression of *IPT*, resulting in a remarkable tolerance to extreme drought conditions in tobacco plants. These findings and the reported role of CKs in sink-source polarization indicated their role in plant responses to drought (Morkunas et al. 2014).

3.3.3 Auxins

Auxins (indoleacetic acid, IAA) play a major role in the development of plant and regulate cell elongation, cell division, differentiation, apical dominance, root initiation, and tropic responses, besides a multitude of other developmental processes. Recent researches have confirmed the chief role of auxins in plant responses under unfavorable abiotic and biotic stress conditions. Various environmental stresses trigger the differential expression of genes involved in various auxin-related pathways. In fact, functional roles of auxin-related genes have provided utilitarian knowledge to establish the key role auxin plays under osmotic stress imposed by drought, salinity, and low temperature conditions (Sharma et al. 2015). Meristematic tissues are the main site of IAA biosynthesis through tryptophan-dependent and tryptophan-independent biosynthetic pathways. The major intermediates identified in tryptophan-dependent IAA biosynthetic pathways are indole-3-acetamide (IAM), indole-3-pyruvic acid (IPA), tryptamine (TAM), and indole-3-acetaldoxime (IAOX). Auxin homeostasis and the concentration and ratio of IAA and IAA derivatives in plant tissues are maintained by its degradation, conjugation to amino acids, and transport; however, these processes are found to be sensitive to abiotic stresses in plants. Two possible mechanisms have been described for the modification of IAA levels in plants under stress conditions: one by alterations in expression of auxin polar transporter gene and the other by deceased the polar transport by some compounds accumulated in response to stress (Upreti and Sharma 2016).

Expression analysis of the auxin-responsive marker IAA2-GUS and the direct auxin transport assay under the response of auxin mutants to cold stress induced inhibition of root growth and gravity response has confirmed that chilling stress mainly targets intracellular auxin transport. The changes in plant growth and development induced by cold stress are tightly linked to the intracellular auxin gradient, which is regulated by the intracellular trafficking of auxin carriers and polar deployment. Under drought stress conditions, some evidence of the involvement of auxin transport carriers has been obtained. PIN proteins, being a primary mediator of auxin transport in plants, were presumed to be involved either directly or indirectly under drought stress. In rice plants, the auxin efflux carrier protein family regulated by a gene, OsPIN3Tt, is found to play key role in rice shoot and root development and is involved in drought stress response. Moreover, *NTM2*, a membrane bound NAC transcription factor, mediates the signaling cross-talk between auxin and salt stress via *IAA30* gene during seed germination in *Arabidopsis*. Researches have confirmed that *NTM2* is a molecular link that incooperates the auxin signal into salinity stress signaling, elucidating the role of auxin in modulating seed germination under salt stress (Morkunas et al. 2014).

3.3.4 Gibberellins

Gibberellins (GAs) is a class of growth hormones, best known for their role in seed germination, stem elongation, leaf initiation, flower and trichome initiation, and flower and fruit development. GAs also known to play a chief role in regulating various processes in plant development and through their influence on photosynthetic enzymes, leaf interception, and leaf area index improve the photosynthetic efficiency and also enhanced the use efficiency of nutrients (Iqbal et al. 2011). After years of researches, a significant knowledge has come to our understanding that defines the role of gibberellins in stress conditions. Under several forms of abiotic stress, growth is restrainted in different plant parts mediated by major GA negative regulators, DELLA proteins (Colebrook et al. 2014). Reduction in endogenous levels of bioactive GAs has been observed under salt stress which coincides with higher accumulation of DELLA proteins. In an experiment conducted, salt stress inhibited growth and developmental processes in wild-type plants, whereas reduction in primary root growth and delayed flowering time and lowered rate of leaf production in salt stress were compromised in a DELLA quadruple loss-of-function mutant (Ryu and Cho 2015). The key components of gibberellin signaling function under stress responses are DELLA proteins, the GA receptor GID1, and the F-box proteins SLEEPY1 (SLY1) and SNEEZY (SNZ). When GA binds to GID1 receptor, it forms a complex with nuclear growth-repressing DELLA proteins, subsequently polyubiquinated and degrade the DELLA protein by E3 ubiquitin-ligase $SCF_{SLY1/GID2/SNZ}$. Therefore, DELLA protein function to restrain plant growth, while GAs promote it by targeting them for destruction (Gómez-Cadenas et al. 2014).

3.3.5 *Ethylene*

Being a gaseous hydrocarbon, ethylene, commonly known as “aging” hormone, is unique among hormones in both plant and animal kingdom. It is considered as potent growth regulator, modulating various developmental processes, mainly senescence of plants as little as 0.01 μ1/1 (Reid 1995). Although ethylene is not only involved in the end stages of plant development, it regulates development throughout the life cycle of the plant. It can regulate the cell size, often restricting cell elongation, and it is also associated with cell division. Aside from these functions, ethylene also controls the formation of root nodules in leguminous plants, enhances root initiation in many plant species, promotes flowering in some species, inhibits the formation of such storage organs as tubers and bulbs, and induces the production of female rather than male in cucurbits (Schaller 2012). Biosynthesis of ethylene increases under several environmental stresses such as extremes of temperature, flooding, drought, high light, radiation, high salt, and heavy metal and organic pollutions, often referred to as “stress ethylene.” Several stresses have been found to initiate the synthesis of ethylene that in turn can either exacerbate the symptoms of stress or initiate the signal cascade resulting in responses that enhance plant survival under adverse conditions. This initial synthesis of ethylene after onset of stress initiates a protective response such as transcription of pathogenesis-related genes and induction of acquired resistance. However, if the stress condition persists, the *ACC* synthase gene (1-aminocyclopropane-1-carboxylic acid) transcribed, and due to additional *ACC*, rise in ethylene synthesis occurs again which in turn showed deleterious effects on plant and induces processes such as abscission, senescence, and chlorosis that may lead to a significant inhibition of plant survival (Gamalero and Glick 2012).

3.4 Conclusion

It has been concluded that different abiotic stresses cause different morphological and physiological symptoms that varies from plant to plant; however some growth and photosynthetic changes are found common in all stresses. A number of MAPs have been found moderately tolerant to some HMs and drought stresses, and some are less tolerant to all kinds of stresses. Accumulation of HMs in some medicinal plants enhance their secondary metabolites, eventually lead to increase their medicinal property and use of these herbal remedies is a major concern of safety for mankind, as increasing concentration of these metals in plants above than safe limit can be hazardous to human health. Noticeably, increase in essential oil yield, alkaloids, and flavonoids have been reported in some medicinal plants under drought stress. However, effects of salt stress on such medicinal plants are more severe than other stresses. Different PGRs play salient role in different abiotic stress conditions and maintain the normal functioning of plants. Numerous signaling pathways are involved in the response mechanisms and enhance the chances of survival of plants in the adverse conditions.

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Chapter 4
Hormonal Regulation in Cell Culture of *Artemisia annua* L. Plant

Mauji Ram

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	Benzylaminopurine
IAA	Indoleacetic acid
IBA	Indole butyric acid
Kin	Kinetin
NAA	Naphthalene acetic acid

4.1 Introduction

Artemisia annua, also known as sweet wormwood, sweet annie, sweet sagewort, or annual wormwood, belonging to the family Asteraceae, is a common type of wormwood that grows throughout the world. It is a crop for the production of anti-malarial and possibly antibacterial agents and natural pesticides. It was originally collected by the Chinese as an herbal medicine and is currently processed by pharmaceutical firms for the production of artemisinin for artemisinin-based combination therapies (ACTs) in the treatment of malaria. ACTs have been shown to have rapid resolution to fever and parasitemia and low toxicity and are well-tolerated. Artemisinin is a sesquiterpene-lactone isolated from the aerial parts of *Artemisia annua* L. plants. Artemisinin has proved to be a dramatically effective anti-malarial against multi-drug-resistant *Plasmodium* spp. (Brown 1995; Duke et al. 1987). Besides being currently the best therapeutic agent against multi-drug-resistant and cerebral malaria causing strains of *Plasmodium* sp., it is also effective against other infectious diseases such as Schistosomiasis, hepatitis B and Lishmmaniasis (Borrmann et al., 2001; Utzinger et al., 2001; Romero et al., 2005; Sen et al., 2007). More recently, it has also been shown to be effective against a variety of cancer cell lines including

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breast cancer, human leukemia, colon cancer, and small cell-lung carcinomas (Efferth et al. 2001; Singh and Lai 2001). Due to its current use in artemisinin based-combination therapy (ACT), its global demand continuously is increasing. However, the relatively low yield of artemisinin in *A. annua* L. plant leaves (0.01–0.8%) is a serious limitation to the commercialization of the drug (Van Agtmael et al. 1999; Laughlin 1994; Abdin et al. 2003). However, cross-pollination in *A. annua* L. plants is a serious limitation to maintain the genetic fidelity and higher artemisinin yield throughout the population.

Micropropagation and organogenesis of different *Artemisia* species have been previously established by using several parts of plants, in order to obtain a large number of plants, such as *A. mutellina* (Mazzetti and Donata 1998); *A. scorpia* (Aslam et al. 2006); *A. vulgaris* (Govindaraj et al. 2008); and *A. absinthium* (Zia et al. 2007). An efficient in vitro method for multiple bud induction and regeneration has been developed in *Artemisia annua*, using leaf, stem, shoot tip, and explants or by using young inflorescence segments (Lualon et al. 2008; Sharma et al. 2008; Ganesan and Paulsamy 2011; Almaarri and Yu 2010; Gonzalez et al. 2013; Tahir et al. 2013; Hailu et al. 2013).

In vitro direct organogenesis of different parts of *Artemisia annua* was investigated, in this research, to obtain a large number of plants true to type. The ultimate goal was the multiplication of the selected clones with high levels of secondary metabolites and the utilization of the protocol in any future genetic transformation of this species.

Tissue culture uses standard protocols with shoot tips of mature field-grown plants (Simon et al. 1990). Shoot tips and lateral buds of *A. annua* L. produced numerous shoots on MS medium and formed 100% roots on half strength Murashige and Skoog minimal organic medium. The medicinal use of *A. annua* in the tropics should be emphasized. There is therefore an urgent need for the conservation and rapid propagation of the seedlings using tissue culture techniques. This experiment was carried out to study growth response of *A. annua* L. plant shoot tip and stem explants in vitro, under different combinations of plant growth regulators, and to develop a protocol for production of clean healthy plantlets for subsequent multiplication. In vitro selection has been proved very effective method for the selection of high artemisinin yielding genotypes of *A. annua* L. plants. Thus we have been able to maintain genetic fidelity and production of quality seed of *A. annua* L. plants.

4.2 Materials and Methods

4.2.1 Plant Materials and Chemicals

This experiment was carried out at Green Technology Department, Ipca Laboratory, Ratlam. Seeds of *A. annua* L. (CIM AROGYA) were purchased from CIMAP, Lucknow. These seeds were used for the preparation of nursery, and 45-day-old seedlings of *A. annua* L. plants were transplanted in the experimental field of Ipca Laboratories Ltd. The leaf and stem explants were selected from high artemisinin yielding *A. annua* L. plant grown in experimental field of Ipca Laboratory Ltd., Ratlam. Explants were washed under running tap water for 30 min and then surface sterilized with 5% teepol for 5–7 min. Further sterilization of explants was done in laminar air flow; 1.0% bavistin (w/v) and 0.25% streptomycin (w/v) were used for 5 min. 5% solution of sodium hypochlorite (v/v) is used for 4 min followed by disinfection with 70% ethyl alcohol for 30 seconds. Explants were then rinsed five times in sterile distilled water before inoculation on media. All chemicals and hormones were procured from HiMedia Laboratories (India) and Sigma-Aldrich (USA). Sucrose and agar were procured from HiMedia Laboratories, India. All the buffers and solutions were prepared by using autoclaved MilliQ water.

4.2.2 Media and Culture Conditions

Basal medium used was full strength Murashige and Skoog (1962). The medium containing 3% (w/v) sucrose, B5 vitamins, and 0.1 g Inositol was augmented with different cytokinins and an auxin. For callogenic study, four auxins; 2, 4-D, IAA, NAA and IBA with different concentrations (0.25–1.5 mg/L) in combination with two cytokinins; Kinetin and BAP (0.25–1.5 mg/L) were used in basal MS. Different concentrations of BAP and Kin (0.25–2.0 mg/L) in combination with amino acids (glutamine 100 mg/L; cystine HCl 5 mg/L; arginine 50 mg/L; asparagine-40 mg/L) and two constant concentrations of NAA (0.05 mg/L and 0.1 mg/L) were tested for the shoot induction from callus and leaf. While different concentrations of IAA IBA and NAA (0.1–2.0 mg/L) were used for root induction. Hormone-free MS medium served as control. The pH of the medium was adjusted to 5.8 prior to the addition of 0.8%w/v agar. 20 ml aliquots were dispensed into jam bottles and autoclaved at 121 °C at 15 lb. pressure for 15 min. Surface-sterilized explants were aseptically inoculated in jam bottles, and the cultures were maintained at 25 ± 2 °C using 16/8 light/dark period, under a light intensity of 3000 lux provided by cool-white fluorescent lamps and 50 to 55% relative humidity.

4.2.3 Statistical Analysis

The experiments were entirely randomized with six replicates for each growth regulator(s) concentration(s). Statistical analyses were carried out by the ANOVA and Duncan's multiple test, at a 0.5% probability level.

4.3 Result and Discussion

Micropropagation is an advanced technique for producing a large number of genetically uniform and pathogen-free plants in limited time and space (Zobayed and Saxena 2003). In vitro clonal propagation of species through tissue culture has been frequently based on the successful adjustment of the type and combinations of plant growth hormones (Murashige 1990; Uranbey et al. 2005).

4.3.1 Callogenic Response

The callogenic response from leaf and stem explants was observed at different plant growth regulators concentration either singly or in combination (Table 4.1). Callogenic response from leaf and stem explants started at the margins or from injuries. Plant growth regulator (PGR)-free basal MS medium also induced callogenic response from both explants where leave explants showed 90% callogenic response, while 73% stem explants produced calli. Nin et al. (1996) reported no callogenic response from leaf explant on PGR-free medium, and explants died after few days. 2, 4-D as callus-inducing hormone produced light green, soft, friable, and compact callus from leaf and stem explants. But at all concentration of 2, 4-D organogenic response was not observed within observation time. Nin et al. (1996) stated that low concentration of 2, 4-D stimulated adventitious root development from 86% of all explants of *A. annua* L. At all concentrations of BAP and Kin, the callogenic response was poor. Very low callus was developed which was green, soft, and compact. Small and few numbers of leaves also emerged, when the callus remained on the same medium for 6 weeks or the callus turned to hard and embryogenic. Callus produced at different concentrations of IAA and IBA was yellowish, soft, and friable, and callogenic response was 100% at lower concentration of both hormones. Nin et al. (1996) reported that callogenesis occurred in 100% of explants, independent of the cytokinins/auxin ratio. But at different concentrations of NAA, light green, soft, and friable callus was observed. At low concentrations of NAA, small shoots emerged, while at higher concentrations callus turned hard and compact. The result shows that media supplemented with BAP either with Kin, NAA, 2, 4-D, or IBA produced 100% callogenic response from both explants. 0.5 mg/l BAP and 0.5–1.5 mg/l NAA in combination produced green, soft, and friable callus from both explants (Figs. 4.1 and 4.2). Ganesan and Paulsamy (2011) observed 98.66% callogenic response from leaf discs with NAA at 0.9 mg/l in *A. annua* L., while Nin et al. (1996) reported best callogenic response with BAP and NAA in the medium for *A. absinthium*, whereas Benjamin et al. (1991) observed callus induction from shoot buds using BAP plus IAA for *Artemisia pallens*. 2, 4-D at varying concentration (0.05–0.25 mg/l) in combination with BAP (0.5 mg/l) also produced light green and soft callus when supplemented in MS medium. When IBA and NAA were com-

Table 4.1 Callogenic response from leaf and stem explant at different growth regulators[a]

Growth regulator	Concentration (mg/l)	Leaves explant		Stem explant	
		Callus formation (%)[b]	Response	Callus formation (%)[b]	Response
2, 4-D	0.25	62.5	++	59.5	++
	0.5	76.0	+++	76.0	+++
	0.75	100.0	++++	100.0	++++
	1.0	100.0	+++	86.0	+++
	1.25	58.0	++	42.5	++
	1.5	52.0	+	32.5	+
IAA	0.25	65.0	++	82.5	++
	0.5	85.0	+++	92.0	+++
	0.75	100.0	++++	100.0	++++
	1.0	100.0	++++	100.0	+++
	1.25	53.0	++	57.0	++
	1.5	44.0	+	41.0	+
NAA	0.25	76.0	++	71.0	++
	0.5	100.0	+++	76.5	+++
	0.75	100.0	++++	100.0	++++
	1.0	100.0	++++	100.0	++++
	1.25	100.0	+++	54.0	+++
	1.5	69.0	+	38.5	+
IBA	0.25	77.0	++	68.0	++
	0.5	88.0	+++	79.0	+++
	0.75	100.0	++++	100.0	++++
	1.0	100.0	+++	100.0	+++
	1.25	68.0	++	53.5	++
	1.5	45.0	+	51.0	+
BAP	0.25	62.0	+	55.0	+
	0.5	78.0	+++	72.0	++
	0.75	85.5	++++	80.0	+++
	1.0	53.5	+++	36.5	+++
	1.25	–	+	–	+
	1.5	–	–	–	–
Kin	0.25	51.0	++	47.0	++
	0.5	64.0	+++	61.0	+++
	0.75	74.0	++++	72.5	++++
	1.0	22.0	++	14.0	++
	1.25	–	+	–	–
	1.5	–	–	–	–
BAP/IBA	0.5/0.5	81.5	++	77.0	++
	0.5/1.0	100.0	++++	100.0	++++
	0.5/1.5	100.0	+++	100.0	+++

(continued)

Table 4.1 (continued)

Growth regulator	Concentration (mg/l)	Leaves explant		Stem explant	
		Callus formation (%)^b	Response	Callus formation (%)^b	Response
BAP/NAA	0.5/0.5	100.0	++++	100.0	++++
	0.5/1.0	100.0	++++	100.0	++++
	0.5/1.5	100.0	++++	100.0	++++
Kin/NAA	0.5/0.5	100.0	+++	100.0	+++
	0.5/1.0	100.0	++++	100.0	++++
	0.5/1.5	100.0	++++	100.0	++++
BAP/2, 4-D	0.5/0.5	87.0	+++	82.0	+++
	0.5/1.0	100.0	++++	100.0	++++
	0.5/1.5	100.0	+++	100.0	+++
Kin/IBA	0.5/0.5	84.0	+++	78.0	+++
	0.5/1.0	100.0	++++	100.0	++++
	0.5/1.5	100.0	+++	100.0	+++
BAP/IBA/Kin	0.5/0.5/1.0	100	++++	100	++++
	0.5/1.0/1.0	100	++++	100	++++
	0.5/1.5/1.0	100	+++	100	+++
BAP/NAA/Kin	0.5/0.5/1.0	100	+++	100	+++
	0.5/1.0/1.0	100	++++	100	++++
	0.5/1.5/1.0	100	++++	100	+++
BAP/2, 4-D/Kin	0.5/0.5/1.0	100	+++	100	+++
	0.5/1.0/1.0	100	+++	100	++++
	0.5/1.5/1.0	100	++++	100	+++
Control		64.0	++	72.0	+++

[a] Rated after 30 days of culture: + = Low, ++ = good, +++ = very good, ++++ = excellent, − = nil
[b] %age response of 6 replicates

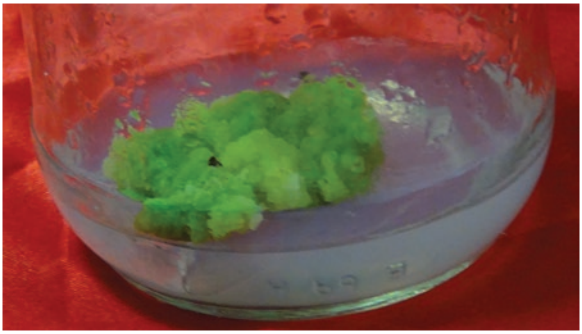

Fig. 4.1 Callus induction from leaf explant of *Artemisia annua* L. plant on MS medium (BAP 0.5 mg/l + NAA 1.5 mg/l)

bined with Kin, the callogenic response was also low, and callus was not good in texture. Xu and Jia (1996) observed best callus result in the presence of 2, 4-D with Kin for *Artemisia sphaerocephala*.

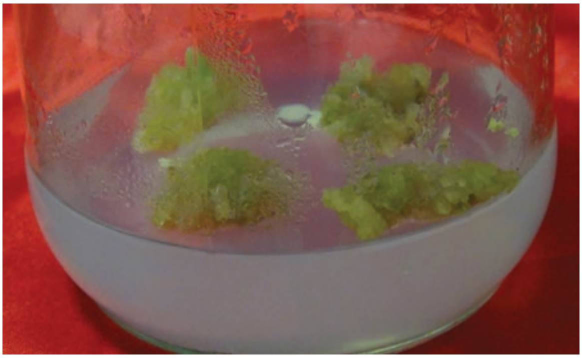

Fig. 4.2 Callus induction from stem explant of *Artemisia annua* L. plant on MS medium (BAP 0.5 mg/l + NAA 1.5 mg/l)

4.3.2 Shoot Induction and Regeneration

Shoots were induced from callus and leaf explants by supplementing various combinations of BAP, Kin, and NAA. All combinations of these plant growth regulators in callus and leaf explant culture could not induce shoot induction (Tables 4.2 and 4.3). The period of shoot induction was 4 weeks, and it varies in different species of *Artemisia* (Liu et al. 2003; Aslam et al. 2006; Govindaraj et al. 2008; Mazzetti and Donata 1998; Zia et al. 2007).

Shoot induction from callus was observed at different concentration of BAP and Kin, alone and in combination with NAA and amino acids (glutamine 100 mg/L; cystine HCl 5 mg/L; arginine 50 mg/L; asparagine 40 mg/L) (Table 4.2). At 1.5 mg/l BAP, 2.16 ± 0.614 shoots emerged, while at 0.5 mg/l no shoot induction was observed. Nin et al. (1996) and Zia et al. (2007) also did not observe any shoot induction at low concentrations of BAP in *A. absinthium*. However, Le (2001) reported that new axillary shoots development was promoted in *Artemisia annua* by addition of BAP in MS medium. The best shoot induction 83.6% (2.83 ± 0.234) was observed on BAP (1.5 mg/l) in combination with NAA (0.05 mg/l) (Fig. 4.3). At different concentrations of Kin, alone or in combination with NAA response of shoot induction was observed very low. At 1.0 mg/l Kin, shoot induction was 56.2% (1.5 ± 0.335) and at higher concentrations response was absent while the callus turned hard, compact and embryogenic. In the present study, direct shoot induction from leaf explant was also carried out. The best shoot induction 85.7% (3.16 ± 0.434) was observed on BAP (1.5mg/l) in combination with NAA (0.05mg/l) (Fig. 4.4). Geng et al. (2001) observed shoot cluster in *A. annua* L. on MS medium supplemented with BAP and NAA. Mackay and Kito (1988) and Nam-cheol et al. (1992) also reported shoot induction on MS medium supplemented with BAP and NAA in different *Artemisia* species. Shoot induction was very low or absent at different concentrations of Kin alone or in combination with NAA. At 1.5 mg/l Kin along with 0.05 mg/l NAA, shoot induction was 65% (2.0 ± 0.724), and at higher concentrations, response was absent (Table 4.3). Vergauwe et al. (1996) reported the shoot regeneration from leaf explants of *A. annua* L. on MS medium with 0.05 mg/l NAA and 0.05 mg/l BAP after 5 weeks of culture. In the present study, results of

Table 4.2 Effect of growth regulators on in vitro shoot induction of *Artemisia annua* from callus on MS medium[a]

Growth regulator	Conc. (mg/l)	Response (%)[b]	Average no. of shoots^{XT}	General description
BAP	0.25	00.0	–	No response
	0.5	28.0	–	No response
	0.75	46.0	0.83 ± 0.234^{d}	1–2 shoots with small green leaves
	1.0	69.2	1.66 ± 0.412^{cd}	1–3 shoots with small green leaves
	1.5	79.0	2.16 ± 0.614^{ab}	2–3 shoots with small green leaves
	2.0	15.0	–	No response but embryogenic callus
Kin	0.25	00.0	–	No response but embryogenic callus
	0.5	21.0	–	No response but embryogenic callus
	0.75	36.0	0.83 ± 0.352^{d}	1–2 shoots with small green leaves
	1.0	56.2	1.5 ± 0.335^{cd}	1–3 shoots with small green leaves
	1.5	41.0	–	No response but embryogenic callus
	2.0	00.0	–	No response but embryogenic callus
NAA	0.1	00.0	–	No response but embryogenic callus
	0.5	00.0	–	No response but embryogenic callus
BAP/NAA	1.5/0.05	83.6	2.83 ± 0.234^{a}	3–4 shoots with green leaves
	1.5/0.1	26.0	$2.16 \pm 0.271b^{bc}$	1–2 shoots with green leaves
Kin/NAA	1.5/0.05	65.0	2.33 ± 0.724^{cd}	2–3 shoots with green leaves
	1.5/0.1	16.5	1.16 ± 0.121^{d}	1–2 shoots with green leaves
LSD			1.366	

Interval of confidence 95%
Values with the different letters on the same column are significantly different
[a] Rated after 30 days of culture
[b] Data are mean of 6 replicates
[c] Mean ± standard error
[d] Mean separation by LSD

shoot induction rate is in agreement with the report of Banyai et al. (2005) who considered 1 mg/L BAP with 0.1 mg/L NAA as the best supplemented medium for leaf explant-derived shoot regeneration. Almaarri and Yu Xie (2010) reported 100 and 66.6% shoot induction in different genotypes of *A. annua* on MS fortified with TDZ (1 mg/l) and BAP (1 mg/l), respectively. Similar results have also been reported by Sujata and Kumari (2007), Sharma et al. (2008), Gonzalez et al. (2013), Tahir et al. (2013), and Hailu et al. (2013).

Table 4.3 Effect of growth regulators on in vitro direct shoot induction of *Artemisia annua* from leaf explants on MS medium[a]

Growth regulator	Conc. (mg/l)	Response (%)[b]	Average no. of shoots[XT]	General description
BAP	0.25	00.0	–	No response
	0.5	28.0	–	No response
	0.75	46.0	0.833 ± 0.324^{d}	1–2 shoots with green leaves
	1.0	69.2	1.5 ± 0.622^{cd}	1–3 shoots with green leaves
	1.5	79.0	2.83 ± 0.615^{ab}	2–3 shoots with green leaves
	2.0	15.0	–	No response but embryogenic callus
Kin	0.25	00.0	–	No response but embryogenic callus
	0.5	21.0	–	No response but embryogenic callus
	0.75	36.0	0.833 ± 0.352^{d}	1–2 shoots with green leaves
	1.0	56.2	2.16 ± 0.335^{cd}	1–3 shoots with green leaves
	1.5	41.0	–	No response but embryogenic callus
	2.0	00.0	–	No response but embryogenic callus
NAA	0.1	00.0	–	No response but embryogenic callus
	0.5	00.0	–	No response but embryogenic callus
BAP/NAA	1.5/0.05	85.7	3.16 ± 0.434^{a}	4–5 shoots with green leaves
	1.5/0.1	26.0	$1.83 \pm 0.271\text{b}^{bc}$	2–3 shoots with green leaves
Kin/NAA	1.5/0.05	65.0	2.0 ± 0.724^{cd}	2–3 shoots with green leaves
	1.5/0.1	16.5	1.66 ± 0.121^{d}	1–2 shoots with green leaves
LSD			1.233	

Interval of confidence 95%

Values with the different letters on the same column are significantly different

[a] Rated after 30 days of culture

[b] Data are mean of 6 replicates

[c] Mean ± standard error

[d] Mean separation by LSD

However, we established an improved protocol for direct shoot regeneration of *A. annua* L. using leaf explants on MS medium supplemented with BAP and NAA resulting in a rapid and high number of shoots per explant in this study. Therefore, this regeneration system might be a useful method for high regeneration efficiency and has commercial advantage due to the shoot regeneration period over a combination of several plant growth regulators. The regeneration system developed in this study will be useful for plant improvement through micropropagation and genetic engineering of *A. annua* L. Moreover, this system can be available for the clonal propagation in order to obtain the strain containing a constant concentration of artemisinin in *A. annua* L.

Fig. 4.3 Shoot induction from callus of *Artemisia annua* L. plant on MS medium (BAP 1.5 mg/l + NAA 0.05 mg/l + glutamine 100 mg/L; cystine HCl 5 mg/L; arginine 50 mg/L; asparagine 40 mg/L)

Fig. 4.4 Direct shoot induction from leaf explant of *Artemisia annua* L. plant on MS medium (BAP 1.5 mg/l + NAA 0.05 mg/l)

4.3.3 Root Induction

Regenerated shoots were subcultured on same medium and multiplied on BAP (1.5 mg/l) in combination with NAA (0.05 mg/l) (Fig. 4.5). Individual shoots were isolated and transferred to elongation medium (half strength MS) for 30 days. Elongated shoots were transferred on rooting medium. Rooting was found very well in the present study (Table 4.4). Six concentrations of auxins (IAA, IBA, and NAA) were tested in full and half strength MS medium. Highest response 85.8% with 0.5 mg/l of NAA in full MS followed by IBA (59.6%) and IAA (55.8%) was observed from shoots. At 0.5 mg/l NAA at full MS and ½ MS, 2–3 roots were observed. When these plants were transferred on the same medium, they produced further roots. Similar findings were observed by Zia et al. (2007) and Mohammad et al. (2014). Plants that produced roots were transferred to pots filled with soil and peat moss (3:1) under high humid condition till maturation of leaves and then transferred to green house.

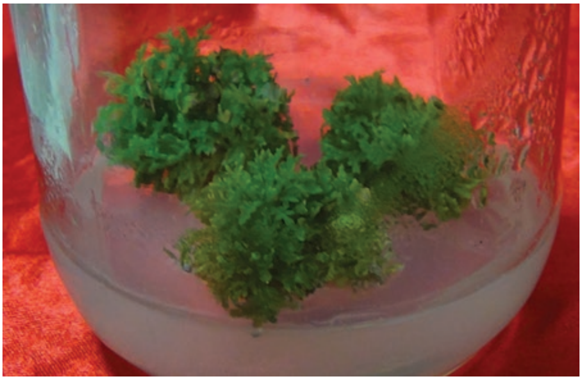

Fig. 4.5 Multiplication of regenerated shoots of *Artemisia annua* L. plant on MS medium (BAP 1.5 mg/l + NNA 0.05 mg/l)

Table 4.4 Effect of growth regulators on in vitro rooting of *Artemisia annua*[a]

		Full MS		½ MS	
Growth regulator	Concentration (mg/l)	Response (%)[b]	Average no. of roots[c]	Response (%)[b]	Average no. of roots[c]
IAA	0.1	–	–	–	–
	0.25	36.0	1.16 ± 0.5	–	–
	0.5	55.8	2.33 ± 0.6	45.8	0.83 ± 0.25
	1.0	23.5	1.33 ± 0.4	–	–
	1.5	–	–	–	–
	2.0	–	–	–	–
IBA	0.1	–	–	–	–
	0.25	23.5	0.83 ± 0.3	21.9	0.66 ± 0.
	0.5	59.6	2.0 ± 0.9	55.4	21.66 ± 0.7
	1.0	–	–	–	–
	1.5	–	–	–	–
	2.0	–	–	–	–
NAA	0.1	–	–	–	–
	0.25	50.6	1.83 ± 0.6	20.2	0.83 ± 0.3
	0.5	85.8	2.5 ± 0.9	72.3	1.83 ± 0.9
	1.0	43.5	1.33 ± 0.5	20.5	0.66 ± 0.2
	1.5	18.7	1.16 ± 0.3	–	–
	2.0	–	–	–	–

[a] Rated after 30 days of culture
[b] Data are mean of 6 replicates
[c] Mean ± standard error

4.4 Conclusion and Summary

It has been proven in several studies including this present one that plant growth regulators exert far reaching effects on plant growth; the precise action depends on the concentrations of the substances present and the sensitivity of the concerned organ. In vitro seed culture is a method for producing improved regenerants under controlled condition, especially under conditions where seeds are scarce and expensive to purchase. Conservation and multiplication can however be more easily carried out on plantlets grown in vitro through selecting plants grown under field conditions. Micropropagation technique has been proven an important tool to maintain the genetic fidelity in *A. annua* L. plants including many others crops. It can also be used to select high artemisinin yielding strains of *A. annua* L. and maintain the purity of lines.

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Chapter 5
Medicinal and Aromatic Plants Under Abiotic Stress: A Crosstalk on Phytohormones' Perspective

Abbu Zaid, Bilal Ahmad, and Shabir H. Wani

5.1 Introduction

Medicinal and aromatic plants (MAPs) have been identified as a great treasure and one of the most important biodiversities bestowed by nature to mankind. MAPs significantly affect human life across the globe. These plants play a significant role in human civilization and have impacted the lives of people in one way or the other since time immemorial. People in ancient times became aware of the pharmaceutical importance of various kinds of MAPs. These plants and their products are considered as a valuable source of ingredients/constituents used in pharmaceutical industries and factories. These plants are rich in various kinds of specialized primary and secondary metabolites (SMs) having useful pharmacological properties. These plants have characteristics like fragrance, flavor, pigment, insecticidal, and therapeutic (Facchini et al. 2012). According to an estimate, there are more than 200,000 to 1,000,000 diverse plant metabolites that are produced by the plant kingdom (Dixon 2003; Afendi et al. 2011). These metabolites help plants to adapt and survive under different abiotic stresses, biotic invasions, and ecological niches (Mazid et al. 2011; Fürstenberg-Hägg et al. 2013; Weng 2014; Lajayer et al. 2017; Zinta et al. 2018; Banerjee et al. 2019). At present, there are more than 80% of the world population that use traditional MAPs (especially plant extracts and essential oils) for their primary health need (Thatoi and Patra 2011). These plants are chiefly

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used for extraction of compounds like vincristine, vinblastine, aspirin, atropine, artemisinin, lobeline, strychnine, berberine, colchicine, digoxin, ephedrine, morphine, menthol, menthone, methyl acetate, physostigmine, pilocarpine, quinine, quinidine, reserpine, Taxol, teniposide, tubocurarine, etc. (Balandrin and Klocke 1988; Lawrence 2006; Ramawat and Goyal 2008). A large number of MAPs have radical scavenging activity. MAPs are also used for extraction of essential oils. The essential oil finds application in detergents, soaps, perfumes, cosmetics, insect repellents, and food processing industries. In addition to their socioeconomic importance to the communities that produce them, these crops also play an important environmental role. These plants are also used in phytoremediation, as an indicator of toxic metal/metalloid pollution in the soil environment (Chaiyarat et al. 2011; Gupta et al. 2013). However, due to various abiotic stresses, growth and productivity of MAPs are affected in the current times.

Growth and reproduction of plants in a complex and dynamic environment is affected by a range of abiotic and biotic pressures. Abiotic stresses like cold, salt, heat, drought, high temperature, and metal/metalloid and biotic stresses like pests, pathogens, and fungal stress limit growth and productivity of crop plants considerably. The abiotic ones are nonliving components and include, among others, wind, mechanical injuries, submergence, light intensity and quality, temperature, anaerobiosis, water availability, deficiency of mineral nutrients, acid conditions, and salt and soil conditions, whereas the biotic system comprises animate entities such as man, herbivores, pathogens, insects, pests, weeds, etc. (Cramer et al. 2011; Kissoudis et al. 2014; Zinta et al. 2016; Cao et al. 2017; Meena et al. 2017; Feller et al. 2017; He et al. 2018). However, plants being sessile are challenged by both biotic and non-biotic pressures simultaneously which act in combination, and the responses of plants to combined stress differ significantly from that induced by each factor singly. The change in one or more of these environmental cues out of their normal ranges induces alterations in a myriad of physiological and biochemical processes, and the condition is referred to as environmental stress in plants. Such alterations cause a serious threat to the sustainability of growth and development of crop plants.

Nevertheless, depending on its nature, the non-biotic stress may cause in plants, among others, water potential reduction, dehydration of cellular contents, hydraulic resistance, ion cytotoxicity, overproduction of reactive oxygen species, inhibition of protein synthesis, disrupted membrane stability, and damage to DNA/RNA and other macromolecules (Gill and Tuteja 2010; Gémes et al. 2011; Konotop et al. 2012; Zhu 2016; Zaid et al. 2018; Zaid and Mohammad 2018; Wani et al. 2018a, b; Ahmad et al. 2018a, b; 2019). According to the report of the Food and Agricultural Organization of the United Nations (2007), there is 3.5% of the total world land area that is unaffected by any abiotic stress factor (http://www.fao.org/docrep/010/a1075e/a1075e00.html). Also, abiotic stress-induced altered metabolism may result in 51–82% of annual agricultural crop yield loss (Bray et al. 2000). In MAPs, various kinds of abiotic stresses reduce the quality and quantity of crop production. A representation showing the role of various phytohormones under stressful and optimal conditions in some selected MAPs is given in Table 5.1.

Table 5.1 Table showing the representative studies of various medicinal and aromatic plants under stressful/normal conditions and their response mediated by various phytohormones

Plant name	Abiotic stress factor/ condition	Phytohormone	Response	Reference
Mentha arvensis	Metal stress	Methyl jasmonate	Decreased plant growth (length of root and shoot, fresh and dry masses of shoot and leaf area); decreased photosynthetic pigments (chlorophyll and carotenoids content) and leaf gas exchange traits and leaf nitrogen, phosphorous, and potassium content; increased activities of ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and osmolytes; stomatal movement inhibition	Zaid and Mohammad (2018)
Mentha piperita	Metal stress	Salicylic acid	Decreased morphological parameters and photosynthetic traits and nitrate reductase and carbonic anhydrase activities; increased synthesis of total phenolics, superoxide dismutase (SOD), and peroxidase (POX) activities and proline content; decreased essential oil content and yield and secondary metabolite in the tested plant	Ahmad et al. (2018b)
Cuminum cyminum	Optimal conditions	Salicylic acid and methyl jasmonate	Salicylic acid (0.01 and 0.1 mM) resulted in a significant increase of plant height, number of branches and umbels per plant, fruit yield, and essential oil yield	Rahimi et al. (2013)

(continued)

Table 5.1 (continued)

Plant name	Abiotic stress factor/condition	Phytohormone	Response	Reference
Mentha piperita	Optimal conditions	Brassinosteroids	Plant fresh and dry weights increased in line with rising concentrations of brassinosteroid application. Essential oil content increased with 0.5 ppm of brassinosteroid application, which also modified essential oil composition. Brassinosteroid application Had positive effects on menthol content, and the highest menthol content was found in plants treated with 0.5 ppm	Çoban and Baydar (2017)
Mentha piperita	Optimal conditions	Melatonin, salicylic acid, and gibberellic acid	Application of SA increased the oil content. A total of 33 compounds were detected in the oil samples treated with graded levels of SA supply with menthone, menthol, methyl acetate, and 1,8-cineole being the major secondary metabolite	Saharkhiz and Goudarzi (2014)
Cymbopogon flexuosus Steud. Wats	Water stress	Salicylic acid	Salicylic acid supply reduces the detrimental effects of water deficit. Water stress reduced growth parameters; however, foliar application of salicylic acid improved the growth parameters—Changes in activities of nitrate reductase and carbonic anhydrase. Electrolyte leakage, proline content, free amino acid, and PEP carboxylase activity increased in response to water-deficit conditions. Content and yield of oil decreased under water stress	Idrees et al. (2010)

(continued)

Table 5.1 (continued)

Plant name	Abiotic stress factor/ condition	Phytohormone	Response	Reference
Cucumis sativus	Salt stress	Salicylic acid	Salinity treatments (0, 60, and 120 mM) on sodium chloride (NaCl) stress negatively affected the growth, chlorophyll content, and mineral uptake. Foliar applications of salicylic acid caused a greater increase in shoot fresh weight, shoot dry weight, root fresh weight, shoot diameter, leaf number, and root dry weight as well as higher plants under salt stress conditions. Leaf water relative content was found to be reduced in response to salt stress, while SA raised these values. Salt treatments increased electrolyte-reduced nutrient content, but plants treated with foliar SA had lower values of electrolyte leakage and high nutrient content	Yildirim et al. (2008)
Mentha piperita	Optimal conditions	Benzylaminopurine, chlormequat, gibberellic acid, indole-3-acetic acid, and indole-3-butyric acid, kinetin, methyl jasmonate, salicylic acid, and triacontanol	Salicylic acid proved best for increasing oil content, oil yield, menthol concentration, and menthol yield. However, benzylaminopurine maximally enhanced menthone content; gibberellic acid menthyl acetate content; triacontanol for menthone yield and again salicylic acid menthyl acetate yield	Khanam and Mohammad (2017)

(continued)

Table 5.1 (continued)

Plant name	Abiotic stress factor/ condition	Phytohormone	Response	Reference
Capsicum annuum	Temperature stress	Salicylic acid	Salicylic acid treatment increased germination rate and potential and lowered high temperature-induced oxidative damage. SA also maintained the integrity of the cell structure under stress conditions. SA lowered the production of reactive oxygen species (ROS), by increasing the activities of protective enzymes under stress conditions. SA also maintained photosynthetic capacity under stress	Zhang et al. (2019)
Artemisia annua	Metalloid stress (boron)	Methyl jasmonate	Boron induced oxidative stress and negatively impacted the stem height as well as fresh and dry masses of the plant, the net photosynthetic rate, stomatal conductance, internal CO_2 concentration, and total chlorophyll content. Foliar application of methyl jasmonate circumvents the B-induced changes in growth and photosynthetic efficiency and increased the activities of antioxidant enzymes. Foliar methyl jasmonate also reduced and enhanced the content and yield of secondary metabolites in the tested plants	Aftab et al. (2011)

In MAPs, the production of secondary metabolites (SMs) is affected by abiotic stress factors. Secondary metabolites are defined as those natural products biosynthesized by an organism which is not important to sustain growth and life. In the plant kingdom, over 200,000 distinct chemical compounds are manufactured, most of which are biosynthesized from specialized metabolism playing significant roles in the interspecies competition (Pyne et al. 2019). The medicinal value and aroma of the plants are due to the presence of distinct types of SMs. However, in contrast to primary metabolites, SMs have very limited distribution in plants. During the past

50 years, comprehensive research on plant SMs has increased manifold as a result of their necessity in day-to-day lives which includes health benefits of plant products (Mulabagal and Tsay 2004; Jamwal et al. 2018). Many of the SMs are found in ppm levels in natural conditions. There are various signaling molecules or elicitors which induce accumulation of various kinds of SMs in MAPs. In plants, SMs are the principal source of pharmaceuticals compounds which play a profound role in the adaptation of plants to various abiotic factors (Rao and Ravishankar 2002; Demkura et al. 2010; Zhou and Memelink 2016; Çoban and Baydar 2016; Nakajima et al. 2019; Osama et al. 2019; Huang et al. 2019). In view of the current scenario, there is an urgent need for increasing awareness and unraveling the adaptive mechanisms that may prove fruitful in engineering resistance in growth and development in one hand and boost the biosynthesis of SMs in MAPs.

Phytohormones are organic signaling molecules, produced in minute concentration that regulates growth and development under stressful and optimal conditions in plants (Fahad et al. 2015; Wani et al. 2016; Sytar et al. 2018; Ullah et al. 2018; Podlešáková et al. 2018; Banerjee and Roychoudhury 2018; Ahanger et al. 2018; Zaid and Mohammad 2018; Ciura and Kruk 2018; Maury et al. 2019). External supplementation of phytohormones is a promising technique to engineer tolerance against various types of abiotic stress and to increase the production of SMs in MAPs. Little literature is available on the role of phytohormones' mediated action under abiotic stress in MAPs. Therefore considering the importance, the present chapter comprehensively reviews the recent updates on the role of various phytohormones in MAPs in conferring abiotic stress tolerance. In the following section, we are discussing the role of various phytohormones in specific MAPs under various abiotic stresses.

5.1.1 Mentha Arvensis

Mentha arvensis, commonly called as menthol mint/Japanese mint/corn mint, is a perennial herbaceous plant belonging to Lamiaceae (Labiatae) family which is widely distributed across Europe, Western and Central Asia, Siberia, North America, and east to the Himalayan region (Lawrence 2006). The genus *Mentha* includes around 30 species (Bhattacharjee 2000). This plant has long been used for extraction of useful SMs which possess antiviral, antifungal, diaphoretic, anti-spasmodic, stomachic, choleretic, antibacterial, carminative, vermifuge, and numerous other medicinal properties (Bhat et al. 2002; Tiwari 2016; Naeem et al. 2017; Rajpoot et al. 2018). Owing to its high adaptability, menthol mint is widely cultivated in tropical and subtropical regions of the world, and menthol is the chief SM which is extracted from it (Chand et al. 2004; Pande et al. 2007). In India, presently 150,000 ha acres of land is under menthol mint cultivation which spreads over the Indo-Gangetic plains, and 20,000 tons of EO is produced (Anonymous 2012). The essential oil obtained from its fresh shoot part is rich in menthol (62–78%), menthone (3.6–19.32%), isomenthone (2.65–3.56%), and limonene (0.98–4.47%)

(Tiwari 2016). Across the globe, menthol mint is commercially grown for its essential oil content and herbage yield (Zheljazkov et al. 2010). The annual essential oil production of *Mentha arvensis* is 22,000 mt; India is its largest producer contributing to nearly 16,000 mt of essential oil per year (Khanuja et al. 2006; Patra et al. 2002).

Research on abiotic stress tolerance in menthol mint is scarce; however, there are some reports which we are discussing in the accompanying section. In the first report on menthol mint, Zaid and Mohammad (2018), in their classical study, showed the regulatory impacts of phytohormone and nutrient supply in reducing cadmium-induced toxic impacts. In their study, they reported that Cd-induced oxidative stress by orchestrating the ROS biosynthesis reduced nutrient contents, imposes stomatal inhibition, and retards growth and photosynthetic traits but increased antioxidant enzyme activity and osmolytes' and endogenous Cd content in menthol mint. However, phytohormone (methyl jasmonate)- and nutrient (nitrogen)-mediated crosstalk restored the damage done by Cd stress and optimized the growth and photosynthesis of menthol mint plants. In response to salinity stress, Bharti et al. (2014) reported the effect of plant growth-promoting rhizobacteria (PGPR) in alleviating salinity (0, 100, 300, 500 mM)-induced changes in growth, oil content, and physiological status in menthol mint. They reported that increasing levels of salt concentration caused a linear decrease in growth, fresh weight, leaf-stem ratio, and oil content and yield of menthol mint var. Kosi. However, the negative effects of salt were convalesced in plants inoculated by the PGPR. In yet another study, Bharti et al. (2016) reported the effectiveness of *Glomus* spp. in improving productivity, oil content, and tolerance against salt-stressed menthol mint plants.

In response to waterlogging stress, a comparative study was performed by Phukan et al. (2014) involving *Mentha arvensis* and *Mentha piperita* plants by subjecting them to sustained waterlogging stress. They found that *Mentha arvensis* exhibited better adaptive characters in contrast to *Mentha piperita* under stress conditions. *Mentha arvensis* plants exhibited a healthy posture and well-flourished vegetative growth under waterlogged stress. Soluble protein content, chlorophyll, relative water, and nitric oxide scavenging activity were comparatively increased in *Mentha arvensis* during stress treatment. The process of peroxidation of lipids was also less and also exhibited vigorous outgrowth of roots under waterlogging stress. In order to further validate the results, quantitative real-time polymerase chain reaction was carried out, which showed the induced expression of proteins in *Mentha arvensis* in comparison to *Mentha piperita*. In salt-stressed *Mentha arvensis*, Bharti et al. (2016) studied the integrated application of *Exiguobacterium oxidotolerans*, *Glomus fasciculatum*, and vermicompost in improving growth, yield, and quality. Their results showed that the multi-microbial inoculation and vermicompost-treated plants recorded the highest fresh weights and oil yield under saline conditions. In a yet recent study, Choudhary et al. (2020) studied the effects of B toxicity on growth traits, oxidative damage indices, antioxidant modulation, and essential oil fingerprinting in *Mentha arvensis*. Their findings suggested that increasing doses of B impose growth and photosynthetic pigment inhibitions but incremented the oxidative damage and activities of antioxidant enzymes. Nevertheless, low dose of B orchestrated the production of essential oil. Thus, from the above literature, it is clear that abiotic stress poses negative impacts on the crop yield of *Mentha arvensis* plants.

5.1.2 Mentha Piperita

Mentha piperita, commonly called as peppermint, is one of the oldest known medicinal plants (Sastri 1950). This plant was adjudged as a medicinal plant of the year in 2004 (Saller 2004). This EO-bearing MAP is of paramount economic importance due to its EO and because of its widespread use in food industry, confectionaries, mouthwashes, toothpastes, herbal preparations, chewing gums, and manufacture of various aromatherapy-related products (MIRC 2010; Santoro et al. 2013; Barros et al. 2015). Oil obtained from peppermint can be externally applied because of its antiseptic, antifungal, rubefacient, antimicrobial, antioxidant, and astringent properties (Ford et al. 2008; Khanam 2014; Kapp 2015). This plant has long been used as a food preservative and in the treatment of diseases like migraines, neuralgia, myalgia, and viral infections (Kline et al. 2001; Mohsenzadeh 2007; Singh et al. 2015). A range of abiotic stress factors alters the growth and development of peppermint plants. These factors are also known to negatively affect yield and quality attributes of this plants. In a recent study, Khanam and Mohammad (2018) studied the applicability of plant growth regulators in ameliorating the ill effect of salt stress (0, 50, 100 or 150 mM NaCl) in *Mentha piperita*. In a factorial randomized design experiment, graded levels of salt stress (0, 50, 100, or 150 mM NaCl) were applied 30 days after transplanting suckers in pots. Salinity decreased growth traits; photosynthetic parameters; contents of nitrogen, phosphorous, and potassium; carbonic anhydrase activity; peltate glandular trichome density; essential oil and SM content; and herbage yield of plants. In contrast, salt stress increased the activities of catalase, peroxidase, superoxide dismutase, and proline content. Foliar spray of phytohormones salicylic acid, gibberellic acid, and triacontanol improved all parameters under salt conditions. The interactive effect of 150 mM NaCl × salicylic acid proved best in maximally increasing antioxidant activities, proline content, trichome density, and EO content.

In yet another study, Çoban and Baydar (2016) carried out an experiment in order to examine the effect of steroidal phytohormone (brassinosteroid, 0, 0.5, 1.5, and 2.5 mg L^{-1})-mediated changes in growth, biochemical properties, and secondary metabolite accumulation in *Mentha piperita* plants under 0, 100, and 150 mM of salt stress. Plant growth traits such as fresh and dry weights of leaves and plants and EO content decreased in line with the elevating level of salinity, while lipid peroxidation, proline, antioxidant enzyme activity, and total phenolic content increased in a salt concentration-dependent manner. They further observed that brassinosteroid applications prevented the death of the plant under high (150 mM) salt stress by boosting the antioxidant enzymes activities and preventing salt-induced oxidative stress. Nevertheless, brassinosteroid application significantly triggered the biosynthesis of essential oil content under salt stress conditions in peppermint plants. In a recent study, Ahmad et al. (2018b) designed a pot experiment to unravel the response of foliar salicylic acid on cadmium-induced photosynthetic, antioxidant changes and essential oil production in peppermint plants. Three doses of cadmium (30, 60, and 120 mg kg^{-1}) were applied to the pots through the soil. The effect of SA under Cd stress was assessed by spraying SA (10^{-4} M) at an interval of 7 days. Results showed that exogenously sourced SA ameliorated Cd-induced growth inhibition, protected photosynthetic traits, and essential oil production against Cd-accrued stress. Salicylic acid improved Cd-induced toxicity by improving photosynthesis through enhancing activities of RuBisCo and carbonic anhydrase and minimizing oxidative stress. In addition, Cd mediated decrease of EO content, and SM concentration was successfully counteracted by the application of SA treatment in peppermint plants.

In a greenhouse randomized complete block design experiment, Amirmoradi et al. (2012) studied the effect of Cd and lead on quantitative and EO traits of peppermint plants. Plants were irrigated with Cd (10, 20, 40, 60, 80, 100 ppm) and Pb (100, 300, 600, 900, 1200, 1500 ppm). Results depicted that with increasing concentrations of Cd and Pb, fresh and dry weights, main stem height, leaf area per plant, leaf number, number of nodes per main stem, and EO of the plant showed a decreasing trend. The results suggested that peppermint plants can tolerate soil challenged with a medium range of Cd and Pb toxicity, and the percentage of EO was not affected by these metals. It is now evident from the above discussion that abiotic stress factors limit the growth and metabolism of peppermint plants adversely. However phytohormone application initiates the cascades of signaling events which restore the altered metabolism and promote optimum photosynthesis and growth under abiotic stress factors.

5.1.3 Catharanthus roseus

Catharanthus roseus, belonging to Apocynaceae family, is an important MAP cultivated mainly for its alkaloids and is used as a traditional herbal medicine globally; its chemical extraction has a role in cancer treatment, because of its anticancer activities. The plant has been called a miracle plant for its use in the prevention of leukemia and cancer treatment. It is also called *Vinca rosea*, *Ammocallis rosea*, and *Lochnera rosea*. The plant is an evergreen sub-herb or herb attaining a height up to 1 m tall. The leaves are oval to oblong, broad glossy green, and glabrous having a short petiole and pale midrib which are oppositely arranged. The flowers have a white to dark pink color encircling a dark red center. The terpenoid indole anticancer alkaloids vinblastine and vincristine are mainly derived from the leaf and stem of the plant and are the most pharmaceutically valued compounds (Moreno et al. 1995; Gueritte and Fahy 2005; El-Sayed and Verpoorte 2007; Sain and Sharma 2013). SMs in *C. roseus* were reported to be tightly affected with various kinds of environmental stress factors such as salt, UV-B, and water and nutrient deficiency

(Peebles et al. 2009; Srivastava and Srivastava 2010; Guo et al. 2014). In addition, growth and development of this important MAP is negatively affected by environmental stress factors. In a study, Jaleel et al. (2007) studied the role of calcium chloride on 80 mM sodium chloride-induced oxidative stress, proline metabolism, and indole alkaloid accumulation in *C. roseus* plants. They found that NaCl induced oxidative stress by increasing lipid peroxidation and H_2O_2, glycine betaine, and proline contents. The highest SM accumulation was recorded in NaCl-treated plants. Antioxidant enzyme activities like those of superoxide dismutase, peroxidase, and catalase were increased in response to salinity. The application of calcium chloride to NaCl-treated *C. roseus* plants showed an increasing trend in total SM content.

Paeizi et al. (2018) investigated the effects of exogenous methyl jasmonate (100 μM) and silver nitrate (50 and 100 μM) on the production of vincristine, vinblastine, ajmalicine, vindoline, and catharanthine in shoots of *C. roseus* plants. A decreasing trend in protein and photosynthetic pigment contents was observed under silver nitrate supply in plants. They found increased contents of alkaloids and expression of genes under the supplied treatments. However, exogenous methyl jasmonate in combination with silver nitrate triggered the maximum yields of these alkaloids. Nevertheless, gene expression levels were found to be the highest under methyl jasmonate and silver nitrate treatments in the tested plants. They concluded their study by observing that phytohormone (methyl jasmonate) and silver nitrate combination is an effective way to elicit SM biosynthesis in *C. roseus* plants. In a metabolomics analysis, Liu et al. (2016) studied the combinational effects of two phytohormones, namely, ethylene and methyl jasmonate, on metabolic profiling of phenolic compounds in *C. roseus*. They identified a total of 34 kinds of phenolic compounds in the plant. The interactive actions of ethylene and methyl jasmonate effectively promoted the biosynthesis of phenolic compounds. In a recent study, Nabaei and Amooaghaie (2019) work out the interactive effect of phytohormones (melatonin and sodium nitroprusside) in *C. roseus* under 200 μM of cadmium stress. Their results showed that Cd negatively affected the germination of seed, root elongation, and seedling fresh and dry weight. The combined applications of phytohormone improved the germination rate of seeds, length, and vigor index of seedlings under Cd stress. Cadmium application decreased the amylase activity and contents of soluble and reducing sugars while increasing the electrolyte leakage in *C. roseus* plants.

5.1.4 Artemisia Annua

Artemisia annua belongs to the Asteraceae family, and the main stem reaches about 2 m in height. The leaves of this MAP are about 2.5–5.0 cm, dissected, and alternate. The inflorescence is typical capitula, and the central flowers are either fertile or sterile. There are non-glandular and glandular trichomes which have been found on the surfaces of leaves, stems, and flowers of this plant. *A. annua* produces 40 volatile compounds and a lot of non-volatile compounds, and artemisinin and other artemisinic compounds are the most important SM which are isolated from this important MAP and used as antipyretic and analgesic having minimal side effects (Ferreira and Janick 1996; Xiao et al. 2016; Zhai et al. 2019). The growth development and yield of this important plant is affected by a myriad of abiotic stress factors. A handful of literature is available on the effect of abiotic stress factors on *A. annua* plant. However there are few reports on phytohormone-mediated reversal of abiotic stress effects in *A. annua* plant. In a pot culture experiment, Aftab et al. (2011) studied the effect of methyl jasmonate (300 μM) in counteracting boron (B) toxicity and artemisinin biosynthesis in *A. annua* plants. They found that B (1 and 2 mM) toxicity generated oxidative stress by increasing endogenous ROS levels and negatively affected height as well as fresh and dry masses of the plant, net photosynthetic rate, stomatal conductance, internal CO_2 concentration, and total chlorophyll content but boosted the activities of antioxidant enzymes, such as catalase, peroxidase, and superoxide dismutase. However, the foliar application of MeJ (300 μM) alleviated B-induced growth and photosynthetic inhibition. Nevertheless, MeJ application enhanced the content and yield of artemisinin in the tested plants.

In order to study the effects of phytohormones (triacontanol and chlormequat), Shukla et al. (1992) designed a randomized complete block design experiment with three replications to study the effect of triacontanol and chlormequat on plant growth, endogenous phytohormones, and artemisinin yield in *Artemisia annua*. They observed that triacontanol (1 and 1.5 mg/L) results in enhanced artemisinin level, plant height, and leaf and herbage yield. In a yet recent experiment, Kumari et al. (2018) applied exogenous salicylic acid (100 μM) to ascertain the modulation of arsenic stress (100 and 150 μM) tolerance in *Artemisia annua*. Their results suggested that As application decreased biomass and photosynthetic potential but resulted in the induction of H_2O_2 and MDA contents. Furthermore, As also enhanced the enzymatic (SOD, CAT, APX, and GR) and non0enzymatic antioxidant. Application of SA considerably reduced the As-induced ROS and ameliorated biomass and chlorophyll inhibition. Combined application of phytohormone (SA) with As increased endogenous SA, artemisinin, and dihydroartemisinic acid levels. The study got a further confirmation by observing the expression levels of key genes involved in artemisinin biosynthetic pathway, viz., *ADS*, *CYP71AV1*, *DBR2*, and *ALDH1* that were found to be upregulated to a maximum extent under SA and As treatments. They concluded their study by observing that SA and As induced tolerance in *A. annua* plants on the one hand and enhanced the biosynthesis of artemisinin on the other. Therefore, in view of the above reports, we can conclude that abiotic stress factors negatively affect the growth and development of this important MAP and phytohormones and in turn circumvent the changes and promote optimum plant metabolism.

5.2 Conclusion and Future Outlook

Medicinal and aromatic plants when exposed to stress conditions experience retardation in growth and development. Abiotic stresses also affect the production of secondary metabolites in MAPs. Stress factors modulate the expression of genes encoding diverse proteins which regulate the metabolism of MAPs. Phytohormones mediate the intimate crosstalk with signaling agents under stress conditions in MAPs which impart stress resistance under stress environments. Phytohormones also alter the production of secondary metabolites under abiotic stresses. Therefore the supplementation of phytohormones in MAPs under abiotic stress factors could act as a double-edged sword sustainably by boosting resistance on the one hand and increasing the production of secondary metabolites on the other.

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Chapter 6
Cytokinin-Mediated Signalling During Environmental Stress in Plants

Ankur Singh and Aryadeep Roychoudhury

6.1 Introduction

Plants are sessile living organisms which cannot escape unfavourable environment by shifting their positions. Harsh environmental conditions are negative factors which adversely affect the growth, biomass and yield of plants. Stress faced by plants may be due to the invading pathogens or infestations of insects collectively known as biotic stress and abiotic stress which occurs due to hostile environmental conditions like drought, salinity, extreme temperature or heavy metal toxicity (Roychoudhury et al. 2013). Harsh conditions lead to oxidative burst in plant tissues which ultimately enhances the formation of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), superoxide ($\cdot O_2^-$) and hydroxyl radicals ($\cdot OH$). These ROS lead to cellular membrane damages and formation of toxic products like malondialdehyde (MDA) and methylglyoxal (Banerjee and Roychoudhury 2018). Plants have several mechanisms which help them adapt and survive under unfavourable conditions. Production of various endogenous signalling molecules helps to coordinate and enhance the functions of the internal defensive pathways which ultimately induce their survival capability under severe environmental conditions.

In addition to the action of other stress hormones such as abscisic acid (ABA), jasmonate and salicylic acid, cytokinin (CK) also plays an important role in regulating the action of protective machineries of plants (Ha et al. 2012). CKs were initially believed to be only involved in cell division and in the regulation of the cell cycle (Schaller et al. 2014), but later it was found that CKs also govern various other functions like inhibition of root growth, maintenance of apical dominance, formation of shoot meristem, growth of lateral buds, expansion and senescence of leaves and nitrogen signalling pathways (Frebort et al. 2011; Giulini et al. 2004; Miyawaki

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et al. 2004; Nooden et al. 1990). Analysis of the interactive pathways involved between CKs and environmental stress has recently begun and is now well characterised (O'Brien and Benkova 2013; Ha et al. 2012).

CKs are derivatives of adenine residue with a substitution at N6 position by isoprenoid or aromatic acid residues (Sakakibara 2006). Based on the reduction or hydroxylation of the side chains, a wide variety of derivatives of CKs are found in nature such as cis-zeatin, trans-zeatin, isopentenyladenine and dihydrozeatin known as isoprenoid CKs. N6-(meta-hydroxybenzyl) adenine is an aromatic CK found in less quantity in plants (Faiss et al. 1997). CKs in plants are mostly found in immature seeds, meristematic regions and young leaves. Endogenous level of CK in plants is maintained by the activity of various enzymes which are mainly responsible for biosynthesis of CKs or alteration of one member of CK family to other members (Letham 1994). Expression of targeted genes can be regulated by CK through the action of a two-component system where transfer of phosphate unit between Asp and His residues helps to transduce the signal which ultimately facilitates plants to provide suitable response against stimuli (Huang et al. 2012).

In this chapter, we summarise the emerging role of CKs in plant systems when exposed to different stress conditions. We begin with the metabolism and signalling pathway of CKs in plants and then cover the different aspects of CKs under various abiotic stresses like drought, salinity, extreme temperature, light, nutrient deficiency and heavy metal stress. We also provide an overview of protective role of CKs under different biotic stresses. For each of these stress conditions, we present an extensive analysis on the role of CK signalling pathway and its interaction with other stress hormones and metabolites which are involved in enhancing the tolerance limit of plants against harsh environmental conditions.

6.2 Metabolic Pathway of CKs

An interesting feature of the CKs is that they are made of purine and differ from adenine and other members of CK family by the presence of various chemical groups at N6 position which also determines their biological activity, transportation and presence in the cells or tissues of plants (Hirose et al. 2008). The two major forms of CKs present in *Arabidopsis* are trans-zeatin and isopentenyladenine (Kiba et al. 2013) which shows acropetal and basipetal movement, respectively, during long-range transportation (Matsumoto-Kitano et al. 2008). CK receptors also have distinctive binding affinity to different CKs involved in the signalling pathway in *Arabidopsis* (Stolz et al. 2011; Romanov et al. 2006). Cis-zeatin, an isoform of trans-zeatin, is less active and is found in some monocots and dicots, and its expression is mostly dependent on the growth stage of the plants (Frebort et al. 2011). Dihydrozeatin which is formed in *Phaseolus vulgaris* by the enzymatic action of zeatin reductase on trans-zeatin (Martin et al. 1989) was found in apical buds and seeds (Mok et al. 1990). In addition, aromatic CKs are also present in plants such as tomato (Nandi et al. 1989), *Arabidopsis* (Tarkowska et al. 2003), pea (Gaudinova et al. 2005) and *Populus* (Strnad 1997) but are less studied, and their presence and activity need to be analysed in other crops. Inactive forms of CKs are synthesised by de novo pathway, and phosphoribohydrolase activity by Lonely Guy (LOG) enzyme is required to make free active base CKs (Sakakibara 2010). Interconversion between active and inactive forms of CKs can be done by the application of adenosine kinase (Schoor et al. 2011). Plants can regulate the activity of N- and O-glucosyltransferases which are involved in the glucosylation of CKs, producing their bioactive forms (Veach et al. 2003).

It was earlier considered that CKs were formed in the roots of plants and transported upward to the shoot, but recent studies have shown that CKs are also locally synthesised in the plant cells. Different members of CKs are present in different plant tissues and in different species which affirms that different CKs have different functions (Hirose et al. 2008), for example, members of isopentenyladenine family of CKs are more found in the seeds of oil palm (Huntley et al. 2002), whereas dihydrozeatin and zeatin are mostly found in soybean seeds (Singh et al. 1988). In addition, members of trans-zeatin family are mostly found in xylem sap, and the members of isopentenyladenine and cis-zeatin family are predominantly present in phloem sap (Hirose et al. 2008). The concentration of CKs is also mediated by the interaction of plants with extrinsic factors such as biotic and abiotic stress (Schafer et al. 2014; Miyawaki et al. 2004; Hashem 2013).

The metabolic pathway of CKs can be divided into three phases: synthesis of CKs by isopentenyltransferase (IPT) enzymes, activation of the synthesised inactive forms by the activity of LOG enzymes and degradation by CK dehydrogenases (CKX). Miyawaki et al. (2004) reported that the presence of *AtIPT* in *Arabidopsis* is required for the production of tRNA- and ATP/ADP-IPTs. IPT produces isopentenyladenine using AMP, ADP or ATP as isoprenoid acceptors for isopentenylation and dimethylallyl pyrophosphate (DMAPP) as the side chain donor generated from methylerythritol phosphate and mevalonate pathways (Sakakibara 2006). Synthesis of isopentenyladenine is initiated from its nucleoside and nucleotide precursors, whereas trans-zeatin are produced either using isopentenyladenine directly or indirectly using the immediate precursors of isopentenyladenine. Sakakibara (2010) reported another pathway involved in the synthesis of trans-zeatin by the activity of isopentenyltransferase using hydroxylated terpenoid as a side chain donor. Isopentenylation of tRNA by AtIP2 and AtIP9 leads to the formation tRNA-IPTs (Miyawaki et al. 2006). Decomposition of isopentenylated tRNAs leads to the formation of cis-zeatin in a very low amount due to longer half-life of tRNAs (Klambt 1992). Bassil et al. (1993) reported that isomerisation of trans-zeatin to cis-zeatin in the presence of a catalyst occurs in beans. Members of the dihydrozeatin family are synthesised from zeatin by zeatin reductase (Gaudinova et al. 2005).

LOG enzyme plays a major role in the activation of isopentenyladenine and trans-zeatin. Phosphoribohydrolase activity of LOG enzymes on the nucleotide 5′-monophosphates unit of CKs is essential for their function (Frebort et al. 2011). CKs may be activated by the LOG-dependent pathway which is considered as the major pathway for CK activation or by the two-step pathway which is independent of LOG enzyme (Tokunaga et al. 2012). LOG independence is not well characterised, and very few studies have been conducted which can elaborate the mechanism of this pathway (Thu et al. 2017). Tokunaga et al. (2012) reported that seven genes encoding LOG enzymes are present in *Arabidopsis*, where most of the functions are performed by LOG7 enzymes. Other homologs of LOG enzymes have been recently discovered in plants like *Prunus persica* and *Populus trichocarpa* (Immanen et al. 2013).

Catabolism of CKs takes place by addition of a sugar moiety by glycosyltransferase reaction or by irreversible dehydrogenation (Zalabak et al. 2013). CKXs cleave the N6 moiety of trans- and cis-zeatin and isopentenyladenine, whereas the aromatic side chain of CKs is cleaved at a lower rate; dihydrozeatin are non-cleavable by this enzyme (Galuszka et al. 2007). CKXs belong to a small family, containing only seven members which are species-specific, and play an essential role in maintaining the homeostasis of the cells (Bilyeu et al. 2001). In *Arabidopsis*, cis-zeatin is mostly cleaved by AtCKX 1 and AtCKX 7, whereas AtCKX 2, AtCKX 3 and AtCKX 4 cannot hydrolyse it (Gajdosova et al. 2011). Another way to maintain the level of CKs in the cells is by glycosylation. O-glucosyltransferases and O-xylosyltransferases are involved in reversible glycosylation of CK residues to O-glucosylated and O-xylosylated forms, respectively. N-glucosyltransferases produce N9-glucosylated or N7-glucosylated CK residues which cannot be degraded by β-glucosidases (Thu et al. 2017).

6.3 CK-Mediated Signalling Pathway and Its Components

6.3.1 Signal Transduction by CKs

CK signalling pathway is a multistep process which involves the phosphorylation of histidine kinase (HK) protein acting as a receptor, followed by the transfer of signal by histidine phosphotransfer (HP) protein and finally receiving the signal and accordingly responding by an ultimate response regulator (RR) (Thu et al. 2017). The signalling pathway starts when stimuli leads to the formation of the CK molecules which binds to the cyclase/histidine kinase associated sensory extracellular (CHASE) domain of the HK receptor. This binding leads to the transfer a phosphoryl group from the histidine residue to the aspartate molecules present in the receptors. The histidine residue present in HP protein then receives the phosphoryl group which ultimately transfers the residue to the aspartate residue of the RR which helps to initiate the transcription of the targeted genes (Fig. 6.1). The signalling pathway is dependent on the HK receptors because it has been reported in *Arabidopsis* that HK receptors are highly specific to the substituted side chain residues of the CKs (Hwang et al. 2012).

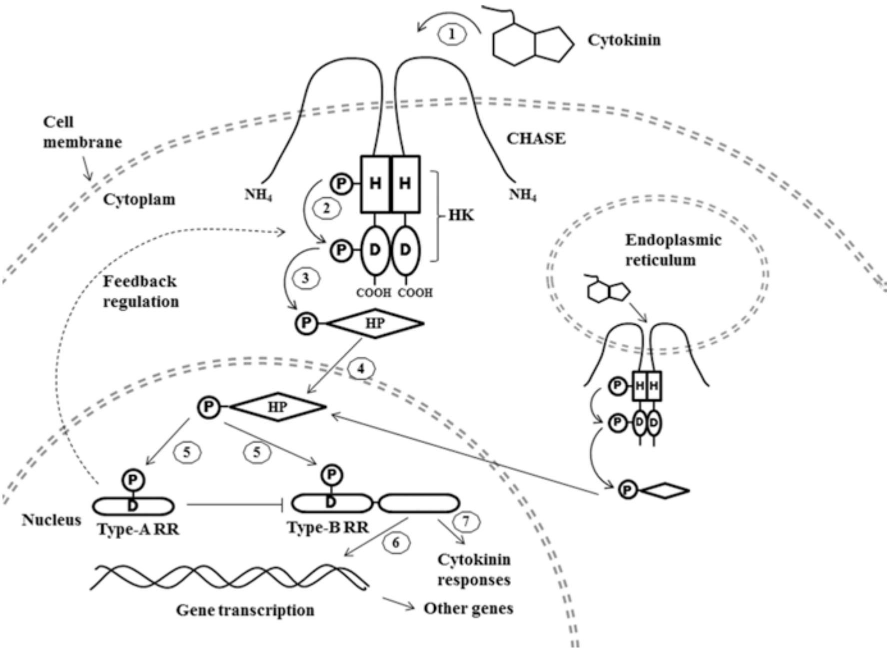

Fig. 6.1 Two-component CK signalling system. The HK receptor for CKs which is activated in plants due to external stimuli is mainly located on the membrane of cells and endoplasmic reticulum. The pathway is initiated by the binding of CKs to the CHASE domain of HK receptor (1), which then gets phosphorylated at the histidine residue and ultimately transfers it to the aspartate residue at the C-terminal of HK receptors (2). The phosphate molecule is then transferred to the HP protein (3) which enters into the nucleus (4). Within the nucleus, it transfers the phosphate molecule to aspartate residue present on the receiver domain of either type-A or type-B RR (5). Type-A RR acts as inhibitor of type-B RR and negatively regulates the HK receptors, whereas type-B RR via its effector domain regulates the gene transcription (6) or other responses mediated by CK signalling (7)

6.3.2 Components of the Signalling Pathway

6.3.2.1 Histidine Kinases (HK)

HK are multi-domain complex receptors of CKs. A CHASE domain present at the N-terminal acts as a sensor domain for CKs which is followed by two or more transmitter domain and lastly two receiver domains having HK activity (Lomin et al. 2012). CKs bind to the CHASE domain which leads to the conformation change of the sensors that initiate the autophosphorylation of the transmembrane domain and finally transferring the phosphoryl group to the receiver domain (Lomin et al. 2012).

6.3.2.2 Histidine Phosphotransfer (HP)

HP proteins are 17 kDa phosphotransmitter proteins which act as a transmitter of signal from HK to RR by transferring the phosphate groups (Shi and Rashotte 2012). Five *Arabidopsis* HPs have been identified by Suzuki et al. (1998). Imamura et al. (2001) reported that HPs are present in cytoplasm and also demonstrated their phosphotransfer activity in vitro. Formation of protoxylem is mediated by another type of HP which acts as an inhibitor of the CK signalling pathway (Mahonen et al. 2006).

6.3.2.3 Response Regulator (RR)

There are 23 RRs present in *Arabidopsis* which can be categorised under four types, type-A, type-B, type-C and pseudo-RRs, based on their domain structure and protein sequences (Mizuno and Nakamichi 2005). Addition of a phosphate group to type-A RR is necessary to stabilise it, whereas phosphorylation of type-B RR is required to enhance their binding ability to DNA that ultimately regulates the expression of target genes and also induces the stability of type-A RR (To et al. 2007). Type-A RR negatively regulates the pathway due to the presence of only a receiver domain (To et al. 2004), whereas type-B RR consists of both an output domain having a glutamine-rich domain which enables them to bind to DNA and a receiver domain (Sakai et al. 2001). Gupta and Rashotte (2012) reported that in *Arabidopsis*, some of the genes which are targeted by type-B RR are under the regulation of type-A RR which shows that signalling mediated by CKs is self-regulated.

6.4 Role of CK Signalling System in Response to Various Abiotic Stresses

Researches differ regarding their view about the role of CKs in plants against stress conditions. Several studies have shown that CKs act as negative regulators in plants in initiating tolerance mechanisms against environmental stress (Ghanem et al. 2008; Hansen and Dorffling 2003; Albacete et al. 2008; Kudoyarova et al. 2007), whereas several other studies have reported a short-term induction in the level of CKs against stress conditions (Alvarez et al. 2008; Pospisilova et al. 2005; Walker and Dumbroff 1981). Nishiyama et al. (2011) have reported higher activity of IPT in *Arabidopsis* for 1–2 hours, whereas the activity of CKXs was lowered for 1 hour after plants were exposed to salt stress. Overall, it can be concluded that initial exposure of plants to stress conditions leads to higher production of CKs which again gradually decrease to the normal level in the course of time.

6.4.1 Drought Stress

Drought stress is experienced by plants due to inadequate or insufficient supply of water which leads to chlorophyll degradation and enhanced production of ROS resulting in higher membrane damage (Nadeem et al. 2019). In addition, drought stress also reduces growth of plants by reducing cell division which results in lower turgor pressure caused by limited water uptake from the surrounding environment (Bhatt and Rao 2005).

Separate studies have reported that drought stress reduces the endogenous content of CKs in plants (Todaka et al. 2017; Nishiyama et al. 2011). Similar effect was noted in *Arabidopsis* where drought stress reduces the expression of IPT and induces the expression of CKXs (Le et al. 2012; Ramireddy et al. 2014). Lowered formation of CKs and thus reduced signalling under drought enhance the formation and sensitivity of plants to ABA (Tran et al. 2007), since ABA is antagonistic to CKs. In addition, reduced level of CKs lowered the shoot length (Werner et al. 2003). Higher synthesis of ABA and reduced shoot length are two known adaptive responses of plants against drought stress (Roychoudhury and Paul 2012).

Plants in which CK signalling pathway was suppressed by mutating the HK receptors or lowering the transfer of phosphate group to HP or by lowering the function of RR showed higher tolerance to drought stress (Nguyen et al. 2016). They also reported that CKs also control the transcription of a large number of ABA-responsive, drought/dehydration genes which enhances the tolerance capacity of plants under water-deficit conditions. Contradictory to these studies, Tran et al. (2007) and Susan et al. (2013) reported that expression of HK in *Arabidopsis* and maize plants, respectively, was upregulated under drought stress. The expression of RR6 was also upregulated in response to drought stress or exogenous application of CKs, suggesting that RR6 may play an important role in improving the yield of rice plants under drought stress (Panda et al. 2018). In addition to the negative role of RR22, recent studies have also demonstrated the positive role of RR22 by maintaining the integrity of the cell membrane and enhancing the expression of drought-responsive gene in *Arabidopsis* (Kang et al. 2013). Higher expression of *IPT* gene enhances the endogenous content of CKs which reduces the effect of drought stress in plants like cotton (Kuppu et al. 2013), tropical maize (Bedada et al. 2016), eggplant (Xiao et al. 2017) and bentgrass (Xu et al. 2016). CKs also negatively affect the water content of plants by lowering root length and increasing stomatal density and conductance. Overexpression of *DREB6.2* genes ultimately induces the expression of *CKX4a* which reduces the endogenous content of CKs to produce transgenic apples which were resistant against drought stress (Liao et al. 2017). Production of ROS is one of the major damaging effects of drought stress in plants. Mytinova et al. (2011) reported that overexpression of *CKX2* from *Arabidopsis* in tobacco plants reduces the endogenous content of CKs in the cells which results in a higher activity of superoxide dismutase, glutathione reductase and ascorbate peroxidase lowering the level of ROS in transgenic plants. Contradictory results were observed in bentgrass and eggplants where overexpression of *IPT* gene induces the endogenous content of CKs which reduces the level of ROS formed in the cells by enhancing the activity of superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase and catalase (Xu and Huang 2017). Thus, the actual signalling process of CKs varies in different plant species when exposed to water-deficit conditions.

6.4.2 Salinity Stress

High salt concentration in the soil is one of the major abiotic stresses experienced by plants. Cell membrane damage reduces the ability to detoxify the ROS content. ROS accumulation and nutrient imbalance are some of the initial symptoms observed in plants when exposed to salt stress (Rahnama et al. 2010). During salt stress, excess deposition of Na^+ ions in the tissues of plants affects the ion balance which may interfere with signalling process (James et al. 2011; Roychoudhury and Chakraborty 2013; Roychoudhury et al. 2013). High salt concentration also interferes with the nutritional uptake from the soil, especially uptake of K^+ required for growth and productivity of plants.

Salinity stress in plants is mostly regulated by ABA. Since ABA acts antagonistically to CKs, it can be concluded that CKs act as a negative regulator of salt stress in plants (Cortleven et al. 2019). Reduced expression of *IPT* gene leads to lower formation of CKs in *Arabidopsis* which was able to adapt more efficiently against salt-stressed environment as compared to wild-type plants (Nishiyama et al. 2011). Plants better adapted against salt stress also showed altered expression of genes which were regulated by ABA. Similar observation was also reported by Wang et al. (2015), who showed that overexpression of *IPT8* gene in *Arabidopsis* leads to higher formation of CKs which ultimately enhances the symptoms of damage in plants maintained under high salt concentration. High-affinity K^+ transporter 1;1 (HKT1;1) is present in *Arabidopsis* which is responsible for removal of Na^+ ions during salt stress in plants. CKs negatively regulates the activity of HKT1;1. Mason et al. (2010) reported that reduced endogenous content of CKs enhanced the expression of HKT1;1 which removed excess Na^+ ions accumulated in the cells during salinity stress ultimately improving the survival capability of plants.

Along with the negative role, positive effect of CKs is also reported against salt stress. Keshishian et al. (2018) reported that salt stress in tomato plants enhanced the formation of CKs which positively affects the tolerance capability of plants. A similar effect in tomato was also demonstrated by Aremu et al. (2014) where they showed that application of INCYDE which acts as a potent inhibitor of CKX and thus increases the CK content of the cells results in better production of flowers and also protected the photosynthetic apparatus. Salt-sensitive cultivars of rice lacking CKX2 showed higher tolerance against salt stress which was ultimately reflected in their grain yield (Joshi et al. 2018).

6.4.3 Extreme Temperature

Plants require optimum temperature for their growth and maximum productivity. Any deviation from this optimal range may cause molecular and physiological damage and can also reduce the yield of plants. Cold stress leads to retardation in development, chlorosis and necrosis of cells and also causes sterility of flowers as noted in rice plants (Jiang et al. 2002). Low temperature causes shortage of water in plants affecting the integrity of plasma membrane (Steponkus et al. 1993). Temperature above the optimal range induces the formation of ROS causing oxidative stress in plants. Collapse of cellular structure in plants may occur when exposed to very high temperature which leads to cell death (Ahuja et al. 2010). High temperature also interferes with the metabolic process by altering the protein structures of the enzymes (Lobell et al. 2011).

The effect of CKs in plants is still uncertain. Maruyama et al. (2014) reported that cold stress decreases the endogenous level of CKs and CK signalling, which reduces the size of plants. *Arabidopsis* containing mutant receptors such as *ahk2*, *ahk3* and *ahk3 cytokinin response element (cre)1* was highly adapted against cold stress, proving that CKs play a negative role during regulation of cold stress (Cortleven et al. 2019). Jeon et al. (2010) reported the activation of CK receptors, and Jeon and Kim (2012) observed that several HP and RR can be activated in the absence of CKs. Higher level of cold tolerance in *Arabidopsis* having mutant *rr5, rr6* and *rr7* was observed (Jeon et al. 2010). In contrast to the above observation, Shi et al. (2012) reported that overexpression of *RR* genes such as *RR22*, *RR15*, *RR7* and *RR5* enhanced the cold adaptability of *Arabidopsis* plants. Along with the classical signal pathway of CKs, several CK response factors (CRFs) have also been linked with protection of plants against cold stress. Jeon et al. (2016) reported that *CRF2* expression can initiate the formation of lateral roots which is directly controlled by RR1 in *Arabidopsis*. Zwack et al. (2016) reported that the expression of *CRF4* can be induced by cold stress, and it acts as a positive regulator of freeze tolerance. Thus, from the above observation, it can be concluded that the activation of the two-component signalling system either in presence or absence of CKs may play an important role in providing protection against cold stress in plants.

Exogenous application of CKs enhances the adaptability of plants against high temperature, whereas excess heat reduces the endogenous level of CKs in the cells (Cortleven et al. 2019). External application of CKs reduces the effect of heat in plants by protecting their photosynthetic machineries (Liu and Huang 2002), increasing the thermo-tolerance of the reproductive tissues in plants like rice, passion fruit and maize (Sobol et al. 2014; Wu et al. 2016; Cheikh and Jones 1994) and enhancing the activity of heat shock protein and antioxidative system in bentgrass (Xu et al. 2010). Skalak et al. (2016) reported that high temperature induces the degradation of endogenous CKs of the cells which helps in the stomatal closure in *Arabidopsis*.

6.4.4 Light Stress

Light is responsible for the survival of life on earth, but excess light also causes damage to the photosynthetic apparatus and bleaching of chlorophyll. Solar radiation is the only source of natural light on earth which can be utilised by plants for photosynthesis, but it also contains ultraviolet light which can damage or mutate the DNA of the cells (Jansen et al. 1998). Excess light can inhibit photosynthesis which leads to excess production of ROS causing damage to the cellular membrane and its components.

Recent studies have demonstrated a direct link between CKs and light through photoreceptor phy B (Chi et al. 2016; Sweere et al. 2001). Plants having a higher level of CKs show better adaptability against light stress and protect the D1 reaction centre of photosynthesis which is severely damaged by excess light (Cortleven et al. 2014). They also reported that CK-mediated signalling system is mostly controlled by HK3 and HK2 and type-B RRs such as RR1 and RR12 in *Arabidopsis*. Other investigations observed that the components of antioxidative system protect photosynthetic apparatus under different stresses like drought (Rivero et al. 2009). This was used as evidence by Cortleven and Schmulling (2015) to support their observation that CKs can protect photosynthetic apparatus in plants when exposed to high light. Low light also acts as a stress stimulus causing an induction in response mechanism in plants. In tobacco and *Arabidopsis*, CKs act as an important signalling molecule transported across the xylem tissue which helps plants adapt in low light environment (Boonman et al. 2009). Carabelli et al. (2007) reported that CKX induces the degradation of CKs in plants which inhibits the formation of leaf primordia, ultimately promoting growth of hypocotyl towards light.

6.4.5 Nutrient Stress

Besides carbon, oxygen and hydrogen which plants easily accumulate from CO_2 and H_2O present in the surrounding environment, plants also require several micronutrients (boron, silicon, iron and selenium) and macronutrients (potassium, nitrogen and phosphorus) for their normal growth and development. CK plays a pivotal role during uptake of these nutrients from the soil.

Boron is an essential micronutrient whose deficiency can lead to the downregulation of genes involved in CK-mediated signalling (Abreu et al. 2014). Pavlu et al. (2018) observed that *boron transporter* (*BOR)4*, a gene which codes for boron transporter, has an expression pattern similar to that of *RR1* and *LOG7* genes. In oilseed rape, a strong correlation was observed between boron concentration and formation and activation of CKs (Eggert and von Wiren 2017). Silicon is the major soil component, and its deposition leads to enhanced activation of various defensive machineries in plants; it also provides structural support to various tissues of plants. Markovich et al. (2017) reported that silicon deposition in the tissues of *Arabidopsis* and sorghum enhanced the synthesis of CKs by upregulating the expression of *IPT7* gene and reduced the process of leaf senescence induced by dark stress via activating the CK signalling pathway. Selenium induces the stress tolerance capability of plants when present in lower concentration (Pilon-Smits et al. 2009). The two major forms of selenium, viz. selenite and selenate, are absorbed by plants through phosphate and sulphate transporters (Schiavon and Pilon-Smits 2017). Thus, it can be said that the pathway responsible for phosphate and sulphate uptake in plants, mediated by CK signalling, acts as a common pathway for the uptake of selenium from the soil.

Nitrogen acts as a major element during the synthesis of CKs. In *Arabidopsis*, the enzymatic activity of IPT3 and IPT5 which catalyses the rate-limiting step of CK synthesis is controlled by the availability of nitrogen during the production of cytochrome P450 and trans-zeatin (Kiba et al. 2013; Kieber and Schaller 2014). CKs transported to the shoot via the root control the nitrate response and key traits such as activity-related traits of meristem (Muller et al. 2015) and leaf size (Rahayu et al. 2005). The activity of IPT3 is also mediated by the presence of inorganic phosphate (Pi), and thus the expression of *IPT3* is downregulated in the absence of Pi (Hirose et al. 2008). Supply of Pi in plants, after facing a situation of phosphorus deficiency, leads to enhanced expression of CRF5, CRF6 and IPT3 (Woo et al. 2012) Exogenous application of CKs lowers the uptake and response of Pi deficiency in *Arabidopsis* and rice (Franco-Zorrilla et al. 2002) by utilising the stored Pi from the internal sources which ultimately reduces the Pi deficiency signalling and symptoms (Lai et al. 2007). Potassium is one of the major macronutrients for plants, and it is added externally in the soil as fertilizer along with nitrogen and phosphorus. Potassium deficiency in *Arabidopsis* lowers the endogenous CK level which results in inhibition of CK-mediated signalling system, ultimately reducing the root length and inducing potassium uptake from the soil (Nam et al. 2012). They also reported that CK deficiency leads to higher accumulation of ROS and enhanced root hair growth along with higher expression of *HAK5* gene which codes for a transporter involved in the uptake of potassium from the surroundings. Hirose et al. (2008) showed that CK formation by IPT3 is directly associated with sulphur deficiency in soil. They also reported that exogenous application of CK enhanced the expression of genes which were regulated by the presence of sulphur, whereas Maruyama-Nakashita et al. (2004) reported that the expression of sulphate transporter in roots was downregulated by CKs.

6.4.6 Heavy Metal Stress

Tight regulation is important for transport and absorption of heavy metals, since higher deposition of heavy metal in plant tissues damages the cell structure and leads to oxidative stress. Along with desired element such as macronutrients and micronutrients, plants also uptake several metals and metalloids like mercury, cadmium, chromium, arsenic and lead which are toxic for plants. Bruno et al. (2017)

and Yang et al. (2017) reported that *Arabidopsis* adapt themselves against cadmium and aluminium toxicity by enhancing the level of CKs along with signalling system mediated by CKs which hamper the growth of the roots. Werner et al. (2010) reported that plants which are deficient in CKs accumulate a higher amount of cadmium in their tissues, whereas a contrasting result was observed in the case of arsenate toxicity where plants were able to tolerate arsenate stress in CK-deficient conditions (Mohan et al. 2016). CK enhances the activity of glutathione-S-transferase (Brenner and Schmulling 2015), which induces the production of thiol compounds which can be utilised by plants against arsenate toxicity (Mohan et al. 2016).

6.4.7 Biotic Stress

Biotic stress in plants is mostly caused by the infection of pathogens like virus, fungus and bacteria or by rodent and insects which severely affect the yield of plants. In recent times, broad studies have been conducted on the role of CKs with regard to such biotic stresses (Albrecht and Argueso 2017). Walters and McRoberts (2006) reported that during biotrophic fungal infection, CKs help the pathogens to multiply within the infected host. CK helps in the formation of green islands which cause the assimilation of nutrition at the infected site and also delay senescence of the leaf. Not only that, CK at the infected site also inhibits photosynthetic metabolism by enhancing the activity of invertase. Infection of plants with geminiviruses hinders the activity of various adenosine kinases (Baliji et al. 2010). The viral infection got boosted up due to higher expression of genes involved in primary CK formation. Increased level of CK helps geminiviruses by promoting cell division, availability of resources and inhibiting senescence of infected leaves. Radhika et al. (2015) reported that *Rhodococcus fascians*, an actinomycete which produces a wide range of methylated CKs, is more stable against plant CKX and helps the pathogen grow more quickly by inducing the cell division of host plants. Kind et al. (2018) observed that the virulence of *Claviceps purpurea* can be reduced by expressing the CK synthesis and *CKX* gene in the pathogen. Various other studies conducted by several groups such as Siemens et al. (2006), Spallek et al. (2017) and Siddique et al. (2015) have also shown the negative effect of CK in plants under different biotic stresses.

6.5 Conclusion and Future Perspectives

It is evident from our discussion that CKs play an important role in abiotic and biotic stress signalling in plants. Manipulating the signalling process involved during stress condition can be beneficial for sustainable growth of plants mostly in the case of biotic stress where lower level of CK helps host plants to better inhibit the growth of the pathogen infection by either degrading the endogenous content of

CKs via enhancing the activity of CKX or by lowering the production of CKs via downregulating the activity of the enzymes like IPT. CKs behave differently for different abiotic stresses, and thus it becomes necessary in the near future to further elaborate CK pathways for better understanding the role of CKs against harsh environmental conditions. Though the interaction of CKs with different stress hormones such as ABA or salicylic acid is known, further studies will help to expand our knowledge about the interaction between these hormones which can be applied in genetic engineering to develop stress-tolerant crop plants with better yield. Thus, it can be concluded that CKs are essential signalling molecules which enhance co-ordination and interaction among different pathways under various biotic and abiotic stresses.

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Chapter 7
Leaf Senescence and Ethylene Signaling

Yasir Yousuf Peerzada and Muhammad Iqbal

7.1 Introduction

During the autumn season, we often observe spectacular colour changes in the leaves of plants growing mainly in the temperate regions of the world. These changes form part of an important developmental phase of plant leaves, which is known as senescence. During this phase, the colour of leaves changes from green to orange, yellow, red and finally brown after which the leaves usually die and are eventually detached from the plant. These visual cues and transformations are accompanied by various active metabolic changes that result in the transfer of most of the stored nutrients including nitrogen, carbon, etc. and minerals from the leaf to other plant parts including the specialized trunk cells (deciduous trees), developing seeds (annual crops such as wheat), actively growing new buds, young leaves and fruits. Senescence, a highly regulated and well-programmed developmental process, indicates cessation of growth in cells, organs or the whole plant (Li et al. 2012; Guo and Gan 2012). It includes active degenerative processes associated with the structural, physiological, biochemical and molecular changes at the cellular, organ and whole plant level. According to its standard definition, "Senescence is an important developmental phase following the completion of growth that may or may not be succeeded by death and is dependent on viability of a cell and the expression of specific genes" (Gan 2007; Lim et al. 2007; Guiboileau et al. 2010; Breeze et al. 2011; Liu et al. 2011; Thomas and Ougham 2014).

The understanding of senescence in plants and of the mechanisms underlying it is important in order to maintain or increase plant productivity and minimize the

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postharvest quality loss throughout the transportation and storage processes. In plants senescence can occur either at the organismal level (whole plant senescence) or at the organ level (Organ senescence). Depending on the organ involved, organ senescence can be classified into three main types including leaf senescence, flower senescence and fruit senescence.

7.2 Leaf Senescence

Leaf senescence is defined as a developmental process during which leaf cells switch from anabolism to catabolism and the cellular components are actively degraded and remobilized (Woo et al. 2019). The process occurs near the end of a leaf's active life and is associated with the dismantling of cell membranes and other cell components, loss of green plastids and an overall decline in the leaf metabolism. It is a primary process which is indispensable for seed production and plant viability in the next generation or season, as it allows plants to recycle leaf nutrients.

7.3 Physiological Changes During Leaf Senescence

The senescence process in leaves occurs in a highly regulated fashion with the cell constituents disassembling in an ordered progression. The first visible indication of leaf senescence is chloroplast degradation, which is accompanied by protein and RNA degradation followed by the transfer of leaf nutrients, including nitrogen, phosphorus, molybdenum, minerals and metal ions, to other parts like fruits, tubers, seeds and more apical leaves (Maillard et al. 2015; Have et al. 2016; Gortari et al. 2018; Ahmad and Guo 2019). Leaf senescence can be regarded as a nutrient mining and recycling process that leads to better nutrient management and an efficient resource economy for the production of new organs and plant fitness. The main physiological changes that occur during leaf senescence are listed below.

- ***Chlorophyll Degradation***

  One of the first visible indications of leaf senescence is the yellowing of leaves, which is caused by degradation of pigment-protein complexes of green plastids and transformation of the constituent chlorophyll molecules into non-green catabolic products. This transformation is caused by the opening of chlorin ring structure (Hortensteiner and Krautler 2011; Mayta et al. 2019; Tamary et al. 2019) through the removal of phytol tail (dephytylation) and central Mg atom (Eckardt 2009).

- ***Protein Degradation***

  One of the most important catabolic processes associated with leaf senescence is protein degradation. There is a considerable decrease in the steady-state levels of proteins during senescence, which is due to decreased protein synthesis as well as enhanced degradation rates. This degradation process, which occurs with the help of temporal expression of proteases belonging to different cellular locations including chloroplasts, mitochondria, nucleus, etc., plays an essential role in the recycling of nutrients, especially nitrogen. The proteins are degraded into amino acids, amides and ammonium (Diaz-Mendoza et al. 2014; Roberts et al. 2012). Thus there is a complex transfer of proteins, peptides and amino acids through cell compartments including plastids, cytosol, vesicles and vacuoles (Roberts et al. 2012; Carrion et al. 2013; Avila-Ospina et al. 2014; Carrion et al. 2014; Diaz-Mendoza et al. 2014). As a result of protein degradation, ammonium is formed, which is re-assimilated into amino acids and subsequently exported to sink tissues including the developing seeds, fruits and tubers via phloem. This indicates the crucial importance of timing of leaf senescence for yield in crop species (Gregersen et al. 2013).

- ***Lipid Degradation***

Besides the breakdown of chlorophyll and proteins, there is a considerable degradation of lipid content of leaves during senescence. As lipids are the components of all organelle membranes, their turnover requires a major transfer of head groups and acyl chains to the degradation sites. The breakdown process starts with the lipolytic reactions releasing fatty acids from membrane lipids, which subsequently undergo oxidation in peroxisomes. More than 80% decrease in the total fatty acid content has been noticed in the leaves of *Arabidopsis* and *Panicum virgatum* (Yang and Ohlrogge 2009).

- ***Changes in Nucleic Acid Metabolism***

There is a substantial change in nucleic acid metabolism during leaf senescence. The nucleotide polymers, particularly RNAs, get degraded and act as a source of phosphorus in a mature leaf. The degradation of nucleic acids is carried out by the over-expression of several nucleases. As a result, the RNA content declines rapidly with the progress of senescence; however nuclear DNA is maintained until late in the process to allow continuation of the specific gene expression and maintain the basic metabolic activities (Roberts et al. 2012; Avila-Ospina et al. 2014; Sakamoto and Takami 2014).

7.4 Factors Affecting Leaf Senescence

Leaf senescence relies on age, and its specific timing is particularly directed by the developmental factors. However, the senescence process, together with its rate and molecular nature, is closely affected by both external factors and internal signals (Fig. 7.1). The external environmental cues that instigate premature leaf senescence include abiotic stress (such as light, temperature and nutrient stress) and biotic stress, whereas internal factors include phytohormones, reproductive growth and developmental stage (Lim et al. 2007). At a particular stage of plant development,

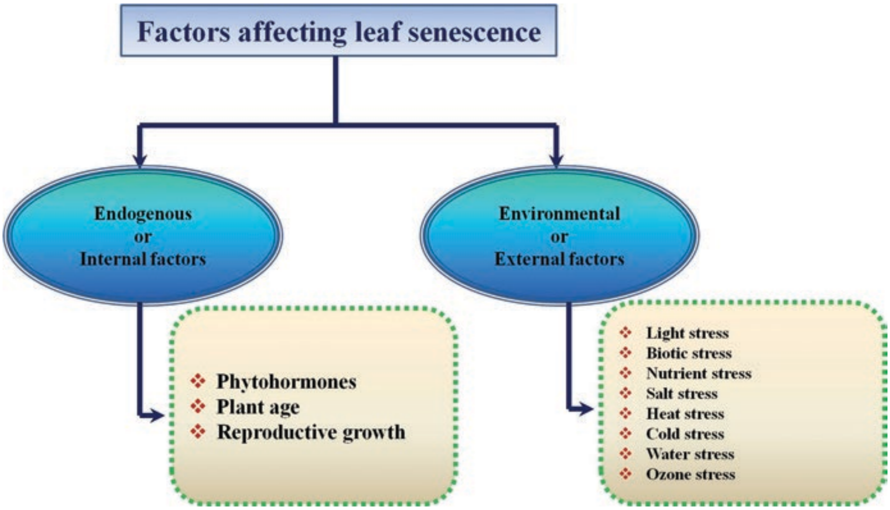

Fig. 7.1 Endogenous and environmental factors affecting leaf senescence

these stresses are capable enough to cause alteration of gene expression and/or physiological functioning. Numerous pathways operate in response to different environmental and internal cues and form a complex of regulatory network for leaf senescence (Breeze et al. 2011).

7.5 Phytohormones and Leaf Senescence

Hormones are low-molecular-weight compounds that operate as chemical messengers to transfer signals from the sites of their synthesis to action sites, where they change the expression of gene/s and/or protein activities. There are different classes of hormones in plants that mediate or influence the signals received from environmental factors (photoperiods, water supply, temperature, atmospheric pollution, etc.) and endogenous developmental programs. Phytohormones are thus essential to translate information about the prevailing environmental conditions into imperative developmental decisions, like timing of germination, flowering or senescence.

Phytohormones play crucial roles at all the three stages of leaf senescence, viz. initiation, progression and the terminal phase. As the hormones exert multiple effects on various developmental and/or environmental processes in a complex manner, it is difficult to identify their exact roles in leaf senescence. Nonetheless, characterization of genetic mutants and the global gene expression analysis, along with other molecular approaches, have provided significant information about the hormonal signaling pathways and their possible roles during leaf senescence. While some of the phytohormones including ethylene, jasmonic acid, abscisic acid and salicylic acid may promote leaf senescence, others like auxin, gibberellic acid, cytokinin, nitric oxide and polyamines may repress it.

7.5.1 Role of Ethylene

Ethylene (C_2H_4), a simplest and smallest plant hormone, is a gaseous unsaturated hydrocarbon alkene. It has been studied for over a century. Dimitry Neljubov, a Russian scientist, was the first to determine that ethylene is a biologically active gas. He also demonstrated that ethylene causes horizontal rather than vertical growth in etiolated pea seedlings (Neljubov 1901). Crocker and Knight (1908) reported that ethylene from illuminating gas causes senescence in carnations. Gane (1934) studied the gasses released by apples and demonstrated that plants synthesize ethylene naturally. Based on a triple response (increase in horizontal growth, inhibition of stem elongation and thickening of stem) exhibited by etiolated pea seedlings, Crocker (1932) developed for the first time a bioassay for ethylene. Following similar findings (inhibition of root and hypocotyl elongation, inflated apical hook and thickened hypocotyl) in *Arabidopsis thaliana*, triple response has been employed as a screening device for the identification of ethylene mutants (Guzman and Ecker 1990). These ethylene mutants have in turn paved the way to unravel a deep understanding of the cellular mechanisms underlying the ethylene signaling transduction process. Ethylene regulates a variety of physiological, metabolic and developmental processes in plants, including seed germination, cell division, cell size, cell differentiation, root hair growth, ripening of climacteric fruits, stress responses, flowering, fruit ripening, sex determination, gravitropism, senescence and abscission (Lin et al. 2009; Ijaz 2016; Dubois et al. 2018).

7.5.2 Ethylene Biosynthesis

Ethylene biosynthesis starts with the conversion of methionine to S-adenosylmethionine catalysed by S-adenosylmethionine synthetase. S-adenosylmethionine is then catalysed into ACC (1-aminocyclopropane-1-carboxylic acid) and MTA (5′-deoxy-5’methylthioadenosine) by ACC synthase (Adams and Yang 1979). This reaction is often the rate-limiting step in the ethylene biosynthesis pathway. ACC is finally converted to ethylene, CO2 and cyanide, by ACC oxidase. Subsequently cyanide is detoxified by its conversion into β-cyanoalanine, a non-toxic compound, via β-cyanoalanine synthase (Wang et al. 2002). MTA is used for the synthesis of methionine via modified methionine cycle (Miyazaki and Yang 1987). In the synthesis of ethylene, the methylthio group is preserved through every cycle utilizing one ATP molecule. This ensures the maintenance of high rates of ethylene biosynthesis even at the low concentration of free methionine (Fig. 7.2).

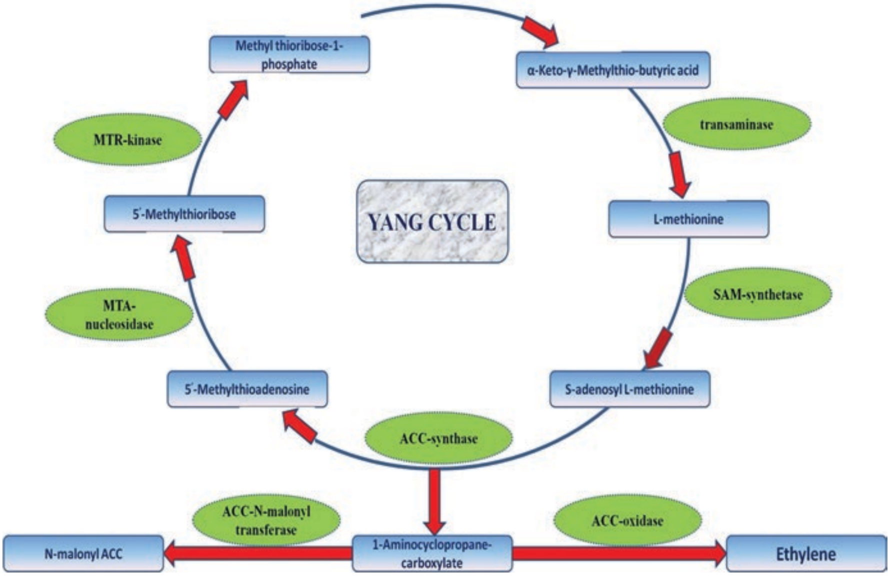

Fig. 7.2 Ethylene biosynthesis pathway (Yang cycle)

7.5.3 Ethylene Receptors

The cascade of ethylene signaling begins with the perception of ethylene interceded by a family of receptors. In general, the ethylene receptors of plants share a common structure. Three conserved transmembrane domains containing the ethylene-binding site are present on amino terminus, followed by a GAF (cGMP-specific phosphodiesterases, adenylyl cyclases, formate hydrogenlyase transcriptional activator) domain that possibly mediates protein-protein interactions and a signal output domain on the carboxy terminus related to histidine kinases and receiver domains. Although signal output domain is always associated with kinase activity, it is frequently (but not always) associated with a receiver domain. N-terminal domain is membrane-bound, while the other two domains form the cytosolic portion (Light et al. 2016).

Based on the phylogenetic analysis and some shared structural characteristics, ethylene receptors in angiosperms have been classified into (a) subfamily-1 receptors and (b) subfamily-2 receptors (Chang and Stadler 2001; Schaller and Kieber 2002). The presence of a conserved histidine kinase domain with all the residues required for activity is the main characteristic feature of subfamily-1. On the other hand, the subfamily-2 receptors possess a diverged histidine kinase domain with no residues critical for activity (Fig. 7.3). Most of the receptors belonging to subfamily-2 contain an additional hydrophobic segment preceding the three conserved transmembrane domains that serve as a signal sequence for their targeted transport to endoplasmic reticulum (ER). The number of receptors varies among species, e.g.

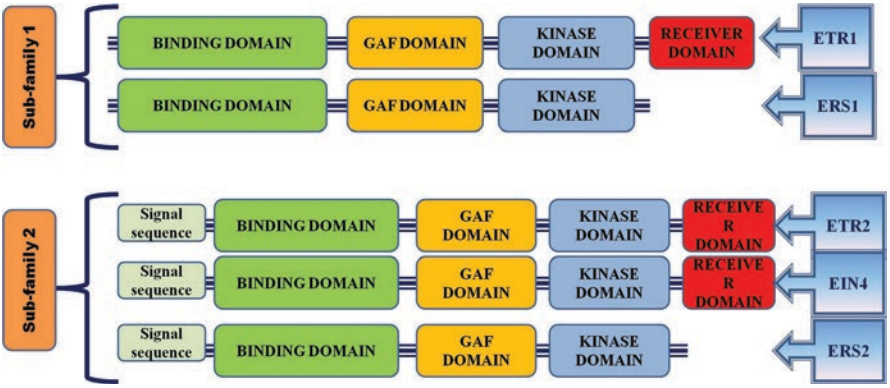

Fig. 7.3 Structural representation of ethylene receptors in *Arabidopsis*

six are found in *Solanum lycopersicum* and only four in *Nicotiana tabacum* (Light et al. 2016). In *Arabidopsis*, five ethylene receptors have been identified among which ETR1 (Ethylene Response 1) and ERS1 (Ethylene Response Sensor 1) belong to subfamily-1, while subfamily-2 is made up of ETR2 (Ethylene Response 2), ERS2 (Ethylene Response Sensor 2) and EIN4 (Ethylene Insensitive 4) (Hua et al. 1998). Although receptors of both subfamilies are present in both monocots and dicots, there exists a structural difference (Klee 2004; Yau et al. 2004). The receiver domains are found only in subfamily-2 receptors of the monocots, in contrast to dicots where they are present in both the receptor subfamilies (Fig. 7.2).

The ethylene receptors form a homodimer via a disulphide bond between Cys-4 and Cys-6, and this receptor homodimer binds one ethylene molecule (Schaller and Bleecker 1995). In addition, there is also a heterodimer formation in receptors, possibly mediated through GAF domain, depicting the role of receptor clustering in signal amplification and crosstalk between the different receptor types in a heterodimer (Gao et al. 2008; Gao and Schaller 2009).

7.5.4 Location of Ethylene Receptors

Since ethylene is a gaseous hormone and can easily diffuse through both hydrophilic and hydrophobic environments, its receptors do not require to be localized on plasma membrane unlike other phytohormones. Using different techniques to identify the location of ethylene receptors at the subcellular levels, they have been found located predominantly on the endoplasmic reticulum. Such studies carried out mainly by Chen et al. (2002, 2007) on native AtETR1 receptor (in *Arabidopsis*) using membrane fractionation and immunoelectron microscopy and by Ma et al. (2006) on native CmERS1 and CmERS1 (in Melon) using membrane fractionation and GFP-tagged expression techniques, respectively, were later confirmed by Dong et al. (2008), Grefen et al. (2008) and Zhong et al. (2008). Although predominantly located on ER, the possibility of the presence of low-level ethylene receptors on the membranes of other organelles including Golgi, plasmalemmma and vacuole may not be ruled out (Hall et al. 2012).

Two key factors that help in modelling the ethylene-binding site are the number and topology of the transmembrane domains of the receptor. Based on the computational methods, Chang et al. (1993), Schaller (1995) and their co-workers predicted the location of N-terminus of ethylene receptors on the non-cytosolic side (lumen side) of ER and C-terminus on the cytosolic side, which was later confirmed by experimental studies carried out by Ma et al. (2006).

7.6 Elements of the Ethylene Signaling Pathway

Apart from the main receptors of ethylene, there are some important elements that play crucial roles in ethylene signal transduction. Based on the triple response assay, mutants that perturb the ethylene response were isolated through genetic analysis. Some of these essential effectors of the ethylene pathway are listed below.

CTR1 (Constitutive Response 1) CTR1 is a serine/threonine kinase which acts as a negative regulator of ethylene signaling. The loss-of-function mutation of this element results in the constitutive ethylene response (Chang et al. 1993; Huang et al. 2003). The C-terminal half of CTR1 contains a kinase domain showing similarity with Raf kinase family (functioning in mitogen-activated protein kinase pathway in animals) and the N-terminal half with a domain that interacts with ethylene receptors forming receptor complex (Gao et al. 2003; Chen et al. 2007; Shakeel et al. 2015). Mutation in the N-terminal domain of CTR1 that prevents its binding to the concerned receptors stops CTR1 from getting localized to the ER and renders it non-functional (Gao et al. 2003; Huang et al. 2003). This in turn results in a constitutive ethylene-response phenotype, signifying the importance of receptor-CTR1 interaction.

EIN2 (Ethylene Insensitive 2) EIN2 is a positive regulator of ethylene signaling pathway that acts downstream of CTR1. The loss-of-function mutation in this protein results in an ethylene-insensitive phenotype (Alonso et al. 1999). There are twelve predicted transmembrane segments on the N-terminal of EIN2. Although N-terminal half shares a sequence similarity with NRAMP (Natural Resistance-Associated Macrophage Protein) cation transporter family, unlike NRAMP, it is not associated with the complementation of metal-uptake-deficient yeast strains, implying its acquired new functionality (Thomine et al. 2000). On the other hand, there is a large soluble C-terminal domain, which is associated with the positive regulation of ethylene response. The over-expression of this C-terminal domain results in a constitutive ethylene-response phenotype (Alonso et al. 1999). In the absence of ethylene, E1N2 is maintained at low levels by the proteosome-mediated degradation via two essential proteins, ETP1 and ETP2. These two proteins belong to EIN2

Targeting Protein (ETP) family of F-box proteins. In the presence of ethylene EIN2, proteins are stabilized by MHL1 and MHL2 proteins (Qiao et al. 2009; Ma et al. 2018). These membrane proteins possibly inhibit the ubiquitination of EIN2. Like CTR1, EIN2 localizes to ER membrane and possibly interacts with the receptors, forming a large complex composed of closely associated ethylene receptors, ETR1 and EIN2 (Bisson et al. 2009; Qiao et al. 2012; Light et al. 2016). Plants with mutated EIN2 have exhibited delayed developmental leaf senescence due to down-regulation of 21 SAGs (Senescence-Associated Genes) that encode the cell wall-decomposing enzymes, including polygalacturonase and pectinesterase enzymes (Buchanan-Wollaston et al. 2005).

The EIN3/EIL (Ethylene Insensitive3-Like) Family of Transcription Factors The EIN3/EIL family of transcription factors is composed of six members (EIN3, EIL1, EIL2, EIL3, EIL4 and EIL5) and functions downstream to EIN2 (Alonso et al. 2003). These transcription factors are the positive regulators of the ethylene signaling pathway. Like EIN2, these transcription factors are present at very low concentrations in the absence of ethylene. The low concentrations are maintained through the proteasome-mediated degradation by the members of EBF family of F-box proteins. In the presence of ethylene, these transcription factors are stabilized, their concentration increases and they bind to regulatory elements of their target genes (Potuschak et al. 2003; Gagne et al. 2004; An et al. 2010; Chang et al. 2013). These transcriptional factors lead the sequence of transcriptional cascade, while several other transcription factors such as Ethylene Response Factor (ERF) TF family regulate numerous subsets of the hormone response (Chang et al. 2013).

7.7 Other Transcription Factor Families

NAC Transcription Factor Family NAC [*N*AM, *A*TAF and *C*UC (No Apical Meristem/Arabidopsis Translation Activation Factor/Cup-Shaped Cotyledon)], one of the largest groups of plant transcription factors, with more than 100 members in *Arabidopsis*, acts downstream of the different hormone-signaling networks including ethylene (Puranik et al. 2012). These NAC proteins are characterized by a NAC domain (InterPro IPR003441) on the N-terminal half and highly variable transcription-regulatory regions (TRRs) on the C-terminal half (Puranik et al. 2012). The NAC domain, which is further subdivided into five well-conserved sub-domains (A–E), is associated with dimerization and DNA binding, whereas the TRR region acts as a transcription regulator (activator or repressor). Global transcriptome profiling reveals that almost more than 30 NAC genes in *Arabidopsis* are over-expressed during natural leaf senescence, and these genes regulate crosstalk between phytohormones and environmental cues, depicting their importance in the regulation of senescence (Breeze et al. 2011). Of these transcriptional factors, ORE1, NAP, ANAC16, ATAF1, ANAC017, ANAC019, ANAC055, ANAC072 and ANAC082 promote leaf senescence, whereas VNI2 and JUB1 (JUNGBRUNNEN1) impede it (Balazadeh et al. 2011; Yang et al. 2011; Wu et al. 2012; Garapati et al. 2015; Kim et al. 2018). ORE1, NAP, ANAC019 and ANAC055 are directly involved in ethylene signaling pathway and function downstream the EIN3/EIL family of transcription factors.

AP2/ERF Transcription Factor Family AP2/ERFs (Apetala2/Ethylene Responsive Factor) are an important group of plant transcription factors characterized by an AP2/EREB (APETALA2/Ethylene Responsive Element Binding Factor) domain, which comprises of 40–70 conserved amino acids associated with DNA binding (Feng et al. 2005; Nakano et al. 2006). This family of transcription factor comprises of four major subfamilies, namely, AP2, RAV (Related to Abscisic Acid Insensitive 3/Viviparous 1), DREBs (Dehydration-Responsive Element Binding proteins) and ERFs (Ethylene Responsive Factors) (Sakuma et al. 2002; Nakano et al. 2006).

WRKY Transcription Factor Family One of the major families of plant-specific transcriptional factors is represented by the WRKY. There is a DNA-binding domain in these WRKY TFs, known as the WRKY domain. It has a conserved WRKYGQK sequence and a zinc-binding motif (Bakshi et al. 2014). WRKY superfamily comprises of 197, 100 and 75 members in *Glycine max*, *Oryza sativa* and *Arabidopsis*, respectively (Rushton et al. 2010; Fan et al. 2015; He et al. 2016; Wei et al. 2016; Zentgraf and Doll, 2019). WRKY domain is approximately 60 amino acid residues long and possesses DNA-binding activity. It often binds to consensus-binding motif TTGAC(C/T) known as W-box elements, although the sequence flanking the W-box regulates the binding selectivity of the transcription factor (Yamasaki et al. 2013). For instance, unlike other WRKY transcription factors, WRKY6 and WRKY11 of *Arabidopsis* show higher affinity toward a G base present upstream of the core motif of W-box (Agarwal et al. 2011).

7.8 Role of Transcription Factors in Ethylene Signaling During Leaf Senescence

EIN3 (Ethylene-Insensitive 3), which works downstream EIN2, directly regulates chlorophyll catabolic genes (CCG), including NYC1 (Non-yellow Coloring1), NYE/SGR (STAYGREEN) and PAO (Pheophorbide A Oxygenase) (Qiu et al. 2015). Chlorophyll degradation, the main marker of senescence, is catalysed sequentially by several different Chlorophyll Catabolic Enzymes (CCEs). During the degradation process, chlorophyll b is first converted to chlorophyll a via two consecutive reactions. The first reaction is catalysed by Chlorophyll b Reductase, which is encoded by NYC1 and NYC1-like (NOL), and the second reaction by Hydroxymethyl Chlorophyll a Reductase (HCAR), respectively (Sato et al. 2009;

Meguro et al. 2011). Subsequently, magnesium atom is removed from chlorophyll a, giving rise to cleavage of phytol tail (Ren et al. 2010), resulting in pheophorbide a, which is further converted to a primary Fluorescent Chlorophyll Catabolite (pFCC). This conversion occurs in two sequential reactions catalysed by Pheophorbide A Oxygenase (PAO) and red Chlorophyll Catabolite Reductase (RCCR), respectively (Pruzinska et al. 2007). To finish, pFCC is converted to phyllobilins, the end catabolites of green plant pigment, chlorophyll (Christ et al. 2013; Krautler 2014). NYC1 encodes chlorophyll b reductase, an enzyme involved in the degradation of chlorophyll b and LHCII (light harvesting complex II). NYE is a main regulatory gene that promotes chlorophyll degradation during senescence possibly by dismantling the photosynthetic chlorophyll-apoprotein complexes (Hortensteiner 2009; Hortensteiner 2013). EIN3 positively regulates the leaf senescence process by activating two main senescence-promoting transcription factors, viz. ORE1 and AtNAP (Kim et al. 2014; Dolgikh et al. 2019).

ORE1, a transcription factor regulated by EIN2, plays several roles in ethylene signaling during leaf senescence. First, it interacts with Golden2-Like1 (GLK1) and Golden2-Like2 (GLK2), required for chloroplast development and maintenance (Rauf et al. 2013). Then, a heterodimer of ORE1 and GLKs is formed, which slows down the transcriptional activity of GLKs, thus leading to leaf senescence (Rauf et al. 2013). Second, ORE1 directly activates the transcription of Chlorophyll Catabolic Genes (Qiu et al. 2015), promoting chlorophyll degradation. Third, it activates BFN1 (Bifunctiona Nuclease1), a bifunctional nuclease that acts on polynucleotides (RNA and DNA) to promote nucleotide and phosphate recovery during the senescence. It also promotes sugar transport by activating SAG29/SWEET15 (Senescence-Associated Gene 29/Sugars Will Eventually Be exported Transporters 15) besides regulating NAC transcription factors via ubiquitination by activating SINA (Seven in Absentia) (Matallana-Ramirez et al. 2013). ORE1 also promotes transcriptional activation of ACS2 (Ethylene Biosynthesis Gene) regulating ethylene biosynthesis by positive feedback (Qiu et al. 2015). Moreover, ORE1 along with NAC2 and ANAC092 activates SAG12 (Jan et al. 2019).

NAP (Nac-Like Activated by AP3/PI) is an important transcription activator that binds to the promoter of abscisic aldehyde oxidase (AAO3) and transactivates it. The over-expression of AAO3 increases levels of the hormone abscisic acid and promotes chlorophyll degradation during senescence (Yang et al. 2014). Besides, it plays a crucial role in the regulation of dehydration in senescing leaves. In addition, it also binds to the promoter region of SAG (Senescence-Associated Gene) 113, a negative regulator of ABA signaling for stomatal closure and control of water loss during leaf senescence (Zhang and Gan 2011). EIN3 directly binds to NAP and increases its expression in the ethylene-induced senescence (Kim et al. 2014; Kim et al. 2015; 2016).

ANAC055 and ANAC019, two important SAGs, play an important role in ethylene senescence response (Hickman et al. 2013; Bengoa Luoni et al. 2019). Studies indicate an EIN2-dependent regulation of these genes (Bu et al. 2008; Kim et al. 2014; 2018). It has been shown that EIN3 can bind to ANAC055 and ANAC019 promoters (Chang et al. 2013). These ANACs more specifically activate Chlorophyll

Catabolic Genes (CCGs) for chlorophyll degradation, VSP1 for temporary storage of nitrogen and BSMT1 for salicylic acid catabolism.

Several AP2/ERF genes activated by ethylene regulate the progression of leaf senescence. AP2/ERF transcription factor family comprising of 146 members acts both as an activator and a repressor of transcription (Mitsuda and Ohme-Takagi 2009; Dubois et al. 2018). Among these is a special subgroup of repressors with the ERF-associated repression (EAR) motifs (including NtERF3, AtERF4 and AtERF8) that positively control the leaf senescence onset in *Arabidopsis* (Ohta et al. 2001; Koyama et al. 2013). The AtERF4 transcription factor acts downstream of EIN2, as is evident from its EIN2-dependent expression in the leaves (Fujimoto et al. 2000). This transcription factor, along with AtERF8, is degraded by a proteasomal-dependent pathway and accumulates within the plant as a result of increasing age (Koyama et al. 2013). ERF TFs act as direct repressors for the expression of ESP/ESR (Epithiospecifier Protein/Epithiospecifying Senescence Regulator) gene, which in turn represses leaf senescence onset (Miao and Zentgraf 2007; Koyama et al. 2013), at the transcriptional as well as post-translational levels (Miao and Zentgraf 2007). Both AtERF4 and AtERF8 inhibit ESP/ESR, thus activating WRKY53.

While many of the WRKY transcription factors have multiple functions in plants, some specific ones are involved in the senescence-regulating processes (Guo et al. 2004). Among these senescence-regulating transcription factors, WRKY53 deserves a special mention, as it is one of the central hubs in the WRKY network regulating early senescence. WRKY53, besides influencing SAGs, also directly targets other WRKY factors regulating their functions. WRKY53 directly regulates the genes associated with the general features of senescence such as remobilization processes and transport (Zentgraf and Doll, 2019). WRKY57, which downregulates the expression of SAG4 and SAG12, is degraded by WRKY53 via an F-box protein. Besides, WRKY53 directly binds to the promoter of SAG12 and upregulates it (Zentgraf and Doll, 2019). Although WRKY network is regulated by different environmental factors and other hormones, it has also been implicated in the ethylene-regulated leaf senescence. In particular, ethylene response of the WRKY53 gene is transmitted via Ethylene Response Factor 4 (ERF4) and Ethylene Response Factor 8 (ERF8), which modulate the expression of the ESR/ESP (Epithiospecifying Senescence Regulator/ Epithiospecifier Protein), a well-known WRKY53 regulator (Koyama et al. 2013).

7.9 Ethylene Signal Transduction Pathway

The ethylene signaling pathway involves both positive and negative regulators. In the absence of ethylene, CTR1 is stimulated (turned *on*) by the receptors, and its kinase activity suppresses the ethylene responses (Shakeel et al. 2013; Merchante et al. 2013; Yang et al. 2015; Ju and Chang 2015). In the presence of ethylene, the binding of receptors to ethylene inactivates CTR1 kinase activity (turned *off*). In fact the complex formation of receptor with CTR1 induces conformational changes that are likely responsible for the regulation of CTR1 kinase activity by the receptors. Although both the subfamilies of ethylene receptors (subfamily-1 and subfamily-2) bind to CTR1, the genetic analysis depicts greater roles of the subfamily-1 receptor/ CTR1 complexes than the subfamily-2 receptor/CTR1 complexes in both the ctr1 regulation and downstream hormone response (Gao et al. 2003; Qu et al. 2007; Shakeel et al. 2015). This differentiating role is attributed to His-kinase activity and the phosphorylation state of the members of subfamily-1 receptors (Ju et al. 2012; Qiao et al. 2012). CTR1 directly phosphorylates EIN2 (putative metal transporter) and maintains it in an inactive state. CTR1 phosphorylates six (Ju et al. 2012) or four (Chen et al. 2011) amino acids in EIN2, but the phosphorylation of two amino acids (Ser645 and Ser924) appears to be more crucial in the context of ethylene signaling (Chen et al. 2011; Qiao et al. 2012; Ju et al. 2012). When ethylene binds to receptors and CTR1 is inactivated, EIN2, which is now in a dephosphorylated state, is subjected to proteolytic cleavage via Ub/26S proteasome. The resulting C-terminal product of EIN2 (EIN2C) or EI2 C-END (CEND) is processed and translocated to the nucleus (Qiao et al. 2012; Ju et al. 2012; Zhang et al. 2014; Zhang et al. 2015; Zhang et al. 2019), where it interacts with ENAP1 (Ein2 Nuclear-Associated Protein1) and promotes histone acetylation of chromatin, thereby smoothening the progress of the DNA binding of EIN3/EIL family members and commencing the ethylene transcriptional response (Zhang et al. 2016; Zhang et al. 2017; Wang et al. 2017). In addition, EIN2C also functions in the cytosol by repressing the expression of IEBF F-box protein (that targets members of the EIN3/EIL family for degradation), thus inhibiting the EIN3/EIL degradation (Li et al. 2015; Merchante et al. 2015) (Fig. 7.4).

7.10 Conclusion

Leaf senescence is a highly regulated developmental process, occurring at the end of a leaf's active life. This developmental phase is manifested by many physiological changes including the degradation of chlorophyll, proteins and lipids along with the changes in the nucleic acid metabolism. The process is very intricate and tightly regulated by different external and internal factors. Ethylene is an important phytohormone which positively regulates leaf senescence. The ethylene signaling process involves different receptors and transcriptional factors, which function in a well-defined way. The main targets of the ethylene signal transduction are the important SAGs which lead to leaf senescence.

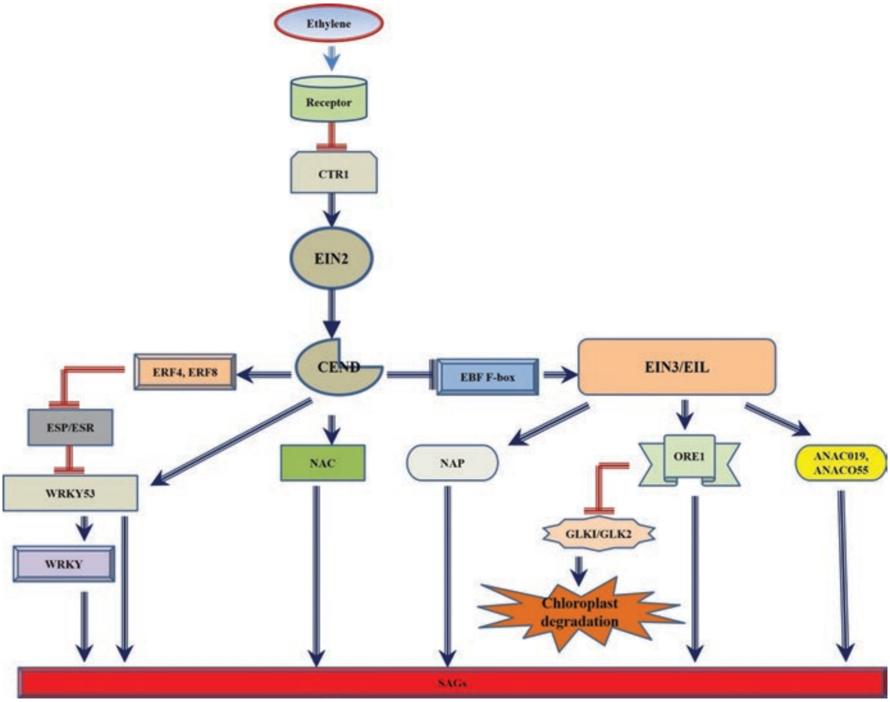

Fig. 7.4 A model for ethylene perception and signal transduction during leaf senescence

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Chapter 8
Methyl Jasmonate and Brassinosteroids: Emerging Plant Growth Regulators in Plant Abiotic Stress Tolerance and Environmental Changes

Haifa Abdulaziz S. Alhaithloul and Mona H. Soliman

8.1 Introduction

With the burgeoning rise in human population, the demand for food, clothing, and shelter is also increasing tremendously, and to meet these challenges, it is quite necessary to accelerate the productivity of crop plants under the challenging circumstances. Plants are sessile and encounter various biotic and abiotic pressures which hamper their growth and productivity to a considerable extent. Due to change in climatic scenario and the threat of global warming, plants are challenged with a myriad of abiotic pressures from early to mature stage during the course of their life cycle (Sadiq et al. 2020). Abiotic pressures like salt stress, drought, and cold and high temperature are known to severely affect growth and productivity of diverse crop plants (Ahmad et al. 2019; Fariduddin et al. 2019; Sadiq et al. 2020). Nevertheless, as a consequence of depletion of resources (water and soil) and erosion of soil, the global land area under cultivation has also been reducing. According to the the 2007 report of the Food and Agriculture Organization (FAO), only 3.5% of the global area remained unaffected by some environmental constraint. The increased global population thus demands balanced production of crop plants, and there is a dire need for engineering new crop cultivars/varieties with improved tolerance to various stresses and more yields with optimum nutritional value.

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In view of these circumstances, productivity of crop plants should be increased so that abiotic stress-induced impacts are minimized to an appreciable extent. Therefore, engineering abiotic stress tolerance is vital for sustainable crop production under the impacts of dwindling natural resources, increase in salt and metal/metalloid challenged environments, and global climate change. Hence, in order to address these requirements of the growing population, it is essential that crop yield per unit area should be increased significantly, and situation should only be achieved/possible with the use of additional/exogenous supplements/inputs. One such approach is the external supplements of phytohormones. Plant hormones are cheap, biodegradable, and highly potent in regulating growth and development of plants and also increasing crop yield under optimal as well as stressed conditions. Plant hormones (phytohormones) are a group of small, active signaling elicitors that regulate the complex process of growth and development in a well-coordinated action. These signaling elicitors are well-known to act either near to their synthesis or are transported to other regions from their sites of biosynthesis to control myriads of physiological, biochemical, and/or molecular responses of crop plants under optimal or stressful environmental pressures.

Jasmonic acid and its conjugates, such as methyl jasmonate (MeJ) and jasmonoyl-isoleucine (JA-Ile), both known as jasmonates (JAs), are one of the important and ubiquitous PGRs found in the plant kingdom (Ghasemi Pirbalouti et al. 2014). JAs were earlier identified to function as growth regulators in diverse plant species (Engelberth et al. 2001). As stated above, MeJ is one of the conjugates of JA are fatty acid derivatives and was initially identified in *Jasminum grandiflorum* L. flowers (Demole et al. 1962). However, a decade later, the first possible isolation of JA was reported from *Lasiodiplodia theobromae* (fungus) culture filtrate (Alderidge et al. 1971). In the early 1980s, processes like inhibition of root growth and senescence promotion in Japan and Germany were observed and were found to be regulated by JAs (Hause and Schaarschmidt 2009). It has also been reported that JAs can either suppress or enhance responses of plants to various stress (Agrawal et al. 2003). In addition, MeJ has also been evidenced to mediate the upregulation of secondary metabolism and stress defense system in plants (Chen et al. 2011). In crop plants, MeJ control vital processes like photosynthesis and antioxidant metabolism (Maserti et al. 2011; Per et al. 2016; Ahmad et al. 2016; Zaid and Mohammad 2018). Nevertheless, JAs exert a significant role in responses of plants to heavy metals (Piotrowska et al. 2009; Poonam et al. 2013; Ahmad et al. 2017; Zaid and Mohammad 2018; Bali et al. 2018), flooding stress (Kamal and Komatsu 2016), drought (Gao et al. 2004; Brossa et al. 2011; Wei-Wei et al. 2011), salt (Dong et al. 2013; Enteshari and Jafari 2013), chilling (Gonzalez-Aguilar et al. 2000), and heat stress (Clarke et al. 2009).

BRs are polyhydroxylated sterol-derived phytohormones which are present in all plant species. Brassinolide is considered as the most active form of BRs (Grove et al. 1979). 28- Homobrassinolide (28-HBL) and 24-epibrassinolide (24-EBL) are the two brassinosteroid isomers. BRs are regarded as classical phytohormones in imparting abiotic stress tolerance in diverse crop plants (Fariduddin et al. 2014a; Ahanger et al. 2018; Gruszka 2018; Jiroutova et al. 2018; Banerjee and Roychoudhury

2018; Sharma et al. 2017; Fang et al. 2019; Planas-Riverola et al. 2019; Ahammed et al. 2020a, b).

Therefore, in view of the above discussion, the present chapter thus focuses on the potent role of these two important phytohormones, viz., MeJ and BRs, in enhancing physiological roles and providing plant tolerance to various non-biological stresses. Furthermore, this review also highlights crosstalk of these two PGRs with other phytohormones in conferring abiotic stress tolerance.

8.2 Methyl Jasmonate and Brassinosteroids and Abiotic Stress: Main Alleviating Factors in Plant Abiotic Stress Tolerance

In the current times, various plant signaling molecules are being utilized to alleviate negative environment-induced stress impacts, and this strategy is now visualized as a potential means for sustainable crop production (Ahmad et al. 2019; Wani et al. 2016; Ciura and Kruk 2018; Islam et al. 2020, Islam and Mohammad 2020). In the following sections, an appraisal is provided to dissect and gain an insight into the potential role of MeJ and Brstds in overcoming major environmental stresses in diverse crop plants. In addition a tabulated review is also given in Table 8.1 showing some of the representative examples of these two PGRs in plants encountering major abiotic stresses.

8.2.1 Salinity Stress

Various pressures, including both biotic and abiotic, correspond to the main factors in reducing the overall productivity of crop plants. Salinity is among the major abiotic stress factors that prevent sustainable development of plants (Muchate et al. 2016; Wan et al. 2017; Etesami and Noori 2019; Fariduddin et al. 2019). Salinity stress in arid and semi-arid regions is imposed by various anthropological activities and is a natural component of the ecosystem (Abrol et al. 1988). In arid and semi-arid regions of the world, salinity is often a result of low precipitation and high temperature (Etesami and Noori 2019). On a global scale, salt stress is known to affect 20% of the total irrigated cultivable land comprising more than 45 million hectares that results in significant reductions in growth and yield of important crops (Qadir et al. 2014; Shrivastava and Kumar 2015). Every year, soil salinity decreases crop yields worth billions of dollars (Shabala and Cuin 2008). In plants salinity results in ionic, oxidative, and osmotic stress which negatively affects the growth and development of plants (Khan et al. 2010; Hasanuzzaman et al. 2014; Fariduddin, et al. 2014b; Sehar et al. 2019; Smoleń et al. 2020). A soil is characterized as saline when the electrical conductivity (ECe) of ions is more than 4 dS m^{-1} (Munns and

Table 8.1 Representative studies showing the effect of BRs and methyl jasmonate in diverse crop plants under different abiotic stress environments

Environmental stress	PGR application	Plant species	Effect	Reference
Cd (150 mg/kg)	MeJ (1 μM)	*Mentha arvensis* L.	Cd reduced plant growth traits, SPAD index photosynthesis, and stomatal movements. MeJ exogenous application ameliorated growth reduction, increases in photosynthesis, activities of antioxidant enzymes and osmolytes and ROS detoxification	Zaid and Mohammad (2018)
Cd (50 μM) concentration	MeJ (10 μM)	*Brassica juncea* L.	Cd stress increased oxidative markers such as electrolyte leakage and malondialdehyde and hydrogen peroxide contents, which were decreased by application of MeJ treatment. MeJ alleviates Cd-induced photosynthetic damages by increasing S assimilation	Per et al. (2016)
B (1 and 2 mM)	MeJ (300 μM)	*Artemisia annua* L.	Application of MeJ reduced B-induced oxidative stress by lowering levels of ROS production and lipid peroxidation level, enhancing antioxidant system, and increasing secondary metabolite accumulation	Aftab et al. (2011)

(continued)

Table 8.1 (continued)

Environmental stress	PGR application	Plant species	Effect	Reference
Pb solution (0, 0.25, 0.50, and 0.75 mM)	MeJ concentrations (0, 0.01, 1, and 100 nM)	*Lycopersicon esculentum* L.	Pb stress caused a decrease in shoot and root lengths. Pb uptake and contents of osmolyte and metal chelating compounds (total thiols and non-protein and protein-bound thiols) were found to be increased with increasing Pb levels. Treatment with MeJ improved shoot and root lengths, reduced Pb uptake, increased chlorophyll and carotenoid contents, and improved gaseous exchange parameters, such as internal CO_2 concentration, net photosynthetic rate, stomatal conductance, and transpiration rate. MeJ also enhanced the enzyme activity of ascorbate-glutathione cycle and reduced H_2O_2 concentration. MeJ primed seeds had increased contents of osmolytes and metal chelating compounds under Pb stress	Bali et al. (2018)

(continued)

Table 8.1 (continued)

Environmental stress	PGR application	Plant species	Effect	Reference
Drought stress (40% field capacity)	MeJ (0.5 mM)	*Dracocephalum kotschyi Boiss*	Plants treated with MeJ had higher fresh and dry weights; low electrolyte leakage, MDA, and H_2O_2, and phenol content; and increased total antioxidant activity and antioxidant power assay. Drought-stressed plants treated with MeJ had more intense and amount of essential oil components	Bidabadi and Sharifi (2020)
As concentrations (50 and 200 µM)	MeJ (0.1 and 1 µM)	*Brassica napus* L.	As (200 µM) was found to be more phytotoxic than 50. MeJ application caused a significant increase in leaf chlorophyll fluorescence and biomass production, reduction in MDA content, lower levels of ROS synthesis, and maintenance of high redox states of reducing molecules (glutathione and ascorbate). MeJ induced the enhanced expression of antioxidants (SOD, APX, CAT, POD) and secondary metabolites (PAL, PPO, CAD) and significantly reduced the As content in both leaves and roots of both cultivars	Farooq et al. (2016)

(continued)

Table 8.1 (continued)

Environmental stress	PGR application	Plant species	Effect	Reference
Drought stress (25% pot water capacity)	24-Epibrassinolide (1 μM)	*Capsicum annuum* L	Oxidative stress biomarkers like electrolyte leakage, MDA level, and H_2O_2 production were significantly declined, while proline and total soluble sugar were increased on subjecting plants with 24-EBR. In addition, 24-EBR maintained all chlorophyll fluorescence parameters for normal photosynthesis	Khamsuk et al. (2018)
Cu (100 mg/kg) and NaCl (150 mM)	24-Epibrassinolide (0.01 μM)	*Cucumis sativus* L.	Plants exposed to simultaneous NaCl and Cu toxicity showed a significant decline in fresh and dry mass, chlorophyll content, activities of carbonic anhydrase, net photosynthetic rate, and maximum quantum yield of the PSII primary photochemistry. Follow-up treatment with 24-EBR showed an improvement in growth, chlorophyll content, carbonic anhydrase activity and photosynthetic efficiency, and activities of antioxidant enzymes, viz., CAT, POD, and SOD, and content of proline. In conclusion, the elevated levels of antioxidants and osmolyte (proline) conferred tolerance to the joint NaCl- and Cu-induced oxidative stress	Fariduddin et al. (2013)

(continued)

Table 8.1 (continued)

Environmental stress	PGR application	Plant species	Effect	Reference
Cd (300, 600 and 1200 µM)	24-Epibrassinolide (10^{-9} mol/L)	*Solanum nigrum* L.	Cd stress reduced plant height, root length, and chlorophyll content but increased MDA content. The external application of 24-EBR enhanced SOD, POD, and CAT activities and also proline and sugar contents and concomitantly decreased MDA content. 24-EBR increased photosynthesis by increasing the chlorophyll content through increasing the expression of synthetic genes and decreasing the expression of degraded genes, accumulation of Cd, and increased *Fv/Fm*	Peng et al. (2020)

(continued)

Table 8.1 (continued)

Environmental stress	PGR application	Plant species	Effect	Reference
Pb doses (1, 10, 100, and 1000 µM)	MeJ (0.01, 0.1, 1, 10, and 100 µM)	*Wolffia arrhiza* L.	MeJ acted in a dose-dependent manner. The highest concentration of 100 µM further aggravated the heavy metal phytotoxicity resulting in an increase in metal biosorption and formation of lipid peroxides and decreased fresh weight and chlorophyll *a*, carotenoid, monosaccharide, and soluble protein content. MeJ applied at 0.1 µM protected *W. arrhiza* against Pb stress by inhibiting the accumulation of heavy metal and promoting optimum plant growth. In addition, MeJ (0.1 µM) upregulated enzymatic and non-enzymatic antioxidants	Piotrowska et al. (2009)
Salt (0, 40, and 80 mM)	Both PGRs-MeJ (0 and 75 mM) and 24-epibrassinolide (0, 5.1, and 3 mM)	*Brassica nigra* L.	Increased salinity concentration decreased chlorophyll concentrations, Hill reaction rate, transpiration rate, and stomatal conductance but increased stomatal resistance and maximum fluorescence. All the traits were improved at the same amounts of salinity stress, with increasing concentrations of MeJ and 24-EBR	Rezaei et al. (2018)

(continued)

Table 8.1 (continued)

Environmental stress	PGR application	Plant species	Effect	Reference
Temperature stress (4 and 44°C)	28-Homobrassinolide (10^{-9}M)	*Brassica juncea* L.	Temperature stress caused by free radicals production suppressed membrane stability and growth and caused cell death in tested plant seedlings. Enhanced MDA, H_2O_2, and the content of NO resulted in enhanced oxidative stress. Activities of SOD, CAT, GPOX, and APOX were enhanced. Priming plants with of 28-HBR maintained the growth of seedlings; enhanced the activities of SOD, CAT, GPOX, and APOX; and reduced oxidative stress caused due to temperature stress. The formation of dead cells was reduced in 28-HBR-treated plants	Sirhindi et al. (2017)

(continued)

Table 8.1 (continued)

Environmental stress	PGR application	Plant species	Effect	Reference
Ni (2 mM)	MeJ (1 nM)	*Glycine max* L.	Ni decreased growth traits and chlorophyll content and increased H_2O_2, MDA, and NADPH oxidase. The application of MeJ improved the chlorophyll content and growth characters. MeJ restored the disturbed chlorophyll fluorescence parameters; increased osmolytes, protein, and sugar contents; minimized the accumulation of oxidative stress biomarkers; and enhanced the expression of antioxidant enzyme activities	Sirhindi et al. (2016)
Salt (100 mM NaCl solution having 10.7 dS m^{-1} EC)	MeJ (2mM JA)	*Daucus carota* L.	Salt stress imposed reduction in root and leaf mass and resulted in the accumulation of Na and Cl ions. There was a differential uptake in accumulation of minerals in salt-tolerant and salt-sensitive genotypes. MeJ had an impact on underlying mechanisms that are involved in improving salt tolerance in salt-sensitive carrot varieties	Smoleń et al. (2020)

BRs brassinosteroids, *PGR* plant growth regulator, *ROS* reactive oxygen species, *MeJ* methyl jasmonate

Tester 2008). The principal salt that causes soil salinity is sodium chloride (NaCl) normally found in irrigation water or seawater (Deinlein et al. 2014). Prolonged and high accumulation of NaCl ions in plants causes disturbed Na^+/K^+ homeostasis, enzyme inhibition, and enhanced production of myriads of reactive oxygen species (ROS), thereby resulting in oxidation of membrane lipids, protein nucleic acids, and other macromolecules (Mudgal et al. 2010; Ahmad et al. 2018), growth inhibition, and reduction in crop productivity (Khan et al. 2009; Fatma et al. 2016; Ahanger and Agarwal 2017; Elbasan et al. 2020). Salinity stress also modulates nutrient acquisition, osmoprotectant accumulation including proline and glycine betaine (GB), redox potential, phytohormone levels and enzymatic and non-enzymatic antioxidants, and components of ascorbate-glutathione (AsA-GSH) cycle, sugar metabolism, secondary metabolites which in turn overcomes the adverse effects of salt-induced ROS (Khan et al. 2010, 2012; Fatma et al. 2016; Siddiqui et al. 2017; Khanam and Mohammad 2018; Medini et al. 2019; Li et al. 2020). Plant stress biologists are thus concerned with chalking out various alternatives to increase the production of crops under salt stress. Both PGRs, viz., MeJ and Brstds, are known to play an alleviating role in diverse crop plants during salt stress environments. In a study, Tavallali and Karimi (2019) applied foliar (0, 0.025, 0.050, and 0.075 mM) MeJ on almond exposed to various concentrations of NaCl (0, 50, 100, and 150 mM). The results revealed that higher levels of salt stress decreased the plants' growth. Foliar MeJ concentrations particularly 0.025 to 0.05 mM alleviated effects of salt stress by increasing photosynthetic potential, activity of antioxidant enzymes, and growth traits, as well as maintaining cell membrane integrity. Nevertheless, MeJ-mediated mitigating effect on the growth of salt-stressed almond plants was attributed to the inhibition of cytokinin. Taken together, their results suggested the interplay of two PGRs (MeJ and cytokinins) in protecting and improving growth of almond rootstocks under salt stress. The cumulative effect of Brstds (24-epibrassinolide) and MeJ under salinity stress (0, 40, and 80 mM) was reported in *Brassica nigra* (Rezaei et al. 2018). The effect of MeJ (0 and 75 Mμ) and 24-epibrassinolide (0, 1.5, and 3 Mμ) on photosynthesis, chlorophyll fluorescence, and leaf stomatal traits was studied in a hydroponic culture. Chlorophyll content, Hill reaction, transpiration rate, and stomatal conductance decreased, but stomatal resistance and maximum fluorescence (Fm) increased with increasing concentration of salinity. However, both MeJ and 24-epibrassinolide (24-EBL) at the same amounts of salt stress improved all these tested traits. The study provided a clue on the conceivable interaction of these two PGRs in enhancing salt stress tolerance in black mustard (*Brassica nigra* L.). Amraee et al. (2019) investigated the priming impact of 24-EBL on extent and pattern of cytosine DNA methylation by adopting the technique of methylation-sensitive amplified polymorphisms (MSAP) to *Linum usitatissimum* L. under salinity (150 mM) stress exposure. Salinity stress decreased the total methylation of CCGG sequences, while 24-EBL (10^{-8} M) priming induced its total methylation. Taken together, their results revealed that 24-EBL seed priming under salinity stress exposure could show a regulatory role in protecting flax plants via epigenetic modification and induction of methylation. The potential of exogenous 24-EBL (1.04 μmol L^{-1}) by soaking and root dipping in alleviating the impacts of salinity (0, 100, 200 mmol L^{-1}) stress on chloroplasts and photosynthesis was studied in *Robinia pseudoacacia* L. plants (Yue et al. 2019). The results suggested that salinity stress reduced photosynthetic potential, chlorophyll content, transpiration rate, stomatal conductance, water-use efficiency, photochemical quenching, and quantum efficiency but increased H_2O_2 content and antioxidant enzyme activities, leakage of membrane, and Na^+ ions in leaves and chloroplasts and also caused a disruption in chloroplast ultrastructure. Treatment of plants with 24-EBL exogenously showed an improved membrane stability index and less Na^+ content, while thylakoid membrane structure was found to be protected.

8.2.2 *Metal/Metalloid Stress*

Metal/metalloid-induced negative impacts in integrative plant physiology are a global emerging problem to sustainable agricultural production (Wani et al. 2018; Zaid et al. 2020). Agricultural soil contamination by metal/metalloid is due to various anthropogenic activities like discharge of effluents from industries and factories, sewage and urban runoff, improper use of pesticides and fertilizers in agriculture and allied areas, release of domestic garbage, military warfare activities, and automobile emissions (Dalvi and Bhalerao 2013; Singh et al. 2016; Ali et al. 2018; Dutta et al. 2020). Metal/metalloid alters plant physiological processes by inducing excess generation of ROS. There is literature available on the significance of MeJ and Brstds in alleviating the metal−/metalloid-induced oxidative stress in plants. Plants treated with either these PGRs were reported to have enhanced growth and photosynthetic and antioxidant traits in several crop plants. MeJ counteracts toxicity of cadmium (Cd, 150 mg/L) (Zaid and Mohammad 2018), As (Farooq et al. 2016, 2018), Cu (Hanaka et al. 2015; Poonam et al. 2013), B (Aftab et al. 2011), Pb (Piotrowska et al. 2009), and Ni stress (Sirhindi et al. 2016) in crop plants. In a hydroponic study involving rice plants, the significance of MeJ (0.25 μM)-induced molecular signaling and tolerance toward As (25 μM) stress was studied (Verma et al. 2020). The results showed that As decreased the overall growth and development of rice. The application of MeJ incremented plant biomass and chlorophyll content, increased antioxidant enzyme activities, and decreased MDA content, electrolyte leakage, and accumulation of total As in root and shoot of rice plants. Exogenous MeJ conferred As stress tolerance by modulating the expression of genes engaged in downstreaming of signaling pathways of JA (*OsCOI*, *OsJAZ*3, *OsMYC2*), uptake of As (*OsLsi1*, *OsLsi2*, *OsNIP1*;1, *OsNIP3*;1), its translocation (*OsLsi6*, and *OsINT5*), and finally its detoxification (*OsNRAMP1*, *OsPCS2*, and *OsABCC2*). On the other hand, BR analogs like 24-epibrassinolide (24-EBL) and 28-homobrassinolide (HBL) are known to ameliorate metal−/metalloid-induced oxidative stress in diverse crop plants. Yusuf et al. (2011) studied the impact of 28-HBr (10^{-8} M) in mitigating boron (0.50, 1.0, and 2.0 mM) stress in *Vigna radiata* L. plants. The levels of boron reduced growth, plant water relations, photosynthetic attributes, activities of nitrate reductase and carbonic anhydrase in a concentration-dependent manner whereas, an enhancement in MDA content, H_2O_2, electrolyte leakage, proline as well as various antioxidant enzymes was observed. Follow-up on treatment with 28-HBr to boron-stressed plants showed an improvement in growth, water relations, photosynthesis, antioxidant enzyme activities, viz., CAT, POX, and

SOD, and proline content. Nevertheless, 28-HBr-induced B stress tolerance was regarded to elevated level of antioxidant enzymes as well as proline content.

In a recent study, Peng et al. (2020) studied the impact of 24-EBL in enhancing Cd tolerance of *Solanum nigrum* L. plants by assessing their growth, photosynthetic activity, antioxidant activities, and Cd concentration. Results revealed that Cd stress reduced plant height, root length, and chlorophyll content but increased malondialdehyde (MDA) content. 24-EBL supply enhanced proline and soluble sugar and promoted photosynthesis by enhancing chlorophyll content and Fv/Fm on one hand and decreasing MDA content on the other. Nevertheless, antioxidant activities like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were found to be increased. EBL-mediated increase in chlorophyll content was attributed to the increased expression of synthetic genes and decreased expression of degraded genes. In yet another study, Talarek-Karwel et al. (2019) studied the potential of 24-EBL (1 μM) in countering Pb (0.01 and 500 μM) stress in *Acutodesmus obliquus*. Pb imposes negative effects in both the growth and development of green algae and induces oxidative stress. However, 24-EBL proved to be effective in countering Pb stress by increasing the number of cells and metabolite contents (e.g., proteins, monosaccharides, chlorophylls, carotenes, and xanthophylls); reducing endogenous Pb, H_2O_2, and MDA content; and increasing activities of CAT, APX, SOD, and GR, ascorbate and glutathione contents, and phytochelatin synthase activity. A hydroponic experiment was performed to unravel the 24-EBL (1 μM)-induced biochemical- and molecular-mediated mechanisms conferring As (100 and 200 μM) toxicity tolerance in *Arabidopsis thaliana* L. (Surgun-Acar and Zemheri-Navruz 2019). Enzymatic activities of SOD and CAT; total antioxidant status; MDA and proline content; expression profiles of *SOD* isoform genes (*Cu-ZnSODs*, *FeSODs*, and *MnSOD*), *CAT* isoform genes (*CAT1*, *CAT2*, and *CAT3*), and heat shock proteins (*Hsp70–4* and *Hsp90–1*); and proline biosynthesis (*P5CS1* and *P5CS2*) were studied. 24-EBL increased SOD and CAT activities and total antioxidant and proline levels but decreased the MDA level increased in As-treated plants. The transcript levels of *CSD1*, *CSD2*, *FSD1*, *FSD2*, P5CS1, *MSD1*, *CAT2*, *Hsp70–4*, and *Hsp90–1* genes were found to be increased by the treatment of 24-EBL, but expression of *CSD3*, *CAT1*, and *CAT3* genes was downregulated by the combined treatment of As and 24-EBL. Their results suggested that 24-EBL exerts an improving effect on the antioxidant defense system at biochemical and transcriptional levels in *A. thaliana* plants to promote As stress tolerance.

In a greenhouse experiment, Zhou et al. (2018) studied the potential of exogenous 24-EBL in alleviating oxidative damage from Co-induced stress in *Vitis vinifera* L. cuttings on root parameters, active oxygen metabolism, contents of osmolytes, antioxidant activities, levels of endogenous phytohormone, and ascorbate-glutathione (AsA-GSH) cycle enzyme activity, viz., monodehydroascorbate reductase (MDHAR), glutathione peroxidase (GR), ascorbate peroxidase (APX), and dehydroascorbate reductase (DHAR) and the contents of the antioxidant ascorbate (AsA) and dehydroascorbic acid (DHA). The results revealed that 24-EBL increased total root length, root surface area, root diameter and volume, and tip number; enhanced the contents of protein and proline; increased activities of SOD, CAT, and

POD and levels of endogenous PGRs abscisic acid, jasmonic acid, and salicylic acid in leaves; and regulated the balance of the AsA-GSH cycle enzymes while significantly decreasing oxidative indicators like H_2O_2, O_2•-, and MDA contents in roots and leaves and contents of glutathione (GSH) and oxidized glutathione (GSSG). In a completely randomized block design study conducted on *Brassica juncea* plants, Fariduddin et al. (2015) studied the mitigating effects of 24-EBL under manganese (Mn)-induced toxicity. 24-EBL protected plants from Mn stress, and EBL-mediated mitigating effect was attributed to improved antioxidant defense system and photosynthetic attributes. The ameliorative role of 24-EBL (0.5 μM) among others was studied under As (100 μM)-induced oxidative stress in *Glycine max* L. (Chandrakar et al. 2018). The results showed that As reduced growth traits like germination percentage, radical length and dry mass, viability, total protein content, and activities of SOD, CAT and APX while increasing sugar and proline content and oxidative stress biomarkers like electrolyte leakage, ROS accumulation, MDA, protein carbonyls, and hydroperoxides. Interestingly 24-EBL treatment played a protective role and caused an improvement in growth trait; viability; total protein, sugar, and proline content; and antioxidant activities while reducing the levels of oxidative stress markers and As accumulation, respectively. Soares et al. (2016) studied the effect of 24-EBL on ROS content, antioxidant defense system, peroxidation of lipid membranes, and Ni uptake in *Solanum nigrum* L. under Ni toxicity. Ni stress resulted in a decline in weight and lengths of shoots and roots, cell death symptoms in the form of chlorotic and necrotic spots, a reduction in photosynthetic pigments, soluble protein content, and RuBisCO contents but increased the enzymatic antioxidant system. By employing reverse transcriptase-PCR analysis, their results revealed that the exogenous application of 24-EBL increased plant tolerance to Ni-induced stress. In a recent work, Xie et al. (2020) applied 24-EBL and found an enhancement in the phytoextraction of Cd to *Amaranthus hypochondriacus* plants grown under Cd-contaminated soil.

8.2.3 Drought Stress

Due to change in climate pattern and the threat to global warming, in present times, drought stress is deliberated as one of the crucial abiotic stresses for declining growth and productivity of plants (Boyer 1982; Vinocur and Altman 2005; Iseki et al. 2018; Kaya et al. 2020). Drought is known to impose alterations in physiological and metabolic processes in plants. The unavailability of soil water causes drought (Ahamed et al. 2020a, b). Moreover, insufficient rainfall or irrigation results in drought. Drought stress is prevalent in arid and semi-arid regions of the world (Farooq et al. 2009; Zargar et al. 2017). Drought stress results in osmotic stress that causes altered cellular activities by disrupting the normal homeostasis of plants (Begum et al. 2019; Raja et al. 2020). Brstds and MeJ are known to play an ameliorating role under drought stress. Aghaee and Rahmani (2019) studied the biochemical and molecular responses of flax plants under drought stress to 24-EBL (10^{-8} M) seed priming. The plants were subjected to 6%, 12%, and 18% polyethylene glycol (PEG)-induced drought stress. Results revealed that drought stress increased the activities of CAT, POD, and SOD and protein, proline, and MDA contents but decreased APX and NR activities. 24-EBL triggered the upregulation of Mn-SOD, POD1, POD3, and ERF gene expression and WRKY 40 transcript levels while causing a reduction in MDA content. In a greenhouse experiment, Wang et al. (2019) tested the effect of 24-EBL in alleviating drought stress damage in *Prunus persica* L. plants. They noted that drought stress decreased the photosynthetic ability but increased the antioxidant enzyme activity, autophagy, and expression of putative autophagy-related genes (*ATG*s). Exogenous 24-EBL (1 μM) decreased the MDA levels but increased SOD, CAT, POD, APX, and GR activities while inhibiting the expression levels of *PpATG*s. Taken together, the results suggested the ameliorative role of 24-EBL treatment under drought stress damage to peach plants via decreasing *PpATG* expression and reducing the number of autophagosomes. The efficacy of 24-EBL (1 μM) on chili pepper under drought (25% pot water capacity) was investigated (Khamsuk et al. 2018). Under 24-EBL application, electrolyte leakage, MDA content, and H_2O_2 production were significantly declined, while proline and soluble sugar content, net photosynthesis rate, stomatal conductance, and intercellular CO_2 concentration were increased. In addition, application of 24-EBL maintained chlorophyll fluorescence parameters $F\text{v}/F\text{m}$, $F\text{v}'/F\text{m}'$, ΦPSII, qP, and ETR for optimum photosynthesis and osmotic maintenance.

In a complete randomized design experiment, the effect of 24-EBL (25 mg L^{-1}) application in regulating photosynthesis and hormonal balance was studied in drought-stressed *Zea mays* L. Results revealed that imposition of drought impaired photosynthetic pigments; photochemical reactions of photosynthesis; net photosynthetic rate; transpiration rate; stomatal conductance; maximum quantum efficiency of PSII photochemistry; electron transport rate; actual photochemical efficiency of PSII; photochemical quenching coefficient; effective quantum yield of PSII photochemistry; activities of RuBisCo, rubisco activase, and carbonic anhydrase; carbohydrate content; as well as concentrations of PGRs. These changes were significantly overcome by exogenous application of 24-EBL to promote growth and photosynthesis of maize plants under drought stress (Talaat 2019). The comparative effect of 24-EBL and 28-HBr in mitigating drought stress in pigeon pea seedlings was investigated (Shahana et al. 2015). Application of both 24-EBL and 28-HBL treatments alleviated drought-induced negative effects to promote germination, growth, and increased dry mass production. The application MeJ has also been evidenced to promote growth and photosynthesis in diverse crop plants under drought stress persistence. In yet a recent study, Bidabadi and Sharifi (2020) applied 0.5 mM MeJ in *Dracocephalum kotschyi Boiss* under drought stress to study the changes in antioxidant defense system and the composition of various active compounds. The results showed that a crosstalk between MeJ and strigolactone played a crucial role in imparting drought stress resistance and optimization of the essential oil production.

8.3 Conclusion and Future Prospectus

The present literature clearly suggests that both PGRs are important growth-regulating agents in diverse crop plants. Environmental stresses disturb the internal redox environment by inducing oxidative stress. BRs and MeJ ameliorated these abiotic stresses by regulating and modulating diverse plant physio-biochemical attributes. PGRs (BRs and MeJ) induced antioxidant defense system and enhanced osmolyte and primary and secondary metabolite accumulation under abiotic pressures. These changes result in the entire recharged (maintained/engineered) plant antioxidant defense machinery due to which the plants are able to detoxify ROS to tolerate various environmental stresses. Figure 8.1 shows a mechanistic elucidation of BR- and MeJ-induced abiotic stress tolerance.

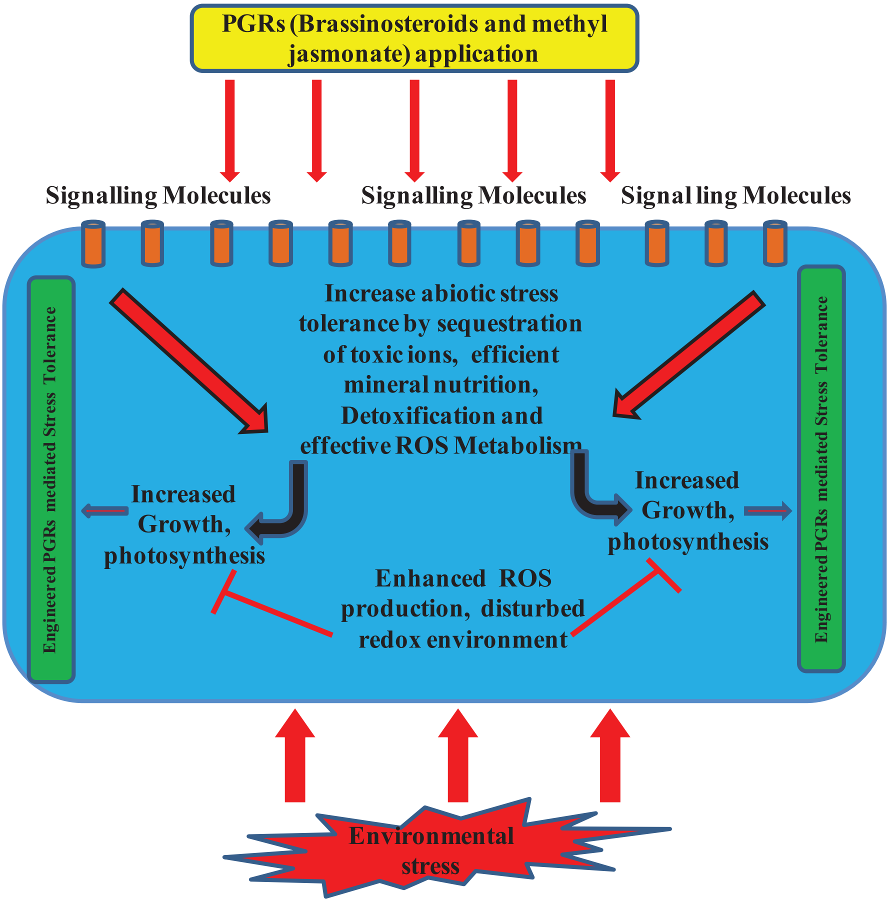

Fig. 8.1 A schematic model showing brassinosteroid- and methyl jasmonate-mediated improved growth and development under various abiotic stresses

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Chapter 9
Brassinosteroids Signaling Pathways in Plant Defense and Adaptation to Stress

Tauqeer Ahmad Yasir and Allah Wasaya

9.1 Introduction

Brassinosteroids (BRs), are a class of important steroidal plant hormone after the classic plant hormones gibberellins, abscisic acid, auxins, cytokinins, and ethylene (Clouse 2011). BRs are phytohormones, which play an active role in diverse aspects of plant growth and developmental processes. They optimize numerous physiological processes in germinating seeds and seedlings like cell division and elongation, root development and differentiation, reproduction, senescence, and photomorphogenesis (Clouse and Sasse 1998; Sreeramulu et al. 2013), as well as a variety of biotic and abiotic stresses (Bajguz and Hayat 2009; Sharma et al. 2013).

Mitchell et al. (1970) screened about 60 species of different plants and were able to find explicit compounds from the pollens of *Brassica napus*, having growth-promoting ability. Analysis revealed that the reactions caused by these compounds were unlike those made by gibberellins. They confidently assumed that the discovered compounds were a novel class of hormones and given the name “brassins.” This assumption was not accepted by other scholars, who contended that activities of the compounds could be caused by gibberellin because of the raw nature of that compound used to obtain brassins (Milborrow and Pryce 1973). Afterward, the US Department of Agriculture headed to refining 4.0 mg of brassins out of 500 pounds of *Brassica* pollens. By this effort, the crystalline structure of purified brassins was identified as the first plant steroidal hormone “brassinolide” (BL) (Grove et al. 1979). At present, about 70 different types of natural brassinolide analogues have been found in tissues of different plant species, which constitute the novel category of plant hormones BRs (Kutschera and Wang 2012). Out of these BRs, the BL, 28-homobrassinolide (28-Homo-BL), and 24-epibrassinolide (24-Epi-BL) are

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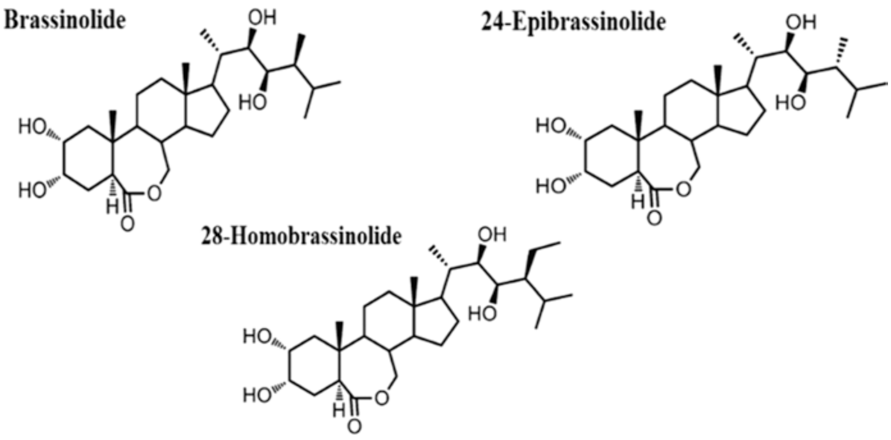

Fig. 9.1 Structure of the three most effective brassinosteroids

considered the most effective BRs extensively used in plant molecular and physiological research (Vardhini and Anjum 2015) (Fig. 9.1).

9.2 Signaling Pathways of Brassinosteroids

To apprehend the BR-mediated contribution in various crucial physiological and molecular functions of plants, widespread research have been directed during last two decades (Zhu et al. 2013). BR signaling encompasses its sensitivity through the receptor in the cell membrane trailed by stimulation of step-by-step reactions involved in phosphorylation, which transmits the signals to the downstream ends causing BR-induced expression of gene (Belkhadir and Jaillais 2015). Current research determine that BR interacts with the signaling mechanisms of other phytohormones at various levels and optimizes the crucial processes responsible for plant growth and development.

When BRs are not present (left side of Fig. 9.2), PM-localized receptors BRI-1 (Brassinosteroid Insenstitive-1) and BAK-1 are diminished by a number of factors, like BKI-1 and BIR-3. Moreover, BIN-2 kinase acts as a destructive controller and phosphorylates BES-1 and BZR-1 TFs to constrain their movement via various mechanisms. BSS-1 establishes a complex along with BES-1 and BZR-1 inside the cytoplasm, whereas THXh-5 decreases BZR-1 in the nucleus and finally deactivates TFs. This is directed to comparatively lessen expression of BR-related genes while enhancing expression of BR-suppressed genes. When BL form of BRs exists, they then attach with the receptor-BRI-1 and co-receptor-BAK-1 to cause BR signaling (right side of the Fig. 9.2). BKI-1 and BIR-3 detach from the complex, allowing conversion of BRI-1 and BAK-1 to phosphorylated and stimulated BRI-1 and BAK-1. BSKs or CDGs are phosphorylated and trigger BSU-1 phosphatase to

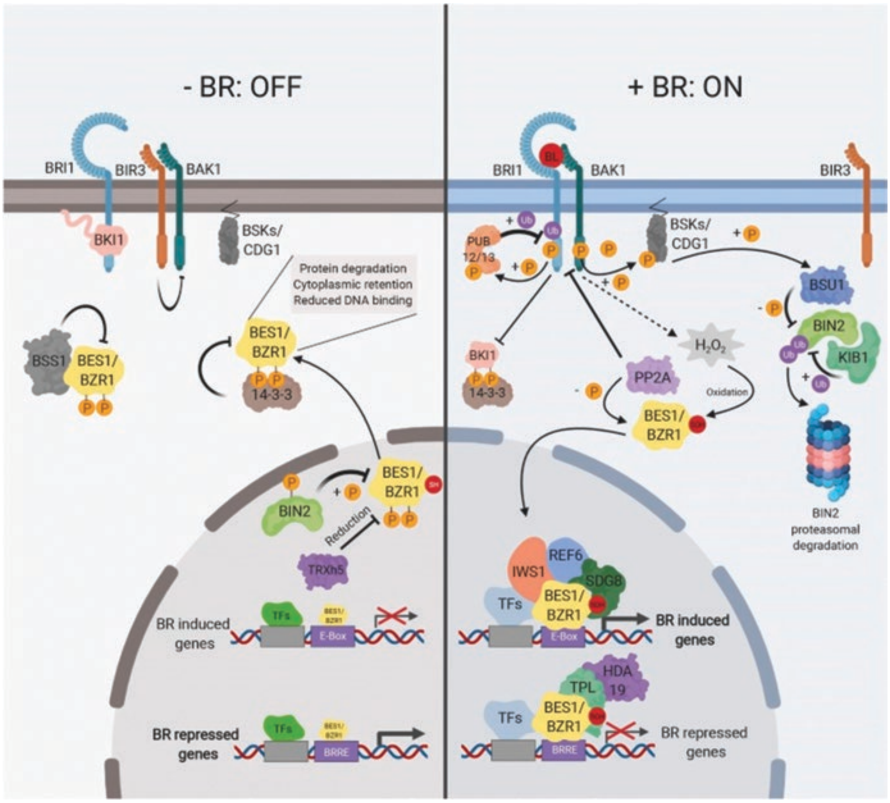

Fig. 9.2 Overview of the BR signaling pathway. (Reproduced from Nolan et al. 2020)

impede BIN-2. De-phosphorylation by PP-2A permits BES-1 and BZR-1 to work with further TFs and co-factors to stimulate BR-related genes and reduce BR-suppressed genes.

9.3 Brassinosteroids Signaling Transduction

BRs are recognized in extracellular spaces in the form of BRI-1 (Brassinosteroid Insenstitive-1), which belongs to LRR-RLK (leucine-rich repeated receptor-like kinase) (Wang et al. 2001). The BR straightly attaches to the 93-amino-acid region found inside the extracellular spaces in the membrane-bound BRI-1 (Sun et al. 2013). This attachment stimulates the development of BRI-1 and BAK-1 (Brassinosteroid Insensitive−1-Associated Receptor Kinase-3), which is also recognized as SERK-3 (Somatic Embryogenesis Receptor Kinase-3). This induces an intracellular phosphorylation transmit cascade (Russinova et al. 2004). This cascade (Fig. 9.3a) ends in increasing the activity and solidity of plant-specific

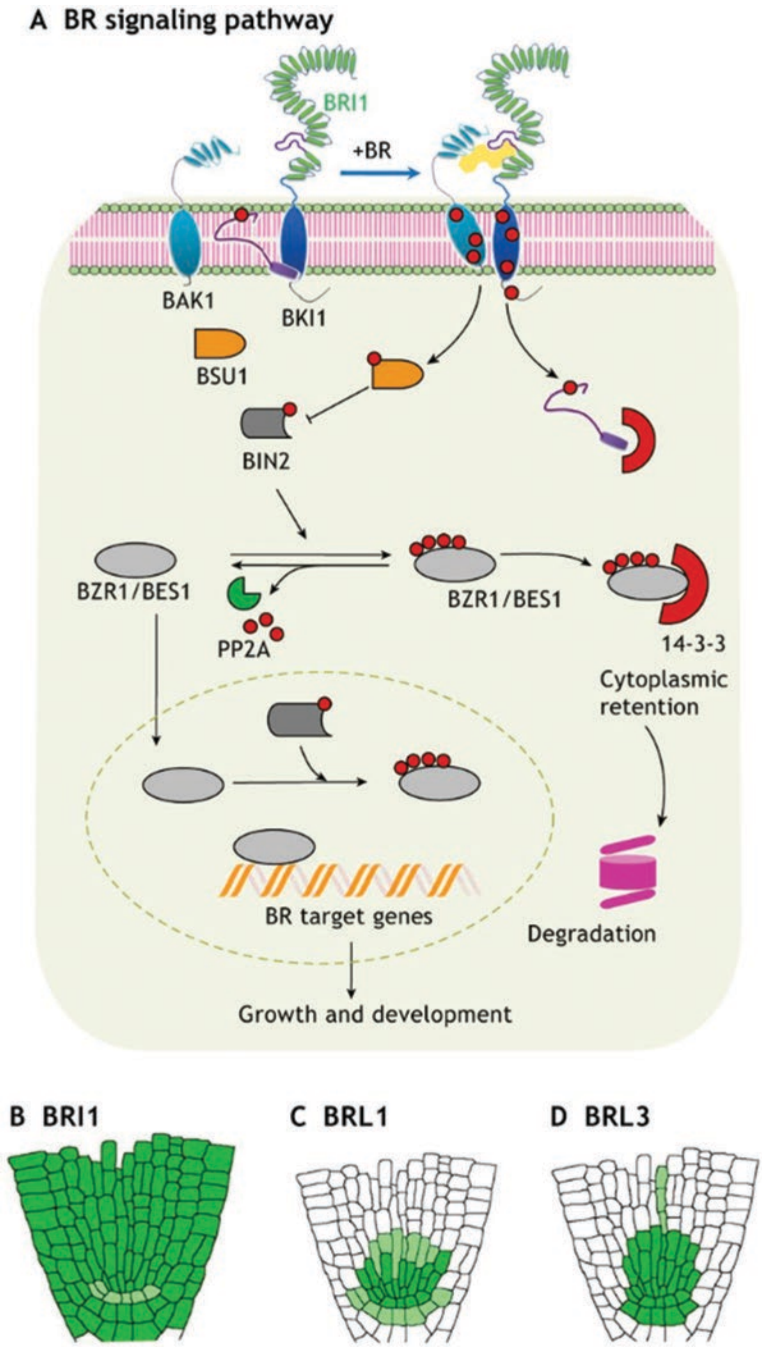

Fig. 9.3 An overview of the BR signaling pathway. (Reproduced from Planas-Riverola et al. 2019)

transcription factors BZR-1 (Brassinozole Resistant-1) (Wang et al. 2002) and BES-1 (BRI-1-EMS-SUPPRESSOR-1) (Yin et al. 2002). In this way, many of the BR-responsive genes are directly controlled and optimize several developmental processes in the plant body (Sun et al. 2010). In the absence of BRs the GSK3-like kinase BIN-2 (Brassinosteroid Insensitive-2) phosphorylates BZR-1/BES-1 type proteins deactivates them, stimulating their attachment to 14-3-3 proteins and leading to cytoplasmic holding and deprivation (Peng et al. 2008). This eventually impedes their capacity to attach DNA and causes inactivation of the signaling pathway.

The extracellular BR-binding region consists of three membrane-localized BRI-1-like homologs (BRL-1, BRL-2, and BRL-3). BRL-1 and BRL-3 are functional BR receptors like BRI-1 and can attach with high affinity to steroid molecules, whereas BRL-2 seems not an efficient BR receptor (Caño-Delgado et al. 2004). Moreover, BRI-1 is articulated nearly ubiquitously within the roots (Fig. 9.3b), while BRLs are located only in a few distinct tissues (Fig. 9.3c, d). In such positions, both BRL-1 and BRL-3 can heterodimerize with the BAK-1 co-receptor, and not by BRI-1, which makes it complex (Fàbregas et al. 2018).

BR signaling transduction has been widely deliberated in the past decade, and a complex BR signal pathway has been established, which plays a key role in overall plant growth and development. BRs can trigger the cell cycle during the process of seed germination (Zadvornova et al. 2005), regulate development of the cell cycle, encourage embellished growth of plants grown hydroponically (González-Garcia et al. 2011), and also regulate propagation of leaf cells and their expansion (Nakaya et al. 2002; Zhiponova et al. 2013).

9.4 Brassinosteroids Signaling in Plant Development

BRs are important for plant growth with its biosynthetic, signaling mutant properties and exogenous application. Majority of developmental processes like seed development and pollen development and flowering time are affected when BR signaling is disturbed (Ye et al. 2010; Jiang et al. 2013). BRs regulate the transport of auxin, which helps in coordination of plant organ responses (Li et al. 2005). Lateral root primordium development is also linked with variation in auxin supply due to BRs (Bao et al. 2004). GSK3-like kinases are responsible for differentiation of cambial cells into xylem vessels, which are the major regulators of the BR signaling pathway and work as downstream machineries of the tracheary element differentiation inhibitory factor signaling pathway (Kondo et al. 2014).

9.5 Brassinosteroids Signaling in Environmental Stresses

The capacity of plants to withstand in stressful conditions, for instance different water regimes, thermal variations, or soil-salinity is subjected to its response and capability to shift among growth stimulation and suppression under critical environments (Bechtold and Field 2018). In order to cope with environmental pressures, the ABA signaling pathway plays an important role (Zhu et al. 2017b). Nonetheless, convincing results direct that the BRs also have a key role in regulating the equilibrium between regular growth and confrontation against environmental risks. Numerous mechanisms have been suggested to elucidate how BR signaling intervenes variation to stress.

9.5.1 Role of BRs in Response to Drought Stress

Previous studies reveal that BR-deficient mutant plants are oversensitive to ABA (Clouse et al. 1996; Li et al. 2001). During drought stress, the production of ABA gets triggered in order to avoid this stressful condition (Cutler et al. 2010). This interpretation gives close association between BRs and ABA pathways (Zhang et al. 2009). The antagonism between BRs-ABA has been studied extensively such as BES-1 and AB-I3 or AB-I5 (Yang et al. 2016) to BIN-2 kinases, that is an adverse regulator of BR signaling and get activated when ABA is present (Wang et al. 2018). Considering BRs-ABA antagonism, it might be anticipated that BRs impede drought-related responses.

Plants prudently organize their progression and pressure responses by considering the period and harshness of drought stress. In moderate water-limited conditions, inhibited growth results into loss in yield and plant fitness, while in severe water-limited conditions, plant growth ceases to ensure its survival by compromising yield (Claeys and Inzé 2013). Several phenomena have been explained that retard BES-1 activity while stress have been imposed. BES-1 is degraded by DSK-2-mediated selective autophagy in drought conditions to obstruct BR-optimized growth. Mutant plants with damaged BES-1 degradation such as DSK-2 decreased existence in drought conditions, which can be reinstated by barring BES-1 expression by using RNAi of BES-1 (Nolan et al. 2017). These outcomes show that the degradation of BES-1 is imperative to check BR-optimized growth in drought conditions to stimulate plant existence.

9.5.2 Role of BRs in Response to Heat Stress

BRs also regulate plant development and stress reactions under different ranges of decreased or increased temperatures. Under high temperatures, BES-1 and BZR-1 mount up and work along with PIF-4 to encourage thermogenic plant growth (Martínez et al. 2018). Increased levels of BES-1 and BZR-1 encourage the conduct of PIF-4, and this increase in PIF-4 permits for the suppression of BR development by swapping BES-1 from a suppressive homodimer to a PIF4-BES-1 heterodimer which triggers transcription factor (Martínez et al. 2018). Increased temperatures also lower BRI-1 concentrations, which harm BR signaling pathway, resulting in an increased growth of roots (Martins et al. 2017). BRI-1 go through ubiquitination and endocytosis and get degraded (Zhou et al. 2018). These are essential for heat-persuaded decrease in BRI-1 production (Martins et al. 2017). Whereas PUB-12 and PUB-13 ubiquitinate BR-I1 after BR discernment (Zhou et al. 2018), and E-3 ubiquitin ligase accountable for ubiquitination, while heat-stress remains to be identified.

9.5.3 Role of BRs in Response to Cold Stress

Plant tolerance to cold stress is also mediated by BR signaling. The BR-mediated elevation of cold stress tolerance with the accretion of vigorous un-phosphorylated types of BZR-1 and BES-1 normalize cold-stress response of the plants (Li et al. 2017). CESTA, which is a controller of BR signaling, also regulates low temperature responses, and as a result, it enhances the actual and attained ultralow temperature tolerance (Eremina et al. 2016). BIN-2 also takes part in BR optimization of low temperature tolerance through promoting phosphorylation of CBF expression-1 in extended exposure to cold, upholding its degradation to decrease CBF generation (Ye et al. 2019).

9.5.4 Role of BRs in Response to Salinity Stress

Salt stress tolerance is also mediated by BR signaling in plants through regulation biosynthesis of ethylene and signaling. In salinity stress, the pre-treatment of BRs encourages the production of ethylene, and thereby BR-signaling, through augmenting the action of 1-aminocyclopropane-1-carboxylate synthase (ACS), which is an enzyme related to ethylene synthesis (Zhu et al. 2017a, b). Contrariwise, ceasing production of ethylene and its signaling machineries prevents the activities of antioxidant enzymes which are induced by BR during salinity stress (Zhu et al. 2017a, b). BR signaling may be facilitated by BRI-1 through constraining the degradation of protein associated with the endoplasmic reticulum to salvage the mutant (Cui et al. 2012).

9.5.5 Role of BRs in Response to Nutrient Stress

The limited availability of nutrients in soil and their supply to the plant hinders ideal root growth. BR signaling recently proved to have regulatory effects on root growth development under low levels of phosphate and iron nutrients (Singh et al. 2018).

During iron deficiency, BR signaling gets activated, promotes root growth, and also affects the distribution of iron in the plant parts. Reduced phosphate concentration enhanced iron accumulation but introverted BR signaling initiation, resulting into accelerated root growth. The BRI-1 adverse controller BKI-1 was considered as the focus point of this signal relationship, the BZR-1 or BES-1, with their straight target LPR-1, a ferroxidase which works at the more downstream steps during this act (Singh et al. 2018).

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Chapter 10
Roles of Hydrogen Sulfide in Regulating Temperature Stress Response in Plants

Aditya Banerjee and Aryadeep Roychoudhury

10.1 Introduction

The gaseous by-product hydrogen sulfide (H_2S) was earlier considered only as a toxic cellular compound whose elimination was necessary for optimum plant survival (Ahmad 2016). H_2S has been reported to efficiently interact with the heme group containing proteins like hemoglobin, myoglobin, and cytochrome oxidase to render them inactive, and this has been accredited as the preliminary reason behind the Permian mass extinction (Li et al. 2016). However, recent advances have illustrated the diverse roles of H_2S as a gasotransmitter and modulator of downstream signaling responses, thus indicating at the physiological plasticity of plants (Banerjee et al. 2018). Previous studies have verified the growth-promoting and ameliorating effects of H_2S during multiple environmental stresses largely responsible for yield and production loss in crops and plants (Li et al. 2016). Hence, H_2S has been considered as a gasotransmitter regulating plant cross-adaptation to suboptimal conditions (Foyer et al. 2016). The mechanism of such cross-adaptation is due to the probable interaction of H_2S with crucial secondary messengers like calcium (Ca^{2+}), hydrogen peroxide (H_2O_2), nitric oxide (NO), and the stress phytohormone abscisic acid (ABA) (Banerjee et al. 2018; Pandey 2015; Wang et al. 2016). These messengers/gasotransmitters along with H_2S have been observed to be major participants of the signaling module regulating heat and cold stress response in plants (Calderwood and Kopriva 2014; Fotopoulos et al. 2015; Guo et al. 2016; Li et al. 2016; Hancock and Whiteman 2016). H_2S donors like sodium hydrosulfide (NaHS) and morpholin-4-ium 4-methoxyphenyl(morpholino) phosphinodithiolate (GYY4137) have been popularly used to promote overall plant growth and develop-

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ment and delay cellular aging (Li 2013; Wojtyla et al. 2016; Zhang et al. 2011). While NaHS supplementation induces short-term bursts of H_2S, long-term generation of the gasotransmitter is initiated upon the application of GYY4137 (Wang 2012; Lisjak et al. 2013).

Climate change has been widely regarded as the major threat to global food security and crop quality (Bita and Gerats 2013). Very high or low temperatures are climatological extremes which inhibit crucial physiological processes and development in plants and crop species (Banerjee and Roychoudhury 2018; Banerjee et al. 2020a, b). Like most abiotic stresses, elevated or low temperature induces the production of reactive oxygen species (ROS) (Bita and Gerats 2013). Due to uncontrolled generation of ROS, the tissues experience severe oxidative stress, resulting in accelerated lipid peroxidation, electrolyte leakage, protein degradation, and growth inhibition (Banerjee et al. 2019a, b; Banerjee and Roychoudhury 2019a, b). Ortiz et al. (2008) reported that increase in temperature could potentially decrease crop yield by 15–35% across Asian and African countries and by 25–35% in nations belonging to the Middle East. Cereal crop cultivation in South East Asia has been predicted to be drastically affected by increases in temperature (Nelson 2009). The worst affected European countries due to temperature increase and loss in agricultural productivity would be Italy, Spain, and Portugal (Bita and Gerats 2013). The sharp decline in crop yield in developing countries like India and China due to climatic fluctuations could aggravate calorie unavailability and global malnutrition (Chhetri and Chaudhary 2011).

Some roles of H_2S in ameliorating the inhibitory effects of temperature stress have been reported. However, these have not been orderly documented altogether in literature in a concise form. Since the above instances show the negative impact of temperature stress on agriculture, the roles of the crucial gasotransmitter H_2S need to be properly understood so that such protective molecules could be used for sustaining agriculture under extreme environmental conditions.

10.2 Understanding H_2S Homeostasis Within Plants

H_2S cause injury to the cell or promote stress protection, and this completely depends on the systemic regulation of H_2S homeostasis in plants (Li et al. 2016). The synthesis of H_2S is initiated by the action of L-cysteine desulfhydrase (LCD) and D-cysteine desulfhydrase (DCD) which catalyze the conversion of cysteine to form H_2S (Banerjee et al. 2018). Sulfite reductase (SiR) catalyzes the production of H_2S from sulfite by reducing ferredoxin (Hancock and Whiteman 2014). Cyanoalanine synthase (CAS) produces H_2S from cysteine in the presence of hydrogen cyanide. The formation of cysteine from O-acetyl-L-serine upon the incorporation of H_2S is mediated by O-acetyl-(thiol)-serinelyase (OAS-TL) (Li 2013). Li et al. (2016) inferred that usually the LCD or DCD enzymes are primarily utilized for the production of H_2S in plants for the regulation of abiotic stress responses and excess H_2S synthesized is excreted through the stomata.

10.3 Regulation of H_2S Signaling During Temperature Stress

The gasotransmitter H_2S regulates the homeostasis of downstream signaling molecules and phytohormones during temperature stress (Banerjee et al. 2018). These together promote physiological plasticity and generate systemic tolerance against temperature stress in plants.

10.3.1 Regulation of H_2S Signaling During Heat Stress

It has been reported that elevated temperature (~35 °C) accelerated H_2S production in tobacco seedlings (Chen et al. 2016). The increased H_2S level in turn induced nicotine biosynthesis and jasmonic acid accumulation (Chen et al. 2016). Christou et al. (2014) reported significant regulation of H_2S synthesis, leading to overall increased H_2S accumulation in strawberry plants subjected to heat stress at 42 °C.

10.3.2 Regulation of H_2S Signaling During Cold/Chilling Stress

Exposure to low temperature (4 °C) triggered the activity of LCD and DCD enzymes and the expression of associated genes in *Vitis vinifera* seedlings which together increased H_2S synthesis by more than twofold (Fu et al. 2013). Exposure to similar low temperature conditions also stimulated H_2S production in *Cynodon dactylon* (Shi et al. 2013). The growth of *Lamiophlomis rotata* plants at high altitudes of 4800 m and 5200 m was supported by high endogenous production of H_2S (Ma et al. 2015). The mechanism of such increased H_2S synthesis was deciphered through proteomic studies which ascertained high abundance of H_2S-synthesizing enzymes like OAS-TL, CAS, LCD, and DCD (Ma et al. 2015). These observations indicated the central role of H_2S in mediating plant systemic adaptation to temperature variations and fluctuations.

10.4 Role of H_2S in Generating Temperature Stress Tolerance in Plants

10.4.1 Role of H_2S in Generating High Temperature Stress Tolerance

H_2S-mediated heat tolerance in tobacco suspension cultured cells has been observed to be regulated by uninhibited entry of extracellular Ca^{2+} into the stressed cells (Li et al. 2012). Heat tolerance in response to exogenous NaHS application was further improved when the suspension cultured cells were treated either with exogenous Ca^{2+} or with the ionophore A23187 (which allowed unrestricted influx of Ca^{2+} into the cells). However, such tolerance was lowered under the influence of Ca^{2+} chelators (ethylene glycol tetraacetic acid), plasma membrane channel blocker La^{3+}, and antagonists of calmodulin proteins. These results altogether clearly illustrated that H_2S accelerated heat tolerance via the Ca^{2+}-dependent signaling pathway in the suspension cultured cells of tobacco (Li et al. 2012). Tolerance to high temperature stress (42 °C) was promoted in strawberry roots pre-treated with NaHS (Christou et al. 2011). The pre-treatment led to lower chlorosis, malondialdehyde (MDA) accumulation, and electrolyte leakage in stressed roots compared to that in the non-treated and stressed roots (Christou et al. 2011). Christou et al. (2014) showed that exogenous treatment of strawberry seedlings with NaHS promoted tolerance against acute heat stress by activating the ascorbate-glutathione (AsA-GSH) cycle and increasing the activity of antioxidant enzymes like ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR). It was also determined that H_2S-dependent thermotolerance was mediated by increased transcript abundance of *heat shock proteins* (*HSP*s) like *HSP70*, *HSP80*, and *HSP90* and aquaporins, viz., *plasma intrinsic proteins* (*PIP*s) (Christou et al. 2014). Yang et al. (2016) showed similar mechanism of H_2S-induced heat stress tolerance in the NaHS-treated wheat seedlings. The NaHS-treated plants exhibited increased activity of enzymatic antioxidants like SOD, CAT, and APX and lower accumulation of oxidative stress parameters like MDA and H_2O_2 compared to those in the non-treated and stressed seedlings (Yang et al. 2016). Li et al. (2013) showed the involvement of the compatible solute proline (Pro) in mediating H_2S-mediated thermotolerance in maize seedlings grown from NaHS pre-treated seeds. It was observed that NaHS pre-treatment stimulated overall Pro biosynthesis by activating Δ^1-pyrroline-5-carboxylate synthetase (P5CS) and suppressing the Pro-degrading enzyme, proline dehydrogenase (PDH) (Li et al. 2013). In another study, it was shown that H_2S-mediated thermotolerance in NaHS-treated maize seedlings involved enhanced accumulation of the osmolyte trehalose. Increased activity of trehalose-6-phosphate phosphatase in the NaHS-treated and heat stressed seedlings supported high endogenous accumulation of trehalose (Li et al. 2014). The crucial growth regulator salicylic acid (SA) which often mediates crosstalk between abiotic and biotic stress signaling in plants has been observed to mediate H_2S-dependent thermotolerance responses (Li et al. 2015).

10.4.2 Role of H_2S in Generating Low Temperature Stress Tolerance

Low temperature stress affects cell survival by reducing lipid mobility/fluidity within membranes. Chilling temperature induces the formation of ice crystals within membranes, leading to disruption and uncontrolled electrolyte leakage (Barrero-Sicilia et al. 2017). Exogenous application of NaHS activated enzymatic antioxidants like CAT, GR, and glutathione peroxidase (GPX) and stimulated the accumulation of reduced glutathione (GSH) in Bermuda grass exposed to freezing temperatures (Shi et al. 2013). Increase in H_2S production due to NaHS treatment stimulated the activity of SOD and lowered the levels of superoxide radical and MDA in *Vitis vinifera* seedlings exposed to low temperature stress (Fu et al. 2013). Luo et al. (2015) showed that fumigation of banana with H_2S increased the activity of SOD, CAT, APX, GR, and phenylalanine ammonia lyase (PAL) which enabled better mitigation of ROS and enhanced the ability of the fruits to maintain peel firmness during cold stress. Ma et al. (2015) showed that exogenous H_2S treatment ameliorated the chilling-induced oxidative damages in *Lamiophlomis rotata* by triggering the accumulation of Pro and soluble sugars. Shi et al. (2015) reported that *Arabidopsis* transgenics overexpressing LCD or treated with NaHS exhibited higher level of chilling stress tolerance due to enhanced production of H_2S. On the contrary, knockdown of LCD drastically lowered the ability of the mutants to withstand chilling temperature (Shi et al. 2015).

The close association between H_2S and auxin has been recently reported during chilling tolerance responses in cucumber seedlings (Zhang et al. 2020). Exogenous NaHS triggered the activity of flavin monooxygenase (FMO) and transcript abundance of FMO-like protein encoding gene *YUCCA2* which together stimulated the accumulation of indole-3-acetic acid (IAA). Increased H_2S and IAA accumulation promoted efficient ROS scavenging and reduced electrolyte leakage in cucumber plants exposed to chilling stress (Zhang et al. 2020). Liu et al. (2019) showed that H_2S promoted the biosynthesis of the triterpenoid secondary metabolite cucurbitacin C (CuC) in cucumber plants subjected to cold stress. The increased production of CuC was due to higher rate of S-sulfhydration of proteins in presence of H_2S which together stimulated the systemic ability of the seedlings to tolerate stress (Liu et al. 2019). Recently Joshi et al. (2020) showed that exogenous NaHS stimulated H_2S production in avocado seedlings which significantly promoted CO_2 assimilation, photosynthetic capacity, and stomatal conductance and mitigated ROS-induced injuries during overnight exposure to frost. Du et al. (2017) reported that H_2S activated mitogen activated protein kinase 4 (MAPK4) during cold stress in *Arabidopsis*, leading to enhanced induction of cold-responsive genes like *C-repeat binding factor 3* (*CBF3*), *cold responsive 15A* (*COR15A*), and *COR15B*. Overall, H_2S-mediated stimulation in the cold signaling pathway led to enhanced low temperature tolerance in *Arabidopsis* seedlings (Du et al. 2017).

10.5 Conclusion

H_2S has gained tremendous importance in the field of stress physiology, since it acts as a crucial gasotransmitter regulating important downstream phytohormone-dependent signaling cascades during oxidative stress responses in plants. Due to rapid climate change, fluctuation in global temperature has become a prevalent phenomenon which drastically affects plant growth and crop yield. The gasotransmitter

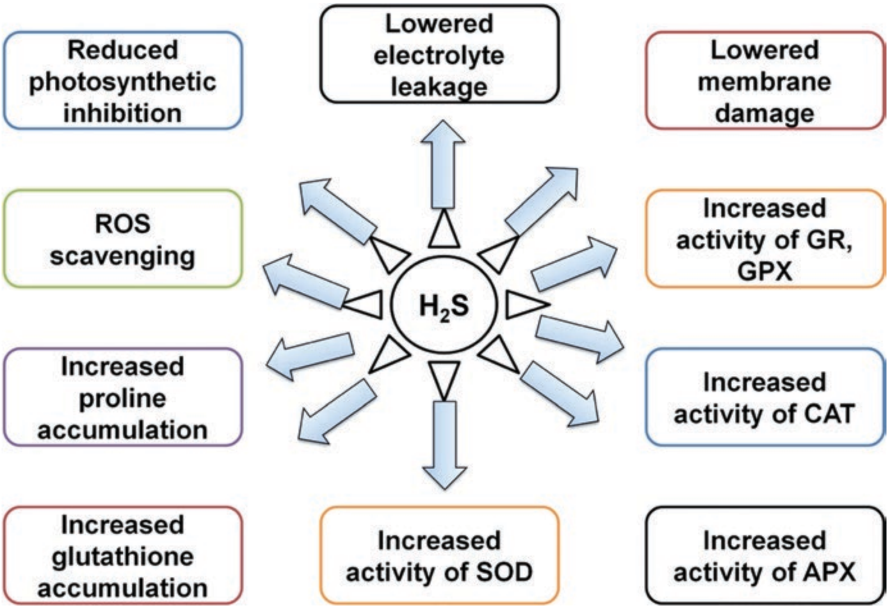

Fig. 10.1 The possible mechanism of H_2S-mediated temperature stress tolerance in plants

H_2S activates the defense machinery and protects the tissues and cells from succumbing to oxidative injuries triggered by both high and chilling temperatures. It has been observed that H_2S interacts with phytohormones like ABA, JA, and SA to mediate signaling responses during such temperature stress. Exogenous treatment of stressed seedlings with the H_2S donor NaHS significantly enhanced the accumulation of osmolytes and important non-enzymatic antioxidants and triggered the activity of enzymatic antioxidants in several plant species (Fig. 10.1). Due to such enhanced antioxidant response, stressed plants could avoid ROS accumulation beyond the critical physiological level and could survive in suboptimal temperatures.

10.6 Future Perspectives

Practical field-level research regarding the outcome of exogenous NaHS on crop improvement during high and low temperature should be undertaken to popularize the utility of H_2S as an important protective gasotransmitter. Research conducted in this area is still in a naive stage and requires exhaustive metabolomic and proteomic investigations which would actually aid in understanding the downstream regulation of H_2S during temperature fluctuations. Next-generation sequencing (NGS) platforms could also be utilized to understand tissue-specific plasticity of transcriptomes under the influence of H_2S donors during temperature stress. Future perspectives could also include epigenomic studies in order to decipher the genome-wide epigenetic alterations responsible for dictating H_2S-induced up-/down-regulation of crucial downstream signaling genes.

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Chapter 11
Physiological, Biochemical, and Molecular Mechanism of Nitric Oxide-Mediated Abiotic Stress Tolerance

Khushwant Singh, Ila Shukla, Ajay Kumar Tiwari, and Lubna Azmi

11.1 Introduction

Nitric oxide is an omnipresent molecule and an extremely diffusible gas (Siddiqui et al. 2011). It has drawn much attention in recent years due to its widespread application in processes linked to normal plant physiology. Due to these properties, it has also been termed as the "molecule of the year." It has been synthesized by plant itself, and it also acts as a gaseous plant growth regulator (Neill et al. 2008). It has been given the name "plant growth regulator" because there are rising evidence reporting its role as mediator of several biological procedures involved in the growth and development of the plant. Seed germination, root organogenesis, photosynthesis, stomatal closure, hypocotyl growth, floral regulation, defense against pathogens, and senescence are the processes included in plant physiology where NO has an extensive role. It has also been reported that nitric oxide is also linked to phytoalexin production and apoptosis (Delledonne et al. 1998). Nitric oxide suppresses growth at higher concentrations, while it provides benefits at lower concentrations. This review puts light on the role of nitric oxide in plants for tolerance of different abiotic stresses (Simontacchi et al. 2013; Wendehenne et al. 2001). Also, the relation between nitric oxide and other phytohormones has been included here. Many

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recent reports have been found about molecular data, regarding NO-mediated PTMs in animals. This has encouraged botanists to find out the effect of these PTMs in plants when exposed to different stresses. Hence, in the last few years, a great deal of effort were put into the identification of the role of S-nitrosylated, tyrosine nitrate, and S-glutathionylated proteins in plant function modulation (Wendehenne et al. 2001). Also, current knowledge reports about the role of nitric oxide in cell signaling in plants by involvement of oxidative and reductive pathways (Crawford 2006). This review also attempts to impart knowledge on the N-end rule pathway responsible for protein stability, which alters the steadiness of group VII ethylene response factors under hypoxic conditions.

11.2 Antiquity of Nitric Oxide

In 1722, when Joseph Priestly first described nitric oxide, it was known as a highly toxic gas and also a component of industrial wastes and exhaust gas. The model concerning the cytotoxicity of free radical substances was changed with the 1980s' discovery about the role of nitric oxide signaling in regulating the cardiovascular system by R.F. Furchgott, L.J. Ignarro, and F. Murad (Nobel Prize winners in Physiology and Medicine 1998) (Delledonne et al. 1998). In 1992, nitric oxide was declared as the Molecule of the Year by the journal *Science*. In that year only, a nitric oxide society was founded, and a scientific journal devoted to nitric oxide was formed. Nitric oxide is a gaseous free radical, and its emission was reported in soybean many years back. Later sunflower and maize were reported to have in vivo and in vitro nitrate reductase (NR)-dependent nitric oxide producing activity (Klepper 1979). Animals possess nitric oxide synthase, and it acts as chief enzyme that catalyzes the in vivo synthesis of nitric oxide. But there is no report of plants having nitric oxide synthase, though it has been proven to be a functional metabolite in plants (Qi and Dong 1999).

11.3 Nitric Oxide Synthesis in Plants

Involvement of NO in various signaling pathways related to plant's growth and development and biotic and abiotic stress resistance requires quick generation of NO in a site-specific manner. Production of NO in plants has been known to occur through various routes which are broadly classified into enzymatic and non-enzymatic mechanism. Enzymatic route of NO production involves three enzymes, including nitrate reductase, nitrate reductase and animals like nitric oxide synthase (NOS), for the production NO in plants. All routes of NO production in plants are shown in Fig. 11.1.

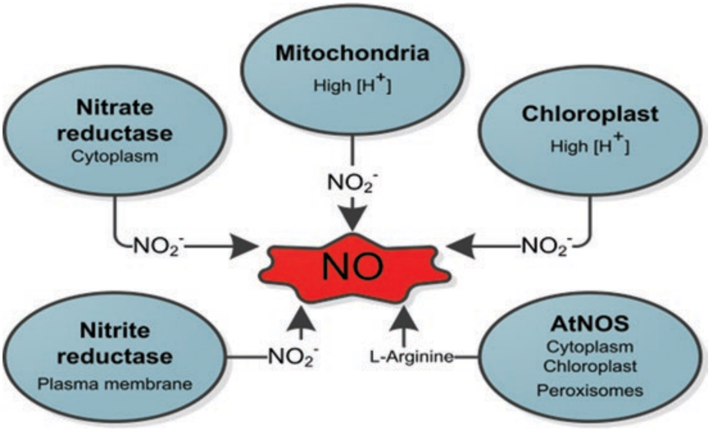

Fig. 11.1 Various routes of NO production in plants

11.3.1 Nitrate Reductase

Nitrate reductase is a homodimeric protein, containing two subunits of 100 kD. Each subunit constitutes a flavin adenine dinucleotide (FAD), a heme protein containing Fe as a metal cofactor, and a Mo-molybdopterin (Mo-MPT). Nitrate reductase facilitates the first step of nitrogen assimilation in higher plants and is reported to exist dominantly in two forms which are NADH-specific NR forms (EC 1.6.6.1) and NAD(P)H-bispecific forms (EC 1.6.6.2). Reaction catalyzes by nitrate reductase is:

$$NO_3^{-} + NADH \rightarrow NO_2^{-} + NAD^{+} + OH^{-}$$

While catalyzing the reduction of nitrate, FAD, Fe, and Mo present in nitrate reductase are cyclically reduced and oxidized. Due to the ability of FAD and Mo in Mo-molybdopterin to attain 3 oxidation states and 2 oxidation states of Fe in heme in collectively results in approximately 12 to 18 oxidized and reduced states of nitrate reductase which are transient in nature and occur in vivo. Under some circumstance, nitrate reductase may also result in the formation of NO (Dean and Harper 1988; Rockel et al. 2002). In *Arabidopsis thaliana*, nitrate reductase was reported to be present in two isoforms, NIA1 and NIA2. Both isoforms were 83.5% identical to each other in terms of their amino acid constituent, but they had some independent sequence regions including the N-terminal region. Nitrate reductase encoded by NIA1 was demonstrated to control NO emission through stomata guard cells during light to dark transition. In a study on double mutant nia1 and nia2, involvement of nitrate reductase in ABA-induced generation of NO was reported in guard cells (Desikan et al. 2002). Involvement of NO in signaling requires its rapid generation during stimulus, and thus a tight control is required on nitrate reductase to show its activity. In a study on *Arabidopsis*, modulation of nitrate reductase activity was demonstrated by phosphorylation of a conserved serine residue (Ser ^{534})

present in it (Rockel et al. 2002). Therefore, removal of the phospho-regulation through substitution of the Ser ^{534} can results in increased NO production by. In another study, the role of 14-3-3 proteins was highlighted in controlling the activity of nitrate reductase (Lillo et al. 2004) probably through its proteolysis (Weiner and Kaiser 1999).

11.3.2 Nitric Oxide Synthase (NOS)

Similar to NOS activity in animals, L-arginine-dependent production of NO has been reported in many plants, which was confirmed by inhibition of NO production with the use of arginine analogues. These inhibitors were also observed to interfere in NO mediated, ABA control over stomata closure. A few examples of the NOS-type activity are shown in Table. 11.1.

Besides the documented NOS-like activity in many plants, no protein homologs to NOS of animals have been yet confirmed in any plant species. In an immunological study, cross-reactivity of anti-mammalian NOS antibodies toward a few plant proteins was demonstrated by Ribeiro et al. (1999). This made researchers to believe that NOS activity in plants is mediated through proteins orthologous to NOS of animals, until a proteomic study was performed in which cross-reacting proteins were confirmed to be heat shock proteins and glycolytic enzymes (Butt et al. 2003). In the same year, a protein (AtNOS1) was identified in *Arabidopsis* reported to have 16% similarity with a 60 kD protein present in neuron cells of *Helix pomatia* (snail). This 60 kD protein was demonstrated to have cross-reactivity to anti-human nNOS antibody and was confirmed for its involvement in NO production in snail's neuron cells. Thus, to find NOS activity of AtNOS1, a mutant *Arabidopsis* was generated through T-DNA insertion technology, and results of this experiment highlighted the central role of AtNOS1 in NO production in *Arabidopsis*. Unlike in mammals, NOS activity in plants did not required BH_4, FAD, FMN, or heme as cofactors. Rather,

Table 11.1 Nitric oxide synthase activity observed in different plant species

Plant species	Tissue	References
Pisum sativum	Leaves	Leshem and Haramaty (1996)
Pisum sativum	Leaf peroxisomes	Barroso et al. (1999)
Pisum sativum	Embryonic axes	Sen and Cheema (1995)
Triticum aestivum	Germ	Kuo et al. (1995)
Nicotiana tabacum	Leaf epidermal cells	Foissner et al., (2000)
Nicotiana tabacum	TMV-infected leaves	Durner et al. (1998)
Mucuna hasjoo	Total extract	Ninnemann and Maier (1996)
Glycine max	Cotyledons	Modolo et al. (2002)
Lupinus albus	Roots and nodules	Cueto et al. (1996)
Zea mays	Root tips and young leaves	Ribeiro et al. (1999)

amino acid sequence analysis revealed NOS of plants were found similar to proteins with GTP-binding or GTPase activity present in bacteria (Guo et al. 2003).

11.3.3 Nitrite Reductase

In plants, a plasma membrane-bound nitrate-NO oxidoreductase (Ni-NOR) has been reported in roots (Stöhr and Stremlau 2006). This enzyme was reported to utilize cytochrome-c as an electron donor to produce NO under in vitro conditions. Due to lesser studies on Ni-NOR, it was not yet fully identified and characterized and needs to be studied thoroughly for its role in the production of nitric oxide.

11.3.4 Non-enzymatic Production of NO in Plants

Apart from the enzymatic production of NO in plants, it is also produced though non-enzymatic mechanisms. For instance, oxidation of N_2O during the nitrification-denitrification cycle results in production of NO as a by-product and is released into the atmosphere (Wojtaszek 2000). It was demonstrated that local acidic condition in the chloroplast and apoplastic spaces where ascorbate is present in abundance promotes chemical reduction of nitrate and produces NO and dehydroascorbic acid (Henry et al. 1997). Another example of reduction of nitrite by ascorbate under acidic pH has been demonstrated in aleurone cells of barley (Beligni et al. 2002). Direct dismutation of nitrate into nitrite or NO under acidic environment is also feasible (StÖhr 2002). In 1994, Cooney et al. reported involvement of carotenoids in the production of NO by reduction of NO_2 in a light-dependent manner. In addition to this biotic as well as abiotic stress conditions, interaction between nitrogen species and reactive oxygen also results in the formation of NO.

11.4 Signaling Pathways of Nitric Oxide

Since the orientation of the present chapter is toward the plant kingdom, thus all details further provided in this chapter are related to plants. To act as signaling molecule, it should have the ability of rapid generation during a need to relay the signal quickly to the target protein and shall be rapidly removed when there is no need of the signal. Uncharged free radical entity with small half-life led to identification of NO as a signaling molecule in plants as well as in animals. Also free radical nature of NO favors its existence in three inter-convertible species which, namely, are the radical ($NO^•$), nitrosonium cation (NO^+), and the nitroxyl radical (NO^-) (Stamler et al. 1992; Wojtaszek 2000). NO is a gaseous molecule with slight solubility in water as well as lipids. This property of NO allows it to diffuse from one cell to another while travelling through their cell membranes and can also perpetuate signals freely within a cell diffusing through cytoplasm. NO is known to convey the signal through various routes, all of which are displayed diagrammatically in Fig. 11.2.

A previous study on *Nicotiana tabacum* demonstrated that NO triggers various cellular events by increasing cytoplasmic calcium ion concentration (Besson-bard et al. 2008). Several other studies displayed similar increase in calcium ion concentration, which was mediated due to NO in the hyperosmotic, abscisic acid, and elicitor transduction pathways (Lamotte et al. 2006; Garcia-Mata et al. 2003; Vandelle et al. 2006; Gould et al. 2003). Many other studies have also highlighted the vital role of NO in regulating activities of nearly all cellular channels involved in calcium transportation (Clementi 1998). NO is known to regulate activity of calcium channels either directly by causing post-translational modifications in them, e.g., S-nitrosylation (Stamler et al. 2001; Ahern et al. 2002), or indirectly by activating guanylate cyclase and/or cyclic ADP-ribose (cADPR) resulting in synthesis of cGMP from ADP-ribosyl cyclase and NAD^+. cGMP acts as a calcium-mobilizing metabolite and therefore helps in relaying signals (Hanafy et al. 2001). In many studies, presence of plasma membrane and intracellular calcium channel inhibitors resulted in inhibition of NO-mediated accumulation of calcium in the cell cytoplasm. This confirmed the involvement of NO in influx of calcium ions from extracellular and intracellular calcium stores (Lamotte et al. 2006; Gould et al. 2003; Vandelle et al. 2006; Garcia-Mata et al. 2003). Besides, ryanodine receptors are the only calcium channels identified to be involved in NO-mediated mobilization of calcium ion from intracellular calcium reserves (Allen et al. 1995; Fliegert et al. 2007). This increased concentration of calcium ion in the cytoplasm modulates

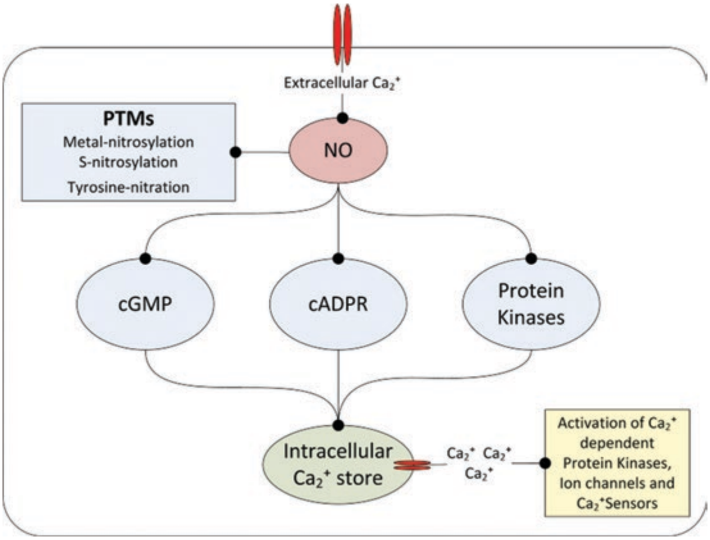

Fig. 11.2 Diagram displaying various routes of signal transduction by NO in plants

activity of calcium-dependent protein kinases (CDPKs), mitogen-activated protein kinases (MAPKs), and calcium-sensitive channels like Na^+ and K^+ channels. NO-mediated induction of protein kinases has been demonstrated in many plants including *Nicotiana tabacum*, *Vicia faba*, *N. plumbaginifolia*, etc. (Sokolovski et al. 2005; Lamotte et al. 2006). In a study on *Nicotiana tabacum*, NO was displayed to induce two protein kinases in it, one of which was identified as NtOSAK (*Nicotiana tabacum* Osmotic-Stress-Activated protein Kinase), a 42 kD protein kinase characterized for its activation under abiotic stress conditions such as hyperosmolarity and high salinity (Lamotte et al. 2006). The second protein kinase which was induced by NO was a 48 kD protein and was identified to be to MAPK (Zhang and Liu 2001). NO and its derivatives are also known to perform their biological actions by modulating activities of various target proteins through three different types of post-translational modification, which includes metal nitrosylation, S-nitrosylation, and Tyr-nitration.

11.4.1 Metal Nitrosylation

Plants contain many types of metalloproteins, including hemoglobin (Hb) which has been an important choice of researchers to study its interactions with NO. Most of these studies were focused on the three types of hemoglobin proteins such as leghemoglobin (a symbiotic hemoglobin present in root nodules of leguminous crops), two classes of non-symbiotic hemoglobin (differing in their affinity for oxygen), and truncated hemoglobin. Pioneering studies on leghemoglobin present in root nodules of alfalfa, cowpea, and soybean demonstrated formation of NO nitrosyl-Lb complex (LbFeIINO) and suggested interaction between ferrous-leghemoglobin and NO produced in nodules (Mathieu et al. 1998). In another study, different forms of leghemoglobin, oxy-leghemoglobin and ferryl-leghemoglobin, were demonstrated to scavenge NO and peroxynitrite under in vitro conditions (Herold and Puppo 2005). During the reaction, oxy-hemoglobin gets converted into met-hemoglobin, which is further recycled into oxy-hemoglobin. Similarly, scavenging mechanism was observed to be utilized by class-I hemoglobin in distinct plants, which catalyzed the conversion of NO to nitrate in a NAD(P)-H-dependent manner. In a mutation study on *Arabidopsis*, it was confirmed that class-1 hemoglobins were also regulated through S-nitrosylation. In other mutagenesis experiments, it was demonstrated that interaction between NO and class-I hemoglobin was not only critical for NO scavenging but also for various physiological conditions like abiotic and biotic stresses (Dordas et al. 2003; Dordas et al. 2004; Perazzolli et al. 2004; Seregélyes et al. 2004).

11.4.2 S-Nitrosylation

S-nitrosylation is a NO-dependent post-translational modification of proteins in which NO is incorporated in the reactive thiol group of cysteine residue to form nitrosothiol. S-nitrosylation is a reversible reaction in which the formation or breakage of the product does not require enzymes, mandatorily. Besides, two enzymes are known to facilitate the forward and backward reactions of S-nitrosylation which are nitrosylase and denitrosylase, respectively. At present thousands of proteins are known in plants which are target for S-nitrosylation, few of these proteins were common in animals which were also characterized for S-nitrosylation. Studies revealed that most of the proteins which were target for S-nitrosylation were involved in major physiological processes including photosynthesis, signaling, genetic information processing, primary and secondary metabolism, cytoskeleton activities, biotic/abiotic stress responses, etc. Few of the proteins which were identified as potential target for S-nitrosylation in previous studies are summarized in Table 11.2.

11.4.3 Tyr-Nitration

Post-translational modification of proteins by covalent addition of a nitro group (-NO_2) to tyrosine residue is generally referred to as tyr-nitration. Similar to S-nitrosylation, no enzymes are required for tyr-nitration (Ischiropoulos et al. 1992).

Table 11.2 Few of the proteins in plants which are known to undergo S-nitrosylation

Function	Target proteins	S-nitrosylation (Cys residue no.)	References
Photosynthesis	Rubisco large subunit	192, 427	Fares et al. (2011), Abat and Deswal (2009)
	Photosystem I apoprotein A2	559	Fares et al. (2011)
Metabolism	GADPH	155, 159	Abat et al. (2008), Wawer et al. (2010)
	Methionine adenosyltransferase 1	114	Lindermayr et al. (2005), Lindermayr et al. (2006)
Oxidative stress	NADPH oxidase	890	Yun et al. (2011)
	Ascorbate peroxidase	32	Tanou et al. (2009), Bai et al. (2011)
	Catalase	230	Ortega-Galisteo et al. (2012)
Signaling	NPR 1	156	Tada et al. (2008)
	Transcription factor	53	Serpa et al. (2007)
	Auxin receptor (TIR1)	140	Terrile et al. (2012)
	Calnexin	108	Fares et al. (2011)

Attachment of the nitro group at ortho position of aromatic ring in tyrosine residue is a two-step process. In the first step, the aromatic ring of the tyrosine residue gets oxidized by one electron, which results in the formation of a tyrosine radical. Electrons required for tyrosine oxidation are provided by either hydroxyl ($OH^{\bullet}$) or carbonate ($CO_3^{\bullet -}$) radicals, which are themselves derived from peroxynitrite ($ONOO^-$) (Yeo et al. 2015; Radi 2013) through three different pathways. The second step of tyrosine nitration involves interaction of tyrosine radical with nitrogen dioxide radical, which eventually produces 3-nitrotyrosine (YNO_2) (Souza et al. 2000). In this reaction, peroxynitrite is one of the most important molecules required for tyrosine nitration as it produces an oxidant required for the production of tyrosine radical. Peroxynitrite is itself produced through a fast reaction between nitric oxide and super oxide anion radical, and this shows that the peroxynitrite belongs to the reactive nitrogen species (RNS) group which is generated from NO (Patel et al. 1999) and also has its relations with oxidative stress responses. A diagrammatic representation of tyrosine nitrosylation and reactions involved in it is shown in Fig. 11.3.

Few of the proteins which were identified to be the target for tyrosine nitration include methionine synthase (Lozano-Juste et al. 2011), photosystem II protein1 (Galetskiy et al. 2011), abscisic acid receptors (Castillo et al. 2015), monodehydroascorbate reductase (Begara-Morales et al. 2015), isocitrate dehydrogenase (Begara-Morales et al. 2013), mitochondrial Mn superoxide dismutase (Holzmeister et al. 2015), etc.

11.5 Abiotic Stress Tolerance and Its Response

Due to immobile nature of plants, they are not capable of changing their place when encountering unfavorable environmental conditions. Unfavorable conditions may be biotic or abiotic. Biotic factors refer to living organisms that are able to threaten plants' viability via direct physical damage or induce diseases in it, and this category of stress factors include organisms like bacteria, virus, nematodes, fungi,

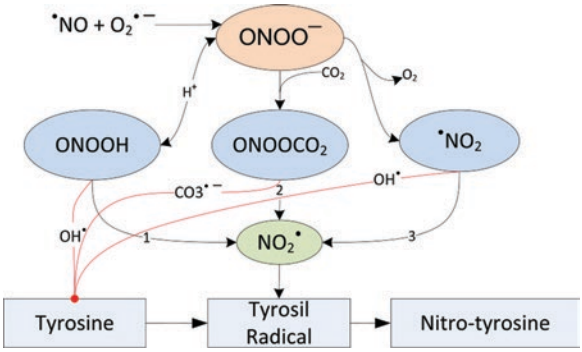

Fig. 11.3 Diagram displaying formation of nitro-tyrosine from tyrosine

insects, etc. (Atkinson and Urwin 2012). In contrast, abiotic factors are non-living factors like high soil salinity, high/low temperature, high/low light conditions, drought, heavy metals, etc. (Wang et al. 2003; Wani et al. 2016; Nakashima and Yamaguchi-Shinozaki 2006). Among these abiotic factors, high salinity and high temperature are the major role players in decreasing crop yield globally (Grattan et al. 2012; Pereira et al. 2014; Hasegawa et al. 2018; Fischer and Edmeades 2010). Regular irrigation, rainfall, and rock weathering are the main reasons why a normal fertile soil is converted into saline soil (commonly known as user soil). Most of the water leaches down the soil and part of it evaporate either directly or indirectly via transpiration by plants into the atmosphere, leaving salt in the upper layer of the soil (Rengasamy 2006). Thus, over a long period of time, salt keeps on accumulating in the soil and stays unfavorable for plant development. High soil salinity results in several plant changes at physiological, biochemical, and molecular levels. High concentration of salt in the soil lowers its water potential, disabling plants to absorb water from the soil and getting dehydrated Hoffman and Shalhevet (2007). As a consequence of dehydration, growth and metabolism of a plant slows down, eventually leading to its death. Due to salinity, water availability to the plant cell may also become limited even if soil is at saturation level, and the phenomenon is known as "physiological drought." High salinity results in reduced leaf area, leaf thickening, and stomatal closure, all of which eventually lead to a drastic reduction in its photosynthetic efficiency (Delfine et al. 1998). In order to maintain normal growth and metabolism under highly saline conditions, plants activate cell signaling pathways including those that lead to synthesis of osmotically active metabolites (amino acids, glycine betaine, sugars, sugar alcohols, etc.), plant hormones (ABA), specific proteins (LEA-type proteins, chaperonins), and certain enzymes that control ion and water flux and support scavenging of ROS (Serraj and Sinclair 2002; Kav et al. 2004; Raghavendra et al. 2010). Exposure of plants to high temperature also leads to various morpho-anatomical, physiological, and biochemical changes, affecting plant growth and yield (Long and Ort 2010). The effect of high temperature on plants growth is complex because plant responses are influenced by various factors such as diurnal temperature range and water stress (Paulsen 1994). Heat stress is also known to reduce germination and early growth and lead to accumulation of H_2O_2 which is able to oxidize various biomolecules in plant cell and leave them inactive (Bhattacharjee 2008). Heat stress is most often associated with drought stress as it impairs physiological processes and plant-water relations of crops (McDonald and Paulsen 1997). Also exposure of high temperature to plants results in a higher production of hydroxyl radical ($OH^{\cdot}$), which is known to initiate peroxidation of thylakoid lipids and thus lead to a decrease in photosynthesis efficiency (Wahid et al. 2007; Mishra et al. 1993).

Most of the pioneering studies were focused on exposure of different plants to a single abiotic stress condition, but their data could not be validated in field conditions, since under field conditions, plants are exposed to different abiotic and biotic stress conditions. This promoted researchers to study plants under combinations of different abiotic stresses, which confirmed that acclimatization done by plants against combinatorial stress conditions was significantly different from that of plants exposed to a single abiotic stress condition (Jiang and Huang 2001; Rizhsky et al. 2004). Similar results of many such studies changed the fashion of experiments, and now most of the abiotic stress studies on plants are done by exposing them to a combination of different abiotic stresses. In a recent study, it was demonstrated that exposure of those seedling to high temperature which were growing on a medium containing high salt concentration resulted in an enhanced thermostability of catalase present in leaves of two varieties of *Vigna mungo*. In contrast when seedlings of those two varieties were exposed to high temperature ion absence of salt catalase in them was observed to be inactivated (Singh and Mishra 2020).

When plants experience abiotic stress conditions, high-energy electrons which are being transported through electron transport chains of chloroplast and mitochondria spill their energy to nearby oxygen molecules, resulting in an uncontrollable generation of various reactive oxygen species (ROS) in plants (Foyer and Noctor 2003; Asada 2006; Foyer and Noctor 2005; Navrot et al. 2007). ROS are oxidizing in nature with a short life span and are known to exist in various forms including superoxide anion radical (O_2^-), hydroxyl radical ($OH^•$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), etc. (Wahid et al. 2007). The presence of reactive oxygen species (ROS) generating system in the plants, together with high percentage of polyunsaturated lipids, particularly in the thylakoid membrane, makes them susceptible to oxidative injury. Superoxide anion radical is one of the most commonly generated reactive oxygen species. Although capable of oxidizing various cellular components directly, it exerts most of its damaging effects by initiating the generation of more reactive species including hydroxyl free radicals (Halliwell and Gutteridge 1989). Other ROS are also detrimental to plants as they are highly efficient in generating damages to biomolecules such as proteins, lipid molecules in plasma membrane, and genetic material (DNA) present in a plant cell Mazid et al. (2011). On interaction with ROS, lipid molecules get converted into harmful lipid peroxide and other lipid abducts, and proteins are damaged which results in the reduction of their activity and possibility of DNA molecules getting mutated (Shah et al. 2001).

During the course of evolution, the higher plants have developed an efficient antioxidant defense system, which helps them to cope with environmental stresses. Antioxidant defense system in plants is categorized into two categories. First category of enzymatic defense system is constituted by various enzymes like superoxide dismutase, catalase, peroxidases, glutathione reductase, DHAR, MDHAR, etc. (Asada and Takahashi 1987). These enzymes are involved in detoxification of different ROS, e.g., SOD dismutates superoxide anion radical into H_2O_2 (Fridovich 1986), which is further detoxified by catalase and various other peroxidases to H_2O and O_2 (Passardi et al. 2004; Asada 2006). Various enzymes involved in detoxification of different ROS are displayed in Table 11.3. It is generally believed that those plant species or their varieties, which contain antioxidant enzymes possessing high tolerance toward different abiotic stresses, are referred to be resistant. In a recent study on seven varieties of *Vigna mungo*, it was demonstrated that the extent of thermostability of different SOD isoforms was not similar among varieties and those varieties with thermotolerant SOD isoforms were considered to be more resistant toward heat stress (Singh et al. 2019). Another category of antioxidant defense system constitutes non-enzymatic molecules like ascorbate, reduced glutathione (GSH), α-tocopherol, carotenoids, flavonoids, etc., which contribute to protection of plants by deactivation/quenching of ROS in multiple ways (Asada and Takahashi 1987). In addition to this, proline and glycine betaine are also well-known for their contribution in coping stress conditions such as high temperature and high salinity. Both of these molecules act as an osmolyte, which promotes the entry of water from soil into plant cells. Besides ROS, when plants are exposed to different stress conditions, there occurs a simultaneous increase of nitric oxide and its derivatives in plant cells (Valderrama et al. 2007). Nitric oxide and its derivative are referred to as reactive nitrogen species (RNS) due to the presence of unpaired electron. RNS are known to exist in various inorganic and organic forms which are themselves sub-categorized into radical and non-radical forms (Corpas et al. 2011). Few important examples of RNS are shown in Table 11.3.

Among RNS, peroxynitrite ($ONOO^{-}$) has been studied most extensively by researchers due to its involvement in various physiological processes in plants. Peroxynitrite is produced in a very fast reaction between NO and $O_2{\bullet}^{-}$, with a rate constant of approximately (10^{10} M^{-1} s^{-1}) (Estévez and Jordán 2002). Due to the co-existence of NO and $O_2{\bullet}^{-}$ in chloroplast, mitochondria, and peroxisomes, these organelles were identified to be the main site of generation in plants (Blokhina and Fagerstedt 2010; Corpas and Barroso 2014). Peroxynitrite, being highly oxidizing in nature, has been demonstrated to cause oxidative damages by interacting with all biomolecules present in the plant cell. Additionally, as described in the previous section of this chapter, RNS species particularly peroxynitrite has been identified to mediate post-translational modifications of various proteins, in turn leading to alterations in the physiological function controlled by them (Szabó et al. 2007; Corpas et al. 2009; Arasimowicz-Jelonek and Floryszak-Wieczorek 2011; Calcerrada et al. 2011; Berton et al. 2012; Szuba et al. 2015). Some researchers proposed that increase in protein nitration could be considered as a biomarker for specific stress conditions in similar to oxidation of proteins, which is utilized as a biomarker for oxidative stress (Corpas et al. 2007; Arasimowicz-Jelonek and Floryszak-Wieczorek 2011).

Table 11.3 Types of reactive nitrogen (RNS) species generated in plants

Type	Inorganic	Organic
Non-radical forms	Nitrous acid (HNO_2) Nitrosonium cation (NO^{+}) Nitroxyl anion (NO^{-}) Peroxynitrite ($ONOO^{-}$) Peroxynitrous acid (ONOOH) Dinitrogen trioxide (N_2O_3) Dinitrogen tetroxide (N_2O_4)	Nitrotyrosine (Tyr-NO_2) Nitrosoglutathione (GSNO) Nitrosothiols (SNOs) Nitro-γ-tocopherol Nitro-fatty acids (FA-NO_2)
Radical Forms	Nitric oxide (^{-}NO) Nitrogen dioxide ($^{-}NO_2$)	Lipid peroxyl radicals (LOO^{-})

There are many previous studies which have demonstrated that the effects of ROS and RNS on plants are tightly regulated by each other. It has been shown that RNS are involved in mediating post-translational modifications of various enzymes such as catalase, superoxide dismutase (Mn, Fe, and Cu/Zn isoforms), peroxiredoxin II E and F, DHAR, MDHAR, ascorbate peroxidase, etc., which are known to be involved in ROS detoxifications, and thus RNS regulate their activity during stress conditions (Ortega-Galisteo et al. 2012; Chaki et al. 2015; Holzmeister et al. 2015; Romero-Puertas et al. 2007; Camejo et al. 2015; Clark et al. 2000; Fares et al. 2014; Begara-Morales et al. 2015). On the other hand, generation of one of the most primary RNS "peroxynitrite" is dependent on the presence of a reactive oxygen species "superoxide anion radical." On observing these regulatory roles of ROS and RNS over each other, Corpas et al. (2013) suggested that using the term nitro-oxidative stress in place of oxidative stress shall be more appropriate.

11.6 Function of Nitric Oxide in Abiotic Stress Tolerance

11.6.1 Salt Stress

Plant suffers osmotic and ionic stress at high salt concentrations. Due to this many chief metabolic processes get affected which in turn limits the growth of plants. Enzyme activities of plants are badly affected under high salinity which leads to alteration in nitrate and sulfate assimilation pathway and a drop in energy. Further, salinity alters the activities of and sulfur & nitrogen demand increases (Vanlerberghe 2013). High salinity leads to oxidative damage due to ROS and causes cellular injury. To avoid such oxidative damage, plants have developed a wide range of defense methods. However, it is a less known fact that plants become tolerant to salinity in the presence of nitric oxide. Arora et al. reported the impacts of exogenous sodium nitroprusside (SNP), a NO donor. It was found that it remarkably reduced the oxidative damage under highly saline conditions. Its effect was observed in rice, cucumber, and lupin seedlings where it enhanced seedling growth and also maize dry weight even under salt stress (Arora et al. 2016). A good stability was observed between carbon and nitrogen metabolism when pretreated with nitric oxide. This effect of nitric oxide was due to increased total soluble protein and enhanced endopeptidase and carboxypeptidase activities in plants under salinity stress (Wojtyla et al. 2016). Salt stress tolerance of roots of red kidney beans was due to the significant role of glucose-6-phosphate dehydrogenase enzyme in NR-dependent nitric oxide production. Prior studies have reported that nitric oxide induces ROS scavenging enzyme activities of CAT, peroxidase (POD), SOD, and ascorbate peroxidase (APX), which in turn reduces membrane permeability, rate of ROS production, and intercellular CO2 concentration under high salinity stress. Nitric oxide also induces the expression of transcripts of genes encoding sucrose-phosphate synthase and Δ′-pyrroline-5-carboxylate synthase, related to stress (Shi and Chan 2014).

11.6.2 Drought Stress

Drought is a key factor responsible for limited crop yield. It has been found that nitric oxide synthesis is dependent on intensity of water deficit, mild or severe. When cucumber roots suffer from mild water deficit (5–10 h), nitric oxide synthesis in root tip cells and the elongation zone surrounding it is slightly increased. But when the duration of this stress increases up to 17 h, it results in rigorous nitric oxide synthesis in the roots of cucumber (Ahmad et al. 2016). Also, pea, wheat, and tobacco have shown enhanced nitric oxide production. Stress due to lack of water induces the production of nitric oxide in mesophyll cells of maize and activity of nitric oxide synthase (NOS) in cytosolic and microsomal fractions of maize leaves. This nitric oxide synthesis was blocked by prior treatment with inhibitors of nitric oxide synthase and nitrate reductase. This suggests that nitric oxide is synthesized from NOS and NR in maize leaves when exposed to water stress. Treatment with nitric oxide synthase and nitrate reductase inhibitors suppressed the activities of chloroplast and antioxidant enzymes present in cytosol, i.e., SOD, and GR. Reduced activities of these enzymes were improved by externally applying NOS and decreasing the buildup of H_2O_2 due to water stress (Delorge et al. 2014). Due to induction of subcellular antioxidant defense, the possible ability of nitric oxide to scavenge H_2O_2 is in some part only (Laxa et al. 2019).

11.6.3 Temperature Stress

Crop productivity is equally affected by temperature variations. Plant injuries due to high temperature include damage due to oxidative stress, damage to membrane, lipid peroxidation, degradation of protein, inactivation of enzymes, and disruption of DNA strands. Also cold stress retards biochemical and physiological processes and ROS homoeostasis in plants (Roychoudhury et al. 2013). Nitric oxide has a unique role in the management of heat and cold stress. Lucerne cells have been reported for increased nitric oxide synthesis at high temperatures, while tomato, wheat, and maize developed cold tolerance when NO is applied externally (Parankusam et al. 2017). NO synthesis was augmented by short-time heat stress in alfalfa. It is likely that this result due to antioxidative property of nitric oxide, which raise adverse effects imposed by the intensification of peroxidative metabolism in cold and heat stress. Heat stress stimulated NO synthesis that could play a role in the induction of cell death in *Symbiodinium microadriaticum* by causing a boost in caspase-like activity (Leterrier et al. 2012).

11.6.4 Ultraviolet Radiation Stress

The injure of the stratosphere ozone layer amplified the radiation of UV-B (250–320 nm) responsible for increase in ion leakage, chlorophyll loss, and reducd efficiency of PS-II photochemistry (Fv/Fm) and the quantum yield PS-II electron transport (ØPS-II), and augmented H_2O_2 and thylakoid membrane protein oxidation. UV-B radiation leads to a boost in nitric oxide and reactive oxygen species in *Arabidopsis* (Shi and Chan 2014). Nitric oxide generated from NOS-like activity acts synergistically with ROS to stimulate ethyl synthesis in defense response under UV-B radiation in leaves of maize (Arora et al. 2016). UV-B induced augmentation of NOS activity in maize hypocotyls, indicating that NO may act as a second messenger and perform antioxidant response to UV-B radiation, and SNP-exposed maize plants exhibited increased activity of glucosidase and protein synthesis.

11.6.5 Heavy Metals Stress

As we know heavy metals contamination affects the biosphere in many places worldwide. Several studies have been conducted in order to evaluate the effects and remedy of different heavy metals concentration on plants (Schützendübel and Polle 2002). NO also plays a vital role in the enhancement of antioxidant enzyme activities and alleviating the toxicity of heavy metals. Exogenous application of SNP reduced copper (Cu) toxicity and $NH4^+$ accumulation in rice leaves (Manara 2012a). The protective effect of SNP on the toxicity and $NH4^+$ accumulation can be reversed by c-PTIO, a NO scavenger, suggesting that the protective effect of SNP is attributable to NO released. These results also suggest that reduction of Cu-induced toxicity and $NH4^+$ accumulation by SNP is most likely mediated through its ability to scavenge active oxygen species. SNP pretreatment significantly reduced O_2- induced specific fluorescence in *Lupinus luteus* roots under heavy metal treatment. Results obtained in this study suggest that antioxidant function of NO may be traced by a scavenging of O_2-, resulting in a decrease of superoxide anion (Yadav 2010). The detoxification and antioxidative properties of NO are also found in soybean cell cultures under cadmium (Cd) and Cu stress. Moreover, NO decreased the aluminum (Al3+) toxicity in root elongation of *Hibiscus moscheutos* (Manara 2012b).

11.7 Conclusion

Nitric oxide is a ubiquitous bioactive molecule with diverse roles in a huge spectrum of physiological processes in plants. Even then, there are much more rigorous studies required to understand the complete range of activities being performed by nitric oxide. A huge amount of work is already done about functions of nitric oxide as a signaling molecule interacting with plant hormones, nutrients, or metals. But still much more is to be searched upon the activities of nitric oxide.

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Chapter 12
Melatonin: Role in Abiotic Stress Resistance and Tolerance

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12.1 Introduction

Plants are exposed to numerous abiotic stresses that limit their growth and productivity in areas which are more stress-prone (Gürel and Avcıoğlu 2001). Due to the persistence of global climate change and the surge in extreme climatic conditions, it is stated that the adverse impact of environmental stress factors on plant production will rise in many areas of the world (Denby and Gehring 2005), as stress factors can concurrently show their harmful effects on plants (Kalefetoğlu and Ekmekçi 2005). Abiotic stress has extended a massive agricultural product loss of about 71% and other stresses of 29% (Boyer 1982), and only 10% of the arable land in the world is estimated to be free from some forms of stress. Thus, abiotic stress factors represent the main restrictive factors of crop growth and production worldwide resulting in more than 50% yield reduction of most products (Mahajan and Tuteja 2005). The impact of abiotic stress factors has been intensified in recent years due to various irrigation techniques and the use of chemical fertilizers which reduced soil improvement.

These abiotic stresses pose a threat to plant growth and productivity due to production of excessive reactive oxygen species (ROS), which causes severe damage to metabolic and cellular processes (Apel and Hirt 2004). Thus, to counteract the

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detrimental effects of stress-induced excessive ROS, plants have evolved different strategies to protect themselves, and the most relevant is the antioxidant defence system, comprising enzymatic and non-enzymatic components that effectively scavenge the ROS and maintain a proper balance within the cells (Meloni et al. 2003). Enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT), and the non-enzymatic antioxidants include glutathione (GSH), ascorbate (AsA) as well as polyphenols and vitamins in plants (Meloni et al. 2003). Furthermore, the amplified synthesis of osmolytes such as soluble protein, sugars and proline also has a role in reducing stress (Shafi et al. 2014, 2015, 2017, 2019). As an alternative method, some exogenously applied healers during plant growth has been used in recent years, and among them, melatonin (Mel) applications have been observed to have a beneficial effect in increasing stress tolerance in the plant (Martinez et al. 2018).

Melatonin (N-acetyl-5-methoxytryptamine) was discovered in 1958 by Aaron B. Lerner and exists universally in all taxonomically distant groups of organisms and plants as an indoleamine neurohormone (Favero et al. 2017). Mel is notoriously considered as critical bioactive molecule affecting plant and animal growth (Janas and Posmyk 2013). An increasing Mel's roles and impacts on various plant cellular metabolic and biological processes have been documented including growth promoters as auxins, antioxidants for ROS and other roles as signal molecules (Paredes et al. 2009), rooting (Park and Back 2012), chlorophyll catabolism (Arnao and Hernández-Ruiz 2009) and stress tolerance (Liang et al. 2015; Zhang et al. 2017a). Plants can also synthesize Mel, and thus Mel plays an indispensable role as an antioxidant or a modulator of growth and development in plants (Hernandez-Ruiz et al. 2005). Mel acts as a broad-spectrum antioxidant against ROS/RNS (Tan et al. 2015) and as a protective agent against various abiotic stress situations (Jiang et al. 2016; Lee and Back 2018; Liang et al. 2019). Moreover, Mel applications have elevated activities of the antioxidant enzyme under abiotic stress conditions (Tan et al. 2007b), and along with acting as an antioxidant agent, Mel induces numerous changes in gene expression and has a significant effect on nitrogen and carbohydrate metabolism and transcription rearrangement (Shi et al. 2015a). Mel's role in the cell signalling mechanisms has been reported as it activates redox-sensitive regulatory pathways and perhaps has a higher antioxidant capacity, and highly effective, even at low concentrations, in protecting against oxidative stress (Tan et al. 2015; Martinez et al. 2018). Thus, it is suggested that Mel affects signal transduction and is also beneficial for dealing with adverse situations, assuming reinforcement against plant stress.

Mel is an eco-friendly molecule which may serve as an economical substitute strategy to induce plant protection and tolerance against various abiotic stresses such as extreme temperatures, salinity, drought, heavy metals, UV radiation and oxidative stress (Qi et al. 2018; Li et al. 2016a). Taking into consideration the new progress in Mel studies in recent years, the activity of Mel in plants has been comprehensively and intensely explored. Being comparatively "novel" in plant biology, Mel has gained a notable interest in the research community due to its diversified biological role as a plant master regulator and defensive roles in capricious environmental conditions. In the current chapter, we have summarized Mel's biosynthesis pathway, response to harsh abiotic conditions and roles in alleviating different stresses and possible mechanisms. Besides, we focus herein the mitigation impacts of exogenous Mel on plant responses to environmental stresses. Particularly, the significance and scope of Mel research in plants are speculated, which might be supportive and insightful for existing research, and the imminent route of Mel study in plants during abiotic stresses is also determined. Some reports on genetic modifications which can enhance Mel synthesis in transgenic plants and improve resistance to adverse conditions is also discussed.

12.2 Melatonin Biosynthesis Pathway in Plant

Mel is a lipophilic and hydrophilic molecule that is distributed in the cytoplasm and lipid membranes (Angel 2007), located in the hydrophilic side of the lipid bilayer, and averts the biological membrane from the lipid peroxidation by directly scavenging the toxic molecules (de Lima et al. 2010). Interestingly, the organization of Mel in lipid membranes depends on its concentration; at low concentrations, the Mel molecules arrange parallel to the lipid tails, whereas at high concentrations, they arrange parallel to the bilayers (Dies et al. 2015). Thus, the location of Mel in the lipid bilayer is speculated to monitor disordering in the hydrophobic tail of the lipid bilayer. Mel biosynthesis in plants has been elucidated with the help of isotope tracer experiments (Murch et al. 2000), and it starts from amino acid tryptophan into 5-hydroxytryptophan or tryptamine and then to serotonin (Fig. 12.1). Mel biosynthesis in plants is also catalysed by four successive enzymatic steps, including tryptophan decarboxylase (TDC), tryptamine 5-hydroxylase (T5H), serotonin N-acetyltransferase (SNAT) and N-acetylserotonin methyltransferase (ASMT)/caffeic acid O-methyltransferase (COMT) (Kang et al. 2011; Dhole and Shelat 2018). The four consecutive enzymatic steps of Mel biosynthesis in plants are as follows (Fig. 12.1):

- The first dedicated enzyme in tryptophan decarboxylase (TDC) catalyses the conversion of tryptophan into tryptamine. TDC is the first committed enzyme for Mel biosynthesis in plants, which catalyses tryptophan into tryptamine or 5-hydroxytryptophan to serotonin. It is a soluble cytosolic protein that exists as a homodimer with a monomer molecular weight of 54,000 and shows high substrate specificity to tryptophan and 5-hydroxytryptophan. TDC has been cloned in several plant species including rice (Kang et al. 2008), pepper (Park et al. 2009), *Catharanthus roseus* (De luca et al. 1989) and tobacco (Di Fiore et al. 2002). TDC serves as a bottleneck in regulating serotonin (precursor of Mel) biosynthesis since TDC expression is very low or negligible.
- The second step in Mel biosynthesis is catalysed by the cytochrome P450 enzyme tryptamine 5-hydroxylase (T5H), which hydroxylates the C-5 position of tryptamine to form serotonin (Fujiwara et al. 2010). T5H is associated with the

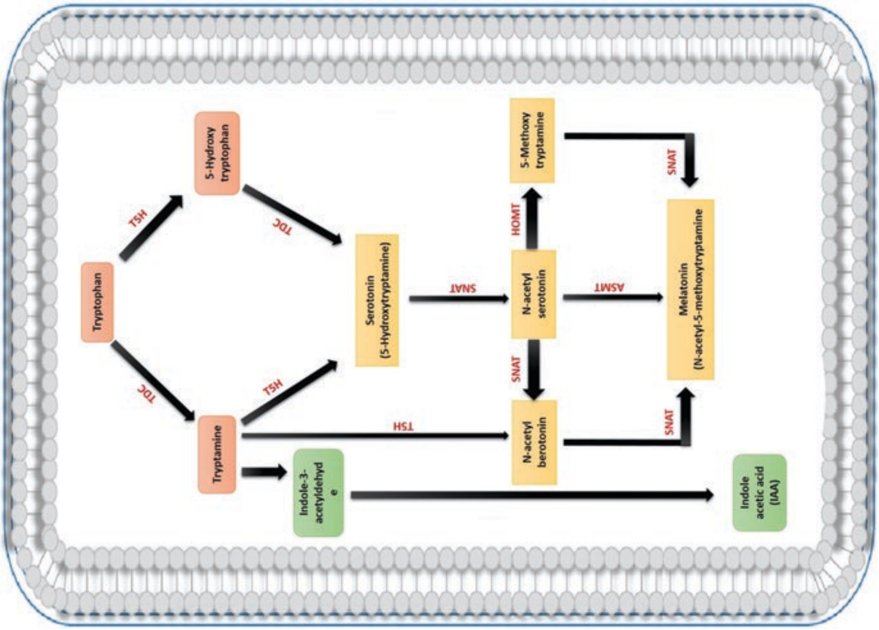

Fig. 12.1 Metabolic pathway of melatonin in plants. TDC, tryptophan decarboxylase; T5H, tryptophan 5-hydroxylase; SNAT, serotonin N-acetyltransferase; ASMT, N-acetylserotonin methyltransferase; HOMT, hydroxyindole-O-methyltransferase

hydroxylation reaction, which is predominantly mediated by cytochrome P450-dependent monooxygenases (P450s) and 2-oxoglutarate-dependent dioxygenases (2-ODDs) in plants. It also catalyses conversion of tryptophan to 5-hydroxytryptophan and *N*-acetyl tryptamine to *N*-acetyl serotonin reactions. T5H has been isolated in rice Sekiguchi lesion (sl) mutants by map-based cloning (Fujiwara et al. 2010). T5H is constitutively expressed in healthy rice (*Oryza sativa*) plants (Kang et al. 2007).

- The final two enzymes in Mel biosynthesis are arylalkylamine N-acetyltransferase (AANAT) and N-aceylserotonin methyltransferase (ASMT). Currently, there are no known AANAT homologous genes in higher plants; however, AANAT has been cloned in the unicellular green alga *Chlamydomonas reinhardtii* (Okazaki et al. 2009).
- The penultimate step in Mel biosynthesis is catalysed by serotonin *N*-acetyltransferase (SNAT) (also known as arylalkylamine *N*-acetyltransferase (AANAT)). It catalyses the transfer of an acetyl group from acetyl-coenzyme A to an array of molecules, such as aminoglycosides and arylalkylamines. The rice SNAT enzyme can accept various amine substrates, such as serotonin, tryptamine and 5-methoxytryptamine, which are catalysed to *N*-acetylserotonin, *N*-acetyltryptamine and Mel. 5-Hydroxy-indole-O-methyltransferase is the last

step enzyme; it catalyses N-acetylserotonin into Mel by O-methyltransferase (OMT) reaction.

- The last enzyme, ASMT, which was known as HIOMT (hydroxy-indole-O-methyltransferase), has been purified in rice via heterologous *Escherichia coli* overexpression (Kang et al. 2013). ASMT is the rate-limiting enzyme of Mel biosynthesis. Subcellular localization shows that SNAT protein is localized in chloroplasts, whereas ASMT is found in the cytoplasm (Byeon et al. 2014a).

Among these four members, a single-copy T5H (Fujiwara et al. 2010) and SNAT gene (Kang et al. 2013) encode the respective enzyme in rice, while each of TDC (Kang et al. 2007) and ASMT (Kang et al. 2011) was constituted by a small gene family in rice. The upregulation of these genes in Mel biosynthesis pathway is beneficial for endogenous Mel accumulation. Among them, the ASMT is the last enzyme of Mel biosynthesis and contained three members, while the T5H and SNAT genes encode single-copy genes (Fujiwara et al. 2010; Kang et al. 2013).

12.3 Melatonin as Abiotic Stress-Alleviating Agent

Melatonin (Mel) is an ubiquitous molecule that serves several biological functions in plant growth, development and responses to abiotic stresses (Fig. 12.2). Mel levels in plants increase significantly under several abiotic stress conditions which are believed to be favourable for stress resistance. Exogenous application of Mel is often associated with a means of ameliorating the harmful effects of stress, and many pieces of evidence have confirmed that Mel can boost resistance against numerous hostile environmental factors, such as cold, drought, salinity, high temperature, ultraviolet radiations and heavy metals (Han et al. 2017). Antioxidative effects of Mel on alleviating different environmental stresses have been widely explored in plants (Wang et al. 2013), under abiotic stress. Recently, a positive indication was observed in plants which accumulate higher levels of Mel when exposed to harsh environmental conditions, and exogenous application of Mel improved tolerance to these stresses (Shi et al. 2015b).

12.3.1 Role of Melatonin in Mitigating Salinity Stress

The hallmark of salinity is the reduction of the water potential and increasing the energy required for the intake of water and nutrients which result in oxidative stress. Salt stress is triggered by the accumulation of Na^+ and Cl^- ions in sensitive plant tissues (Flowers et al. 2010), and it has been reported that high concentrations of salt perturb the morphological and physiological processes in plants and prevent growth (Cirillo et al. 2016). Salinity surges ROS formation and stimulates oxidative stress (Gao et al. 2008), which causes extensive injury to cell membranes and other

Fig. 12.2 Versatile roles of melatonin in alleviating abiotic stress conditions

biomolecules (Arora et al. 2008). Plant physiology at both plant and cellular levels is hampered by osmotic and ionic stress caused due to salt stress. Accumulation of Na^+ under salt stress of plants leads to ion imbalances and toxicity, but with K^+ content declining, the effect of salt stress is reversed by exogenous Mel which promotes the absorption of K^+, suggesting that Mel can regulate ion homeostasis or gene expression by mitigating the damage caused by stress (Zhan et al. 2019; Wu 2018). Some of the reports of salinity stress alleviation by Mel are as follows:

1. The positive roles of Mel in salt stress tolerance have been progressively evolved either by exogenous application of Mel or genetic modification of Mel synthesis enzymes (Kanwar et al. 2018). Indeed, exogenous Mel applications improved growth, photosynthetic capacity, antioxidant activity and chlorophyll content but decreased the ROS level and oxidative injury in cucumber grown under salinity stress conditions (Wang et al. 2016a). In *Cucumis sativus*, Mel treatment under salinity conditions increased seedling growth, nutrient intake and nitrogen metabolism (Zhang et al. 2017c). Similarly, Ke et al. (2018) proved that Mel

pretreatments eased the negative impact of salinity stress by regulating polyamine metabolism in wheat by inducing enzyme activity for ROS scavenging. In another study, it was concluded that Mel solutions enhanced germination and seedling growth in rice under salinity conditions, which was attributed to reducing the contents of Na^+ and Cl^- in roots and leaves (Li et al. 2017c). The results of Jiang et al. (2016) indicated that exogenous Mel treatments on salt-stressed maize plants caused a prominent improvement in growth, photosynthetic capacity, antioxidant enzyme activity and homeostasis, where Mel concentration was six times higher in roots compared to the control under stress conditions, which indicates that Mel plays an imperative role in the amelioration of stress conditions (Arnao and Hernández-Ruiz 2009). Dawood and El-Awadi (2015) indicated that exogenous Mel applications mitigated the negative effects of salinity in *Vicia faba* by enhancing plant biomass, relative water content, photosynthetic activity, phenolic matter and plant nutrient uptake and reduced the Na^+ and Cl^- content. Zhou et al. (2016b) investigated influences of Mel treatments on photosynthetic activity in tomato under salinity conditions and established that Mel treatments mitigated the deleterious effects of salinity by improving plant growth and photosynthetic capacity.

2. Mel treatments also showed a major effect on lipid metabolism with K^+/Na^+ homeostasis in a potato grown under salinity stress (Yu et al. 2018). Mel applications mitigated the deleterious effects of salinity on photosynthetic capacity by reducing oxidative stress, improving antioxidant enzyme activity in watermelon roots along with inhibition of stomatal closure and heightened light energy absorption and electron transport in photosystem II (Li et al. 2018a). Liang et al. (2015) treated plants with Mel to determine its effect on physiological and biochemical properties in rice grown under salinity stress, and they further revealed that Mel treatments decreased or inhibited chlorophyll damage and the transcripts of senescence-associated genes, thus improving salinity tolerance. Further, Mel treatments reported improved tolerance to salt stress and K^+/Na^+ homeostasis in potato, increasing K^+ and decreasing NaCl concentration (Yu et al. 2018).
3. Mel plays a main role as an antioxidant in H_2O_2 scavenging and enhancing activities of various antioxidant enzymes to improve salt tolerance in alfalfa (Cen et al. 2020). SNAT and ASMT genes from alfalfa are respectively overexpressed into the alfalfa genome, and the elevated Mel content in transgenic plants promoted transgenic plant growth. Kaur and Bhatla (2016) reported that in sunflower seedlings exposed to salt stress, exogenous Mel restored root growth and hypocotyl elongation. Similarly, Mel treatments on roots of watermelon mitigated salt stress damage in photosynthetic capacity and oxidative stress, improving redox homeostasis and antioxidant enzyme activity (Li et al. 2018a).
4. Exogenous Mel was able to effectively ameliorate salt stress damage and promote cotton seed germination by improving the physiological activity of cotton seeds by maintaining the K^+/Na^+ balance in vivo and promoting the content of Mel and osmotic regulation substances (Chen et al. 2020). Yin et al. (2019) reported that exogenous Mel application increased NaCl tolerance in tomato by

reducing chlorophyll; by balancing the distribution of photosynthetic electrons, thereby suppressing ROS production; and by promoting the activities of enzymes involved in the ASC-GSH cycle to enhance ROS scavenging.

12.3.2 Role of Melatonin Against Drought Stress

Among abiotic stresses, drought represents a major environmental constraint which limits plant growth and development (Cui et al. 2017) by inducing a wide range of morphological, physiological and anatomical disruptions in plants (Cui et al. 2017). It also causes a severe reduction in leaf area, degrades photosynthetic pigments and degrades chlorophyll, which in turn decreases photosynthesis and reduces canopy size, resulting in a lower yield and finally leading to total crop loss (Liang et al. 2019). Drought stress also stimulates the overproduction of ROS (Liang et al. 2019), and if toxic levels of ROS are not removed, they cause severe oxidative damages to cell membranes, proteins, enzyme activity, RNA and DNA (Cui et al. 2017). The mitigating potential of Mel application for drought stress is by maintaining plant growth, improving the photosynthetic characteristics and enhancing activities of antioxidant enzymes in maize seedlings (Ahmad et al. 2019).

1. Mel is a new plant growth regulator reported to alleviate the oxidative damages caused by drought stress (Wei et al. 2015). The protective role of Mel in maize roots and leaves under drought stress was observed through comparing ROS accumulation, the abilities of antioxidant enzymes and photochemical capacities (Huang et al. 2019). Drought stress led to the rise in ROS, severe cell death and degradation of the D1 protein, which were mitigated by the Mel application. The application of Mel improved the photosynthetic activities and alleviated the oxidative damages of maize seedlings under drought stress. Huang et al. (2019) concluded that signals of exogenous Mel received by roots could affect the stress responses of leaves, and the Mel signals perceived by leaves also led to changes in physiological metabolisms in roots under stress (Huang et al. 2019).
2. Increased antioxidant activity in different plants grown in drought stress has been associated with the Mel content (Li et al. 2016b). Drought has induced the significant accumulation of ROS in the maize leaves as well as higher levels of EL (ethylene) and MDA, which are important oxidative damage indicators of the integrity of cell membranes (Liu et al. 2015a). Mel treatments in *Brassica napus* L. mitigated the deleterious effects of water deficit on plant growth by decreasing H_2O_2 levels and increasing antioxidant enzyme activity and osmotic solutes (Li et al. 2018b). Increase in photosynthetic capacity and stress-related phytohormones was associated with the endogenous Mel content under water deficit conditions. Exogenous Mel treatments have resulted in enhanced photosynthetic capacity and water use efficiency due to increased indole acetic acid (IAA) and zeatin and decreased H_2O_2 and aminocyclopropane-1-carboxylic acid (ACC) production (Li et al. 2017a).

3. Fleta-Soriano et al. (2017) proved that Mel treatments enhanced photosystem II (PSII) resulting in a preserving factor in maize under drought stress. Maize plants recovered from drought stress by augmenting the Fv/Fm ratio, which could have a defensive effect in plants subjected to water deficit conditions. Cui et al. (2017) verified that Mel applications alleviated the deleterious effects of drought stress in wheat by increasing antioxidant activity and decreasing ROS and membrane injury. Further, Mel assisted in maintaining PSII function under stress and delayed the typical reduction in chlorophyll content. In kiwifruit seedlings, Mel application alleviates the negative effects of drought stress and increased the leaf area per plant, improving the content of chlorophyll and carotenoid compared with the untreated control plants (Liang et al. 2019), watermelon (Li et al. 2017b) and tomato plant (Liang et al. 2019). Recent research indicated that Mel is effective in the process of the PSII repair by maintaining the protein availability of D1 in tomato under salt stress (Zhou et al. 2016b). Mel played a vital role in maintaining photosynthetic efficiency by regulating the repair cycle of PSII and preserving chlorophyll pigments, resulting in the improvement of photosynthesis in the plants grown under stress conditions (Wang et al. 2016a; Cui et al. 2017).
4. Mel is an extremely efficient antioxidant that can effectively suppress H_2O_2 production through enhancing activities of antioxidant enzymes (CAT, POD and APX) under drought stress and regulating various physiological process in plants (Li et al. 2015; Kaya et al. 2019). Mel application promoted some antioxidant enzymatic activities of maize roots and leaves under drought conditions, especially with the root-irrigation method. Previous studies showed that application of exogenous Mel could induce endogenous nitric oxide generation (Zhao et al. 2018), which has emerged as an important signalling molecule in plants, activating ROS scavenging enzymes under drought conditions. Moreover, Wang et al. (2013) proved that Mel had an ameliorative effect on drought stress by increasing antioxidant activity. Similarly, mitigation of deleterious effects of drought stress could be attributed to its ROS scavenging functions by improving antioxidant enzyme activity and photosynthetic efficiency (Meng et al. 2014). Mel played an important role in the detoxification of reactive oxygen and free radicals and functions as an antioxidant in living organisms.
5. Ma et al. (2018) showed that Mel application elevated Mel biosynthesis gene (TDC1, SNAT1 and COMT) expression, resulting in mitigation of leaf senescence caused by water deficit in *Agrostis stolonifera*. Mel applications reduce the expression of the ABA synthetic gene (MdNCED3) and increase the expression of catabolic genes (MdCYP707A1 and MdCYP707A2), thus reducing the level of ABA under dry conditions (Li et al. 2015). Drought increased the expression of genes related to drought stress and decreased the production of abscisic acid (ABA), which leads to the closure of stomata.
6. Drought stress causes inhibition of seed germination and root vitality; however, pretreatment with Mel significantly reverses this inhibition (Zhang et al. 2013, 2014a, b). Mel application enhanced levels of endogenous growth factors, which resulted in the formation of denser roots (Zeng et al. 2018), increased root growth

in cherry (Sarropoulou et al. 2012) and improved the vigour of cucumber roots under drought stress (Zhang et al. 2013).

12.3.3 Against Heavy Metal Stress

Generally, metals are the elements indispensable for normal survival of plants; however, the presence of some metals in the root region has toxic effects which harm plant growth and yield, e.g. aluminium (Al), cadmium (Cd), chromium (Cr), zinc (Zn), copper (Cu), lead (Pb) and nickel (Ni) (Prasad and Strazalka 2002). They can easily accumulate in plants which result in plant growth and nutrient uptake inhibition (Brune et al. 1995); these heavy metals stimulate ROS formation in plants which causes a decrease in chlorophyll and photosynthesis rate, increases ethylene level, slows down root and shoot growth, reduces CO_2 fixation and limits the sugar transport leading to oxidative stress (Buchanan et al. 2000). A study revealed that Mel and its precursors can bind to several toxic metals: Al with Mel, tryptophan and serotonin; Cd with Mel and tryptophan; Cu with Mel and serotonin; Fe^{3+} with Mel and serotonin; Fe^{2+} with tryptophan only; Pb with Mel, tryptophan and serotonin; and Zn with Mel and tryptophan. Electrochemical studies show that Mel can bind to both Cu^{2+} and Cu^{+} which cause free radical damage (Parmar et al. 2002). It is accepted that Mel affects biological systems either through direct quenching of free radicals or by chelation of toxic metals (Romero et al. 2014).

1. The plants exposed to heavy metals have been shown to induce Mel biosynthesis for alleviating stress effects (Tal et al. 2011). Tan et al. (2012) pointed out that Mel treatments elevated the phytoremediation capacity of pea under Cu stress. Many studies have documented that exogenous Mel treatments reduced the toxic impact of various heavy metals such as Cd, Al, Cu, Vn, Ni, etc. via increasing antioxidant activity, photosynthetic capacity and root growth and regulating antioxidant-related gene expression and Mel biosynthesis in several plants (Nawaz et al. 2018). In green algae, exogenous Mel can relieve Cd-induced stress (Tal et al. 2011), and Tang et al. (2015) stated that foliar Mel applications enhanced the photosynthetic capacity of eggplant under Cd stress. A substantial increase in antioxidant enzyme activity along with low ROS levels was related to the treatment of Mel-stimulated Cd tolerance in tomato. These Mel applications mitigated Cd-stimulated oxidative stress by both Cd sequestration and transfer of Cd from the cytosol to the vacuole and cell wall (Hasan et al. 2015) or increasing the levels of non-enzymatic and enzymatic antioxidants. Similarly, Gu et al. (2017) reported that exogenous Mel treatments mitigated the negative effect of Cd on alfalfa growth by reducing Cd accumulation and re-establishing the micro RNA-mediated redox homeostasis. They further suggested that Mel could regulate expression of ion channel genes in crops against Cd stress.
2. Ni et al. (2018) demonstrated that Cd stimulated the expression of Mel-related genes and enhanced the endogenous Mel content in wheat. However, exogenous

Mel treatments mitigated Cd toxicity on plant growth and increased ascorbate peroxidase (APX) and superoxide dismutase (SOD) activity. They reported that Mel had an important role in keeping H_2O_2 homeostasis by modulating antioxidant activity. Mel treatments in spinach alleviated increased lipid peroxidation and restricted growth properties, chlorophyll and carotenoid content and photosynthetic activity by reducing ROS levels and increasing antioxidant activity (Moussaa and Algamal 2017). Further, in *Solanum lycopersicum* L., Cd stress-induced Mel biosynthesis and external application of Mel conferred plant resistance to Cd toxicity (Hasan et al. 2015).

3. Higher Cu contamination levels are toxic to pea plants; however, Mel application has significantly enhanced their tolerance to Cu contamination and increased their survival rate (Tan et al. 2007a), and Mel treatment has improved seed germination and seedling growth of cabbage in the presence of $CuSO_4$.
4. Sarafi et al. (2017) established that excessive boron (B) decreased photosynthesis and dry matter in pepper, and exogenous Mel treatments eradicated visible B toxicity symptoms along with increased nutrient uptake, sugar accumulation, photosynthetic activity, antioxidant capacity and reduced ROS and membrane permeability.
5. Zhang et al. (2017b) verified exogenous Mel treatments on mitigating Al-induced phytotoxicity in *Glycine max*. They documented that the effect of Mel on Al stress was dose-dependent, and Mel improved root growth, reduced H_2O_2 content and increased antioxidant enzyme activity. Al toxicity triggers Mel accumulation by upregulating two NSI-like variants of acetyltransferase that may be involved in Mel biosynthesis. Further, optimal concentrations of Mel improved resistance to Al stress by enhancing antioxidant enzyme activity and increasing malate and citrate exudation in soybean (Zhang et al. 2017b).
6. Exogenous Mel application can make water hyacinth even more useful for phytoremediation as it is a powerful antioxidant capable of elevating their tolerance to pollutants and removing additional contaminants. Hyacinth (pollutant-tolerant plant) can tolerate contamination of wastewater generated from industrial and agricultural sources (Munavalli and Saler 2009), such as nitrogen and phosphorus (Jayaweera and Kasturiarachchi 2004), the heavy metal mercury (Riddle et al. 2002) and the carcinogen arsenic (Misbahuddin and Fariduddin 2002) with the help of Mel and its metabolite N1-acetyl-N2-formyl-5--methoxykynuramine (AFMK).
7. The heavy metals Cd, Pb and Zn induced a rise in Mel levels in green microalgae *Ulva* sp. (Tal et al. 2011). Exposure to Cd induces a significant rise in Mel levels in the algae, while Pb and Zn exposure also induced an increase in Mel levels, but to a lesser extent. This study suggests that Mel participates in environmental stress adaptation.

12.3.4 Against Temperature Extremes

Plants require an optimal temperature at every stage of growth, and this requirement may vary between species and even varieties. The temperatures below the optimum negatively affect plant growth and ultimately yield. It is observed that low and high temperatures slow down seed germination and emergence, limit water and nutrient intake, increase disease damage, negatively affect flowering and seed and fruit formation and finally cause plant death (Pierce 1987). Hot climate plant species are very sensitive to low temperatures (Decoteau 2000) and affect the whole metabolic system of the cell and even cause water stress (Kratsch and Wise 2000) and damage in cell membranes, which also affects sugars, phenolics, phospholipids, protein and ATP (Lyons 1973). Low temperature stimulates ROS level enhancement in plant cells which may lead to lipid peroxidation and oxidative modifications in proteins and nucleic acids (Fan et al. 2014). Similarly, high temperature can deleteriously affect germination and output in many plant species, negatively affect food intake and deteriorate membrane stability, resulting in necrotic spots similar to water stress symptoms in leaves, eventually leading to premature deaths (Hall 2018). In the generative development period, high temperature, flower dust germination, fertilization, flowering and seed and fruit formation can cause a substantial yield loss.

1. A drastic variation in light, temperature and various environmental stress agents may increase Mel levels. Arnao and Hernández-Ruiz (2009) determined the Mel level in barley roots and lupin (Arnao and Hernández-Ruiz 2019) under naturally or artificially induced adverse conditions. Byeon and Back (2013) reported that rice seedlings at high temperatures show enhanced Mel synthesis due to an increase in serotonin N-acetyltransferase and N-acetylserotonin methyltransferase activities.
2. Cell membrane systems are the primary sites of chilling injuries as low temperature leads to changes in cell membrane, cell structure and cell wall composition (Kratsch and Wise 2000). This low temperature-induced shrinkage and disruption of carrot cell plasma membranes were almost completely alleviated by Mel treatment which indicates that Mel helps plants to cope with severe environmental conditions by maintaining membrane integrity. The protective effect of exogenous Mel during chilling stress was also reported in mung bean (*Vigna radiata* L.), highly vulnerable to chilling (Hung et al. 2007). Moreover, it has been reported that exogenous Mel treatments helped to protect plants from temperature extremes (Tan et al. 2000), and several studies indicated that the antioxidant capacity of Mel could strengthen plants subjected to cold and heat stresses (Arnon and Hernandez-Ruiz, 2018; Janas and Posmyk 2013). It was reported that Mel supplementation induced Mel biosynthesis and upregulated genes under cold stress conditions (Bajwa et al. 2014). Uchendu et al. (2013) reported that Mel significantly improved the recovery of cryopreserved shoot tips of American elm (*Ulmus americana* L.)
3. Several reports have revealed that Mel treatments significantly alleviate the deleterious impact of high- or low-temperature stresses on plants by upregulating or

downregulating genes modulating polyamine metabolism, increasing chlorophyll and heat shock protein synthesis and affecting the ABA and cytokinin pathway and ROS scavenging (Tan et al. 2007b; Zhang et al. 2017a). Lei et al. (2004) suggested that Mel enhanced carrot cell survival due to induced putrescine and spermidine biosynthesis under cold stress.

4. Bałabusta et al. (2016) determined that osmo-primed cucumber seeds with Mel had higher superoxide dismutase (SOD) activity which can detoxify ROS under low-temperature stress. Exogenous Mel treatments under chilling stress reduce photoinhibition by enhancing non-photochemical quenching via induction of violaxanthin de-epoxidase activity in tomato (Ding et al. 2017). Alam et al. (2018) established that Mel-treated tall fescue plants had lower ROS electrolyte leakage and malondialdehyde levels and higher chlorophyll, total protein and antioxidant enzyme activities compared to control plants under high-temperature stress. They also showed that exogenous Mel treatments improved thermotolerance. In another study, maize seeds were primed with Mel to determine the priming-induced changes under chilling stress, where Mel regulated Mel-associated proteins in seeds exposed to a lower temperature and enhanced plant tolerance (Kołodziejczyk et al. 2016).
5. Foliar Mel application of *Lolium perenne* plants had more biomass, chlorophyll content and photosynthetic capacity compared to control ones under heat stress. Further, Mel applications also caused amplified endogenous Mel and reduced ABA content (Zhang et al. 2017a), decreased the H_2O_2 and MDA content of pepper seedlings but increased the SOD and CAT enzyme activities under chilling stress (Korkmaz et al. 2017). Xu et al. (2010b) also reported that exogenous Mel applications in cucumber under high-temperature stress caused a significant increase in enzymatic antioxidants and non-enzymatic antioxidants resulting in decline in ROS levels and lipid peroxidation. Lei et al. (2004) have reported that Mel applications reduce cold-induced apoptosis root cell suspensions in carrot.
6. Posmyk et al. (2009) investigated that osmo- and hydro-priming with Mel application improve germination in cucumber under cold stress conditions, and Mel treatments protected cell membranes against peroxidation in cucumber seeds during chilling stress, but high levels of Mel caused oxidative changes in proteins. Shoot explants grown in Mel-enriched media exhibited increased regrowth as Mel can significantly reverse the inhibitory effects of light and high temperature on germination of photosensitive and thermosensitive *Phacelia tanacetifolia* Benth seeds (Tiryaki and Keles 2012).
7. The mitigating role of Mel in two Bermuda grass (*Cynodon dactylon*) genotypes under lower temperatures was reported, where the effects of Mel differed between genotypes, which were attributed to differential adaptive responses to lower temperatures due to the differentiation of photosystem capacity, antioxidant enzyme activity and metabolic homeostasis (Hu et al. 2016).
8. The heat tolerance of tomato seedlings positively increased Mel pretreatment by improving their antioxidant defence mechanism, inducing ASC-GSH cycle and reprogramming the polyamines' metabolic and nitric oxide biosynthesis

pathways. These attributes facilitated the excess ROS scavenging and increasing stability of the cellular membrane, thus mitigating heat-induced oxidative stress (Jahan et al. 2019).

12.4 Mechanism of Stress Alleviation by Melatonin

Mel is known as a plant growth regulator which induces tolerance to stress in plants and thus can be used to increase the plant productivity positively under abiotic stress conditions. It improves plant growth such as shoot and root biomass and increases seed germination under extreme stress conditions. Mel's role in tolerance to stress may be associated with different mechanisms such as regulation of enzymes involved in photosynthesis, improved development of antioxidant system, proline and carbohydrate metabolism, the ASC-GSH cycle, downregulating or upregulating stress-related genes and elevating osmolyte levels (Fig. 12.3). The mechanisms by which Mel alleviates different abiotic stress conditions in plant are as follows (Fig. 12.3).

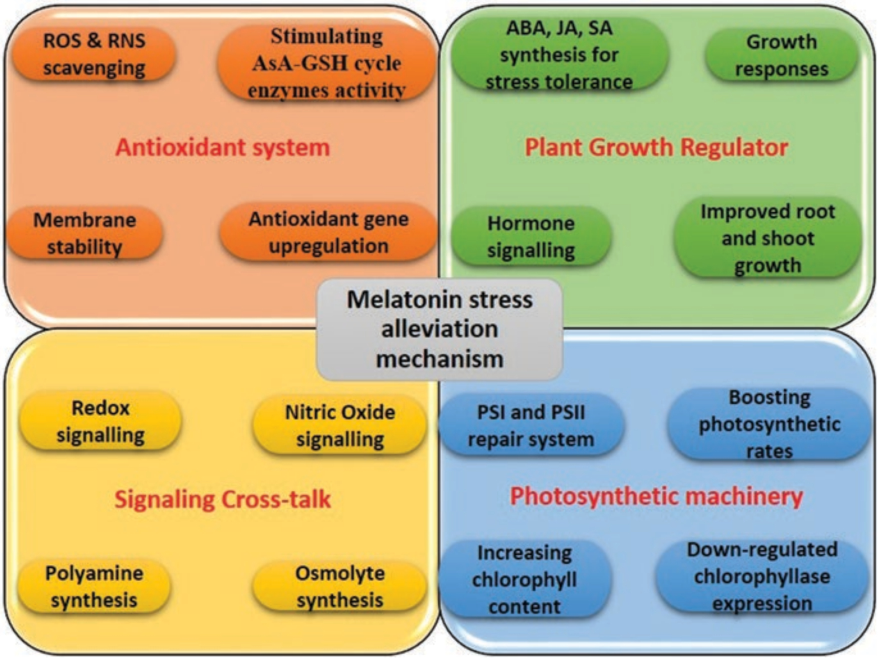

Fig. 12.3 Mechanism of melatonin activities in plants as a growth regulator, a bio-stimulator and an antioxidant

12.4.1 *Mel Activates Antioxidant Systems in Response to Stress*

Mel's intricate relationship with the antioxidant system has been recognized because of its ability to regulate stress signalling mechanisms (Fig. 12.4). Cellular responses to stress are predominantly started by the interaction of the extracellular material

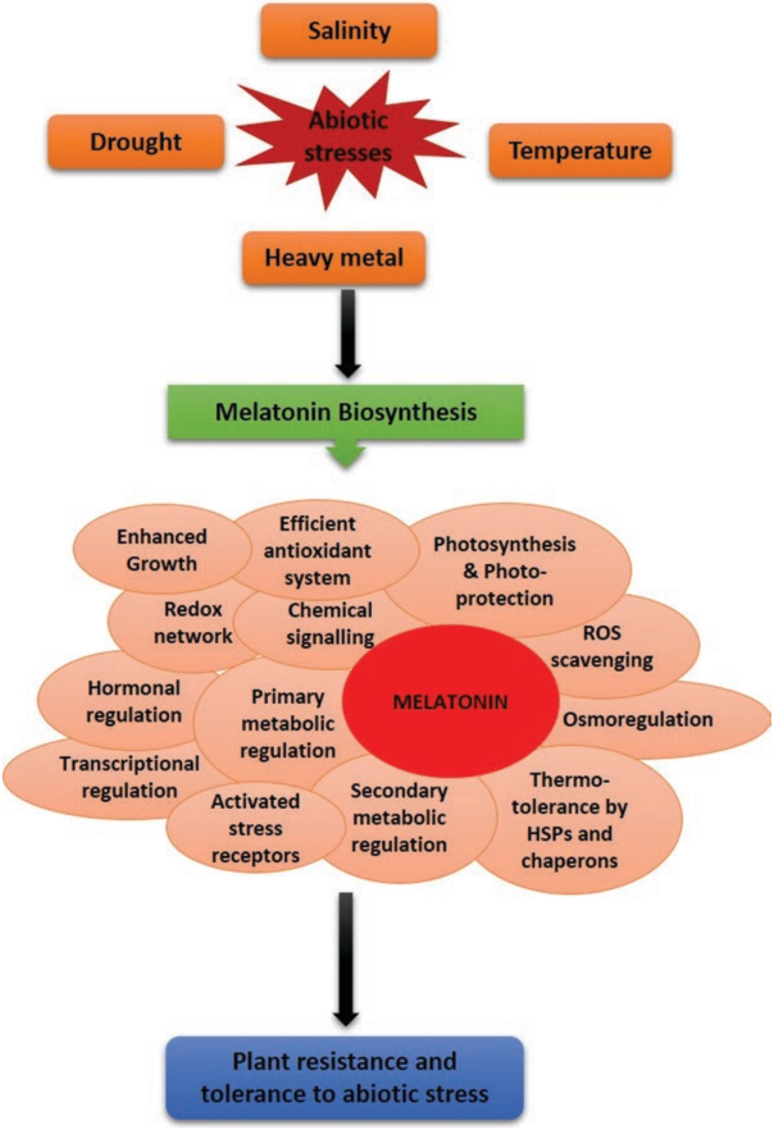

Fig. 12.4 Melatonin-mediated abiotic stress response in plants

with a plasma membrane protein, which means that stresses are first perceived by plant cells via membrane-bound receptors. This signal is then transduced and amplified downstream, causing generation of secondary messengers including calcium and ROS. There are numerous evidences which confirm the broad-spectrum role of Mel as a direct antioxidant which can efficiently scavenge ROS (Fig. 12.4). Allegra et al. (2003) have documented a detailed knowledge of Mel chemistry and molecular interactions with ROS. Mel also can improve cellular antioxidant defence mechanisms by stimulating the production of endogenous antioxidants, such as vitamin C (ascorbate), vitamin E and glutathione which further alter cellular signalling and trigger redox-sensitive regulatory pathways (Zhang et al. 2013). The theory that the exogenous Mel applications can reduce the oxidative damage caused by stress is by directly ROS scavenging and enhancing activities of antioxidative enzymes and antioxidants (Li et al. 2012). In higher plants, the AsA-GSH cycle is an important antioxidant system protecting against oxidative stress, and Mel treatment leads to the maintenance of higher contents of ascorbic acid (AsA) and GSH (Wang et al. 2012). Moreover, as reported by Siddiqui et al. (2019), Mel could be responsible for maintaining the high ratios of GSH/GSSG and ASC/DHA in tomato.

Moreover, Mel receptors act independently (Tan et al. 2002), and their bioactive metabolites influence the exchange of Mel with ROS and reactive nitrogen species (RNS) (Schaefer and Hardeland 2009). The other mechanism of Mel in scavenging ROS and RNS is by non-receptor-mediated activities, thus improving antioxidant capacity, preventing cells, tissues and organisms from oxidative stress (Xu et al. 2010a). Mel is also known to scavenge the superoxide (O^{2-}) (Poeggeler et al. 1996). The interaction between Mel and ROS in plants indicates the function of Mel as an effective antioxidant through both direct and indirect mechanisms (Fig. 12.4). Poeggeler et al. (2002) stated that Mel is five times more effective than glutathione (GSH) in neutralizing hydroxide (OH-) and 15-fold more effective than the exogenous scavenger mannitol. Melatonin has greater antioxidative potential and consists of an electron-rich indole moiety and two side chain groups: 5-methoxy group and 3-amide group. The high resonance stability, electroreactivity and low activation energy barriers make Mel a potent free radical scavenger. The side chains also have a significant contribution to the antioxidative properties of the molecule. Carbonyl moiety present in the functional group (N-C=O) of C3 amide side chain has a key role in the quenching of more than one reactive oxygen species. After the interaction of Mel with ROS, the nitrogen present in the carbonyl group of the Mel molecule leads to the formation of a new five-membered ring. Mel acts as a direct antioxidant and is proficient in lowering the levels of reactive oxygen compared to ascorbic acid. Mel metabolites, such as 3-OHM, AFMK and 2-hyxdroxyMel, act by utilizing influential antioxidants, which promotes the antioxidant capabilities of Mel (Tan et al. 2007b). Mel acts as a mediator in different antioxidant pathways, for example, the ASC-GSH cycle, peroxidases, superoxide dismutase and catalase through varied mechanisms, resulting in abiotic and biotic stress responses in the plant (Tan et al. 2015).

Genome-wide expression profiling analysis reveals that Mel plays a potent role in scavenging intracellular H_2O_2 levels to extend leaf longevity and enhance salt stress. It regulates the expression of TFs, including bZIP, NAC and MYB TFs, which, in turn, directly mediate expression of H_2O_2 scavenging enzyme-encoding genes or via transcriptional cascade to regulate its transcription. This is supported by the observation of Lang et al. (2015) that exogenous Mel significantly inhibits intracellular H_2O_2 levels under salt stress condition. Many genes related to redox homeostasis were identified among the transcripts that were altered by Mel. Gene expression of oxidative stress alleviating enzymes including peroxidases, glutathione peroxidase, haloperoxidase and thioredoxin reductase was induced in Mel-rich rice and Mel-treated plants (Zhang et al. 2014a, b).

12.4.2 Melatonin Improves Plant Photosynthesis Under Stress

Photosynthesis improvement is manifested by increased PN, the maximum quantum efficiency of photosystem II and total chlorophyll content (Wang et al. 2016a). Moreover, Mel elicits the photosynthetic activity by upregulating the expression of photosystem I and photosystem II genes (Wei et al. 2015). Interestingly, Mel application enhanced tomato salinity tolerance by reducing the ROS production by balancing the distribution of photosynthetic electron flux, facilitating the PSII repair by maintaining the abundance of PSII genes (PsbO and D1) and promoting the ability of the donor and acceptor sides of PSII to deliver electrons. In radish seedling, chlorophyll a, chlorophyll b and total chlorophyll contents increased upon Mel treatment under salt stress (Jiang et al. 2017). Mel also enhanced rice seedlings' salt tolerance by decreasing chlorophyll's degradation rate (Liang et al. 2015). Exogenous Mel's protective roles in photosynthesis were also observed in soybean, apple and tomato (Li et al. 2012; Zhou et al. 2016b). Mel plays a pivotal role in the regulation of several specific stress-related genes. It maintains the photosynthetic process by inhibiting the degradation of chlorophyll and proteins and regulating the metabolism of sugar and nitrogen (Erland et al. 2015; Arnao and Hernández-Ruiz 2009). Mel may preserve chlorophyll content by significantly downregulating the expression of chlorophyllase (CLH1) in *Arabidopsis* and inhibiting transcript levels of pheide a oxygenase (PAO) enzymes involved in chlorophyll degradation (Weeda et al. 2014; Wang et al. 2013). Therefore, Mel, in conjunction with antioxidant enzymes, protects chlorophyll content in leaves, boosts photosynthetic rates, delays biosynthesis of metabolites and modulates stress genes to form an efficient system that protects plants from harsh environments. Overall, exogenous Mel improves photosynthesis by effectively alleviating chlorophyll degradation and stomatal closure caused by salt stress, therefore enhancing salt stress tolerance (Fig. 12.4).

12.4.3 Melatonin Regulates Plant Hormone Metabolism

Plant hormones are important signals for plant growth and development (Zhang et al. 2014a, b). Mel widely participates in stress responses via crosstalk with various phytohormones (Fig. 12.4), such as indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acids (GA), cytokinins (CK) and ethylene, jasmonic acid (JA), salicylic acid (SA) and brassinosteroids (BR) (Arnao and Hernández-Ruiz 2019). This crosstalk of Mel with other phytohormones occurs via regulating the expression of its upstream and downstream genes. Mel modulates the salt tolerance of grapevines by enhancing ET biosynthesis via regulating the transcripts of ACS1 (Xu et al. 2019). Exogenous applications of Mel alleviate oxidative damage induced by salt stress by enhancing the expression of genes related to ABA and GA biosynthesis (Zhang et al. 2014a, b). The potential involvement of Mel under different stress conditions was observed in transgenic rice plants (Park and Back 2012), where Mel treatment boosted the root growth and development followed by abscisic acid and methyl jasmonic acid treatments (Park et al. 2013).

Mel promotes ethylene biosynthesis and salt tolerance through the regulation of expression of MYB108A and ACS1 which function as a transcription factor and a key gene involved in ethylene production, respectively (Xu et al. 2019). Further, exogenous application of Mel in *Arabidopsis* alters a large number of genes related to stress tolerance and upregulates transcript levels for many stress receptors and most genes in salicylic acid, jasmonic acid, abscisic acid and ethylene pathways (Weeda et al. 2014). Additionally, there is a significant crosstalk between Mel and other plant growth regulators, as exogenous Mel application enhances drought priming-induced cold tolerance and drought tolerance by modulating ABA levels in apple and barley, respectively (Li et al. 2015; Li et al. 2016b). Furthermore, Mel interacts with calcium and ROS signalling networks, as well as with auxin signalling, and is also involved in other metabolic pathways (Erland et al. 2015).

Mel molecule shares chemical similarities with IAA, both using tryptophan as a substrate in their biosynthesis pathways (Fig. 12.1) (Wang et al. 2016b). It is reported that Mel acts as a growth regulator and exhibits auxin-like activities (Pelagio-Flores et al. 2012). Under stress conditions, the growth-promoting effects of melatonin are higher compared to those in control plants (Arnao and Hernández-Ruiz 2018b). Mel has been proposed to regulate lateral root formation through an IAA-independent pathway in *Arabidopsis* (Pelagio-Flores et al. 2012). In contrast, others suggest a certain relationship between Mel and IAA, as an increase in endogenous IAA content was observed in *Brassica juncea* (Arnao and Hernández-Ruiz 2018a) when treated with exogenous Mel. Furthermore, the application of low concentrations of IAA increases endogenous melatonin levels.

Various studies have shown that Mel mediates ABA biosynthesis and metabolism regulation, thus decreasing ABA content under stress conditions. As in two drought-stressed *Malus* species, Mel selectively downregulates MdNCED3 (ABA biosynthesis gene) and upregulates MdCYP707A1 and MdCYP707A2 (ABA catabolic genes) (Li et al. 2015). Similarly, in perennial ryegrass, exogenous Mel downregulates ABA biosynthesis genes under heat stress, thereby decreasing ABA content (Zhang et al. 2017a). Interestingly, Mel can also activate the expression of cold-responsive genes to improve plant cold stress tolerance in an ABA-independent manner. This suggests that both ABA-dependent and ABA-independent pathways might be involved in Mel-induced cold tolerance (Fu et al. 2017). Thus, exogenous Mel can also alleviate salt stress by regulating ABA biosynthesis and catabolism, CsNCED1 and CsNCED2-ABA synthesis-related gene transcript levels were reduced in Mel-pretreated seeds, and genes related to ABA catabolism were significantly increased, thus leading to a decreased ABA content.

12.4.4 Melatonin Promotes Ion Homeostasis Under Salt Stress

Ion homeostasis refers to the ability of living organisms to maintain stable ion concentrations (Figs. 12.3 and 12.4) under extreme conditions (Amtmann and Leigh 2010). Na^+, K^+, Ca^{2+} and H^+ are major intracellular ions (Amtmann and Leigh 2010). In salt-stressed plants, Na^+ can enter into plant cells, which at high concentrations is harmful to cytosolic enzymes (Fukuda et al. 2011). Therefore, regulation of K^+ and Na^+ concentrations to maintain high K^+ and low Na^+ cytosolic levels has a significant impact on salt-stressed plants (Fukuda et al. 2011). Mel treatment could significantly reduce Na^+ accumulation and increase K^+ content in maize seeds under salt stress (Jiang et al. 2017) as Mel might control the expression of ion channel genes (MdNHX1 and MdAKT1) under salinity and maintain ion homeostasis and thus improve salinity resistance in plants (Meloni et al. 2003). Mel significantly increased K^+ and decreased Na^+ contents in shoots of maize seedlings, leading to a significantly higher K^+/Na^+ ratio in shoots under melatonin-mediated salinity (Jiang et al. 2016). Improved ion homeostasis may be related to the upregulation of several genes, such as NHX (a vacuolar Na^+/H^+ exchanger), SOS (Salt Overly Sensitive) and AKT. Under salt stress, MdNHX1 and MdAKT1 transcript levels were greatly upregulated by Mel, which is consistent with the relatively high K^+ levels and K^+/Na^+ ratio in Mel-pretreated *Malus hupehensis* seedlings (Li et al. 2012). Similarly, NHX1 and SOS2 expressions were higher in Mel-treated rapeseed seedlings compared to control plants, which correlated with lower Na^+/K^+ ratio (Zhao et al. 2018).

12.4.5 Melatonin Mediates NO Signalling Pathway

Nitric oxide (NO) is an important messenger and ubiquitous signalling molecule (Figs. 12.3 and 12.4), which participates in various plant physiological processes and responds to abiotic and biotic stresses (Arora and Bhatla 2017). Various studies have reported that Mel, via its interaction with NO, plays vital roles in plant stress responses such as NO acts as a downstream signal for Mel-mitigated salt stress in tomato seedlings (Liu et al. 2015b), and exogenous Mel significantly induces the accumulation of polyamine-mediated NO in the roots of *Arabidopsis* under Fe deficiency conditions and increases the plants' tolerance to Fe deficiency (Zhou et al. 2016a). In rapeseed seedlings, the possible roles of NO in Mel-enhanced salt stress tolerance have been reported, where stress induces the increase in Mel content and NO serves as the downstream signal. These data suggest that NO is involved in the maintenance of ion homeostasis equilibrium in plant salt stress tolerance. NO is also involved in the improvement of the antioxidant systems triggered by Mel (Zhou et al. 2016a) as in sunflower Mel regulates salt tolerance by supplementing with NO to modify the expression of Cu/Zn-SOD and Mn-SOD genes (Arora and Bhatla 2017). Further, high-temperature stress-induced damage was suppressed by Mel, by coordinating with the PAs (polyamines) and NO biosynthesis pathways, which helps to detoxify the ROS levels. These outcomes provide novel insight into the crosstalk that exists among Mel, PAs and NO to inhibit thermal stress (Jahan et al. 2019).

12.4.6 Melatonin Enhances the Production of Osmolytes

Osmotic regulators or osmolytes play an important role in maintaining intracellular stability and protecting cells from abiotic stress-related toxicity (Figs. 12.3 and 12.4). Jahan et al. (2019) established that salt stress leads to K^+/Na^+ imbalance, destroys cell membrane integrity and reduces potassium retention, while exogenous Mel effectively relieved this effect, by promoting the proline accumulation. Further, Mel applications substantially enhanced the proline and soluble protein concentration in maize seedlings under drought stress presenting the potential efficacy of Mel to effectively cope with the drought stress. Earlier studies have reported that the application of Mel to *Citrus aurantium* L. and *Brassica napus* L. under abiotic stress significantly improved the concentrations of compatible solutes (Kostopoulou et al. 2015). A higher concentration of Mel when applied as foliar spray enhanced the gas exchange parameters, improved protein and proline concentration and stimulated sucrose biosynthesis pathway by regulating sucrose-related enzymes and genes (Zeng et al. 2018).

12.4.7 Melatonin Regulates Polyamine Metabolism

Polyamines (PAs) are small aliphatic polycations that have been found in almost all living organisms (Figs. 12.3 and 12.4) as they play vital roles in plant growth and development and in responses to various biotic and abiotic stimuli (Sánchez-Rodríguez et al. 2016). Spermidine (Spd), putrescine (Put) and spermine (Spm) are the three main polyamines in plants (Sánchez-Rodríguez et al. 2016). Both exogenous polyamines and modulating endogenous polyamine contents effectively enhance plant stress tolerance (Sánchez-Rodríguez et al. 2016). Mel plays a key role in polyamine-mediated signalling pathways (Figs. 12.3 and 12.4) under various abiotic stresses, such as salt stress, cold stress and oxidative stress, and iron deficiency tolerance (Ke et al. 2018). Polyamines mediate the Mel-induced salt stress tolerance of *Malus hupehensis*, as Mel application significantly upregulated the expression of six polyamine synthesis-related genes, including SAMDC1, SAMDC3 and SAMDC4 and SPDS1, SPDS3, SPDS5 and SPDS6. Moreover, Mel-treated *Malus hupehensis* exhibited more polyamine accumulation compared to the untreated seedlings (Gong et al. 2017). Exogenous Mel also modulates PA and ABA metabolisms of cucumber seedlings during chilling stress as Mel-related cold tolerance improvement is consistent with the increased PA content (Zheng et al. 2017). PA modulation by Mel under a salt stress response was also described by Ke et al. (2018), where they show that Mel treatment increases PA content by accelerating the conversion of arginine and methionine to polyamines in wheat seedlings and Mel suppresses polyamine metabolism enzymes such as PAO (polyamine oxidase) and DAO (diamine oxidase) activities, thus improving salt stress tolerance (Ke et al. 2018).

12.4.8 Melatonin Role in Stress Signalling

Mel alters the expression of genes involved in the signal transduction. RNA-seq analysis revealed that 6 stress receptors and 14 genes involved in calcium-dependent signalling are upregulated in response to Mel (Weeda et al. 2014). Many transcription factors have been identified as being upregulated by Mel treatment, and the majority of these are stress-related transcription factors including WRKY, MYB, NAC domain-containing proteins and zinc finger-related transcription factors (Zhang et al. 2014a, b). Besides, Mel may be involved in the regulation of most stress signalling transduction pathways in receptor-dependent or receptor-independent manners and the expression of genes involved in plant tolerance to abiotic stresses (Arnao and Hernández-Ruiz 2018b, 2019).

12.5 Regulatory Mechanism of Melatonin

Mel is a multi-regulatory molecule that coordinates a wide range of biological functions that are required for normal plant growth and development (Fig. 12.4). It is also an essential element in the successful ecological adaptation to abiotic-stressed environments. This regulatory role of Mel in all of these functions is closely associated with multiple changes in gene expression, which are the result of Mel acting alone or in combination with other environmental stressors. Some of the regulatory mechanisms of melatonin are as follows:

- Mel acts as a modulator of gene expression or as a signalling molecule that triggers extensive transcriptional reprogramming (Shi et al. 2015a). Plants metabolize Mel into 2-hydroxyMel (2-OHMel) in the presence of Mel 2-hydroxylase (M2H) genes, which is a major Mel metabolite in plants (Byeon et al. 2015). Furthermore, the direct proportional induction patterns of the Mel biosynthesis pathway and metabolic genes in response to Cd treatments indicate that Mel synthesis is tightly linked to its metabolism (i.e. 2-OHMel) (Byeon et al. 2015). Because of the elevated catalytic efficiencies of M2H enzymes (Byeon et al. 2015), compared to those of other Mel biosynthetic enzymes, the concentration of 2-OHMel in plants exceeds that of Mel (Byeon et al. 2015), suggesting that it has a key physiological role. 2-OHMel may have a regulatory role in mediating tolerance to multiple simultaneous abiotic stressors in rice plants, in part through induction of the transcription factors Myb4 (Lee and Back 2016).
- Many regulatory elements of the Mel-related defence signalling network have been investigated (Shi et al. 2015c; Lee and Back 2016). Recently, an AtPMTR1, the first receptor for phytoMel, was discovered in *Arabidopsis* (Zhan et al. 2019), and this finding elucidated Mel's function as a plant hormone (Arnao and Hernández-Ruiz 2019).
- Mel also interacts with other signalling pathways like mitogen-activated protein kinase pathways (MAPKs) which are required for Mel-mediated defence responses in plants (Lee and Back 2016), and nitric oxide is required for Mel-enhanced tolerance against salt stress in rapeseed seedlings (Zhao et al. 2018).
- Mel modifies the expression of various plant stress defence-related genes, stress receptors, plant stress defence-related transcription factor genes and stress-associated calcium signals, reflecting its pleiotropic physiological roles in plants (Weeda et al. 2014; Zhang et al. 2014a, b). NHX1 and AKT1 are ion channel genes which possibly contribute to the maintenance of ion homeostasis, and Li et al. (2012) have reported that the expression of NHX1 and AKT1 is greatly upregulated, thus improving salinity stress in plants exposed to exogenous Mel (Li et al. 2012).
- Weeda et al. (2014) identified apoptosis-associated genes that were generally downregulated by Mel treatment, which suggested another role of Mel-related anti-apoptotic process. Mel may exert its anti-apoptotic action through polyamines (PAs) as polyamines are also implicated, directly or indirectly, in pathways regulating programmed cell death.
- Mel acts as a vital messenger in plant stress responses against various stresses which can cause a rapid and massive upregulation of Mel production in various plants (Tan et al. 2012, 2014). Further, in *Arabidopsis*, transcription factors including zinc finger protein 6 (ZAT6) (Shi and Chan 2014), auxin resistant 3 (AXR3)/indole-3-acetic acid inducible 17 (IAA17) (Shi et al. 2015d), class A1 heat shock factors (HSFA1s) (Shi et al. 2015e) and C-repeat-binding factors (CBFs)/drought response element-binding 1 factors (DREB1s) (Shi et al. 2015c) are involved in Mel-mediated signalling. AtZAT6- activated CBF pathway is essential for Mel-mediated freezing stress response (Shi and Chan 2014); HSFA1s-activated transcripts of HSFA2, heat stress-associated 32 (HSA32),

heat shock protein 90 (HSP90) and HSP101 may contribute to Mel-mediated thermotolerance (Shi et al. 2015e); AtCBFs-mediated signalling pathway and sugar accumulation may partially be involved in Mel-mediated stress response (Shi et al. 2015c) and C-repeat-binding factors (CBFs)/drought response element-binding factors (DREBs); a cold stress-responsive gene, COR15a; a gene encoding the expression of a transcription factor involved in freezing and drought stress tolerance, CAMTA1; and transcription activators of ROS-related antioxidant genes, ZAT10 and ZAT12, following cold stress (Bajwa et al. 2014). Thus, these transcription factors may play important roles in Mel-mediated stress responses in plants.

- Nitric oxide is required for Mel-enhanced tolerance against abiotic stresses, which might downregulate miR398 expression to activate ROS scavenging enzymatic activities and promote the expression of related genes and finally scavenging intracellular ROS (Gu et al. 2017).

12.6 Genetic Modification in Melatonin Changes Stress Tolerance in Plants

Exogenous treatment or ectopic overexpression of Mel biosynthetic genes (Fig. 12.5) can also improve the resistance and tolerance against various abiotic stresses, all of which leads to ROS generation. The cloning and expression of Mel synthesis genes have proven that the plants have the necessary enzymatic machinery for Mel biosynthesis. This elucidation of Mel biosynthesis pathway genes has assisted in

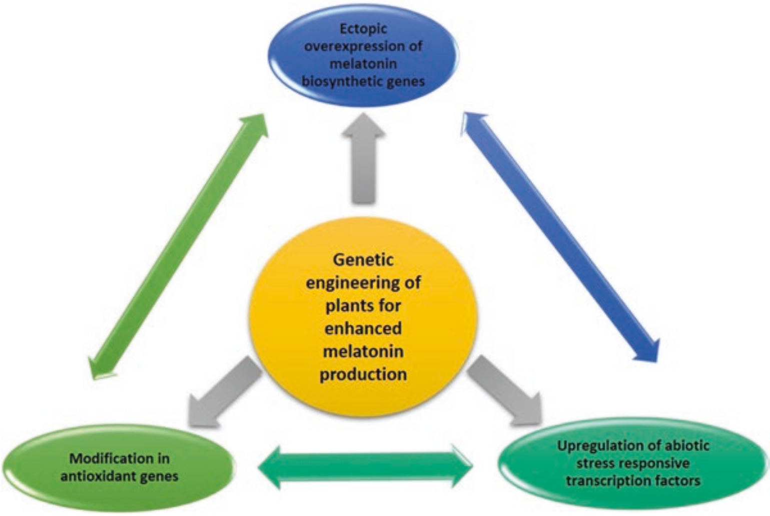

Fig. 12.5 Genetic approaches to enhance melatonin production for sustainable agriculture

uncovering the regulation mechanisms of Mel synthesis in plants and then tempering of Mel biosynthesis pathway gene expression (mostly SNAT and ASMT). This proved to be an efficient way to modulate endogenous Mel and to further reveal the Mel regulation pathway in response to various stress conditions (Fig. 12.5).

Some of the latest work on this aspect is as follows:

- The TDC (first Mel biosynthesis gene) has been overexpressed in rice (Byeon et al. 2014b). Kang et al. (2009) reported that transgenic rice that overexpresses TDC showed delayed senescence of leaves, while suppression of TDC by RNA interference (RNAi) produced lower serotonin levels and stimulated the senescence process. Mel accumulation is seed-specific in TDC3 transgenic lines; transgenic seeds exhibited Mel concentrations 31-fold higher than those in wild-type seeds. Further, Mel intermediate level upregulated in both homologous and ectopic TDC overexpression lines (Kanjanaphachoat et al. 2012).
- Okazaki et al. (2009) have isolated an AANAT (gene homologue is absent in the plant genome) from the green alga *C. reinhardtii* and introduced it into the tomato genome. AANAT gene ectopic overexpression in tomato and rice positively resulted in higher Mel content (Kang et al. 2013). Transgenic rice expressing the human serotonin N-acetyltransferase gene (SNA/AANAT) and ovine AANAT exhibited high levels of Mel and provided cold stress tolerance (Byeon et al. 2014a). Transgenic Micro-Tom tomato overexpressing the homologous ovine AANAT and HIOMT genes displays loss of apical dominance and enhanced drought tolerance (Wang et al. 2014). Transgenic plants of *Nicotiana sylvestris* expressing an AANAT gene and a HIOMT gene have enhanced production of Mel having a protective effect against UV-B-induced DNA damage (Zhang et al. 2012).
- Transgenic rice seedlings expressing ovine SNAT (the rate-limiting enzyme in Mel biosynthesis) exhibit enhanced seminal root growth (Park and Back 2012). In an *Arabidopsis* AtSNAT mutant, endogenous Mel content was lower than that in wild-type ones, and AtSNAT mutant was more salt hypersensitive than the wild-type ones (Byeon and Back 2013). High temperatures increased Mel levels by increasing the activities of the final two enzymes in Mel synthesis (Byeon and Back 2013) for high-temperature defence. Overexpression of ovine AANAT and HIOMT genes in switchgrass has resulted in improved growth performance and salt tolerance (Huang et al. 2017).
- N-Acetylserotonin methyltransferase (ASMT) (the most important enzyme in Mel biosynthesis) plays a rate-limiting role in the Mel production. Pan et al. (2019) have studied the role of ASMT in pepper subjected to different abiotic stresses. They documented that several CaASMT genes in leaf were induced when plant growth is affected by heat and cold, whereas under heat stress, the three genes (CaASMT01, 11 and 12) are downregulated in different stages, and under cold stress whileas, three CaASMT genes (CaASMT01 and 09) were slightly induced. These observations showed that three CaASMT genes have changed under heat and cold treatments, suggesting their role in plant response to abiotic stresses. Furthermore, rice ASMT mRNA was induced upon abscisic

acid (ABA) and methyl jasmonic acid treatments, suggesting the potential involvement of Mel in response to various stresses (Park et al. 2013). The role of ASMT genes in heavy metal stress was also reported by several workers, e.g. upregulation of AtASMT expression was closely associated with Cd-induced Mel synthesis in *Arabidopsis* (Byeon et al. 2016), and then the expression of acetyltransferase NSI-like transcript variants NSI-X1 and NSI-X2, homologous to *Arabidopsis* serotonin N-acetyltransferase (AtSNAT), was significantly increased by Al stress in soybean roots. The possible role of apple MzASMT9 was investigated in *Arabidopsis* under salinity stress, where transcript levels were upregulated, and Mel levels were also increased by the ectopic expression of MzASMT9, thus leading to enhanced salt tolerance in transgenic *Arabidopsis* (Back et al. 2016). Mel pretreatment under salt stress has increased relative expression level of TDC, SNAT and ASMT genes, leading to the production of endogenous Mel (Back et al. 2016).

- Transcriptomic profiling of Bermuda grass, done by Shi et al. (2015b), identified 3933 transcripts that were more differentially expressed in Mel-treated plants than controls. Further analyses with mathematical modelling have revealed that genes involved in hormone metabolism, nitrogen metabolism, transport, major carbohydrate metabolism, metal handling, tricarboxylic acid (TCA)/org transformation, redox and secondary metabolism were upregulated after Mel application. This study has provided evidence of the protective roles of exogenous Mel in the Bermuda grass in response to abiotic stresses, partially via activation of antioxidants and modulation of metabolic homeostasis. Comparative metabolomics and transcriptomic analyses were also performed to identify differentially expressed metabolites and genes after exogenous Mel treatment (Shi et al. 2015f) in Bermuda grass responses to multiple abiotic stresses. These analyses revealed that the core mechanisms of Mel could involve major reorientation of carbohydrate and nitrogen metabolism and photosynthetic machinery.
- The expression levels of certain stress elements such as C-repeat-binding factors (CBFs)/drought response element-binding 1 factors (DREB1s) have increased significantly by exogenous melatonin treatment under various abiotic stresses. These stress elements have further improved and upregulated endogenous Mel levels which have further increased the transcript levels of multiple stress-responsive genes, including COR15A, RD22 and KIN1 (Shi et al. 2015c). Similarly, the transcriptomic analysis was performed in cucumber roots with or without Mel treatment under salt stress, and the results showed that many transcription factors including MYB, NAC and WRKY and the ethylene-responsive transcription factor were more differentially expressed in Mel-treated plants compared to control plants under salinity (Zhang et al. 2014a, b).
- The effect of Mel on the expression of ROS scavenging genes under salinity stress in cucumber was investigated, and it was observed that the Mel application stimulated the expression of an antioxidant enzyme system (Cu-ZnSOD, Fe-ZnSOD, POD and CAT) under salt stress (Zhang et al. 2014a, b). Similar results were also observed in rapeseed, where antioxidant defence-related genes

such as APX, Cu-ZnSOD and MnSOD were involved in Mel-induced salt stress tolerance (Zhao et al. 2018). In tomato seedlings under salt stress, Mel significantly improved TRXf gene expression which helps in redox regulation of many physiological processes (Yu et al. 2018). The role of Mel in alleviating salinity stress by regulating hormone biosynthesis and metabolism gene expression was also reported, e.g. Mel induced the expression of GA biosynthesis genes (GA20ox and GA3ox); however, the ABA catabolism genes, CsCYP707A1 and CsCYP707A2, were upregulated, and the ABA biosynthesis gene CsNECD2 was downregulated by Mel in salt-stressed cucumber seedlings (Zhang et al. 2014a, b).

12.7 Conclusion

Melatonin is a pleiotropic molecule present in different parts of the plant with amphiphilic properties, and Mel biosynthesis holds a crucial function in plants for surviving against various abiotic stresses. Mel's role in mitigating abiotic and biotic stress is either directly through scavenging ROS and RNS or indirectly through improving the photosynthesis system, scavenging toxic molecules, stimulating plant growth regulators or triggering antioxidant activities in plants (Figs. 12.2, 12.3 and 12.4). Nevertheless, endogenous Mel levels in plants are insufficient to protect plants against severe stress conditions. Thus, exogenous Mel application has shown notable coping mechanisms against harsh stress conditions by boosting plant antioxidant system, increasing photosynthesis, enhancing levels of protective metabolites and phytohormones and increasing ROS and RNS scavenging systems. Thus, the physiological and molecular activities of Mel in plants specify that Mel is an essential molecule in improving the growth and productivity of field crops, especially where biotic and abiotic stress is a limiting factor. However, there are still various major issues yet to be explored, and there is still a lacuna of information available regarding the genes and core pathways that are precisely regulated by Mel. To conclude, there is a mammoth research potential for improving our understanding of Mel impact in basic life functions across plant kingdoms and the creation of new approaches to advance progress in plant cultivation and sustainable agriculture.

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Chapter 13
Strigolactones: A Novel Carotenoid-Derived Phytohormone – Biosynthesis, Transporters, Signalling, and Mechanisms in Abiotic Stress

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Abbreviations

ABA	Abscisic acid
AM	Arbuscular mycorrhizal
APX	Ascorbate peroxidase
BRs	Brassinosteroids
CAT	Catalase
CCO	Carotenoid cleavage oxygenase
CK	Cytokinin
ET	Ethylene
GAs	Gibberellins
GPX	Glutathione peroxidase

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HI	Harvest index
JA	Jasmonic acid
KAR	Karrikins
LRs	Lateral roots
MDA	Malondialdehyde
NAA	1-Naphthylacetic acid
NCED	Nine-cis-epoxycarotenoid cleavage dioxygenase
NO	Nitric oxide
NPA	1-Naphthylphthalamic acid
PAs	Polyamines
PHs	Phytohormones
POD	Peroxidase
ROS	Reactive oxygen species
SA	Salicylic acid
SLs	Strigolactones
SOD	Superoxide dismutase

13.1 Introduction

Crops are habitually endangered by diverse abiotic stresses concurrently during their growth period which unpleasantly disturb their growth and development, leading to limit the output of field crops (Hussain et al. 2018a; Zandalinas et al. 2018; Abdel Latef et al. 2019, 2020). Environmental factors, such as temperature, precipitation, relative humidity, and carbon dioxide (CO_2), are considered strategic constituents that control the efficiency of a crop (Wu et al. 2018; EL Sabagh et al. 2020). Several estimations forecasted that on an inclusive scale, a proliferation in land use of ~100 Mha with a trebling of intercontinental trade is prerequisite by

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2050 for meeting the future food difficulties of 9.8 billion people without affecting any substantial alteration in currently harvested cropland (Pastor et al. 2019). The growing demand for increased agricultural productivity together with the high cost of agricultural inputs and the declining trend in farmers' livelihood cause economic problems in conventional farming (Uphoff et al. 2006; Daniel 2018), indicating that feeding the growing populations with utilization of existing agricultural land and traditional methods will be a big challenge (Koohafkan et al. 2012). Besides these aspects, soil fertility and productivity are not only significant to viable for food production but also key thoughts in poverty mitigation and the enhancement the income of poor farmers (Heger et al. 2018). As a result of the changing climate and also excess and imbalanced use of agricultural inputs, soil health is declining in many regions, particularly intensive agricultural farming (Jiang et al. 2020; Middendorf et al. 2020; Stewart et al. 2020). Several important agricultural inputs, such as synthetic fertilizers and pesticides, are already practiced over and imbalanced, which lead to pollutant the agroecological conditions such as diminishing soil physical and chemical properties, degrading arable land, increasing greenhouse gas emissions (GHGs) and also generating other ecological complications (Lesk et al. 2016; Shah and Wu 2019).

Crops that are grown under open environments often pass through periods of abiotic stresses since tolerant plants pass through a series of physiological and metabolic activities to mitigate such adversities of the abiotic stresses (Kukal and Irmak 2018; Abdel Latef et al. 2018; Zandalinas et al. 2018; Hussain et al. 2018b). In the process, PHs play a vital role by signalling plant organs under changeable environmental conditions as they activate different variations in gene expression and modify growth and developmental processes and metabolic activities for better adaptation to a particular environment (Rameau et al. 2019; Raza et al. 2019a; Hossain et al. 2020). Several growth regulators such as auxins, ET, CK, GAs, ABA, BRs, JA, SA, plant peptide hormones, PA, NO, and newly established SLs and KARs show crucial roles for increasing the survival ability of plants to hostile environmental conditions. Among these growth regulators, SLs are one of them, which influence different physiological and metabolic activities of plants through providing signals for arbitrating required changes as an adaptive measure to stress under changing environment (Umehara et al. 2008; Waters et al. 2017; Yang et al. 2019). SLs communicate with other microorganisms and opportunistic weeds under the genera *Striga* and *Orobanche* and influence growth and development of plant root and shoot. Lately, SLs were established as host detection and hyphal-branching signals for AM fungi. The chapter discussed on the biosynthesis, signalling, governing, and physiobiochemical roles of SLs in numerous abiotic stress conditions.

13.2 The Adverse Effect of Abiotic Stress on the Sustainable Crop Production System

Globally, agricultural productivity is harshly affected by the extreme events of the changing climate (Porfirio et al. 2018; Mar et al. 2018; Rastogi et al. 2019; Raza et al. 2019b). In the future, the production of chief crops is expected to decrease globally due to global warming as a result of climate change (Tebaldi and Lobell 2018; Bonan and Doney 2018). Zhao et al. (2017) observed yield reductions in wheat, rice, soybean, and maize of about 6, 3.2, 3.1, and 7.4%, respectively, as a consequence of climate change. Moreover, the estimated potential yield losses due to abiotic stresses such as drought, salinity, heat stress, low-temperature stress, and others were 17, 20, 40, 15, and 8%, respectively (Ashraf and Harris 2005). Similarly, Wang (2013) documented that yield reductions due to the hostile effect of environmental stress were 65.8, 82.1, 69.3, and 54.1% for maize, wheat, soybeans, and potatoes, respectively.

13.2.1 Heat Stress

The rise in global temperature as a result of climate change poses a substantial risk for growth and development of crop plants ultimately affecting crop productivity (Schlenker and Roberts 2009; Priya et al. 2019; Raza et al. 2019b; EL Sabagh et al. 2020). Fluctuation of temperature between optimum levels in all plant growth stages of limit the production of food crops (Hussain et al. 2019). For example, a 1°C rise in global mean temperature reduced the mean yield to 3–10% in wheat (Brown 2008; Asseng et al. 2015; Ray et al. 2015), 8.3% in maize (Lobell and Field 2007; Lobell et al. 2011), 2.6% in rice (Easterling et al. 2007), and 7.8% in sorghum (Otto et al. 2017; Kjellstrom et al. 2018). Easterling et al. (2007) found that a 2 °C increase in temperature causes a 7% reduction in yield, while a further increase in temperature by 4 °C decreased the yield to 34% in wheat. Mean temperatures above 25 °C (minimum 15 °C at night and maximum 35 °C during the day) lead to heat stress in chickpea, leading to 20–70% yield reductions through flower drop and pod abortion (Kumar et al. 2016). During the reproductive stage, day/night temperature above 32/20 °C reduced the yield of lentil; for example, a temperature above 35 °C for 6 days reduced the yield of lentil to about 70% in south-eastern Australia in 2009 (Delahunty et al. 2015).

13.2.2 Drought Stress

Similar to heat stress, drought stress also poses a considerable risk to crop productivity, thereby threating the food and nutritional security of the increasing population (Hussain et al. 2019). The severity of drought which affects plant yield and development depends on the growing environment, soil types, and growth stages of affected plants and species. For example, as a result of drought stress, a yield reduction of 13% has been reported in potato, while yield reduction in rice, maize, and common beans ranges between 24 and 92% (Farooq et al. 2009). Daryanto et al. (2016) observed that a water reduction of 40% from the optimum level reduced the yield of maize and wheat to 40 and 21%, respectively. Moreover, a yield reduction of 1–30% was also recorded in wheat if the crop faces drought stress at post-anthesis; if wheat plants face drought at flowering and grain-filling stages, the reduction increased up to 92%. About 42% lower yield of barley was recorded as a result of uneven grain size due to drought stress (Alghabari and Ihsan 2018). Drought stress at the flowering stage has seriously changed the yield of *Vigna mungo* L. from 57% to 31%; but when drought stress took place at the reproductive stage, a yield reduction of 26% was recorded. In the case of cowpea, yield reduction can differ, sometimes between 34% and 68%, based on the pheno-phases (Farooq et al. 2017). Soybean yield reduction was recorded at 42% when drought stress took place at the grain-filling stage (Maleki et al. 2013). Drought stress also leads to early leaf senescence in *Brassica juncea* plants (Alamri et al. 2020).

13.2.3 Salinity Stress

Salinity is the major abiotic stress that distresses almost 20% of cultivated land and 33% of irrigated land, resulting in decreased crop productivity, thus threatening the global food security (Machado and Serralheiro 2017; Abdel Latef et al. 2019; Okon 2019). Around 20% of arable land and 50% of crop land globally are salt-stressed and are expanding to about 10% annually (Flowers 2004; Kumar et al. 2017). Soil salinity distresses 19 million ha of land in sub-Saharan Africa (Tully et al. 2015). Crop production losses due to soil salinity ranged from 10% to 70% (Qureshi et al. 2019). Salinity not only affects plant growth and development through limiting photosynthesis and water-use efficiency but also alters the physiological activity and ion harmfulness in plants, thus decreasing crop production (Shahid et al. 2018).

13.2.4 Waterlogging

Waterlogging at the tillering stage of wheat decreased the grain yield by 41.5% (Ghobadi et al. 2016). In wheat, 20 days of waterlogging at 3–4-leaf stage caused a yield reduction between 90% and 95% (Pampana et al. 2016a), while in barley, the reduction was recorded between 85% and 90% (Masoni et al. 2016). Contrasting growth stages, waterlogging at 25–45 days after emergence (DAE) of barley yield caused final seed mass about 25%, if at 65–85 DAE led to reducing the grain yield by 75% (de San Celedonio et al. 2014). An investigation conducted by Wollmer et al. (2018) revealed that a 14-day waterlogging in rapeseed led to reduction of seed mass between 75% and 85%, whereas waterlogging for 5 days at the beginning of flowering in field pea decreased the seed production by about 38% (Pampana et al. 2016b).

13.3 Role of Strigolactones to Alleviate the Adverse Effects of Abiotic Stress

PHs control numerous developmental activities in plants that lead them to acclimatize to survive against various abiotic stresses, thus creating inherently engineered plants with better yield. In the last decade, the detection of SLs delivered new prospects to explore the hormonal directive of plant growth and adaptation to ecological restrictions. In the last few years, a significant amount of experiments has established the decisive aspects of SLs in the adaptation and alteration of the plant responses to various abiotic stresses (Bonneau et al. 2013; Liu et al. 2015; Hu et al. 2018; Visentin et al. 2020; Ling et al. 2020). Thus, in this section, we have documented the possible role of SLs under several abiotic stresses in crop plants.

13.3.1 Irregular Temperature

Temperature is one of the most vital ecological factors which controls the physiological behavior and distribution of living organisms and is essential for plant growth and development. Thus, plants need a combination of optimal temperature to perform normal physiological, morphological, biochemical, and cellular processes under stressful environment (Basbouss-Serhal et al. 2016; Raza et al. 2019a, b, 2020). Seed development in plants is exposed to ideal temperature condition, e.g., seed development in *Arabidopsis* is repressed by alleviated temperature (Chiu et al. 2012; Basbouss-Serhal et al. 2016).

PHs like CK, GA, and ABA are involved in seed germination; ABA is known to be a negative stimulator, whereas GA and CKs are both positive stimulators of seed development under unfavorable conditions (Davies 2010; Miransari and Smith

2014). Thus, SLs are recognized to stimulate seed development in parasitic root weeds, as well as in other higher plants. Seed development in SLs-defective *Arabidopsis* mutant plants under alleviated temperature is modulated by the GR24 treatment. Besides, GR24 treatment reduced the ABA and GA proportion and increased CKs concentrations. They also showed that GR24 suppresses the transcriptional process of *NCED9*, a vital enzyme responsible for ABA biosynthesis (Tsuchiya et al. 2010). Likewise, during warm stratification, SLs rescued the seed dormancy in broomrape plants via decreasing the ABA concentrations (Lechat et al. 2015).

Recently, SLs have been documented to stimulate chilling stress tolerance in *Arabidopsis* and pea plants, respectively. In pea plants, under dark-chilling stress, biomass growth was reduced in the SLs-signalling mutant (*rms3*) plants and the SLs-synthesis mutant (*rms5*) shoots. Notably, parallel consequences were also found for the SLs-synthesis mutant (*max4*) in *A. thaliana*. Below dark-chilling stress, photosynthetic carbon acclimatization was repressed in *rms* mutant in *Pisum sativum* plants and *max* mutants in *Arabidopsis*. Results specify that SLs plays a critical role in tolerance to dark-chilling in both *Arabidopsis* and pea plants. Besides, a remarkable decrease in leaf area was noticed under dark-chilling stress in the existence of GR24, suggesting that SLs signalling helps in adapting to chilling stress by stimulating the leaf growth under low-temperature stress (Cooper et al. 2018). The regulatory function of SLs in root development has been described in a perennial grass species (e.g., tall fescue) (Hu et al. 2019) and also in rice plants (Sun et al. 2019) under stress condition. Tall fescue and rice plants were supplemented with GR24, 1-naphthylacetic acid (NAA), 1-naphthylphthalamic acid (NPA) and a combination of these supplements under controlled environment in a growth chamber (Hu et al. 2019; Sun et al. 2019). The density of secondary lateral roots (LRs) was reduced as a result of the supplementation of NPA, but the use of NAA improved the quantity of LRs, and GR24 excluded the consequence of NAA on secondary LRs growth (Hu et al. 2019; Sun et al. 2019). Remarkably, SLs assisted in root elongation under stress and healthy condition and improved temperature reluctance of root development. GR24-stimulated root growth was due to intensification in cell numbers, upregulation of cell cycle-associated transcripts and downregulation of auxin transport associated with root caps. Therefore, results suggesting that the practical effect of SLs in stimulating crown root development under both stressed and healthy plants might be due to the modulation in cell partition and as well as the interference of auxin transporter genes (Hu et al. 2018; Hu et al. 2019; Sun et al. 2019).

13.3.2 Drought and Salinity

Among numerous environmental stresses, water deficit condition and soil salinity are the two significant factors affecting plant germination and developmental processes and reducing crop yield around the globe (Raza et al. 2019a, b, 2020;

Raza 2020). SLs have been included in the emerging list of PHs involved in stress-mediated signalling pathways stimulated under various biotic and abiotic stresses, for ET, ABA, JA, SA, etc. (Mostofa et al. 2018; Raza et al. 2019a). Several loss-of-function/mutant/knockout and exogenous SLs supplemented experiments have discovered that SLs play a crucial part in overcoming drought and salinity stresses in rice, rapeseed, and *Arabidopsis* (Van Ha et al. 2014; Ma et al. 2017; Haider et al. 2018).

Therefore, the relationship between SLs and ABA signalling concerning water stress was examined in tomato plants using ABA lacking mutants, definite enzyme inhibitors, and LC-MS/MS system. Handling of controlled vegetation with the NCED inhibitor (abamine SG) and the unprocessed ABA lacking mutants shows decreased SLs and ABA concentrations. Further, expression investigation of transcripts tangled in the biosynthesis of SLs revealed the downregulation of *LeCCD7* and *LeCCD8* transcripts in all mutant plants, suggesting that these two genes are associated with SLs production, and there is an association between SLs production and ABA concentrations, and the vital character of ABA in the modulation of SLs biosynthesis is anticipated (López-Ráez et al. 2010).

Recently, Van Ha et al. (2014) reported the necessary controlling sign of SLs in response to salinity and drought stress in *Arabidopsis* plants. *Arabidopsis* mutants (*max*) showed hypersensitivity to both stresses, which was correlated with shoot-related instead of root-related traits. Exogenously applied SLs saved the drought-sensitive germination phenotype in SLs-biosynthesis mutant genotypes (*max3–4*) but not in a SLs-responsive mutant genotype (*max2*). Notably, SLs increased drought tolerance in WT plants via the ABA signalling pathway, showing a lesser sensitivity of the entire mutant plants to ABA than WT plants, thereby suggesting the encouraging part of SLs in response to stresses. Further indication in *max2* mutant assisting ABA-mediated SLs response arises from improved transcription rates and stomatal thickness and modulation in ABA-mediated stomatal conductance. Microarray examination of *max2* mutant plants displays downregulation of ABA importation transcripts (*ABCG22* or *ABCG40*), CK catabolism transcripts (*CKX1–3* and *5*), positive managers of ABA and osmotic pressure (*CIPK1*), and abiotic stress-receptive gene (*AtNAC2*) (Van Ha et al. 2014). In another study, Bu et al. (2014) reported that *max2* mutant plants under drought condition show a thinner cuticle and bigger stomata aperture. The qRT-PCR analysis disclosed that drought stress reduced the expression of ABA-inducible genes, as well as ABA-responsive *RD29A*, *RD29B*, and *KIN1*. Notably, this expression pattern is *max2* definite and not detected in further SLs signalling pathway transcripts, indicating that *max2* work as a mutual factor of several signalling pathways, e.g., *max2* is correspondingly elaborated with karrikin pathways. The expression of *max2* is modulated by *ABI3* and *ABI5* genes involved in ABA signalling, documenting that *max2* works downstream of ABA signalling. Recently, the affiliation among SLs and ABA was documented under drought stress in rice. They showed that drought stress at once induces the SLs generation in the roots and ABA biosynthesis and the expression level of SL generation transcripts in the shoots. Notably, mutant plants (*d10*) possess higher ABA level in shoots as compared to WT plants, and a parallel pattern was noticed for SL-perception genotype (*d3*), under the control condition. The SLs and ABA biosynthetic pathways are associated with each other via *OsD27* gene that acts a crucial part in the directive of SLs and ABA level in rice under stress condition (Haider et al. 2018).

Under salinity, lettuce plants increase the SLs biosynthesis that helps in symbiosis formation and alleviates salt stress (Aroca et al. 2013). In contrast, osmotic stress reduced SLs concentrations in tissues and root biomass in lotus. Pretreatment with SLs repressed the osmotic pressure-induced ABA rise in roots via downregulation of ABA biosynthetic transcript (*LjNCED2*). Throughout osmotic stress, SLs concentration reduced to allow a rise in ABA in the underground parts such as the roots. The SLs absorption and impact of ABA concentrations differed in roots and shoots in relation to a stressful environment (Liu et al. 2015). Another study was performed using WT tomato plants grafted onto SLs-depleted and self-grafted WT plants under drought stress. Results display that SL leads to drought adaptation in shoots mainly due to the SLs-exhausted plants were oversensitive to drought, and stomata show sensitivity to ABA. Notably, SLs generation was blocked in roots under drought stress, suggesting that SLs work as an indicator mediator under a stressful environment (Visentin et al. 2016).

However, the foliar application of SLs in the form of GR24 increased drought tolerance in grape plants. GR24-treated genotypes displayed higher resistance to drought due to lesser electrolyte leakage, ROS generation and stomatal conductance; elevated RWC, chlorophyll, and MDA contents; and improved photosynthesis rate. Notably, GR24-treated plants increased the ABA level and decreased the IAA and ZR level both in roots and leaves under drought stress. Findings recommended that foliar supplementation of SLs could reduce the harmful impact of drought stress by regulating morpho- and physiological contents and by improving the activities of several antioxidant defense systems (Min et al. 2019). In contrast, Ling et al. (2020) described the protecting role of SLs on photosynthetic and physiological parameters of rice plants under salinity. Rice plants were treated with GR24 under salinity, and notably higher GR24 level enlarged the plant tallness and length of root and reduced the contrasting effects of salinity on plant growth and photosynthesis rate. GR24 treatment also increased the chlorophyll contents, transpiration rate, stomatal regulation, and CO_2 levels; and the activities of the defensive systems of antioxidants were also increased under salinity.

In a recent study, the association between SLs and miR156 is reported under drought stress in tomato plants. Transgenic seedlings, overexpressing miR156, show that SLs treatment led to lesser stomatal closure and alleviated ABA sensitivity. Notably, exogenously applied SLs were enough for miR156 accumulation in leaves; on the other hand, endogenous SLs were essential for miR156 induction under drought stress. Results showed that there is a strong link between miR156 and drought stress based on SLs treatment. They suggested that miR156 is a key mediator for ABA-dependent pathways responsible for stomatal regulation led by SLs treatment (Visentin et al. 2020). SLs receptor gene *MdD14* was studied in apple, and the posttranslational changes of *MdD14* were stimulated by GR24. Further, the ectopic analysis of *MdD14* in a model plant (*Arabidopsis thaliana*) inhibited the shoot branching and hypocotyl elongated phenotype, and it also boosts the survival ability to salt and drought stresses (Yang et al. 2020). Previous studies have shown that SMXL6, SMXL7, and SMXL8 proteins unnecessarily repress SLs signalling in plant evolution and development. Therefore, Li et al. (2020) reported that SMXL6, SMXL7, and SMXL8 proteins negatively regulate drought tolerance in *Arabidopsis* plants. They presented that SMXL6, SMXL7, and SMXL8 mutant seedlings showed more survival ability to water deficit condition as compared to WT plants. Further, the physiological analysis showed that mutant plants have higher leaf exterior temperature and decreased cuticle permeability, including drought-prompted water loss and damage to the cell membrane, than WT plants. Mutant plants also showed improvement in anthocyanin production under drought stress, increased depollution potential and improved sensitivity to ABA in cotyledon opening and germination inhibition analysis. In short, SMXL6, SMXL7, and SMXL8 could provide an innovative way forward to enhance drought resistance in plants.

13.3.3 Abiotic Stress-Induced Oxidative Stress

Plants generate ROS in several cell components under several stresses, i.e., biotic and abiotic stresses. ROS are useful signalling molecules in plants that play a dynamic role in stress adaptation and tolerance and the stimulation of plant germination and developmental process (Hasanuzzaman et al. 2020). Nevertheless, ROS are imagined to perform a dual task in plant molecular biology. They are considered as vital signalling transduction molecules and as damaging by-products of aerobic metabolism that gathered in cells in the course of various environmental stresses (Gomes et al. 2014; Xia et al. 2015; Mhamdi and Van Breusegem 2018). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and apoplastic peroxidases are the main foundations of ROS production in crop plants. Thus, ROS act as a crucial secondary messenger molecule modulating gene expression, helping in resistance and adaptation to different stresses (Hasanuzzaman et al. 2020). Plant hormones are recognized to stimulate plant growth and developmental mechanisms and adapt to stresses by stimulating the ROS generation via NADPH oxidases programmed by *RBOH* genes (Sagi and Fluhr 2006). According to Lin et al. (2007), SLs signalling has also been connected with ROS production, but ramblingly. The connection between ROS and SLs arises from the discovery that *FHY3* works as a harmful modulator of *RBOH* gene. Notably, *FHY3* is considered as a primary element of PHs signalling and the circadian clock, elaborated in the far-red bright reactions. On the other hand, *FHY3* overwhelms both shoot branching and roots in a double mutant (*FHY3-max2*) of *Arabidopsis* plants, indicating that *FHY3* works in terms of suppression of *max2* gene (Ouyang et al. 2011).

Koltai et al. (2011) described that the loss of function of *FHY3* gives rise to the enhanced look of *RBOH* gene, which is accountable for the suppression of shoot splitting. However, *RBOH* has been revealed to modulate branching in *Solanum lycopersicum*, where the expression of *RBOH* enhanced the shoot branching.

Therefore, Xia et al. (2015) represented the correlation flanked by ROS and SLs under drought and salinity stresses, where *max2* mutants displayed enhanced sensitivity to both stresses and reduced ABA signalling together with the impact on stomatal conductance and expression of stress-related transcripts. ROS act as a secondary messenger throughout ABA responses, and *RBOH* gene is unquestionably elaborated in SLs-dependent root and shoot splitting stimulation as well as in other stresses. Nevertheless, the transcriptome study in *Medicago truncatula* root has revealed that the stimulation of NADPH oxidases below nutrient deficiency (P and N) causes the regulation of SLs biosynthesis transcripts (Bonneau et al. 2013). Nonetheless, Kong et al. (2017) revealed that the hampering of NADPH potential and scavenging of H_2O_2 remarkably decreased SLs-persuaded salinity resistance and reduced SLs amounts. Interestingly, H_2O_2-persuaded SLs gathering was convoyed by improving resistance to salinity, suggesting that higher H_2O_2 level resulting from increased NADPH oxidase activity stimulated SLs-induced tolerance to salinity in AM fungus.

13.3.4 Karrikins (KARs) Like Strigolactones (SLs) Are Able to Alleviate the Adverse Effect of Abiotic Stress

KARs are somehow similar to SLs because they both contain essential butanolide moieties. However, KARs are not formed by plants itself but are produced by fire or burning of carbohydrates (Flematti et al. 2004; Flematti et al. 2011; De Cuyper and Goormachtig 2017). Consequently, KARs originated in smoke from sweltering plants and play a vital part in triggering the development of dormant seeds. Although smoldering arises from the boiling of plant residue, it has been revealed to disturb certainly seed development of over 1200 crop species from 80 diverse genera, together with *Arabidopsis thaliana* (Chiwocha et al. 2009). To influence seed development, the pyran moiety of KARs is essential, though minor adjustments can be observed (Flematti et al. 2010; Flematti et al. 2011).

Nonetheless, KARs and SLs have unalike origins, and both molecules share a remarkably analogous insight and signalling mechanisms, together with *max2* (Umehara et al. 2008; Nelson et al. 2011; Zhao et al. 2018). A genetic inspection of *karrikin-insensitive* (*kai*) mutant plants shows that KARs signalling molecules involve *max2* role in seed germination (Nelson et al. 2011). Two KARs molecules (*KAR1* and *KAR2*) are recognized to inspire seed development in *Arabidopsis*, endorsing the development of dormant seeds of *Landsberg erecta* plants together with the addition of GR24. Both GR24 and KARs molecules prevent hypocotyl length in WT plants including *max1*, *max3*, and *max4* plants throughout photomorphogenesis. In contrast, obtained phenotype persisting natural in *max2* seedlings. Notably, *max2* is vital for the initiation of a primary transcriptional marker of KARs in *A. thaliana* as well as *STH7*, *KUF1*, and *KUOX1*. Both KARs and GR24 stimulate the expression levels of all these transcripts. In parallel, no improvement was noticed in *max2* plants. Besides, KARs molecules also stimulate the transcript levels of *max4*, *IAA1*, in a *max2*-based method in *Arabidopsis* plants (Nelson et al. 2011).

The SMXL transcript family comprises eight members, in which diverse specificities and events have been anticipated with efficient isolation into KARs and SLs signalling molecules. Some associates of subclade1, such as SMAX1 and SMAX2, are associated with the KARs signalling factors, while associates of subclade4, comprising SMXL6, SMXL7, and SMXL8 proteins, regulate SLs signalling factors (Stanga et al. 2013; Soundappan et al. 2015). Similarly, associates of subclade2 (SMXL3), and subclade3 (SMXL4 and SMXL5), take part in the phloem advancement independent of KARs and SLs molecules (Wallner et al. 2017). Additionally, D53 accumulates in the SMXL6, SMXL7, and SMXL8 phylogenetic clades in rice plants (Soundappan et al. 2015; Zhou et al. 2016). Interestingly, three proteins from SMXL family, including SMXL6, SMXL7, and SMXL8, negatively regulate drought tolerance in *Arabidopsis* plants (Li et al. 2020). According to recent reports, DWARF14 protein is crucial for SLs-dependent reluctance of shoot-branch elongation system. Notably, the DWAER14 ortholog of *Arabidopsis*, *AtD14*, is recognized as a vital element for SLs responses at seedling and adult stages of plants. Moreover, the *AtD14* paralog KAI2 is mainly associated with KARs responses, not with SLs responses. Mutational analysis of KAI2 and *AtD14* displays a diverse unit of *max2* phenotype, and transcript levels of *AtD14* and KAI2 are steady with the potential to rejoin to either SLs or KARs at various growth and developmental stages. Finally, it has been suggested that KAI2 and *AtD14* are tangled in the stimulation of KARs and SLs signalling induced by *max2* (Waters et al. 2012). According to Conn and Nelson (2016), KAI2 receptors are observed to have a strange signal which is not required for SLs or KARs, and they assumed that KAI2c distinguish KAI2 ligand that could be an unexplored plant growth regulator.

13.4 Biosynthesis, Localization, Distribution, Transport, and Regulatory Mechanisms of Strigolactones in Plant Cells Under Abiotic Stress

13.4.1 Biosynthesis, Transport, and Biosynthetic Enzymes of Strigolactones in Plant Cells

13.4.1.1 Biosynthesis Localization of Strigolactones

There are several research reports which state that plant root contains and produces high levels of SLs during exudation, but it produces low levels of SLs in other tissues such as hypocotyl, stem, and leaves (Matusova 2005; Xie et al. 2010; Ruyter-Spira, et al. 2013). SLs were found to be produced from the shoot of wild-type scions grafted on the rootstock of *Arabidopsis* and pea mutants (Morris et al. 2001;

Turnbull et al. 2002). The transcriptions of SLs biosynthetic genes have been identified in several plants in aboveground tissues and around axillary buds (Al-Babili and Bouwmeester 2015). However, the transcripts of the individual biosynthetic genes do not always follow the same pattern. It was found that transcription of the *Arabidopsis* CCD7 (MAX3) gene is highest in the roots (Booker et al. 2004; Schmitz and Harrison 2014), while the appearance of rice and tomato CCD7 is maximum in the aboveground tissues and fruitlets in the undeveloped green stage, respectively (Vogel et al. 2010). Although the exact reason of this pattern is not recognized, it may be thought that SLs biosynthetic enzymes also contribute to other pathways showing different localization, as CCD7 provides improvement of pigments such as mycorradicin and cyclohexanone, accruing in mycorrhizal roots (Vogel et al. 2010). Several studies that investigated CCD8 genes reported that they have relatively higher expression levels in roots (Sorefan et al. 2003; Snowden et al. 2005; Foo et al. 2005; Arite et al. 2007; Ledger et al. 2010; Liang et al. 2010; Guan et al. 2012; Kohlen et al. 2012; Pasare et al. 2013). In *Arabidopsis*, CCD8 (MAX4) gene expresses high appearance in the tip of roots, styles, and stigmata of matured florets and siliques, low appearance in nodal tissues around buds, and no appearance in the buds themselves (Sorefan et al. 2003; Bainbridge et al. 2005). *Arabidopsis* MAX1 is universally articulated in vascular-linked tissues and axillary sections of leaves and flowers. In roots, it is identified simply in the dominant cylinder above the distinction zone. This localization is dependable with the perception of MAX1 performing on a transportable substrate transported via the xylem (Booker et al. 2005), like carlactone. In rice, CCD7 appeared in vascular bundles throughout the plant, whereas CCD8 appeared in roots' vascular cells (not in root tips), nodes, internodes, and inflorescences (Arite et al. 2007).

13.4.1.2 Transport of Strigolactones (SLs)

Transport of SLs is a convenient mechanism as it permits adjustment and precisely regulates the root to shoot (branching) proportion in relation to rhizosphere circumstances. Several studies reported the inhibition of branching due to the long-distance transport of a compound (Napoli 1996; Beveridge 1997; Foo 2001; Turnbull, et al. 2002). This inhibition of shoot branching in SLs-scarce scions by wild-type rootstocks proves that SLs and its end product are transported from root to shoot, evidently via the xylem (Kohlen et al. 2011). In an earlier study, SLs orobanchol and orobanchyl acetate have been detected in the xylem sap of *Arabidopsis* and tomato (Kohlen et al. 2011). Under abiotic stress condition such as phosphate-deficient condition, the level of orobanchol was found to be increased due to the involvement of SLs (Umehara et al. 2010; Kohlen et al. 2011). In the rhizosphere, SLs are released following an active transport system (Kretzschmar et al. 2012). In petunia, the reliable transporter *PhPDR1* has been recognized in a shade for PDRs (pleiotropic drug resistance efflux pumps belonging to the ATP-binding cassette membrane protein superfamily of transporters, which consume ATP) that release arbuscular mycorrhizal-promoting factors, such as SLs, into the soil. SLs levels in the roots of

PhPDR1 mutants were similar to those of wild-type plants, but they were much lower in root exudates (Kretzschmar et al. 2012). SLs biosynthesis is not limited to the roots, and in rice, its responsible gene expression is also found in the stem vascular bundle (Arite et al. 2007; Lin 2009). It is found to be expressed in the cambial region of the vascular bundle and xylem-associated parenchyma cells in the stem of *Arabidopsis* (Booker et al. 2005). In vascular cambium, induced meristematic activity is also reported, leading to increased secondary growth, thereby indicating the SLs-mediated architectural responses to environmental conditions (Agusti et al. 2011). In another study, signalling of SLs localization in the vascular cambium is found to have a stimulatory secondary growth in plants (Agusti et al. 2011) that has a potential role in abiotic stress adaptation. SLs have a direct function on tip growth and mitochondrial activity in AM fungi, which is also not likely to be mediated by auxin. However, the tip growth of xylem fibers is known to occur in the cambial and radial-expansion zones (Mellerowicz 2001). It is also known to be involved in other tip growth-related processes such as in root hairs (Koltai, et al. 2010; Kapulnik 2011) and rhizoid elongation (Delaux et al. 2012). SLs-induced stimulation of root hair growth also involves an auxin-independent component (Kapulnik 2011). The intrinsic mechanism of SLs show possibilities in the management of unfavorable environmental conditions.

13.4.1.3 Strigolactones (SLs) Biosynthetic Enzymes

SLs biosynthesis occurs from β-carotene, isomerized from a trans to a cis configuration by the β-carotene isomerase involving enzyme DWARF 27 (D27) and then the creation of 9-cis-aldehyde by the enzyme carotenoid cleavage dioxygenase 7 (CCD7) and the production of carlactone by the enzyme carotenoid cleavage dioxygenase 8 (CCD8) from 9-cis-aldehyde (Alder, et al. 2012; Bruno et al. 2014). CCD8 has high stereospecificity and, therefore, determines the stereochemistry of all carlactone-derived SLs (Seto 2014). The CCD7, an associate of the ubiquitous carotenoid cleavage oxygenase (CCO) enzyme family that also comprises 9-cis-epoxycarotenoid-cleavage dioxygenase (NCED) associated in ABA biosynthesis (Walter and Strack 2011).

In the next step, CCD8, an additional member of the CCO family, converts the CCD7 product 9-cis-b-apo-100-carotenal by catalyzing an arrangement of yet non-understood reactions into carlactone, an SL that lacks the typical B-C-ring structure (Alder et al. 2012; Al-Babili and Bouwmeester 2015). However, CCD8 catalyzes also a common cleavage reaction by converting a different substrate, all-trans-bapo-100-carotenal, into all-trans-b-apo-13-carotenone (Alder et al. 2008). In *Arabidopsis*, carlactone is converted by a CYP450, more axillary growth 1 (MAX1), into carlactonoic acid (Abe et al. 2014). In rice, the MAX1 homolog carlactone oxidase catalyzes the formation of 4-deoxy-orobanchol from carlactone, which can be converted by a further MAX1 homolog, the orobanchol synthase, into orobanchol (Zhang et al. 2014). It is assumed that 5-deoxystrigol, the parent molecule of the second family of canonical SLs, is also formed from carlactone by a yet unidentified CYP450 enzyme (Zhang et al. 2014). It was showed that rice enzyme OsDWARF27(OsD27) catalyzes the alterable alteration of all-trans- into 9-cis-b-carotene in vitro, on behalf of the first b-carotene isomerase described so far (Alder et al. 2012). The OsD27 action is compulsory for SLs biosynthesis since it delivers the CCD7 substrate 9-cis-b-carotene. However, the phenotype of the d27 mutant is not as severe as the ones of d17 (CCD7) and d10 mutants (CCD8) (Ishikawa et al. 2005), demonstrating that the OsD27 isomerase action can be judiciously remunerated by light isomerization (Jensen et al. 1982; Aman et al. 2005) or by other enzymes. The latter assumption is based on the presence of two OsD27 homologs in the rice genome (Waters et al. 2012), which may encode enzymes with OsD27 activity. Recently, substrate specificity is also reported in CCD7 enzymes and the cleavage of different 9-cis-configured carotenoids, including 9-cis-zeaxanthin, 9-cis-lutein, and 9-cis-cryptoxanthin (Bruno et al. 2014). In another study, cytochrome P450 CYP722C has been recognized as an important enzyme that catalyzes the response of B-C-ring closing prominent to orobanchol, the most predominant canonical SLs. The shortest alteration of carlactonoic acid to orobanchol without passing through 4-deoxyorobanchol is catalyzed by the recombinant enzyme (Wakabayashi et al. 2019).

13.4.2 Regulatory Mechanisms of Strigolactones Signalling During Abiotic Stress

13.4.2.1 Trafficking

It has been well documented that auxin level in the root tips is varied with the SLs signalling and thus affects the lateral root development, size of prime root meristem and root hair length (Koltai 2014). The presence of auxin transporter and PIN protein localization determines the auxin flux in the plasma membrane of plants' cell. Additionally, filamentous actin (F-actin) determines the PIN protein localization as its trafficking is slowed down by F-actin stabilization (Shinohara et al. 2013). In *Arabidopsis* mutants *max2*, *eir1*, *der1*, and *tir3*, the mechanism of SLs was determined by the root elongation test. The increased endocytosis in G24-treated WT plants is found as it is maintained by ARA-7 (Plant Rab5 small GTPases)-labeled vesicles and condensed F-actin bundling. However, in Brefeldin A (BFA) bodies, mutant plants demonstrate a higher accumulation of PIN2 and a better polar localization, where BFA acts as a vesicular transport inhibitor. Furthermore, higher PIN2 transcription, endocytosis, and the restructuring of actin cytoskeleton are also observed in BFA bodies. *Arabidopsis* mutants *der1* and *tir3* show the hyposensitivity to GR24 in respect to root hair elongation (Pandya-Kumar et al. 2014). A similar trend of observations was found at the plasma membrane of *Arabidopsis* shoots when watching the levels of PIN1. This PIN1 removal from the plasma membrane is accelerated by SLs, resulting in the shoot branching phenotype. The clathrin-mediated xylem parenchyma cells deplete the level of PIN1 from the plasma membrane (Shinohara et al. 2013). Therefore, it can be concluded that PIN protein localization, transcription, translation, and trafficking are positively regulated by SLs through the reorganization of actin cytoskeleton modulating auxin distribution, where auxin positively correlates with SLs biosynthesis (Hayward et al. 2009).

13.4.2.2 Transcription

The correlation between SLs and PHs together with ABA and CK is evident by comparative transcription study of *max2* and *WT Arabidopsis* plant under water-deficient and normal conditions. The downregulation of *AtNAC2* (NAC TF) is exhibited in *max2* plants by ABA, CIPK1 (CBL-interacting protein kinase 1), and auxin signalling (He et al. 2005). Additionally, AtNAC2 is involved in lateral root development, also known as SLs-regulated mechanism. ABA-induced genes, *ABCG22* and *ABCG40*, demonstrate lower expression in *max2* plant, particularly under drought stress because of reduced stomatal closing and enhanced transpiration rate that supports the constructive controlling mechanisms of ABA with drought signalling (Osakabe et al. 2014). CKs can mitigate drought stress and CRX genes, which encode CK oxidase dehydrogenase for CK catabolism and are regulated by SLs. CRX genes including *CKX1*, *CKX2*, *CKX3*, and *CKX5* are downregulated in *max2* plants (Reguera et al. 2013; Ha et al. 2014). Recently, Jiang et al. (2013) identified a class I Clp ATPase named *DWARF53* (D53), and its structural analysis showed the existence of EAR (Ethylene-responsive element-binding factor-associated Amphiphilic Repression) motifs. These EAR motifs interact with topless-related (TPR) transcription co-processor and regulate the plant gene expression including SLs-responsive genes (Kagale and Rozwadowski 2011). However, the model is not sufficient as D53 binds weakly to TPR proteins (Zhou et al. 2013). Moreover, EAR motifs also interact with the proteins that contain CTLH domain. These CTLH domain-containing proteins have been used in the identification of cytoskeleton and endocytosis (Kagale and Rozwadowski 2011). Auxin localization and transportation through trafficking and cytoskeleton rearrangement are regulated by SLs signalling.

13.4.2.3 Proteolysis

Proteolysis is a posttranslational regulatory mechanism that maintains cellular homeostasis with the involvement of RING-finger E3 ligases comprising anaphase encouraging complex and Skp1-Culin-F-box protein complex. The leucine-rich repeat F-box protein, i.e., ORE9/MAX2/RMS4/D3, is required for SLs signalling mechanism. This protein recruits the subunit of SCF-type ubiquitin E3 ligase and α-/β-fold hydrolase D14/D88/HTD2 of rice and DAD2 of *Petunia*, which might play the role of a SLs receptor (Chevalier et al. 2014). Both *max2* and *d14* mutants support the function in SLs perception pathway and act analogous to GA signalling (Waters et al. 2012). GA binds to GIBBERELLIN INSENSITIVE DWARF1 (GID1) to promote the formation of GA-GID1-DELLA complex, which is identified by the SCF^{SLY1}/^{GID2} complex, comprised with an F-box protein SLEEPY1 (SLY1) (Gallego-Bartolomè et al. 2012). Besides these, there was a good similarity between GA receptor GID1 and D14/DAD2 proteins. These GA receptors and D14 belong to the α-/β-hydrolase family and F-box family which consisted of SLY1/GID2 and MAX2/D3 proteins. Hence, D14 and MAX2/D3 are functioning in SLs signalling, similar to that of GID1 and SLY1/GID2 in GA signalling. Furthermore, a rice DELLA protein, SLENDERRICE1 (SLR1), also has the potential to target the SLs signalling (Zheng et al. 2014). Nakamura et al. (2013) supported the hypothesis that the binding of D14 with SLs is a crucial mechanism for SLs signalling. The overexpression of GA2-oxidase in rice improves the tillering ability and shrinks the GA levels, which is in harmony with the phenotype in SLs-deficient mutant plant (Lo et al. 2008). Rice D53 is a Clp ATPase, a family of proteins that mainly functions in protein degradation and disintegration (Jiang et al. 2013). This D53 is a target of SLs response and acts as a negative regulator of SLs signalling (Zhou et al. 2013).

13.5 Current Progress in the Molecular Basis of Strigolactones to Abiotic Stresses

SLs are small carotenoid-derived PHs that play significant roles in various plant developments. Current researches recommended that, in addition to the roles in hindering shoot branching, moderating root construction, and encouraging leaf senescence (Ruyter-Spira et al. 2013), SLs could also act as regulators in plant's responses to environmental stresses, such as drought (Ha et al. 2014), salinity (Ma et al. 2017), and dark-chilling stress (Cooper et al. 2018). Several observations have been conducted that explains the molecular basis of SLs to abiotic stress tolerance (Table 13.1). It has been reported that SLs-depleted *Arabidopsis thaliana* and *Lotus japonicas* showed higher sensitivity to drought and salinity stress as compared to their wild-type counterpart (Ha et al. 2014; Liu et al. 2015). The drought-sensitive phenotype of SLs-deficient mutants like max3 and max4 is found to be rescued by foliar use of GR24, a synthetic SLs analog, thus improving the drought and salt tolerance of wilted plants (Ha et al. 2014) and also the cultivated plants (Visentin et al. 2016). In *Arabidopsis*, high-temperature stress upregulated the appearance of SLs synthesis gene (max3) designated to play a significant role in the plant's survival against heat stress (Marzec and Muszynska 2015).

Recently, it has been stated that GR24 helped to elongate crown root under high-temperature stress in tall fescue (Hu et al. 2018). SLs also act as signalling molecules for plant interactions with microbes. They stimulate nodulation in the legume-rhizobium interaction process (Soto et al. 2010; Foo and Davies 2011). It can be revealed that SLs create a significant assembly of signalling molecules and are crucial controllers of plants to survive in changing environmental conditions. SLs have potentiality to be used in agriculture for several dedications comprising as

Table 13.1 Recent studies involving the molecular mechanism of SLs to abiotic stress in different plant species

Plant species	Abiotic stresses	Phytohormones	Molecular mechanism	References
Arabidopsis thaliana	Water deficit and salinity	ABA and CK	SLs upregulate the appearance of max2–3, max2–4, max3–11, max3–12, max4–7, and max4–8 headed to progressive reactions to both salinity and water deficit	Ha et al. (2014)
Arabidopsis thaliana	Water deficit	ABA	Max2 plays an important role during SLs biosynthesis in plant response to water deficit	Bu et al. (2014)
Solanum lycopersicum	Water deficit	ABA	Low levels of SLs biosynthetic genes (SlCCD7) in roots act as constituents of the systemic signal of water deficit stress	Visentin et al. (2016)
Solanum lycopersicum	Water deficit and AMF	ABA	AMF induces SLs biosynthesis under water deficit and improves water deficit tolerance	Ruiz-Lozano et al. (2016)
Festuca arundinacea	PEG-induced water deficit stress		Water deficit inhibition of tiller development and growth in grass species are associated with SLs accumulation and signalling	Zhuang et al. (2017)
Brassica napus	Salinity		Salinity depresses the shoot and root growth, whereas GR24 improves the growth under salt stress	Ma et al. (2017)
Oryza sativa	Water deficit	ABA	SLs biosynthesis/perception interferes with ABA formation, and D27 plays a crucial role in determining ABA and SL content	Haider et al. (2018)
Sesbania cannabina	Salinity and AMF	ABA	ABA is regulating the induction of salt tolerance by SL in AMF seedlings	Ren et al. (2018)
Oryza sativa	Phosphate and nitrate deficiency	Auxin	SLs are involved in NO-activated elongation of seminal root under nitrogen and phosphate deficiency conditions	Sun et al. (2016)
Solanum lycopersicum	Water deficit	ABA	SLs act as a molecular link between water deficit and miR156 action on stomata of tomato	Visentin et al. (2020)
Arabidopsis thaliana	Water deficit	ABA	SMXL6, 7, and 8 act as negative regulators of water deficit resistance and that disruption of these SMXL genes in crops may provide a novel way to improve their drought resistance	Li et al. (2020)

(continued)

Table 13.1 (continued)

Plant species	Abiotic stresses	Phytohormones	Molecular mechanism	References
Arabidopsis thaliana	Water deficit and salinity resistance	ABA	SsMAX2 regulate osmotic, water deficit, and salt stress resistance in *Arabidopsis*, and MAX2 can be a genetic target to improve stress tolerance	Wang et al. (2019)
Festuca arundinacea	Heat stress	Auxin	A synthetic analog of SLs such as GR24-induced leaf elongation was associated with an increase in cell numbers, upregulated expression of cell cycle-related genes, and downregulated expression of auxin transport-related genes in elongating leaves	Hu et al. (2019)
Vitis vinifera	PEG-induced drought	ABA, IAA, GR	Foliar application of GR24 ameliorates the adverse effects of drought enhancing its regulation of stomatal closure through ABA or ROS, and modulation of chlorophyll components and photosynthesis, as well as activation of the antioxidant defense	Min et al. (2018)
Arabidopsis thaliana	Freezing stress		The identified gene tbl29/esk1 suppressor mutation reduces the expression of the MAX4 gene, affecting the biosynthesis of methyl carlactonoate (MeCLA), an active SLs	Ramırez et al. (2018)

inducers of suicidal seed germination of parasitic plants (Vurro and Yoneyama 2012). SLs act as plant hormones that are involved in inhibition of shoot branching and better response to abiotic stresses. In this respect, ABA can be the strategic controller of plant responses to environmental stresses and is linked with many developing processes (Vishwakarma et al. 2017). It is evident that the biosynthetic pathways of SLs and ABA are functionally associated and had a strong correlation between them (Roychoudhury and Banerjee 2017). Recently, a multifaceted cross talk between SLs and ABA during drought acclimatization has also been reported (Liu et al. 2015; Visentin et al. 2016). The β-carotene isomerase D27 (DWARF 27) is reported to be a linker regulating SL and ABA homeostasis and hence determining drought tolerance in plants (Haider et al. 2018).

13.6 Conclusion

From the discussion of the present chapter, it may be highlighted that the extreme events of abiotic stresses affect the food production across the globe, which ultimately threaten the food and nutritional security of the increasing populations. To meet the adverse effects of abiotic stresses, scientists and policy-makers are trying to find solutions to mitigate or eradicate them. The plant growth regulator can regulate numerous progressive routes that acclimatize plant against many abiotic stresses and constructing them an appropriate objective for creating naturally concocted crop plants with better yield. The discovery of SLs delivered new prospects to reconnoitre hormonal directive of plant improvement and familiarization to ecological restrictions. The understanding of SLs, through learning its localization of biosynthesis, transport, signalling, regulation, and defensive mechanisms against the hostile environment will be helpful to improve the crop production under changing climate, leading to substantial food and nutritional security of the increasing population.

Conflict of Interest Authors declare no conflict of interest.

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Chapter 14
Role of Soluble Sugars in Metabolism and Sensing Under Abiotic Stress

Shadma Afzal, Nidhi Chaudhary, and Nand K. Singh

14.1 Introduction

Soluble sugars (mono- and disaccharides) play a main role in the assembly and functioning of all living cells. Their origin gives the impression to be firmly connected with prebiotic and primary biotic evolution (Hazra et al. 2019). In plants, sugars are the metabolic substrates involved in actively modulating several metabolic processes at different stages of growth and development (Alagbe et al. 2020). Synthesis of sugar is the crucial process taking place in a plant through photosynthesis by carbon dioxide reduction while the solar energy is exploited for oxidation of water molecule (Taiz and Zeiger 2002). Glucose (Glc) has been reported to affect germination, floral transitioning, cell wall development and senescence (Dekkers et al. 2004). Glc promoted cell division, root architecture and elongation. It also controls the secondary metabolite synthesis (glucosinolates and phenolic compounds) (Wei et al. 2011). On the other hand, sucrose (Suc) assists in cell expansion and starch synthesis (Gibson 2005). The plant sugar status regulates the innate and ecological (external) factors that affect the overall progress (Sedigheh 2019). Sugar starvation in plants affects various pathways such as it increases the synthesis of α-amylase (Gill et al. 2003). On the contrary, it also leads to decline in respiration rate, protein synthesis, sugar metabolism and nitrate assimilation (Rolland and Sheen 2005). Also, the enzyme activity and synthesis increase for catabolic processes, viz. fattvy acids, amino acids and proteins (Scott 2008).

The recent rise in global population, decrease in freshwater levels and continuous increase in cultivable land deterioration pose severe environmental stresses. Cumulatively, the aforementioned factors cause threats to worldwide agricultural

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production and food security (Dutta et al. 2018). Songstad et al. (2017) reported up to 70% reductions in global production due to abiotic stresses. As sessile organisms, plants have to tolerate drastic environmental changes as well as a diverse array of abiotic stressors (Singh et al. 2018). These abiotic stresses consist of temperature changes, ultraviolet-B radiation (UVBR), high light intensity, soil salinity, drought, flooding, chilling, freezing, heavy metals and nutrient deficiency or excess that discretely or combined negatively impacts both crop yield and quality (Khan et al. 2019). Abiotic stresses directly or indirectly impact plant growth, photosynthetic cycle, carbon partitioning, carbohydrate and lipid homeostasis, osmotic metabolism, protein synthesis and gene expression (Rosa et al. 2009). Therefore, plants have developed an extensive range of adaptative strategies to evade the impacts of environmental stresses. Plants in general are regularly subjected to a combination of diverse abiotic stresses (Gratão et al. 2005). Hence, plant responses to combined stresses are exclusive and cannot be applied independently to each stress. Plants' responses to abiotic stresses are complicated phenomena as it can be synergistically or antagonistically adapted (Chaves et al. 2002).

The molecular mechanisms underlying these responses are barely identified. The lack of necessary progress is due to the fact that tolerance to abiotic stress is affected by coordinated and differential expression of gene networks. Plant strategies to deal with abiotic stresses typically involve either stress avoidance mechanism or tolerance mechanism. A common response to abiotic stresses is the accumulation of sugars and other compatible solutes (Sami et al. 2016). Soluble sugars also act as signalling molecules and help in regulating several processes of plant development (Loreti et al. 2001). The sugar signalling pathways may interact with stress pathways to form a complex network for regulating plant metabolic responses (Tran et al. 2007). Soluble sugars might act as negative modulators of plant sensitivity and cell responses to stress-induced distant signals. Numerous stress reactive genes have been described to be activated by Glc, thus signifying the role of soluble sugars in such responses (Price et al. 2004). The response of soluble sugars in plants to salt stress is complex. It is attained through a series of biochemical reactions and molecular mechanisms, ultimately leading to differential expression of stress-related genes (Espasandin et al. 2018; Lu et al. 2018). The differential regulation of 31 genes corresponded to carbohydrate content as well as metabolic enzymes in *Arabidopsis* under salt, cold and drought stresses (Loreti et al. 2005). Soluble sugars by acting as osmoprotectants can alleviate the adverse effects of salinity on plants. Rosa et al. (2009) reported a substantial rise in Glc, Suc and Fru levels in plants during salinity stress. The exogenous application of Glc can decrease Na^+ accumulation and increase K+ uptake, thus continuing ion homeostasis under stress. Sugars played a dynamic role in carbon storage, osmotic defence and free radical scavenging (Nemati et al. 2011). Trehalose (T6P) is a non-reducing disaccharide of Glc, which plays an important physiological role as an abiotic stress protectant (Paul et al. 2018). By acting as an osmoprotectant, it can possibly stabilize biomolecules (dehydrated enzymes, proteins and lipid membranes) under abiotic stress (Denver and Ullah 2019).

Sugar sensing can be defined as the interaction amid a sugar moiety and a sensor protein in such a way that a signal can be produced, which in turn can stimulate signal transduction cascades resulting in various cellular responses. The sugar level in plant cells can be sensed by the sensor proteins. Hexokinase (HXK) enzyme is reported to have a functional role of a Glc sensor (Moore et al. 2003). Different Glc sensing processes are reported that regulate different genes and pathways in plants. These are the HXK-dependent and HXK-independent systems. In the former system, HXK is an active signalling mediator, whereas in the latter system, the receptor is unknown. There is also a metabolite-reliant system which is dependent on glycolysis pathway downstream of HXK (Rolland et al. 2006). As signalling molecules, sugar impacts the plants at all growth phases beginning from germination. As hormones, it can act as prime messenger and control signals, hence regulating gene expression.

Reactive oxygen species (ROS) are partially reduced forms of oxygen and act as both signalling and damaging entities in plants (Møller and Sweetlove 2010). ROS such as hydroxyl radical ($^{\bullet}OH$), superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are continually generated as by-product of aerobic metabolism in different plant organelles (viz. mitochondria, peroxisomes and chloroplasts) (Miller et al. 2010). Under abiotic stress, plants tend to accumulate higher ROS, and this might be due to a disruption of equilibrium between ROS generation and scavenging at different cellular locations (Bolouri-Moghaddam et al. 2010). ROS can act as signals triggering stress (by oxidizing proteins, lipids and nucleic acids) and defence pathways. In plants, ROS generation and photosynthetic mechanism are interlinked (Peshev and Van den Ende 2013). Stress-induced ROS accretion in plants is counteracted by two processes: firstly, inhibition of ROS development and, secondly, ROS scavenging by enzymatic or non-enzymatic antioxidants (Gill and Tuteja 2010). Soluble sugars appear to be involved in ROS-generating metabolic pathways. However, specifically, those sugars that interact with membranes can act as innate ROS scavengers (antioxidant) in plants (Peshev and Van den Ende 2013). They can correspondingly feed on NADPH-generating metabolic pathways such as the oxidative pentose phosphate (OPP) pathway and in so doing contribute to the ROS scavenging cascade (Khan et al. 2019).

The objective of this chapter is to review topical evidences on what method plants can sense and strategically respond to abiotic stresses via sugar-sensing mechanisms. The different functions of Glc, Suc and other sugars will be deliberated. We have also assessed the dual position of soluble sugars on the basis of our current knowledge on ROS production in plants. ROS evasion and scavenging will be discussed with respect to abiotic stress in plants with specific consideration on the role of sugars as antioxidants. Sugar responses to different abiotic stresses and biochemical and genetic evidences for diverse sugar-sensing mechanisms are analysed and summarized. Finally, this could provide a background for the developmental strategies for crop tolerance enhancement to abiotic stress conditions.

14.2 Physiological Role of Sugars in Plants

Soluble sugars guarantee an adequate flow of energy and building materials to carry out specific developmental activities in plants (Fig. 14.1). Glc ($C_6H_{12}O_6$) is a simple sugar and the most abundant monosaccharide. Fru (fruit sugar) is a ketonic monosaccharide, where it is frequently bonded to a Glc molecule to form the disaccharide Suc in plants. Suc ($C_{12}H_{22}O_{11}$) is a common sugar produced naturally in plants. Mannitol is a six-carbon acyclic sugar alcohol that can act as osmoprotectant to counter ROS and as a carbon storing compound. T6P is a naturally occurring non-reducing disaccharide. It has a crucial role in plant metabolic processes and acts as a reserve carbohydrate in plants (Stephanie et al. 2010). Sink formation, carbon

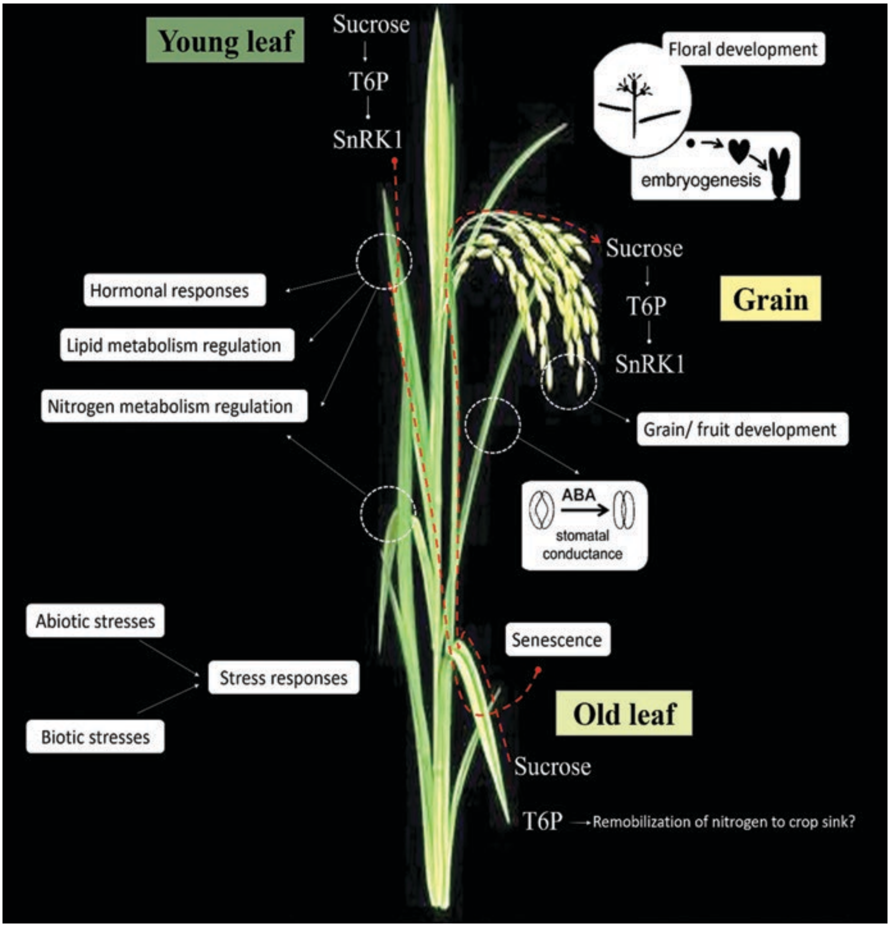

Fig. 14.1 A diagrammatic representation of the role of soluble sugar in plant growth and development. See text for details. *SnRK1* Snf1-related kinase, *T6P* trehalose, *ABA* abscisic acid

metabolism and sugar build-up have significant functions in vegetative plant development, possibly through sugar signalling cascade.

14.2.1 Germination and Seedling Development

In the process of seed germination, the chief reserved compounds (carbohydrates, lipids and proteins) are mobilized by hydrolytic enzymes which in turn are used in seedling growth (Soltani et al. 2006). The utilization of these reserve substances continues till the seed turn into autotrophic seedling (Pritchard et al. 2002). The high activity of cell wall invertase creates high hexose concentration that helps embryo advancement by promoting rapid cell division. This has been observed by high-resolution histographical mapping in emerging cotyledons (Borisjuk et al. 1998). Exogenous treatment of Glc negatively affects germination in a concentration-dependent ($1.5 < 2.5 < 5\%$) manner (Zhao et al. 2009). The Glc might function as a "morphogen" possibly due to sugar-mediated regulation of cyclin D gene (Riou-Khamlichi et al. 2000). In the transition phase, the embryo shifts from a predominant mitotic phase to differentiation determined by cellular enlargement due to Suc uptake surge and the creation of embryo sink strength. Elevated concentrations can lead to obstruction in germination as well as can inhibit the early stages of seedling growth (Price et al. 2003). However, the process is reversible, as the growth continues when the Glc concentration decreases (Gibson 2005). The effect is seen in *Vigna radiata* where with increase in Glc content, the germination rate and height of sprouts are decreased (Dewi 2015).

Arabidopsis seed growth shows a similar pattern, but the only difference is that its storage material is mainly lipids, and T6P metabolism also has a crucial role during embryogenesis. The inhibitory action of Glc at higher level on the development of seedling is also well studied. *Arabidopsis* seedlings at higher Glc concentration are incapable of developing green colour and also fail to develop expanded cotyledons or leaves (To et al. 2003). Researchers reported that a loss-of-function mutation in the putative APETALA2 (AP2)-type TF intensified the seed mass and yield. The increase in cell size and count in the mutant embryos is linked with the elevated hexose to Suc ratio during embryo development (Jofuku et al. 2005). Hypocotyl elongation is inversely proportional to the Glc concentration (Kushwah and Laxmi 2014). Glc intermediated regulation of seed germination might be an outcome of its capacity to mitigate the inhibitory impacts of abscisic acid (ABA) on germination in a concentration-dependent way. Additionally, few ABA and Glc signalling mutants demonstrate standard germination kinetics (Price et al. 2003), signifying the role of specific signalling pathways in germination and differential reaction to sugars conditional on the developmental phase. An essential role of ABA in plant sugar metabolism was validated by the description of Glc insensitive5 and Glc insensitive6/Suc uncoupling6/sugar insensitive5 as mutant alleles of ABA3 and the gene that codes the AP2-type TF ABI4, respectively (Leon and Sheen 2003). ABA response element (ABF2) is an essential component of sugar signalling, and overexpression of

ABF3–4 also upsurges Glc sensitivity (Kim et al. 2004). Exogenous Glc explicitly improved the expression of ABA synthesis and signalling genes along with the endogenous levels (Cheng et al. 2002). Hence, it is proposed that Glc-specific build-up of ABA is essential for sugar signalling in early seedling growth.

14.2.2 Carbon and Nitrogen Metabolism

Glc is a minor photosynthetic product; however, its exogenous treatment can modify the photosynthetic cycle and its associated traits. In the presence of Glc, the rate of electron allocation to photosystem I (PSI) increases. In turn, Glc hydrolysis to NADH and NADPH (reducing powers) also increases which are vital for several biosynthetic reactions (Rineau et al. 2013). The rate of chlorophyll molecule synthesis also increases in the incidence of Glc. It was reported that excised, etiolated bean leaves showed enhanced chlorophyll synthesis on external application of Glc (Prasanna et al. 2004). The effect of Glc on the photosynthetic process is concentration dependent. With increasing concentration of Glc, the chloroplast development is suppressed and finally inhibited. This inhibition is due to Glc- and Suc-mediated repressed transcription of photosynthetic genes (Sinha and Roitsch 2002). The rate of oxygen evolution and electron transport also declines in Glc presence. RuBisCO is the vital enzyme in photosynthesis that increases with increasing Glc concentration (Spreitzer and Salvucci 2002). Carotenoids are photoprotective pigments that also act as precursors for ABA biosynthesis in plants. It is reported that carotenoid synthesis is decreased by the Glc treatment probably due to repression of genes coding for carotenoid pathway enzymes (Stephanie et al. 2010). β-Carotene is the amplest form of vitamin A present in fruits and vegetables. On the contrary, the concentration of β-carotene rises in plants after Glc application (Krinsky and Johnson 2005). Exogenous application of Glc can increase the expression level of glycolysis, pyruvate metabolism, Krebs cycle (energy-generating mechanisms) and respiration (energy-consuming mechanism). It is reported that the presence of Glc can increase respiration but decreases starch in potato (Stephanie et al. 2010). This response is similar to overexpression of invertase enzyme (Alagbe et al. 2020). Plant assimilates nitrates into other organic compounds, and their reduction into nitrite is catalysed by nitrate reductase. It is reported that Glc could affect enzyme nitrate reductase by enhancing the accumulation of nitrate in roots of corn (Oaks 1994). NRT2.1 is a nitrate transporter whose protein levels and transferring capacity are augmented in the presence of Glc. However, the augmentation is independent of HXK1-mediated stimulation (Jong et al. 2014).

14.2.3 Plant Growth and Development

Soluble sugars are capable of regulating overall plant growth and development. Genes that translate proteins like AGPase and Snf1-related kinases (SnRK) in apical meristem assist as markers for initial leaf expansion (Pien et al. 2001). Shoot maturation is separable into juvenile, adult nonreproductive and reproductive phases. By repression of the small subunit of RuBisCO, shoot morphogenesis is delayed, whereas the shoot to root ratio is increased (Tsai et al. 1997). Glc suppresses miR156 (transition of juvenile phase to adult phase) expression up to 50% (through HXK1-dependent pathway) due to a decrease in miR156 genes (Yang et al. 2013). Sugars are also reported to regulate the development of organs. *Arabidopsis* seedlings (dark-grown) showed adventitious root formation initiation by exogenous Suc application (Takahashi et al. 2003). Hence, it is established that Glc treatment may result in suspending the transition process in plants. In the root meristematic zone, Glc concentration increases the rate of meristematic cell differentiation along with its transport to the elongation and differentiation region. The effect is that the Glc concentration is inversely proportional to the root length (Lorenz et al. 2003). Additionally, initiation of flowering is allied with starch utilization and elevation in leaf carbohydrate transfer to the shoot apical meristem, signifying that sugars in the phloem are critical components. It is reported that Suc accessibility boosts morphogenesis and flowering in *Arabidopsis* even in dark conditions. Exogenous Suc (1%) treatment induces the late-flowering phenotype of different mutants, whereas at higher concentration of sugars, the transition is delayed by extension of vegetative phase (Roitsch and Gonzalez 2004). *Arabidopsis* sugar-insensitive mutant (glz2) showed late flowering, entirely sterile gynoecium and abnormal flowers and fruits (Chen et al. 2004). The essential function of precise carbon allocation in reproductive advancement arises from antisense SnRK transgenic barley and tobacco male sterility phenotypes with tissue explicit antisense repression of a cell wall invertase (Ruuska et al. 2002). An associated delay in stimulation of LEAFY advocates that sugars can regulate the expression of floral meristem identity genes (Ohto et al. 2005).

14.2.4 Senescence

Leaf senescence is the last step in the plant's life cycle in which the metabolites are remobilized and transported into the developing organs from old leaves (Fig. 14.1). Senescence can be stimulated by different conditions, and numerous signalling pathways are intricate in the regulation. Possibly, it is the result of integration of environmental and sugar signalling. Leaf senescence was induced in *Arabidopsis* by Glc application (2%) in combination with low nitrogen supply (Wang et al. 2008). Amino acid amount decreased in senescing leaves, but the sugar content was increased in numerous plant species (Jongebloed et al. 2004). In *Arabidopsis*, the contents of Glc and Fru (hexoses) upsurge, whereas Suc content remains stable in senescing leaves (Wingler 2007). Data analysis of Affymetrix GeneChip assay confirmed that variations in gene expression during Glc-stimulated senescence are representative of developmental senescence. Sugars can be supposedly synthesized by gluconeogenesis from fatty acids. Senescence during drought stress is beneficial as it not only reutilizes nutrients but also averts excessive water loss, thus improving plant water balance (Lim et al. 2007). On the contrary, in another study, researchers reported that delayed ageing can enhance drought tolerance in tobacco plants (Rivero et al. 2007). Stessman et al. (2002) reported hexose accumulation in senescing leaves of *Arabidopsis*. Glc, Fru, Suc and other osmolytes are reported to get accumulated during cold treatment (Kaplan et al. 2007). In another report, Jongebloed et al. (2004) displayed that phloem obstruction due to callose deposition may perhaps result in an age-dependent sugar accumulation. The ABA is an imperative signal in plant retort to stresses like cold, drought and salinity. The phytohormone synthesis and signalling has identified a vital component in sugar signalling. *Arabidopsis* mutants in ABA synthesis or signalling did not show developmental seizure in response to high-sugar levels (Rolland et al. 2006). A few cold response genes can be induced by ABA and sugar signalling; therefore, a feedforward loop might occur enhancing stress acclimation by sugar aggregation (Masclaux-Daubresse et al. 2007). Furthermore, exogenous Glc application did not induce senescence in cold-grown *Arabidopsis* as in higher temperatures. This controlling interaction might be significant in the prevention of premature senescence.

14.3 Sugar as a Signalling Molecule: Signal Transduction Cascades

In order to coordinate the metabolic processes in plants and respond appropriately to the altering environmental changes and energy demands, plants have developed an array of mechanisms to sense diverse sugar signals (Gupta and Kaur 2005). The signal then begins the signalling transduction cascade that leads to different cellular responses. Plant HXK (HXK) has been proposed as a dual-functioning enzyme with both regulatory and catalytic functions (Xiao et al. 2000). Plants have two different HXK-Glc sensing and signalling pathways, which catalyse via the phosphorylation of Glc, Fru and other molecules (Wei et al. 2020). First is the HXK-dependent pathway and the second is the HXK-independent pathway. The HXK-dependent signalling pathway requires phosphorylation of sugars, whereas the HXK-independent signalling pathway does not require phosphorylation. Sugar analogues that are phosphorylated by HXK were able to activate downregulation of photosynthetic genes. Additional breakdown of sugar phosphates was not important to cause suppression, as 2-deoxyglucose (2-DG) and mannose cannot undergo further processing after phosphorylation and this might likewise cause downregulation. Moreover, mannoheptulose, a competitive inhibitor of HXK, can inhibit the suppression caused by 2-DG (Gupta and Kaur 2005). Moreover, 3-oxy-methyl-glucose (3-o-MG) cannot diminish the suppression by 2-DG since it cannot also be phosphorylated by HXK (Gupta and Kaur 2005). The epimer of Glc is mannose, which is phosphorylated by HXK to mannose-6-phosphate, which only gradually enters glycolytic metabolism (Smeekens 2000). *Arabidopsis* plant has Glc analogue such as 2-DG and mannose which inhibits seed germination through HXK signalling cascade. HXK-independent substrates such as 3-o-MG and 6-deoxyglucose have no effects on seed germination, whereas the HXK inhibitor mannoheptulose impedes the mannose-induced inhibition of seed germination.

In an experiment, the role of *Arabidopsis* HXK (AtHXK1 and AtHXK2) in the sugar-induced repression of photorespiration (gene) and early seedling development is shown (Xiao et al. 2000). The metabolic enzyme (AtHXK1) acts as a Glc sensor by modifying the HXK levels or with a heterologous yeast, viz. YHXK that give a primary catalytic function. This concept is supported by a five times increase in hexose phosphorylation activity, but no inhibition of seedling development in plants ectopically expressing YHXK2. Plants overexpressing AtHXK1 show both an increased hexose phosphorylation activity and an inhibition of germination and early seedling development. It was reported that AtHXK1 had an important signalling function which was not altered by YHXK2 (Jang et al. 1997). Some genes, involved in carbon and nitrogen metabolism, were reported to be independent of HXK (Sheen et al. 1999).

The standard definition of HXK is that it acts as a dimeric cytosolic enzyme crucial for glycolysis. HXK is a Glc sensor, where intracellular sugar (Glc) signals are sensed, or it has regulatory and catalytic functions or whether the ATP/AMP ratio is the actual signal (Bolouri-Moghaddam et al. 2010). HXK activity could be involved in cell expansion, cell division and ROS signalling in plant organelles such as mitochondria and chloroplasts (Alagbe et al. 2020). Mitochondrial HXK (mtHXK) activity has an important role in producing ADP to maintain oxidative phosphorylation, thereby controlling ATP generation-associated limitation of oxidative respiration and successive H_2O_2 generation. In maize roots, mtHXK is supposed to be connected to sugar synthesis that is needed for phenylpropanoid, flavonoid and cellulose biosynthesis (Bolouri-Moghaddam et al. 2010). The mtHXK enzyme in plant performs an antioxidant action in mitochondria. Glc signalling through HXK downregulates a branch of the ethylene-signalling pathways that induce germination and cotyledon and leaf development (Smeekens 2000). HXK and SnRK are already regarded as conserved sugar signalling components regulating energy homeostasis, survival, abiotic stress resistance and longevity. Additionally, invertase-related sugar signals appear to be very important during plant defence reactions (Bolouri-Moghaddam et al. 2010). A significant role for HXK-dependent sugar signalling in leaf senescence has also been reported (Bolouri-Moghaddam et al. 2010). Figure 14.2 shows a diagrammatic depiction of sugar-sensing mechanisms in plants.

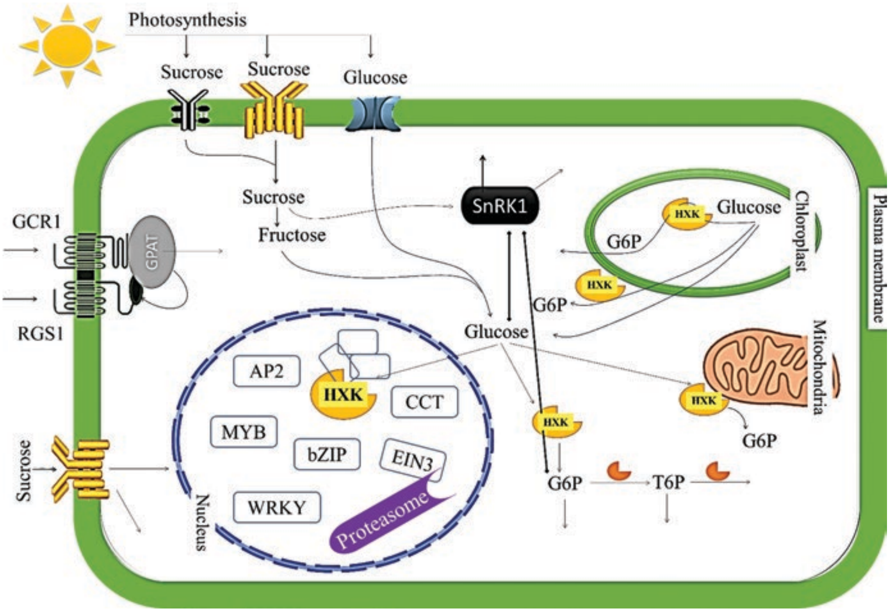

Fig. 14.2 A diagrammatic representation of sugar-sensing mechanisms in plants. Sucrose and glucose are sensed by transporter homologues at the plasma membrane. The HXK glucose sensor is linked with the outer membrane of plastids (mitochondria and chloroplast) and cytoplasm. HXK is also found in the nucleus with high-molecular-weight complexes; here it regulates transcription and proteasome-mediated breakdown of the EIN3 transcription factor. G-protein-coupled receptor signalling by RGS1 and GCR1 is intricate in glucose-mediated plant development, but by the hexokinase-independent pathway. SnRK1 protein in plant has an imperative function in sugar and starvation cascade; however, its regulation by sugars is still uncertain. Downstream of SnRK1, significant regulatory effects for T6P (trehalose) are reported. Lastly, several transcription factors controlling the sugar-regulated transcription are located in the nucleus. See text for additional details

14.4 Abiotic Stresses

Since the last few decades, the existence of plants under abiotic stress is a wide area of research. These abiotic stresses directly or indirectly distress the concentration, metabolism, transport and storage of sugars as well as the sugar signalling pathways (Cramer et al. 2011). Abiotic stress in plants can also reduce the growth and yield below the optimum levels. Sugars are firstly involved in several metabolic activities; it controls numerous genes specially those of Suc metabolism and photosynthesis. Secondly, accumulation of soluble sugars enhances plant survival under abiotic stresses, such as salt, heat, chilling and drought (Mohammadkhani and Heidari 2008). Other forms of carbohydrate such as raffinose family oligosaccharides (RFOs), T6P and fructans also function as protectants against different types of abiotic stresses. A set of genes involved in sugar metabolism influenced under

Table 14.1 Set of genes involved in sugar metabolism in plants that are upregulated and downregulated by glucose and under abiotic stresses

S. no.	Genes upregulated	Genes downregulated
1	Sulphur assimilatory genes	Amino acid degradation
2	Ribosomal proteins	Trehalose metabolism
3	Lipoxygenase	Storage proteins
4	Nucleotide synthesis	Gluconeogenesis
5	Secondary metabolism methyltransferases	Aldolases
6	Protein kinase	Glutaredoxins
7	Protein targeting	Fructose bisphosphatase
8	Lipid transfer proteins	Lipid degradation
9	Glutathione S-transferase	Light-mediated signalling
10	Secondary metabolism flavonoid synthesis	Ubiquitin conjugation
11	Phosphoenol pyruvate carboxylase	Ethylene synthesis
12	Nucleotide sugar transferases	Pentose phosphate pathway

Li et al. (2006), Seki et al. (2002)

glucose concentration and abiotic stresses in plants are shown in Table 14.1. The role of sugars under diverse abiotic stresses has been described further in details.

14.4.1 Water Stress

Drought is a major limiting factor in plant development and productivity. In a study conducted with a variety of plants, it was validated that drought or flooding induced conversion of hexoses, for instance, Suc and starch into polyols (sugar alcohols) (Mohammadkhani and Heidari 2008). Former reports stated that during drought stress, sugars protect the cells by two mechanisms. Primarily, the -OH groups of sugars may replace water (H_2O) to sustain hydrophilic interactions in proteins and membranes during dehydration. Thus, sugars make hydrogen bonding with proteins and membranes and inhibit protein denaturation. Secondly, sugars are an extensive contributing factor to vitrification, i.e. the synthesis of a biological glass in the cytoplasm of dehydrated cells. In numerous plant species, it was reported that raffinose (Raf), verbascose and stachyose accumulated at the time of desiccation of seeds as well as accumulated in the leaves when the plant was exposed to abiotic stress (Sami et al. 2016). These sugars have a significant function during stress in membrane protection and ROS scavenging. Soluble sugars also prevent dehydration of membranes and maintain the turgidity of leaves. Drought is amongst the major factors that significantly downregulate photosynthesis (Sami et al. 2016). Glc induces stomatal closure and enhances plant's adaptability under drought stress (Osakabe et al. 2014). Another significant component of carbohydrate metabolism is sucrose-phosphate synthase (SPS) present in plant leaves. Gupta and Kaur (2005) reported that SPS activity was decreased in leaves of plants subjected to drought stress.

During meiosis water stress induced male sterility and prevent starch accumulation but enhanced the accumulation of soluble sugars (Pressman et al. 2002).

14.4.2 Salt Stress

Salt stress not only modifies nitrogen assimilation, carbohydrate levels, cell division and plant growth but also modifies the types of carbohydrates synthesized and transferred by the source tissues (Sami et al. 2016). Osmotic stress has an effect on the carbohydrate source (photosynthesis) as well as the mobilization of carbohydrate stock (Henry et al. 2015). Increased concentration of salt leads to ionic imbalance, declines stomatal conductance and causes reduction of leaf growth (Sami et al. 2016). Salinity induces alterations in processes such as energy metabolism, protein synthesis, ion transport and protein fate (Cramer et al. 2011). Osmotic imbalance subsequent from salinized soils or drought can be calamitous for plants like maize, chiefly due to its effect on early kernel development (Henry et al. 2015). Osmotic stress induces kernel abortion by diminishing photosynthesis and evapotranspiration in maize. Osmotic stress also reduces Suc-sugar levels, transcription levels, Suc-degrading enzyme activity and starch in the kernels. The accumulation of sugar serves as carbon storage and osmoprotectant, maintains osmotic homeostasis as well as scavenges free radicals to mitigate the damaging effects of salt stress (Sami et al. 2016). Plants try to reduce their osmotic potential by enhancing mineral ion content and synthesis of compatible solutes to enhance water uptake under stress (Nemati et al. 2011). The synthesized and accumulated total soluble carbohydrates in the cytosol are also important solutes during salinity stress. Total soluble carbohydrates induce better osmotic adjustment and maintain turgor for growth under salinity. Plants and bacteria are reported to produce fructans (polyfructose molecules) that have a crucial part in adaptation to osmotic tension due to their high soluble characteristic.

14.4.3 Cold Stress

Cold stress causes substantial dysfunctions at the cellular level; these comprise disruption of membranes, generation of ROS, protein denaturation and accretion of toxic by-products (Yuanyuan et al. 2009). Development of cold tolerance in higher plants enhanced by the accumulation of a diverse variety of soluble sugars such as Glc, Fru, Suc, stachyose and Raf (Sami et al. 2016). It has been reported that these sugars function as osmoprotectants and also protect the cellular membrane from damage, induced by dehydration and freezing (abiotic stress) through interaction with the lipid bilayer. In addition, T6P sugar also serves as osmolyte and stabilizes protein, lipid and membrane. In rice, higher concentration of T6P is detected under cold stress. Some oligosaccharides like fructans and Suc induce resistance from crystallization and then enable glass formation within the cell and protection of membrane phospholipids under freezing. In other words, the accumulation of soluble sugars contributes to the enhancement of cryo-stability of cell membranes. This enhanced membrane stability is a prerequisite for dehydration or cold tolerance because freezing causes destabilization of cellular membranes which is the initial cause of damage in plants. Polyols like glycerol, sorbitol, Suc and mannitol are osmoprotectants in insects, exposed to freezing algae and certain halophytic plants (Rathinasabapathi 2000).

14.4.4 Heat Stress

In heat stress, the accumulation of soluble sugar and starch in the pollen grains was markedly lesser than that under normal conditions (Pressman et al. 2002). Abiotic factors such as high temperature and water stress affect SPS activity (Lafta and Lorenzen 1995). The enhancement in SPS activity is transferred to the entire plant level from source leaves at high temperature (heat stress). In this experiment, the changes in SPS activity at high temperature coincided with altered enzyme concentrations of carbohydrate metabolism in developing tissues. It was also associated with increased carbohydrate partitioning in young leaves and stems. High temperature reduced the plant growth and leaf expansion. Heat stress affects more the tuber growth than the shoot growth (Feil and Lunn 2018). High-temperature-treated leaves reported higher levels of Suc at the edge of the photoperiod as compared to the leaves from the cold treatment (low temperature), whereas starch concentration was reported to decline in the plant leaves after high-temperature treatment (Feil and Lunn 2018). A paper proposes that heat stress-induced depletion in sugar transport to reproductive tissue leads to loss of gametophyte growth (Pressman et al. 2002).

14.5 Abiotic Stress-Induced Oxidative Stress and Scavenging in Plants

Abiotic stress at cellular and tissue levels commonly induces an oxidative stress. It is defined as a disproportion between ROS synthesis and scavenging via antioxidants like Glc (Keunen et al. 2013). Under physiological conditions, ROS such as $^1O_{2,}$ $O_2^{\bullet -}$, $^{\bullet}OH$ and H_2O_2 are generated as by-products of oxidative metabolism in different plant organelles. ROS plays a dual role as signalling and damaging compounds (Sami et al. 2016). Increased ROS accumulation under abiotic stress is life-threatening as it leads to oxidization of proteins, lipids and nucleic acids (Hu et al. 2012). In addition, it affects the gene expression, plant cell growth, cell membrane integrity, hormone signalling and programmed cell death (Fraire-Velázquez and

Balderas-Hernández 2013). Salt stress affects the plant's metabolism in several ways such as ion toxicity, production of ROS and osmotic stress (Hu et al. 2012). ROS are highly reactive and can seriously impair normal metabolism causing photooxidative damage and leading to lipid peroxidation. Drought stress leads to an induction of high accumulation of ROS, which damages the biomolecules. Researchers stated that the shortage of water in cucumber cells caused lipoxygenase activity, membrane injury and lipid peroxidation affecting normal growth (Morsy et al. 2007; Hu et al. 2012). Soluble sugars are already testified to improve the antioxidative mechanisms as it is involved in ROS scavenging, thus contributing to an overall environmental stress tolerance (Keunen et al. 2013). In another way, soluble sugars can also funnel NADPH-producing metabolic pathways (i.e. OPP pathway), which can scavenge the ROS. Moreover, sugars and sugar alcohols have been widely accepted as an antioxidant system quenching ROS against various kinds of stresses, regulating the osmotic alteration and providing membrane protection (Sami et al. 2016; Hu et al. 2012).

14.5.1 ROS Scavenging in Plant Chloroplast

Under standard as well as stressed conditions, ROS is generated as the by-product of high photophosphorylation (oxidizing metabolism) or rapid electron flow in organelles such as chloroplasts and peroxisomes (Keunen et al. 2013). In the thylakoid membrane, photosystem I and II reaction centres account for high levels of ROS in the chloroplast. Soluble sugars can be involved in ROS producing photorespiratory pathway which is a major producer of H_2O_2. Environmental stresses, for instance, salinity, excess light, drought and metal exposure, enhance chloroplast ROS production. It can also utilize the OPP pathway producing reduced power for glutathione (GSH) production and contribute to ROS quenching (Cramer et al. 2011). Glucose-6-phosphate dehydrogenase (G6PDH) enzyme catalyses the first reaction in this pathway. This enzyme activity can be vital in regulating the ROS decontamination capacity along with redox potential in the chloroplast. Moreover, limited sugar supply may interrupt respiratory metabolism, thereby increasing ROS creation at the level of the electron transport chain (ETC). Sugars such as galactinol (Gol), RFOs, fructans and sugar alcohols (inositol, mannitol, sorbitol) are suggested to function as antioxidants and possess ROS quenching capacities (Keunen et al. 2013). Commonly, they have higher scavenging affinity for $^{\bullet}OH$ as compared to $^1O_2$. Nishizawa et al. (2008) analysed that Gol and Raf had $^{\bullet}OH$ scavenging ability and showed similar antioxidant capabilities to GSH. Moreover, the researchers also reported that their concentrations are appropriately ranged to protect plant cells from oxidative damage. Interestingly, Raf concentration can directly scavenge $^{\bullet}OH$ radicals as compared to ascorbate (AsA) and GSH in chloroplasts of stressed plants. In chloroplast stroma, ascorbate-glutathione (AsA-GSH) cycle is present that is fully functional to neutralize H_2O_2 produced by the difference of $^1O_2$ catalysed by superoxide dismutase (SOD) (detoxifying ROS).

Some water-soluble antioxidants such as carotenoids and tocopherols are highly abundant in chloroplasts. Tocopherols efficiently reduce $^1O_2$ and are located in the thylakoid membranes. They are also reported to scavenge lipid peroxides precisely under abiotic stress conditions (Keunen et al. 2013). On the other hand, carotenoid pigments protect the photosynthetic apparatus from photo-oxidation. Raf was shown to protect chloroplast from photorespiration and chloroplast membranes against desiccation, high temperature and freezing stress (Keunen et al. 2013). The oxidized RFO radicals might be regenerated by reducing antioxidants such as AsA or flavonoids. The overexpression of galactinol and raffinose synthase in transgenic *Arabidopsis* plants enhanced the Gol and Raf concentrations and showed effective oxidative stress tolerance and ROS scavenging capacity (Keunen et al. 2013). Peroxiredoxins (Prxs) are thiol-based peroxide reductases that are capable of reducing several substrates specifically ranging from peroxynitrite to H_2O_2 (Van den Ende and Valluru 2009). Fructans can protect plants against water stress by stabilizing and activating other specific antioxidative defence mechanisms. Additionally, sugar availability can increase AsA biosynthesis, possibly due to an enhanced respiration rate (Bolouri-Moghaddam et al. 2010). In tobacco, mannitol is reported to protect ferredoxin, GSH, thioredoxin and the thiol-regulated enzymes against $^{\bullet}OH$ (Keunen et al. 2013).

14.5.2 ROS Scavenging in Plant Mitochondria

In non-photosynthetic cells, mitochondria are the core sites of ROS generation. This might be due to electron outflow at complexes I and III in the ETC (Keunen et al. 2013). Proactive energy-dissipating systems are present in plant mitochondria, which are able to considerably reduce the rates of ROS generation at the ETC (Hussain et al. 2019). In this situation, the function of the alternative oxidase (AOX) bypassing ETC III and IV complexes is confirmed to reduce ROS creation (Møller 2001). Plant uncoupling proteins are another proactive energy-dissipating structure that reduces the ROS production through fine-tuning the membrane potential of plant mitochondria (Keunen et al. 2013). Soluble sugars showing protective effects against oxidative or abiotic stress have been mostly owed to signalling cross-talks that could stimulate explicit ROS scavenger (Singh et al. 2019). However, sugars, AOX, uncoupling proteins and their allied metabolic enzymes may perhaps also act as ROS scavengers at the level of the mitochondrial ETC. However, it was recently proposed that the synergistic interaction of sugars and phenolic compounds is part of an integrated redox scheme, contributing to ROS scavenging and stress tolerance. The mitochondria-associated HXK (mtHXK) have catalytic activity which regulates detoxification of ROS and consecutive signalling pathways, thus generating antioxidant defence systems in plants (Keunen et al. 2013). The inner mitochondrial membrane contains galactono-glactone dehydrogenase enzyme which catalyses AsA synthesis. In the biosynthesis of AsA, SOD and sucrosyl oligosaccharides (SOS) act as antioxidants and detoxify ROS. In plant mitochondria, GSH levels could also cause cell survival during environmental stress, as GSH protects mitochondrial DNA and proteins from being oxidized. The enzyme mtHXK can play an important role in producing ADP to support oxidative phosphorylation (Bolouri-Moghaddam et al. 2010).

14.5.3 ROS Scavenging in Plant Peroxisomes

Similar to other plant organelles described earlier, ROS is also generated in peroxisomes as a by-product of its oxidative metabolism (Keunen et al. 2013). In the peroxisomal matrix, $O_2^{\bullet-}$ is produced by xanthine oxidase enzyme, and in membranes, it is produced via NADPH-dependent ETC reactions. In peroxisome, APX and MDAR are located on the membrane facing the cytosol. Peroxisomes are subcellular single-membrane organelles where H_2O_2 is generated by the activities of flavin oxidases and catalase (CAT) enzymatic components (Keunen et al. 2013). Peroxisomal ROS are also produced by various factors such as metals, xenobiotics, soil salinity and abiotic stresses. The presence of CAT in peroxisomes has been unequivocally recognized. The CAT enzyme dismutases H_2O_2 into O_2 and H_2O however, no external sources of reducing equivalents are needed for the reaction catalyzed by this enzyme. Three isoforms of enzyme i.e. CAT1–3 are reported to be present in the peroxisomal matrix of *Arabidopsis.* Correspondingly, the presence of Prx in plant peroxisomes could furthermore enhance the H_2O_2 quenching system in peroxisomes (Corpas et al. 2003). Experimental information suggests that only *APX3* could have a significant role in the peroxisomal ASC-GSH cycle. Numerous components of the AsA-GSH cycle have already been reported to be present in the peroxisomal membrane and matrix. The CAT enzyme was found to coordinate with the AsA-GSH cycle. Until now, there are no reports that could clearly indicate the involvement of soluble sugars such as Glc and Suc in peroxisomal antioxidant defence.

14.5.4 Emerging ROS Scavenging Systems in Plant Vacuoles

Abiotic stresses can cause an enhancement of cytosolic H_2O_2 concentration that can enter the plant vacuole via aquaporin or diffusion throughout the tonoplastic membrane (Singh et al. 2017). Class III peroxidases are reported to be associated closely with the inner membrane of tonoplast. Lipid peroxidases can attack H_2O_2 and convert it into $^{\bullet}OH$ radicals via the hydrolytic cycle, under stress conditions. Vacuolar sugars or sugar-like compounds can deeply insert itself amid the hydrophilic headgroups of the tonoplast membranes, thus stabilizing them under abiotic stress and playing a crucial role in scavenging these radicles. NADPH oxidases are enzymes present at the tonoplastic membrane which might be the major producer of ROS (cytosolic NADPH), as reported by some proteomic studies (Keunen et al. 2013).

The initial product of this enzyme is $^1O_2$, which is detected in the tonoplast. This $^1O_2$ could be converted to the less toxic H_2O spontaneously or by the activity of tonoplast-associated SOD (Van den Ende and Valluru 2009). Vacuoles accumulate a complex of anthocyanins, phenolics and malate (strong antioxidant compounds), which impacts the potential to buffer the redox state inside the cells (Keunen et al. 2013). Flavonoids such as anthocyanins and flavonols concentrate in the plant vacuoles and play versatile roles in plant metabolism. Phenolic compounds and fructans might function in a synergistic way to detoxify the excess vacuolar H_2O_2 and redox regulation processes. Vacuolar sugars and sugar alcohols are crucial players in antioxidative stress defence (Stoyanova et al. 2011). In addition, fructans were suggested to be acting directly as ROS quencher in the vicinity of the tonoplastic membrane (Singh et al. 2015). GSH, RFOs, fructans and SOS are supposed to protect biological membranes by detoxifying ROS in vacuoles and chloroplast or indirectly activating the antioxidative defence system (Van den Ende and Valluru 2009).

14.6 Possible Mechanisms of Sugar-Mediated Abiotic Stress Tolerance in Plants

Carbohydrates synthesized by photosynthesis are the building block and energy generator that supports plant growth. Under abiotic stress conditions, major plant growth is prevented, but photosynthesis and accumulation of fructans, disaccharides (Suc, T6P) and RFOs are slightly permitted. RFOs such as Raf, verbascose and stachyose are produced in the cytosol (Keunen et al. 2013). In addition to RFO accumulation, the activity of enzymes and gene expression involved in the metabolism are strongly connected with abiotic stress responses (Afzal et al. 2019). Under stress conditions, fructans can interact with apoplast cell membranes by directly forming hydrogen bonds (Livingston et al. 2009). RFOs and fructans are sucrosyl oligosaccharides, and their associated enzymes together interact with ROS scavenging cascades (Keunen et al. 2013). Carbohydrates and carbohydrate-containing biomolecules (soluble sugars) play a leading role in the cellular redox equilibrium due to their proximate associations with mitochondrial respiration, photosynthesis and fatty acid β-oxidation (Keunen et al. 2013). Small water-soluble sugars and the enzymes linked with their synthesis and signalling pathways are generally believed to be allied with oxidative stress and are capable of scavenging ROS. Any imbalance in the redox equilibrium of plant cells requires induction of specific antioxidant or antioxidant enzymes which represses ROS. The possible role and mechanism of sugar-mediated abiotic stress tolerance in plants have been described further in details (Table 14.2).

Table 14.2 Sugar compounds that can improve abiotic stress tolerance in different plant species

S. no.	Plant species	Gene	Gene function	Family/type	Sugar compound	Abiotic stress	Reference
1	*Arabidopsis thaliana*	Trehalose-6-phosphate synthase and phosphatase	Biosynthesis of trehalose in plants	Disaccharides	Trehalose	Temperature fluctuation, drought, salinity	Miranda et al. (2007)
2	*A. thaliana*	Galactinol synthase	Catalyses the initial and rate-limiting step of raffinose biosynthetic pathway	Raffinose family oligosaccharides (RFOs)	Galactinol	Oxidative stress, drought, chilling, salinity	Nishizawa et al. (2008)
3	*A. thaliana*	Stachyose synthetase	Catalyses the primary and rate-limiting reaction of RFO biosynthetic pathway	RFOs	Raffinose	Oxidative stress, salinity, chilling, drought	Nishizawa et al. (2008)
4	*A. thaliana*	UDP-glucose 4-epimerase	Catalyses the reversible change of UDP-glucose to UDP-galactose	RFOs	Raffinose	Freezing, drought, salinity	Liu et al. (2007)
5	*A. thaliana*	Mannose-6-phosphate reductase	Crucial enzyme in mannitol biosynthesis	Sugar alcohols	Mannitol	Salinity	Zhifang and Loescher (2003)
6	*Nicotiana tabacum*	Trehalose phosphorylase	Regulates starch and sucrose metabolism	Disaccharides	Trehalose	Drought	Han et al. (2005)
7	*N. tabacum*	Trehalose synthase	Catalyses reversible conversion of maltose and trehalose	Disaccharides	Trehalose	Salinity and drought	Zhang et al. (2005)
8	*N. tabacum*	Levansucrase (fructosyltransferase)	Levan synthesis	Fructans	Fructans	Freezing	Parvanova et al. (2004)
9	*N. tabacum*	Sucrose: Sucrose 1-fructosyltransferase	Catalyses a reversible interconversion	Fructans	Fructans	Freezing	Li et al. (2007)
10	*N. tabacum*	Mannitol-1-phosphate dehydrogenase	Enzyme in fructose and mannose metabolism	Sugar alcohols	Mannitol	Oxidative stress	Shen et al. (1997)

S. no.	Plant species	Gene	Gene function	Family/type	Sugar compound	Abiotic stress	Reference
11	*Lycopersicum esculentum*	Trehalose-6-phosphate synthase	Trehalose synthesis	Disaccharides	Trehalose	Oxidative stress, drought, salinity	Cortina and Culianez-Macia (Cortina and Culiáñez-Macià 2005)
12	*Oryza sativa*	Sucrose: Sucrose 1-fructosyltransferase	Catalyses a reversible reaction	Fructans	Fructans	Chilling	Kawakami et al. (2008)
13	*O. sativa*	Hexose reductase	Involved in fructose and mannose metabolism	Sugar alcohols	Mannitol	Salinity and drought	Pujni et al. (2007)
14	*O. sativa*	Sucrose: Fructan 6-fructosyltransferase	Enzyme catalyses fructosyl group transfer from sucrose to different acceptors	Fructans	Fructans	Chilling	Kawakami et al. (2008)
15	*Petunia x hybrida cv Mitchell*	α-Galactosidase	Hydrolysis of terminal α-galactosyl moieties from glycolipids and glycoproteins	RFOs	Raffinose	Cold stress	Pennycooke et al. (2003)
16	*Petunia x hybrida (Hook) Vilm. cv. Mitchell*	Hexose reductase	Enzyme contributes to fructose and mannose metabolism	Sugar alcohols	Mannitol	Freezing	Chiang et al. (2005)

14.6.1 The Defending Role of Sugar and its Associated Metabolic Enzymes Under Abiotic Stress

Fructans are Suc-derived Fru polymers supposed to be produced in the plant vacuoles. The presence of fructans is reported in only about ≤15% of all angiosperms. Fructans such as inulin show a stronger interaction with apoplast or tonoplast membranes as compared to levan-type fructans. Fructans can inhibit lipid condensation and cessation of the transition phase by decreasing the molecular motions of the lipid headgroups. RFOs show analogous physiological characteristics in plants and were reported to be involved in dehydration and desiccation stress tolerance in seeds. Thus, both RFOs and fructans are supposed to protect cellular membranes under stress maintaining cellular integrity (Keunen et al. 2013). Some soluble carbohydrates function as osmoprotectant carbohydrates (e.g. Suc, T6P, fructans, Raf and Gol) (Van den Ende and Peshev 2013). Suc is supposed to function as an osmoprotectant, stabilizing/protecting biological membranes and maintaining cell turgor pressure (Morsy et al. 2007). T6P can function also as an osmolyte and keep stabilizing membranes and proteins (Hernandez-Marin and Martínez 2012).

Soluble sugar may contribute to the enhancement in cryo-stability of cellular membranes by maintaining membranes in their appropriate phase which is essential for survival under abiotic stress (Krasensky and Jonak 2012). Sugars keep membrane surfaces “hydrated” and inhibit membrane fusion by inserting into the space between phospholipid biomolecules (Van den Ende and Peshev 2013). Hence, soluble sugars can function as cryoprotectant for protecting the membrane in the dry state or dehydrated state. In the glass forming process, T6P not only crystallizes but also undergoes vitrification. It is the only sugar that can remain in glasslike state during dehydration stress (Fernandez et al. 2010). Raf may be more effective in membrane stabilization than disaccharide or monosaccharide (Morsy et al. 2007).

ROS accumulation has been shown to be inhibited by dysfunction of G6PDH, suggesting that the protective role of sugars was dependent on OPP activity. Soluble sugars can replenish NADPH, required for monodehydroascorbate reductase and glutathione reductase activities (Singh et al. 2016). Keunen et al. (2013) reported the roles of Gol and RFOs in ROS homeostasis that have a potential function as neutral invertase and HXK functioning in chloroplasts to prevent ROS build-up in stressed plants. Oligogalacturonides were reported to stimulate the metabolic and enzymatic antioxidative defence system in alfalfa roots, which can be potentially connected with sugar signalling in plant defence against oxidative stress (Keunen et al. 2013). In addition, sugars can stimulate the synthesis of auxins that places sugars at a pivotal position in the regulatory processes driving plant development, under normal as well as under environmental stress conditions (Van den Ende and Peshev 2013).

14.6.2 Sugars as Antioxidants in Plants

Small water-soluble sugars such as Glc, Suc, sugar alcohols and Gol are proven to function as antioxidants in plants (Keunen et al. 2013). Bolouri-Moghaddam et al. (2010) hypothesized that sugars or sugar-like complexes such as Suc (present in the vacuoles of sugar beet and sugar cane plants) might function as an antioxidative compound inhibiting lipid peroxidation. This might be due to high concentration of H_2O_2 produced under stress (Van den Ende and Peshev 2013) or by directly quenching the $^{\bullet}OH$ and $^{\bullet}OOH$ radicals that are formed around the membranes (Hernandez-Marin and Martínez 2012). Mannitol could not reduce $^{\bullet}OH$ radical generation in the chloroplast, but it could enhance the scavenging ability, thus defending the cells against oxidative damage. In addition, mannitol accumulation had no toxic effects on plants (Van den Ende and Peshev 2013).

In an experiment, it was reported that in *Arabidopsis* plants, oligosaccharides' concentration such as Gol and Raf was increased in stressed conditions (Krasensky and Jonak 2012). Accordingly, α-galactosidase is reported to be inhibited in *Petunia* plant, causing increased Raf synthesis and freezing tolerance (Pennycooke et al. 2003). The *Arabidopsis* plant contains seven Gol (GolS) genes, one of which is induced by cold (GolS3) and the other two by drought (GolS1 and GolS2). In addition, overexpression of drought caused GolS1 and GolS2 genes in the plant to enhance Gol and Raf levels, as well as showed decreased transpiration rates to improve drought stress tolerance. These plants exhibit more effective oxidative stress tolerance and ROS scavenging capacity than wild-type plants (Van den Ende and Peshev 2013). Nishizawa et al. (2008) evaluated the $^{\bullet}OH$ scavenging ability of oligosaccharides (Raf and Gol) in vitro and indicated similar antioxidant scavenging abilities for both metabolites as compared to GSH. Interestingly, Raf levels in stressed plants are comparable with AsA and GSH, signifying that this water-soluble sugar can unequivocally neutralize $^{\bullet}OH$ radicals in chloroplasts (Moller et al. 2008).

Production of $^{\bullet}OH$ radical is highly dangerous in the vicinity of vacuolar membrane (Stoyanova et al. 2011). Thus, the presence of sugar metabolites can intensively interact with vacuolar membranes, such as fructans, and this could be critical for stabilization of membrane under stress (freezing tolerance) (Van den Ende and Peshev 2013). The accumulation of water-soluble sugar can be considered as true antioxidant capable of quenching ROS. It is found that inulin and stevioside are superior scavengers of both $O_2^{\bullet -}$ and $^{\bullet}OH$ and are more effective than Suc and mannitol (Stoyanova et al. 2011). T6P also plays a role in ROS scavenging in vitro (Van den Ende and Peshev 2013). Krasensky and Jonak (2012) analysed some genetically engineered plants and reported that increased T6P metabolism can positively influence stress tolerance, but the exact role of T6P during abiotic stress remains unclear. Suc-treated plantlets were able to sense altering ROS levels and in response could trigger efficient scavenging and antioxidant systems (Ramel et al. 2009).

14.6.3 Effect of Sugars on Proline Accumulation

Under abiotic stress, amino acid metabolism plays an essential role in stimulation of stress tolerance in plants. As a result, different amino acids (proline (Pro), nonprotein amino acids, minor amino acids and branched-chain amino acids) tend to accumulate in plants (Kaur and Asthir 2015). Pro is a proteinogenic amino acid reported to function both in plant metabolism (as molecular chaperone) and defence (osmoprotectant) (Woodrow et al. 2017). In plants, Pro synthesis takes place by using two pathways, primarily glutamate pathway and secondarily ornithine pathway (activated in chloroplasts) (Suprasanna et al. 2016). Synthesis of Pro in the cytoplasm or chloroplast during unfavourable conditions stabilizes the low NADPH to $NADP^+$ ratio, normalizes the redox balance by maintaining the electron flow between photosynthetic excitation centres and overall protects the photosynthetic apparatus from photoinhibition and damage (Dar et al. 2016). Accumulation of Pro has been described in various studies in response to different types of abiotic stresses, viz. salinity, drought, high temperature and metal toxicity (Vives-Peris et al. 2017). Surekha et al. (2014) reported a four times rise in Pro accretion in *Cajanus cajan* (transformed with *V. aconitifolia P5CSF129A*) as a response to salinity stress as compared to the wild type. In another report, the transformation of *P5CS* gene from *V. aconitifolia* in transgenic rice and chickpea plants showed a higher increase in Pro content (up to five times) as compared to non-transformed ones (Karthikeyan et al. 2011). In a topical study, Li et al. (2019) reported a transgenic *Arabidopsis* expressing salt-tolerant *IbRAP2–12* gene from sweet potato. The plant was found to accumulate higher Pro content with lesser ROS generation under abiotic stresses as compared to the wild counterpart. It is still not clear whether accumulation of Pro is an adaptive strategy, a stress response or just an indication. Pro is reported to stabilize membranes and proteins, scavenge ROS, act as an antioxidant and induce expression of salt stress-responsive genes (Carillo 2018). Pro metabolism in the mitochondria under unfavourable conditions can contribute to additional oxidative respiration that may produce energy for continuing plant development (Kaur and Asthir 2015). It also functions as a sugar remobilization signal for restabilizing plant growth, thus causing beneficial effects on the growth (Woodrow et al. 2017). Nevertheless, there are species explicit variances in Pro accumulation strategies and sugar signalling crosstalk in plants under abiotic stress (Dar et al. 2016).

14.6.4 Sugar and Phytohormone Interaction

Sugars can likewise crosstalk with prevailing phytohormone signalling systems to control significant developmental processes in plants like germination and seedling and tuber growth (Zouari et al. 2016). Plants malfunctioned in ABA or ethylene signalling and sensitivity show altered sugar retort phenotypes. Hence, an enormous overlay between sugar, ethylene, ABA signalling and regulating metabolic processes has been reported (Gibson 2005). ABA biosynthesis and ABA-insensitive mutants are also reported to be insensitive to elevated Glc concentrations (Dekkers et al. 2008). Identification of mutants during sugar response screening also displayed defect in ABA metabolism functioning, thus proving a connection amid them. Ethylene signalling pathways are also thoroughly associated with the sugar and ABA sensing interactions. Ethylene mutants, ethylene insensitive 2, ethylene receptor 1 and ethylene insensitive 3 are Glc hypersensitive, whereas constitutive triple response 1, which is a negative regulator of ethylene signalling, is reported to be Glc insensitive (Araújo et al. 2010). Glc and ABA act provocatively during the process of germination and seedling growth. Here exogenous Glc allows germination of wild-type seeds on otherwise inhibitory ABA concentration (Leon and Sheen 2003). On the other hand, ABA upsurges the Suc stimulation and expression of starch biosynthetic genes (Rook et al. 2001). In another study, a splicing factor (SR45) acts as a negative regulator of sugar signalling during initial development. It is involved in the repression of Glc-induced ABA build-up and downregulation of ABA biosynthesis genes (Carvalho et al. 2010). In maize seeds, the signal stimulus is regulated by soluble sugars in the emerging embryo, and the stimulus binds to regulatory elements for ABA and sugars equally (Niu et al. 2002). ABA concentration is improved in transformed ethylene precursor 1-aminocyclopropane-1-carboxylic acid phenocopy gin mutants and wild-type seedlings (Dahiya et al. 2017).

14.7 Conclusions and Future Perspectives

Sugars are acknowledged to partake vast and diversified functions in plants. Sugars are universally present in plants as an essential carbon and energy source. Sugar moieties act as nutrients and regulate vital functions, viz. growth, metabolism and stress tolerance responses. It overall participates in the development of plant from embryogenesis to senescence. In addition to being an imperative energy source and primary metabolite, the focus of investigation has now been shifted to the role of sugar as a regulatory and signalling molecule. Genes are differentially expressed depending upon the plant sugar status. The work in this specific area of research highlights the diversity of developments associated with sugars and their necessity in plants. The regulatory networks to which it is associated with physiological processes like photosynthesis, germination, reproduction and senescence are also explained. Fascinatingly, sugar signalling functions at the gene level in relation with different phytohormones. The role of sugars as signalling moieties is clear when the plant is under environmental stress. Under abiotic stress, plants produce sugars (osmoprotectants) for scavenging ROS and preservation of homeostasis. The sensor proteins of plant cells help in identifying status of sugar in the cell.

Sugar signalling in circadian rhythm and developmental mechanisms has also been conferred. The molecular specifics of signal transduction cascades and their interaction with other mechanisms can be discovered by using an amalgamation of genomic proteomic and genetic methods. Novel genomic tools such as microarray and clustering analysis offer a comprehensive view on the transcript aspects regulated by diverse sugar responses that can help in identification of new regulatory components and target genes. The signal created by signal transduction cascades can stimulate Ca^{2+}, calmodulins, mitogen-activated protein kinases and protein phosphatases and lead to apt gene expression. In spite of the prevailing research on this subject, there remains a lack of information, as countless key problems would be addressed if we might recognize the controlling complexity and the mechanisms involved in sugar homeostasis, cellular and subcellular distribution as well as long-distance translocation. New molecular sensors and FRET (fluorescence resonance energy transfer)-based imaging have the capability to picture and quantify the accurate location and level of different sugar moieties as well as metabolites in alive cells. Sugar signalling study under abiotic stress will remain to be a fascinating research area for many years to come.

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Chapter 15
Natural Polysaccharides: Novel Plant Growth Regulators

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15.1 Introduction

Crop production plays a critical role in an agricultural-based economy like India. The agriculture sector still decides the fate of 70% of its rural population (FAO 2015). Apart from cereal crops, India is a major exporter of crops with commercial standards. Presently, various innovative methods for crop enhancement are being sought to meet the rising demand of exponentially growing population. Fertilizers, PGRs, minerals and metal nanoparticles have proved their elicitor effect on various crops (Tripathi et al. 2016; Ahmad et al. 2019). However, their side effects and toxicity have always been a concern. Therefore, much recent research has been directed to explore some more sustainable and eco-friendly growth elicitors. Natural polysaccharides (NPs), besides being growth enhancers, are also antimicrobial, non-toxic, biocompatible and cheaper with no negligible side effects (Hu et al. 2005; Campo et al. 2009; Yan and Chen 2015). This makes NPs farmer-friendlier and a more sustainable option. Moreover, due to its greater biocompatibility, NPs legend with cell membrane and regulate membrane permeability. Various sources have asserted their eliciting effect on various crops with ample data to support (El-Mohdy 2017; Rabêlo et al. 2019; Saucedo et al. 2019). Figure 15.1 indicates different natural polysaccharides, i.e. chitosan, carrageenan and sodium alginate, and their growth-regulating attributes besides their biological properties.

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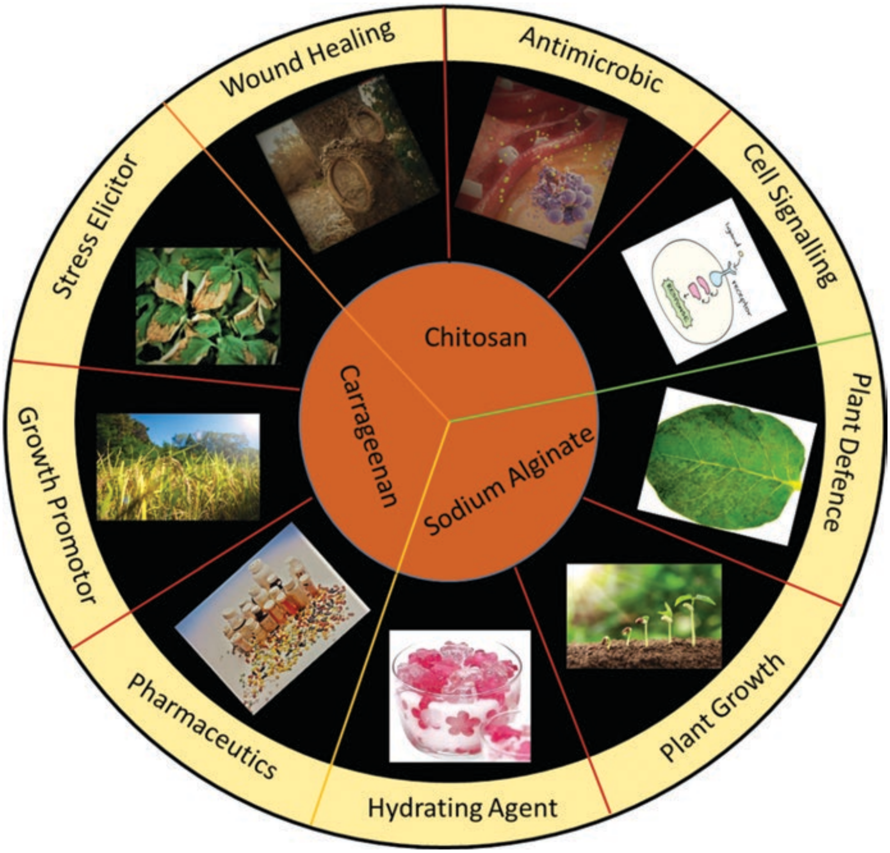

Fig. 15.1 Pictorial representation of the commercial applications of carrageenan, chitosan and sodium alginate along with their effect on plants

15.2 Chitosan

Chitin [(1,4)-2-acetamido-2-deoxy-β-D-glucan] is the second most abundant molecule in nature (Yan and Chen 2015) and is found primarily in crustacean shells, insects and fungi (Yan and Chen 2015; Jia et al. 2016; Turk 2019). Chitosan is a deacetylated chitin polymer that contains β-(1 → 4)-linked D-glucosamine and N-acetyl-D-glucosamine subunits (Malerba and Cerana 2016). Chitosan and its derivatives can further enrich chitosan properties due to their different physicochemical properties (size, density, surface area, etc.) enabling them to cross-talk with the cell wall and membrane more efficiently (Kim and Rajapakse 2005; Muley et al. 2019a). Multiples studies have reported that chitosan imparts a general trend of positive influence on plant growth and overall productivity (Pichyangkura and Chadchawan 2015; Malerba and Cerana 2016; Rabêlo et al. 2019). Figure 15.2

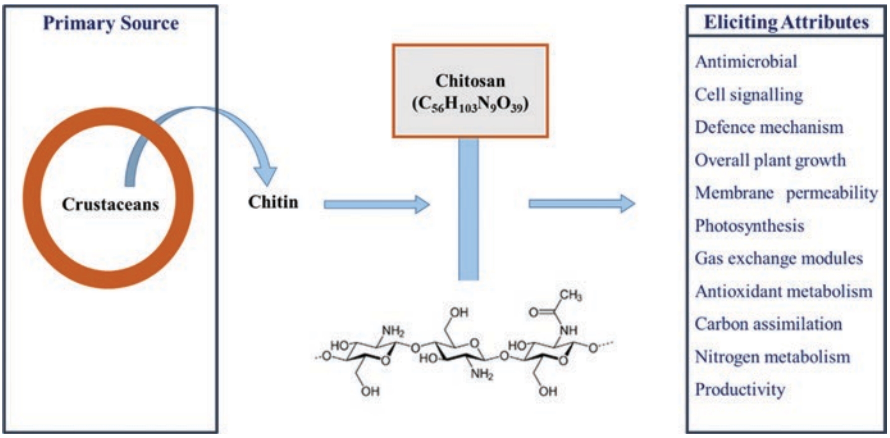

Fig. 15.2 Chitosan and its accompanied biological properties

traces the primary source of chitosan as well as induced responses in the biological systems.

15.2.1 Biological Activities of Chitosan

Chitosan has been reported to encompass a wide array of biological activities including antimicrobial, antitumor, antidiabetic, immunity-enhancing and wound-healing properties (Hayashi and Ito 2002; Xing et al. 2008; Zeng et al. 2008). Antimicrobial activities of chitosan chiefly comprise its antifungal and antibacterial properties (Xing et al. 2008; Meng et al. 2010). These properties, however, depend on multiple factors such as species of the microbe, concentration, deacetylation rate and molecular weight of chitosan or the pH of the solution itself (Xu et al. 2007; Xing et al. 2008; Yen et al. 2009). Chitosan can also constrain the formation of fungal spore, germ tube as well as mycelia (Meng et al. 2010). Various studies have suggested that chitosan can be used in food preservation and packaging industry given its antimicrobial potential (Chien and Chou 2006). Moreover, chitosan is cheaper, biocompatible, biodegradable and non-toxic and thus can be used in diverse fields including preservation and packaging of edible items (Chien and Chou 2006; García et al. 2015). Chitosan has been found effective against various economically important fungal strains including *Alternaria*, *Fusarium*, *Penicillium*, *Phytophthora* as well as *Botrytis* ((Meng et al. 2010) and the references therein). Therefore, chitosan can also be used as food preserver. Chitosan can keep food products away from fungal spoilage and thus elongate their shelf life (Liu et al. 2007), a decisive step in global food security itself.

Chitosan has been found effective against numerous bacteria as well (Du et al. 2009; Badawy et al. 2014). Chitosan solution has antipathogenic activity against the spread of many crop-threatening bacterial strains including *Xanthomonas* (Li et al. 2008). Keeping in view its action against fungi, it could be hypothesized that chitosan might inhibit bacterial biofilm formation and its further development by interacting with lipid bilayer and destabilizing bacterial membrane. As chitosan solution is generally prepared in acids given its low solubility in neutral or basic media, it can be argued that these properties might perhaps be more associated with acidic solvents. Thus, the extent of chitosan efficacy against such microbes is debatable. Recently, few chitosan derivatives with higher solubility in water were prepared and checked for various activities (Badawy and Rabea 2012; Tan et al. 2013; Badawy et al. 2014). It seemed more plausible that antimicrobial properties are to be more attributed to chitosan rather than the acidic solvent itself.

15.2.2 Role of Chitosan in Plant Growth Regulation

Chitosan has been reported as a growth promoter and signalling molecule in plants (Wang et al. 2015; Malerba and Cerana 2016; Muley et al. 2019b). Chitosan may also indulge in a complicated cascade of signal transduction that results in positive modulation of photosynthesis and multiple other related phenomena (Zhang et al. 2018). Chitosan being an important plant signalling molecule may target the nucleus and chloroplast (Pichyangkura and Chadchawan 2015; Rabêlo et al. 2019). Multiple genes associated with light reaction including those encoding for chlorophyll a/b binding protein and oxygen-evolving protein complex could be enhanced with chitosan application (Chamnanmanoontham et al. 2014). This might stabilize photosystem II and increased its efficiency and result in enhanced photosynthetic productivity. Similar regulatory effect was demonstrated in maize (Rabêlo et al. 2019), mint (Ahmad et al. 2019), potato (Muley et al. 2019b) and wheat (Zou et al. 2015).

Chlorophyllase is a crucial enzyme that catalyses the degradation of chlorophyll. Chitosan can suppress the expression level of the genes encoding for chlorophyllase resulting in increased photopigment content (Zhang et al. 2018). Chitosan is also capable of upregulating the translation of transcripts associated with photosynthesis as well as of those pertaining to the metabolism of carbon, nitrogen and amino acid (Zhang et al. 2018). Increased C- and N-assimilation plays a crucial role in source-sink potential and in the biosynthesis of growth- and yield-related molecules such as proteins and phenols (Chamnanmanoontham et al. 2014).

Plant mitochondria and chloroplasts produce different kinds of reactive oxygen species (ROS) and reactive nitrogen species (RNS) including peroxides and superoxides during various physiological processes under normal environment (Turk 2019). These compounds, collectively known as oxidants, have the tendency to damage lipid membrane via lipid peroxidation or alter membrane permeability via electrolyte leakage (Gupta et al. 2018; Zehra et al. 2020). Antioxidants are a group of compounds produced by plants as a counter-mechanism to regulate such phenomena. This cross-talk between oxidant and antioxidant signalling cascades

contributes to ROS pathway and is directly linked with plant innate immunity (R
et al. 2002; Gupta et al. 2018; Kohli et al. 2019).

Multiple studies (El-tantawy 2009; Chatelain et al. 2014; Wang et al. 2015; Ahmad et al. 2019; Muley et al. 2019b) have established that chitosan in different forms could enhance overall growth and yield in various crops. Chitosan also influences expression level of multiple glycolysis-related enzymes that might provide more energy to the plant (Chamnanmanoontham et al. 2014). However, chitosan might also exhibit some other different responses in different plants due to the fact that these responses chiefly depend on plant species and the concentration of chitosan used (Pongprayoon et al. 2013). As a general spectrum of chitosan effect on various plant phenomena, it has been reported to improve the biosynthesis of photosynthetic pigments, i.e. chlorophyll and carotenoids (Ahmad et al. 2017). Similar eliciting effects are also exhibited by source-sink potential through providing more efficient mineral uptake and their assimilation (Ahmad et al. 2017). Chitosan also plays a decisive role in plants during adverse environmental conditions. It upregulates antioxidant metabolism and ROS pathway and assists in enhanced production for various enzymatic as well as non-enzymatic antioxidants to resist the cellular damage (Chandra et al. 2015). These regulations, ultimately, help the plant to exhibit enhanced growth and overall production and also to survive in a stressful environment (Muley et al. 2019b; Rabêlo et al. 2019). Table 15.1 emphasizes on particular

Table 15.1 Eliciting effects of chitosan and its derivatives on various plant species

Plant	Eliciting effects on	Reference
Tomato	Plant height, fresh and dry weight, number of branches and leaves and marketable yield	El-tantawy (2009)
Coffee	Photosynthetic pigments, mineral nutrients (N, P, K, Ca and mg), overall growth and productivity	N. A. Dzung et al. (2011)
Maize	Root and shoot growth, antioxidant metabolism, leaf number, ear length and grain yield	Choudhary et al. (2017)
Chilli	Shoot biomass, chlorophyll content, fruit number and fruit weight	P. D. Dzung et al. (2017)
Common bean	Plant height, number of leaves and branches, leaf area, fresh and dry biomass, content of mineral nutrient (N, P, K), total carbohydrate and protein and productivity	Abu-Muriefah (2013)
Mint	Plant biomass, chlorophyll and carotenoid contents, activities of CA and NR enzymes, mineral nutrient (N, P and K) status and oil yield	Ahmad et al. (2017)
Rice	Expression level of genes pertaining to various physiological phenomena, overall plant growth and productivity	Chamnanmanoontham et al. (2014)
Tea	Antioxidant metabolism and plant immunity	Chandra et al. (2015)
Soybean	Photosynthetic modules, e.g. net photosynthetic rate and stomatal conductance	W. M. Khan et al. (2002)
Wheat	Overall plant growth, antioxidant capacity, sucrose and starch content and regulation of miRNA and mRNA expression profiles	Zhang et al. (2018)

phenomena that were associated with application of chitosan and its derivatives in different plants of economic importance.

15.3 Carrageenan

Carrageenan is generic name for the water-soluble and sulphated linear polysaccharides mainly found in the cell walls of various red algae (Mercier et al. 2001). It is composed of D-galactose and 3,6-anydrogalactose units joined through α-1,3 and β-1,4-glycosidic linkage (Di Rosa 1972; Necas and Bartosikova 2013). Carrageenan can however vary based on the number and position of the sulphate groups and the content of 3,6-anhydrogalactose units (Hashmi et al. 2012; Necas and Bartosikova 2013). Kappa (one sulphate group), iota (two sulphate groups) and lambda (three sulphate groups) are three commercially utilized carrageenan variants (De Ruiter and Rudolph 1997). Higher sulphate ester levels confer lower solubility temperature and thus weaker gel strength (Necas and Bartosikova 2013). These sulphate groups make carrageenan chemically active, giving it various biological properties. Figure 15.3 unfolds carrageenan from its primary source along with its interaction with multiple plant phenomena.

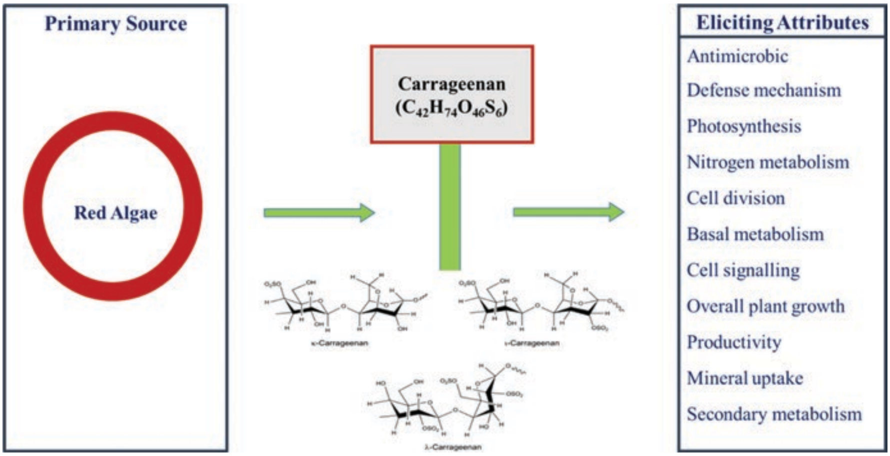

Fig. 15.3 Carrageenan and its accompanied biological properties

15.3.1 Biological Activities of Carrageenan

Carrageenans (CGs) consist of numerous biological properties including induction of experimental inflammation and inflammatory pain. Aside from these functions, they found to have several potential pharmaceutical formulations including antitumour, antihyperlipidemic, immunomodulatory and anticoagulant activities (Morris 2003; Zhou et al. 2004; Campo et al. 2009). Recent researches have demonstrated that carrageenan is an extraordinarily potent infection inhibitor of a wide range of genital human papillomaviruses (HPVs), and it is also indicated that HPV transmission may be cured by carrageenan-based sexual lubricant gels (Buck et al. 2006). However, questions about the safety of CG uses as food additive and pharmaceutical adjuvant have been raised. Besides, its long-term safety is a major concern as CG is used as an inducer of inflammatory responses in laboratory animals for the investigation of anti-inflammatory drugs (Li et al. 2014).

Several research studies also mentioned the anti-HIV properties of CG, but their usual mode of action in anticoagulant is considered to be an adverse reaction when used as a therapeutic drug for AIDS (Necas and Bartosikova 2013). Although all kinds of CGs possess antioxidant activity, λ carrageenan exhibited the highest antioxidant and free radical scavenging activity. A positive correlation has been observed between sulphate content and antioxidant activity (Rocha De Souza et al. 2007). A few CGs are found to affect strong macrophage activation, while some restrict macrophage functions. An experiment conducted on Fischer 344 rats, feeding on foods containing 15% kappa/lambda CGs from *Gahnia radula*, showed a cholesterol-reducing effect (Zia et al. 2017).

15.3.2 Role of Carrageenan in Plant Growth Regulation

To achieve crop protection, by activating or eliciting their natural defence system to introduce desired resistance, is the most effective way and an environmentally safer approach to the problem. The strong elicitors narrated in literature are diverse in nature including oligosaccharides, polysaccharides, peptides, proteins and lipids, and it has been confirmed that polysaccharides purified from seaweeds as well as derived oligosaccharides play a significant role in plant defence responses (Bi et al. 2011). Carrageenans are considered to play a significant role in plant signalling and defence under several adverse environmental conditions (Mercier et al. 2001). Several experiments have been conducted to scrutinize the elicitor activity of carrageenans. *Hypnea musciformis*, a rich source to obtain kappa carrageenan, has been evaluated as an elicitor or inducer of plant defence responses in terms of phytoalexin synthesis and induced browning and resulted as a potent plant protector as well as growth-promoting agent in plants (Arman and Qader 2012). Carrageenans and their oligomeric form, the oligocarrageenans (OCs), modulate the activity of different plant defence pathways, including jasmonate, salicylate and ethylene signalling pathways which in turn induce plant defence responses against viruses, viroids, bacteria, fungi and insects (Shukla et al. 2016).

Various endogenous and environmental factors such as light, hormones, temperature and nutrient availability affect plant growth and development. Moreover, it has been observed that marine algae oligosaccharides stimulate plant growth. Various treatments of oligocarrageenans K, L and I exhibited increased growth of commercial tobacco plants by enhancing photosynthesis, basal metabolism, nitrogen assimilation and cell division, as well as that of other plants of commercial interest, and enhanced protection against tobacco mosaic virus (TMV) infection in tobacco plants (Castro et al. 2012). In addition, accumulation of several phenylpropanoid compounds (PPCs) with microbial activity increased by oligocarrageenans improves protection against viral, fungal and bacterial infections in tobacco plants. Moreover, OCs induce the level of essential oil and increase cellulose content and some PPCs with antimicrobial activities, indicating that defence against pathogen may also be cured (González et al. 2013). A red macroalga, *Kappaphycus alvarezii*, has a great economic importance due to its production of kappa carrageenan. It produces and accumulates photoprotective compounds such as carotenoids and mycosporin-like amino acids (MAAs), which absorb UVR energy directly or indirectly (Schmidt et al. 2010).

Liquid extracts of seaweeds have been reported to enhance the growth of plants, increase yield and quality, improve resistance to disease and pest, increase mineral uptake from soil and antioxidant properties and amend resistance to abiotic stresses (salinity, drought, heavy metal stress and extreme temperatures). Carrageenans are the best characterized seaweed elicitors that have the potential to activate disease resistance in plants and animals (Mousavi et al. 2018). OC kappa enhances C-, N- and S-assimilation and improves growth-promoting hormone content and growth in pine trees; therefore, it may account for useful biotechnological tool to increase growth in pine forests (Saucedo et al. 2015). Several research experiments have been conducted and analysed the effects of carrageenan on growth and secondary metabolite status in plants. Several physiological and biological activities such as plant growth, physiological attributes, herbage yield and content and yield of alkaloids (vincristine, vinblastine) of periwinkle and the content and yield of essential oil in mint improved after foliar application of the degraded marine polysaccharides (Naeem et al. 2012a, 2015a).

It is well studied that growth and development in plants, algae, mammals and nematodes is controlled by the kinase target of rapamycin (TOR). It is a key regulatory kinase of the TOR pathway and is a phosphoinositol-related kinase (PIK) having protein serine/threonine protein kinase activity. In *E. globulus* trees, the stimulation of growth induced by OC kappa, by the activation of TOR pathway and increased expression of genes encoding protein involved in photosynthesis and enzymes of basal metabolism, has been reported (Saucedo et al. 2019). Table 15.2 weights on the eliciting effects of carrageenan and its derivatives on growth and physiology of various crops.

Table 15.2 Eliciting effects of carrageenan and its derivatives on various plant species

Plant	Eliciting effects on	Reference
Mung bean	Disease resistance and marketable yield	Gatan et al. (2019)
Fennel	Shoot length, plant biomass, photosynthetic pigments, activities of CA and NR enzymes, contents of N and P, overall productivity	Hashmi et al. (2012)
Maize	Plant length; number of pods, branches and leaves; and secondary metabolism	Bi et al. (2011)
Pine	Assimilation of carbon, nitrogen and Sulphur, contents of auxin and gibberellin and basal metabolism	Saucedo et al. (2015)
Peanut	Seed germination, flowering, plant height, pod length and number, seed weight and total yield	Abad et al. (2018)
Tobacco	Photosynthesis, basal metabolism and overall productivity	Castro et al. (2012)
Basil	Shoot length, leaf area, phenolic and antioxidant content and defence system	Mousavi et al. (2018)
Tasmanian blue gum	Plant height, photosynthesis, levels of sugar and trehalose, expression of transcripts pertaining to glucose, basal and secondary metabolism	Saucedo et al. (2019)
Lemongrass	Photosynthetic pigment, osmotic and turgor potential, antioxidant metabolism, overall growth and oil yield	Singh et al. (2017)
Chickpea	Defence system and overall growth promotion	Arman and Qader (2012)

15.4 Sodium Alginate

Alginates are natural polysaccharides chiefly derived from marine brown algae (Phaeophyceae) (Mollah et al. 2009; Khan et al. 2011). Commercial varieties of alginate are extracted from seaweed, including giant kelp *Macrocystis pyrifera*, *Ascophyllum nodosum*, *Ecklonia maxima*, *Sargassum sinicola* and various types of *Laminaria* (Hernández-Carmona et al. 2013). Sodium alginate ($NaC_6H_7O_6$) is the sodium salt of alginic acid and is composed of poly-β-(1, 4) D-mannuronic acid and poly-α-(1, 4) L-guluronic acid (Xu et al. 2006; El-Mohdy 2017). It ranges from white to yellowish brown and have filamentous, granular and powdered forms and is widely used in pharmaceutical, biotechnological and food sectors (Mollah et al. 2009; Khan et al. 2011). Annexed Fig. 15.4 represents sodium alginate and its chief source. Consecutive effects of sodium alginate and its derivatives on various plants and microbes are also illustrated in the same figure.

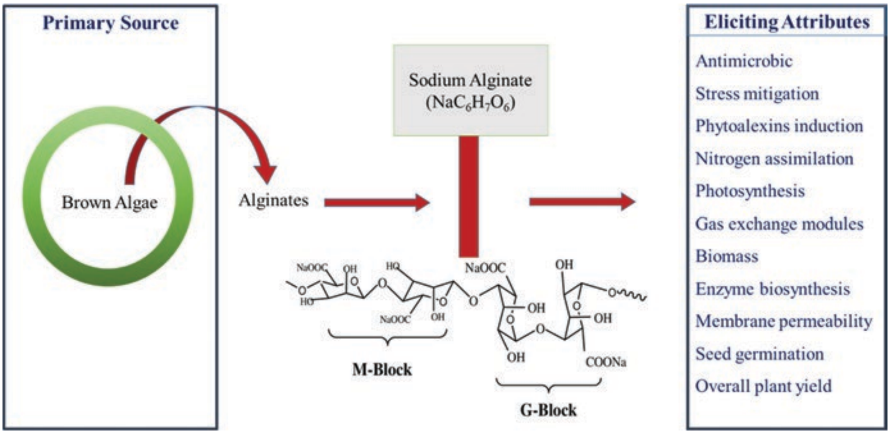

Fig. 15.4 Sodium alginate and its accompanied biological properties

15.4.1 Biological Activities of Sodium Alginate

Sodium alginate plays a major role in the structural component of the cell wall and intercellular matrix in organisms from Phaeophyceae and *Laminaria* (Hernández-Carmona et al. 2013). This marine polysaccharide consists of residues of mannuronic acid (M-block) and guluronic acid (G-block) (El-Mohdy 2017). Monomers are arranged in three types of block structure. These blocks may be homopolymeric block (M-block, G-block) or heteropolymeric block (MG-block). MG-block is known for its most flexible chain formation while M-block for its strong immunostimulating property (Pegg 2012). The fraction of mannuronic acid (M-block) and guluronic acid (G-block) of sodium alginate showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* (Hu et al. 2005).

Marine polysaccharides are highly reactive and peculiar compounds with thermo-reversible gel formation ability and have widespread use in pharmaceutical industry and bioengineering products (Hien et al. 2000; Aftab et al. 2014). Alginates are also exploited in drug delivery and as hydrogels for immobilizing cells and enzymes due to their mild conditions of cross-linking through bivalent cations (Ca^{2+}) (Russo et al. 2007; Liu and Li 2016). These characteristics can also be further altered by chemical modification, blending and integrating biodegradable additives which allows to tailor the final properties of the polysaccharides and opens the doors to wider applications, particularly in pharmaceutical area (Gomez d'Ayala et al. 2007).

Commercially, sodium alginates are also exploited in gel formation given their efficient and rapid water-absorbing property, sometimes absorbing multiple times of its own weight in water (Jamaludin et al. 2017). Moreover, carbohydrates like sodium alginate, chitosan, carrageenan, cellulose and pectin help in recycling bioresources and reducing environmental pollution. These carbohydrates in various forms can induce different kinds of biological activities including antimicrobial activity and phytoalexin induction (Kume et al. 2002).

15.4.2 Role of Sodium Alginate in Plant Growth Regulation

Sodium alginate in various forms and concentrations can impart a general trend of improved overall vegetative growth in different crops. SA has the potential to enhance plant height, biomass (both fresh and dry weight), number of tillers and leaves as well as leaf area (Hien et al. 2000; Iwasaki and Matsubara 2000; Kume et al. 2002; Hu et al. 2004; Hegazy et al. 2009; Mollah et al. 2009; Qureshi 2010; Sarfaraz et al. 2011; Naeem et al. 2015b). In addition to overall growth, sodium alginate renders stimulating effects on seed germination as well (Jamsheer 2010; Khan et al. 2011). The effect of alginate-derived oligosaccharide concentration on α- and β-amylase activities in different germination stages of maize seeds enhanced the seed germination by increasing the activities of several enzymes beneficial for germination (Hu et al. 2004). These SA-induced effects could also be attributed to the sodium alginate's interaction with plant cell signalling and its perceived regulation of gene expression (Khan et al. 2011).

These SA-induced responses can be understood by the fact that application of SA can induce multiple physiological and biochemical changes in plants. On a molecular level, SA is capable of regulating the biosynthesis of various enzymes (Ma et al. 2010; Khan et al. 2011) and the references therein) including those pertaining to nitrogen and carbon metabolism. Nitrate reductase (NR) is a key enzyme in nitrogen metabolism that assists in the first step of nitrogen assimilation in plant system through conversion of nitrate into nitrite. Thus, an efficient NR enzyme provides enough raw materials for the synthesis of various structural and functional biomolecules including amino acids and lipids. Various studies reported the direct influence of SA on the NR activity where it was found that SA can significantly ($p \leq 0.05$) upregulate the activity of NR enzyme in various crops of economic importance, e.g. fennel (Sarfaraz et al. 2011), lemongrass (Idrees et al. 2012) and mint (Naeem et al. 2012b). As a result, SA assist in maintaining a higher nitrogen content that ultimately influences the content of photosynthetic pigments (i.e. chlorophyll and carotenoid) through amino acid, protein and lipid biosynthesis (Idrees et al. 2012). Another key enzyme in carbon metabolism is carbonic anhydrase (CA). SA has also been reported to upregulate the CA activity in different crops (Luan et al. 2003; Khan et al. 2011; Naeem et al. 2015b).

Another aspect of SA-induced physiological response can be observed on plant gas exchange modules. Net photosynthetic rate (P_N) and stomatal conductance (g_s) can play a decisive role in determining overall plant growth and productivity. SA positively influence both P_N (Luan et al. 2003) and g_s (Naeem et al. 2015b) possibly because of the SA-induced photosynthetic pigment content (Mollah et al. 2009; Sarfaraz et al. 2011) and enhanced membrane permeability (Khan and Srivastava 1998). Due to its regulating effects on membrane permeability as well as on protoplast formation, alginate and its derivatives have also been labelled as endogenous elicitors (Akimoto et al. 1999). Similar regulating effects of SA on secondary metabolism were noted otherwise where the content of secondary metabolites exhibited positive correlation with sodium alginate application (Idrees et al. 2011; Khan et al. 2011; Naeem et al. 2015b).

Some other miscellaneous SA-induced effects on plant system includes enhanced water-use efficiency (Idrees et al. 2011); phosphoenolpyruvate (PEP) carboxylase activity and protein content (Idrees et al. 2012); content of nitrogen, phosphorus and potassium (leaf NPK); and phytoalexin induction (Mollah et al. 2009; Khan et al. 2011; Sarfaraz et al. 2011; Idrees et al. 2012). Sodium alginate has also been attributed to provide resistance against various disease and adverse environmental conditions, thus contributing in plant defence system by ameliorating antioxidant metabolism and reactive oxygen species pathway (Hien et al. 2000; Liu et al. 2009; Ali et al. 2014). The combination of *Alteromonas macleodii* (common marine bacterium), as exogenous elicitor, and alginate oligomers, acting as both endogenous elicitor and scavenger of active oxygen species, reportedly minimized the cell growth inhibition and enhanced 5′-phosphodiesterase production in periwinkle (Aoyagi et al. 2006).

Various researches have demonstrated that SA is significantly potent in imparting an eliciting effect on the overall productivity and plant yield. SA can enhance the weights of seed and capsule in opium (Khan et al. 2011), oil production in lemongrass (Idrees et al. 2012) and herbage yield in periwinkle (Naeem et al. 2015b) along with the productivity of barley, carrot, cabbage, maize, peanut rice, tea and tomato (Hien et al. 2000; Hu et al. 2004; Hegazy et al. 2009; Liu et al. 2009). Table 15.3 represents plant growth, productivity and immunity promotion in different crops by sodium alginate and its derivatives.

Similar to chitosan and carrageenan, sodium alginate is also an efficient plant growth regulator which not only hampers yield loss during adverse environment but also promotes overall plant growth and productivity. Although the exact mechanism for these effects is not yet fully known, in the light of current advancements, we can assume that these NPs interact extensively with various vital physiological processes which play a decisive role in determining the fate of overall plant growth and productivity (Chamnanmanoontham et al. 2014). A general idea for this mechanism can be understood by annexed Fig. 15.5., where a hypothetical model for oligomeric action of chitosan, carrageenan and sodium alginate in plants is portrayed.

15.5 Conclusion and Future Prospective

Marine polysaccharides (chitosan, carrageenan and sodium alginate) in various forms seem to enhance overall plant growth and productivity. It is now known that these marine polysaccharides can act as a signalling molecule which interact with plant physiology in a complex cascade mechanism and produce these favourable effects (Mercier et al. 2001; Khan et al. 2011; Shukla et al. 2016). While all the three

Table 15.3 Eliciting effects of sodium alginate and its derivatives on various plant species

Plant	Eliciting effects on	Reference
Maize	Seed germination, α- and β-amylase activity, overall growth and productivity	Hegazy et al. (2009), Hu et al. (2004)
Peanut	Shoot growth, plant biomass and defence mechanism	Hien et al. (2000)
Mint	Shoot growth, biomass and essential oil yield	Naeem et al. (2012b)
Periwinkle	Plant height, leaf area index, fresh weight and dry weight, increased enzyme activities and photosynthetic rate	Luan et al. (2003)
Red amaranth	Shoot growth, leaf number, leaf area, dry weight, chlorophyll and carotenoid contents and phytoalexin induction	Mollah et al. (2009)
Opium poppy	Plant length; dry weight; chlorophyll and carotenoid contents; activities of CA and NR enzymes; cell signalling; weights of seeds, capsule and crude opium; and alkaloid content	Khan et al. (2011)
Fennel	Contents of chlorophyll and carotenoid, efficiency of carbon and nitrogen assimilatory enzymes, leaf NPK content, plant height, leaf size, biomass and overall plant productivity	Sarfaraz et al. (2011)
Lemon-scented eucalyptus	Plant growth, fresh and dry weight, leaf number and size, photosynthetic pigments, leaf NPK and essential oil production	Ali et al. (2014)
Faba bean	Biomass, germination, plant height, leaf width and seed yield	El-Mohdy (2017)
Lemongrass	Shoot growth; leaf and tiller number; dry weight; photosynthetic pigments; leaf NPK content; activities of CA, NR and PEP carboxylase; protein content; and essential oil yield	Idrees et al. (2012)

reviewed polysaccharides, i.e. chitosan, carrageenan and sodium alginate, have a general positive influence on plant overall growth and productivity, they possess few attributes that make a distinction among them. While chitosan can be used in preservation and safety of food and dairy products, sodium alginate can be exploited as a gelling or hydrating agent (Piculell 1995; Chien and Chou 2006; Liu et al. 2007). Similarly, carrageenan can be extensively used in pharmaceutical and drug development industries.

Although this review assessed the role of chitosan, carrageenan and sodium alginate on plant biology, there are few questions still left to be answered. Further investigations could reveal important insights about the exact mechanism responsible for such NP-induced responses in various crops. Additionally, critical evaluation of such responses through transcriptomics, epigenetics, radiation biology and bioinformatics might give us a better understanding of the cross-talk of NPs with other signalling pathways in various crops.

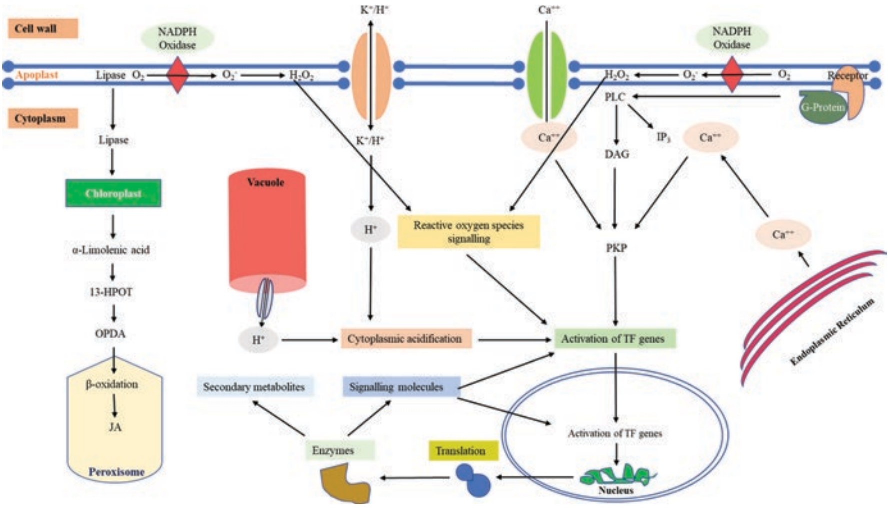

Fig. 15.5 Hypothetical model for the mechanism of carrageenan, chitosan and sodium alginate in oligomeric form. (13-HPOT, 13-hydroperoxy-linolenic acid; OPDA, 12-oxophytodienoic acid; DAG, diacylglycerol; IP_3, inositol triphosphate; JA, methyl jasmonate; PKP, protein kinase phosphatase; PLC, phospholipase C; TF, transcription factors)

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Chapter 16
Role of AM Fungi and PGPR in Alleviating Stress Responses and Inducing Defense Mechanism

Zeenat Mushtaq, Shahla Faizan, Basit Gulzar, and Humira Mushtaq

16.1 Introduction

Soil is a dynamic organization that supports the growth of plants. In soil ecosystem, plants are influenced by large number of biotic as well as abiotic stresses. The main abiotic stresses including salinity, drought, heavy metal, and temperature have harmful effects on plant growth and productivity (Ahmad and Prasad 2012; Mantri et al. 2012), and biotic stresses include viruses, bacteria, pests, etc. The negative impacts of biotic as well as abiotic stresses on agricultural productivity have occurred on global scale. Abiotic stresses are said as chief causes of physiological disorders of plants, hormonal imbalances, and yield reduction of agricultural crops. It was estimated that about 3.6 billion ha of world's dry land agriculture has been adversely affected by salt stress and other soil problems (Riadh et al. 2010). Application of chemical fertilizers and improper irrigation to agricultural fields had increased the soil salinization worldwide and caused about US$ 27.3 billion annual loss in crop production (Qadir et al. 2014). Due to the anthropogenic activities, climate change also took place and increased the global temperature from 1.8 to 4.0 °C (IPCC 2007) which is more vulnerable to plant growth and development (Lobell et al. 2011). Thus, plants are confronted with different stresses which affect their growth, physiological activities, and productivity.

Rhizosphere resides numerous microbes responsible for various root activities and metabolism in plants. These microbes include plant growth-promoting bacteria,

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fungi, protozoans, and algae. These microbes either freely or symbiotically influence positive effects on plants (Hayat et al. 2010; Saharan and Nehra 2011). Plant growth-promoting bacteria such as *Rhizobium*, *Pseudomonas*, *Bacillus*, *Azospirillum*, *and Azotobacter* are few bacteria that play a significant role in enhancing plant growth and productivity under stressed conditions (Mushtaq et al. 2020a; Nadeem et al. 2010). These bacteria induce the nitrogen fixation, siderophore production, solubilization of mineral elements, production of phytohormones, defense against pathogens, and disease resistance (Berg 2009). Besides PGPR, arbuscular mycorrhizal fungi also inhabit plant roots and showed symbiotic association with them. AMF penetrate the cortical cells of plant roots and form the special structure known as arbuscules which are involved in nutrient extractions. They increase the root surface area and enable the plants to absorb water as well as nutrients efficiently from large soil area (Fig. 16.1). AMF symbiosis with plants also provides them defense against abiotic stresses.

16.2 Stresses Affecting Plants

Stress is defined as an external condition or sudden changes in environment that adversely affect the plant growth by changing the cell metabolism, gene expression, and physiological activities and reduction of crop yield. Primarily stress in plants can be divided into two main types, viz., biotic stress and abiotic stress.

16.2.1 Biotic Stresses in Plants

Plant stress which is raised by living beings, particularly insects, viruses, nematodes, weeds, and some bacteria and fungi, is said as biotic stress. Biotic stress directly divests the nutrients of its host plant,causes different types of diseases in plant, damages it as well as reduces the crop yield, and in extreme conditions causes death of the plants. In agriculture, biotic stress can become major cause of pre-harvesting and post-harvesting losses.

16.3 Role of PGPR in Alleviating Biotic Stress in Plants

Various strategies are being employed to improve the plant growth and productivity under stress conditions. During the last few decades, soil microbes as of few discussed in Table 16.1 have been employed to increase the agricultural crop production, sustainability and induce stress tolerance in plants (Kumar and Verma 2018). These microbes are heterogeneous group of bacteria present in the root zone of plants. They are generally called as plant growth-promoting bacteria (PGPR) as

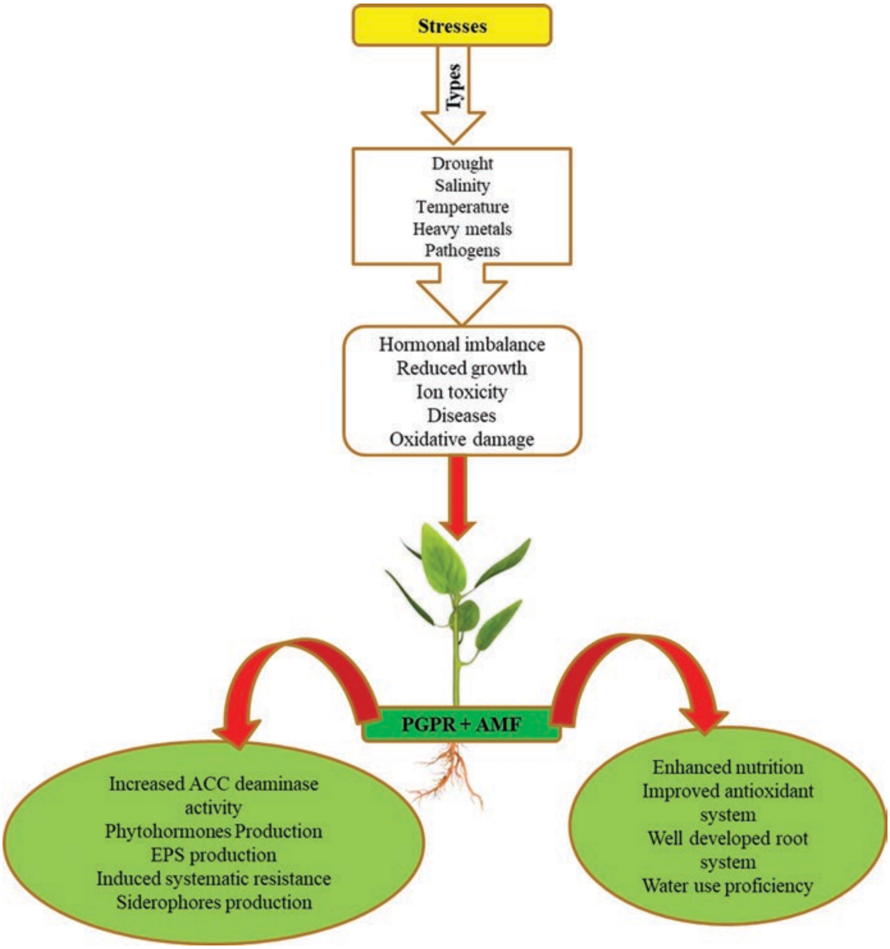

Fig. 16.1 Schematic diagram representing the effects of various stresses on plants and role of PGPR and AMF in alleviating stress in soil

they were found to increase the growth and development of plants under both stressed and non-stressed conditions and also maintained the soil health (Vimal et al. 2017). PGPR belonging to various genera such as *Rhizobium*, *Pseudomonas*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, etc. exhibited different strategies for alleviating stress in plants. PGPR colonize the rhizosphere of leguminous plants as well as non-leguminous plants with numerous biologically important functions. These microbes dynamically participate in enhancing the growth and development of plant through mobilization of nutrients, production of phytohormones which induce resistance against phytopathogens, production of exopolysaccharides and signal molecules (Tanaka et al. 2015; Dakora et al. 2015; Ansari et al. 2017). Some PGPR also secrete biosurfactants which target the phytopathogens and altered their

Table 16.1 Some plant growth-promoting rhizobacteria involved in alleviating stresses in plants

Stress	PGPR	Plant	Functions	References
Drought	*Trichoderma hamatum* DIS 219	*Theobroma cacao*	Improved net photosynthesis and stomatal conductance	Bae et al. (2009)
	Glomus mosseae	*Z. mays*	Increased plant biomass, chlorophyll content, soluble sugar and P uptake	Feng et al. (2002)
	Glomus intraradices	*Sorghum bicolor*	Increased the crop production up to 17.5%	Alizadeh et al. (2011)
	Glomus mosseae	*Vigna radiata*	Increased seed yield and P, N, and protein content and improved water use efficiency	Habibzadeh et al. (2012)
Salinity	*Glomus intraradices*	*Dianthus caryophyllus*	Increased number of flowers per plant	Navarro et al. (2012)
	Glomus mosseae	*Lycopersicon esculentum*	Enhanced fresh weight of fruits and yield production	Abdel-Latef and Chaoxing (2011)
	Glomus clarum	*Capsicum annum*	Enhanced dry biomass of root and shoot as well fruit yield	Kaya et al. (2009)
	Pseudomonas sp.	*Triticum aestivum*	Increased percent germination and root as well shoot growth	Egamberdieva and Kucharova (2009)
	Azotobacter sp. *C5 and C9*	*Zea mays*	Increased chlorophyll content and root dry weight	Rojas-Tapias et al. (2012)
	Bacillus licheniformis	*Capsicum annum*	Increased nutrient uptake and reduced Na content	Siddikee et al. (2011)
Heavy metal	*Glomus intraradices*	*Pisum sativum*	Decreased the harmful impact of cd	Rivera-Becerril et al. (2002)
	Cicer arietinum	*Mesorhizobium RC3*	Decreased the chromium content and increased nitrogen content in roots and shoots	Wani et al. (2008)
	Trifolium pratense	*Brevibacillus* sp.	Ameliorated lead toxicity and improved plant growth	Vivas et al. (2003)
	Brassica juncea	*Variovorax paradoxus*	Improved plant growth under toxic concentration of cd content	Belimov et al. (2005)
Pathogens				
	Carica papaya	*Bacillus* sp.	Protection from pathogen attack of *Meloidogyne incognita*	Jaizme-vega et al. (2006)
	Solanum lycopersicum	*B. coagulans*	Protection against *Meloidogyne incognita*	Serfoji et al. (2010)

(continued)

Table 16.1 (continued)

Stress	PGPR	Plant	Functions	References
	Fragaria ananassa	*Glomus* spp.	Reduced the attack of *Verticillium dahliae*	Tahmatsidou et al. (2006)
	Triticum aestivum	*Pseudomonas fluorescens*	*Strongly inhibited the growth of pathogenic fungi M. nivale*	Jaderlund et al. (2008)

membrane permeability as well as cell lysis. Siderophore-producing bacteria also protect the plants from pathogen attack by constraining the availability of iron (Zloch et al. 2016). Under stress conditions microbes that secrete rhizobitoxine stop the production of ethylene and promote the growth and development of plants. Signal molecules such as lipochitooligosaccharides and lumichrome also act as growth regulators in plants and were found to increase the growth and biomass production in plants as well as induced the defense against pathogens (Tanaka et al. 2015). It was observed that certain strains of *Bacillus* produced thuricin 17, which is a proteinaceous toxin substance and promotes the germination and growth of seeds under stressed conditions (Subramanian and Smith 2015). PGPR like *Pseudomonas*, *Bacillus*, and *Penicillium* produce antibiotics such as pyrrolnitrin, aminopolyol, kanosamine, colistin, circulin, polymyxin, etc. that can be implicated as potent biocontrol agents (Beneduzi et al. 2012). They synthesize hydrolytic enzymes such as chitinase, protease, and lipase and break the cell wall of pathogens (Maksimov et al. 2011).

16.3.1 Abiotic Stresses in Plants

Abiotic stresses can create more than 50% loss in yield production in plants mainly in arid and semi-arid regions (Shrivastava and Kumar 2015) put forth that abiotic stresses can create more than 50% loss in yield production in plants mainly in arid and semiarid regions.

Among all stresses in plants, salt stress is said as the most dangerous global problem that hinders plant growth and development via osmotic stress, ionic toxicity, nutritional imbalance and deficiency (N, P, K, Ca, Fe, and Zn) and the production of reactive oxygen species (Mushtaq et al. 2020b; Isayenkov 2012). Plants respond salt stress via two ways viz: ion-independent growth retardation and ion-dependent growth retardation. The firsts way usually occurs in plants within minutes to days and inhibits the cell expansion as well as triggers stomata closure (Rajendran et al. 2009). A second way took place from several days to weeks and slows the metabolic activities of plants and early senescence (Roy et al. 2014). Increased concentration of salt ions in plant affects almost all types of plant development, i.e., from germination till harvest (Netondo et al. 2004). At reproductive stage salt stress obstructs microsporogenesis, elongation of stamen, and senescence of embryos. Salt stress reduces the production as well as transportation of growth hormones and also declines the synthesis of photosynthetic pigment content (Ashraf 2004).

Similar to salt stress, drought stress is also one of the major factors affecting the plant growth and development adversely. Change in global temperature and increased atmospheric carbon dioxide level cause uneven precipitation which leads to drought stress. Drought stress primarily shrinks root growth and then all other growth stages of plants (Rahdari and Hoseini 2012) by changing the physiological and biochemical characteristics such as stomatal conductance, pigment as well as water content, transpiration rate and also disrupts the photosynthetic apparatus. It severely hinders the translocation of nutrients because water-soluble nutrients such as Ca, Si, and Mg are usually diffused from soil to plant parts with the help of water present in the rhizosphere (Vurukonda et al. 2016). It was observed that drought stress is a major threat to leguminous plants as drought obstructs the nitrogen-fixing processes in legumes (Serraj 2009) by reducing nitrate uptake and eventually the activity of nitrate reductase which is an important enzyme of N_2 fixation (Caravaca et al. 2005). Drought also decreases the nodule growth in legumes. Water deficiency in plants also leads to oxidative damage by increasing the formation of oxygen species such as hydrogen peroxide, superoxide radicles, singlet oxygen, and hydroxyl radicals. These free radicals damage the biomolecules and cause lipid peroxidation of membranes (Nair et al. 2008). Water defeciency also increases the ethylene foremation in plants that restricts the plant growth and development (Ali et al. 2009).

Unprecedented accretion of heavy metals has the ability to interact with DNA, proteins, and membrane lipids of plants. Heavy metals are inorganic persistent chemicals with density more than 5 g cm^{-3} having various hazardous effects on all living beings. Our earth contains two kinds of metals, which are commonly grouped in two categories, i.e., essential micronutrients and nonessential elements (Tangahu et al. 2011; Rascio and Navari-Izzo 2011). Metals in very minute quantity are essential for growth and metabolic activities of plants; however, increased quantity of them is toxic to plants and inhibits their growth and development by denaturing the proteins, formation of sulfhydryl bonds with protein molecules, and disrupting the activities of various enzymes involved in different metabolic processes (Hossain et al. 2012; Farid et al. 2013). Increased concentration of heavy metals also enhanced the generation of reactive oxygen species as well as cytotoxic compounds such as methylglyoxal in plants, which creates the oxidative stress within plant cells (Rellán-Álvarez et al. 2006; Sharma et al. 2012).

Plants are frequently exposed to varying temperatures both spatially and temporally. Temperature stress has severe effects on both cultivated and wild plants. In equatorial areas, temperature reaches up to 60 °C during daytime, whereas in polar regions during winter season, temperature dips up to −60 °C. Both high and low temperature can hamper the plant growth and development critically. Temperature fluctuations affect the genetic material of cell, change the membrane fluidity, and alter the structure of proteins and enzymes in plants. High temperature in plants increased the membrane fluidity, electrolyte leakage and elevated the levels of ROS, thus leading to cell death (Zinn et al. 2010). Low temperature or freezing stress also results in retarded growth and development in plants. Freezing stress results in degradation of starch granules in pollen grains and obstruction of pollen germination and pollen tube elongation (Shinada et al. 2013). It was also observed that temperature fluctuations result in wilting, chlorosis, or necrosis in plants (Ruelland and Zachowski 2010).

16.4 Role of PGPR in Alleviation of Abiotic Stress in Plants

Plant growth-promoting bacteria are probable agents for alleviating abiotic stresses like salinity, drought, heavy metals, and temperature because of their metabolic and genetic abilities (Gopalakrishnan et al. 2015). These microbes increases nutrient supply to plants, phytohormone production, bioaccumulation of inorganic compounds, bioremediation of heavy metals and also maintain soil properties etc. (Zhuang et al. 2007). *Rhizobium*, *Paenibacillus*, *Azospirillum*, *Burkholderia*, *Pantoea*, *Achromobacter*, *Microbacterium*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Methylobacterium*, and *Variovorax* are the most common bacteria which are reported to provide tolerance against various abiotic stresses (Grover et al. 2011). Microbes inhabiting the soil and plants influence the plant growth and development through their metabolic activities and interaction with host plant (Schmidt et al. 2014). These microbes colonize the rhizosphere of plants and promote the growth by inducing the production of phytohormones like indole-3-acetic acid, gibberellic acid, and cytokinins, synthesis of enzymes such as ACC deaminase that reduces the ethylene level in roots, production of exopolysaccharides, accumulation of osmolytes, and inducing systemic resistance (Dimkpa et al. 2009; Timmusk et al. 2014).

Bacterial phytohormone production and their capability to induce endogenous hormones play the important part in increasing tolerance against abiotic stresses. Dimkpa et al. (2009) observed that *Azospirillum* secreting IAA increased the drought tolerance in plants. Tomato plants inoculated with A. brasilense produce nitric oxide under drought conditions, which act as signalling molecule in IAA inducing mechanism and enhances the development of adventitious root formation in these plants (Molina-Favero et al. 2008). Under drought conditions maize and wheat plants inoculated with *A. brasilense* showed enhanced relative water content, increased root growth, and proline accumulation in foliage than non-inoculated plants, which was accredited to the formation of IAA by bacterial inoculants (Arzanesh et al. 2011). Soil microbes such as *Bacillus thuringiensis* and *Pseudomonas chlororaphis* were found to reduce the production of volatiles metabolites in plants under drought stress and resulted higher photosynthesis and plant biomass productivity (Timmusk et al. 2014). In wheat seedlings, water deficiency alters the composition of phospholipids,enhances phosphatidylcholine and decreases phosphatidylethanolamine, however, wheat seedlings inoculated with *Azospirillum* showed the positive results by preventing these unusual changes (Dimkpa et al. 2009). Drought stress also enhanced the ethylene production in plants which resulted in reduced growth of roots and shoots. In the ethylene biosynthetic pathway, S-adenosylmethionine is converted to aminocyclopropane-1-carboxylate by enzyme 1-aminocyclopropane-1-carboxylate synthase. It was observed that bacteria which produce ACC deaminase degrade the ACC in plants. The degradation of ACC by ACC deaminase diminishes the harmful effects of ethylene, increases the supply of nitrogen as well as energy to plants, ameliorates the stress, and endorses plant growth (Glick 2005).

Zahir et al. (2008) observed that inoculation of pea plants with *Pseudomonas fluorescens* which is an ACC deaminase-producing bacterium increased the root length of these plants and enhanced the water uptake from soil under drought conditions. PGPR like *P. fluorescens*, *Bacillus polymyxa*, *P. jessenii*, *Arthrobacter nitroguajacolicus*, etc. increased accumulation of osmolytes such as proline, betaines, sugars and various other amino acids in plants under drought stress. Osmolytes are compatible solutes, reduce the drought stress in plants by maintaining the water status of plant cell, protecting the cell membrane and proteins from degradation, and finally increasing the growth of plants (Shintu and Jayaram 2015; Armada et al. 2014). Formation of exopolysaccharides (EPS) by PGPR has been shown to increase the water and nutrient uptake in plants under water-deficit conditions. Soil microbes secrete EPS as slime material that form the protecive layer around the soil aggrigates, increases the water holding capicity of soil and eventually protects the microbes as well as plant roots from dessication under drought stress (Sandhya et al. 2009). In plants, drought stress also increases the generation of reactive oxygen species which induces oxidative damage of lipids, proteins, genetic material and impairs the normal functions of cells. It was reported that plants inoculated with PGPR showed significantly low oxidative damage than non-inoculated plants. These microbes increase the activity of enzymatic and nonenzymatic antioxidants and thus protect the plants from oxidative damages by scavenging the harmful reactive oxygen species and enhancing the drought tolerance of plants (Gusain et al. 2015).

Plant growth-promoting bacteria belonging to different genera have been testified to provide resistance to plants against salt stress. These bacteria include *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Azotobacter*, *Bacillus*, etc. (Grover et al. 2011). It was observed that several crop plants such as lettuce, tomato, bean, and pepper inoculated with PGPR showed enhanced growth under saline conditions (Yildirim and Taylor 2005). These microbes decreased the salt stress and increased the growth and productivity in plants via different mechanisms, few of which are described in this section. PGPR produce phytohormones such as auxin, cytokinins, gibberellins, and volatile compounds. Auxins, for example, indole-3-acetic acid and indole-3-butyric acid, are synthesized by *Actinobacteria*, *Streptomyces* sp., *Nocardia* sp., and *Frankia* species (Barreto et al. 2008; Péret et al. 2008; Dimkpa et al. 2008; El-Tarabily and Sivasithamparam 2006). These microbes were found to regulate the growth and development of plants under salt stress by increasing the formation of lateral roots as well as hypocotyl length, thus increasing the plant's access to soil nutrients (Zhao et al. 2001). It was reported that different strains of Bacillus produce cytokinin that played important role in promotion of plant growth under salt stress (López-Bucio et al. 2007). Bacterial strains such as *Bradyrhizobium*, *Rhizobium*, *Azospirillum*, and *Bacillus* were reported to produce gibberellin and promote the root and shoot development and yield production in wheat and rice under saline environment (Yanni et al. 2001; Fulchieri et al. 1993). The inoculation of plants with PGPR increased the production of enzymatic and nonenzymatic antioxidants that help them to overcome the oxidative stress. Bharti et al. (2016) reported that wheat plants under salt stress inoculated with *Dietziana tronolimnaea* STR1 exhibited increased gene expression of enzymatic antioxidants than plants that were not inoculated. Under saline conditions plants treated with PGPR revealed reduced ionic flux and electrolyte leakage. Kang et al. 2014; Fazal and Bano 2016 observed that inoculation of maize and cucumber plants with *Pseudomonas*, *Rhizobium*, and *Burkholderia* reduced the electrolyte leakage in these plants under salt stress. Thus plant growth-promoting rhizobacteria are the important agents that can retain the integrity of plant cell organelles as well as cellular membranes and help them to alleviate the deleterious effects of salt.

Temperature stress either high or low leads to hormonal imbalances and deficiency of water in cells and tissues of plants due to the high rate of evaporation, thus distressing the plant growth. However, PGPR such as *Pseudomonas putida*, *Burkholderia phytofirmans*, and various other strains of *Pseudomonas* have high temperature tolerance, and the plants treated with these microbes showed increased temperature resistance (Ali et al. 2009). Similarly various cold-tolerant microbes such as *Exiguobacterium acetylicum*, *Pantoea dispersa*, *Serratia marcescens*, *Pseudomonas lurida*, and *Pseudomonas fragi* have also been reported that are involved in the amelioration of deleterious effects caused by cold stress in plants (Chen et al. 2007; Selvakumar et al. 2012).

The accumulation of heavy metals in soil leads to heavy metal stress in plants. It was observed that PGPR have the efficiency for transformation of heavy metals in the plant's rhizosphere and thus alleviating the harmful effects of these metals on plant growth and productivity. These microbes mainly produce siderophores and other metal-chelating substances and protect the plants from these hazardous heavy metals (Dimkpa et al. 2009).

16.5 Role of AMF in Alleviating Stress in Plants and Mechanism of Action

Arbuscular mycorrhizal fungi (AMF) enable host plant to grow vigorously under stressful conditions by utilizing various mechanisms that lead to increased photosynthetic efficiency and nutrient and water uptake through root system from the soil. Numerous studies revealed that AMF symbiosis enhanced the resistance of various plants against stresses like salinity, drought, heavy metals, temperature, and pathogens by bringing about various changes in the morphological as well as in physiological charecters of host plants (Salam et al. 2017; Hashem et al. 2015). The different mechanisms that AMF are involved to increase the plant growth and productivity and provide resistance against stresses are phytohormone production, ACC deaminase activity, production of volatile compounds, improved nutrient and water uptake, antioxidant defense system, and induction of systemic resistance (Ahanger et al. 2017; Rodriguez et al. 2008).

There is a strong indication of drought stress amelioration by AMF symbiosis in various crop plants such as maize, barley, wheat, and soya bean (Ruiz-Lozano et al. 2015; Yooyongwech et al. 2016). The tolerance of plants toward water deficiency could be initially due to huge area of soil explored by radial hyphae of fungi and plant roots (Gutjahr and Paszkowski 2013). Plant-AMF symbiosis can regulate numerous morphological, physiological, as well as biochemical processes in plants. Various reports revealed that AMF colonization can modify morphological variations to enhance drought resistance of the host plant. Liu et al. (2016) reported that AMF inoculation changes the root morphology of trifoliate orange plants by increasing its root length, number of lateral roots, and root surface area under drought conditions. These morphological changes help the plant to absorb more water and nutrients from the soil, thus possibly increasing the drought tolerance of the host plant (Comas et al. 2013). The morphological changes induced by AMF inoculation may be due to the regulation of endogenous phytohormone production and polyamine metabolism (Wu et al. 2012). AMF colonization also modifies the physiological properties of host plants by enhancing the nutrition acquisition especially N, P, Ca, K, Zn, and Mn of plants through root system which resulted in enhanced leaf water relations, stomatal conductance, gas exchange, and transpiration rate (Mena-Violante et al. 2006). It was observed that AMF colonization can expedite ABA responses that adjust stomatal conductance and carry other physiological activities of host plant under drought stress (Ludwig-Müller 2010). AMF inoculation also increased the production of phytohormones as well as osmolytes and induced the antioxidant defense system to drought stressed plants. Higher accumulation of proline due to AMF inoculation has been observed in plants such as *Macadamia tetraphylla*, *Oryza sativa*, and *Prunus persica* under water-deficit conditions (Ruíz-Sánchez et al. 2011; Yooyongwech et al. 2013; Tuo et al. 2015), which provides these plants the greater capacity of osmotic adjustment against drought stress. Various researchers have reviewed that AMF inoculation increased the production of antioxidant defense enzymes and protect the plants against oxidative damage, thus increasing drought tolerance.

Efforts are being made to discover possible ways of accomplishing enhanced crop production under salt conditions (Abdel Latef and Chaoxing 2014). AMF inoculation improves the physiological activities such as water absorption capability, nutrient uptake, accumulation of osmolytes (proline, betaine), carbohydrate composition like mannitol and sucrose, stomatal conductance, and transpiration rate in plants under salt stress conditions (Jahromi et al. 2008; Daei et al. 2009). *Antirrhinum majus* inoculated with AMF showed enhanced growth and water use efficiency under saline conditions. Peanut plants inoculated with AMF showed increased chlorophyll content than non-inoculated ones. Under salt conditions, excess generation of reactive oxygen species took place that causes the oxidative damage to plants. However, it was observed that symbiotic association of AMF with host plants increased the production of enzymatic antioxidants such as SOD, POD, CAT, and

APX and protected the plants against salt stress. AMF colonization can effectively increase the levels of growth regulators.

AMF colonization plays a key role in mitigating the heavy metal contamination of plants. These fungi colonize the host root system, increase the root surface area, accumulate the metal cations, and prevent their entry to the plants (Joner et al. 2000). Under heavy metal stress, plants colonized with AMF produces the protein family called glomalines that can precipitate the metal ions and secrete chemical substances such as lactic acid, malic acid, etc. which have the capability of forming complexes with heavy metals present in plants, thus reducing their quantity (Gomathy et al. 2011). It was observed that colonization of wheat plants with Funneliformis mosseae under cadmium stress resulted its enhanced growth, photosynthetic pigment content, increased biomass production, and less cadmium content in root system (Shahabivand et al. 2012). Similar report was observed in wheat cultivars under excess boron concentration by Sonmez et al. (2009).

16.6 Role of AMF in Mitigating Biotic Stress

The arbuscular mycorrhizal fungi symbiosis infers notable changes in the physiology of host plant. AMF symbiosis has the ability to reduced the soil borne pathogen attack as well as diseases caused by these pathogen in plants and thus can be used as potent biocontrol agent for plant diseases (Bagy et al. 2019). AMF colonization makes noticeable alterations in root system of host plant, modifying the dynamics of pathogens and altering the microbial abundance with the help of stimulation of exudates having antagonistic action to various root pathogens (Barea et al. 2005). AMF colonization enhanced the nutrient uptake of plants and makes the host plant stronger as well as resistant, compensating for the attack triggered by phytopathogens (Tahat et al. 2010). AMF symbiosis also increased the synthesis of photosynthetic activity of host plant and increased the demand of carbon compounds which may also inhibit the growth of phytopathogens. The important change that AMF symbiosis brings is defense mechanism. AMF inoculation also increased the production of secondary metabolites such as phytoalexins, proteins, chitinases, glucanases, phenols and enzymes involved in phenylpropanoid pathway, and hydroxyproline-rich glycoproteins that can act as biocontrol agents (Jung et al. 2012; Siddiqui 2006).

16.7 Conclusions

Biotic and abiotic stresses are the severe constraints to agricultural productivity. PGPR and AMF play an important role in conferring tolerance and adaptation of plants to different stresses and have the prospective part in resolving future food safety problems. The interaction of plants with PGPR and AMF under stress conditions not only affects the plant but also modifies the soil properties. The mechanisms such as maintaining water status, phytohormone production, siderophore synthesis, antioxidant defense system, and inducing resistance against pathogens in plants by AMF and PGPR inoculation play important role in ensuring the better growth of plants under abiotic as well as biotic stress conditions. The development of stress-tolerant agricultural crops through crop breeding and genetic engineering is indispensible but is both costly and time-taking process. However, PGPR and AMF inoculation to ameliorate abiotic as well as biotic stresses in plants opens a new window to researchers and may be considered as good tool in alleviating stresses. Thus, future research is required in terms of documentation of the proper types of microorganisms and field assessment of potential organisms in diverse range of crops.

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Chapter 17
Arbuscular Mycorrhizal Fungi: The Natural Biotechnological Tools for Sustainable Crop Production Under Saline Soils in the Modern Era of Climate Change

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Abbreviations

ABA	Abscisic acid
AMF	Arbuscular mycorrhizal fungi
APX	Ascorbate peroxidase
AsA	Ascorbate
CAT	Catalase
DHAR	Dehydroascorbate reductase
FAO	Food and Agricultural Organization
GEF	Global Environment Facility
GIPs	GlpF-like intrinsic proteins
GR	Glutathione reductase
GSH	Glutathione
HIPs	Hybrid intrinsic proteins

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JA	Jasmonic acid
NIPs	NOD26-like intrinsic proteins
PIPs	Plasma membrane intrinsic protein
PHs	Phytohormones
Put	Putrescine
ROS	Reactive oxygen species
SIPs	Small basic intrinsic proteins
SLs	Strigolactones
SOD	Superoxide dismutase
Spd	Spermidine
Spm	Spermine
TIPs	Tonoplast intrinsic proteins
UN	United Nations
XIPs	X-intrinsic proteins

17.1 Introduction

The population of the world is increasing rapidly. The world's population is predicted to rise 9.7 billion by the year 2050 (UN, 2017). To feed this increased population, an increase of 70–100% of overall food production will be needed. Total demand for cereal foods for both human food and animal feed will be around 3 billion tonnes by 2050 (Borlaug 2007; Guillon et al. 2012). Furthermore, global climate change poses a serious threat to crop production due to the steady increase in biotic and abiotic stresses. Therefore, novel approaches are needed for promoting sustainable agriculture for achieving this targeted food production.

Degradation of land and deterioration of the environment are two major problems in agriculture. Scientists recently warned that 24 billion tons of fertile soil are being lost per year, largely due to unsustainable agriculture practices (Möhner and Klein 2007; Karamesouti et al. 2015; GEF 2020). It is estimated that about 25% of the total global land area has been degraded resulting in substantial economic impacts on agricultural livelihoods and national economies especially in lower-income countries (Le et al. 2016; Nkonya et al. 2016). If this trend continues, 95% of the Earth's land area will become degraded by 2050 (GEF 2020). Land degradation resulted in deterioration or loss of the soil productivity, which is directly linked to higher food price, climate change, environmental hazard, and the loss of biodiversity and ecosystem services (GEF 2020). The alarming pace of land degradation is contributing to a dramatic decline in the productivity of croplands and rangelands worldwide (GEF 2020).

Soil salinity is also considered as the most important abiotic stress which is responsible for land degradation particularly in arid and semi-arid regions, lead to cause a major challenge to sustainable agriculture (Al-Karaki 2006; Wicke et al. 2011; Mohamed et al. 2019). Soil salinity has been recognized as the most important problem for crop production worldwide (FAO 2008). However, the Mediterranean Basin (Nedjimi et al. 2006), Australia, Central Asia, the Middle East and Northern Africa (Yensen 2008; FAO 2008) are considered as the emerging regions in the risk of increasing levels of salinization (Fig. 17.1). Leaching of excessive salts or desalinizing seawater for use in irrigation (Muralev et al. 1997; Lal 2015; Gomiero 2016) is an important method employed to combat salt stress. Though successful, these approaches are costly and beyond the economic means of developing nations (Cantrell and Linderman 2001; Gomiero 2016). To deal with saline soils and minimize crop loss, new salt-tolerant crop plants (Glenn and O'Leary 1985) developed through classical breeding (Cuartero and Fernandez-Munoz 1999) and genetic engineering have been considered (Wei-Feng et al. 2008). For example, a transgenic *Arabidopsis thaliana* carrying a peroximal-type ascorbate peroxidase (APX) gene from barley showed higher salt tolerance than the wild type (Wei-Feng et al. (2008). Besides these approaches, several lines of evidence indicate that arbuscular mycorrhizal fungi (AMF) promote plant growth and enhance salinity tolerance by employing various mechanisms including enhanced nutrient acquisition by AMF-colonized plant roots (Al-Karaki and Al-Raddad 1997; Muthukumar et al. 2017). In fact, the AMF facilitate host plants to grow vigorously under stressful conditions by mediating a series of complex communication events between the plant and the fungus. Nearly 90% of plant species including flowering plants, bryophytes and ferns can develop interdependent connections with AMF (Zhu et al. 2010; Ahanger et al. 2014). The AMF form vesicles, arbuscules and hyphae in roots and also spores and hyphae in the rhizosphere. Formation of hyphal network by the AMF with plant roots significantly enhances the access of roots to a large soil surface area, causing improvement in plant growth (Bowles et al. 2016). Although a large body of literature is available on the enhancement of growth and

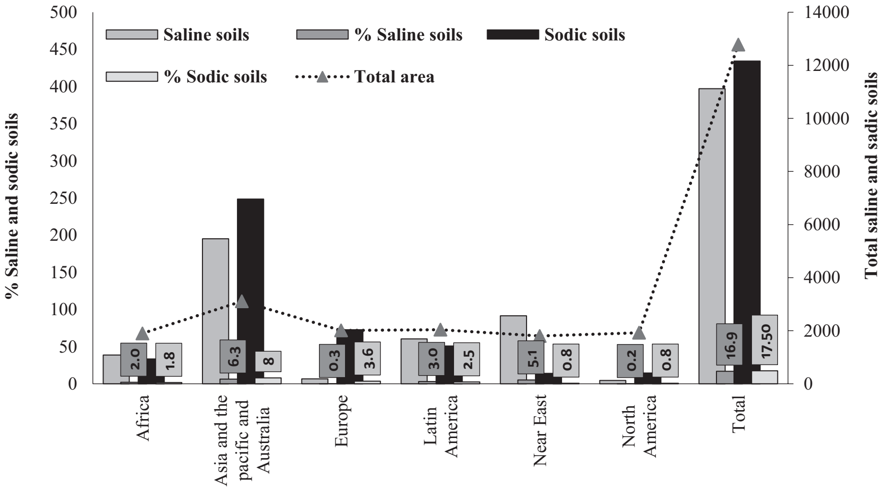

Fig. 17.1 Total saline and sodic soils and their per cent coverage in all continents

yield of crop plants in saline soils, no comprehensive review has so far been published. This chapter reviews the occurrence of AMF in saline soils and their effects on growth and salinity tolerance in host plants. The alleviation of salt stress in plants by AMF and their underlying molecular mechanisms including changes in biochemical, physiological, and molecular properties in host plants to alleviate salt stress are also discussed. Furthermore, the knowledge gap and areas of further research for better understanding the plant-AMF symbiosis were identified and discussed in this review.

17.2 Consequences of Soil Salinity for Sustainable Crop Production

According to FAO's Land and Plant Nutrition Management Service, over 6% of the world's land is affected by soil salinity. Of the current 230 Mha of irrigated land, 45 Mha are salt-affected (19.5%), and of the 1500 Mha under dryland agriculture, 32 Mha are varying levels of salt-affected (2.1%) (FAO 2016; Table 17.1). Table 17.1 also provides the updated information on the technical production potential of biomass in salt-exaggerated soils that were estimated per grid cell in Table through multiplying the available salt-affected area by the yield corresponding to the climate and soil characteristics of the grid cell (Wicke et al. 2011).

Salt-affected land is assumed to be available if it is not classified as forest, wetland, unsuitable areas (e.g. urban areas) or (inter)nationally protected areas. Agricultural land is not excluded in the potential assessment because conversion to a forestry plantation can reduce the risk of further degradation of the land and may even help improve the soil (Bensel 2008; Acharya and Kafle 2009). However, the use of agricultural salt-affected land for biomass production may not be desirable for various reasons, most importantly food insecurity and (in)direct land use change. Therefore, the fraction of the technical potential originating from agricultural land is distinguished. Numerous causes are involved in soil salinity comprising natural or human-induced processes that result in the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth. Natural causes such as the microscopic salt particles carried by the wind to inland from the oceans are a primary cause of soil salinity. Some anthropic causes include irrigation with saline water (Yensen 2008; Stringer 2012; Osman 2014).

The direct effects of salt on plant growth may involve:

(a) Reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought. To counteract this problem, plants must maintain lower internal osmotic potentials to prevent water movement from roots into the plant soil (Jahromi et al. 2008).

(b) Toxicity of excessive Na^+ and Cl^- ions towards the cell. The toxic effects of soil salinity include disruption to the structure of enzymes and other macromolecules in plants' cell, damage to cell organelles and plasma membrane and

Table 17.1 Technical biomass production potential by type and severity of salt-affectedness, land use/cover and regions globally (Data source: Wicke et al. 2011; FAO 2016)

Types of soil salinity	Severity	Land use/ cover	East Asia	South East Asia	Japan	Middle East	Former USSR	East Africa	West Africa	South Africa	North Africa	East Europe	West Europe	South America	Central America	USA	Canada	Oceania	Total
Salt-affected land (Mha)			98	58	0	176	126	56	83	22	161	2	1	84	5	77	7	169	1128
Salt-affected land excluding forest, wetlands, unsuitable & high biodiversity areas (Mha)			83	50	0	158	117	43	76	19	157	1	1	57	4	58	5	144	971
			Global technical biomass production potential (EJ y^{-1})																
Saline	Slight	Agriculture	246	0	0	135	2344	21	0	27	2	1	1	982	1	25	0	453	5502
		Shrub/herb	129	0	0	511	3305	661	0	812	267	3	0	354	73	204	0	122	21,832
		Bare areas	96	0	0	506	579	69	0	1	139	0	0	15	0	0	0	82	1590
		Total	163	0	0	115	6227	751	0	840	409	4	1	1350	75	206	0	128	28,924
	Moderate	Agriculture	5	0	0	12	28	56	0	13	1	0	0	4	12	0	0	0	132
		Shrub/herb	22	0	0	113	57	345	0	488	28	0	0	107	143	0	0	0	1303
		Bare areas	30	0	0	126	61	71	0	0	69	0	0	31	0	0	0	0	388
		Total	57	0	0	252	146	471	0	501	98	0	0	142	155	0	0	0	1823
	Very	Agriculture	7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	8
		Shrub/herb	2	0	0	0	1	0	0	19	0	0	0	0	0	0	0	0	22
		Bare areas	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
		Total	9	0	0	0	1	0	0	20	0	0	0	0	0	0	0	0	31
	Extreme	Agriculture	0	0	0	0	4	0	0	0	0	0	0	1	0	0	0	0	5
		Shrub/herb	0	0	0	0	7	1	0	0	0	0	0	2	0	0	0	0	9
		Bare areas	0	0	0	0	9	0	0	0	0	0	0	1	0	0	0	0	9
		Total	0	0	0	0	19	1	0	0	0	0	0	4	0	0	0	0	24
	Total		70	0	0	140	6394	1223	0	136	506	4	1	1496	229	206	0	128	30,800

(continued)

Table 17.1 (continued)

Types of soil salinity	Severity	Land use/ cover	East Asia	South East Asia	Japan	Middle East	Former USSR	East Africa	West Africa	South Africa	North Africa	East Europe	West Europe	South America	Central America	USA	Canada	Oceania	Total
Sodic	Slight	Agriculture	17	367	0	0	233	72	39	135	107	38	12	410	1	166	237	348	2243
		Shrub/herb	40	44	0	0	668	2087	69	132	33	0	1	300	0	237	245	931	4796
		Bare areas	34	0	0	0	403	5	4	0	0	0	0	27	0	0	0	91	564
		Total	90	411	0	0	1304	2164	112	267	140	38	13	737	1	403	482	137	7603
	Moderate	Agriculture	378	0	0	1	806	224	221	18	212	54	0	1349	0	187	117	860	4583
		Shrub/herb	129	0	0	3	855	605	179	80	65	2	0	949	0	260	61	501	8224
		Bare areas	3	0	0	0	238	0	0	0	0	0	0	6	0	0	0	90	341
		Total	509	0	0	4	1899	829	400	98	277	57	0	2304	0	447	178	596	13,147
	Very	Agriculture	0	0	0	0	0	17	0	0	0	0	0	480	0	0	0	0	497
		Shrub/herb	0	0	0	0	0	163	0	0	3	0	0	128	0	0	0	0	294
		Bare areas	0	0	0	0	0	0	0	0	0	0	0	24	0	0	0	0	24
		Total	0	0	0	0	0	179	0	0	3	0	0	632	0	0	0	0	814
	Extreme	Agriculture	0	36	0	0	0	8	0	2	0	0	0	11	0	0	0	0	78
		Shrub/herb	0	3	0	0	0	14	0	39	8	1	0	3	0	0	0	0	69
		Bare areas	0	0	0	0	0	0	0	0	12	0	0	2	0	0	0	0	14
		Total	0	39	0	0	0	22	0	42	20	21	0	16	0	0	0	0	160
	Total		600	450	0	4	3204	3195	511	407	439	116	13	3688	1	849	661	733	21,725

Saline-sodic	Slight	Agriculture	0	0	0	0	0	109	2	0	0	0	0	0	0	0	0	0	111
		Shrub/herb	0	0	0	0	0	154	0	0	0	0	0	0	0	0	0	0	154
		Bare areas	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
		Total	0	0	0	0	0	263	3	0	0	0	0	0	0	0	0	0	267
	Moderate	Agriculture	35	0	0	0	20	9	0	1	1	9	8	6	0	0	0	0	88
		Shrub/herb	8	0	0	0	21	167	0	51	27	0	0	350	0	0	0	0	625
		Bare areas	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	6
		Total	43	0	0	0	46	176	0	52	28	9	8	357	0	0	0	0	718
	Very	Agriculture	0	0	0	22	0	0	47	7	1	1	0	0	0	0	2	0	840
		Shrub/herb	2	0	0	40	0	47	12	13	18	1	0	0	0	15	1	0	172
		Bare areas	0	0	0	48	0	0	5	0	11	0	0	0	0	0	0	0	66
		Total	2	0	0	111	0	47	64	20	30	1	0	0	0	15	3	0	1078
	Extreme	Agriculture	38	76	0	80	80	11	58	42	30	19	14	1	18	3	1	0	557
		Shrub/herb	21	2	0	117	168	32	165	79	61	5	1	3	12	26	0	48	755
		Bare areas	3	0	0	180	126	0	3	7	18	0	0	1	0	0	0	0	348
		Total	62	78	0	378	375	43	226	128	109	25	15	6	30	28	1	48	1660
	Total		107	78	0	488	420	529	293	199	168	35	23	363	30	43	5	48	3723
Total			240	528	0	189	1001	4947	804	196	111	155	37	5547	260	296	665	201	56,248

disruption of photosynthesis, respiration and protein synthesis (Feng et al. 2002; von Braun and Gerber 2012; Van Lynden et al. 2004).

(c) Nutrient imbalance in the plant body caused by impaired nutrient uptake and/or transport to the shoot leading to ion deficiencies (Adiku et al. 2001).

17.3 Importance of Arbuscular Mycorrhizal Fungi for Sustainable Crop Production

Plants, in their natural environment, are colonized both by myriad of external and internal microorganisms. Some microorganisms, particularly beneficial bacteria and fungi, can improve plant performance and improve yield under stressful environments (Creus et al. 1998). The AMF is associated with the roots of most of the terrestrial plant species (Hejiden et al. 1998). The AMF penetrate the cortical cells of the roots of a vascular plant as root obligate biotrophs that exchange mutual benefits with plants species including many crops for providing with water, nutrients and pathogen protection, in exchange for photosynthetic products (Smith and Read 2008; Berruti et al. 2016). The AMF is characterized by the formation of unique structures, arbuscules and vesicles by fungi of the phylum *Glomeromycota*. They help plants to capture nutrients such as phosphorus, sulphur, nitrogen and micronutrients from the soil. It is believed that the development of the AMF symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of the vascular plants. The plant-AMF symbiosis is a highly evolved mutualistic relationship found between fungi and plants (Schüßler and Walker 2010). In this context, biological processes such as AMF application to alleviate salt stress would be a better option, since it has been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by employing various mechanisms, such as enhancing nutrient acquisition (Al-Karaki and Al-Raddad 1997), producing plant growth hormones, improving rhizospheric and soil conditions (Lindermann 1994), altering the physiological and biochemical properties of the host (Smith and Read 1995) and defending roots against soil-borne pathogens. Besides, AMF can improve host physiological processes like water absorption capacity of plants by increasing root hydraulic conductivity and favourably adjusting the osmotic balance and composition of carbohydrates (Ruiz-Lozano 2003). This may lead to increased plant growth and subsequent dilution of toxic ion effect (Juniper and Abbott 1993). These benefits of AMF have prompted it to be a suitable candidate for bio-amelioration of saline soils.

17.4 Arbuscular Mycorrhizal Fungi Are a Natural Ameliorator of Salt Stress

Naturally, a wide range of soil microorganisms are associated with plants which influence their growth and development. Some of these microorganisms help plants to survive under adverse conditions. The AMF belonging to the phylum *Glomeromycota* are an important group of fungi that are associated with most of the cultivated field crops (Smith and Read 2008; Bagyaraj 2011, 2014). The influence of AMF symbiosis on plant growth mainly depends on the ability of fungi to take up and transfer limiting soil nutrients, especially phosphorus (P) to plant roots in exchange for plant photosynthates (Desai et al. 2016). The role of AMF to alleviate soil salinity through improving plant's tolerance to salinity has been extensively reviewed in the recent and past (Ruiz-Lozano et al. 2015). Despite the common occurrence of AMF, plants in saline habitats like the mangroves often trend to be non-AMF (Brundrett 2009). The average density of spores in saline areas is reported to be low or absent observed by some researchers (Carvalho et al. 2001) to high as in non-saline soils (Aliasgharzadeh et al. 2001; Landwehr et al. 2002). Similarly, Wilde et al. (2009) reported that AMF spore populations of 36–951/10 g in soil from salt marshes at the Dutch Island of Terschelling and Schreyahn in northern Germany. The occurrence of AMF in salt marsh plants has also been reported by many authors (Carvalho et al. 2001; Hilderbrandt et al. 2001).

17.4.1 Alleviates Salt-Induced Oxidative Stress

Soil salinization is a serious environmental constraint for crop production throughout the globe, particularly the arid and semi-arid regions (Giri et al. 2003; Hameed et al. 2014; Muthukumar et al. 2017). According to Wang et al. (2004), 50% of the land will be lost by salinity before the end of the twenty-first century. Na^+Cl^- is responsible for both hyperionic and hyperosmotic stress in plants due to the accumulation of Na^+ ions, which impairs many cellular processes in plants such as photosynthesis and respiration and also affects the functions of the cell plasma membrane. Increased Na^+ accumulation alters the basic structure of the soil and results in decreasing soil porosity, consequently reducing soil aeration and conductance of water (Porcel et al. 2012). Its accumulation also creates low water potential in the soil and hampers the uptake of water and mineral nutrients (Porcel et al. 2012).

Both enzymatic and non-enzymatic antioxidants are present in plant cells, which mediate the scavenging of ROS and provide protection against oxidative damage (Ahmad et al. 2010, 2012). Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Apel and Hirt 2004). The non-enzymatic antioxidants such as ascorbic acid (AsA), tocopherols, phenols and thiols in combination with other antioxidants and phytohormones contribute to improve tolerance of plant against Na^+Cl^- stress (Ahmad et al. 2010; Ahmed et al. 2015; Rasool et al. 2013; Hashem et al. 2015).

In natural ecosystems, many microorganisms such as bacteria and fungi can colonize the roots of almost all plant species. The AMF are ubiquitous as compared to other organisms inhabiting the rhizosphere. They enhance plant growth and development by helping plants in the nutrient uptake and improving the rhizospheric soil health (Al-Khaliel 2010). The AMF improve several physiological processes in host plants including water absorption potential by increasing the hydraulic conductivity of roots (Ruiz-Lozano 2003). These changes improve the growth of plants and subsequently recede the toxic ionic effect induced by salinity (Juniper and Abbott 1993). The positive role of AMF in mitigating Na^+Cl^- stress is also reported in *Ocimum basilicum* (Hashem et al. 2016) and *Solanum lycopersicum* (Balliu et al. 2015). Symbiotic association of AMF with plants opens new alternatives for pyramiding strategies against salt stress (Dodd and Pérez-Alfocea 2012).

Although arbuscular mycorrhizal fungi have been shown to increase crop yield under saline soils (Daei et al. 2009), nevertheless, results on the influence of salinity on AMF formation and function are often contradictory. Some studies have shown that soil salinity reduces root colonization by AMF and increases plants' mycorrhizal dependency (Tian et al. 2004; Sheng et al. 2008). In contrast, it has also been shown that AMF colonization either remains unaffected (Yamato et al. 2008) or even increased under salt stress (Aliasgharzadeh et al. 2001). Increased soil salinity has also been shown to adversely affect the production of extra-radical hyphae of AMF strains that are sensitive to salinity (Juniper and Abbott 2006; Evelin et al. 2009). The extent to which salinity reduces AMF colonization depends on the stage of the association such that inhibition is more prominent during early stages of the symbiosis development than during the later stages (McMillen et al. 1998). For example, salinity inhibited early colonization of plant roots by *Gigaspora decipiens* more than by *Scutellospora calospora* (Juniper and Abbott 2006). It has been shown that AMF alleviate salt stress in some plants through modifications in physiological mechanisms (Evelin et al. 2009; Porcel et al. 2012). However, the adjustment of osmotic potential by settling down of soluble sugars in mycorrhizal fungal parts has been suggested to protect the plant from salinity (Soliman et al. 2012). For instance, trehalose present in the spores and extra-radical mycelium enables AMF to colonize host plants even under high salinity (Schubert et al. 1992). Several studies have reported that salt stress induces modifications in plants even at ultrastructural levels (Andrea and Tani 2009). Recently, Evelin et al. (2013) showed that the ultrastructural changes in AMF-inoculated fenugreek (*Trigonella foenum-graecum*) plants exposed to four different levels of salinity were less than the non-mycorrhizal plants. Studies have also shown that some AMF can adapt to different environmental conditions better than others (Stahl and Christensen 1991). Thus, the varied observations reported by different workers may partly reflect the differences between the fungi used and their ability to adapt to various environments. Nevertheless, most of the studies examining mycorrhiza and soil salinity to date have not considered these differences.

Arbuscular mycorrhizal fungi-mediated enhanced salt stress tolerance has been shown for many crops including chilli (*Capsicum annuum*) (Çekiç et al. 2012), Chinese milk vetch (*Astragalus sinicus*) (Peng et al. 2014), pepper (*Piper nigrum*) (Turkmen et al. 2008; Kaya et al. 2009), fenugreek (Evelin et al. 2012), corn (Sheng et al. 2008, 2011), bajra (*Pennisetum glaucum*) (Borde et al. 2017), tomato (Hajiboland et al. 2010) and clover (*Trifolium alexandrinum*) (Gharineh et al. 2009). Like drought stress, an increased P uptake mediated by AMF has been suggested to alleviate saline stress (Tian et al. 2004). However, in certain cases, salinity tolerance of mycorrhizal plants appears to be independent of P concentration (Feng et al. 2002). Both differences in the ability between AMF to obtain P from the soil and their ability to adapt to changing edaphic conditions (del Val et al. 1999) could reason for varied sensitivities of AMF to salinity. Therefore, it might be expected that an isolate originating from saline soil would have higher adaptability and a greater capacity to promote plant growth under saline environments. Several studies investigating the role of AMF in protection against salt stress have demonstrated that the symbiosis often results in increased nutrient uptake, accumulation of an osmoregulator, an increase in photosynthetic rate and water use efficiency. Figure 17.2 is showing the mechanism of AMF for alleviating the adverse effect of salt stress in plants. During AMF symbiosis, it creates an appressorium (ap) on the surface of the

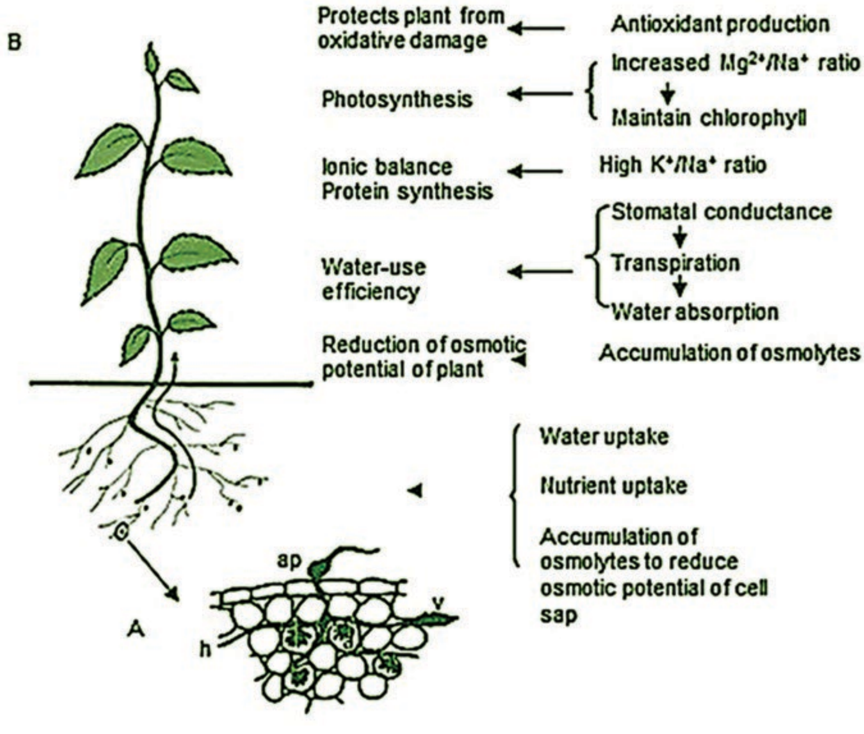

Fig. 17.2 Physiological mechanism of AMF symbiosis for enhancing salt tolerance in plants

plant roots and then enters into the root cortex through expanding its hyphae (h). The hyphae then form arbuscules (a) and vesicles (v) in the root cortex. It is well-noted that during salinity stress, there is a limitation to uptake of water and nutrients. However, AMF + plants symbiosis helps plants for improving water and nutrient uptake under salt stress. The underlying diverse mechanisms involved in enhancement of the plant's tolerance to salinity by the symbiosis of AMF are discussed in the following sections.

17.4.2 AMF Influence to Nutrient Uptake to Cope with Soil Salinity

The AMF has encouraging influence on the uptake and composition of plant nutrients under salt stress through enhancing the uptake of nutrients. AMF primarily controls the supply of nutrients to the root system (Giri and Mukerji 2004; Evelin et al. 2009) and then promotes them for transportation (Al-Karaki 2000; Sharifi et al. 2007). AMF dependency upsurges with the increasing level of salt concentrations (Giri and Mukerji 2004). Since the dependency is differed based on fungus isolates and also species of salt-affected plant (Tian et al. 2004). Table 17.2 shows several earlier findings on the effect of salinity on the nutrient uptake of mycorrhizal plants.

AMF-mediated plants could survive against salt stress through alleviating its adverse effects on nutrient uptake in plants, which are discussed in the following subsections.

17.4.2.1 Nitrogen

Salinity inhibits the uptake of available nitrogen (N) through affecting several phases of N metabolic rate, including NO_3^- uptake and reduction, and protein synthesis (Frechill et al. 2001). Use of AMF can assist to better absorption of N in the host plant under stress condition. Giri and Mukerji (2004) documented a greater uptake of N in shoots of AMF-*Sesbania grandiflora* and AMF-*S. aegyptiaca* plants than non-AMF-plants. It is due to the formation of widespread hyphae and extra-radical mycelia which help to uptake N as NO_3^- and integrated it via nitrate reductase, situated in the arbuscule-containing cells (Kaldorf et al. 1998; Govindarajulu et al. 2005). The formation of obtainable N (NO_3^- or NH_4^+) forcefully influences the accumulation of Na^+ under soil salinity that leads to reduction of ions' toxicity in plants (Giri and Mukerji 2004).

Table 17.2 Several earlier findings related to the nutrient uptake of mycorrhizal plants under saline condition

§Level of salinity	Plant species	AMF	% Symbiosis of AMF with plants	References
1.4–7.4dSm^{-1}	*Lycopersicon esculentum*	*Glomus mosseae*	49.6–36	Al-Karaki (2000)
2–12dSm^{-1}	*Lactuca sativa*	Mixture of *Glomus*, *Acaulospora* and *Entrosphora* spp. procured from (a) saline playa or (b) non-saline vegetable farm	(a) 43 0.0–26.2 (b) 34 0.8–29.9	Cantrell and Linderman (2001)
2–12dSm^{-1}	*Allium cepa*	Mixture of *Glomus, Acaulospora* and *Entrosphora* spp. procured from (a) saline playa or (b) non-saline vegetable farm	(a) 61 0.7–38.8 (b) 28 0.8–18.0	Cantrell and Linderman (2001)
0–13.19dSm^{-1} (0–100 mM)	*Zea mays*	*Glomus mosseae*	70–80	Feng et al. (2002)
0–6.10dSm^{-1} (0–3 g kg^{-1})	*Gossypium arboreum*	*Glomus mosseae*: (a) GM1 from non-saline soils; (b) GM2 from saline soils	(a) 38 + 3 to 15 + 2 (b) 46 + 5 to 21 + 3	Tian et al. (2004)
40.2dSm^{-1}	*Tamarix chinensis, Phragmites communis, Suaeda glauca, Aeluropus littoralis* var. *sinensis* and *Cirsium setosum* (in Yellow River Delta, China)	Mixture of *Archaeospora, Acaulospora* and *Glomus*	0.2–9.5	Wang et al. (2004)

§The range of salinity within brackets is the actual salt concentrations used by the authors

17.4.2.2 Phosphorus (P)

Soil salinity significantly reduces the absorption of mineral nutrients, especially phosphorus (P). Under soil salinity, the phosphate ions are generally hastened with other ions including Ca^{2+}, Mg^{2+} and Zn^{2+}, leads to unable form for plants. As a result, P fertilization is essential for proper growth and development of cultivated plants under salinity stress (Cantrell and Linderman 2001; Shokri and Maadi 2009). However, AMF + plants symbiosis could enhance the uptake of P through enabling by the form of widespread hyphae that permit them to travel more soil volume than the non-AMF-plants (Ruiz-Lozano and Azcón 2000). It is assessed that exterior hyphae of AMF could allocate ˃80% of P requirements in plants (Matamoros et al. 1999; Giri et al. 2007).

Improved P nutrition in AMF-inoculated plants may improve their growth rate through increasing antioxidant production and enhanced nodulation and nitrogen fixation in legumes (Rinaldelli and Mancuso 1996; Garg and Manchanda 2008). Another earlier study conducted by Cantrell and Linderman (2001) also revealed that enhancement of P though the encouragement of AMF may reduce the adverse effect of ion toxicity in salinity by upholding cell membrane integrity, thus inhibiting ions from intrusion in metabolic pathways of plant's growth.

17.4.2.3 Calcium (Ca^{2+}) and Magnesium (Mg^{2+})

Calcium (Ca^{2+}) acts as a secondary messenger in the cell. During salt stress, Ca^{2+} concentration is increased to transduce signals. Studies revealed that AMF colonization strongly influences Ca^{2+} in the plant cell. Cantrell and Linderman (2001) reported increased Ca^{2+} uptake in AMF-lettuce. A higher Ca^{2+} concentration in AMF-banana was recorded than in non-mycorrhizal banana plants (Yano-Melo et al. 2003). Moreover, high Ca^{2+} was also found to enhance colonization and sporulation of AMF (Jarstfer et al. 1998). Besides these benefits, high Ca^{2+} has a beneficial effect on toxic effects of Na^+Cl^- by facilitating higher K^+/Na^+ selectivity leading to salt adaptation (Rabie and Almadini 2005). However, in contrast to the reports above, Giri et al. (2003) reported that Ca^{2+} concentration remains unchanged in shoot tissues of AMF and non-AMF-*Acacia auriculiformis* plants.

Under salt stress, chlorophyll biosynthesis is hampered which leads to limiting the light harvesting during photosynthesis. However, earlier study documented that AMF-symbiosis could improve Mg^{2+} uptake by plants and improve the biosynthesis of chlorophyll under salt stress (Giri et al. 2003; Giri and Mukerji 2004). Enhancement of Mg^{2+} uptake raises the chlorophyll absorption in the plant cell and, hence, expands the efficiency of photosynthesis ultimately increasing crop yields.

17.4.2.4 Balancing Na^+ and K^+ Ratio

Excessive Na^+ concentration forced plants to uptake excess Na^+, causing to decline for uptaking other essential mineral nutrients including K^+. Both Na^+ and K^+ ions are essential in plant metabolism as obligatory elements for numerous cellular activities. Among these ions, K^+ stimulates a variety of enzymes and acts a significant role in protein synthesis and stomatal opening and closure. Better level of K^+ concentration in plants' cells is prerequisite during protein synthesis as K^+ is mandatory of tRNA to the ribosomes (Blaha et al. 2000). However, excessive Na^+ ions can limit the functions of these activities (Giri et al. 2007). Therefore, a balanced proportion of Na^+:K^+ is essential for several biological processes of plants, since excessive Na^+ or K^+ under soil salinity can affect many metabolic pathways (Giri et al. 2007).

AMF-plant can reverse the adverse effect of salinity on K^+ and Na^+ nutrition in plants through enhancing K^+ uptake under salinity (Sharifi et al. 2007; Zuccarini and Okurowska 2008) and averts Na^+ translocation to shoot tissues. Contrarily, better concentration of K^+:Na^+ proportions in plants' cell is also important for influencing the ionic equilibrium of the cytoplasm or Na^+ efflux from plants as reported by several researchers: Founoune et al. (2009), Parida and Das (2005), Ouziad et al. (2006) and Colla et al. (2008). While Al-Karaki (2000, 2006) revealed that the inferior concentration of Na^+ in the AMF-plants may also be important in some plants.

17.4.2.5 Chloride Ions

Under salinity stress, cells of plant root uptake Cl^- from the soil solution through H^+/Cl^- symporters and also through anion channels. For extending from xylem to shoot, Cl^- ions move root cell by a simplistic pathway through exact anion channels. During excessive soil salinity, Cl^- accumulation enhance prominently, though it leftovers persistent in the roots (White and Broadley 2001). The high Cl^- concentration in plant tissue is generally toxic to plants which limits the productivity of crop under saline soils (Xu et al. 2000). This difficulty can be resolved to some extent by the application of AMF, which can reduce the accumulation of Cl^- ions by AMF-mediated host plant (Cantrell and Linderman 2001; Zuccarini and Okurowska 2008).

17.4.3 Physiological Modifications

The AMF symbiosis can alleviate the harmful effects of salinity stress by engaging various physiological mechanisms, which are discussed in detail in the following subheadings:

17.4.3.1 Plant Growth and Biomass

Plant growth and development of plants suffer as a consequence of soil salinity, particularly due to the unable form of mineral nutrients and the lack of energy to mitigate the noxious effects of Na^+Cl^- (Sharifi et al. 2007). However, AMF-mediated host plants could recover the hostile effect of salinity by improving their survival ability (Sannazzaro et al. 2007; Zuccarini and Okurowska 2008). Al-Karaki (2000) observed higher number of shoot and root with their dry weight in AMF-tomato plants which leads to a higher number of fresh fruit and yield than the non-AMF-plant. Similar result was reported by Giri et al. (2007) that AMF-*Acacia nilotica* seedlings had vigorous root and shoot than the non-AMF-seedlings under salinity stress. Colla et al. (2008) found an improved growth, yield and quality of fruits of

AMF (*Glomus intraradices*)-mediated *Cucurbita pepo* plants when exposed to salinity stress.

17.4.3.2 Chlorophyll Content and Fluorescence

The accumulation of salt causes a decline in chlorophyll content (Sheng et al. 2008) due to destruction of specific enzymes which are accountable for the synthesis of photosynthetic pigments (Murkute et al. 2006). A decrease in the accumulation of mineral nutrient particularly Mg^{+2} reduced the chlorophyll concentration in the leaf (reported by El-Desouky and Atawia 1998). Several earlier findings revealed that AMF-host plants could ameliorate the adverse effect of salinity stress, by enhancing chlorophyll content in leaves of AMF-plants (Zuccarini 2007; Colla et al. 2008; Sheng et al. 2008), which indicates that salt does not or less interferes with chlorophyll synthesis in AMF-mediated plants than in non-AMF-plants (Giri and Mukerji 2004). The hostile effect of salinity was counterbalanced and inhibited in host plants in the presence of AMF (Giri et al. (2003). Similarly, under salt stress conditions, AMF-inoculated plants achieved higher levels of photosynthesis even greater than non-inoculated plants (Zuccarini 2007).

Chlorophyll fluorescence is the indicator of photosynthetic efficiency. It is estimated as the ratio between variable and maximum fluorescence (Fv/Fm) (Sheng et al. 2008; Zuccarini and Okurowska 2008). The ratio Fv:Fm measures the capacity of the primary photochemistry of PSII, which itself is particularly sensitive to a variety of environmental stress-inducing factors (Figueroa et al. 1997; Maxwell and Johnson 2000). Salt stress could destroy the PSII reaction centre and disrupt electron transport in the photosynthetic apparatus of the plants. This toxic influence of salinity on the PSII reaction centre could be mitigated by AMF symbiosis (Sheng et al. 2008; Zuccarini and Okurowska 2008).

17.4.3.3 Photosynthesis

Soil salinity results are reduced crop growth and productivity (Munns et al. 2006). The effects of salt stress on photosynthesis can be highlighted by primary or secondary effects. The primary effect includes reduced CO_2, which leads to change in the photosynthesis metabolism by limiting the stomatal functioning and mesophyll (Hajiboland et al. 2010). The secondary effect arises from the oxidative stress and can extremely distress leaf photosynthetic machinery. The potential role of AMF-host plants symbiosis on photosynthesis under salt stress has been focused in many earlier studies (Huang et al. 2010; Soliman et al. 2012; Abd-Allah et al. 2015; Lin et al. 2017; Muthukumar et al. 2017). Generally, an enhancement in photosynthetic effectiveness has often been reported in AMF-mediated plants under salt stress (Ruiz-Lozano et al. 2012; Beltrano et al. 2013).

17.4.3.4 Relative Permeability

AMF-mediated host plants are empowered to uphold a greater electrolyte concentration by preserving the enhanced veracity and constancy of the membrane (Garg and Manchanda 2008; Kaya et al. 2009). Subsequently, the electrical conductivity of AMF-mediated plants' roots was found greater than the non-AMF-plants' roots (Garg and Manchanda 2008). For example, AMF-*Cajanus cajan* roots presented a developed virtual permeability than the non-AMF-plants under various levels of soil salinity (Garg and Manchanda 2008). The electrolyte leakage in leaves of AMF-inoculated *Capsicum annuum* has been found relatively lower than non-AMF-inoculated plants, indicating that AMF-colonized plants achieved a much lower root plasma membrane electrolyte permeability than the non-AMF-plants (Kaya et al. 2009). The improved membrane stability has been credited to AMF-mediated enhancement for the uptake of P and excessive production of antioxidant (Feng et al. 2002).

17.4.3.5 Reduction in Electrolytic Leakage

The cell membrane of plants acts an adaptable role in the uptake and transport of ions under natural and environmental conditions. The membrane of a cell is initially facing the hostile effect of environment. Salt stress induces a change in membrane potential and permeability which reduces the membrane's ability to take up and retain solutes (Khatkar and Kuhad 2000). Like other abiotic stresses, salt stress prompts severe electrolytic leakage in cell membrane of plants. However, electrolytic leakage in cell membrane levels is varied based on saline-sensitive and saline-tolerant cultivars (Khatkar and Kuhad 2000). They also reported that saline-tolerant plants could maintain the electrolytic leakage in cell membrane levels under salinity stress. The electrolytic leakage in the cell plasma membrane of AMF-colonized plants was few than non-AMF-plants (Ebrahim 2014). In other studies, two AMF (*Glomus* spp.)-colonized wheat cultivars showed a reduced cell plasma membrane electrolytic leakage than non-AMF-colonized plants under different levels of soil salinity (Talaat and Shawky 2014a, b).

17.4.4 Biochemical Mechanisms

Salt-induced changes in plants are primarily due to water deficit rather than specific effects of salts (Javid et al. 2011). To neutralize this osmotic effect, plants accumulate several enzymatic and non-enzymatic organic solutes such as proline, glycine, betaine, soluble sugars, pinitol and mannitol (Ruiz-Luzano et al. 2012). Besides these, a number of nitrogen-containing compounds accrue in plant cells under salinity stress such as proteins, betaines, amino acids, amide and polyamines (Rabie and Almadini 2005). The accumulation of these organic solutes and nitrogen-containing compounds in the saline condition generally varies with plant species and stress severity (Rabie and Almadini 2005). These compounds are normally present in low concentrations when the plant is not under salt stress, while tolerance plants could be enhanced under salinity (Feng et al. 2002).

Several earlier findings on salt-induced oxidative stress and its mitigation strategies through AMF-colonized plants are discussed in Table 17.3.

A detailed description of several important compounds is discussed in the following subheadings.

17.4.4.1 Antioxidants

Plants have numerous defensive appliances for mitigating salt-induced excessive production of ROS (Yamane et al. 2004). Tolerant plants could mitigate the adverse effect of stress through the accumulation of antioxidants and a N-containing solutes (Jiang and Zhang 2002). Major antioxidant enzymes in plant cells are SOD, CAT, APOX, GR (Ghorbanli et al. 2004; Wu et al. 2006), dehydroascorbate reductase (Wu et al. 2006), monodehydroascorbate reductase (Ghorbanli et al. 2004), guaiacol peroxidase, oxidized glutathione (Wu et al. 2006), glutathione peroxidase and the enzymes involved in the ascorbate-glutathione cycle (Alguacil et al. 2003). The non-enzymatic compounds that scavenge reactive oxygen species include carotenoids, glutathione, tocopherols and ascorbic acid (Wu et al. 2006). Several studies suggested that AMF-colonized plants could alleviate the adverse effect of salt stress by accumulating antioxidant enzymes in plants' cells (Zhong Qun et al. 2007). The greater activities and actions of SOD, peroxidase and APOX in AMF-colonized soybean plants than in non-AMF-plants, while the events of CAT and polyphenol peroxidase persist unaffected in both AMF- and non-AMF-colonized soybean plants (Ghorbanli et al. 2004). Alguacil et al. (2003) also stated the enhancement events of CAT, APOX and SOD in AMF-colonized *Olea europaea* and *Retama sphaerocarpa* plants.

17.4.4.2 Proline

Proline is one of the most common osmolytes involved in salt stress tolerance of plants. This compound maintains the osmotic stability of the cell and also keeps the cells from harmful effects of salinity (Ashraf and Foolad 2007; Sannazzaro et al. 2007). Many studies have reported higher concentrations of proline in AMF-colonized plants than in non-AMF-plants under saline conditions (Sannazzaro et al. 2006). Proline enhancement has been also found in plants colonized by AMF. For example, AMF-*Vigna radiata* plants were reported to have higher proline content than non-AMF-plants (Jindal et al. 1993). Sharifi et al. (2007) also reported a higher proline concentration in AMF soybean than the non-AMF-plants at different salinity levels. Contrarily, low concentrations of proline have also been reported in AMF than non-AMF-plants under soil salinity (Jahromi et al. 2008; Sheng et al. 2011).

Table 17.3 AMF is a natural resource to mitigate salt-induced oxidative stress by encouraging the production of antioxidants in several plant species

Levels of salinity	Crop species	AM fungus	Plant organ	Non-enzymatic antioxidants		Enzymatic antioxidants						References
				AsA	GSH	CAT	APX	SOD	POX	MDA	GR	
0–1.0% NaCl	*Lycopersicon esculentum*	*Funneliformis mosseae*	Root			+	+	+	+	+		He et al. (2007)
0–100 mM NaCl	*Capsicum annum*	*Funneliformis mosseae*	Shoot Root					+ +	+ +	− −		Abdel-Latef and Chaoxing (2014)
2,4 g NaCl kg^{-1}	*Lactuca sativa*	*Rhizophagus irregularis*	Shoot			+			+			Kohler et al. (2009)
100–300 mM NaCl	*Pennisetum glaucum*	*Rhizophagus fasciculatus*	Root Shoot			+ +		+ +	+ +			Borde et al. (2017)
4–8 dSm^{-1}	*Cajanus cajan*	*Funneliformis mosseae*	Nodules			+		+	+			Manchanda and Garg (2011)
2.2–12 dSm^{-1}	*Lycopersicon esculentum*	*Funneliformis mosseae*	Leaves			+	+	+	+	+		Abdel-Latef and Chaoxing (2014)
0.9–7.1 dSm^{-1}	*Lycopersicon esculentum*	*Funneliformis mosseae*	Leaves Root	+ +		+ +		+ +	+ +	+ +		Huang et al. (2010)
4.7,9.4 dSm^{-1}	*Triticum aestivum*	Mixture of *Glomus* spp.	Leaves	+	+	+		+	+		+	Talaat and Shawky (2014a, b)
6,12 dSm^{-1}	*Glycine max*	*Funneliformis mosseae*	Nodulated roots			+		+	+			Younesi et al. (2013)
3.13–9.38 dSm^{-1}	*Triticum aestivum*	Mixture of *Glomus* spp.	Leaves			+			+			Talaat and Shawky (2014a, b)
0–100 µmol m^{-2} S^{-1}	*Zea mays*	*Rhizophagus irregularis, Claroideoglomus etunicatum, Septoglomus constrictum*	Shoot			+		+				Estrada et al. (2013)

+, increase; −, decrease

These variations among studies were attributed to the different plants and AM fungi species involved, including genotypes of varied levels of saline tolerance (Ruiz-Lozanao et al. 2012).

17.4.4.3 Betaines

Accumulation of betaines in plants under salt stress is a common occurrence. Betaines are quaternary ammonium compounds which are N-methylated derivatives of amino acids. Once formed, they are seldom metabolized (Duke et al. 1986). This can, therefore, be used as an effective indicator of salt stress (Duke et al. 1986). Betaines are not merely non-toxic cellular osmolytes, but they can also stabilize the structures and activities of enzymes and protein complexes and maintain the integrity of membranes against the damaging effects of excessive salt (Gorham 1995). Accumulation of betaines under salt stress is found to increase when the plant is colonized by AMF. It was found that at higher salinity levels, the glycine betaine content of AMF-plants was about twofold greater than that of non-AMF-plants (Al-Garni 2006). Wheat cultivars colonized by a mixture of *Glomus* spp. accumulated more glycine betaine than non-AMF ones under varying levels of soil salinity (0.1–0.9 dSm^{-1}) (Talaat and Shawky 2014a, b). Higher concentrations of glycine betaine under salt stress (4–8 dSm^{-1}) have also been reported in root nodules of pigeon pea when the plants were colonized with mycorrhiza (Manchanda and Garg 2011).

17.4.5 Phytohormones and Others to Alleviate Salinity Stress

17.4.5.1 Strigolactones (SLs)

Strigolactones are apocarotenoid phytohormones produced in roots of both monocot and dicot plants (Xie et al. 2010). These compounds are also found in root exudates and are known to regulate the above- and below-ground plant processes, including AMF symbiosis (Kohler et al. 2015). Among phytohormones, SLs have categorized novel carotenoid-derived PHs, although primarily SLs were recognized as a host-derived stimulating substance for germination of parasitic weeds under the genera *Striga* and *Orobanche*. Lately, SLs were established to be host-detection and hyphal-branching signals for AMF (García-Garrido et al. 2009; Kohler et al. 2015). Furthermore, increased SLs production is also known to promote AMF development and symbiosis establishment in different plant species (López-Ráez et al. 2011).

17.4.5.2 Abscisic Acid and Jasmonic Acid

Abscisic acid (ABA) and jasmonic acid (JA) are the two endogenous PHs that are recognized to rise with growing salinity (Javid et al. 2011). Increased concentrations of ABA in response to salinity have been reported in plant species like mustard (He and Cramer 1996), common bean (Cabot et al. 2009), maize (Cramer 2002) and rice (Kang et al. 2005). However, the concentrations of ABA were found either lower or were almost similar compared to non-AMF-plants in AMF-colonized plants.

The JA is an important cellular regulator involved in defence mechanisms under various biotic and abiotic stresses including salinity (Cheong and Choi 2003). Increased concentrations of JA in plants' tissue in response to salt stress have been reported in crops like tomato (Pedranzani et al. 2003), barley (Kramell et al. 2000) and rice (Kang et al. 2005). However, the effect seems to depend on the AMF species involved, concentration, timing and soil nutritional conditions (Gutjahr and Paszkowski 2009). In spite of contradictory results, most of the studies suggested that JA signalling is important for the development of AMF symbiosis (Gutjahr and Paszkowski 2009; Landgraf et al. 2012), whereas Ludwig-Müller et al. (2002) found the negative effect for AMF development.

17.5 Conclusion

The agricultural land degradation is one of the foremost threats to the food security of the ever-increasing population in the world. It is estimated that about 25% of the total global land area has already been degraded. However, 24 billion tons of fertile soil is being lost per year as a result of unsustainable agriculture practices. If this trend continues, 95% of the Earth's land area will become degraded by 2050. Factors which are responsible for land degradation, soil salinity has been recognized as the most important one particularly in arid and semi-arid regions, leading to cause a major challenge to sustainable agriculture. To deal with saline soils and minimize crop loss, development of new salt-tolerant crop plants through classical breeding and genetic engineering have been considered as a plaussible solution. Besides, several lines of evidence indicate that AMF promoted plant growth and enhance salinity tolerance by employing various mechanisms including enhanced nutrient acquisition by AMF-colonized plant roots. The chapter highlights and summerizes the essential role of AMF for alleviating the adverse effect of soil salinity. A better understanding the molecular cross-talks between plant and AMF will be helpful for ensuring sustainable crop production under the saline environment in the era of future climate change.

Conflict of Interest Authors declare no conflict of interest.

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Chapter 18
PGPR-Assisted Bioremediation and Plant Growth: A Sustainable Approach for Crop Production Using Polluted Soils

Muhammad Ashir Hameed, Zia Ur Rahman Farooqi,
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18.1 Introduction

Agriculture is the science and the practice of growing food crops, animals, and other life forms which are driving human population. It is difficult to determine exactly how, where, and when farming started. From myth to ecology, the perspective of origin is diverse. Agricultural development cannot be progressed overnight but can spread to every part in the world. The process of evolution turned the wild plants to domesticated and cultivable varieties (Zeder 2009). The interaction between soil living organism, earth, and plant is the base of agriculture. Microbes have the vital role in maintaining our agricultural and natural biomes, which has only increased their research interest (Bishnoi 2015). Using microbes in plant growth stimulation and the concepts of using microorganisms to maintain agricultural production for future is the need of time. Middle Eastern farmers began to work on the farms 6000 BC. They neither understand the chemistry nor the important role of bacteria, but they grow beans and grains and practiced crop rotation too. Later on, it was explored that microorganism present in the roots of certain plants (legumes) can fix atmospheric nitrogen by root colonization in rhizosphere. Soil fertility was improved by cultivation of legume crops (Vessey 2003). In addition, it was also noted that crop rotation and growing intermediate crops helps to replenish soil fertility loss and improve its structure (Havlin et al. 2016).

Plant growth-promoting rhizobacteria (PGPR) are the group of variety of bacteria known to promote plant growth by colonizing upon roots (Kloepper 1978). PGPR are categorized into two groups on the basis of their associations with plants:

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(i) symbiotic bacteria and (ii) free-living PGPR (Khan 2005). Much have been worked on the mechanism and principle of the association between PGPR and plants, which is generally considered to be a rhizosphere effect (Compant et al. 2005; Glick 1995; Hall 2002; Hallmann et al. 1997; Lucy et al. 2004; Sturz et al. 2000; Welbaum et al. 2004). PGPR generally works in three distinct ways, i.e., synthesis of specific plant compounds and promoting the absorption of various nutrients and as biocontrol (Guo et al. 2004; Jetiyanon and Kloepper 2002; Raj et al. 2003).

Large quantities of toxic waste are produced and released into the ecosystem as a result of rapid industrialization and modernization across the world. There are more than 40,000 polluted sites in the United States according to the US-EPA report. Western European countries have more contaminated places in relatively lesser area. Multiple methods including chemical, physical, and biological have been developed for soil remediation. The biological processes are used up in bioremediation to remove harmful substances in the world (Gianfreda and Rao 2004). Compared to a variety of physicochemical remediation methods, it offers the following obvious advantages: cost-effective, appropriate, and comprehensive deprivation of organic toxins and no any collateral damage to the site materials or the native living organisms (Timmis and Pieper 1999).

Lucy et al. (2004) concluded that use of PGPR for organic and inorganic pollutant remediation is promising. These organic and inorganic pollutants remain in the system for a long period and pose a hazard to human healthiness. These include fossil fuel residues like total petroleum hydrocarbons, industrially used polychlorinated biphenyls and polychlorinated aromatic hydrocarbons, halogenated compounds like pesticides, and heavy metals like cadmium (Cd), arsenic (As), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) (Hussain et al. 2019).

18.2 Interaction Zone of PGPR

The portion of the plant which remains under the influence of soil are roots (Dobbelaere et al. 2003). It can be the dynamic, extensive, and dense environment for the interactions of plant-microbe so that plant roots can absorb the required amounts of nutrients. Compared to other soils, this area is rich in nutrients released by microbes, and the number of bacterial populations is 100 times higher in this zone. Plant root excretes a variety of organic acids, sugars, vitamins, plant iron, nucleosides, mucus, and signs to attract microbial community (Badri et al. 2009; Bais et al. 2006; Drogue et al. 2013; Lakshmanan et al. 2014). The rhizosphere microbiome refers to the consequent microbial population living under the influence of the roots (Chaparro et al. 2013), and their composition differs from other bacterial population in the adjacent soil because these bacteria can compete for the release of nutrients and may create a defense mechanism (Bulgarelli et al. 2013; Chaparro et al. 2013).

Through root exudates, root system can regulate nearby microbial communities in the soil, promote useful symbiotes, and improve the physicochemical conditions of soil ultimately enhancing growth (Bais et al. 2004). Plant's growth stage and genotypes are highly associated with the root length of root system, root exudate composition, and the composition of the root zone microbial biodiversity (Berg and Smalla 2009; Bulgarelli et al. 2013; Chaparro et al. 2013). Root exudate released can produce a niche which affects the microbes of the root zone, thus changing the diversity and composition of microbial community in precise way (Grayston et al. 1998). Therefore, plant growth stages and type of soil are the vital factors for the presence of the bacterial community in a certain niche in the rhizosphere (Berendsen et al. 2012; Berg and Smalla 2009; Broeckling et al. 2008). Rhizosphere is crucial for the nutrients, quality, and health of plants, and microbial activity also acts an essential role in carbon sequestration (CS) to drive nutrient cycle of the soils. Therefore, rhizosphere becomes a dynamic natural environment with strong interactions between plants and microbes, which influences plant growth and also acts as a protective buffer against soil pathogens. Pathogens can be divided into advantageous, harmful, and neutral categories due to their consequences on plant growth in the rhizosphere (Dobbelaere et al. 2003). In a negative interaction, phytopathogenic rhizobium emits phytotoxic constituents such as hydrogen cyanide or ethylene and therefore hurts plant physiology and growth. To overcome these pathogens, PGPR have an optimistic effect on plant development through direct processes (such as nutrient solution, N_2 fixation, growth regulator production, etc.) and through indirect processes (such as stimulating mycorrhizal growth and competitiveness) (Bashan and De-Bashan 2010).

Presence of microorganisms is essential for soil fertility whether they are playing positive role in plant life cycle or not (Brimecombe et al. 2007). PGPR are further divided into rhizosphere bacteria, which promote the extracellular growth, and endophytic bacteria, which promote plant growth in the cell (Martínez-Viveros et al. 2010). Rhizospheric bacteria include *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Micrococcus*, *Pseudomonas*, *Serratia*, etc. (Gray and Smith 2005). While the bacterial class present within the cells and called endophytic bacteria or PGPR (Natural Rhizobium, Slow Rhizobium, and Mesozoic Rhizobium) belong to *Frankia specie*. All of them coexist with higher plants to fix N_2 (Verma et al. 2010). The endophytic bacteria or PGPR represents a diversity of soil microorganisms that can persuade growth of plant through direct or indirect effects.

Direct models comprise (I) production of plant hormone (Vacheron et al. 2013; Ryu et al. 2003); (II) reduction in ethylene content (Glick et al. 1998); (III) extraction of macro and micronutrients from insoluble sources for plants (Delvasto et al. 2006; Rodríguez et al. 2006; Boddey et al. 2003); and (IV) stimulating disease resistance systems like induction systemic resistance (ISR) (Van Loon 2007). Endophytic PGPR can be used as biocontrol material that reduce disease (Compant et al. 2005), stimulate symbiotic enzymes (Burdman et al. 2000) and protect plants by breaking down xenobiotics (Jacobsen 1997).

18.3 PGPR: Rooting for the Sustainable Agriculture

Like 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production which promotes plant growth, PGPR remediates the organic pollutants (Belimov et al. 2005; Burd et al. 1998; Reed and Glick 2005; Safronova et al. 2006). PGPR promote growth of plants by producing organic contaminants like iron carriers, auxins, and antibiotics (Belimov et al. 2001; Burd et al. 2000; Glick 2003; Patten and Glick 1996) by stimulating transfiguration methods. For instance, nitrogen binding and absorption of sulfur, nitrogen, phosphorus, magnesium, calcium and their delievery to plants is the responsibility of PGPR (Bashan and Levanony 1990; Belimov and Dietz 2000; Okon and Labandera-Gonzalez 1994; Leong 1986).

Pishchik et al. (2005) used PGPR to remediate Cd tin soil and plants and checked the mechanism. These events started with plant hormone production (IAA and ethylene) and absorption of ions in the roots. A possible description of the mechanism could be that the production of these compounds is not stimulated by heavy metals in organically contaminated systems or that they play no role in the breakdown of organic pollutants, but these are all concentration-dependent processes (Dell'Amico et al. 2005), since rhizobium bacteria survival at high concentration of heavy metal is difficult. The chelation was affected due to the presence of pH and bacterial species (Robinson et al. 2001). Dell'Amico et al. (2005) reported that many diverse microbial communities can tolerate high concentrations of heavy metals when living with rhizosphere soils and rhizosphere levels, although mechanism of heavy metal tolerance, potential of metal translation capacity, and PGPR resistance need more investigation. Figure 18.1 describes the possible mechanisms for plant growth promotion through PGPR.

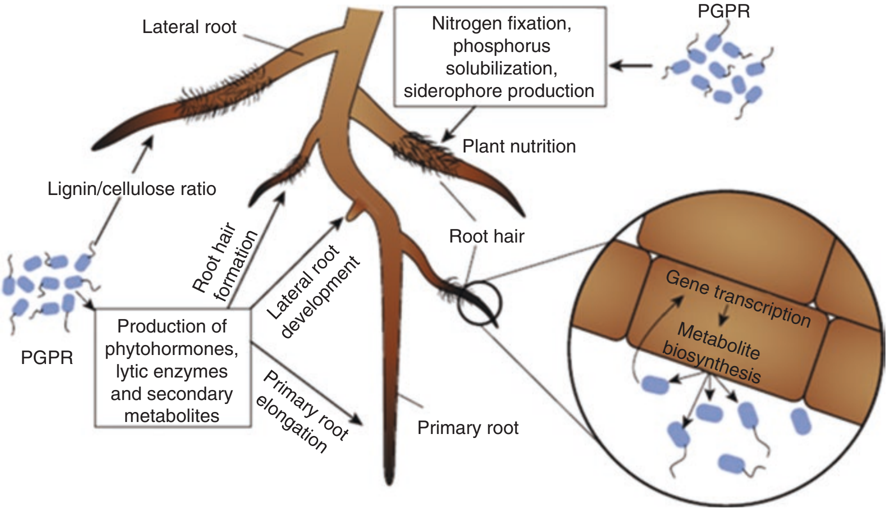

Fig. 18.1 Different modes of action by PGPR during plant growth

18.4 PGPR as Biofertilizers

In addition to moisture supply, temperature and nutrients are the world's most limiting environmental factors for plant development. PGPR promotes growth of plants by boosting the storage, availability, or absorption of these factors in the soil/rhizosphere.

18.4.1 N_2 Fixation

Nitrogen (N_2) content in the atmosphere is around 78%; it is highly inert and cannot be used for growing plants which is also the building block of proteins, nucleic acid, etc. By biological nitrogen fixation (BNF) process, atmospheric N_2 is fixed by N-fixing microorganisms from elemental nitrogen to ammonia (Kim and Rees 1994). N-fixing bacteria are mostly (I) symbiotic N-fixing bacteria i.e. rhizobia family, and (II) non-symbiotic which includes cyanobacteria *azospirobacteria, azotobacteria* and *azotobacter-diazotrophic bacteria* etc. (Bhattacharyya and Jha 2012). Signaling procedure was identified (Long 2001; Henson et al. 2004), and molecular attributes that determine host symbiosis host particular characteristics (Young et al. 2003). Symbiotic N fixation accounts for 80%, and the rest derives from free-living N fixation. Therefore, BNF is an economical and eco-friendly substitute to the high fertilizer solutions used in agriculture today (Adesemoye et al. 2009). Table 18.1 gives an overview for PGPR advantages for plants.

18.4.2 Phosphorus Solubilization

Phosphorus (P) is the most important macronutrients (Vance et al. 2003) that are essential for the improvement and growth of plants. The decline in P use by crops is a global problem. The availability of P decreases by 30–40% of the acreage (Vance et al. 2003). Phosphorus presents in two insoluble key forms in the soil: mineral forms like hydroxylapatite and apatite as well as organic forms such as inositol

Table 18.1 PGPR and their mechanisms to supply nutrients to plants

Term	Definition	Mechanism
Bioenhancers	Substance which contain live microbes which when applied to the seed, plant, or the soil colonize the rhizosphere or the interior of the plant and promote the growth through increased supply or availability of primary nutrients to the host plant	Biological nitrogen fixation, utilization of insoluble forms of phosphorus
Phytostimulators	Microorganisms with the ability to produce or change the concentration of growth regulators such as IAA, GA, cytokinins, and ethylene	Production of phytohormones (auxins, cytokinins, and GA)

phosphates (phytic acid soils), phosphate monoesters, phosphate diesters, and phosphate triesters. Soil biogeochemical cycle as well as plant's growth is greatly influenced by the solubilization and mineralization of phosphate by phosphate-solubilizing bacteria (PSB) (Jeffries et al. 2003; Richardson 2001; Rodríguez et al. 2006). Most significant PSB genera have multiple species like *Azotobacter*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Serratia*, etc. (Bhattacharyya and Jha 2012). Therefore, some specific PSB species reported for specific crop such as pulses, legumes, and numerous vegetables are *Rhizobium leguminosarum*, *Bacillus circulans*, *Pseudomonas chlororaphis*, etc. (Antoun et al. 1998; Cattelan et al. 1999; Chabot et al. 1998; Zaidi et al. 2009).

In contrast, mineralization of organophosphates happens through the production of several phosphatases that catalyze the hydrolysis of phosphates (Glick 2012). The solubilization and mineralization of phosphate can exist in the same bacterial strain (Guang-Can et al. 2008). Furthermore, it can increase the ability of plants to absorb P by inoculating PGPR strains that increase P which offers a promising method of recovering insoluble P reservoirs, thereby diminishing the P fertilizers' exterior uses in the soil (Zaidi et al. 2009).

18.4.3 Absorption of Iron

Iron (Fe) is a critical micronutrient because it is a cofactor for different redox-active enzymes, required for numerous physiological processes. Plant and microorganism have developed specific mechanisms to get the Fe requirement, for chelating insoluble Fe by chelating Fe carriers and taking up complexes based on specific receptor proteins of the outer membrane (Sharma and Johri 2003). Different types of Fe carrier are used like hydroxamates, phenol catechol esters, and carboxylic acid esters (Podile and Kishore 2007). The production of Fe carriers in bacteria is encouraged by Fe levels, while in acidic soils the solubility and availability increase so that the protective effect is reduced. Microbial Fe carriers in the rhizosphere are generally related to biological control activities and should not be used for plant nourishment (Vessey 2003). Iron is necessary for cell metabolism and development. Therefore, Fe, which is produced by iron carriers, plays a key role in regulating the ability of the bacteria to colonize in roots and compete with other microbes for the competitiveness of Fe (Crowley 2006).

PGPR, which produces iron carriers, can avoid the spread of pathogens by isolating Fe^{3+} around the roots, as this bacterial Fe chelation is believed to isolate the limited Fe supply in the roots, making it impossible for pathogenic fungi to use them with limited growth (Loper and Henkels 1999). Recent researches have undoubtedly exposed that Fe nutrient in plants stimulates the microbial population structure in the rhizosphere (Yang and Crowley 2000). The competition for Fe in *Pseudomonas* has been thoroughly investigated, and by evaluating the impacts of disinfected pyridine with a synthetic Fe chelator or using a synthetic Fe chelator, the pyridone Fe produced by numerous *Pseudomonas* species is proved effective in controlling *Pythium*, and *Fusarium* (Duijff et al. 1994). That is why, there is more competition of Fe in rhizosphere to be used by these microbes against pathogens and use as Fe carriers (Ongena et al. 1999). Several environmental factors also influence the Fe carrier synthesis (Duffy and Défago 1999).

18.5 PGPR as Phytostimulator

The plant biochemical, physiological, morphological, and hormone synthesis processes are affected due to the low concentration of severely regulated plant hormones (Fuentes-Ramirez and Caballero-Mellado 2005). Research show that PGPR can stimulate growth of plants by producing auxin (indole acetic acid) (Spaepen et al. 2007), GA (Bottini et al. 2004), and cytokinin (Timmusk et al. 1999) by regulating high concentrations of endogenous ethylene in plants (Glick et al. 1998). Figure 18.2 describes the ways by which PGPR acts to promote plant growth.

18.5.1 Auxin Production

The auxin (indole-3-acetic acid/IAA) is produced by microbes. It is reported that 80% of the microorganisms isolated from different cultures in the rhizosphere can produce and release auxin as inferior metabolites (Patten and Glick 2002). In general, IAA excreted by rhizobacteria can interfere with the development of many

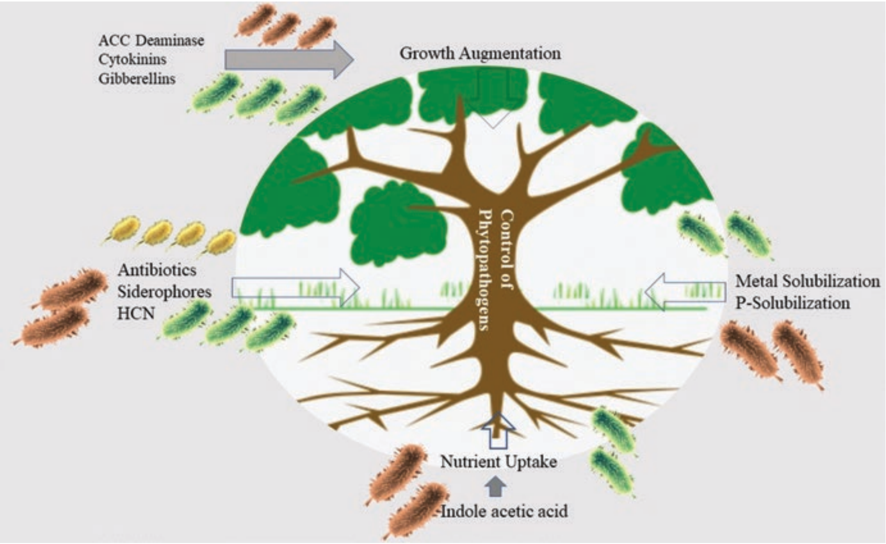

Fig. 18.2 Plant growth promotion by PGPR

plants, since maintaining IAA secreted by soil bacteria can change the endogenous reserve of plant IAA (Spaepen et al. 2007; Glick 2012). IAA also acts as a signaling molecule that influences each other and influences gene expression in numerous microbes (Spaepen and Vanderleyden 2011). Also, downregulation of IAA as signal transduction is related to the plant's defense mechanism against various plant pathogens, and it has been shown that IAA or IAA, which are produced by exogenous use of pathogens, increases plant sensitivity to bacterial pathogens (Spaepen and Vanderleyden 2011). The unusual complexity of the IAA biosynthesis, transport, and signaling pathways reflects the diversity of this function (Santner et al. 2009).

In general, IAA influences the division and variation of plant cells, stimulates germination of seeds and tubers, increases the xylem and root growth, controls the process of the vegetative development, initiates adjacent and random root development, and influences photosynthesis and the biosynthesis of various metabolites and stress resistance. IAA formed by rhizobium disturbs the physiological processes by modifying the auxin pool. Exogenous IAA controls numerous processes of plant growth. A lower IAA concentration stimulates the elongation of the primary root, while a higher IAA content promotes the formation of side roots, shortens the length of the primary root, and increases the root hair development (Patten and Glick 2002; Spaepen et al. 2007; Vacheron et al. 2013). Besides these, root surface and length are enhanced by the bacterial IAA, which means that plants can more easily receive soil nutrients. Also, the IAA rhizosphere bacterium can relax the cell wall of the plant, thereby promoting the growth of root exudates, supplying more nutrients to sustain the growing of rhizosphere bacteria (Glick 2012). Rhizobium IAA was recognized as an effector molecule in plant-microbe interactions against pathogens and plant stimulation (Spaepen and Vanderleyden 2011).

IAA is generally produced from tryptophan by rhizobium, and varying concentrations of amino acids based on plant genotypes are found in root exudates (Kamilova et al. 2006). The capability to produce IAA has been proven in numerous symbiotic and free-living bacterial species (Tsavkelova et al. 2006). Auxin synthetic rhizobacteria are currently the most researched plant hormones (Spaepen et al. 2007; Tsavkelova et al. 2006). From tryptophan, rhizobia synthesis IAA in several ways. Pathogenic bacteria synthesize IAA primarily via the indole acetamide route, while PGPR uses the acidic indole pyruvate route (Patten and Glick 2002). *Azospirillum* is considered one of the most intensive producers of IAA including the PGPR species.

18.5.2 Gibberellin

GA is a different group of plant hormones that are tangled in all stages of a plant's growing from growth to senescence, and the most noticeable physiological consequence of GA is the helping plant's developmental process by stem elongation. Other functions associated with GA plant growth comprise to regulate dormancy, flowering, flower development, and leaf and fruit senescence. (Spaepen and

Vanderleyden 2011). The production of GA has been reported in numerous PGPR, such as xylose-oxidized *Achromobacter*, *Acinetobacter, Azotobacter*, *Bacillus*, *Spirulina*, *Agrobacterium*, and *Rhizobia* (Bottini et al. 2004; Belimov et al. 2001). The mechanism by which GA stimulates plant growth remains unclear. Fulchieri et al. (1993) speculated that GA can increase root hair density at the root zone in assembly with nutrient and water intake.

18.5.3 Cytokinin

Cytokinin stimulates the cell division of plants, regulates the distinction of the root meristem, induces root hair growth, but prevents the lateral root formation and the primary root extension (Riefler et al. 2006). It has been shown that inoculating plants with cytokinin-producing bacteria can stimulate sprout growth and shrink of root-sprout ratio (Arkhipova et al. 2007). They perform a significant character in the growth and development of nodules. It is known that symbiotic N-fixing bacteria, *Azospirillum*, and mycorrhizal fungi, together with other growth-promoting substances, generate cytokinins in the rhizosphere and the production of cytokinins has been documented in numerous PGPR-like *B. japonicum*, *B. licheniformis*, and *P. fluorescens* (Perrig et al. 2007; Timmusk et al. 1999).

18.5.4 Stimulation of Ethylene Level Under Stress Conditions

A higher ethylene content induces foliage and cellular processes, resulting in faster growth and aging of stems and roots and reduced plant growth accomplishment (Li et al. 2005). Under various environmental stresses such as cold, drought, flooding, transmission of pathogens, and the presence of heavy metals, plants react with the synthesis of 1-aminocyclopropane-1-carboxylic acid ester (ACC) as a precursor of ethylene (Glick 2012). Certain of the ACC is secreted in the rhizosphere, reabsorbed through the roots, and transferred to ethylene in the roots. This enrichment of ethylene has a downward spiral effect because weak root growth reduces the ability to retain water and nutrients and increases stress. Therefore, PGPR can break ACC throughout the rhizosphere, which may interfere with this waste cycle and help restore nutritious root systems and eliminate environmental stress. The main mechanism by which rhizosphere bacteria break down ethylene is ACC deaminase. This enzyme can reduce or prevent certain adverse effects of high-level ethylene content (Glick et al. 1998).

Deaminase plays the role of direct ethylene precursor in higher plants ACC and breaks it down to α-ketobutyric acid and ammonia (Glick et al. 1998; Grichko and Glick 2001; Mayak et al. 2004). *Achromobacter* is also involved with ACC when it act to plant roots (Govindasamy et al. 2008), *Pneumocystis* (Li et al. 2005), *Bacillus* (Ghosh et al. 2003), *Enterobacter* (Li et al. 2005), *Pseudomonas* (Govindasamy et al. 2008) and *Rhizobium* (Duan et al. 2009). Several studies have indicated that plants receiving treatment with PGPR that synthesize ACC deaminase have improved their resistance against stress environmental conditions. For example, Grichko and Glick (2001) inoculated *Enterobacter cloacae* along with tomato seeds. This inoculation expressed ACC deaminase activity and introduced resistance in plant against *P. putida*. Ghosh et al. (2003) discovered ACC deaminase activities carried out in three *Bacillus* species (*Bacillus circulans DUC1*, and *B. difficile DUC2*). That accelerated root elongating in *Brassica napus*. Mayak et al. (2004) examined tomato plants inoculated with *piechaudii* bacteria in accordance with the water and salt stress conditions as well as reported substantial increases in the fresh and dried weight of grafted plants.

18.6 PGPR as an Efficient Tool for Environmental Cleanup

Accompanied with physiochemical restoration, phytoremediation has the following advantages: (I) maintains the natural properties of the soil; (II) mainly draws energy from sunlight; (III) has influence on microbial community; (IV) is low cost; and (V) has the ability for rapid growth (Huang et al. 2004). Despite these compensations, certain plants have a less tolerance against soil contamination, which restricts the competence of the degradation values which are not enough for sensible soil remedy, and in accordance with Huang et al. (2004), the adding of PGPR can increase organic pollutants (PAHs and various PAHs) by improving the development and survival of plants in heavily contaminated soil, stimulating plant growth, and accumulating a greater root biomass. Excess ethylene due to stress can inhibit plant growth (Morgan and Drew 1997).

PGPR reduces ethylene production in stressed plants by synthesizing 1-aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase), which consumes aminocyclopropane carboxylic acid (ACC), a direct precursor of ethylene, thereby increasing plant growth (Reed and Glick 2005; Safronova et al. 2006). Given various environmental pollutants such as total petroleum hydrocarbons (TPH), the efficiency of remediation technologies can still be low despite the low simultaneous use of PGPR and facilities. A mixture of PGPR and particular pollutant-mortifying bacteria is the most efficient (Ajithkumar et al. 1998). Huang et al. (2005) suggested that a multi-process phytoremediation system (MPPS) be developed. They are using the PGPR and polluting bacteria to remedy TPH. In such a system, certain polluting microbes can be selected based on the type of pollutant. By efficient use of the PGPR, it is evident that PGPR promotes growth of plants and increases plant tolerance against pollutants simultaneously.

18.6.1 Organic Pollutant Bioremediation

Although PGPR was first used to promote growth of plant and the biological disease control of plant, considerable attention has been recently compensated to use of PGPR for biological remediation (Huang et al. 2004; Huang et al. 2005; Narasimhan et al. 2003). In contrast to inorganic compounds, microbes can combine with plants for organic compounds to be degraded and even be mineralized (Saleh et al. 2004). Therefore, finding effective ways to break down and mineralize organic compounds may lead toward a sustainable approach. So far, bacteria that can degrade various organic pollutants like the polychlorinated biphenyls (PCB) have been kept in isolation from several sites and pathways (Brazil et al. 1995). However, the majority cannot persist near starving soil conditions, comprising the rhizosphere (Normander et al. 1999). Several existing techniques are being developed to enhance the proficiency of degradation as well as the tolerance of bacteria against pollutants in the soil.

18.6.2 Microbes-Assisted Heavy Metal Bioremediation

The remediation of heavy metals by using plants may consist of plant extraction, root filamentation, plant stabilization, and plant volatilization (Glick 2003). Many plants have recently been discovered that can tolerate and accumulate high metal concentrations. These plants are defined as hyperaccumulators. The ideal bioremediation hyperaccumulator must be characterized by rapid growth and large amounts of biomass (Nie et al. 2002). In practice, however, numerous hyperaccumulators grow slowly and are hindered in the presence of elevated heavy metal concentrations. Instead, heavy metal has impacts on the soil microbes in many ways: (I) it can have an advantage to decrease in microbial biomass (Brookes and McGrath 1984; Fliessbach et al. 1994); (II) it reduces the bacterial biodiversity (Chaudri et al. 1993; Koomen et al. 1990); and (III) it changes microbial population structure (Frostegård et al. 1993; Frostegård et al. 1996; Gray and Smith 2005). Sandaa et al. (1999) suggested that the occurrence of lower concentrations of heavy metals can lead to a considerable reduction in microbes. Microorganisms have been used for bioremediation due to chelation ability and sensitivity with respect to heavy metals (Hallberg and Johnson 2005; Kao et al. 2006; Umrania 2006). While most of the literature has been published on remediation, the microbial composition remains unclear (Dell'Amico et al. 2005). Rhizospheres have elevated concentrations of root exudates and attract more bacteria than clumpy lands (Penrose and Glick 2001). Instead, those are the bacteria (including PGPR) which promote plant growth. It turns out that this plant bacterial system removes heavy metals more effectively than its ingredients.

18.7 Conclusion

According to the United Nations, 9.6 billion people are likely to be seen on Earth by 2050, and the planet will reach 11 billion or more by 2100. If we consider that we have not made any significant progress, by 2050 we will need 70% of our food to reduce waste, reduce pollution, slow population growth, or stop consumption of animal products (FAO 2010). Given the limited resources, feeding a growing population is the greatest challenge, which is exacerbated by the decline in irrigation water supplies and other environmental problems. Since the birth of civilization, we humans have carried out a variety of activities which influence our environment, whether positive or negative. The need to increase crop yields and greed have led to overexploitation of soil ecosystems, and due to human activities, such as intensive farming, monoculture, and the use of agrochemicals, we have not used the same conventional agricultural methods to achieve this. This prevailing condition degrades the environmental quality further and disrupts the functioning of the ecosystem.

We must minimize adverse interactions with the surrounding environment. The management of ecological and anthropological resources is essential. To solve a problem, we shouldn't ask another one; it's even bigger and difficult to handle. Maintaining PGPR's beneficial services has a beneficial effect on plant productivity and the functioning of the ecosystem, and we should encourage their successful implementation in key agricultural systems. Plant-promoting rhizobium has a variety of plant-specific activities. By remediating contaminants and combating plant pathogens as biopesticides, it has shown potential for biological remediation. It has shown amazing results in various plant studies. By optimizing and adapting to the most important soil conditions, the production efficiency of specific PGPR can be further improved. Further investigation and awareness of PGPR-mediated plant stimulation mechanisms will pave the way for the search for more effective rhizobium strains that can play a role in a variety of agroecological conditions.

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Chapter 19
Rhizobia: A Potent Tool for Amelioration of Drought Stress in Legumes

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19.1 Introduction

In the present scenario, climate change is the problem of global concern. It is also considered as the major factor for global warming, rising of temperature and uneven distribution of rainfall. Due to these factors, the crop plants experience different types of abiotic stresses. In many areas, rainfall is becoming less predictable due to increasing temperatures, and Rajasthan is one of the examples of such area in India. Climate change is considered as a prime threat for the global agricultural sustainability in the present century. Plants react to such stress and modify their metabolism in different ways resulting in change in physiological, morphological, cellular, biochemical and molecular level. These changes may be due to production of

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different compatible solutes, organize proteins and change cellular structures, regulating cell turgor by osmotic alteration and modification of the antioxidant arrangement to reinstate the cellular redox balance.

Climate change, water scarcity, food shortage and population growth are major consequence at global concern, which we are currently facing. Drought stress imposes a regular challenge for different agricultural crops which is also a major constraint for agriculture productivity at global level. It has been predicted that the intensity and severity of drought stress for crop production will increase in near future. Generally, legume crops are very highly sensitive to drought stress. The reproductive and vegetative stage of legumes is more sensitive than any other growing stage. The severe drought in legume crops is responsible for remarkable production loss although some moderately drought-tolerant crops can bear drought stress only up to a certain extent.

The world total production of pulses is around 95.98 million tonnes (Anonymous 2019). The year 2016 was celebrated as 'Year of Pulses', mainly focused on the high nutritional value of food which is an essential component of a balanced diet for all human populations. The havoc of global warming has led us to a present situation where there is a constant hurdle of aberrant weather situations and inconsistent global climatic changes. All of these are combined effects that adversely affected food production all over the world. In recent years, our country has faced many such situations where we had to deliberately import the food grains to feed the population and specially the pulses. Among all the weather aberrations, drought stress is by far the most difficult situation that affects yield and quality in the crops. The main reasons of drought include either the water supply to the plant root is inhibited or the evapotranspiration has exceeded the rainfall. Under drought conditions available soil moisture does not meet potential evapotranspiration demands. These situations prevail when there are insufficient soil moisture and rainfall during the growing season for healthy ripening of crops which results in severe stress for crop and plant wilting (Choudhary and Vijayakumar 2012). Drought affects the yield by putting an impact on membrane integrity, osmotic balance, water relations and photosynthetic ability. Different methodologies have been devised to mitigate drought conditions in different crops. Some of them include anti-transpirants, growth regulators, altering plant population, genetic manipulation and other mechanical and chemical methods. But most of them are expensive and non-feasible. Legumes can be a very feasible alternative to the existing major crop in the region which performs the dual function – optimize the production and maintain the fertility of soils. Drought mitigation using legumes not only helps plants to tolerate drought conditions but also maintains a sustainable equilibrium and healthy microbial population in the soil-plant-atmosphere system which is the need of the hour. Further, we cannot afford any more of the biomagnified chemical toxic residues. Pulses are very important part of our food; these are generally grown in harsh environments. Pulse crops face a number of abiotic stresses during their life cycle which affect the plant growth by changing the physiology and morphology (Chamoli and Verma 2013) (Fig. 19.1).

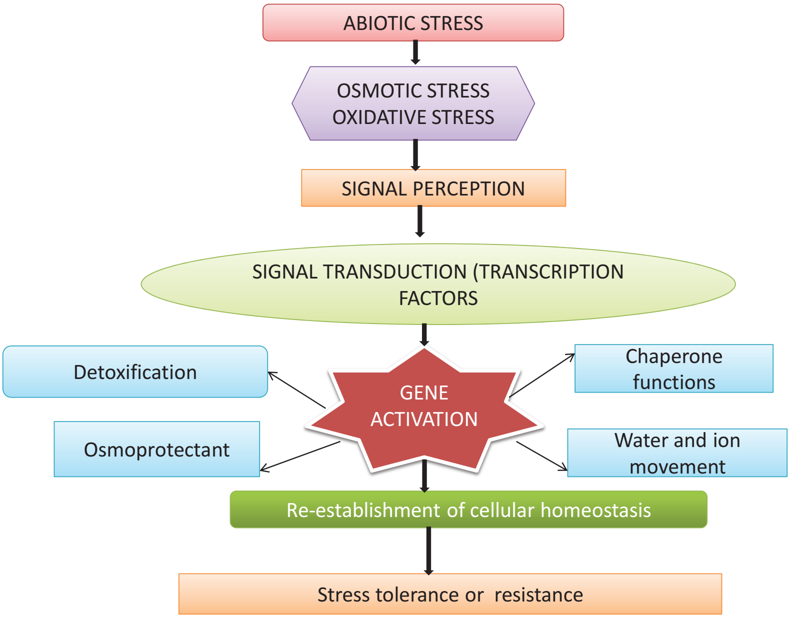

Fig. 19.1 Abiotic stresses affecting the physiology of legumes

19.2 Physiology of Legumes Under Drought Stress

Stresses in legumes are considered as cascades of disturbed, morphological, metabolic, biochemical and molecular modifications in plants (Shinozaki and Yamaguchi-Shinozaki 1997). Plants have adapted to respond to adverse conditions by cross-wired metabolic pathways in order to reprogram the progress of development (Hirt 1997). Stress-mediated reactions are intended to restore homeostasis and to repair damaged proteins. In addition to plant tolerance to biotic stress, which is mostly based on monogenic characteristics, the genetically diverse response to abiotic stress is on multigene basis which is very difficult to control and develop. Plant biotechnology techniques related to abiotic stress tolerance are based on the gene expression involved in signaling and regulatory process or encoding of genetic proteins which is responsible for stress resistance or enzymes responsible for the production of functional and structural metabolites (Park et al. 2004; Apse and Blumwald 2002; Rontein et al. 2002).

19.3 Effects of Drought on Legumes

Drought has its direct effect on yield production of legumes. The extent of the effects of drought depends on the stage at which legume crop encountered drought stress. Reduction in yield was more when plants remained under drought conditions during their reproductive stages as compared to vegetative stages. Legume yield depression as related to drought is also influenced by the type and texture of the soil in which the cultivation has been taken. In medium-textured soils, the effects of drought are more pronounced as compared to those planted in coarse or fine-textured soils (Daryanto et al. 2015). Yield reduction and water reduction in different legume species have been depicted in Fig. 19.2.

Under drought conditions, in alfaalfa the concentration of all free amino acid Increased in shoots, roots and nodules which resulted in severe plant growth reduction. These free amino acids such as alanine, c-aminobutyric acid (GABA) and proline build up evidently in plants under moisture stress (Hanower and Brzozowska 1975; Handa et al. 1983). Xylem sap concentration of several amino acids increases in soybean plants under water deficit (Serraj et al. 1998). Species that transport high concentrations of ureides are less resistant to drought. However, species with less or no ureide have N_2 fixation that was relatively less drought sensitive (Sinclair and Serraj 1995). Less solubility of ureides (Sprent 1980) is important in association with high ureide concentrations with less drought tolerance. Ureide concentration increases in nodule, root and shoot tissues under drought stress conditions.

(a) **Nodule water economy:** Factors responsible for phloem flow also have large effect on nodule physiology. The degree of N2 fixation depends on the rate of flow in the phloem, in turn on the hydrostatic pressure of the leaves which is sensitive to differences in leaf water potential (Nobel 1991). Hence, the water economy of nodules is less resistant to changes in plant water status.

(b) **Drought tolerance of N_2 fixation:** In CO_2-enriched plants (Serraj et al. 1998), water balance is enhanced at specific soil moisture content which allows more

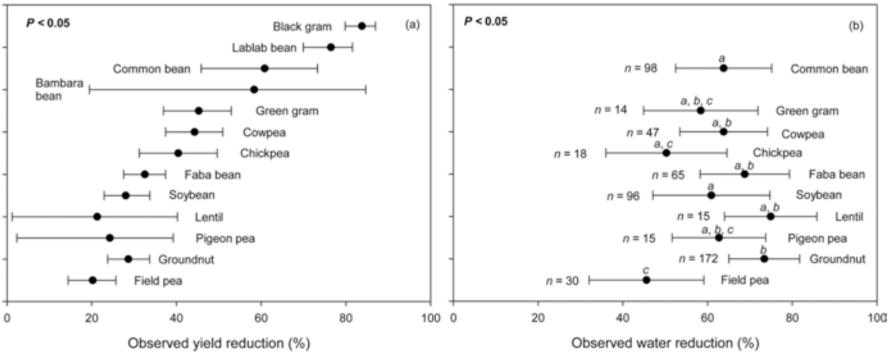

Fig. 19.2 Yield reduction (**a**) and water reduction (**b**) in different legume species. (Adapted from Daryanto et al. 2015)

flow rates of phloem from leaves to the nodules. Due to low rate of transpiration of CO_2-enriched plants as compared to plants of ambient CO_2, the water potential in the leaves and phloem may be less negative than the plants under ambient CO_2. The hydrostatic pressure of leaves and the volumetric flow rate of the phloem would be increased to the nodules. In CO_2-enriched plants, more photosynthesis may contribute to more sugar content of phloem, which would increase pressure potential of leaves and the flow rate of phloem. Enhanced flow of phloem to the nodules enhances transport of carbon to nodules resulting in more nodule growth and N_2 fixation under soil water deficits (Serraj et al. 1998).

(c) **Changes in cell volume and concentration of solutes:** Drying conditions have very little effect on nodule activity. Water supply from phloem plays very important role. Lenticel collapse and triggering of reduction in oxygen diffusion of nodules are due to loss of turgor in cells around lenticels on the nodule surface (Pankhurst and Sprent 1975). In soybean nodules under severe drought stress, reduction in external diameter and loss of lenticels were reported (Davis and Imsande 1988). Water deficit conditions in faba bean resulted in a loss in the turgor of cells in the nodule cortex and alteration in nodule structure which turned into deformed outer cortical cell walls (Guerin et al. 1990). Legumes tolerant to drought are *Prosopis*, *Mimosa* and *Acacia*. Rhizobia may withstand and successfully multiply under these conditions, but their ability for infection and nodulation may be affected.

(d) **Effect of drought on nodulation:** Drought affects adversely the nitrogen fixation in soybean nodules. Studies have been conducted on oxygen role related to nitrogenase activity influenced by drought (Purcell and Sinclair 1995). In acacia, nodule number per plant decreased by 38, 39, and 100% in the first season and 42, 51, and 100% in another season at 7, 14, and 21 days of irrigation as compared to control, respectively (Shetta 2015). Root nodules were thicker, larger, and more tolerant to infection with *Rhizobium* (Miettinen et al. 1988).

(e) **Effect of drought on chlorophyll content:** Chlorophyll content of seedlings under drought periods decreases mainly due to the decrease in number of leaves. Reduction in chlorophyll content is directly related to reduction in leaf area due to drought stress. These findings are the same as the results of Kozlowski (1982).

(f) **Effect of drought on biomass production:** There is a decrease in stem height and diameter of woody trees under drought stress conditions (El-Juhany and Aref 1999). This also resulted in reduction of shoot and root dry weight in *Acacia origena* (Shetta 2015).

(g) **Effect of drought on nutrient content of legumes:** Even short-term drought stress on the nitrogen economy of N_2-fixing faba bean causes an accumulation of amino acids in the shoots (Plies-Balzer et al. 1995).

19.4 Drought Management by Legumes

Legume plants have at least two ways to resist drought: (i) drought avoidance via efficient stomata regulation and (ii) drought tolerance via osmotic adjustment which usually allows root growth to proceed under drought condition (Nunes et al. 2008; Chaves et al. 2002). Sucrose synthase of nodule and carbon metabolism are important aspects of response of nitrogen fixation under drought (Gordon et al. 1997). Decrease in nitrogen fixation under drought is directly related to decline in photosynthesis (Huang et al. 1975). More export of photosynthates from leaves to roots and nodules determines increased tolerance of nitrogen fixation to drought in soybean (Purcell et al. 1997). Ureids are major transporting products from nodules to shoots which increase dramatically when plant is subjected to drought (Sinclair and Serraj 1995). This depression in nitrogen fixation as a result of physiological responses of nitrogenase activity includes mechanisms such as carbon shortage, oxygen limitation, etc. All these mechanisms are sensitive to the water flow of phloem in nodule as a result of turgor pressure in leaves causing changes in nitrogenase activity. Hence, this confirms the relation between nitrogen fixation and soil drying. Nitrogenous signal associated with nitrogen deposition in shoot and nodules is there in legumes so that nitrogen fixation is influenced under drought conditions (Serraj et al. 1998). A high water stress resistance is attributed not only to the large root system but also to the leaf water retention ability. Both the strengthened water uptake by the more developed root and diminished water loss from the leaves would likely contribute to the maintenance of water potential under soil water deficit conditions.

In legumes nodule is the site of nitrogen fixation, the reason for fertility of agricultural soils. These nodules can be determinate or indeterminate. Both types of nodules have three kinds of tissues: central zone, inner cortex and outer cortex. Carbohydrates and other photosynthates diffused to nodule proliferation site from phloem to the cortex, while products of N fixation are transported to the shoot via xylem. Substantial decrease in nodule weight in both common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) under drought has been observed (Smith et al. 1988). Reduced nodule numbers were also observed in faba bean and common bean (Sangakkara et al. 1996). Nodule number and dry weight decreased sharply after severe drought (Sinclair et al. 1988). *Rhizobium*-legume symbiosis is sensitive to drought. However, compared to host plants, rhizobial strains are quite resistant to soil desiccation and can survive in water films surrounding soil particles (Williams and De Mallorca 1984). Fast-growing strains of rhizobia are more sensitive to drought as compared to slow-growing (Sprent 1971). Development and movement of rhizobial bacteria in soil decrease under soil dehydration conditions and reduce the infection processes (Hamdi 1970). When rhizobial bacteria are exposed to osmotic stress, it alters bacterial membrane lipopolysaccharide which is very important for host-rhizobia recognition process. It also affects the root infection thread inhibited by low water status. It is very effective to rotate non-legume crops with legume crops to reduce the requirement for nitrogen fertilizer amendment. More than 50% of agriculture land of various geographical regions is expected to be under drought and salinity by 2050 (Wang et al. 2001). Plant response to abiotic stress is related to many genes and biochemical-molecular mechanisms which is schematically presented in Fig. 19.1. Marker traits responsible for drought stress tolerance have also been identified which may be utilized in breeding programmes for enhancing tolerance.

19.5 Adaptation Mechanism of Rhizobia Under Drought Stress

Performance and activity of *Rhizobium* symbiosis are adversely affected by drought stress (Kirda et al. 1989). The symbiotic rhizobia are rod-shaped, gram-negative bacteria that facilitate the development of symbiotic root nodules involved in the production of symbiotic colonies of different colors, shapes, and sizes. All partners of symbiosis and processes are sensitive to osmotic stress. However, the resilient capacity in combating the drought stress is varying, and the rhizobial strains show comparatively more resistance to such water deficit stress having the capacity to thrive in their water films around soil colloids (Williams and De Mallorca 1984).

Fast-growing rhizobia, however, are less resistant to moisture deficit than those of slow-growing strains (Sprent 1971). Growth and activity of these bacteria in the rhizosphere decrease as drought-like conditions prevail which limits the establishment of nodulation by reducing the infection processes. When rhizobial bacteria are exposed to osmotic stress, it alters bacterial membrane lipopolysaccharide which is very important for host-rhizobia recognition process. In adaptation mechanism under water stress environment, two distinct responses are observed among the microorganisms: one is normal stress response and another is response towards specific stress. The first type of response is generally regulated by monogene or few regulatory genes (Bremer and Kramer 2000) and extends towards numerous stresses of environment irrespective of the initial stimulation to any specific stress (Hecker and Volker 1998). Though this type of response enables survival of affected cell, but even after survival, normal growth under stress conditions is not possible (Bremer and Kramer 2000). Thus, there is a distinct relationship between response of cell and regulators, giving another manifestation to individual cell response to stress and survival for sufficient durations (Hengge-Aronis 1999). Stress owing to components of environmental and management aspects often puts constraints for growth and productivity of leguminous plants in symbiotic nitrogen fixation (Bargaz et al. 2013; Farissi et al. 2011).

19.6 Drought Stress Tolerance in Plants

19.6.1 Germplasm Screening and Conventional Breeding

Environmental conditions often play vital role in survival and multiplication of symbiotic strain of rhizobia. Under such conditions, evaluation of available germplasm assumes importance against environmental factors for identification of suitable genotypes (Farissi et al. 2011; Bargaz et al. 2013). Moisture deficit stress effects particularly in legumes are assessed on the basis of performance of aerial parts of the plant, particularly at reproduction stages. Sufficient studies and researches are not available for below-ground plant parts including roots and nodules. Very little information is available on symbiotic association between rhizobia and host plant under moisture stress conditions (Ferguson et al. 2010).

19.6.2 Biotechnological Approach

Molecular approaches are utilized for characterization of gene expression by drought stress (Kandasamy et al. 2009; Yuwono et al. 2005). Their physiological roles are related to PGPR induced tolerance. At the transcriptional level, symbiotic inoculation with PGPR *Paenibacillus polymyxa* B2 improves the host capacity in combating negative impact of drought stress by augmenting the effect of stress countering gene (Timmusk and Wagner 1999). Similarly pepper plants in response to inoculation with *B. licheniformis* K11 under moisture stress conditions produced and expressed proteins primarily responsible for improving the stress tolerance. Out of these proteins, specific genes of Cadhn, VA, sHSP and CaPR-10 exhibited more than 1.5 times stress tolerance in treated plants compared to untreated plants (Lim and Kim 2013).

19.6.3 Biological Approach

Soil samples treated with PGPR showed enhanced water holding capacity and decreased rate of evaporation. Thus, such treatments with PGPR reduced the extent of dehydration through improved water supply and thus moderate the imposed stress upon root under moisture stress conditions (Zheng et al. 2018).

19.6.4 Augmentation in Drought Tolerance Capacity of Legumes Through Rhizobial Association

19.6.4.1 Metabolite and Osmolyte Production

Tolerance or adaptation in plants in response to moisture stress correlated well with the capacity of such plants to modify their metabolism which is manifested in the formulation of various metabolites including sugars and protein derivatives (Yancey et al. 1982; Close 1996). It has been reported that production of various osmolytes by the plants under the incidence of drought stress often play complementary role which enhance plant growth (Paul et al. 2008). Higher proline production is an indicator of drought stress tolerance, and its elevated production is facilitated by augmentation in its biosynthesis and response to drought (Sandhya et al. 2010).A positive relationship between rhizobial inoculation and proline production indicates its role under drought stress (Gusain et al. 2015). Trehalose which is a non-reducing disaccharide helps in stabilizing dehydrated enzymes and membranes, and its biosynthesis imparts osmoprotection (Yang et al. 2010).

Inoculation of *P. vulgaris* plants with suitable *Rhizobium* strain improves plant capacity to drought stress through expression of favourable gene (Suarez et al. 2008). Macroarray analysis of 7200 expressed sequence from symbiotic nodules of host plants strain overexpressing trehalose-6-phosphate synthase gene revealed regulation of genes involved in stress tolerance, carbon and nitrogen metabolism, suggesting a signaling mechanism for trehalose (Suarez et al. 2008). It is a well-established fact that trehalose plays a critical role as signaling molecule in plants (Paul et al. 2008). Synthesis and accumulation of glycine betaine (GB) are very crucial in enhancing plant resistance towards stress, and choline was found to plays a role in GB synthesis (Zeisel 2006; Zhang et al. 2010). Increased concentration of GB was induced by PGPR strains under stress conditions regulating plant stress responses by reduction of water loss (Nadeem et al. 2010; Bashan et al. 2014). Likewise, some plant growth-regulating compounds such as polyamines have been reported to have definite role in root growth and mitigating moisture stress through increased activity (Vurukonda et al. 2016). Plant growth-promoting rhizobacteria induced plant drought tolerance which has been depicted in Fig. 19.3.

19.6.4.2 Molecular Level Adjustment

A symbiotic relationship between host plant and rhizobia is initiated by the release of flavonoids by roots of host plants in close vicinity to rhizosphere, attracting suitable *Rhizobial* strain to establish relationship with production of Nod factors (Kondorosi et al. 2013). Nod factors are recognized with the help of Lys M receptor-like kinases located on epidermal cells of roots and their binding which results in genetic and metabolic signaling cascades mediated by cell-specific nuclear Ca^{+2} oscillations (Charpentier and Oldroyd 2013). These cascades of signals stimulate

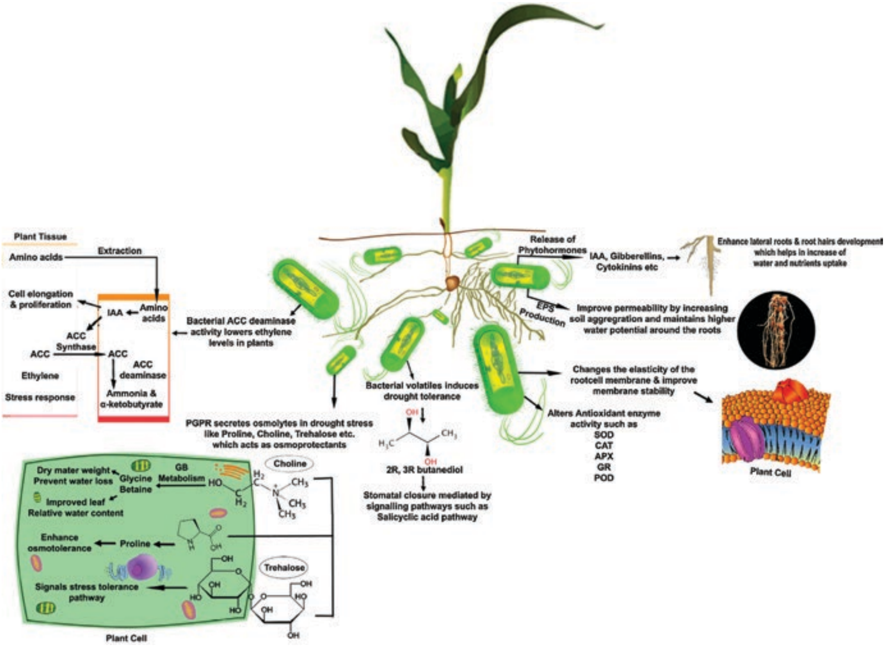

Fig. 19.3 Plant growth-promoting rhizobacteria induced plant drought tolerance. (Adapted from Vurukonda et al. 2016)

the division of cortical cells within infected root area with the formation of composite structures resulting from the symbiotic associators (Gage 2004). The infection thread of bacteria permits rhizobia penetration deep into the dividing cells which results in new formation, the N-fixing 'nodule' giving shelter to suitable rhizobial strain for replication within nodule (Oldroyd et al. 2011; Oldroyd 2013). Inside infected cells, encapsulated rhizobia enter into plant-derived membrane initiating the formulation of the symbiosome (Oldroyd 2013). The symbiosome provides regulation on nutrient movement and stimulates symbiotic activity under stress conditions.

Facultative symbiosis is triggered by nitrogen deficiency in host plant. During the process of symbiosis, bacteria differentiate into an endosymbiotic form (bacteroids) enabling conversion of N_2 to NH_4. Such conversion needs energy that comes from photosynthetic energy. This symbiotic nitrogen fixation process is facilitated by nitrogenase enzyme. Regular oxygen supply for respiration of bacteria is necessary and same is expressed as protein leghaemoglobin.

19.6.4.3 Hormone Level Adjustment

Plant phytohormones like indole acetic acid, gibberellic acid, ethylene, abscisic acid and cytokinins are produced by plants, which regulate the growth and development of plants (Barea and Brown 1974; Egamberdieva 2013). These hormones also play a key role to impart tolerance or capacity to survive under stress conditions in plants (Skirycz and Inze 2010; Fahad et al. 2015). Further, PGPRs synthesize hormones which regulate growth of plants against environmental stresses (Glick and Pasternak 2003).

Auxin

IAA is very auxin, involved in stimulation of growth and development of plants. Different plant species inoculated with IAA-producing bacteria have been found to enhance root growth resulting in higher removal of nutrient and water (Mantelin and Touraine 2004), enabling plants to counter moisture stress (Egamberdieva and Kucharova 2009). Productions of hormones through bacterial inoculation stimulate endogenous hormonal level of plants, thus increasing their ability to tolerate stress (Dobbelaere et al. 1999; Cassan et al. 2001). Rhizobial strain capable of producing gibberellins could enhance soybean plants to grow under moisture stress conditions (Sang-Mo et al. 2014). Under acute stress conditions, stress hormone like abscisic acid is synthesized to counter stress, and higher level of abscisic acid is associated with preventing cellulose dehydration (Kaushal and Wani 2015), because ABA has active role in water loss by regulating closure of stomata and mechanism of signal transduction under stress (Yamaguchi-Shinozakiaib and Shinozaki 1994).

Volatile Organic Compounds

Volatiles cause tolerance induction when plants are exposed to different levels of stresses (Holopainen and Gershenzon 2010). Such volatiles produced under stress act as signals for priming and respond systemically both in the same and in the plants of vicinity area (Heil and Bueno 2007).

ACC Deaminase

Ethylene also regulates the activity of plant tolerance, and synthesis of ethylene is regulated by stress caused by biotic and abiotic factors (Hardoim et al. 2008). In the biosynthetic pathway of ethylene, S-adenosyl methionine (S-AdoMet) is converted to 1-aminocyclopropane-1-carboxylate (ACC) which is the immediate precursor of ethylene by 1-aminocyclopropane-1-carboxylate synthase (ACS). With the incidence of stress, endogenous level of ethylene acts as regulatory substance for homoeostasis in plants, curtailing growth of plants. Further, the deleterious effect of ethylene decreased, and amelioration of plant stress and promotion of plant growth are modified by ACC bacteria (Glick 2005). Fresh and dry weights of both tomato and pepper seedlings are enhanced appreciably by ACC deaminase-producing PGPR: *Achromobacter piechaudii* ARV8 which decreased the ethylene level under drought stress (Mayak et al. 2004). Rhizobacteria residing at water-stressed sites are more adaptive to such stress environments in comparison to inoculants isolated from sites of available moisture conditions (Mayak et al., 2004). ACC deaminase-inoculated plants produced more seed yield, seed number and seed nitrogen and restored nodulation which was depressed in drought stress conditions (Dodd et al., 2005). The inoculants which produce ACC deaminase could fight the drought stress under pot and field conditions (Arshad et al. 2008). Inoculations with such rhizobacteria enhanced the root and shoot growth and also increased lateral root number of wheat plants compared to uninoculated. Favourable effects on growth of root and shoot enabled plants to extract more water and nutrients (Shakir et al. 2012). Increased nodulation due to symbiotic activity resulted in reduction of drought-induced ineffective nodulation and less nitrogen content of seed. Higher population of bacteria in root zone increased yield and nutrient content of plants under moisture stress conditions by local and systemic hormone signaling mechanism. Thus, contribution of bacterial-root association plays a vital role in obtaining economically higher yield in dryland agriculture (Belimov et al. 2009).

Nutrient Solubilization and Uptake

The combined bacterial inoculation with *Sinorhizobium meliloti* TII7 and *Sinorhizobium medicae* SII4 enhance the plant response to iron deficiency stress caused by oxidative reactions by way of producing enzymes having antioxidant activities in genotypes of Medicago truncatula: A17, TN8.20 and TN1.11. The symbiotic relationship between host legume and rhizobia is sensitive to Fe deficiency. Under extreme deficiency of Fe, nodule formation and activity are adversely affected as Fe is essentially required for the proteins which are instrumental for production of nitrogenase, nitrogenase reductase, and leghaemoglobin.

19.7 Current Research Scenario in Microbial Inoculants

Contemporary genomic researches have provided a better understanding of the rhizobium-legume interaction at molecular level. Several genomic approaches have been employed to define and demonstrate the involvement of rhizobial genomes in the symbiotic events. The genomes of two rhizobial species, namely, *Mesorhizobium loti*, the symbiont of several *Lotus* species, and *Sinorhizobium meliloti*, the symbiont of alfalfa, have now been completely sequenced, which have revealed interesting information about the genome evolution and structure, plant-microbes communication and physiological diversity among the microsymbionts of legumes.

However, for legumes, numerous expressed sequence tags representing tens of thousands of different genes involved in root nodule formation and nitrogen fixation from three major legume species, *Glycine max*, *Medicago truncatula* and *Lotus japonicus*, are in the public domain. Currently, biological research is directed to understand gene expression and function involved in rhizobia-legume interaction. In this context, proteomics with continually evolving set of novel techniques to study all facets of protein structure and function is being considered as a promising and effective tool in the postgenomic era to explore further the intricacies of symbiotic process (Musarrat et al. 2010).

Recent advances in this arena include the discovery of specific rhizobial species, emphasis on effects of climate change and adaptation to various abiotic stresses like high temperature, drought and salinity on plants. In the recent two decades in genetics and biochemistry, nitrogenase is encoded by a set of operons, which includes regulatory genes such as nif LA and structural genes such as nif HDK and other supplementary genes (Rao 2014).

Determination of both the components of nitrogenase (Mo-Fe protein and Fe protein) has already been done using crystallography. In *Azotobacter vinelandii*, it was found that the subunits of the nitrogenase Fe-Mo protein have similar polypeptide folds (Kim and Rees 1992).

19.7.1 Non-conventional Nitrogenases

Alternative nitrogenases were discovered more than 25 years ago in *Azotobacter vinelandii* which offer unconventional revealibility to use vanadium instead of molybdemum in an environment lacking molybdenum. *Streptomyces thermoautotrophicus* was found to be able to fix dinitrogen, but is unusual in having three proteins, a heterotrimeric molybdenum-containing dinitrogenase (St1), a homodimeric manganese-containing superoxide oxidoreductase (St2) and another heterotrimeric molybdenum-containing carbon monoxide dehydrogenase (St3 or CODH) (Ribbe et al. 1997). These proteins completely differ from the known nitrogenase components and are insensitive to oxygen abundance as well.

19.7.2 Root Nodulation in Cereals

There is signaling in the process of bacteria-host recognition and legumes. Plant secretes flavonoids that activate the bacteria to secrete nodulation (Nod) factors that promote nodule formation within the plant. Recently, Myc factors, which are involved in this recognition signaling, are between 70 and 90% of terrestrial plants (including cereals), and arbuscular mycorrhizal endosymbiotic fungi were discovered to be very similar structurally to Nod factors (Stoltzfus et al. 1997).

19.8 Future Prospects

Mechanism of plant-microbe interactions in the rhizosphere is difficult and complex to understand. Mechanisms involved in microbial communities of the interactions are all the more complex. To optimize plant adaptation to different stresses including drought requires us to understand the involved phenomenon and to improve the ability of soil microbes for stress alleviation in the crops individually or in an interaction. A range of molecular techniques should be elaborated and made available and be applied to define the molecular bases of the plant-microbe interactions (Barea 2015). Long-term experiments in soil exposed to drought and other abiotic stresses enable us to understand the actual underlying reasons as to why the water deficit in soil causes such adverse effects. Thus, microorganisms can be used to demonstrate the beneficial effect when inoculated in the stress-affected crops.

There is an ultimate need to investigate stress markers such as proline and/or phytohormone synthesis at increasing stress (PEG or salt) levels in the axenic medium. Bacterial cultures may help in selecting the more stress-adapted tolerant strains (Marulanda et al. 2009). Region-specific bio-inoculants need to be screened and isolated according to their characteristics linked with the environment in which they can survive, tolerate and effectively function in adversely stressed conditions (Bisen et al. 2015; Rakshit et al. 2015).

19.9 Conclusion

Adaption of legume production technology is substantially affected by various stresses, and drought is one of the major among them. Adaptive traits for both rhizobia and legumes for survival under moisture stress are of great significance which falilitates inhanced use of rhizobia as inoculants for legumes under stressed environment. This is simple and cost-effective approach for increasing legume production under stressed environment. Thus, it can be concluded that efficient, effective and drought-tolerant rhizobia may have vital role in production of leguminous crops and pulses. More research and investigations are to be needed on proteomics and metabolomics level to find out the mechanisms and the scientific reasons responsible for the survival under stress conditions. However, more research is required to investigate judicious combinations of inoculants and plant species for sustaining soil health and environment.

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Chapter 20
Understanding the Role of Bacterial Fertilizers in Stressed Agriculture: Actions, Mechanisms and Future Prospects

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20.1 Introduction

The increased dependency of modern farming on the excessive use of inorganic fertilizers has resulted in the emergence of several ecological problems related to soil degradation, greenhouse effect, smog and water contamination. There is a dire need for feasible agronomic practices with less energy and environmental hazards for efficient crop production and reduced cost for the increasing population of human beings at a global level. Therefore, biofertilizers are the viable sources for large-scale crop production that maintain soil structure and biodiversity of cultivated land (Thomas and Singh 2019). Mahdi et al. (2010) studied that accessibility and uptake of inorganic compounds for floras are increased by the use of fertilizers that contain living organisms and are well-known as biofertilizers. Their activities affect the soil environment as supplemental substances for crop plants are produced. Natural fertilizers (dung, etc.) are also included in the biofertilizers which are made in plant-available forms due to their symbiotic interaction with plants (Sujanya and Chandra 2011). When biofertilizers are applied as soil or seed inoculants, they increase in number tremendously and improve crop productivity by participating in the nutrient cycling process (Singh et al. 2011a, b).

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There are some processes like phosphate solubilization, N_2 fixation, synthesis of antibiotics, the release of plant growth regulators and biodegradation of biomass in the soil which keep the soil environment enriched in all the useful macro- and micronutrients by the use of biofertilizers (Sinha et al. 2014). They provide resistance against water stress and enhance the accessibility of mineral substances for plants. Synthetic and organic fertilizers directly supply nutrients to crop plants, but biofertilizers do not supply any nutrients directly to plants; thus, in this sense biofertilizers are different from inorganic and organic fertilizers. Basically, they are the pure cultures of fungi and bacteria, comparatively simple and cheaper in cost. Higher growing percentage and yield are attained by the usage of biofertilizers in rice as compared to inorganic fertilizers (Alam and Seth 2014). Therefore, biofertilizers help to solve the problem of food and feed shortage for tremendously increasing population at a global level when agriculture sector is facing different environmental abiotic stresses like salt stress, water deficit and heavy metals stress (Suhag 2016). Bacterial fertilizers could be used as a nutrient-rich source with cheaper production cost.

20.2 Types of Bacterial Fertilizers

Bacterial fertilizers are ready to use for improving soil fertility and strengthening crop plants. Commonly, two groups of microorganisms containing symbiotic association such as *Azolla* spp. and *Rhizobium* spp. and nonsymbiotic association such as *Azospirillum* spp. and *Azotobacter* spp. can be used as microbial inoculants in biofertilizer (Bashan and Holguin 1997). *Rhizobium* and *Azospirillum* are included in the process of biological nitrogen (N) fixation. They have the synergetic association with the plants in which both members attain an advantage from each other. Biological nitrogen fixation minimizes the use of inorganic or synthetic fertilizers, improves plant development and production thereby decreases the cost of production and ecological hazards (Rao 2014). Microorganisms involved in the phosphorus availability to plants include phosphate-solubilizing bacteria and fungi. The mycorrhizal fungi make the mutual correlation with plants having the potential to improve plant development under ordinary as well as environmental abiotic stresses by improving the soil structure (Rabie and Almadini 2005). They promote various plant hormones such as gibberellic acid and IAA which play an essential part in plant evolution. Microorganisms are vital in phosphorus (P) cycle by the transmission of P from mineral and living soil parts and producing the plant-accessible phosphorus (Oberson et al. 2001). Organic acid production and protein extrusion are the two microbial processes which help in phosphate solubilization. Bacterial fertilizers including *Rhizobium* and phosphate-solubilizing microbes are extremely used and beneficial in increasing plant-available N_2 and phosphorus through their synergistic association (Singh et al. 2011a, b). Many bacterial fertilizers which are marketable are primarily dependent on the PGPR. The several processes such as increase of root surface area, iron sequestration by the production of bacterial siderophore, biological nitrogen fixation and phosphate solubilization are very important for improving the plant growth. *Bacillus* and *Pseudomonas* produce indole acetic acid and gibberellins. They employ a beneficial impact on plant development and may be used as bacterial fertilizers in modern and sustainable cultivation. *Pseudomonas* inoculation is not only useful in the degradation of organic nitrogenous compounds but also helps in the better circulation of N and P in soil. Bacterial fertilizers provide protection to the plants from soil-borne infections through inhibition or controlling of pathogenic organisms (Suhag 2016).

20.3 Bacterial Fertilizers Versus Abiotic Stresses

Bacterial fertilizers are the rich and sustained suppliers of substances and plant hormones for plants. Use of bacterial fertilizers is more effective, economical and eco-friendly on a long-term basis. Abiotic stress conditions like heat stress, salt stress, water stress etc. can reduce the plant growth and production in dry and semi-arid areas (Hassan and Bano 2015). A summary of impact of abiotic stresses and significance of bacterial fertilizers is given in Fig. 20.1.

20.3.1 Water Stress

Water stress is a critical abiotic stress in arid regions which affects plant development and yield. Hong and Jj-yun (2007) explained that it increases the number of reactive oxygen species (ROS) due to more energy stored in stressed plants because they get extra sunlight than is used in the process of photosynthesis. Thalooth et al. (2006) studied that increase in osmotic pressure in the rhizosphere is the inhibitory result of water deficit on plant growth and development which declines the elongation and division of cells and causes the reduction in the transfer of nutrients from rhizosphere to the plant roots. Water deficit causes the reduction of net assimilation or photosynthetic rate which is related to the decrease in leaf area. These are the factors which decline the dry matter deposit in stems of the various plants (Ghanbari Zarmehri et al. 2013). Drip irrigation technology is used for the efficient use of water to overcome this severe problem of water shortage (Manchanda and Garg 2008), but its implementation is mostly prohibited due to its higher cost of application and availability of salty water which causes the problem of blockage in the pipe which is being used for irrigation. However, the use of bacterial fertilizers containing microbes tolerant to drought stress are eco-friendly and less costly (Venkateswarlu and Shanker 2009). These microbes improve the plant growth and development through exopolysaccharide (EPS) synthesis, the formation of thick membrane, going to dormant condition and osmolyte accumulation. Appropriate amount of nutrients and good suitable ecological conditions are also provided by the microbes for the healthy plant growth (Farooq et al. 2009; Porcel et al. 2014). When plants are

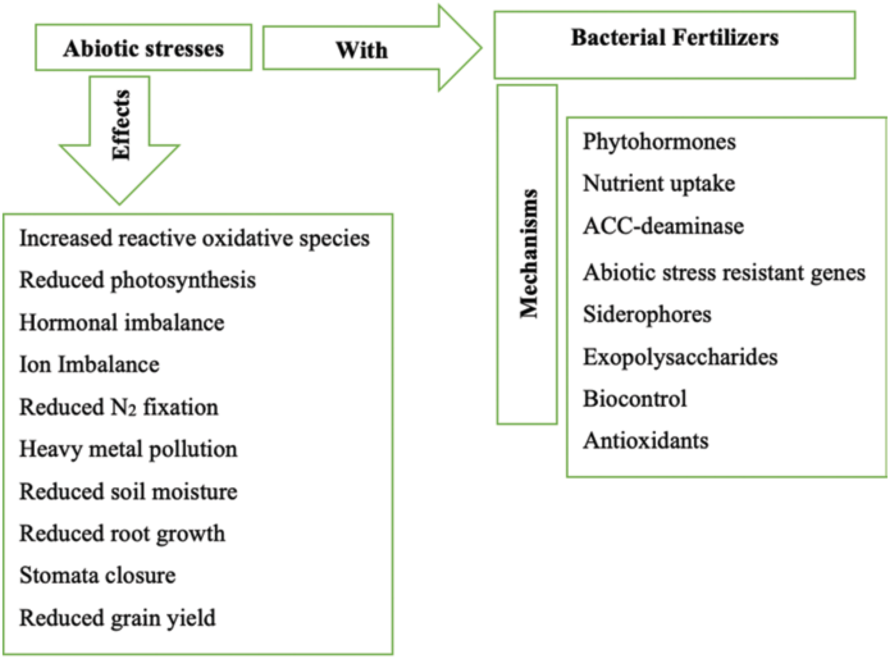

Fig. 20.1 Impact of abiotic stresses and significance of bacterial fertilizers

inoculated with EPS-producing bacteria, they become more tolerant under drought stress conditions (Sandhya et al. 2009). The integrity of the enzymes and membrane penetrability are sustained due to the accumulation of compatible solutes like proline and trehalose by the bacteria during water stress conditions (Ortiz et al. 2015).

20.3.2 Salt Stress

Salt-affected soils are becoming a significant challenge under irrigated lands, and their area is growing day by day. Salt stress limited the growth of plants, so the saline soils have low agricultural potential (Glick et al. 2007). Salts in the form of ions are added to the soils through irrigation water and fertilizer application and naturally through the weathering of minerals in soils. Leaching of ions is decreased from soil profile due to insufficient precipitation (Blaylock 1994). It results in saline soils due to the salt build-up layer in soil. Salinity after water stress is the main ecological aspects decreasing the crop efficiency due to the sensitive nature of plants to salt stress. Crops grown on saline soils are facing the problem of osmotic stress, nutrient toxicities and reduced crop productivity. To solve this problem, good quality-irrigation H_2O is used for the removal of salts from the rhizosphere. The implementation of partial root-zone drying technology and drip irrigation can improve the efficiency of water in irrigated agriculture (Manchanda and Garg 2008). The implementation of these technologies in irrigated agriculture is frequently restricted because it costs more and good-quality irrigation resources are unavailable. Therefore, the most effective, less expensive, eco-friendly and simply adaptable method is required (Venkateswarlu and Shanker 2009) which is the use of bacterial fertilizers for salinity stress management in modern and sustainable agriculture. Useful microorganisms enhance the plant growth both directly and indirectly under saline soils. PGPR directly stimulate the plant development by the provision of phytohormones, soluble phosphorus and fixed nitrogen. Other microorganisms improve plant growth indirectly by imparting safety to the plants against soil-borne infections (Shrivastava and Kumar 2015).

20.3.3 Heavy Metal Toxicity

The metallic components with a concentration higher than 4 g/cm^3 are known as heavy metals. These heavy metals are toxic at low concentrations and also nondegradable. Heavy metal-contaminated soils are increasing day by day due to the intensive farming, anthropogenic activities and continued industrialization (Duruibe et al. 2007; Ma et al. 2016a, b). Several techniques are used for the remediation of massive metal effects but are very expensive and damaging to soil structure (Glick 2010). A sustainable method is required to remove the toxic effects of heavy metals on the soil environment. Commonly, it is the simple technique which makes use of plants (directly) and/or microorganisms (indirectly) to remove the toxic pollutants from the soil is called phytoremediation. This method is cost-effective on a sustained basis for the removal of heavy metals (Chirakkara et al. 2016). In modern agriculture, microbes in the form of bacterial fertilizers are used to increase the effectiveness of phytoremediation. Microorganisms are an appropriate and highly responsive indicator of heavy metal stress than other living organisms (Broos et al. 2004; Chen et al. 2014). Use of bacterial fertilizers for helping the heavy metal remediation is preferred because of its eco-friendly nature and lower cost for sustainable agriculture. Microbes are involved in promoting the plant growth and development by several different ways such as metal complexation, enzymatic detoxification and volatilization. They are also involved in releasing of plant growth controllers like IAA and lowering of ethylene concentration and suppress the disease to increase the crop production (Glick 2010). Verma et al. (2013) and Ahmad et al. (2011) explained that phosphate solubilization, nitrogen fixation, siderophore production and nutrient mobility increase the plant growth and also assist for the removal of heavy metal pollutants. Bioaccumulation by microorganisms especially *Proteobacteria* is potentially used to remove the excessive amounts of lead (Pb) and antimony (As) from heavy metal-contaminated soils (Zhang et al. 2015).

20.3.4 Heat Stress

Rodell et al. (2009) described that temperature stress is increasing day by day due to globally changing climatic conditions. This abiotic stress is dominant in dry climatic regions. Both heat stress (HS) and chilling temperature conditions are very important factors limiting crop production and making the food insecure worldwide (Alam et al. 2017). Heat stress increase the fluidity of the plasma membrane and is also one of the most important abiotic stresses that cause more changes in phytohormone concentration. Under heat stress conditions, the concentration of jasmonic acid (JA) is increased. The impact of heat stress is highest in dehydrated areas. It causes an increase in the rate of transpiration, lowering of photosynthetic activity and reducing of cell division and plant growth. Temperature is essential for regulating the functioning of microbes under extreme temperature environments. Several effective mechanisms are involved by the microbes to save nucleic acid from living under heat stress conditions. Psychrotrophic microbes grow maximum at or above 15 °C. Plants use various mechanisms to reduce temperature stress including osmolyte production and production of various essential enzymes. The production of heat shock proteins (HSP) is enhanced under severe heat stress conditions which provide protection to the microbes from heat. HSP and reactive oxygen species (ROS)-scavenging enzymes are the most important effective proteins (Qu et al. 2013; Kotak et al. 2007). These microbes have maximum growth at 45–50 °C. Under the severe heat stress conditions, they enhance photosynthesis and help in efficient nutrient and water uptake. The strategy which expresses the HSP gene is adopted for high temperature. The concentration of accumulated trehalose in microbes increases tremendously under heat stress which provides protection to them from heat injury and oxidative stress conditions (Li et al. 2009). It maintains protein stabilization in cells. It decreases the induced heat stress protein denaturation and gives protection to the proteins against denatured proteins due to heat stress. Use of bacterial fertilizers inoculated with thermotolerant phosphate-solubilizing bacteria is very important. These microbes help in the transformation of insoluble phosphate to soluble form through acidification to promote plant growth (Kumar and Verma 2018).

20.3.5 Chilling Stress

Chilling or cold conditions are becoming the most important abiotic stress, reducing crop productivity and causing the food insecurity worldwide. Change in the cell membrane and reduced photosynthetic activity are the significant effects of temperature stress especially cold stress (Rodell et al. 2009; Alam et al. 2017). Temperature plays a role in affecting the different parts of the plasma membrane – like cold stress makes it more rigid (Qu et al. 2013; Kotak et al. 2007). Bacterial fertilizers inoculated with cold-tolerant microbes are used to overcome this chilling stress conditions that cause the reduction of plant growth and development. The enzymatic features of microbes help in adaptation to low-temperature environmental conditions. There are two groups of microbes on the basis of their growth: psychrophilic microbes grow maximum at or below 15 °C, but psychrotrophic microorganisms lie plentifully at or above 15 °C. It is studied that trehalose plays most important role actively against the conditions of freezing and desiccation. Yadav et al. (2014) explained that *Arthrobacter nicotiana* and *Pseudomonas cedrina* are the two effective microbes having the multifunctional ability of plant growth promotion. The PGPRs isolated from root nodules of chickpea plants grown in low-temperature areas (Meena et al. 2015), can be used as bacterial fertilizers in low-temperature regions (Meena et al. 2015) which may be used as bacterial fertilizers in low-temperature regions. Furthermore, It is explained that the psychrophilic bacteria isolated from Antarctica express antimicrobial activity (Javani et al. 2015).

20.3.6 Nutrient Deficiency

Nutrients which are essential in huge quantities for plants life cycle are called macronutrients, but some which are necessary for plant growth but require in small amounts are known as micronutrients. Phosphorus is the second most limiting nutrient after nitrogen for plant growth and development. It is important in all metabolic processes like energy transfer, photosynthesis, respiration, etc. (Khan et al. 2010). Phosphorus is found in abundant amount both in living and mineral forms. It is the important limiting factor after nitrogen, but it is not available to plant uptake due to its insoluble forms (Rengel and Marschner 2005). Its availability is restricted to plants due to its low solubility and soil fixation due to calcareousness of the soil. Poor availability of phosphorus reduces the root growth and ultimately decreases plant growth and development. There are two processes like phosphorus sorption on the soil surface and phosphate precipitation by Al^{3+} and Fe^{3+} (Havlin 1999). Chemical fertilizers are added to overcome the problem of phosphorus deficiency in the soil. Tilman et al. (2001) explained that their cost of production is high and they have adverse environmental impacts on soil health and cause the degradation of soil. High P levels cause the eutrophication in groundwater that increases the growth of algal bloom (Schindler et al. 2008). The efficiency of phosphatic fertilizers is not more than 30% due to their fixation in the form of $Ca\ (PO_4)_2$ in alkaline and calcareous soils (Lindsay et al. 1989). So, there is a need to introduce the use of environment-friendly and cost-efficient source which makes the insoluble phosphorus soluble and available to plants. Bacterial fertilizers inoculated with phosphate-solubilizing bacteria are the best option for sustainable agriculture. P solubilization includes the production of organic acids and the liberation of various enzymes. Phosphorus-solubilizing bacteria provide assistance in the P cycle like mineralization and immobilization, sorption and desorption, etc. (Sharma et al. 2013a, b).

20.4 Mechanisms of Biofertilizers Under Abiotic Stresses

20.4.1 Production of Phytohormones

Badri and Vivanco (2009) studied that, naturally, root exudates are the biochemical compounds released by the plant roots to the soil. The composition of root exudates varies according to the biotic such as bacteria and fungi and abiotic stress conditions like soil salinity, soil temperature and soil pH. Root secretions are commonly comprised of various amount of sugars, amino acids and phytohormones such as auxins (Bais et al. 2006). These hormones promoting plant growth are called phytohormones like salicylic acid (SA), indole acetic acid (IAA), abscisic acid (ABA) and jasmonic acid (JA). Their concentration in root exudates increased under salt or heat stress conditions. Root exudation of phytohormones especially in stress conditions increases the transport of nutrients through the membrane of roots in a passive way by different mechanisms like vesicular transport, diffusion and ion channels. Root exudation also uses the proteins situated in the plasma membrane of the roots to transport the compounds through active transport (Baetz and Martinoia 2014). Phytohormones play an essential role as facilitators in numerous activities even under the non-living environmental conditions. Research and scientific literature give infrequent or rare information on phytohormone secretion for plant growth promotion (Vives-Peris et al. 2017).

Letham (1969) explained that there are five critical different types of phytohormones including auxins, cytokinins (CKs), gibberellins, ethylene and abscisic acid (ABA). Auxin was the first hormone identified in plants, is very important to raise tropic responses, promotes cellular elongation, suppresses the leaf abscission, promotes the adventitious root growth and controls the process of ageing and dormancy of seeds. Cytokinins are similar to adenine in molecular structure, but they are important in active cell division. Lateral bud development is increased by the cytokinins and leaf senescence is delayed. Gibberellins are more common in the kingdom Plantae and are found in young shoots and seeds in abundant amount and play an important role to stimulate the cell division and elongation. So, they control the plant stem elongation (Yamaguchi 2008). Ahmad and Prasad (2011) studied that ethylene is the only gaseous phytohormone and its synthesis is enhanced in plant tissues when they are in stress conditions. It is actively involved in the fruit ripening and initiates the abscission of leaves and fruits. Abscisic acid (ABA) is mainly involved in the cell growth inhibition, but it does not initiate the abscission as its name indicates. It promotes the seed dormancy, and ABA concentration is increased immediately to close the stomata when leaves are facing the drought stress conditions (Wilkinson et al. 2012).

20.4.2 Nutrient Uptake Through Natural Processes

There are two most crucial processes like phosphate solubilization and nitrogen fixation that are contributing to plant nutrient uptake. The process of nitrogen fixation capable for the translocation of the N_2 in the whole biological environment is the most important biological process after the process of carbon fixation through photosynthesis (Wani et al. 2016). The symbiotic bacteria like *Rhizobium* sp. and nonsymbiotic bacteria like *Enterobacter* sp. are involved in the conversion of atmospheric nitrogen (N_2) into amonium, ammonium and nitrate ions (Szilagyi-Zecchin et al. 2016). Phosphorus-solubilizing bacteria like *Bacillus* sp. and *Pseudomonas* sp. in rhizosphere soil convert the insoluble phosphorus into a form which is soluble and readily available to plants (Szilagyi-Zecchin et al. 2016). Solubilization and mineralization process is carried out with the help of these phosphate-solubilizing bacteria like *Bacillus* sp. (Milošević and Govedarica 2001; Gopalakrishnan et al. 2015). Such bacteria produce organic acid and several extracellular enzymes for the dissolution of rocks. All these processes help in the nutrient dissolution in soil solution, and providing them to the plant roots (Sharma et al. 2013a, b).

20.4.3 Induction of Abiotic Stress Resistance Genes

In the agriculture sector, abiotic stress is becoming the major challenge for research scientists and agriculturists because soil salinity increases the shortage of water leading to drought stress, and increases temperature which leads to heat stress (Zhang et al. 2000). Extra water on the soil surface leads to waterlogging and anoxia stress, low temperature is causing chilling stress, and heavy metal toxic stress has a negative effect on the overall processes involved in plant development and grain production in almost all important crops growing in fields. In the recent scenario, to overcome this major challenge, plant genetic engineering is a scientific discipline which makes the production of plant species tolerant to numerous abiotic stress conditions (Dhariwal et al. 1998). Different essential and regulatory proteins are encoded by the various types of genes over the last several years and are used to introduce transgenic plants which have ability to tolerate the effects of abiotic stress conditions. The use of controlled gene is appreciable for introducing crop plants tolerant to abiotic stress. It is a beneficial and most efficient method which involves the introduction of a single controlling gene leading to the changed expression of several different essential genes which leads to a wide range of organized altered responses. In research of plant genetic engineering, the work on inducible promoters is increasing in recent years. Abiotic stress-induced promoters have poor efficiency of gene expression as compared to other promoters. Therefore, there is a need to manipulate the regulatory genes in such a way that the efficiency of the abiotic stress-responsive promoter is enhanced without affecting the induction promoters negatively. Such promoters can be used with vectors in future having more advantages on regular work of plant alteration (Katiyar-Agarwal et al. 1999).

20.5 Conclusions and Future Aspects

In modern irrigated agriculture, excessive use of synthetic fertilizers destroyed the soil nutrient status which makes it inappropriate for crop production. Excess use of these fertilizers causes severe environmental hazards like waterlogging, underground water pollution, land erosion, etc. and health issues for the living organisms. Moreover, due to changing climatic conditions, abiotic stresses like soil salinity, drought, heavy metal toxicity, etc. are becoming a significant challenge, affecting all the processes associated with the crop production and, thus, causing the problem of food insecurity for tremendously increasing global population. Therefore, the use of bacterial fertilizers is the best technique which stimulates the microorganisms naturally present in soil and is gaining more importance for the raising of crops due to its cheaper cost and environmentally friendly nature. These fertilizers provide essential nutrients to the soil which restore the natural soil fertility and also provide protection to the soil against soil diseases, salt stress and drought stress. The scope of stress plant genetic engineering in future will be the combination of enhanced efficiency and abiotic stress-induced expression of the transgenes. The production of abiotic stress-tolerant crop plants as a result of plant genomic engineering is a more important but more expensive and lengthy process. So, the application of microbes as bacterial fertilizers to reduce the abiotic stresses in plants could be the most efficient and eco-friendly option, and its availability could be in less time. There is a need for further research and development which makes the mechanisms of the actions of bacterial fertilizers understandable and easier. Further, it is important to isolate and find out the best and competent bacterial strains and use them with suitable carrier material for making farming practices more economical on a sustainable basis.

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Chapter 21
Bioreactor Upscaling of Different Tissue of Medicinal Herbs for Extraction of Active Phytomolecules: A Step Towards Industrialization and Enhanced Production of Phytochemicals

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21.1 Introduction

A wide range of plant-based drug molecules are the products of plant's secondary metabolism. These phyto-bioactives are synthesized via plant's 'secondary metabolism' because of their cardinal contribution in plant's defence responses for acquiring ecological fitness rather than in the 'primary metabolism'-driven growth sustaining processes. As a consequence, the biosynthesis of these secondary metabolites must adjust and accommodate with their primary metabolic pathways (Rao and Ravishankar 2002). Coordinated regulation between these processes is a necessity to survive and act as source for these metabolites for human usages. These phytomolecules are always in short supply because they are often produced as and when required by the plant and in quantities that are just sufficient to prevent or combat a threat/stress situation faced by the plant. Since these molecules are invariably expensive to be synthesized from the point of view of the metabolic energy budgeting of a plant cell, their biogenesis is often tightly regulated at the levels of enzymes and corresponding genes associated with their synthesis (Verpoorte et al. 2002). This intriguing clash of interests between plants to keep the cellular concentration of a given secondary metabolite to a bare minimum for their own use and human desire to extract that metabolite in larger quantity for their own industrial use in drugs and aromas has revolutionized the biotechnology industry. Majority of plant secondary metabolites are synthesized via complex, interlinked biogenetic

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pathways. Our understanding of the architecture, organization and regulation of these pathways at cellular and molecular levels is getting refined, with the advancement in the biotechnological tools (Verpoorte et al. 2002).

Plant cell, tissue and organ cultures have long been viewed as viable renewable resource of industrially important plant natural products. Some of the exclusive advantages that cell and tissue culture-based production technology provide include:

(i) Control of product supply independent of the geographical availability of plant.
(ii) Avoidance of seasonal fluctuations.
(iii) Uniform growth under defined environment.
(iv) Shorter growth and production cycle free of batch-to-batch variation.
(v) Possible options for biotransformation through feeding and elicitation.
(vi) Simpler cellular organization avoids structural constraints and neighbouring cell interference.
(vii) Ease with which cultivation conditions can be altered and optimized to up- or downregulate the pathway expression.
(viii) Sterility ensures that biogenesis of target molecule is not mediated through associated microflora prevalent under field conditions.
(ix) Biosynthetic steps expressed at low level or for very limited time in intact plants can be prolonged.
(x) Industrial upscaling in bioreactor is possible.
(xi) Easy downstream/extraction processing (Bhojwani and Dantu 2013).

To upscale the secondary metabolite-producing cell suspensions, bioreactors were developed initially, which were later used to upscale somatic embryos, hairy root cultures and even intact plants also (Weathers et al. 2010). Previous studies showed the higher accumulation of desired secondary metabolites in plant cell culture in comparison to the intact native plant systems. Five- to tenfold increase in the yield of shikonin, ginsenoside, anthraquinones, ajmalicine, rosmarinic acid and berberine was observed in the callus/cell suspension cultures of *Lithospermum erythrorhizon*, *Panax ginseng*, *Morinda citrifolia*, *Catharanthus roseus*, *Coleus blumei* and *Coptis japonica*, respectively, in comparison to the native plant system (Ruffoni et al. 2010). Interestingly, multiple-fold increase in artemisinin (*Artemisia annua*), gentiopicroside (*Centaurium erythraea*) and genistin (*Genista tinctoria*) was recorded in the bioreactor-grown shoots in comparison to intact plant materials (Krol et al. 2020).

An essential step towards successful commercialization of plant cell culture-based metabolite production is the transfer of biosynthetic processes from shake flasks to bioreactors. Maintaining the biosynthetic potential of the plant cell cultures during such transfer is not always straightforward or translatable (Georgiev et al. 2009). The main difficulties arise due to dependence of the biosynthesis of target compounds on several interrelated factors, ranging from physiological characteristics of the cultured cells to their genetic stability, sensitivity to mechanical stress, variations in the fluid dynamics, mass transfer and heat transfer in different cultivation systems (Georgiev et al. 2013). Due to incompatibilities between the physiological/nutritional requirements of metabolite-producing cells and the operational parameters of the bioreactors at different experimental scales, the choice of reactor always suffers with compromises. Further, it is impossible to design an ideal type of bioreactor to cultivate a particular type of plant cell for a specific purpose (Georgiev et al. 2009). The present chapter summarizes the work associated with bioreactor upscaling, bioreactor environment influencing factors, commonly used bioreactor types and finally the success stories of bioreactor-based industrialization of medicinal plant cell culture.

21.2 Bioreactor Upscaling: Sustainable Production by Different Medicinal Plant Tissue Types as a Future

For commercial drug production, a huge number of medicinal plants are erratically exploited from the native wild plants. In order to conserve this natural wealth and to meet the increasing demand for phytoceuticals, plant cell culture-based upscaled production and industrialization have become imperative today. Though a wide range of phytochemicals have been detected in plant cell cultures, only few large-scale industrial procedures related to medicinal plants, e.g. shikonin, berberine, ginsenosides, paclitaxel and rosmarinic acid, could be advanced to commercial scale (Rao and Ravishankar 2002). This has been largely due to low and/or inconsistent productivity of many cultures, poor resolutions of enzymes and genes involved in the pathway, tissue- or organ-specific synthesis and/or accumulation and expensive downstream extraction and processing. This lack of expected success can also be attributed to fragmentary information concerning the multi-enzyme conversions in the secondary metabolic pathways and employment of tissues that were not specifically selected or engineered to perform the desired metabolic task.

Even after the selection of ideal or engineered tissue for upscaling, maintaining the same physiological and metabolic state of tissue for desired metabolite production under bioreactors is a difficult task. The bioreactors are provided with sustainable conditions for plant cell growth and desired product formation. An ideal bioreactor for plant cell-based production must have scale-up capability, long-term sterility along with homogenous aeration and low shear sensitivity (Fig. 21.1). It should be reasonably maintained with ease of handling. Economic feasibility is another important factor associated with successful upscaling in the bioreactors (Georgiev et al. 2009).

Majorly the medicinal plant tissue employed for upscaling comes under three categories, namely, plant cell suspensions, hairy roots and intact plants. Out of these, plant cell suspensions have highest potential for industrialization, due to their automated replenishment of nutrients, aeration, temperature and light control and pH maintenance. One of the limiting factors associated with plant cell suspensions is the aggregation of cell. Sometimes it is essential for the biosynthesis of desired metabolite production as in plant cell suspensions as the production is not

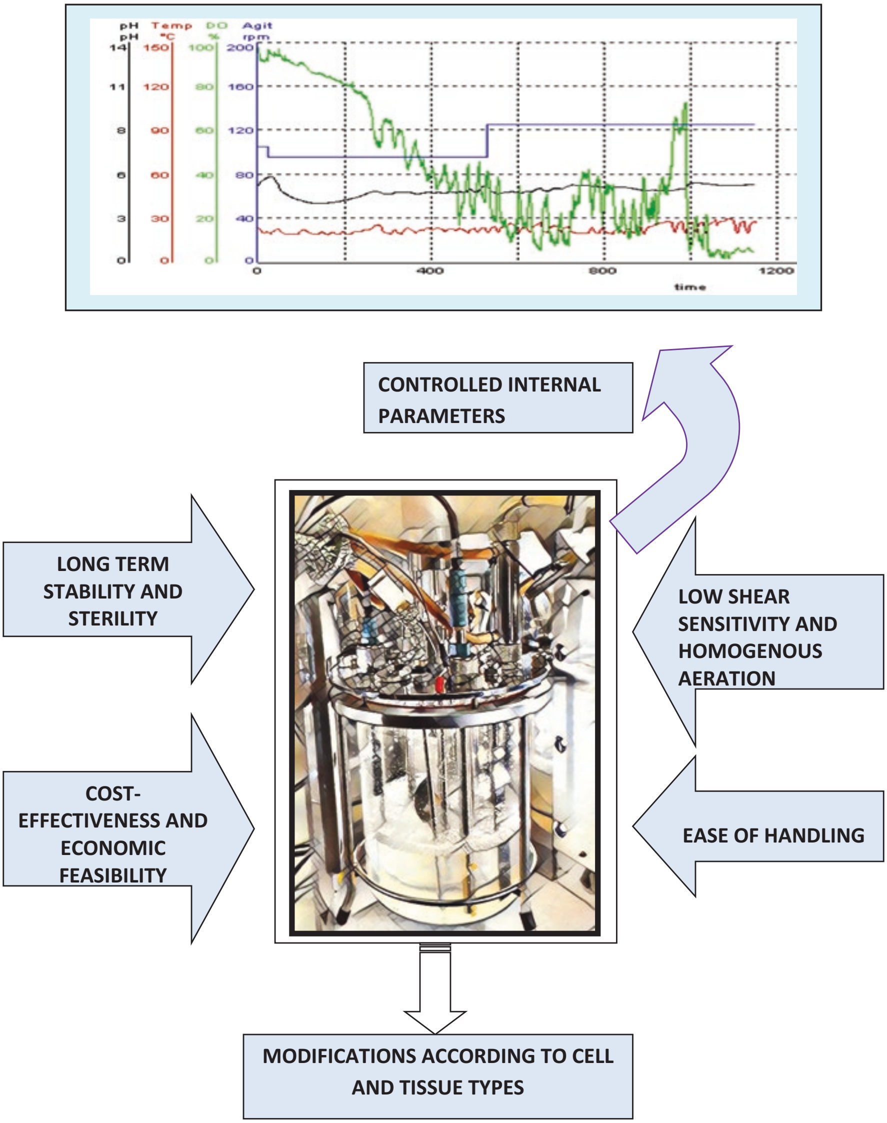

Fig. 21.1 Prerequisites for the bioreactors to be used in plant cell culture upscaling

associated with the growth. This leads to sedimentation and irregular mixing which can turn into uneven allotment of catalytic reaction. Even distribution of nutrients from liquid/gaseous phase to cells and efficient oxygenation are needed for better growth and can be provided by homogenous mixing only. Most of plant-based commercially produced phytoceuticals are processed from bioreactor-grown cell suspensions.

Hairy roots are the other options to produce desired phytomolecule at high concentrations. Because of the shear sensitivity and unnecessary tissue damage during mechanical stirring, the upscaling of hairy root cultures in bioreactor has always been an operational problem (Sevon and Oksman-Caldentey 2002; Zhao and Verpoorte 2007). The oxygen demand is not uniform for the whole tissue in hairy roots; it is found to be higher in meristematic root tip zone in comparison to the zone of elongation and maturation. Therefore another hairy root upscaling-associated difficulty is the heterogeneity in oxygen demand of different growth-staged roots. The third complexity associated with the hairy roots is the unfavourable nutrient gradient within the bioreactor environment due to tendency of growing in tufts.

Due to dedifferentiated and less organized structure of cell suspensions and hairy roots, respectively, in comparison to the intact plants, some of the secondary metabolites are not synthesized in them. Here comes the role of upscaling of intact plants: e.g. hypericin (*Hypericum perforatum*) is stored in the specialized secretary glands present on the leaves. Similarly artemisinin is accumulated in the glandular trichomes on the leaf surface of *Artemisia annua*. Improper mixing, growth in bunches, localized nutrient supplementation and demand of desired physiological states of specialized organs are the main limitations associated with bioreactor upscaling of intact plants (Georgiev et al. 2013).

21.2.1 Bioreactor Upscaling Studies in Various Medicinal Plants

The scale up study from bioreactors is of immense importance as it provides the ideal estimate regarding quantification of certain metabolite from a plant system source. Not only this, the upscale level studies in bioreactors also provide a platform for growth kinetic studies and indicate the biomass accumulation in the plant system on different days of harvesting. For these purposes various kinds of bioreactors are in use depending upon the tissue types, aeration required, mesh size, type of media as well as the capacity of the bioreactors. Different bioreactors are in use since the time the upscaling idea has been approached to researchers and various kinds and types of bioreactor were used such as stirred tank, bubble columns, reactors with temporary immersion system, balloon-type bioreactors and airlift bioreactor to name a few depending upon the plant system and their sources used. As far as the plant sources are concerned, many types of the explants are upscaled in bioreactor which totally depended on the condition that which type of secondary metabolites is produced in optimum form in plant source. Keeping this objective in mind, various starting materials such as suspension cultures, hairy roots, adventitious roots, protocorms, transgenic roots and microshoots have been used and bioreactors have been modified according to the respective plant material. Plant cell suspensions as such on expressing transient gene have been upscaled in bioreactor efficiently as transient expression of protein mediated by *Agrobacterium* has been assessed by

O'Neill et al. (2008) in *Nicotiana glutinosa* cell suspension culture, and yield was found to be increased with co-culturing with the *A. tumefaciens* strain.

In the similar manner, other simple strategies such as elicitation with biotic or abiotic factors were also used in different plant systems such as *Withania somnifera*, *C. roseus* and *V. minor* plant to enhance the productivity of certain secondary metabolites and upscale these systems at bioreactor level. One significant plant study of this kind is elicitation study of *W. somnifera* in which cell suspension cultures of the plant system were challenged with elicitors such as cadmium chloride, chitosan and aluminium chloride. Simultaneously the precursor feeding was also performed with precursor like mevalonic acid, cholesterol and squalene, and the study was conducted at shake flask as well as bioreactor level. The study resulted in enhanced withanolide concentration in combination of chitosan and squalene (Sivanandhan et al. 2014). Elicitation strategies have been used as a potential tool in bioreactor level studies by Verma et al. (2014a). Authors have revealed the improvement in sanguinarine production which is an important alkaloid of *Papaver somniferum* with the employment of abiotic and biotic elicitors. The latex-less variety of *P. somniferum* resulted in maximum accumulation of sanguinarine on using biotic elicitor, namely, *Trichoderma harzianum* extract. The upscaling of these plant cell suspensions in 5 L stirred tank bioreactor resulted in maximum sanguinarine production.

The another major example of transgenic expression of gene and upscaling in bioreactor is the overexpression of *C. roseus* tryptophan decarboxylase and strictosidine genes in different plant system of *V. minor*. The *rol* gene-integrated plant cell suspensions of *V. minor* were successful in showing transgenic expression of TDC and STR genes. The selected cell lines that were upscaled in stirred tank bioreactor exhibited twofold increment in total alkaloid content as well as showed the presence of vincamine (Verma et al. 2015).

The hairy root culture is another important preferred source of plant system used in bioreactor as this system has added advantages of auxotrophic nature, fast growth and genetic stability which are fulfiling the criteria for bioreactor level studies. The potencial of variety of medicinal plants has been assessed especially those plants which have higher production of desired secondary metabolites in plant roots or which can enhance production of secondary metabolites in the hairy root cultures. Such plant systems have been targeted for bioreactor upscaling provided hairy roots were taken as explants. One such example is the production of beta-OH-verbascoside from *Harpagophytum procumbens* in stirred tank as well as shake flask cultures (Georgiev et al. 2010). In this particular plant system, this report has provided the basis of potential use of *H. procumbens* as biofactory for production of important secondary metabolite such as verbascoside, leucoseptoside A and martynoside. A range of medicinal plants have been assessed for their medicinally important molecules. One such example is *Artemisia annua* which is known to produce the antimalarial secondary metabolite artemisinin. The scale up level studies in hairy roots of *A. annua* was conducted with an effort to enhance the biomass production, in the mist bioreactor. The hairy root cultures of *A. annua* in a mist reactor showed improved growth rates when compared to shake flask studies (Sivakumar et al.

2010). A comparative study was conducted by Jaremicz et al. (2014) to check the tropane alkaloid production in the hairy roots of *Hyoscyamus niger* in spray bioreactor and bubble column bioreactor. The hybrid bubble column/spray bioreactor was found suitable for the hairy root growth and secondary metabolite production as maximum anisodamine yield was observed in this type of bioreactor although bubble column reactor was more effective in generating those lines which were having better hyoscyamine, scopolamine and cuscohygrine.

Distinctively the upscaled production level of biopesticides has also been tested in bioreactors as the production of azadirachtin which is a widely accepted biopesticide has been upscaled in stirred tank bioreactors. The suspension cultures of *Azadirachta indica* were raised from callus culture, and it resulted in high biomass accumulation as well as azadirachtin production from it (Srivastava and Prakash 2010). In some studies hairy root cultures have been tested in different modified bioreactor types to suit the growth of the transformed roots. The hairy roots of *Beta vulgaris* have been efficiently raised in bubble column bioreactor with the approach of batch and fed-batch production. This study resulted in significant production of betalain pigment (Pavlova et al. 2007).

In the recent years, temporary immersion system (TIS) has been widely used in raising the different plant systems that are better suited to grow on TIS-based principles. The plant form bioreactor has been used as TIS in date palm plant system under controlled light conditions to assess bud formation and shoot multiplication through direct regeneration process. This approach resulted in increment in shoot numbers, and the length of the shoots was also found to be improved (Nayyef et al. 2019). The temporary immersion bioreactors were also used to micropropagate *Spathiphyllum* plant, where it registered improved growth and resulted into micropropagation of plant with better rooting abilities (Kacar et al. 2020).

As discussed above, the various modifications of the bioreactors have been done for better production of desired compounds. One such example is the production of galanthamine in the shoot culture of *Leucojum aestivum* where modifications of the glass-column bioreactor were conducted with introduction of internal sections (Georgiev et al. 2012). These modifications led to highest dry mass and galanthine production in *L. aestivum* but also galanthine production were highest in this modified column bioreactor with internal sections.

Hypericum perforatum is known for the production of hypericin and hyperforin which are assumed to play a key role in treatment of mild to moderate depressions. This prized medicinal plant has been studied under in vitro system as different level, and Wu et al. (2014) discussed the effect of airlift bioreactor on the growth of adventitious roots of this plant system as well as its effect on hypericin production. The hypericin productivity was found to be improved in balloon-type bioreactors when air volume in the bioreactor was increased with addition of IBA.

Some of the studies conducted in medicinal plants on bioreactor upscaling of different tissue types are given in Table 21.1.

Table 21.1 Medicinal plants with their yield assessments under bioreactor cultivation

S. No	Plant system	Morphogenic level	Bioreactor type	PGRs/elicitors used	Biomass/product yield	Reference
Cell suspensions/somatic embryos						
1.	*Nicotiana glutinosa*	Cell suspension	51 L stirred tank bioreactor	5 mg/l 2,4-D and 0.2 mg/l Kn	Yield of approximately 1.1 mg through co-culture with an auxotrophic strain of *Agrobacterium tumefaciens*	O'Neill et al. (2008)
2.	*Vitis vinifera*	Somatic embryos	Airlift bioreactor		Normal elongation in the transition from heart-shaped to the torpedo-shaped and finally to advanced developmental stages, with radicle emergence and whole plant generation	Tapiaa et al. (2009)
3.	*Harpagophytum procumbens*	Cell suspension	3 L stirred tank reactor and a 1 L glass-column bioreactor (operated with pulsed aeration)	0.2 mg/l 2,4-D	165.42 mg verbascoside/l/day (in the pulse-aerated column reactor)	Georgiev et al. (2010)
4.	*Azadirachta indica*	Cell suspension	Stirred tank bioreactor	2.0 mg/l NAA and 0.2 mg/l BAP	Biomass accumulation of 95.8 g/L and highest azadirachtin production of 380 mg/l	Srivastava and Prakash (2010)
5.	*Catharanthus roseus + Rauwolfia serpentina+ Vinca major*	Cell suspension	5 L stirred tank bioreactor	2.0 mg/l NAA and 0.2 mg/l Kn	A 20- and 40-fold increment in the biomass	Verma et al. (2012a)
6.	*Catharanthus roseus*	Cell suspension	7 L stirred tank bioreactor	2.0 mg/l NAA and 0.2 mg/l Kn	Cell biomass yield enhanced to 30-fold if 30 mg/ l 5-MT is added to vessel	Verma et al. (2013)

S. No	Plant system	Morphogenic level	Bioreactor type	PGRs/elicitors used	Biomass/product yield	Reference
7.	*Withania somnifera*	Cell suspension	7 L airlift bioreactor	Picloram (1 mg/l), KN (0.5 mg/l), L-glutamine (200 mg/l) and 5% sucrose Combined treatment of chitosan (100 mg/l) and squalene (6 mM) along with 1 mg/l picloram, 0.5 mg/l KN, 200 mg/l L-glutamine and 5% sucrose	Withanolide A (7606.75 mg), withanolide B (4826.05 mg), withaferin A (3732.81 mg), withanone (6538.65 mg), 12 deoxywithanstramonolide (3176.63 mg), withanoside IV (2623.21 mg), withanoside V (2861.18 mg)	Sivanandhan et al. (2014)
8.	*Papaver somniferum*	Cell suspension	5 L stirred tank bioreactor	2,4-D and *T. harzianum* culture filtrate and shikimate as elicitor	Sanguinarine (0.025 ± 0.004% dry wt.)	Verma et al. (2014a)
9.	*Vinca minor*	Cell suspension	5 L stirred tank bioreactor	10% v/v *Trichoderma harzianum* culture filtrate	Threefold increase in biomass	Verma et al. (2014b)
10.	*V. minor*	Cell suspension *(transgenic TDC and STR overexpressing genes)*	5 L stirred tank bioreactor	–	Vincamine (2.7 ± 0.3 and 0.005 ± 0.001% dry wt.)	Verma et al. (2015)
Hairy roots/adventitious roots/roots						
11.	*Atropa belladonna*	Hairy roots	30 L stirred tank bioreactor	–	Tropane alkaloids 1490 mg, atropine (5.4 mg/g dry wt.)	Lee et al. (1999)
12.	*Panax ginseng*	Hairy roots	Wave bioreactor	–	28-fold increase in initial root fresh weight, with biomass of 284.9 g/l and a ginsenoside content of 145.6 mg/l	Palazon et al. (2003)

(continued)

Table 21.1 (continued)

S. No	Plant system	Morphogenic level	Bioreactor type	PGRs/elicitors used	Biomass/product yield	Reference
13.	*Hyoscyamus muticus*	Roots	Trickle-bed root culture reactors	–	Tissue concentrations as high as 36 g dry wt./l (752 g fresh wt./l)	Ramakrishnan and Curtis (2004)
14.	*Solanum chrysotrichum*	Hairy roots	Novel modified mesh-draught with wire-helixes 2 L reactor (MR)	–	Growth rate of 0.112/d	Caspeta et al. (2005)
15.	*Beta vulgaris*	Hairy roots	3 L bubble column reactor	–	Betalain 330.5 mg/l	Pavlov et al. (2007)
16.	*Echinacea purpurea*	Adventitious roots	1000 L airlift bioreactor	2 mg/l IBA	Chlorogenic acid 5 mg/gm dry wt. Chicoric acid 22 mg/gm dry wt. Caftaric acids 4 mg/gm dry wt.	Wu et al. (2007)
17.	*Nicotiana*	Hairy roots	Mist reactor	–	Total mouse interleukin-12 concentration 5.3 μg g – 1 FW	Liu et al. (2008)
18.	*Glycyrrhiza glabra*	Hairy roots	5 L capacity stirred tank bioreactor	–	310 g of root biomass (20 times more from initial inoculum)	Mehrotra et al. (2008)
19.	*Salvia miltiorrhiza*	Hairy roots		Hyperosmotic stress (OS, created with 50 g/L sorbitol) and a yeast elicitor (YE, polysaccharide fraction of yeast extract)	Tanshinone content of roots was increased to 18.1 mg/g dry wt. (or 1.8 wt.%) and the volumetric tanshinone yield to 145 mg/l, which were about 100-fold and 70-fold	Wu and Shi (2008)
20.	*Harpagophytum procumbens*	Hairy roots	3 L stirred tank reactor	–	Verbascoside (1.12 mg/g dry wt.)	Homova et al. (2010)

S. No	Plant system	Morphogenic level	Bioreactor type	PGRs/elicitors used	Biomass/product yield	Reference
21.	*Bupleurum falcatum*	Adventitious roots	Modification of internal configuration of a bubble column, airlift and stirred tank reactor	5 mg/l IBA	500–600 mg/L of saikosaponin-a and saikosaponin-d	Kusakari et al. (2011)
22.	*Echinacea purpurea*	Adventitious roots	Balloon-type bubble bioreactor (1000 L)	–	5.1/0.15 final density (g Dry wt./l)/productivity	Baque et al. (2012)
23.	*Panax quinquefolium*	Hairy roots	Nutrient sprinkle bioreactor	–	Ginsenoside content 6 mg/g dry wt.	Kochan et al. (2012)
24.	*Catharanthus roseus*	Hairy roots	5 L stirred tank bioreactor.	–	9–11-fold increment in biomass accumulation Twofold enhancement in ajmalicine (0.029% dry wt.)	Verma et al. (2012b)
25	*Hyoscyamus niger*	Hairy roots	Hybrid bubble column/spray bioreactor	–	Anisodamine content 0.67 mg/g dry wt.	Jaremicz et al. (2014)
			Bubble column reactor		Scopolamine (5.3 mg/g dry wt.), hyoscyamine (1.6 mg/g dry wt.) and cuscohygrine (26.5 mg/g dry wt.)	

(continued)

Table 21.1 (continued)

S. No	Plant system	Morphogenic level	Bioreactor type	PGRs/elicitors used	Biomass/product yield	Reference
26.	*Vinca minor*	Hairy roots	5 L stirred tank bioreactor.	Fortification with secologanin (10 mg/l) along with tryptophan (100 mg/l), naproxen (8.4 mg/l), hydrogen peroxide (20 μg/l) and acetic anhydride (32.4 mg/l)	Vincamine production (0.017 ± 0.001% dry wt.)	Verma et al. (2014c)
27.	*Vinca minor*	Hairy roots	5 L stirred tank bioreactor	10% v/v *Trichoderma harzianum* culture filtrate	Vincamine production (0.015 ± 0.002% dry wt.)	Verma et al. (2014b)
28.	*Atropa belladonna*	Hairy roots	1.5 L bioreactor	–	Scopolamine production (1.59 mg/g dry wt.)	Habibi et al. (2015)
Shoots/ microshoots/protocorms						
29.	*Artemisia annua*	Shoots	Inner-loop mist bioreactor		Dry weight of biomass and artemisinin production in the bioreactor reached 13.4 and 46.9 mg/l, respectively	Liu et al. (1998)
30.	*Stevia rebaudiana*	Shoots	Roller bioreactor	0.1 mg/l BAP + NAA	1.5-fold increase in the number of shoots	Bondarev et al. (2003)
				Gibberellic acid	Shoots and root elongation	
				3% sucrose	Optimal steviol glycoside production	
31.	*Hypericum perforatum*	Plantlets	Balloon-type bubble bioreactor system		Hyperforin content was increased in plantlets grown at 45 g/l sucrose under CO_2-non-enriched conditions	Zobayed et al. (2003)
32.	*Centaurium erythraea*	Shoots	5 L mist trickling bioreactor	IAA + BAP	Secoiridoid accumulation (303 mg l – 1) Dry weight increment from 0.54 g to 18.3 g (28th d)	Piatczak et al. (2005)

S. No	Plant system	Morphogenic level	Bioreactor type	PGRs/elicitors used	Biomass/product yield	Reference
33..	*Dendrobium candidum*	Protocorms	3 L airlift balloon-type bioreactor		10 g/l inoculum volume, 1600 lx light intensity and 0.2 air volume were favourable for the growth of protocorms and the production of dendrobine	Yao et al. (2012)
34.	*Leucojum aestivum*	Shoots	1 L temporary immersion system modified bioreactor vessel which was used as an airlift mode		Galanthamine (19.416 mg)	Schumann et al. (2013)
35.	*Leucojum aestivum*	Shoots	Modified bubble column bioreactor with internal sections	NAA + BAP	Dry biomass (20.8 g/L) and galanthamine (1.7 mg/L) were achieved	Georgiev et al. (2012)
36.	*Securinega suffruticosa*	Shoots	Bubble column bioreactor	BAP + NAA + IP	Securinine 3.25 mg/g dry wt. and allosecurinine 3.41 mg/g dry wt.	Raj et al. (2015)
37.	*Rhododendron tomentosum*	Microshoots	RITA® bioreactor	Aphid extract and lysate of *P. carotovorum*	Increased the volatile fraction content by 14%, while Ni2+ and ergosterol by 8%	Jesionek et al. (2018)

(continued)

Table 21.1 (continued)

S. No	Plant system	Morphogenic level	Bioreactor type	PGRs/elicitors used	Biomass/product yield	Reference
38.	*Schisandra chinensis*	Microshoots	Plant form bioreactor	BAP+ NAA + yeast extract elicitor	Salicylic acid (110.15 mg/100 g DW), neochlorogenic acid (106.05 mg/100 g DW) and cryptochlorogenic acid (69.39 mg/100 g DW). The total content was equal to 362.58 mg/100 g DW and was 1.05-fold higher than in extracts from the non-elicited microshoots	Szopaa et al. (2019)
39.	*Phoenix dactylifera*	Bud and shoot from direct organogenesis	Temporary immersion system (TIS) using the plant form bioreactor with LED lights	2 IP	52.25 shoots	Nayyaf et al. (2019)
40.	*Spathiphyllum* (‘Chico’ genotype)	Microshoots	Temporary immersion bioreactor	BAP + IBA	28.50 (number of leaves)	Kacar et al. (2020)

21.3 Factors Affecting Biomass and Yield of the Medicinal Plant Tissue Upscaled in the Bioreactors

For optimum growth and desired secondary metabolite production, the selection of suitable tissue type is a very important criterion to employ bioreactor upscaling. In *Vinca minor*, transgenic cell line (PVG3) overexpressing *tryptophan decarboxylase* (TDC) and *strictosidine synthase* (STR) was selected on the basis of its twofold increase in total alkaloid content and registration of vincamine presence (0.003 ± 0.001% dry wt.). On optimizing bioreactor upscaling process, vincamine was further enhanced to the level of 0.005 ± 0.001% dry wt. (Verma et al. 2015). Similarly, two hairy root lines of *Catharanthus roseus* were upscaled in bioreactor. First line (PG2) with highest biomass accumulation (GI = 326.73) showed 9–11-fold increment in biomass accumulation with two-fold increment in ajmalicine. The other line (PI3) with highest catharanthine content registered the production of major unknown alkaloid (Verma et al. 2012b).

Once the optimized tissue type is recognized, other constraints related to large-scale bioreactor technology are optimizing uniform growth and metabolite production, culture stability, desirable trait expression and product yield cost. The main factors dealing with the uniform growth and metabolite production are maintenance of pH, temperature, agitation, oxygen supply and nutrient uptake.

21.3.1 pH

Plant tissue under culture conditions is dependent on the absorption of the nutrients from the supplemented media; therefore, pH (which is usually set to be 5.8) of the media plays a crucial role as all the salts are maintained in a buffered form at 5.8. During autoclaving it drops by 0.3–0.5 units due to hydrolysis. Throughout the culture cycle, it changes due to oxidation and differential ion uptakes and sometimes substances released by growing tissue (Bhojwani and Dantu 2013). In a typical culture cycle under bioreactor shown in Fig. 21.1, a drop in pH to 4.0–4.5 took place in the initial 2–3 days in cell suspensions that can be due to ammonium uptake and cell lysis-based acidification. Gradually the pH increased after a few days due to uptake of nitrates and acquires a stable stage of pH 5.0–5.5. It was observed that in *Daucus carota* the bioreactor-grown somatic embryos were produced at highest rate at pH 4.3, but their further differentiation was poor. In contrast, at pH 7, the lower rate of embryo production associated with further development to plantlet stage was observed. Those results can be related to alternations in ammonium ion uptake (Jay et al. 1994).

21.3.2 Temperature

The growth of the plant tissue and biosynthesis of desired tissue are influenced by temperature. Optimal temperature for growing plant species under in vitro conditions ranges from 23 to 30 °C depending upon the species. The metabolite synthesis and growth factors can be associated with key enzymes and their varied temperature optima. A study by Zhong and Yoshida (1993) showed optimal anthocyanin production at 25 °C which was remarkably reduced at 28 °C. Similarly optimum TDC activity and associated ajmalicine production at 27.5 °C were observed by Ten Hoopen et al. (2002) in *Catharanthus roseus* cell suspension cultures. In bioreactors, temperature can be controlled by modifying thermal jackets or circulating water outside the vessel.

21.3.3 Agitation

The agitation phenomenon is considered as one of the destructive elements, i.e. shear stress-creating factor in the plant cell culture upscaling in bioreactors. In the plant cell bioreactors, agitation can be created through mechanically driven impellers or through aeration and bubble formation. The plant cells have large vacuole that can accommodate 95% of the cell volume. Vacuoles have less viscous fluid as compared to cytoplasm due to storage of the secondary metabolites, waste product and water. Therefore plant cells are much sensitive to the shear stress caused by agitation which may lead to biomass degradation and low secondary metabolite yield. Sometimes lowering the agitation resulted in formation of aggregates. Aggregation is another associated problem due to improper mixing. Adequate mixing is required to ensure the uniform supply of nutrients and controlled temperature, pH and substrate concentration. In the study conducted by Verma et al. (2012b), authors ensure the possibility of physically separating the cultivation and the agitation space to reduce the tissue damage in *C. roseus* hairy roots under stirred tank bioreactor. They plated the root inoculum on a plastic mesh tied on the surface of the liquid medium inside the bioreactor. This further ensures the continuous stirring nutrient solution bathing of the proliferating roots. It resulted in better biomass accumulation in comparison to the free-floating roots in the medium inside the bioreactor. Use of an improper impeller type can also exert undesirable hydrodynamic forces leading to poor cell viability and metabolite production due to shear mechanical shocks (Huang and Mc Donald 2009). The Rushton-type impellers were found to be better in comparison to marine-type impeller in *C. roseus* cell suspension upscaling in stirred tank bioreactor, as they pursue a radial flow pattern for cell dispersion in the medium with adequate oxygen mass transfer and lower shear stress (Verma et al. 2013). Cataloguing of the cell morphology- and cell viability-related plant cell suspension rheology is necessary to estimate the extent of shear stress they can sustain. It can help in optimizing bioreactor design and overcoming technical problems.

21.3.4 Oxygen Supply

In a bioreactor, oxygen in gaseous phase above the medium, oxygen in the bubble form inside the medium and dissolved oxygen constitute the total oxygen supply. Air spargers fitted at the base of bioreactor release oxygen, but the part that is dissolved in the water is the oxygen that is available to the plant cells. To improve fluid mixing and adequate oxygen supply, forced aeration is needed in bioreactors. Although plant cells require less oxygen in comparison to microbial cells due to lower metabolic rate and slow doubling time, the depletion of oxygen supply leads to lesser biomass and secondary metabolite yield. Higher supply than required is also detrimental to cell growth due to the stripping of volatile compounds necessary for growth (Georgiev and Weber 2014). Inadequate mixing and high biomass density are the critical conditions responsible for lowering of dissolved oxygen supply. A report by de Feria et al. (2003) determined the effect of 50% and 80% dissolved oxygen concentration on somatic embryos of *Coffea arabica.* It was found that 80% dissolved oxygen induced globular and heart-shaped somatic embryo differentiation, but for production of torpedo-shaped somatic embryos, 50% dissolved oxygen concentrations were needed. The dissolved requirement of each type of cell is different; therefore, for each tissue it must be optimized and facilitated adequate supply.

Sometimes, shearing smash up, breakdown of cell wall and cell debris accumulation have been caused by uninterrupted aeration, mixing and circulation. Cell debris (consisted of polysaccharides) accumulation consequently leads to foaming, cell aggregation and adhesion at culture vessel wall and crust formation at upper half of the vessel. It exaggerates the blockage problem due to limited circulation and resulted in more cell debris formation. Therefore, for proper oxygen supply, higher rate of aeration is required on increment of biomass (Ziv 2000).

21.3.5 Nutrient Uptake (Role of PGRs)

Plant cells growing in vitro in shake flasks or bioreactors are subjected to many types of stress during the growth and development. To combat and manage the posed stress, plant receives the signals through PGRs or other signaling molecules which are dissipated in the plant at the time of stress. The response of plant cells to stress is the combination of external supplementation through the medium (PGRs like gibberellins, abscisic acid, auxins, cytokinin, brassinosteroids/signaling molecules like salicylic acid, jasmonates, ethylene) and internal biosynthesis of the same inside the plant cells (Tuteja et al. 2010). As bioreactor upscaling subjects the plant system to a level of stress in terms of variable pH, high temperature, high agitation and depleted or elevated oxygen. The PGRs and signaling molecules both internally produced or externally supplemented play crucial role in biomass enhancement and secondary metabolite production. The supplemented PGR consumption and uptake is faster in liquid medium due to better exposure of the medium. The nutrient level in bioreactors fitted with condensers to prevent dehydration is affected by the absorption rate and cell lysis. Aggregation is the main limitation of nutrient supply due to improper mixing. As batch culture also sometimes leads to nutrient depletion, fed-batch culture can provide better platforms for growth as medium is frequently replenished. Choi et al. (1999) reported the improved production of berberine in *Thalictrum rugosum* plant cell suspension grown in bioreactors where fed-batch culture was used.

The shoot malformation, hyperhydricity, meristemoid cluster or bud induction with arrested growth of leaves are the major bottlenecks of growing plants in liquid medium under bioreactor cultivation. These alternations can be correlated to flawed timing of normal sequence of signals in differentiation process known to execute in vivo. The utilization of high level of cytokinin and other growth retardants that can inhibit gibberellins biosynthesis can overcome problem of reduced shoot and leaf growth and favour proper meristematic bud formations (Ziv 2000).

21.4 Types of Bioreactor Frequently Used in Medicinal Plant Upscaling

Plants grow in vitro at varied morphogenic levels ranging from protoplast to cell suspensions to organ culture and plantlets. They can be dedifferentiated cellular form or highly differentiated secretary gland type. They can be highly shear-sensitive fragile tissue or shear-resistant hard tissue. Each morphogenic stage is having its own prerequisites in terms of oxygen supply, temperature/pH requirement and nutrient supplementation essential for growth and development. Dealing with variety of parameters, it is hard to design ideal bioreactor for plant cells. Therefore, diverse bioreactor configurations have been made to accommodate more suitable type of tissue (Fig. 21.2). Broadly bioreactors can be classified into (i) mechanically driven bioreactor systems which include stirred tank and rotating drum reactors and (ii) pneumatically driven bioreactors like airlift reactors and bubble column reactors and bed bioreactors that include mist reactors and trickle-bed reactors (Georgiev et al. 2013). Temporary immersion systems are the one laboratory-scale class of bioreactors that is developed for differential tissue. The single-use or disposable bioreactors are one of the recently developed bioreactor types which are highly applicable and economic also. Few of the bioreactor types that are highly used in medicinal plant upscaling are discussed as follows.

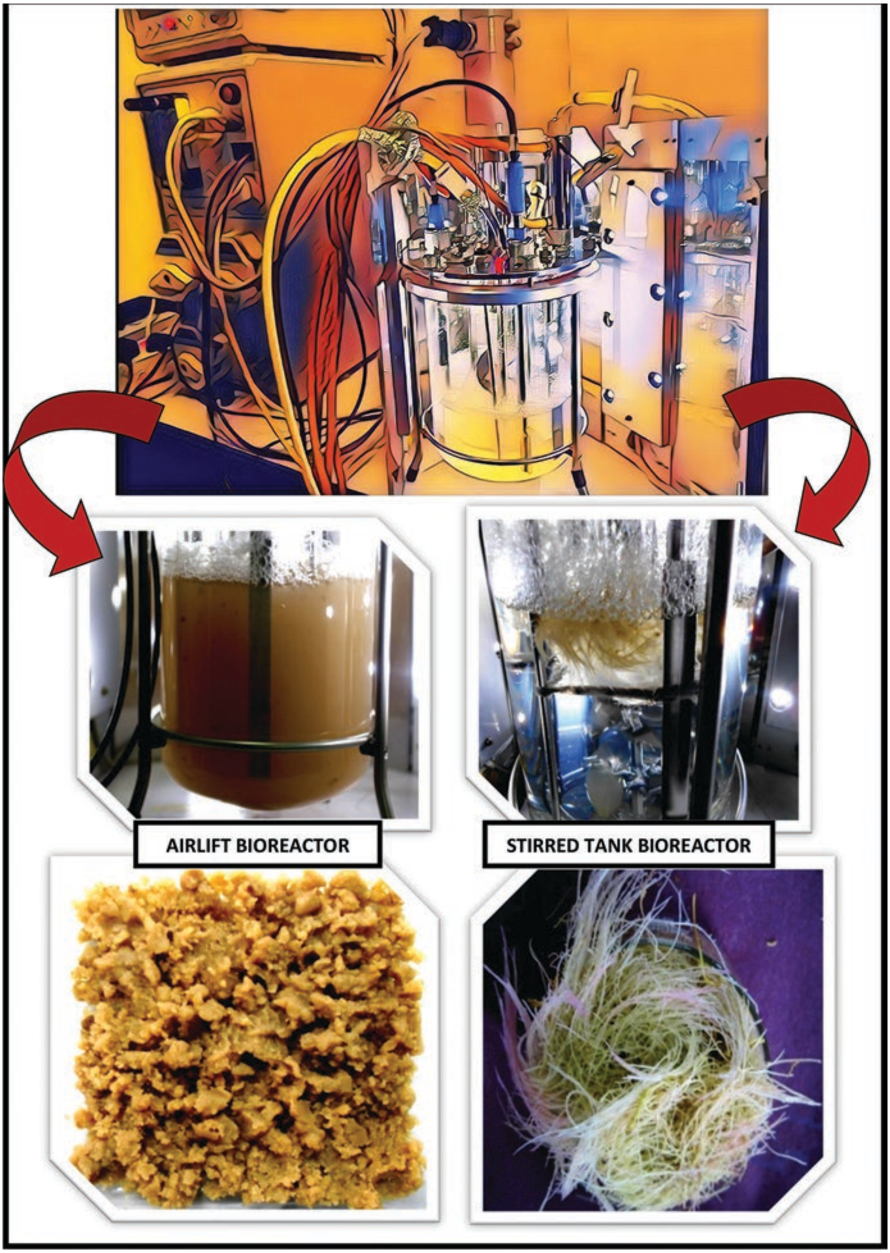

Fig. 21.2 Modification of same reactor as airlift for cell suspensions and stirred tank for hairy root upscaling

21.4.1 Stirred Tank Bioreactor

Stirred tank reactors (STRs) are the most conventional type of bioreactor and have been widely used in cell culture-based industries. In STRs mechanical stirring has been performed by impellers. Impellers (fitted with motor) along with baffles constitute the agitation system of STRs. Impellers provide better mixing either through radial flow (along the radius of the bioreactor vessel, i.e. liquid flows towards the vessel wall) or axial flow (along the axis of the bioreactor vessel, i.e. liquid flows downwards to the bottom of the vessel). Baffles enhance mixing efficiency by putting turbulence in the liquid flow. Radial flow impellers (e.g. Rushton type) are exerting more shear force in comparison to the axial flow impellers (e.g. marine type). STRs are known to have higher fluid mixing and adequate oxygen transfer ability with the ease of using alternative impellers. The limitation of using STRs is maintaining stability and sealing of baffles/shafts, implication of higher shear force and high power consumption (Georgiev et al. 2013). STRs are recommended to high cell density plant cell cultures.

21.4.2 Airlift Bioreactor

The airlift is a kind of gas-liquid dispersion bioreactor where aeration, mixing and circulation of fluid have been carried out without the movement of mechanical parts. As plant cells are more prone to shear forces due to larger size and highly sensitive cell walls and require less oxygen than microbial cells, airlift bioreactors are most applicable to shear-sensitive low-density plant cell suspension cultures. In airlift bioreactors, air is sparged at the bottom of the culture vessel which rises up along the vessel keeping medium in a flow. The airlift bioreactors have some benefits over STRs as they have relative simple construction, low shear force, reasonably high biomass and heat transfer and comparatively high yields with low input rates (Ziv 2000). Rising up of plant cells along with the high aeration bubbles and eventually trapping in the foam and lysis due to nutrient depletion is the factor associated with shortcoming of using airlift bioreactors.

21.4.3 Temporary Immersion Bioreactor

To overcome the problem of hyperhydricity along with improving the nutrient supply, bioreactors operated with temporary immersion system (TIS) have been used specifically to grow plants and organ cultures. In the TIS system, the cultivated propagules used are immersed in liquid medium for adequate time (depending upon the plant tissue) followed by exposure to gaseous environment in a closed vessel (Ruffoni et al. 2010). RITA® system (Recipient for Automated Temporary

Immersion – RITA®, Vitropic, France) is a TIS-based small-scale bioreactor. The apparatus consist of upper and lower compartments which are interconnected. The lower compartment contains the liquid medium, while the upper one is having plant propagules. When overpressure is applied to lower compartment, the liquid medium is pushed to the upper compartment through the interconnection, and on dropping of pressure, it comes back to lower compartment. As a result the propagules in the upper compartment are temporarily immersed for definite period of time on flooding up of upper compartment. The frequency and time period of immersion can be optimized depending upon the plant system. It offers a simpler way to upscale the plants/shoot/somatic embryo cultures at low cost.

21.4.4 Mist Bioreactor

Bed bioreactors are the liquid-dispersed or gas-phase closed systems in which nutrient medium along with ambient air is dispersed or tricked in spray form or droplet form constituting mist. The unused medium is recirculated in respective form (Khan et al. 2018). Mist bioreactor is a kind of bed reactor generally applicable to hairy root cultures. In the mist system, roots are anchored to a horizontal stainless steel mesh on which nutrient medium is delivered in the form of droplets (mist) from the nozzles. Roots are exposed to humidified air on this closed system. The drained unspent medium is recirculated. The droplet size of the mist bioreactors ranges from 0.01 to 10 μm. In comparison to liquid-phase bioreactors, the mist bioreactor overcomes the problem of oxygen mass transfer up to a higher extent with lowering down of shear stress making environment favourable for hairy root cultures.

21.4.5 Disposable Bioreactors

Use of disposable bioreactors/single-use bioreactors revolutionized a bioreactor industry and upscaling workflow. They are low-cost, one-time use cultivation vessels providing the reduced risk of cross-contamination between batches, ease of handling, no cleaning and sterilization cost and time-effective bioreactor cycles. ProCellExTM system of Protalix Biotherapeutics, Israel, producing glucocerebrosidase from *Daucus carota* cell cultures is the encouraging example of using disposable cultivation vessels at industrial level (Wolfson 2013). The disposable bioreactors are now available in many working modes like stirred, wave, orbital, oscillating with vibrating disk, etc. by many branded companies. The containers possibly could be pillow, cylindrical or cubical shape provided the type of tissue to be upscaled (Georgiev et al. 2013). Even after the huge success and applicability of disposable bioreactors, testing protocols standardization, sensor system improvement and reducing the risk of cultivation containers are the future targets for the expansion of single-use bioreactor industry.

21.5 Commercialization of Bioreactor Upscaled Medicinal Plant Cell Cultures by Different Companies/Laboratories

Whole plant from natural environment is no more a viable source of extraction of valuable secondary metabolites due to the destruction of natural habitats with lot of environmental and geographic instabilities. The potential alternative of production platform is provided by the plant cell, tissue and organ cultures which are performed under strict controlled physical and chemical conditions. The first criterion for industrialization is selection of high-yielding cell/plant/hairy root/transgenic line which also has a capability to grow under huge bioreactors. Although, since the last five decades, potential cell types in a variety of the medicinal plants have been reported, only few rose to the level of industrialization as there are a variety of technical difficulties that have to be resolved on a wide scale before the production of useful plant secondary metabolites under bioreactor conditions (Ruffoni et al. 2010). A number of companies/laboratories filed patents for upscaling of 'high value low volume' secondary metabolites for bioreactor upscaling in large volume, but the success stories are very few in the last three decades. The very first success was achieved by Mitsui Petrochemical Industry Co. Ltd., Japan, which produces shikonin from *Lithospermum erythrorhizon* root cell suspension grown in 750 L jar fermenter. Similarly large-scale production of ginseng/ginsenosides from *Panax ginseng* cells has been achieved by Nitto Denko Co. Ltd., Japan, and CBN Biotech Company, South Korea. *Taxus chinensis* cell culture-based production of anticancerous drug Taxol and Paclitaxel has been executed by Samyang Genex, South Korea, and Phyton Biotech, Germany. Phyton Biotech managed to be the world's biggest cGMP plant cell culture facility. They have specifically designed the bioreactors to meet the requirements of plant cells in culture conditions. The total production competence of the taxanes runs is up to 880,000 L/year. The Phyton Biotech is a worldwide supplier of chemotherapeutic biochemicals including paclitaxel, docetaxel APIs (active pharmaceutical ingredients) and taxane. Most of the plant cell-based reports are carried out in classical stirred tank or airlift bioreactors. Using ProCellExTM system, a novel bioreactor system, consisted of large flexible polyethylene bags, to which growth medium and air were supplied from a central system under sterile conditions, Protalix Biotherapeutics, Israel, is producing glucocerebrosidase from *Daucus carota* cell cultures that are used against Gaucher's disease. Greenovation GmbH Heilbronn, Germany is a bryophytes-based company. It is known for producing galactosidase (used against Fabry disease) from protonema of *Physcomitrella patens* using single-use bioreactors. Details of the above industrialization set-ups are given in Table 21.2. For commercialization of plant cell culture-based processes, overcoming upscaling technicalities and the development of more reliable methods for bioprocess monitoring with smoothening of shake flask to bioreactor path are the focused targets to be achieved.

Table 21.2 Commercialization of bioreactor upscaled medicinal plant cell cultures by different companies/laboratories

S. No	Company/laboratory	Bioreactor	Plant	Morphogenic level	Product	Product activity
1.	Mitsui Petrochemical Industry Co. Ltd. Japan	750 L airlift fermenter	*Lithospermum erythrorhizon*	Root cell suspensions	Shikonin	Antimicrobial, anti-inflammatory and anti-tumour
2.	Nitto Denko Co. Ltd. Japan	20,000 L jar fermenter	*Panax ginseng*	Cells	Ginseng	Promoting immune and central nervous system function, relieving stress, antioxidant
3.	Samyang Genex, South Korea	35,000 L bioreactors	*Taxus chinensis*	Cells	'Genexol' (paclitaxel)	Anticancerous
4.	Phyton Biotech, Germany	75,000 L aerated stirred tanks	*Taxus chinensis*	Cell suspension	Taxol	Anticancerous
5.	CBN Biotech Company, South Korea	10,000 L; 13-ton high-tech cultured tank	*Panax ginseng*	Adventitious roots	Bioactive ginsenoside	Anti-tumour, anti-inflammatory, anti-oxidation and inhibition of cell apoptosis
6.	Protalix Biotherapeutics, Israel	ProCellExTM system: a novel bioreactor system, consisted of large flexible polyethylene bags, to which growth medium and air were supplied from a central system under sterile conditions	*Daucus carota*	Cell suspension	Glucocerebrosidase	Used against Gaucher's disease
7.	Greenovation GmbH (Heilbronn, Germany	Single-use bioreactors	*Physcomitrella patens*	Protonema	Galactosidase	Used against Fabry disease

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