Mirza Hasanuzzaman *Editor*

Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives II

Mechanisms of Adaptation and Stress Amelioration

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This book is dedicated
to
My Mother
To me, she was a great inspiration, a great teacher, and a great philosopher.

Preface

Impact of climate change is expected to be broadly negative, including reduced water availability, salinity, flood, and infestation of pests and diseases. Due to the significant climate change over the centuries, the incidence of various abiotic stresses such as salinity, drought, extreme temperature, atmospheric pollutions, and metal toxicities regularly affect plant life and productivity. Many crops perform only at 30% of their genetic potential under adverse environmental conditions. The predictable loss of crop production is as much as 70% in an average and might be 100% in extreme cases. The resulted economic loss caused by environmental stress is a great concern in agriculture.

To sustain productivity against the environmental stresses, the crucial importance is to know and understand the plants-specific responses to the different environmental factors. Plant ecophysiology is the science of interaction of plants with the environment, and the vital underlying acclimation and adaptation processes. The off-putting effects of abiotic stresses result in alteration in plant metabolism and physiology, which challenge survival, productivity, reproductive biology, and reproducibility. These adverse effects result from structural and functional alteration of cellular components of plant. Structural alterations of cellular organelles due to environmental stresses cause alteration in physiological processes, such as water entrance and transportation, nutrient uptake, chloroplast functioning, photosynthetic efficiency, mitochondrial activity, vacuolar structure and function, and the altered structure of nucleus cause genetic modification. The physiology and adaptive mechanisms of plants are greatly varied in different species and genotypes. The ability of various plant groups to tolerate the extremes posed by natural conditions and/or chemically rich environments involves morphological and physiological adaptation as well as changes in ecological behavior to sustain in relatively protected niches within an extreme environment.

To survive under environmental extremity, plants respond at the molecular, cellular, and physiological level, which involves a complex network supporting perception and transmission of stress signals, which subsequently initiate a plethora of responses. Against different kinds of stress-induced responses, there are two broad outcomes: programmed cell death (PCD) or stress acclimation. The PCD is considered a lethal effect whereas acclimation often leads to adaptation to certain adverse environmental stresses, which sustain plant survival and productivity. A deeper understanding of the mechanisms underpinning plant stress adaptation may offer novel opportunities to develop crop plants with an enhanced ability to tolerate environmental fluctuations, which are the focal points of concern of plant ecophysiological study. In modern concept, the survival mechanism and potential of plants are not left behind as a natural process. Rather how the adaptation process can be enhanced is a great concern of scientists of the related fields. In present perspectives, scientists are manipulating the surrounding environment of target plants so that the plant can be less affected by natural environmental stresses. Use of a broad range of exogenous phytoprotectants including plant nutrients, trace elements, phytohormones, and signaling molecules, probiotic microorganisms to improve adaptation processes of plants are being explored day by day. Scientists are going through the genetic manipulation and biotechnological processes to sustain plant productivity under the adverse environmental conditions. Much progress has been gained in the last few decades in the area of plant ecophysiology research and on their adaptive mechanisms. Although there are numerous publications in journal and proceedings, there is a scarcity of a comprehensive book dealing with both ecophysiology and adaptive mechanisms of plants under climate change.

This is the second volume of the two-volume book, *Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives* that provides current state-of-the-science knowledge of plant ecophysiology, with particular emphasis on plant responses and tolerance mechanisms as well as remediation techniques. This volume will provide the reader with a wide spectrum of information, including vital references. This is done through 32 chapters written by hundreds of experts in the field of Botany, Plant Physiology, Ecology, Crop Science, and Environmental Sciences, ultimately aiming to become a useful information tool for plant biologists, crop scientists, ecologists, plant breeders as well as a guide for students in the field of Plant Science, Agriculture and Environmental Sciences.

I like to give special thanks to the authors for their outstanding and timely work in producing such fine chapters. Our profound thanks also go to Mr. Sayed Mohammad Mohsin, Dr. M.H.M. Borhannuddin Bhuyan, Ms. Khurshida Parvin, Dr. Kamrun Nahar, Khussboo Rahman, Khadeja Sultana Sathi, and Mr. Abdul Awal Chowdhury Masud, for their critical review and valuable support in formatting and incorporating all editorial changes in the manuscripts. I am highly thankful to Ms. Lee, Mei Hann, Editor (Editor, Life Science), Springer, Japan, for her prompt responses during the acquisition. I am also thankful to Sivachandran Ravanan, Project Coordinator of this book, and all other editorial staffs for their precious help in formatting and incorporating editorial changes in the manuscripts.

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About the Editor

Mirza Hasanuzzaman is Professor of Agronomy at Sher-e-Bangla Agricultural University in Dhaka, Bangladesh. He received his PhD in *Plant Stress Physiology and Antioxidant Metabolism* from the United Graduate School of Agricultural Sciences, Ehime University, Japan, as a recipient of a scholarship from the Japanese Government (MONBUKAGAKUSHO). Later, he completed his postdoctoral research at the Center of Molecular Biosciences, University of the Ryukyus, Okinawa, Japan, as a recipient of the Japan Society for the Promotion of Science (JSPS) postdoctoral fellowship. Subsequently, he received the Australian Government's Endeavour Research Fellowship for postdoctoral research as an Adjunct Senior Researcher at the Tasmanian Institute of Agriculture, University of Tasmania, Australia. Mirza Hasanuzzaman has supervised 20 MS students. His current work is focused on the physiological and molecular mechanisms of environmental stress tolerance. Prof. Hasanuzzaman has published over 120 research publications in peer-reviewed journals. He has edited 12 books and written 45 book chapters on important aspects of plant physiology, plant stress responses, and environmental problems in relation to agricultural plants. According to Scopus®, Prof. Hasanuzzaman's publications have received roughly 4800 citations with an h-index of 37 (As of April 2020). He is an editor and reviewer for more than 50 peer-reviewed international journals and was a recipient of the

"Publons Peer Review Award 2017, 2018 and 2019." He has been honored by different authorities for his outstanding performance in different fields like research and education and has received the World Academy of Sciences Young Scientist Award (2014). He has attended and presented 25 papers at international conferences in many different countries (the USA, the UK, Germany, Australia, Japan, Austria, Sweden, Russia, Indonesia, etc.). Prof. Hasanuzzaman is an active member of 40 professional societies and is currently the Acting Research and Publication Secretary of the Bangladesh JSPS Alumni Association. He is also a fellow of The Linnean Society of London.

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Chapter 1
Salinity Stress Management in Field Crops: An Overview of the Agronomic Approaches

Abdul Majeed and Saira Siyyar

Contents

Abstract Crop plants have defined roles in the global food supply. Cereals, pulses, beans, and grains are particularly important for their nutrients and easy availability for consumers who cannot purchase meat and dairy products. Thus, elevated production of field crops for addressing the food and energy demands of the human population is crucially necessary, and it remains one of the emerging areas of interest for agronomists. In global agriculture, a significant proportion of cultivated land is affected by soil salinity. The problem has devastating effects on crops' growth, yield, and production. The adversities of salinity stress on crops become even worse in regions with low rainfall and high evaporation rate and where substandard irrigation practices are common. Soils enriched with salinity affect the growth, physiology, and production by triggering water deficit conditions, ionic toxicity, oxidative stress, and alteration in metabolic events. To reduce the agronomical, physiological,

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and biochemical damages on crops imposed by high salinity levels, development of salt-tolerant varieties and modulation in agricultural practices seem to be ideal strategies as they would lead to attaining high yields of crops under a stressful environment. In this paper, a comprehensive review is presented about the problem of salinity and updates about management strategies in crop plants employing agronomic approaches.

Keywords Agricultural sustainability · Salt-tolerant crops · Ionic toxicity · Osmotic abnormalities · NaCl · Biotechnology

1.1 Introduction

Rice, maize, wheat, potato, pulses, beans, and several other crops are essential food commodities which are actively playing their role in global food supply and contributing to the energy needs of millions of people throughout the world. A drastic increase in human population (estimated to exceed 9 billion by 2050) will require more food and energy (Edgerton 2009; Cole et al. 2018); hence, greater efforts are required to enhance the crop productivity and reduce the gap between current and required yields. Concurrently, several biotic and abiotic challenges in global agriculture persist which hinder the production and yields of crops on which humans rely for their food and energy. Excessive burden and infestation of plant pathogens, weeds, and other pests (Korres et al. 2016; Savary et al. 2019), prevailing and anticipated changes in climate (Hasegawa et al. 2018), ozone and heavy metal pollution (Shahid et al. 2015; Tian et al. 2016), heat and drought (Daryanto et al. 2017; Fahad et al. 2017), and salinity (Parihar et al. 2015; Parmoon et al. 2018; Majeed et al. 2019) are important factors which influence negatively the production of crop plants and are expected to impart their effect on food supply in the future.

Salinity which currently affects more than 20% of agricultural land and which is expected to pose potential challenges to land degradation and agricultural production in the future is a leading global abiotic stress that affects general plants and cultivated crops adversely (Pitman and Läuchli 2002; Butcher et al. 2016). Impact of salinity on crops is direct as well as indirect. Direct and abrupt effects include osmotic stress which results from concentrated soil with salts, while the indirect and relatively slower effect is the accumulations of Na^+ and Cl^- ions inside tissues, which cause ionic toxicity (Munns and Tester 2008). Both effects correspond to physiological and growth abnormalities in challenged crops resulting in either delayed or inhibited germination, growth, and yields (Munns and Tester 2008; Hanin et al. 2016). It is widely suggested that hyper-salinity levels induce ionic toxicity, oxidative stress, and abnormalities in stomatal functionality, respiration, and mineral uptake (Han et al. 2015a, b; AbdElgawad et al. 2016). Earlier studies have clearly demonstrated that higher salt concentration affected metabolic and physiological functions, germination, growth, development, and yields in wheat

(Saboora et al. 2006; Wahid et al. 2007; Mohamed et al. 2017), rice (Zeng and Shannon 2000; Shereen et al. 2005; Vibhuti et al. 2015; Kumar and Khare 2016), maize (Akhtar et al. 2015b; AbdElgawad et al. 2016; Waheed et al. 2016), tomato (Li et al. 2015; Gharsallah et al. 2016; Zhang et al. 2016), potato (Akhtar et al. 2015a; Hu et al. 2016), sorghum (Miranda et al. 2016; Nxele et al. 2017), and several other crops. Thus there is a greater need for devising methods, which could lead to minimal adversities of salinity on crops of agronomic importance.

Sustainable measures to manage the salinity problems are achievable through successive efforts which may encompass modulation in agricultural practices and biotechnological approaches though the specified methods may have variable advantages and disadvantages depending on the type of salinity management method, responding crop species, agricultural area, and level of salinity. Modification in agronomic practices may efficiently contribute to the management of salinity stress to a varying degree. The practices may include sequencing and cultivation of relatively tolerant crop species on saline soil (Sharma and Minhas 2005), appropriate irrigation designs in saline environment (Qadir and Oster 2004; Majeed and Muhammad 2019), residue retention (Devkota et al. 2015), and employing seed priming with different priming materials and growth-promoting bacteria (Majeed et al. 2018; Majeed and Muhammad 2019). Development of salt-tolerant varieties of crops using breeding and biotechnology can further strengthen our grips on yield gap because tolerant varieties would exhibit anticipated production and yield in a salty environment (Fita et al. 2015). Identification of salinity tolerance genes and their adaptation in a specific crop, while their introgression to major crops from candidate donor is a challenging task in the field of biotechnology and progress in this field, can yield promising results toward salinity management. In this chapter, salinity and its effects on crops and different approaches exploiting agronomic tools for addressing salinity stress are discussed.

1.2 Salinity and Its Causes

Naturally, soils have different organic and inorganic materials which originate from death and decomposition of organisms and weathering processes of parent materials, respectively. The composition of organic and inorganic substances widely differs in different soils, which depends on several factors ranging from the abundance of biotic communities to depth of soil and locations. Generally, soils with fluctuating biological activities of organisms will have substantial amounts of organic matter than barren soils. Similarly, soils with considerable bulk of parent rocks and those located near coastal areas will comprise abundant minerals and salts. The presence of salt and minerals is a natural prerequisite for fertile soil and proper growth of plants; however, excessive concentrations of salts (especially NaCl) result in hyper-ionic solution in soils which hinders a conducive environment necessary for plants' establishment, growth, and development. On the basis of electrical conductivity (EC), soil is said to be affected with salinity if EC exceeds 4 dS m^{-1} (Munns and Tester 2008; Butcher et al. 2016).

The origin of agricultural salinity stems from naturally weathering materials as well as from intensive activities of human beings. Naturally, weathering processes of rocks containing Na, Ca, Mg, SO_4, and other salts add to soil salinity when a significant amount of such salts accumulate in water/soil. However, this type of salinity is more pronounced in arid regions as the water carrying these salts evaporates leaving salts in soils (Chhabra 1996). Changing patterns of rainfall in many areas are also a leading cause of salinity. Infrequent rainfall coupled with temperature fluctuations often causes drought conditions in soils which in most instances are linked with high soil salinity (Salehi-Lisar and Bakhshayeshan-Agdam 2016). To a significant extent, fossil salts (resulting from previous salt deposit or entrapments), the flow of salts from oceans to coastal areas, and low water table in some regions may also cause natural salinity and soil degradation (Chhabra 1996). Causes of salinization originating from human activities include excessive irrigation practices, land degradation, changing vegetation patterns, and poor drainage schemes (Parihar et al. 2015). Fields located near industrial zones and mining sites are prone to anthropogenic salinity because water entering into fields from such sites may carry industrial wastes and salts which lead to salinity as well as other sources of pollution. Cutting and burning of trees and other plants for domestic and commercial purposes lead to barren land, accumulation of CO_2 in the atmosphere, high evaporation rate, and reduced rainfall, which are the leading causes of salinity (Majeed and Muhammad 2019). Land cleanup and irrigation indirectly cause the rise of water table which allows salts to migrate to the upper portion of the soil making it saline (Munns and Tester 2008).

1.3 Plants' Responses to Salinity Stress

Soil salinity, whether of natural or anthropogenic origin, adversely affects plants' germination, growth, and physiological functions. Accumulation of salts in soil causes osmotic, physiological, and metabolic abnormalities both in soil and within the challenged plant (Fig. 1.1). Classical causes of salinity-induced growth and germination abnormalities in exposed plants are osmotic stress, nutrient imbalance, and toxicity of ions, which trigger water deficiency, reduced nutrient uptake and distribution, and abnormal metabolism, respectively (Gorham et al. 2010).

First and essentially drastic effects of salinity include osmotic stress which corresponds to reduced water availability in soil. High salinity results in hyperaccumulation of salts in soil with consequent changes in the water status. Water scarcity drastically affects the germination of yet-to-germinate seeds as well as the growth of already established seedlings and plants. Since water is an essential component for the germination initiation, salinity-induced osmotic stress either completely hinders the germination of the sensitive plant species or delays the processes in tolerant

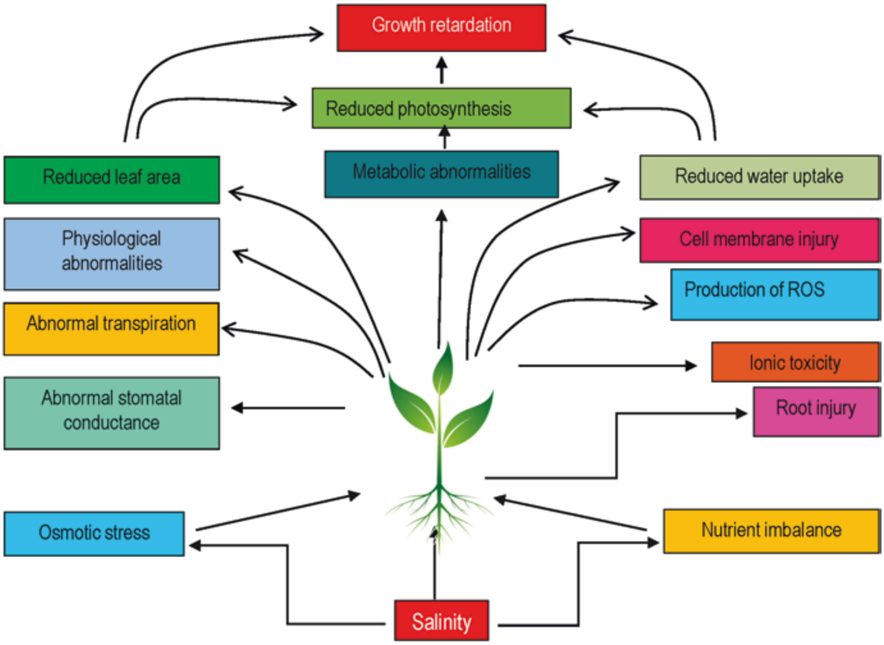

Fig. 1.1 Effect of salinity stress on physiology and growth of challenged plant. *ROS* reactive oxygen species

species. For already established plants, osmotic stress results in lower water absorption by the plant which leads to physiological wilting (Kordrostami and Rabiei 2019). Root zone saturated with salts can draw water from roots resulting in wilting and even in death of those plants which are not tolerant of salinity.

Another abnormality associated with hyper-saline conditions is a nutritional imbalance in soil and plant tissues (Gorham et al. 2010). Research on nutritional status of different plants under salinity stress has established that different nutrients such N, NO_3^-, P, Zn, Mn, etc. significantly declined in target plants due to lower uptake because in soil the compositional status and availability become imbalanced due to interactive effects of Cl^- and Na^+ ions with different minerals (Hu and Schmidhalter 2005). In studies such as those conducted by Santos et al. (2001), Grewal (2010), Wang et al. (2012), Yousif et al. (2010), and Garg and Bhandari (2016), salinity-induced nutritional imbalance in different plants was observed. The deficiency in nutrient uptake or mobilization inside host tissues results in metabolic disorders and impaired enzymatic activities which affect vital processes such as photosynthesis, respiration, and transpiration consequently leading to reduced growth and yield of the challenged plants.

Ionic toxicity, induced by salinity, is a leading factor, which severely affects several structural components and biochemical processes of plants' tissues. Hyperaccumulation of specific ions in shoots due to high salts in soils results in ionic toxicity which injures cells and tissues with negative effects on cellular machinery that promotes metabolic and physiological activities of plants (Sairam and Tyagi 2004; Machado and Serralheiro 2017). Excessive quantities of Na^+ and Cl^- in cytosol are toxic to many of the cellular processes including imbalanced ions (due to competitive replacement of Na with K, Mg, Ca, etc. and $Cl^-$for NO_3, PO_4^-, etc.) which have consequential effects on enzyme activity, senescence, and photosynthesis (Zörb et al. 2019). Läuchli and Grattan (2007) ascribed that accumulation of Na and Cl ions usually occurs in older leaves which formidably reduces the rate of photosynthesis and causes abnormalities in transpiration. Greater accumulation of these ions in leaves also results in leaf death and senescence. Like other stressful conditions such as drought, flooding, heat stress, and pathogenic interactions, salinity is also known to cause production of reactive oxygen species (ROS) in plants through a variety of mechanisms which lead to suffocation of enzymes, DNA and protein damages, cell membrane injury, disturbance of pigment systems, and lipid peroxidation (Bose et al. 2014). The net results of salinity stress are reduced growth and production output of challenged crop plants (Plett and Møller 2010).

1.4 Effect of Salinity on Crop Plants

Crop plants are variable in their response to salinity stress. Crops on the basis of their tolerance to salinity may be halophytes or glycophytes. Halophytes are salt-tolerant while glycophytes do not exhibit tolerance to salinity beyond a certain level. Even some crops may show extreme sensitivity to salinity and are unable to survive at salt concentration beyond the threshold level. Generally, most of the cultivated crops which cannot tolerate salinity beyond threshold levels are glycophytes although some authors describe them as moderately salt-tolerant. Important cereals such as maize, rice, and wheat, most of the vegetables, ornamentals, and other domestic crops are glycophytes which exhibit salinity tolerance at variable ranges, e.g., 1–8 dS m^{-1} (Kataria and Verma 2018). Among cereals, wheat and barley are regarded as comparatively salt-tolerant while rice is a sensitive glycophyte (Munns and Tester 2008). Salinity level of 100–200 mM has been described as formidable for most of the domestic crops which retards their growth and productivity or even their survival (Kataria and Verma 2018).

Glycophyte crop plants like other non-cultivated plants show similar responses to salinity stress. In the first instance, the higher salt concentration in soil decreases water availability causing osmotic stress. Under osmotic stress, crop glycophytes either cannot absorb water or they take up a very little amount of water resulting in wilting or even death of the challenged crop plants. Surviving glycophytes under osmotic stress exhibit further abnormalities in their growth and physiology due to several secondary effects such as alteration in transpiration and photosynthetic processes. Salinity-induced osmotic stress can significantly alter the concentration of carbohydrates and their metabolism which essentially affect processes such as photosynthesis, respiration, and translocation (Kerepesi and Galiba 2000). Deficiency in water status directly disturbs photosynthesis because of disturbance in cellular turgidity and availability of CO_2 to Rubisco—a key enzyme in photosynthesis (Chaves et al. 2009).

Besides osmotic problems, salinity also induces imbalance in nutrients both in the soil and in plant tissue. Depending on the types of salts, available nutrients in the soil may experience imbalance due to competitive interactions between anions and cations present in salts and in nutrients. Hu and Schmidhalter (2005) suggested the interaction of salt ions with several nutrients present in soil which leads to deficient availability and uptake of N, Ca, K^+, P, Zn, Mn, etc. by crop plants. These and several other minerals are crucial for vital processes and for overall growth and development of crops. Several enzymes and metabolic processes work properly in the presence of important minerals whose deficiency leads to severe abnormalities in enzymes and metabolic events. Respiration, photosynthesis, and transpiration under imbalanced nutritional distributions in plant tissues are affected to drastic extents. Zörb et al. (2019) outlined that higher concentration of Na and Cl ions in plant tissues is toxic to the cytosol and cellular components besides their role in preventing the uptake and distribution of beneficial minerals such as K^+, Ca^{2+}, and Mg^{2+}. Finally, specific ion effect or ionic toxicity induced by salinity stress in crop plants corresponds directly to cellular, membranal, and molecular damages or indirectly by causing oxidative stress in crop plants. The production of ROS as a result of salinity stress creates oxidative stress which is lethal for cells as well as for their components including membranes, DNA, proteins, and lipids (Rivera-Ingraham et al. 2016). Osmotic stress, nutritional imbalance, and ionic toxicity either alone or in combination make it hard for glycophyte crops to grow and develop normally; consequently retarded growth and production occur.

1.5 Agronomic Practices for Salinity Stress Management

The problem of salinity corresponds to substantial yield losses of major crops, and some studies estimate that yield losses of important crops and vegetables may reach 50% (Shrivastava and Kumar 2015). To attain maximum yields of crop plants and make the available maximum of the cultivated land, dedicated efforts are needed to manage soil salinity. The success of salinity management depends on (1) the site where salinity is prevailing, (2) the crop species, (3) the concentration of salinity, and (4) other prevailing stresses. Salinity management approaches should be multifaceted, and only employment of a single remediating approach seems less effective particularly in the current scenario of climate change (Fig. 1.2).

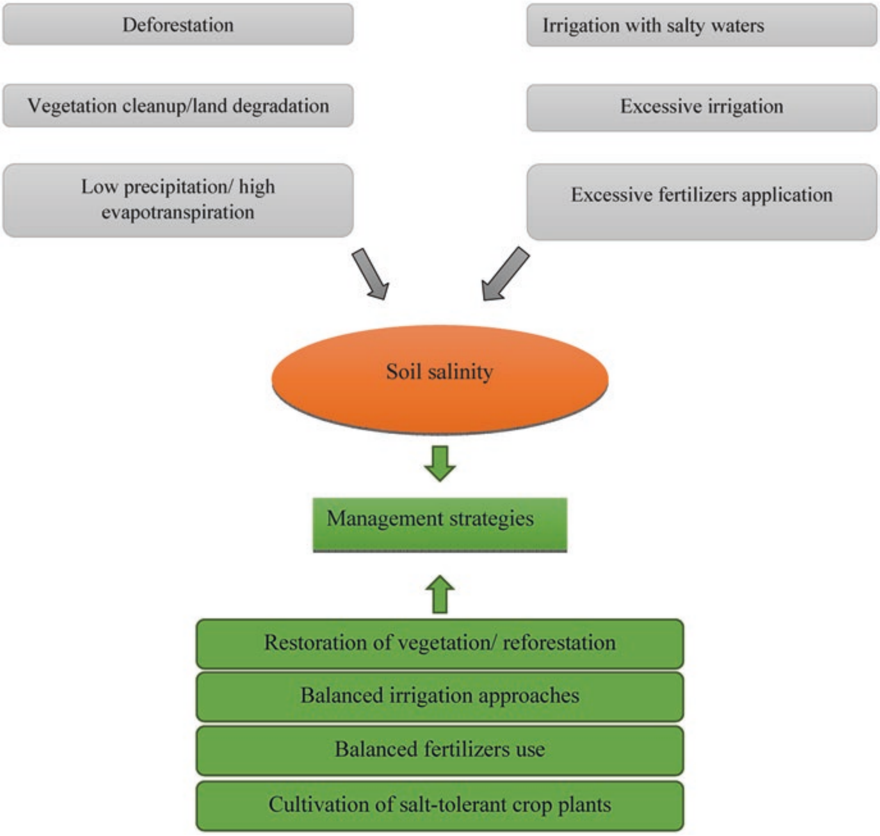

Fig. 1.2 Causes of agricultural salinity and mitigation methods employing agronomic practices

1.5.1 Changes in Irrigation Pattern

Excessive irrigation of soil and irrigation with substandard water are the leading causes of salinity in cultivated lands. In areas where natural precipitation is not adequate, crops are watered with extensive irrigation both from natural (rivers, water bodies, etc.) and built resources (streams, canals, wells, ponds, etc.). Water used for irrigation from such sources is regarded as freshwater if it does not contain any appreciable amount of salts or other pollutants. Balanced and well-designed irrigation with freshwater does not pose a significant threat of salinity to irrigated land. However, when proper scientific approaches are not observed in irrigation or water containing salt is used for irrigation purposes, lands become salinized. Irrigation practices cause salinization by introducing salts to irrigated water (if a sufficient concentration of salts is present in irrigated water) directly or by raising the water table which communicates with root zone and brings with it salts from mineral deposits (Hillel 2000).

The problem of irrigated salinity is prominent in developing countries because of the scarcity of freshwater and lack of well-suited irrigated systems there (Umali 1993). A technical report compiled by the author described that rise in groundwater and irrigation with saline water promoted soil salinity in arid regions in the developing countries. In arid and semi-arid areas, the rate of evapotranspiration is higher which leads to water evaporation leaving salts behind in the cultivated zone of soil (Kirzhner et al. 2008). In most of the developing countries, irrigation water flows through simple streams and canals which are not lined thus resulting in substantial leaching of water that leads to the rise of water table. Moreover, water flowing through saline areas may dissolve salts in it, and when such water is served for irrigation, soil will become salinized. Although, in such areas, excessive irrigation seems to lower the water evaporation rate and consequently reduce salinity, however, in practice, without adopting suitable measures, the salinization problem will worsen.

In order to minimize or prevent irrigation-induced salinity, sustainable measures in arid and semi-arid lands are necessary to be practiced. These include the use of freshwater sources not contaminated with salts, provision of proper drainage and lining of streams and canals, and frequency of irrigation strictly focusing on soil's conditions and prevailing climate. The lining of canals and streams prevents seepage of water and minimizes the chances of water table rise. Discouraging the use of heavy machinery for irrigation in soils is a helpful technique because it would reduce poor drainage and leaching. Groundwater may be balanced by lining water canals, by improving percolation, and by installing pump wells (Smedema 1990). Fayrap and Koç (2012) proposed the use of drip irrigation as an efficient way to decrease irrigation-induced salinity and waterlogging in irrigated systems. Machado and Serralheiro (2017) also favored the use of surface drip, subsurface drip, and furrow irrigation in soils where potent salinization as a result of salinity is evident. Improvements in irrigation system by canal lining and installation of tube wells are ideally approached to prevent irrigation-induced salinity; the processes, however, require huge financial resources and technical expertise which without the help of governments and other financial institutions are not possible.

1.5.2 Restoration of Vegetation Cover and Reforestation

In tropical, arid, and semi-arid regions, the evaporation rate is higher in warmer seasons which causes reduced water availability in soils. The problem becomes worst when rainfall is infrequent. Rich vegetation in those areas can be a check for water loss because plants hold water as well as recycle water by transpiration process. In recent years, diversification and increase in human needs have resulted in substantial deforestation activities. Trees and other plants including herbs and shrubs are removed for domestic burning, commercial, and medicinal purposes. The large-scale removal of plantation leaves the land barren making it prone to accelerated evapotranspiration. Moreover, cleaning up vegetation for building houses, factories, and other commercial installments results in land degradation. The net result is water loss on such soils which tends to cause accumulation of more and more salts in these soils.

To prevent salinization induced by deforestation, there is an urgent need to stop removal of trees and other plants and minimize commercial activities on lands, which are potentially exposed to high evaporation of water and subsequent salinity. In many studies, a positive correlation of salinity with deforestation and efficient control of the problem with reforestation have been observed which were attributed to the role of plants in climatic dynamism (Miura and Subhasaram 1991; Heaney et al. 2000; Bari and Croton 2002; Harper et al. 2012). Schofield (1992) comprehensively reviewed the benefits of tree plantation and vegetation restoration in salinity control in Australia. Heaney et al. (2000) suggested that re-vegetation is a cost-effective and environment-friendly strategy to manage salinity and water scarcity problems. Bari and Croton (2002) also revealed favorable effects of reforestation on minimizing salinity and salt loads in Western Australia. Majeed and Muhammad (2019) regarded deforestation and overgrazing as the indirect causes of salinity because these activities could stimulate changes in rainfall and ultimately soil salinization.

1.5.3 Soil Amendments and Appropriate Use of Fertilizers

Soil's physical and chemical attributes have a greater influence on water status and associated salinity. Proper maintenance of cultivated land and addition of healthy substances to it could be efficient practices to prevent the problem of salinity. Rough preparation and improper leveling may result in accumulation of salts in a specific portion of soil which could serve as an additional source of salinization in a given cultivated area. Moreover, application of different organic amendments is also useful to maintain soil's health and control salinity problems. The healthy effect of different amendment approaches on controlling salinization and improving soil's health is well established. Badia (2000) reported that straw amendment into saline soil resulted in reduced electrical conductivity and improved mineralization of the affected soil. Saline soil amended with organic manure imparted stimulatory effect on growth and physicochemical properties of barley suggesting the role of amendments in ameliorating the adverse effects of salinization (Liang et al. 2005). Tejada et al. (2006) have evaluated the role of chicken manure and cotton grid compost in soil improvement. They demonstrated that amendments significantly contributed to soil's physical, chemical, and biological traits. Lakhdar et al. (2009) reviewed the importance of different municipal solid wastes in recovery of salinized soil. They proposed that compost with little inorganic and high organic contents could be used as cost-effective remediation for recovery of salinized soil. Diacono and Montemurro (2015) described the role of composts, waste products, and farmyard manure in soil salinity management. They favored the use of certain composts and amendments for stabilizing salt-affected soils. In more recent studies, different amendment techniques such as municipal solid waste and organic and inorganic composts have been found effective in improving soil's properties and minimizing the adverse effects of salinity on different crop plants (Mbarki et al. 2018; Alcívar et al. 2018; Chávez-García and Siebe 2019).

For attaining maximum yields for crops, fertilizers' application in most of the cultivated soils is widespread, and farmers do not care about the subsequent hazards caused by synthetic fertilizers. Although the use of fertilizers improves soils' nutritional contents and makes them available to the crops grown, imbalanced input of huge quantities of fertilizers also causes severe deterioration in soil besides contributing to environmental pollution. The problem of extensive fertilizer application is very common in developing countries where the majority of the farmers are uneducated and they are unaware of the potential risks associated with heavy fertilizer application. Han et al. (2015a, b) documented that excessive use of nitrogen fertilizers induced salinity and sodicity in soils under greenhouse conditions. Machado and Serralheiro (2017) have argued that extensive fertilizer application is one of the causes of secondary salinization in agricultural lands. They pointed out that the quantity, schedule of application, and method of application of fertilizers are important determinants in their consequent effects on soil salinity. There is ample space for utilization of biofertilizers and plant growth-promoting bacteria as alternative choices to conventional fertilizers in managing soil salinity and a diverse range of pollution (Bhardwaj et al. 2014; Majeed et al. 2018). Salinity caused by fertilizer use can be managed by educating the farmers about salt contents of fertilizers, their required quantities, and the schedule of application. Farmers must know the nutrient deficiency of their soils. Soil analysis could be carried out to determine which types of fertilizers are suitable for specific soils and their role in salinity induction. Although these are difficult tasks, with the help of agricultural institutions and experts, these problems can be resolved to promising extents.

1.5.4 Selection of Appropriate Crop Plants

When farmers have no option but to cultivate crops on salinized soil, the selection of suitable crops could offer a promising solution to minimizing yield losses in crops induced by salinity. Although it is evident that most of the domestic crops are affected by salinity stress, still variation does exist in different crops for their responses to salinity. Determination of the tolerance level of glycophytic species to salinity stress is the first step in selecting the crop for a given salinized land. Agricultural and research organizations can screen and approve domestic crop species for cultivation in saline conditions. Studies conducted by Glenn et al. (2013) demonstrated that *Salicornia bigelovii*, *Atriplex lentiformis*, and *Distichlis palmeri* could tolerate moderate salinity and their cultivation under mild saline soils was recommended. Several cultivars of wheat, maize, and barley are now available which exhibit tolerance to salinity and could be used as alternatives to other sensitive crops in salinized soils (Ashraf et al. 2012). In developing countries, several organizations are working actively to guide farmers about the use of a specific crop in a specific environment. The Pakistan Agricultural Research Council (PARC), Indian Agricultural Research Institute (IARI), Bangladesh Agricultural Research Institute (BARI), and Sri Lanka Council for Agricultural Research Policy (SLCARP) are among the leading research organizations in South Asia which are actively engaged in addressing agricultural issues including salinity.

1.6 Conclusion

Soil salinization caused by natural and extensive human activities remains the leading abiotic stress in agriculture which has a range of adverse effects on crop plants. Osmotic imbalance, nutritional abnormalities, and ionic toxicity become apparent in a saline environment which induces damages at micro- and macro-level to plant physiology, biochemistry, and overall growth. Crop plants serve domestic purposes and they provide raw as well as processed material for consumption by human beings and other animals. Being the acknowledged sources of energy and food, production enhancement of crop plants is vital for maintenance of food, feed, and energy. Since soil salinity imparts adverse effects on the growth, physiology, and final production output of crops, management of saline environment through appropriate techniques could help in reducing salinity-induced crop losses. First-hand and feasible approaches include modification of agronomic practices. Irrigation, fertilizer application, and deforestation have been identified as agents of soil salinity. Updating the current irrigation systems and giving attention to drainage systems could be effective strategies to overcome the salinity problems. Balanced use of fertilizers and appropriate schedule of application would contribute to minimized salinity and improved soil health. Strict monitoring of deforestation and promotion of replantation not only help in climatic stability but also reduce soil salinization. Cultivation of salt-tolerant cultivars of crop plants in a saline area offers advantages of minimized losses of crops than using sensitive species. Although salinity control is a complex and costly strategy, by the involvement of individuals and agricultural organizations and with governmental support, its drastic effects can be reduced substantially.

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Chapter 2
Improving Cotton Crop Tolerance to Drought Stress Through Molecular Approaches

Tayyaba Shaheen, Muhammad Junaid Bilal, Usman Ijaz, and Nazia Nahid

Contents

Abstract Cotton is an essential source of raw material for the fiber and food industry. Limited availability of water for irrigation results in reduced growth of plants. Water scarcity is an upcoming dilemma globally that needs attention to develop strategies to overcome the situation. Development of plant varieties that can withstand the limited availability of water is the dire need of the day particularly in the case of economically vital crops. Cotton crop being the backbone of the economy of the country needs significant attention. There are many strategies to improve the varieties including conventional and modern approaches. Conventional approaches are being utilized for the last many years. However, these are time consuming and laborious. Molecular approaches are accurate, time effective, and of broad spectrum as compared to conventional approaches. Over the years many genes and QTLs related to drought stress have been detected and utilized in cotton to enhance its drought tolerance. Additionally, the genome-editing approaches can hold the promise for the improvement of mechanisms related to drought stress directly or indirectly. This chapter will cover the studies conducted so far to explore and improve the ability of cotton to combat drought stress and recent advances in genomics

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research for cotton crop having emphasis on genetic linkage maps, genetic engineering, and gene/QTL mapping for drought stress tolerance.

Keywords Cotton · Drought · Molecular markers · Conventional breeding · Genetic engineering

2.1 Introduction

Cotton possesses a dominant spot in world economics as a source of natural fiber and is also known as "white gold." Cotton provides 35% of the total fiber used globally (Abdelraheem et al. 2019). Around the world, textile industries mostly depend on cotton. The word "cotton" denotes the spinnable fiber (lint)-producing species (Azhar et al. 2010). It similarly contributes as oilseed source in the production of bioenergy. There are four domesticated species of genus *Gossypium* (Malvaceae); *G. barbadense*, *G. hirsutum*, *G. herbaceum*, and *G. arboreum* are important for textile fiber (Brubaker et al. 1999) and also considered as cultivated cotton (Sethi et al. 2014). *G. hirsutum* has substituted the other cotton species *G. barbadense*, *G. arboreum*, and *G. herbaceum* in cultivation (Sahito et al. 2015).

Several abiotic stresses (temperature, drought, salinity, and osmotic stress) have harmful effects on plant growth and development (Agarwal et al. 2013; Zhu 2002). The diplomatic Intergovernmental Panel on Climate Change suggested that these stresses will exert harmful effects on the growth of plants in the future (Hirayama and Shinozaki 2010). Crop production is highly sensitive to environmental conditions (Pasha et al. 2015) such as drought which causes reduction in crop yield (Ahmad et al. 2015). Development of better-yielding cultivars under drought stress is a major challenge for plant breeders, due to limited water availability for agricultural crops (Messmer and Stamp 2010). Exploration of crop genome for drought resistance is supportive of the development of drought-resistant cultivars (Ahmad et al. 2018).

Production of cotton is affected by several abiotic and biotic factors. Constant exposure to environmental stresses including limited water availability, heat, and soil salinity affects its yield (Rodriguez-Uribe et al. 2014). Water deficit or drought is the major yield-limiting factor in cotton (Lou et al. 2016; Deeba et al. 2012). Cotton being a drought-sensitive crop does not proficiently consume water (Saeed et al. 2011; Iqbal et al. 2013). Several investigations on the drought-receptive stress management in cotton have exerted many negative impacts of drought stress on crops depending on the length, time of stress, severity, and developmental stages of plants (Rodriguez-Uribe et al. 2014).

Plants are capable of managing the biotic and abiotic stresses using complex mechanisms (Almeselmani et al. 2011). Identification of stress signals and signal transduction to stimulate the response of stress-related genes are also included in them (He et al. 2016). The plant physiology, biochemistry, and proteomics are affected due to limited water stress. Plant photosynthesis mechanism is usually damaged the most (Deeba et al. 2012; Lou et al. 2016). Boll formation is usually most affected due to drought. Cotton needs 10% of water at early vegetative stage, but at flowering time, it needs 50–60% of water of the overall water requirement. Understanding stress-related genes whether they are upregulated or downregulated under water stress is significant (Maqbool et al. 2009). Genetic manipulation to enhance drought resistance may help to reduce yield loss. Genomic techniques including molecular markers can be harnessed to explore drought-related traits, i.e., relative water content, cell membrane stability, and excised leaf water loss (Nguyen et al. 2000; Jenkins et al. 2000). DNA markers coupled with QTL mapping can be used to reveal the molecular basis for the drought-associated traits (Yong-Sheng et al. 2009). In past decades, various drought-associated genes have been identified in cotton (Babar et al. 2009; Saranga et al. 2004; Saeed et al. 2011). Manipulation of the plant genomes using transgenic technologies has the capability to support the revolutionary changes in cotton crops. Selection of suitable promoters is significant for expression of transgenes. In addition, promoters can control transgenic expression at different levels that can considerably accelerate the transgenic techniques (Hou et al. 2016). The advent of modern techniques like CRISPR/Cas9 can further facilitate the genetic manipulation of crops. This chapter covers the efforts to enhance drought tolerance in cotton.

2.2 Cotton Breeding Strategies for Drought Stress Management

Development of a plant having favorable characteristics by crossing two plants is termed as breeding (Iqbal et al. 2013). Outcome of the process of breeding is the development of varieties with superior traits. Efforts to utilize recent techniques in breeding like biochemistry, genomics, cell biology, and marker-assisted breeding have also been done. In the previous years, conventional breeding methods have been utilized in cotton to increase drought tolerance. Selection of cotton plants performing well under water shortage is the basis of breeding linked with drought tolerance. Particular traits are considered for the selection of the best-performing cotton varieties under water deficit conditions. Physiological traits like osmotic adjustment and gaseous exchange and anatomical traits like plant water status measurement and root characteristics are considered as the traits of significant importance in desiccation tolerance of cotton (Tuberosa and Salvi 2006; Lopez et al. 1995; Ullah et al. 2008).

Introduction, mutagenesis, selection, and hybridization are the main breeding methods accompanied by other methods which can be utilized to develop new varieties/hybrids with improved yield and better fiber quality (Chaudry and Guitchounts 2003; Poehlman and Sleper 1995). Due to complex genetic inheritance, the growth of cotton plants by conventional breeding has shown a slow development (Boopathi et al. 2015).

Various classical breeding methods have been utilized to increase drought tolerance in plants (Abdelraheem et al. 2015). One of them is “direct selection,” which is the screening of germplasm and selection of the highest-yielding genotypes under drought stress conditions. However, this process is laborious due to the complex nature of drought tolerance-associated traits with less heritability under stress, because these traits are not simply influenced environmentally but also by genotype and genotype-environment interaction. Another approach is indirect selection, which is established on secondary traits. In cotton, efforts to increase the yield for secondary traits against water stress condition have also been made. Finding the relationships between yield and secondary trait is the prerequisite of this approach. Ranking of genotypes by testing the germplasm against drought stress and non-stress conditions to evaluate the yield reduction is the third technique used in conventional breeding. Although conventional breeding has been utilized for many years and many superior cultivars were produced using its techniques, the molecular approach itself coupled with conventional methods can be more effective to improve crop varieties.

2.3 Molecular Approaches to Combat Drought Stress

2.3.1 Molecular Breeding Methods

Molecular breeding is defined as genetic manipulation made at DNA level to enhance desired characteristics in animals and plants through genomic selection, gene alteration, genetic engineering, and marker-assisted selection. Molecular breeding or marker-assisted breeding is the approach of molecular biotechnology, generally using DNA markers in association with genomic and linkage group to enhance and change animal and plant desired traits based on genotypic assays (Jiang 2013). In addition, numerous novel approaches include marker-assisted backcrossing (MABC), genomic selection (GS), marker-assisted selection (MAS), genome-wide selection (GWS), and marker-assisted recurrent selection (MARS) (Ribaut et al. 2010; Crossa et al. 2017). MAS is remarked as a powerful methodology and novel approach for plant genetic perfection, and until date, it is extensively applied in multiple crops (Xu 2010; Jiang 2013). From the last decades, DNA-based marker approaches like restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-nucleotide polymorphism (SNP), and DArT have become accessible (Kumar et al. 2016). From the mentioned markers, RFLP, AFLP, and RAPD are mostly active for diversity analysis and marker-assisted selection related to pulses, but conventional breeders do not favor their use in MAS because of the handling difficulties, use of radioactive element, poor reproducibility, and necessity of a skilled person for developing these markers (Gupta et al. 2010).

Conventional breeding strategies are not very useful due to limitations on genetic advancement and limited knowledge about complex but economically valuable traits (Bolek et al. 2016). Recent advances in transcriptomics, proteomics, basic metabolomics, and genomics strategies merged with molecular marker-assisted breeding have considerably contributed in expanding the competency of cotton breeding. Detection of genetic diversity using DNA polymorphism is the most powerful development in molecular genetics. This approach enables to precisely target the genes and QTLs linked with production of cotton fiber and agronomically/economically significant traits (Boopathi et al. 2015). One of the key factors which reduce the crop production is drought stress. It can be overcome by molecular breeding approaches for the genetic enhancement of cotton and crop production (Saleem et al. 2015).

2.3.1.1 DNA Markers and Marker-Assisted Selection for Drought Stress

Molecular markers are predominantly useful in breeding of plants. They can select agronomically valuable genes to precisely locate their position on genetic maps (Lateef 2015). Molecular markers are beneficial for the identification of genetic diversity in different plant species and development of desired traits and QTL analysis and marker-assisted selection (Bertrand et al. 2008; Qin et al. 2015).

The efficiency and accuracy of conventional plant breeding can be enhanced through marker-assisted selection due to the enormous potential of DNA markers (Bertrand et al. 2008). DNA markers like AFLP, RFLP, RAPD, and microsatellite SSRs proved very advantageous in breeding meant for dehydration tolerance (Iqbal et al. 2013). DNA markers have given a base to study the different traits relevant to drought tolerance because they disclose the genetic and molecular basis (Yong-Sheng et al. 2009). SSR markers are highly efficient to identify polymorphism (Lin et al. 2010; Dongre et al. 2011) and for cotton genome mapping and marker-assisted selection (Frelichowski et al. 2006; He et al. 2007).

Currently, functional genomic components (gene and EST sequences) are accessible in various plant species, e.g., *A. thaliana* and *O. sativa* (Kumar et al. 2009). These resources, which will improve the development of functional markers in cotton, like use of the resistant gene analogues and resistant candidate gene sequences, have been transformed into molecular markers comprising RGA polymorphism. However, next-generation sequencing (NGS) equipped with high-throughput genotyping is being significantly utilized. It will help in large-scale screening of segregating entities and low-cost selection of required phenotypes (Nadeem et al. 2018). It will make marker-assisted selection to be most efficient, cheap, as well as useful in the future.

Javaid et al. (2017) elaborated that development of cotton varieties that can withstand adverse environmental conditions is an effective strategy to enhance the yield of cotton. Thirty SSR primers were used to detect genetic diversity in 22 cotton plant genotypes for tolerance during drought condition. Eleven SSR primers out of the 30 were able to distinguish the different cotton genotypes, ensuring 37% of the primers were enlightening. In total, 41 alleles were identified; the amount of alleles per locus ranged from 1 to 6, while the allelic diversity was observed at 0.40. The genetic diversity between 22 genotypes of cotton was low. Sang et al. (2017) studied drought tolerance in upland cotton (*G. hirsutum* L.) and molecular markers linked with the drought tolerance traits to find out elite allelic variation related with drought resistance traits. A total of 191 cultivars were treated with PEG 6000-induced stress. Seventy-four pairs of SSR primers were employed to find the genetic variation of the natural population. These were performed based on the analysis of population structure to correlate the genotypic data with the drought tolerance traits at germination stage and discover the molecular markers associated with the drought tolerance traits.

Ditta et al. (2018) studied the diversity of genomes and structure of population in cotton germplasm involving 132 diploid, such as *G. klotzschianum*, and allotetraploid, such as *G. ekmanianum*, *G. darwinii*, *G. barbadense*, *G. stephensii*, and *G. tomentosum*, cotton accessions. Overall 111 genomic simple sequence repeats (gSSRs) and expressed sequence tag (EST) were used which resulted in 382 polymorphic loci; analyzing molecular alteration revealed highly important variation between six identified groups. An uncharacterized gene, associated with tryptophan aspartic acid (W-D) repeats, was associated with genomic markers and was determined to be unregulated under salt and drought stress environments. The characterization of and variation in genes, genetic diversity, and novel germplasm assortment will be valuable in finding the genetic structure of cotton germplasm.

Sang et al. (2017) analyzed drought tolerance of upland cotton (*G. hirsutum* L.) and molecular markers linked with drought tolerance traits to discover top allelic variation related with drought resistance traits. A total of 191 cultivars were treated with PEG 6000-induced stress. Seventy-four pairs of SSR primers were employed to detect the genetic variation of the natural population. These were performed based on the analysis of population structure to correlate the genotype data with the drought tolerance traits at germination stage and discover the molecular markers linked with the drought tolerance traits. They could identify 46 drought-resistant materials and 15 molecular markers related to drought tolerance, which can be utilized for marker-assisted breeding of drought-resistant cotton (Sang et al. 2017).

2.3.1.2 QTL Mapping for Drought Stress

QTL mapping is one of the basic tools, which helps to explore the complex traits like drought stress resistance. The genetic factors regulating the phenotypic variations, which are known for quantitative characteristics, are termed as QTLs (Tuberosa and Salvi 2006). Natural genetic variability of a crop can be used for subsequent MAS, direct selection, and QTL mapping (Ashraf et al. 2008;Ashraf 2010). The parts of polygenes have been broadly assessed using conventional techniques, but QTL mapping and DNA markers made it easy to analyze complex traits (Ashraf 2010).

Quantitative trait loci (QTLs) are used for enhanced crop development through genetic mapping techniques under water stress conditions (Iqbal et al. 2013). The QTLs proffered an idea about marker-assisted selection (MAS) (Morgante and

Salamini 2003). QTLs have been mapped by researchers for morphological traits, physiological traits (Saranga et al. 2004; Saeed et al. 2011; Peitong et al. 2005; Liang et al. 2014), earliness (XianLiang et al. 2008; Li et al. 2013), fiber traits (Said et al. 2013; Islam et al. 2014), and also yield (Babar et al. 2009; LiFang et al. 2010). Drought tolerance could be enhanced by utilizing induced physiological traits associated with drought tolerance. (Bartels and Sunkar 2005; Xoconostel and Ortega 2010). In drought condition, the conservation of water content in leaves of a plant is a vibrant adaptation. Limited water loss from leaves helps in maintaining the optimum level of water in the plant. Cell membrane stability is a central target of stress under stressed conditions (Levitt 1972). Water stress-tolerant genotypes generally maintain the structure of the cell membrane through drought stress (Bajjii et al. 2001). Plant selection to increase tolerance in stress is extremely hard because of environmental conditions and genotype-environment interaction (Schuster 2011). Improving drought stress in a hydroponic environment using polyethylene glycol is useful to assess plants due to uniform stress imposed to populations. Many studies (de Brito et al. 2011; Ren et al. 2011;Saleem et al. 2015) were directed for QTL analysis of drought-tolerant traits involving excised leaf water loss, relative water content, and stability of cell membrane.

Fiber quality, physiology, productivity, and drought-tolerant traits involving leaf water loss, stability of cell membrane, and relative content of water have been studied by QTL mapping in cotton (Saranga et al. 2001; Saleem et al. 2015). Physiological variations were discovered to succeed because of QTLs related with osmotic adjustment tolerance (Tuberosa and Salvi 2006). Levi et al. (2009a, b) described QTLs, which were linked to physiological parameters in two water shortage-tolerant species. QTL mapping has frequently been used for family mapping in cotton and to study traits like resistance to heat and drought stress. These studies have made it possible now to select high-quality QTL alleles with linked genetic markers in marker-assisted selection (MAS) for cotton breeders (Saranga 2009).

Other important alleles can be explored by genomic analysis of wild tetraploid cotton (*G. mustelinum*, *G. tomentosum*, and *G. darwinii*) (Saranga et al. 2004). These wild species have potential use as a genetic resource; these are being utilized to improve the cultivated cotton in different breeding programs (Mehetre and Aher 2004). Owing to their several barriers to gene flow in cotton, the usage of interspecific crosses in breeding has been constrained (Jiang et al. 2000). Zheng et al. (2016) constructed a dense genetic map between a wild-type cotton, *G. tomentosum*, and an upland cotton, *G. hirsutum* (CRI-12), from an inter-specific cross, using 1295 SSRs. The map is used to identify QTLs for traits linked to drought tolerance at the flowering stage, full boll stage, and budding stage. An inter-specific cross between upland and wild cotton population employed for QTL mapping was the very first effort.

In a cross of *G. barbadense* cv. F-177 and *G. hirsutum* cv. Siv'on, a subset of 33 QTLs were recognized under drought conditions. From these, 5 QTLs were recognized for various physiological traits, 17 for fiber quality, and 11 for plant productivity. Maximum QTLs were present on chromosome 2, 6, and 14 (Saranga et al. 2001). *G. hirsutum* cv. Siv'on and *G. barbadense* cv. F-177 near isogenic lines were produced by exchange of QTL for yield-related and drought traits (Levi et al. 2009a, b). Furthermore, mineral and metabolite analyses showed that *G. hirsutum* cv. Siv'on gave improved levels of metabolites under drought and well-watered conditions as compared to *G. barbadense* cv. F-177, which only gave an evident adjustment to drought (Levi et al. 2011). Moreover, under salt and drought stress, 106 microsatellite markers were analyzed on 323 *G. hirsutum* germplasms; a total of 15 markers were linked to drought tolerance. Twelve markers exhibited negative allele effects and other markers presented positive allele effects for the drought tolerance (Jia et al. 2014). These QTLs may be used for development of cotton varieties having drought tolerance.

Said et al. (2013) reported 1223 QTLs in *Gossypium* and meta-analyzed these QTLs for yield, yield-associated morphological and fiber quality traits, drought tolerance, and disease resistance in tetraploid cotton. QTL hotspots for fiber quality traits were found in the same regions as clusters; it was observed that clusters might also develop hotspots. QTL clusters and hotspots found in the same place confirmed that multiple QTLs are associated with same traits and develop hotspots.

Wang et al. (2015) analyzed 178 recombinant inbred lines produced by a single seed descendant by crossing *G. hirsutum*. cv Jimian5 and *G. hirsutum*. acc DH962. A genetic map was created using 644 polymorphic loci. Twenty-six QTLs were spotted that were associated with yield components and fiber quality. Saleem et al. (2015) studied drought-tolerant traits under osmotic stress by crossing drought-susceptible (FH-1000) and drought-tolerant (B-557) cotton varieties. EST-SSR primers and 524 SSRs were used and a genetic linkage map was built. By mapping primers, two QTLs were determined that have linkage to relative water content. These QTLs were mapped on chromosome 12 and 23. These QTLs may be helpful to develop cotton cultivars with tolerance to water-deficient condition. Some of the efforts to identify QTLs for drought tolerance have been summarized in Table 2.1.

2.3.2 Drought-Responsive Genes Studied in Cotton

Tolerance to abiotic stress is an intricate phenomenon as these stresses may arise simultaneously and affect plant growth at different developmental stages (Park et al. 2003). Therefore, the basic mechanisms for adaptation and tolerance to abiotic stress have been the area of comprehensive research (Cho et al. 2018). Among these stresses, drought stress is the major problem that influences survival, geographical distribution, and productivity of agricultural crops.

Several TFs and *cis*-elements not only play a role as molecular regulator for expression of genes but also function as end point for signal transduction pathway during drought stress responses (El Esawi & Alayafi 2019; Gujjar et al. 2014). The primary events in abiotic stress adopted by plants are the recognition of stress signals and activation of the signal transduction pathways, which ultimately lead to activation of several stress tolerance genes (Qian et al. 2019). In addition, ABA-independent regulons are further divided into types: (1) the zinc-finger homeo-domain regulon

Table 2.1. List of studies to identify QTLs linked with drought-responsive traits in cotton

Markers used	Traits studied	No. of QTLs identified	Cotton species	Reference
RFLP	Plant productivity and physiological traits	161	*G. hirsutum*	Saranga et al. (2001)
SSR	Drought-resistant traits	Three QTLs for seed cotton, three QTLs for osmotic potential	*G. hirsutum*	Babar et al. (2009)
SSR	Physiological traits (osmotic potential and osmotic adjustment), yield and its component traits, plant architecture traits	Seven QTLs (osmotic potential 2, osmotic adjustment 1, seed cotton yield 1, number of bolls/plants 1, boll weight 1, and plant height 1) were identified. There were three QTLs (qtlOP-2, qtlOA-1, and qtlPH-1) detected only in water-limited conditions. Two QTLs (qtlSC-1 and qtlBW-1) were detected for relative values. Two QTLs (qtlOP-1 and qtlBN-1) were detected for well-watered treatment	*G. hirsutum*	Saeed et al. (2011)
SSR, EST-SSR	Relative water content, excised leaf water loss, drought tolerant traits	Two QTLs for relative water content, one QTL for excised leaf water loss	*G. hirsutum* L.	Saleem et al. (2015)
SSR	Chlorophyll content, plant height, leaf area, leaf number, leaf fresh weight, leaf dry weight, boll weight, number of bolls per plant, and number of fruiting branches	67 and 35 under water-limited and well-watered conditions	*G. hirsutum X G. tomentosum*	Zheng et al. (2016)

(ZF-HD) and NAC transcription factor and (2) CBF/DREB (cold binding factor/dehydration-responsive element binding) regulon (Saibo et al. 2009; Hoang et al. 2017). In cotton, many *cis*-elements have been recognized, which respond to drought stress including dehydration-responsive binding element (DREB1, DREB2); C-repeat binding factor regulons play a role in ABA-independent gene expression (Lenka and Bansal 2019), while ABA binding factor (ABF) and ABA-responsive element (AREB) are the major transcription factors activated in abiotic stress response. Moreover, various regulons, such as NAC (N-acetylcarnosine) and MYB (myeloblastosis)/MYC (myelocytomatosis) regulons, regulate gene expression in an ABA-independent manner (Guo et al. 2017). Many studies described that NAC, AREB/ABF and DREB1/CBF, DREB2, and APETLA2 (AP2)/EREBP regulons play an important role to combat drought stresses in plants (Chinnusamy et al. 2004;

Sunkar et al. 2007). One of the elements is the GCC box, present in most pathogen-related gene promoters which combat against ethylene (Gu et al. 2000). The second is C-repeat CRT/DRE (dehydration-responsive element) motif, which is engaged in drought resistance gene activation (Andeani et al. 2009). LEA gene family was identified and characterized in cotton (Magwanga et al. 2018). Moreover, *Gossypium* MYB transcription factor, *GbMYB5* gene, also positively participates in cotton plant, to combat against drought stress (Chen et al. 2015) (Table2.2).

Sugars perform a key role in a pyramid of metabolic processes throughout the life span of plants, that is, from the initial stage to the reproductive stage. There is an interplay between phytohormone signaling and sugar signaling such as abscisic acid, ethylene, auxins, and gibberellins. This phenomenon is associated with the plant growth process, carbon allocation, and subsequently dehydration stress. Trehalose, a non-reducing sugar, accumulates in many organisms including bacteria, fungi, and plants under drought stress. In plants, trehalose protects the biological molecules against drought stress and stimulates stress signaling pathways. A genome-wide analysis of stress-related gene family of TPS (trehalose-6-phosphate synthase) was conducted in cotton. A significant higher number of upregulated genes in *Gossypium hirsutum* as compared to *G. arboreum* and *G. raimondii* was observed (Mu et al. 2016).

Table 2.2. List of some recently reported genes in cotton species linked to drought stress

Gene identified	Cotton species	References
GHSP26	*G. arboretum*	Maqbool et al. (2009)
GhDREB *overexpressed in wheat*	*G. hirsutum*	Gao et al. (2009)
GhDi19-1, *GhDi19-2*	*G. hirsutum*	Li et al. (2010)
GhMPK16	*G. hirsutum*	Shi et al. (2011)
GhWRKY17 *overexpressed in Nicotiana benthamiana*	*G. hirsutum*	Yan et al. (2014)
GhRAV1 gene *overexpressed in A. thaliana*	*G. hirsutum*	Li et al. (2015)
GbMYB5	*G. barbadense*	Chen et al. (2015)
NCED	*G. arboretum*	de Souza et al. (2016)
GhERF38	*G. hirsutum*	Ma et al. (2017)
MYB-related, *C2H2*, *FAR1*, *bHLH*, *bZIP*, *MADS*, and *mTERF*	*G. barbadense*	Zhou et al. (2016)
AP2/EREBP superfamily	*G. hirsutum*	Liu and Zhang (2017)
Late embryogenesis abundant (LEA) gene family	*G. hirsutum*, *G. arboreum*, and *G. barbadense*	Magwanga et al. (2018)
GhWRKY33 *overexpressed in A. thaliana*	*G. hirsutum*	Wang et al. (2019)

Generally, drought stress triggers the accretion of reactive oxygen in plants, which produces oxidative deficiency at cellular level or disrupts cellular physiological homeostasis (Ashraf and Foolad 2007). The proline accumulation in several tissues of plants is considered as the main response to drought and other stresses. It plays the role of a shielding agent for the function of enzymes, an osmolyte, and a free radical scavenger. Accumulation of proline was detected in drought-tolerant species of *Gossypium*. Drought-induced expression of drought-responsive genes such as ERF (ethylene-responsive factor), DREB (dehydration-responsive element binding protein), EREB (ethylene-responsive element binding protein), WRKY6 (WRKY transcription factor 6), FeSOD and copper/zinc superoxide dismutase, MAPKKK17 (mitogen-activated protein kinase kinase kinase 17), ZFP1 (CCCH-type zinc finger putative transcription factor), PRP5 (proline-rich protein), and Δ1P5CR (Δ1-pyrroline-5-carboxylate reductase) has been observed in various plant species. These genes could be employed for illustrating drought stress-tolerant cotton genotypes (Hasan et al. 2018).

In a study, expression levels of *WRKY6*, *ERF*, *FeSOD*, *DREB*, *ZFP1*, *ERFB*, *MAPKKK17*, *P5CR*, *PRP5*, and *CuZnSOD* genes were found higher in *G. hirsutum* as compared to *G. barbadense* and *G. arboreum* under drought conditions (Hasan et al. 2018). In *G. arboreum* L. the gene *GHSP26* was identified to be active in drought stress by controlling cellular metabolism as it participates in folding of protein and prevents protein denaturation (Maqbool et al. 2009). Many studies have been directed to identify and analyze drought-responsive genes in cotton (Maqbool et al. 2009; Gao et al. 2009; Li et al. 2010; Shi et al. 2011; Yan et al. 2014; Li et al. 2015; Chen et al. 2015; de Souza et al. 2016; Zhou et al. 2016; Ma et al. 2017; Liu and Zhang 2017; Magwanga et al. 2018; Wang et al. 2019) (Table2.2). Some genes like *GhDREB*, *GhWRKY17*, *GhRAV1*, and *GhWRKY33* from cotton have been transferred and overexpressed in other plant species to enhance drought tolerance in these species (Gao et al. 2009; Yan et al. 2014; Li et al. 2015; Wang et al. 2019).

2.3.3 Transgenic Approaches to Combat Drought Stress

Although cotton is cultivated in both arid and semi-arid areas, where drought stress is dominant, cotton is not considered as a drought-tolerant crop (Shamim et al. 2013; Kerr et al. 2018). Global climate changes can cause more severe environmental stresses. Consequently, it is vital to develop enhanced stress-tolerant crops to defend global agricultural production.

Transgenic technology is an effective method that has been used to enhance drought tolerance by producing drought-resistant plants. Numerous analyses were steered to discover the drought stress response of plants at the cellular level, which resulted to classifying a massive range of stress tolerance genes (Farooq et al. 2009). Analyzing the stress-associated genes at molecular level is important for researchers, for developing stress-tolerant plants by transgenic methods (Shinozaki and Yamaguchi-Shinozaki 2007). The TF family plays an important role to combat abiotic stress in plants. In cotton, overexpression of GhABF2, a bZIP TF gene, provided drought tolerance by stimulation of ABA-related gene. *GhABF2* overexpressed in cotton also resulted to higher yield as compared to wild-type cotton (Liang et al. 2016). Overexpression of *AtRAV1/2* and *AtAB15* genes resulted in enhanced drought tolerance in cotton (Mittal et al. 2014). OsNAC*1*, a participant of NAC TF gene family, was overexpressed in cotton resulting in enhanced drought tolerance by reducing transpiration rate and enhancing rooting system as compared to wild-type cotton plant (Liu et al. 2014; Yu et al. 2016). Overexpression of *Arabidopsis* HOMEODOMAIN GLABROUS11 improved drought stress tolerance (*HDG11/AtEDT1*) gene in cotton, which displayed an enhanced drought tolerance (Jan et al. 2019). High proline content in leaves, a larger rooting system, and ROS-scavenging activity were detected in cotton (Ashraf et al. 2018). In addition, overexpression of *GhWRKY17* gene in cotton also amended drought tolerance in cotton (Yan et al. 2016).

Various studies demonstrated drought resistance in transgenic cotton containing stress-associated genes that are crucial in plant physiology (Ullah et al. 2017). For example, when *Thellungiella halophila TsVP* H^+-PPase gene was overexpressed in cotton, this resulted in high photosynthetic rate and improved drought tolerance (Lv et al. 2009). The overexpression of *A. thaliana AtNHX1* gene, a H^+/Na^+ antiporter, in cotton resulted in high yield and improved drought tolerance in field and greenhouse conditions as compared to wild-type cotton (Umezawa et al. 2006). In addition, Shen et al. (2015) co-expressed the *AtNHX1* and *AVP1* genes in cotton, and cotton co-expressing these genes displayed an enhanced drought tolerance as compared to wild-type cotton. Transgenic cotton co-expressing *AtNHX1* and *AVP1* genes produced 24 and 35% more fibers than wild-type cotton under dryland and low-irrigation environments, respectively (Shen et al. 2015).

Currently, Mishra et al. (2017) conclude that overexpression of *OsSIZ1* gene (SUMO-E3 ligase) in cotton enhances fiber yield and drought and heat resistance under dryland agriculture system. In *G. arboreum* L., the *GHSP2* gene, a heat-shock protein, is involved in drought tolerance. In another study, overexpression of *GHSP2* gene in *G. hirsutum* resulted in increased drought stress tolerance (Maqbool et al. 2009). Moreover, Yue et al. (2012) overexpressed the *LOS5/ABA3* gene in cotton which is implicated in ABA synthesis to improve drought tolerance. Overexpression of *LOS5/ABA3* gene in cotton resulted in increased drought tolerance and formed 13% more biomass than wild-type cotton plants (Yue et al. 2012).

Overexpression of *ScALDH21* gene, taken from *Syntrichia caninervis*, resulted in improved proline and soluble sugar content and higher peroxidase activity and drought tolerance under greenhouse conditions (Yang et al. 2016). In addition, when *osmotin* gene from *N. tabacum* was overexpressed in cotton, it resulted in improved sequestration of sugars and ions into vacuoles, decreased water potential, and enhanced drought tolerance in dryland environment (Wu et al. 2015). The transgenic methods appear to be impactful and robust methods to enhance crop tolerance under drought stress conditions.

Another research stated that *Arabidopsis* has a homeodomain transcription factor (*AtEDT1/HDG11*) which has been verified to expressively enhance drought tolerance in tobacco, *Arabidopsis*, rice, and tall fescue (Qin et al. 2013). *AtHDG11* also enhanced salt and drought tolerance in *G. hirsutum*. Transgenic cotton substantially enhanced drought tolerance in stress conditions with enhanced root system. Proline content, soluble sugar content, and enzyme activity are also increased in transgenic plants. The stomatal density of the leaf is reduced while the size of epidermal cells of the leaf is increased in transgenic cotton. Moreover, transgenic cotton showed enhanced tolerance against drought resulting to higher yield and enhanced agronomic performance in the field under drought stress environment. These results validated that *AtHDG11* is a capable candidate for cotton to develop tolerance against drought (Yu et al. 2016) (Table 2.3).

Table 2.3. Success stories of genetically modified cotton with enhanced yield under drought conditions

Name of gene used	Source of gene	Beneficial traits of transgenic plants under drought stress	Effect on yield	References
GHSP2	*G. arboreum*	Improved drought tolerance	Increased 14%	Maqbool et al. (2009)
TsVP	*Thellungiella halophila*	Higher rate of photosynthesis and relative water content	42–61%	Lv et al. (2009)
Osmotin	*N. tabacum*	Higher relative water content and proline level	57.6% more bolls	Parkhi et al. (2009)
AtLOS5 gene transgenic	*A. thaliana*	Increased abscisic acid level and drought tolerance	Increased 13% biomass	Yue et al. (2012)
SNAC1	*O. sativa*	Enhanced proline content and root development	31% more bolls	Liu et al. (2014)
(ABI3)/Viviparous1 and AtABI5 transcription factor co-expression	*A. thaliana*	Enhanced drought stress adaptation	Increased biomass	Mittal et al. (2014)
AVP1 and AtNHX1	*A. thaliana*	Enhanced sequestration of ions and sugars into vacuole, reduced water potential	Increased 20%	Shen et al. (2015)
AtEDT1/HDG11	*A. thaliana*	Low stomatal density, increased ROS-scavenging enzymes	43% higher seeds	Yu et al. (2016)
ScALDH21	*Syntrichia caninervis*	Increased soluble sugar and proline content, higher peroxidase activity	Yield increased	Yang et al. (2016)
OsSIZ1	*O. sativa*	Improved fiber quality and drought tolerance	Increased 12%	Mishra et al. (2017)

2.4 Modern Techniques Used in Cotton to Combat Drought Stress

To combat drought stress, several modern approaches have been developed. One approach is to initiate transcriptional factors, which control the stress-related expression of genes, which permits plants to acclimate to the adverse conditions and trigger the repair mechanism to repair the damage caused by stresses (Mittler and Blumwald 2010). Another approach comprises rapid fluctuations in cellular metabolisms, i.e., changes in ion transporter metabolism and changes in enzyme activities, which permit cellular homeostasis to be restored in stress environments (Dong 2012).

CRISPR/Cas9 is the most advanced genome-editing tool which is widely used in animals and plants because of its versatility, simplicity, high efficiency, and low cost (Shan et al. 2014; Wu et al. 2014). CRISPR/Cas9 technique has transformed genome editing as it employs short RNA molecules to identify the target sequence instead of large polypeptides as used in TALENs and ZFNs (Bortesi and Fischer 2015; Gao et al. 2015). CRISPR/Cas9 system has been effectively used for genome editing in different model plants and crops such as wheat, rice, *Nicotiana*, sorghum, *Arabidopsis*, tomato, poplar, and maize (Fan et al. 2015; Mao et al. 2016). Recently, many studies have demonstrated the usage of CRISPR/Cas9 approach in cotton targeting GFP, GhARG, GhCLA1, and MYB25 for genome editing signifying that CRISPR/Cas9 can be successfully used for genome editing in cotton (Li et al. 2017; Chen et al. 2017; Janga et al. 2017; Wang et al. 2017a, b, c). CRISPR/Cas9 has been successfully utilized to produce mutagens in cotton by editing different genes such as *GhEF1*, *GhPDS*, and *GhCLA1* (Gao et al. 2017). This technique can be utilized to induce mutations in drought-responsive genes, which could efficiently combat against drought and other abiotic stresses. All the abovementioned methods can be used to develop tolerance against drought individually and in combination with each other (Fig. 2.1).

2.5 Conclusion

Drought is the most devastating stress among abiotic stresses, which negatively affects the yield of many economically important crops including cotton. Water scarcity is the most challenging upcoming dilemma of this century. Conventional breeding methods have been utilized for many years to develop resilient crops; however, these methods have their own limitations. With the advent of modern methods including genetic manipulation of crops, molecular markers, and transgenic technologies, crop breeding is revolutionized. A lot of research has been conducted worldwide to produce drought-tolerant cotton varieties, which are very promising. Furthermore, genome-editing techniques like CRISPR/Cas9 can be utilized to develop drought-tolerant crops without harnessing transgenic technology.

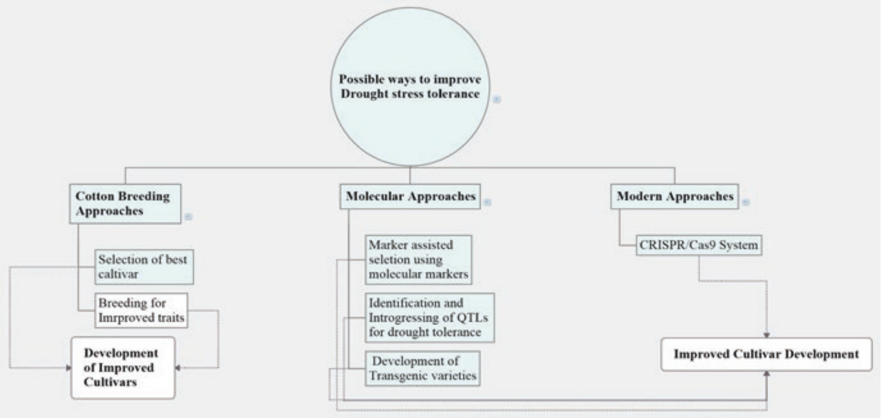

Fig. 2.1 Possible ways to improve drought tolerance in cotton

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Chapter 3
Mechanisms of Plant Adaptation and Tolerance to Heat Stress

Zhong-Guang Li

Contents

Abstract During the entire life cycle, plants always encounter various stresses due to their nonmobile trait. Among these stresses, heat stress is a major stressor limiting cellular anabolism and catabolism, seed germination, seedling growth, development, geographical distribution, production, and even survival. Looking for the useful methods to boost thermotolerance and expounding the mechanism of plant adaptation and tolerance to heat stress is of vital importance for agricultural production. Heat stress commonly leads to different injuries at molecular, metabolic, physiological,

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biochemical, and even whole plant levels depending on the intensity of and endurance to high temperature. The adaptation and tolerance of plants to heat stress also is a sophisticated chemical and biological event, implicating heat stress perception, signaling transduction, gene expression, and physio-biochemical changes. This chapter discusses the heat injury, heat stress perception, signaling transduction, and mechanisms of adaptation and tolerance of plants to high temperature, which lays the foundation of further understanding the mechanisms of heat injury and heat tolerance and acquiring genetically modified plants with heat tolerance and even multiple stress tolerance.

Keywords Calcium signaling · Signaling network · Response and adaptation · Heat stress · Heat tolerance

3.1 Introduction

During the whole life cycle, plants unavoidably encounter all kinds of abiotic (extreme temperature, drought, flooding, and salt and heavy metal stress) and biotic (insects, parasites, nematodes, weeds, bacteria, fungi, and viruses) stresses in nature due to their nonmobile nature. Among these stresses, heat stress (approximately 10–15 °C above the optimal growth temperature) adversely affects cellular anabolism and catabolism, seedling growth, development, geographical distribution, and production and even leads to death, which is a key limiting factor for plant survival (Wahid et al. 2007; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019). As a whole, heat stress commonly gives rise to direct and indirect damage at different levels. The direct damage refers to the destruction of biomembrane integrity (manifesting as the leakage of electrolytes), fluidity, and cellular compartmentalization, as well as protein denaturation (showing up to the loss of protein functions); the indirect damage, also known as secondary stress, is implicated in oxidant stress (indicating the excessive accumulation of reactive oxygen species (ROS)), methylglyoxal (MG) stress (reflecting in the overproduction of MG), and osmotic stress (showing a reduction in osmotic potential and water potential) (Wahid et al. 2007; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019).

To cope with direct and indirect injury induced by heat stress, plants have developed a rigorous and efficient mechanism, which is implicated in the perception of the heat stress, signaling transduction, expression of genes related to stress, and alternation in physiology and biochemistry (Wahid et al. 2007; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019). These adaptive changes are involved in the restoring and rebuilding of biomembrane and cellular compartmentalization, the increase in phase transition temperature of the biomembrane, the biosynthesis of stress proteins (e.g., heat shock proteins

(HSPs)), the enhancement of antioxidant system (such as enzymatic and non-enzymatic antioxidants), the activation of MG-scavenging system (covering glyoxalase I, II, and III (Gly I, II, and III)), and the accumulation of osmolytes (e.g., amino acid (proline, Pro), trimethylammonium (glycine betaine, GB), nonreducing sugar (trehalose, Tre), and total soluble sugar) (Wahid et al. 2007; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019).

In this chapter, in the light of the current progress on plant adaptation and resistance to heat stress, the mechanisms of heat injury and heat adaptation and tolerance were discussed. The contents cover heat injury, heat stress perception, signaling transduction, and the adaptation and tolerance of plants to high temperature, which lays the foundation of understanding the mechanisms of heat injury and heat tolerance and acquiring genetically modified plants with heat tolerance and even multiple stress tolerance.

3.2 Heat Injury in Plants

Heat stress, which is a 10–15 °C increase in environmental temperature, commonly leads to heat injury at molecular, cellular, tissue, organ, and whole plant levels. The damage at molecular level is the basis for others. Heat stress overproduces the reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH), which in turn leads to, to varying extents, oxidative stress, that is, the damage of biomacroelements such as proteins, nucleic acids, and lipids: (1) the inhibition of sensitive enzymes; (2) the degradation of chlorophyll, namely, “bleaching”; (3) the peroxidation of lipids; (4) the damage of DNA, including base alternations and strand breaks; and (5) protein modifications, including specific amino acid substitute, polypeptide breakage, and protein aggregation, denaturation, and susceptibility to proteolysis (Fig. 3.1; Wahid et al. 2007; Mittler et al. 2012; Bita and Gerats2013; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019).

Besides oxidative stress, heat stress damages the biomembrane, which in turn causes the destruction of biomembrane integrity, fluidity, and cellular compartmentalization, showing an increase in ion leakage (Wahid et al. 2007; Mittler et al. 2012; Bita and Gerats2013; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019). Additionally, heat stress commonly combines with drought stress, generating osmotic stress, that is, the lack of water in plant cells due to the massive evaporation of water by transpiration. In addition, similar to oxidative stress, under heat stress conditions, plants can inevitably generate excessive MG, which in turn results in MG stress, also known as carbonyl stress. MG can rapidly combine with bases (e.g., guanine), amino acids (such as cysteine, arginine, and lysine), and phospholipids, which in turn produce advanced glycation end products (AGEs). The production of AGEs on nucleotides, proteins, and phospholipids commonly leads to inherent mutagenesis and cell apoptosis, protein misfolding, and the loss of membrane lipid bilayer, respectively (Fig. 3.2; Wahid et al. 2007; Mittler

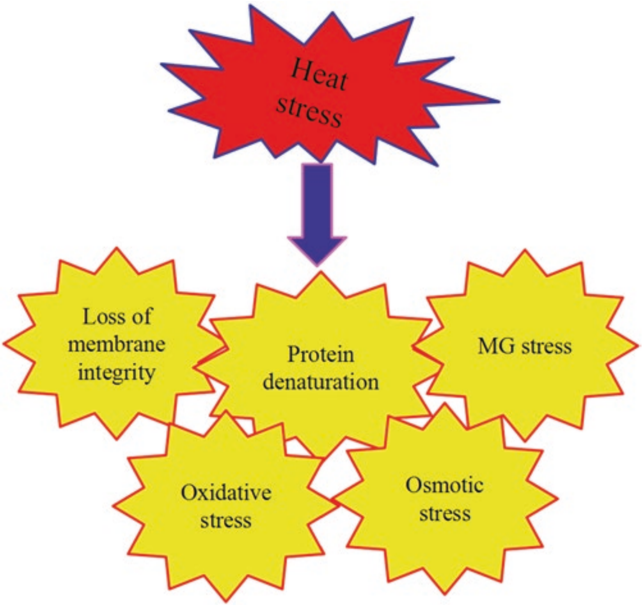

Fig. 3.1 Heat injury in plants. Heat stress results in the destruction of biomembrane integrity, denaturation of protein, and oxidative, methylglyoxal (MG), and osmotic stress in plants

et al. 2012; Bita and Gerats 2013; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019).

3.3 Heat Stress Perception and Signaling Transduction in Plants

Besides heat injury adaptation, plant adaptation and resistance to high temperature is a sophisticated chemical and biological event, involving many steps, that is, heat stress perception, signaling transduction, gene expression, physiological and biochemical change, and anatomical and morphological adaptation (Figs. 3.2 and 3.3).

3.3.1 Heat Stress Perception

Plants can perceive the extracellular and intracellular signaling at structural (such as cell wall, plasma membrane, cytoskeleton, and organelles) and molecular (such as proteins, DNA, RNA, and phospholipids) levels (Saidi et al. 2011; Qu et al. 2013; Dickinson et al. 2018; Sajid et al. 2018). In general, the plasma membrane is considered as a major heat sensor, which is involved in the formation of inherent

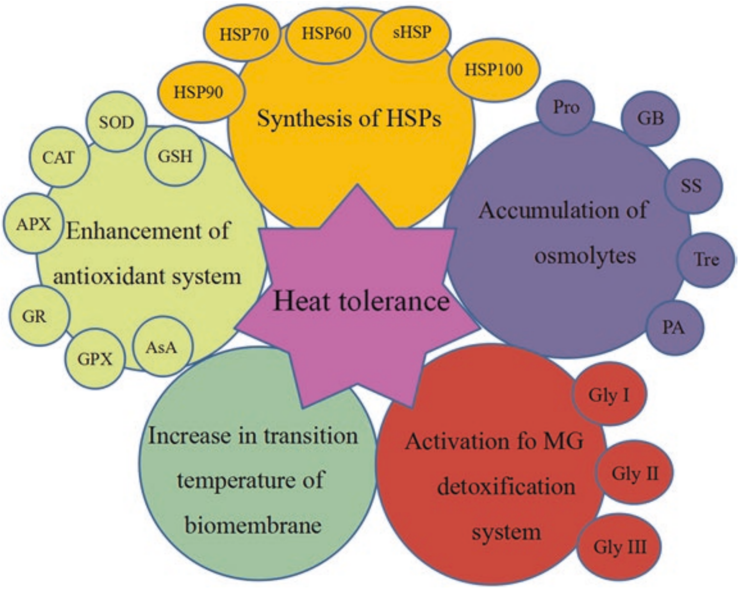

Fig. 3.2 Signaling transduction and crosstalk in the plant adaptation and tolerance to heat stress. Heat priming and/or chemical priming calcium (Ca^{2+}), hydrogen peroxide (H_2O_2), nitric oxide (NO), hydrogen sulfide (H_2S), methylglyoxal (MG), and plant hormone (PH) signaling, as well as their interactions in plants, which in turn trigger the plant response and tolerance to high temperature

and acquired heat tolerance in plants. Modifying the physical properties (such as the fluidity) of the biological membrane can trigger Ca^{2+} signaling in the cytoplasm and induce the expression of genes related to heat tolerance such as heat shock factors (HSFs) and heat shock proteins (HSPs). For example, benzyl alcohol (membrane fluidizer, stimulating an increase in temperature) triggers a similar cascade of Ca^{2+} signaling and HSP expression; conversely, dimethylsulfoxide (rigidifying agent) reduces the heat-mediated Ca^{2+} signaling and the expression of HSPs, further supporting the existence of plasma membrane-associated thermo-sensors that can perceive and respond to heat stress by the change in the fluidity of the membrane (Saidi et al. 2011; Sajid et al. 2018). In addition, plasma membrane-associated ion channels (such as Ca^{2+} channels) and enzymes (such as NADPH oxidase (NOX) and nitric oxide synthase (NOS)) may be heat stress sensors, which trigger Ca^{2+} signaling, reactive oxygen species (ROS, mainly hydrogen peroxide (H_2O_2)) signaling, and nitric oxide (NO) signaling, as well as interactions among these signaling molecules, thus forming a signaling network related to heat tolerance in plants (Price et al. 1994; Keller et al. 1998; Gong et al. 2001; Hu et al. 2007; Saidi et al. 2011; Ma et al. 2012; Qu et al. 2013; Demidchik et al. 2018; Sajid et al. 2018). Also, heat stress can be sensed by organelles (such as mitochondria and chloroplast), followed by triggering ROS signaling cascades and inducing the formation of heat tolerance by regulating transcription regulatory and metabolic networks (Qu et al. 2013).

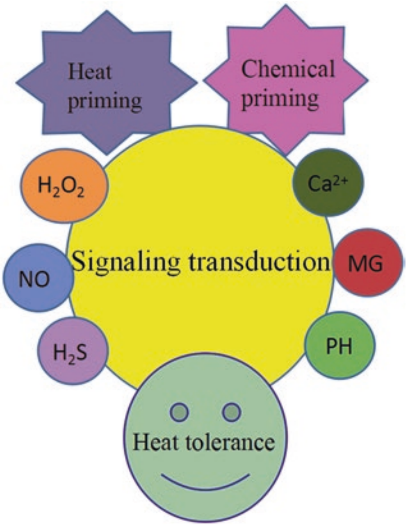

Fig. 3.3 The possible mechanisms of plant response and tolerance to heat stress. Plant adaptation and tolerance to heat stress is implicated in the synthesis of heat shock proteins (HSPs), such as small HSPs, HSP60, HSP70, HSP90, and HSP100; the enhancement of antioxidant system, including enzymatic antioxidants (such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX)) and non-enzymatic antioxidants (such as reduced glutathione (GSH) and ascorbic acid (AsA)); the accumulation of osmolytes such as proline (Pro), glycine betaine (GB), trehalose (Tre), soluble sugar (SS), and polyamine (PA); the activation of methylglyoxal (MG) detoxification system (glyoxalase I (Gly I), glyoxalase II (Gly II), and glyoxalase III (Gly III)); and an increase in transition temperature of biomembrane

During the day, chloroplast can perceive heat stress, which in turn generates light-dependent chloroplast signaling (unknown), followed by inducing the expression of genes associated with heat tolerance such as HSFs and HSPs and finally increasing the heat tolerance of plants (Dickinson et al. 2018). During the night, no light-dependent chloroplast-to-nucleus signaling is generated, HSF and HSP expressions are low, and the plants show a low survival rate under heat stress (Dickinson et al. 2018). Further studies showed that phytohormone phytochrome B (phy B) not only was a photoreceptor but also a thermal sensor (Song et al. 2017). Phy B acted as a molecular switch to turn on or turn off the expression of genes associated with heat tolerance such as HSFs and HSPs, thus improving the heat tolerance under light conditions, indicating the crosstalk between light signaling and temperature signaling in the acquirement of thermotolerance in plants (Song et al. 2017).

Recently, numerous studies have found that epigenetic regulation takes part in the perception of high temperature in plants (Ohama et al. 2017; He and Li 2018; Vu et al.2019). High-temperature sensing followed by triggering signaling induces the transcriptional cascades, which in turn form transcriptional and post-translational regulatory network transcription factors (TFs). Among TFs, HEAT SHOCK

TRANSCRIPTION FACTOR A1s (HsfA1s) is the key transcriptional regulator of the heat stress response (Liu et al. 2013; Ohama et al. 2017). In addition, acetylation, methylation, phosphorylation, and ubiquitination can occur on nucleosomal histones (He and Li 2018). Among these modifications, the acetylation of histone and trimethylation of histone H3 lysine-4 (H3K4me3) are closely associated with the expression of active genes, while histone deacetylation and histone H3 lysine-27 trimethylation (H3K27me3) commonly result in gene repression. This modification process is minutely regulated by histone acetyltransferases and deacetylases (He and Li 2018). Also, DNA methylation (including RNA-directed DNA methylation) at various defense-associated genes participates in the perception and memory of heat stress in plants (Ohama et al. 2017;He and Li 2018). In addition, non-coding RNAs (ncRNAs), such as small interfering RNAs (siRNAs), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), have been found to take part in the sensing of heat stress by the transcriptional and post-transcriptional modifications, which in turn regulate gene expression (Zhao et al. 2016).

The perception of heat stress by the above cellular structures (plasma membrane, chloroplast, and mitochondria) and/or molecules (NOX, NOS, phy B, HsfA2s, and DNA) triggers the following signaling cascades (Ca^{2+}, H_2O_2, NO, H_2S, and MG), followed by inducing the formation of thermotolerance in plants.

3.3.2 Ca^{2+}Signaling

Calcium in the form of ion (Ca^{2+}) plays a very important structural, metabolite, and signaling role in seed germination, seedling growth, development, and adaptation and tolerance to abiotic and biotic stresses including heat stress (Dodd et al. 2010; Campbell 2018; Costa et al. 2018; Konrad et al. 2018). In plant cells, Ca^{2+} is mainly stored in intracellular and extracellular Ca^{2+} pools. The former includes vacuole, endoplasmic reticulum, mitochondrion, chloroplast, and Golgi apparatus; the latter refers to the cell wall. In general, the concentrations of Ca^{2+}(~ 1~10 mM Ca^{2+}) in Ca^{2+} stores (including intracellular and extracellular Ca^{2+} pools) are 1000~10,000 times than that of cytosol (~100 nM Ca^{2+}), producing a huge Ca^{2+} gradient (Dodd et al. 2010; Campbell 2018; Costa et al. 2018; Konrad et al. 2018). This gradient lays the foundation of triggering Ca^{2+} signaling in plants. Generally, Ca^{2+} concentration in the cytosol can maintain homeostasis; when the Ca^{2+} channels are opened by environmental stress (such as heat shock) and/or chemical stimuli (such as abscisic acid), Ca^{2+} swarms into cytosol at the velocity of 10 μM/channel/s and generates Ca^{2+} signatures with different intensity, duration, and amplitude by the coordination of Ca^{2+} channels with Ca^{2+} pumps and Ca^{2+}/H^+ transporters. The process of the formation of Ca^{2+} signatures is known as Ca^{2+} coding (Dodd et al. 2010; Campbell 2018; Costa et al. 2018; Konrad et al. 2018; Sajid et al. 2018).

And then, different Ca^{2+} signatures are decoded by Ca^{2+} sensors or receptors, such as calmodulin (CaM), calmodulin-like proteins (CMLs), calcium-dependent protein kinases (CDPKs), calcineurin B-like proteins (CBLs), and CBL-interacting protein kinases (CIPKs). Afterward, Ca^{2+} signaling is sequentially transmitted and further triggers the formation of thermotolerance of plants (Dodd et al. 2010; Campbell 2018; Costa et al. 2018; Konrad et al. 2018). In tobacco seedlings, heat shock could trigger Ca^{2+} signaling, followed by improving the heat tolerance of seedlings (Gong et al. 1998a). The effect of heat shock was reinforced by exogenous Ca^{2+}, while it was abolished by ethylene glycol-bis (b-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA, Ca^{2+} chelator), $LaCl_3$ (plasma membrane Ca^{2+} channel blocker), ruthenium red (intracellular Ca^{2+} channel blocker), and neomycin (phospholipase C inhibitor) (Gong et al. 1998a).

3.3.3 H_2O_2Signaling

Analogous to other signaling molecules (Ca^{2+}, NO, H_2S, and MG), H_2O_2 has dual function, namely, cytotoxic agent at high concentrations and messenger molecule at low concentrations (Gill and Tuteja 2010; Sewelam et al. 2016; Kolupaev et al. 2017; Mhamdi and Breusegem 2018; Mohanta et al. 2018). As mentioned above, H_2O_2 derived from superoxide anion free radical (O_2^-) can be rapidly generated when the electron transfer in chloroplasts and mitochondria is disturbed by heat stress. In addition, during the process of the photorespiration, peroxisomes can inevitably form H_2O_2 by the degradation of glycolate. Similarly, NADPH oxidase (NOX)-bound plasma membrane, also known as respiratory burst oxidase homolog (RBOH), cell wall pH-dependent peroxidase (CWPOD), and xanthine oxidase (XOX) also can actively synthesize H_2O_2, which serves as the response signaling to heat stress (Gill and Tuteja 2010; Sewelam et al. 2016; Kolupaev et al. 2017; Mhamdi and Breusegem 2018; Mohanta et al. 2018).

H_2O_2 can activate Ca^{2+} channels located in the plasma membrane, converting H_2O_2 signaling into Ca^{2+} signaling, or react with a sulfhydryl group to lead to the sulfhydration (sulfenic acid, sulfinic acid, and sulfonic acid) of proteins in a dose-dependent manner, which in turn regulates the activation of proteins (e.g., sensor, receptors, transcription factors), followed by modifying redox homeostasis in plant cells (Pei et al. 2000; Gill and Tuteja 2010; Sewelam et al. 2016; Kolupaev et al. 2017; Mhamdi and Breusegem 2018; Mohanta et al. 2018). Thus, the crosstalk of H_2O_2 with other signaling molecules further induces the acquisition of plant adaptation and tolerance by the transcriptional regulatory network and physiological and biochemical changes. In maize seedlings, heat shock triggered an increase in endogenous $H_2O_{2,}$ followed by inducing cross-stress resistance to extreme temperature (heat and chilling), drought, and salt stress (Gong et al. 2001). In the formation of cross-stress adaptation induced by heat shock, the effect of heat shock could be replaced by exogenous H_2O_2 treatment (Gong et al. 2001), indicating that H_2O_2 plays a signaling role in the formation of cross-stress tolerance in plants.

3.3.4 NO Signaling

As a signaling molecule like NO, it should be rapidly synthesized when it is needed, but abolished when it is not. In higher plant cells, numerous pathways take part in the synthesis of NO, including reductive and oxidative routes (Domingos et al. 2015; Santolini et al. 2017;Sami et al. 2018; Wany et al. 2018). In reductive pathways, nitrite (NO_2^-) can be transferred to NO via nitrate reductase (NR), deoxygenated heme proteins (located in plasma membrane, peroxisomes, cytosol, and endoplasmic reticulum), electron transport chain in mitochondria, and acidic conditions. In the oxidative pathways, NO can be produced by NO synthase (NOS) that oxidizes L-arginine, by the release from *S*-nitrosoglutathione (GSNO) via the catalysis of GSNO reductase (GSNOR), and by the metabolism of polyamines and hydroxylamines (unknown mechanisms) (Domingos et al. 2015; Santolini et al. 2017; Sami et al. 2018; Wany et al. 2018). Among the synthetic pathways of NO, NR and NOS are considered as key enzymes in higher plants (Domingos et al. 2015; Santolini et al. 2017; Sami et al. 2018; Wany et al. 2018).

NO from different pathways regulates stem cell homeostasis, breaking of dormancy, seedling growth, development, sexual reproduction, and adaptation and tolerance to abiotic and biotic stress including heat stress by the post-translational regulation (*S*-nitrosylation, tyrosine nitration, and *S*-glutathionylation) and interplay with other messenger molecules (including plant hormones) (Domingos et al. 2015; Santolini et al. 2017; Sami et al. 2018; Wany et al. 2018). In alfalfa and *Symbiodinium microadriaticum*, heat stress triggered NO signaling (Leshem 2000; Bouchard and Yamasaki2008, 2009), while NO donors (sodium nitroprusside and S-nitroso-N-acetylpenicillamine) remitted the growth suppression and the decline in cell viability under heat stress by alleviating ion leakage, H_2O_2, and malondialdehyde (MDA) accumulation (Song et al. 2017). In maize seedlings, sodium nitroprusside treatment enhanced the survival rate of seedlings under heat stress by interacting with messenger molecule H_2S (Li et al. 2013). In addition, under high temperature, the seeds of mutant-type *Arabidopsis* (ΔAtGLB3: a homologue of bacterial truncated hemoglobin) did not germinate, while wild-type seeds did and maintained a high germination rate, suggesting the signaling role of NO in the response and adaptation of plants to high temperature (Hossain et al. 2010).

3.3.5 H_2S Signaling

H_2S, NO, carbon monoxide (CO), and ammonia (NH_3) are collectively known as gasotransmitters, which govern seed germination, cellular anabolism and catabolism, seedling growth and development, as well as plant response and adaptation to environmental stresses (Calderwood and Kopriva 2014; Hancock and Whiteman2014; Aroca et al. 2018; Banerjee et al. 2018; Hancock 2018). In general, H_2S can be actively synthesized and maintain homeostasis as a signaling molecule in plant cells by numerous biosynthetic pathways. In higher plants, H_2S is mainly synthesized by enzymatic pathways, which include L/D-cysteine desulfhydrase (LCD/DCD), sulfite reductase (SiR), cyanoalanine synthase (CAS), and cysteine synthase (CS) (Li 2013;Li et al. 2016).

Under environmental stress including heat stress, LCD plays a key role in the generation of H_2S, followed by triggering signaling cascades, gene expression, and physiological and biochemical networks related to adaptation and tolerance to heat stress by post-transcriptional modifications (persulfidation, also known sulfhydration) of thiol group in proteins (Calderwood and Kopriva 2014; Hancock and Whiteman 2014; Aroca et al. 2018; Banerjee et al. 2018; Hancock 2018). In tobacco seedlings, heat stress activated LCD activity, followed by accumulating endogenous H_2S and maintaining its high level under heat stress (Chen et al. 2016). In addition to these, heat stress led to a significant increase in endogenous H_2S content in strawberry seedlings compared with control plants without heat stress treatment (Christou et al. 2014). Also, an increase in H_2S was noted in plants pretreated with NaHS (Christou et al. 2014; Zhou et al. 2018), followed by improving the adaptation and tolerance to heat stress (Li 2013; Li et al. 2015, 2016; Kolupaev et al. 2017).

3.3.6 MG Signaling

Similar to Ca^{2+}, H_2O_2, NO, and H_2S signaling, MG is now emerging to have a messenger molecule function except for its toxicity, which is involved in cellular anabolism and catabolism, seedling growth and development, plant reproduction, and adaptation and tolerance to adverse environments (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). Usually, plant cells can produce MG by enzymatic and non-enzymatic pathways. The former refers to the catalysis of aminoacetone, acetol, and DHAP by monoamine oxidase (MAO), cytochrome P450 (CP450) oxidase, and MG synthase, respectively, to produce MG; the latter includes the auto-oxidation of glucose, ketone bodies, and lipids, as well as the removal of phosphate groups of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP) by β-elimination, to produce MG (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018).

In general, in plant cells, MG can maintain homeostasis by the coordination of glyoxalase system (including Gly I, Gly II, and Gly III) and non-glyoxalase system (NADPH-dependent MG reductase (MGR), aldehyde dehydrogenase (ADH), aldo-keto reductase (AKR), and MG dehydrogenase (MGDH)) (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). Under environmental stress conditions, MG signaling can be rapidly triggered and form downstream signaling cascades, modifying transcriptional regulatory network and metabolic process, thus producing adaptation and tolerance to abiotic and biotic stress including heat stress (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). In addition, exogenously applied MG to maize seedlings stimulated the LCD activity, followed by inducing the accumulation of endogenous H_2S and then increasing the survival rate of maize seedlings under heat stress, suggesting that MG could improve the thermotolerance of plants (Li et al. 2018a, b). Parallelly, exogenous H_2S treatment also enhanced the content of endogenous MG in maize seedlings and the acquirement of thermotolerance, while the H_2S-induced thermotolerance was reduced by aminoguanidine (AG) and *N*-acetyl-cysteine (NAC; both are MG scavengers), further indicating the crosstalk between MG and H_2S signaling in the development of thermotolerance in plants (Li et al. 2018a, b).

3.3.7 Plant Hormone

To survive under environmental stress, plants need to redistribute and assimilate energy to modify plant growth, reproduction, and adaptation to stress. Plant growth, reproduction, and adaptation to stress commonly are regulated by the combined action of plant growth-promoting phytohormones (e.g., auxin (AUX), gibberellin (GA), and cytokinin (CTK)) and plant growth-retarding phytohormones (such as abscisic acid (ABA) and ethylene (ETH)) (Kumar 2016; Arnao and Hernández-Ruiz2018; Ciura and Kruk 2018; Per et al. 2018; Sami et al. 2018; Verma et al. 2016). In addition, besides the traditional five plant hormones (AUX, GA, CTK, ABA, and ETH), some newly emerging plant hormones (e.g., salicylic acid (SA), jasmonate (JA), brassinosteroid (BR), and melatonin (MEL)) also take part in the whole life cycle, such as cellular anabolism and catabolism, seedling growth and development, and plant reproduction, response, and adaptation to abiotic and biotic stresses including heat stress (Kumar 2016; Arnao and Hernández-Ruiz 2018; Ciura and Kruk 2018; Per et al. 2018; Sami et al. 2018; Verma et al. 2016). During the acquisition of heat tolerance, a complex signaling network is formed among plant hormones and other messenger molecules like Ca^{2+}, H_2O_2, NO, H_2S, and MG, further regulating gene expression, cellular metabolism, physio-biochemical processes, and morphogenesis (Arnao and Hernández-Ruiz2018; Ciura and Kruk 2018; Per et al. 2018; Sami et al. 2018; Verma et al. 2016).

Pretreatment with ABA, a stress hormone, significantly improved the thermotolerance of maize seedlings; this improvement was reinforced by exogenous Ca^{2+}, whereas it was impaired by EGTA (Ca^{2+}chelator), La^{3+} and verapamil (plasma membrane Ca^{2+}channel blockers), and calmodulin antagonists (W7 and chlorpromazine (CPZ)), indicating that Ca^{2+}signaling mediated ABA-induced heat tolerance (Gong et al. 1998b). Similarly, treatment with salicylic acid (SA), 1-aminocyclopro pane-1-carboxylic acid (ETH precursor), and ABA also could increase the tolerance of *Arabidopsis* seedlings to high temperature by alleviating oxidative damage (Larkindale and Knight2002). On the contrary, the *Arabidopsis* mutants etr-1 (ethylene-insensitive) and abi-1 (ABA-insensitive) and a transgenic line (expressing *nahG* to inhibit SA synthesis) showed more sensitivity to heat stress (Larkindale and Knight 2002). In addition, other plant hormones like AUX, GA, CTK, JA, and BR have shown to play critical roles in adaptation and tolerance of plants to heat stress (Kumar 2016; Sharma and Laxmi 2016; Ahammed et al. 2016; Ku et al. 2018;

Sadura and Janeczko 2018). These data suggest that all of the plant hormones are involved in the acquirement of thermotolerance in plants.

3.4 Plant Adaptation and Tolerance to Heat Stress

Plants are more sensitive to temperature change than humans; they can detect temperature change as small as 1 °C and activate adaptation and tolerance mechanisms (Saidi et al. 2011;Sajid et al. 2018). As mentioned above, plant heat adaptation and tolerance is a complex chemical and biological process, implicating alternations in signaling, molecular, metabolic, and physiological and biochemical networks. In other words, plant heat adaptation and tolerance is involved in the synthesis of heat shock proteins (HSPs), the stimulation of antioxidant system and MG detoxification system, the biosynthesis of osmolytes, and the change in composition and saturation of membrane lipids (Fig. 3.3), which are discussed as follows:

3.4.1 HSPs

As mentioned above, heat stress commonly results in the loss of enzyme activation, the denaturation of proteins, and even protein aggregation (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Anaraki et al. 2018; Li et al. 2018a, b; Mishra et al. 2018). Thus, the acquisition of heat tolerance needs to alleviate these detrimental effects. The adverse effects can be remitted by molecular chaperones, mainly heat shock proteins (HSPs). According to the size of molecular mass, HSPs are classified into small HSPs (sHSPs), HSP60, HSP70, HSP90, and HSP100 (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Li et al. 2018a, b; Mishra et al. 2018).

Ubiquitin, an sHSP with 8.5 KD molecular mass, can help degrade unsuitable proteins; it has bound enzymes. In addition, the activity of sHSPs is independent from ATP, which distinguishes them from other HSPs (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Li et al. 2018a, b; Mishra et al. 2018). Also, sHSPs are regulated by the plant growth substances (including plant hormones and plant growth regulators) and ROS in plant thermotolerance. Traditionally, it is considered that the sHSPs only can hold back irreversible unfolding and/or aggregation by binding to folded or denatured proteins, Interestingly, recent reports found that sHSPs, such as sHSP18.1 and sHSP16.6, also can bind to denatured proteins and help further refolding by HSP70/HSP100 complexes accompanied by ATP (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Li et al. 2018a, b; Mishra et al. 2018).

HSP60 is also known as chaperonin, which assists in protein folding, such as plastid protein Rubisco. In addition, HSP60 takes part in the folding and aggregating of proteins that are transported to chloroplasts and mitochondria (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Li et al. 2018a, b; Mishra et al. 2018). Also HSP60 can bind different types of proteins before folding to inhibit their aggregation. In plants, HSP70 can prevent the aggregation and folding of newly synthesized proteins during their transfer to their final location, which is a faithful chaperone. In addition, HSP70 and sHSPs (such as sHSP17.6 and sHSP18.1) as molecular chaperones, or a part of guidance complex import, participate in the acquirement of thermotolerance and the development of cross-stress adaptation to temperature stress (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Li et al. 2018a, b; Mishra et al. 2018). Additionally, besides being a molecular chaperone, the HSP90 exhibits a critical role in carrying out messenger protein function and trafficking, innate immune response, and pathogenic resistance (Park and Seo2015). The unique function of HSP100 can react with aggregated proteins to help them redissolve and degrade irreversibly damaged polypeptides (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Li et al. 2018a, b; Mishra et al. 2018).

In *Arabidopsis*, under high-temperature conditions, the expressions of HSPs and heat-stress-associated 32-kD protein (Hsa32) were significantly upregulated, followed by increasing the tolerance to high temperature (Charng et al. 2006; Wu et al. 2013). On the contrary, compared to the wild types, the HSP mutants were susceptible to high temperature (Charng et al. 2006; Wu et al. 2013). Besides heat tolerance, HSPs also take part in the development of other forms of stress tolerance such as cold, heavy metal, drought, high light, salt, and flooding tolerance (Vierling 1991; Al-Whaibi 2011; Xu et al. 2012; Li et al. 2018a, b; Mishra et al. 2018).

3.4.2 Antioxidant System

In general, heat stress results in oxidative stress, that is, biomacromolecule (proteins, lipids, and nucleic acids) and biomembrane damage due to the overaccumulation of ROS (Foyer and Shigeoka 2011; Wahid et al. 2007). Thus, the development of thermotolerance is closely related to the stimulation of antioxidant system. Antioxidant system includes enzymatic antioxidant (including the enzymes related to ascorbic acid-glutathione (AsA-GSH) cycle) and non-enzymatic antioxidant systems. Enzymatic antioxidants are composed of ROS-scavenging enzymes (such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione-*S*-transferase (GST), peroxiredoxin (Prx), and thioredoxin (Trx)). According to their dissolution characteristics, non-enzymatic antioxidant systems are classified into water-soluble (ascorbic acid (AsA), glutathione (GSH), and polyphenol) and lipid-soluble (α-tocopherols, carotenoids, flavonoids, and retinoids) antioxidants (Foyer and Shigeoka 2011; Saeed et al. 2012).

SOD, converting O_2^- into H_2O_2, is the first line of antioxidant defense; H_2O_2 is decomposed into H_2O by CAT, APX, or GPX in different cellular compartments; GR catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH); DHAR and MDHAR reduce both DHA and MDHA to AsA, respectively. Prx and Trx regulate the activity and function of target proteins by modulating disulfide bridges in their redox-active sites. Water-soluble and lipid-soluble antioxidants can scavenge O_2^-, H_2O_2, •OH, and singlet oxygen ($^1O_2$) in different cellular compartments. Therefore, ROS can maintain homeostasis, acting as signaling function, in plant cells by orchestrating the role of enzymatic antioxidant and non-enzymatic antioxidant system (Foyer and Shigeoka2011; Saeed et al. 2012).

3.4.3 Osmolytes

Osmolytes, also known as osmotic adjustment substances, osmoprotectants, or compatible solutes, include Pro, GB (also known as N,N,N-trimethylglycine), Tre, sorbitol, soluble sugars, polyamines, gamma-aminobutyric acid (GABA), and so forth (Szabados and Savouré 2010; Slama et al. 2015; Iqbal et al. 2016; Figueroa-Soto and Valenzuela-Soto 2018; Hasanuzzaman et al. 2019). These osmolytes play a key important role in the alleviation of abiotic and biotic stress including high temperature by osmotic adjustment in plants. In addition, osmolytes take part in maintaining membrane integrity, stabilizing proteins (acting as small chaperone), alleviating toxicity, protecting cellular components, scavenging ROS, defending antioxidant compounds, and balancing cellular redox (Szabados and Savouré 2010; Slama et al. 2015; Iqbal et al. 2016; Figueroa-Soto and Valenzuela-Soto 2018; Hasanuzzaman et al. 2019).

In plants, the enzymes related to osmotic adjustment (such as Δ1-pyrroline-5-carboxylate synthase (P5CS), P5C reductase (P5CR), ornithine-δ-aminotransferase (OA), choline monooxygenase (CMO), NAD$^+$-dependent betaine aldehyde dehydrogenase (BADH), trehalose-6-phosphate synthase (TPS), trehalose-6-phosphatase (TP), and glutamate decarboxylase (GAD)) are the key enzymes in the generation of Pro, GB, Tre, and GABA; their activities and gene expression are commonly upregulated under abiotic and biotic stress including heat stress (Szabados and Savouré2010;Slama et al. 2015; Iqbal et al. 2016; Figueroa-Soto and Valenzuela-Soto2018; Hasanuzzaman et al. 2019).

3.4.4 MG Detoxification System

As mentioned above, MG, an analogue to other signaling molecules, has dual role (signaling molecule at low concentration and toxic agent at high dose) in seed germination, seedling growth and development, and plant reproduction, response, and adaptation to environmental stress (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). Thus, the homeostasis of MG must be maintained in plant cells, and this homeostasis is closely linked to the development of environment stress tolerance including thermotolerance (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). MG homeostasis is commonly regulated by MG detoxification system, which incorporates glyoxalase system and non-glyoxalase system. The glyoxalase system refers to Gly I, Gly II, and Gly III; the non-glyoxalase system covers MGDH, MDR, ADH, ALR, and AKR (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). Many studies reported that the amount of MG eliminated by non-glyoxalase system is less than 1%. Therefore, glyoxalase system plays a critical role in the balance of MG in plant cells, which is the first line of defense against MG stress (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018).

During the process of MG detoxification, Gly I and Gly II scavenge excessive MG accompanied by GSH (as cofactor) by two-step reactions, that is, MG can rapidly react with GSH and produce intermediate product hemithioacetal (HTA), and then Gly I catalyzes the conversion of HTA to S-D-lactoylglutathione (SLG). Afterward, Gly II converts the reaction so that SLG transforms into D-lactate and recovers GSH, followed by maintaining redox homeostasis and MG balance in plant cells (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). In addition, Gly III can directly catalyze the transformation of MG into D-lactate in one step without GSH. D-Lactate enters into tricarboxylic acid cycle, followed by regulating the balance of energy and redox by producing NADH/$FADH_2$ (Kaur et al. 2014, 2016; Hoque et al. 2016; Li 2016; Mostofa et al. 2018). Our previous results have shown that MG pretreatment could activate the action of a rate-limiting enzyme (LCD) in H_2S biosynthesis in maize seedlings followed by accumulating endogenous H_2S and then by improving the heat tolerance of maize seedlings; this improvement was reinforced by exogenous H_2S, while it was reduced by H_2S scavenger hypotaurine and inhibitor DL-propargylglycine (Li et al. 2018a, b). In addition, H_2S-induced heat tolerance in maize seedlings also was reduced by MG scavengers aminoguanidine and *N*-acetyl-*L*-cysteine, respectively (Li et al. 2018a, b). The data imply that the formation of thermotolerance in plants is implicated in the interaction of MG and H_2S. Further experiments found that the MG and H_2S interaction-induced thermotolerance in maize seedlings was achieved by the combined action of glyoxalase system and antioxidant system (data not shown).

3.4.5 Alternation in Composition and Saturation of Membrane Lipids

According to the fluid mosaic model, under normal growth conditions, the biological membrane maintains a liquid crystal state with a given fluidity and rigidity, which has been achieved by multiple functions of plant cells (Wahid et al. 2007; Ruelland et al. 2009; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Barrero-Sicilia et al. 2017; Niu and Xiang 2018; Kollist et al. 2019; Margutti et al. 2019). Thus, the liquid crystal state of the biological membrane plays a very important role in cellular anabolism and catabolism, seedling growth and development, and plant response and adaptation to adverse environments (Wahid et al. 2007; Ruelland et al. 2009; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Barrero-Sicilia et al. 2017; Niu and Xiang 2018; Kollist et al. 2019; Margutti et al. 2019). The fluidity of the biological membrane is affected by a lot of factors, including internal (biological composition and characteristics, such as saturation) and external (environments, such as temperature) factors. In general, heat stress increases the fluidity of the biological membrane and even leads to the liquefying of the biological membrane, while a low temperature reduces fluidity and results in solidifying. These cause the loss of biological membrane integrity and cellular compartmentalization and even plant death (Wahid et al. 2007; Ruelland et al. 2009; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Barrero-Sicilia et al. 2017; Niu and Xiang 2018; Kollist et al. 2019; Margutti et al. 2019).

Plants must remain the integrity of the biological membrane by altering the composition and saturation of membrane lipids. Phospholipids with shorter chains have higher fluidity due to them being less stiff and less viscous, which maintain the membrane's liquid crystal state and facilitate plants to adapt to low temperature (Wahid et al. 2007; Ruelland et al. 2009; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Barrero-Sicilia et al. 2017; Niu and Xiang 2018; Kollist et al. 2019; Margutti et al. 2019). Adversely, sphingomyelin can improve the melting points of cell membrane, followed by decline in the fluidity of cell membrane, which is in favor of the acquirement of thermotolerance in plants (Wahid et al. 2007; Ruelland et al. 2009; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Barrero-Sicilia et al. 2017; Niu and Xiang 2018; Kollist et al. 2019; Margutti et al. 2019). In addition, under heat stress, cholesterol can increase the melting points of cell membrane and maintain the liquid crystal state, which in turn enhance the tolerance of plants to heat stress. On the contrary, under cold stress, cholesterol also can prevent membrane lipids from clustering and stiffening, followed by increase in the resistance of plants to low temperature (Wahid et al. 2007; Ruelland et al. 2009; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Barrero-Sicilia et al. 2017; Niu and Xiang 2018; Kollist et al. 2019; Margutti et al. 2019). More importantly, the saturation of membrane lipids is a key factor that affects membrane melting points. The saturated phospholipids increase the melting points of cell membranes, which in turn improves the heat tolerance of plants. In addition, the unsaturated phospholipids reduce the melting points of biological membranes, followed by increase in the cold tolerance in plants. Therefore, the alternation in composition of cell membranes (an increase in long-chain lipids and cholesterols, as well as a decrease in sphingomyelin) and an increase in the saturation of biological membranes induced by heat shock or chemical treatments are closely associated with the development of heat tolerance in plants (Wahid et al. 2007; Ruelland et al. 2009; Mittler et al. 2012; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Barrero-Sicilia et al. 2017; Niu and Xiang 2018; Kollist et al. 2019; Margutti et al. 2019).

3.5 Conclusion and Perspective

Heat injury and heat adaptation is a sophisticated chemical and biological process, involving all the aspects of morphology, anatomy, physiology, biochemistry, and molecules. Heat injury is usually manifested in the destruction of biological membrane integrity and cellular compartmentalization, the denaturation of proteins and enzymes, the damage of biomacromolecules (e.g., proteins, DNA, RNA, polysaccharides, and membrane lipids) induced by oxidative stress and carbonyl stress (such as MG stress), a reduction in osmotic and water potential of the cells triggered by osmotic stress, and so forth (Wahid et al. 2007; Mittler et al. 2012; Bita and Gerats 2013; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019). Accordingly, plant perception, response, and adaptation to heat stress also have exhibited an elaborate and complex mechanism that is being uncovered. The mechanism includes the perception, triggering, and signaling transduction of heat stress; the restoring and rebuilding of biomembrane and cellular compartmentalization by altering the composition and saturation of membrane lipids, as well as the ratio of proteins to lipids; the gene expression and the biosynthesis of HSFs and HSPs; the activation of antioxidant system composed of enzyme and non-enzyme system, as well as MG-scavenging system consisting of glyoxalase system (Gly I, Gly II, and Gly III) and non-glyoxalase system (MGDH, MGR, ADH, and AKR); the biosynthesis of osmolytes (Pro, GB, Tre, PA, total soluble sugar); and so on (Wahid et al. 2007; Mittler et al. 2012; Bita and Gerats 2013; Hasanuzzaman et al. 2013; Asthir 2015; Hemmati et al. 2015; Kollist et al. 2019).

Although heat injury and heat adaptation in plants has largely developed in recent years, the exact mechanism is still waiting to be expounded. In view of the rapid development of omics (such as genomics, transcriptomics, proteomics, and metabolomics) and genome editing technology (especially clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9)) (Abdelrahman et al. 2018), a new signaling member is to be found and the signaling network in the acquirement of thermotolerance will be updated; the detailed molecular mechanism of heat injury and heat adaptation will be illustrated; genetically modified plants with heat tolerance and even multiple stress resistance are about to be developed and utilized in the future.

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Chapter 4
Molecular Mechanism of Plant Adaptation and Tolerance to Cold Stress

Dipinte Gupta, Anamika Gupta, Kritika Yadav, and Rajiv Ranjan

Contents

Abstract The alarming increase in the rate of population is one of the foremost concerns of the agriculture sector. In particular, cold stress often limits the productivity of economically important crop species largely due to acute dehydration in plants. To cope with these stress conditions, plants respond in a variety of levels by modulating physiological and molecular changes in their system. Characteristic

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features of changes occur during cold stress in plants comprising increasing concentration of oxygen radicals, osmotic regulation, and protein kinase and lipid signaling cascade. Each stress response requires a specific combination of these single reactions regulated by transcription factor and cold-responsive genes. By increasing the activity of such regulatory genes, the plant can be enabled to tolerate cold stress. Various scientific reports suggest the combinatorial regulation of these genes along with transcription to engineer efficient cold stress tolerance in plants. This chapter focuses on the basic molecular and genetic approaches used for generating cold stress tolerance in plants.

Keywords Abiotic stress · Chilling injury · Plant adaptation · Cell signaling · Molecular approach

4.1 Introduction

Cold stress is one of the major temperature-dependent abiotic stress factors which cause detrimental effect on the growth and yield of crop plants. Due to these stress factors, the agricultural yield of plants decreases to up to 50% (Vij and Tyagi 2007). Owing to their consequences, plants have developed the ability to adapt to these unfavorable environmental conditions. They respond to different stress factors, through their physiological, biological, cellular, and molecular signaling pathways (Nakashima et al. 2009), which result in changes in the plant growth and developmental conditions, physical and chemical concentrations of the cell membrane, enzyme conformation and affinity, and photosynthesis conditions; affect intracellular water temperature, nucleic acid and protein synthesis, and water and nutrient balance; and lead to electrolyte leakage and impairment of photosynthesis (Guy 1990; Tuteja et al. 2011; Sicilia et al. 2017).

Chilling injury and freezing injury are the terms often used for describing cold injury; however, these similar-sounding terms are distinct in the sense; thus they can be classified by two conditions, i.e., temperature below freezing (freezing temperature <0 °C) and temperature above freezing (chilling/non-freezing temperature 0–15 °C). Similarly, on the basis of this classification, plants adapt themselves in two different manners; firstly, stress from low temperature causes membrane viscosity, retarded metabolism, and delayed energy dissipation, leading to radical formation and oxidative stress in plants. Second, stress by freezing of water causes dehydration in plants by protoplast volume shrinkage, development of negative turgor pressure, concentration of cellular solutes, abolition of metabolic processes, change in membrane potential, and disintegration of the membrane bilayer.

Each plant has its own optimum temperature ranges for its proper growth and development. Plants can be divided into two types on the basis of their survival in cold stress, i.e., tolerant and susceptible. Plants belonging to the tropical and subtropical region are susceptible to low temperature. In contrast, plants belonging to the temperate region are cold stress-tolerant plants because they have the ability to survive at very low temperature by the process of cold acclimatization. The process by which the plants can adjust their metabolism to cold stress and increase their tolerance in response to low non-freezing temperature is known as cold acclimatization. Due to cold acclimatization, many physiological, biological, and cellular changes occur, viz., reprogramming of gene expression, modification in the metabolism (Chinnusamy et al. 2010; Rihan et al. 2017), increased transcription, and increased stability (Xin and Browse 2000). Acclimatization also helps in membrane modification, increasing the production of abscisic acid, antioxidants, and osmolytes (sugars and proline). The economically important crop plants like rice, maize, tomato, soybean, and cotton and fruit crops like banana, papaya, mango, grapes, and orange are chilling sensitive. Chilling-sensitive plants have a higher transition temperature while chilling-resistant plants have a lower transition temperature (Tuteja et al. 2011). The temperature at which the semi-fluid state of the membrane changes into a semicrystalline state is known as transition temperature. Chilling stress causes various phenotypic symptoms such as reduction in leaf expansion, wilting, chlorosis, necrosis, etc. Freezing stress also induces membrane damage due to acute dehydration associated with freezing (Steponkus 1984; Guy 1990).

A wide range of cold stresses are found in temperate areas which differ in the timing and intensity of low temperature. Plant productivity is more affected by cold stress when it occurs during reproductive stage (Sanghera et al. 2011). The ability of plants to survive better under low temperature is known as cold tolerance or cold hardening. By developing the tolerance against cold stress, plants can increase chlorophyll accumulation and achieve reduced sensitivity to photosynthesis, membrane stabilization, protein and enzyme protection, optimized sugar metabolism, and re-established homeostasis (Thakur and Nayyar 2013).

The world population is continuously increasing, and the food production is decreasing due to the effect of various stresses. The best way to increase the crop yield in a limited land area is by preventing the loss in crop yield due to adverse environmental conditions, which necessitates the development of stress-tolerant superior crop varieties which may be assisted by combinatorial use of plant breeding, genetic engineering, QTL mapping, and omics approaches. Here we intend to present a general overview of various approaches established for cold acclimatization in plants.

4.2 Cell Signaling During Cold Acclimatization in Plants

To understand how plants avoid the damages caused by low temperature, it is mandatory to understand how plants get sensitized to cold. Decrement in temperature generates a series of regulatory molecules to activate a defensive mechanism in plants which includes production of osmotic factors and modification of transcriptional pathways. Signaling transduction pathways (Fig. 4.1) start from sensing of

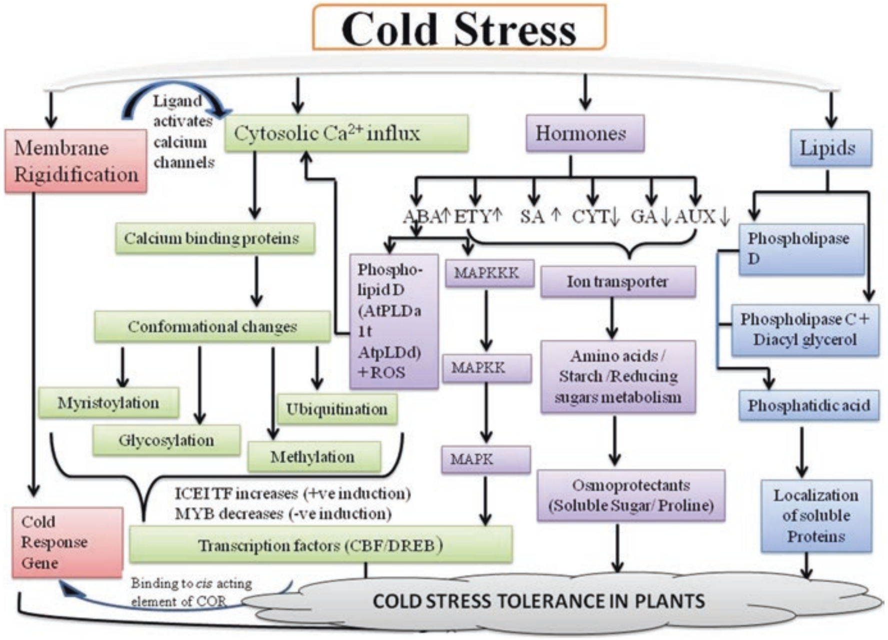

Fig. 4.1 Systematic representation of signaling cascade during cold stress. *ABA* abscisic acid, *ETY* ethylene, *SA* salicylic acid, *CYT* cytokinin, *GA* gibberellins, *AUX* auxin, *ICEI inducer of CBF expression 1, CBF* C-repeat binding factor, *DREB* dehydration responsive element-binding protein

cold by receptors such as receptor-like kinase (RLK) and G protein-coupled receptor (GPCR) and generation of messenger molecules such as reactive oxygen species (ROS), inositol phosphates, etc. These messengers instigate the influx of calcium ions inside the cell. ROS and ABA lead to phosphorylation of proteins. Calcium influx is also responsible for the phosphorylation of proteins which further targets proteins or transcription factors which are engaged in generating cold-responsive genes. A series of regulatory molecules involved in sensing events are discussed as follows:

4.2.1 Calcium Ion Channels

On exposure to cold treatment, the cytosolic concentration of Ca^{2+} increases. Cold stress sensing in plants leads to loss of cell membrane fluidity and cytoskeletal reorganization causing rigidification which is followed by influx of cytosolic Ca^{2+} that causes regulation of COR genes (Guo et al. 2018). The Ca^{2+} signatures are sensed by Ca^{2+} sensors like CaM-binding transcription activators (CAMTAs), CaM (calmodulin), and Ca^{2+}dependent protein kinases (CDPKs). So it is possible that

Ca^{2+} channels and associated electrophysiological changes are responsible for the relaying of low-temperature sensitization of plants (Joshi et al. 2018). Diacyl glycerol kinase (DAGK) activity is also a primary event on exposure to cold stress, also associated with the rigidification of membrane. Diacyl glycerol acts as a messenger for the calcium ion influx (Ma et al. 2015). Studies have shown high expression of DAGK in *Arabidopsis* under cold stress (Joshi et al. 2018). Researchers had found the involvement of rice trans-membrane proteins, COLD1 (CHILLING TOLERANCE DIVERGENCE 1) and RGA1 (RICE G PROTEIN SUBUNIT1), in cold signaling which primarily occurs through fluctuating calcium ion concentration and related physiological changes in plants. COLD1 and RGA1 are localized in the plasma membrane and endoplasmic reticulum (Ma et al. 2015). This complex of COLD1-RGA1 causes the influx of Ca^{2+} which further leads to the activation of *COR* gene. Researchers are trying to find out whether COLD1 acts as a permeable channel for Ca^{2+} or as a facilitator of Ca^{2+}-permeable channel activity (Ma et al. 2015).

Calcium influx also causes the activation of phospholipase C (PLC) and phospholipase D (PLD) which leads to the production of IP_3 and phosphatidic acid. IP_3 increases Ca^{2+} signatures by stimulating IP3-gated calcium channels. It was reported that cytosolic influx of Ca^{2+} ion during stress is required for *COR6* and *KIN1* (Knotted1), cold-responsive genes in *Arabidopsis* (Knight et al. 1996; Monroy et al. 1993; Monroy and Dhindsa1995). Catala had reported higher transcripts of COR gene and C-repeat binding factor (*CBF*)/dehydration-responsive element-binding (*DREB*) proteins in calcium exchanger 1 (*cax1*) mutant plants which signifies that cystolic Ca^{2+} acts as a signature molecule of cell signaling for inducing *CBF* transcription factors and *COR* (cold-responsive) genes during cold stress conditions (Catala 2003).

4.2.2 Reactive Oxygen Species as Signaling Component

Reactive oxygen species (ROS) also plays an important role in signal transduction to regulate COR genes. Accumulation of ROS like superoxide, hydrogen peroxide, and hydrogen radicals takes place under chilling stress. Reactive oxygen species (ROS) induces oxygen scavengers in plants which leads to cold stress in plants. *Arabidopsis Frostbite1* (*fro1*) mutant of complex I of the electron transfer chain showed constitutively higher accumulation of ROS (Joshi et al. 2018). Accumulation of high ROS results in low expression of *COR* gene and hypersensitivity toward chilling stress. SnRK2 and MAPK families of protein kinases are also involved in regulation of C-repeat binding factor (CBF)-dependent or CBF-independent cascade of transcriptional regulation involved in cold acclimatization and freezing tolerance (Chinnusamy et al. 2007; Miura and Furumoto 2013; Shi et al. 2015; Zhao et al. 2015).

4.2.3 Mitogen-Activated Protein Kinase (MAPK) Signaling

In MAPK signaling, post-translational phosphorylation of substrates generates a cascade which acts on downstream sensor and receptor molecules and converts signals into cellular responses (Liu and Zhou 2018). Typically, three protein kinases MEKK (or MAPKKK), MKK, and MAPK form a series of cascades such as MEKK-MKKMAPK (Li et al. 2017; Zhao et al. 2017). In *Arabidopsis*, phosphorylation of ICE1 occurs through MPK3 and MPK6 to constrain CBF expression and freezing tolerance, whereas another series of cascades is found to promote freezing tolerance by antagonizing the MPK3/MPK6 pathway (Zhao et al. 2017).

4.2.4 Lipid Molecule Signaling

In response to cold stress, phosphatidic acid is generated rapidly and functions as a secondary messenger (Meijer and Munnik 2003). In normal condition, this phosphatidic acid constitutes a small portion of membrane lipids (Munnik 2001). The function of phosphatidic acid is the selection and regulation of enzymatic activities of targeted proteins.

4.3 Physiological Changes Adapted in Plants for Defense Against Cold Stress

Physiological and cellular metabolism of plant system is affected by low temperature and it depends on the intensity and time or duration of exposure. Plants such as potato, tobacco, and maize have limited capability to survive at below freezing temperature; on the contrary, leaves of cabbage, lettuce, and spinach have the capacity to develop tolerance against below freezing temperature (Wanner and Junttila 1999). Survival and geographical distribution of plants are affected by the low temperature. The pattern of gene expression and protein product changes, when it is exposed to low temperature. The reproductive stage of plants is very sensitive to low temperature. The several physiological and cellular changes which are affected by cold stress are as follows:

4.3.1 Effect on Membrane Rigidity

The cell membrane is an important part of a plant cell. The membrane is a fluid mosaic in nature and it forms a phospholipid bilayer, sandwiched between the proteins. In 1972, Singer and Nicholson proposed the model of cellular membranes

(Singer and Nicholson 1972). The movement in the plasma membrane is because of its own activation energy which is also a temperature-dependent activity. When the membrane is exposed to below optimum temperature, the process of phase transition takes place in which the transition of liquid crystalline to gel phase occurs. Due to this transition, the membrane movement gets slow and the membrane becomes more static rather than dynamic or rigid (Vigh et al. 2007). Membrane fluidity causes configurational changes in the membrane proteins which start the signaling cascade and cause the unfolding of proteins (Pastore et al. 2007).

The first event in the cold signaling is that cell membrane starts to become rigid on exposure to cold. It is considered as the first line of defense in response to cold stress (Denesik 2007). The more fluid state of the cell membrane protects the cell from the effect of low temperature. It helps to maintain the cell shape and prevent cellular component from water loss. So, the membrane rigidity can reduce the collapsing of cells during the extracellular freezing by providing the negative pressure to the cells. Several studies have revealed that *COR* expression is initiated in plants at 25 °C due to rigidity of cell membrane while it is repressed by fluidization of cell membrane at 4 °C. A group of scientists had developed a method for measuring the fluidity of membrane. Fluidity is related to the amount of desaturated fatty acids. FAD2 encodes *oleate desaturase* which is important for the maintenance of fluidity of membrane. Impairment of FAD2 leads to some physiological changes in plants like leaf number and hypocotyl length. This shows that reduced cell membrane fluidity is an important event in sensing chilling stress (Khodakovskaya et al. 2006).

4.3.2 Changes in Cytoskeleton

Multimeric polypeptides are affected by changes of optimum temperature to low temperature. The temperature drop causes the depolymerization of microtubules and actin microfilaments (Thakur and Nayyar 2013). According to Pokorna et al. (2004), the microtubules get disassembled when they are exposed to 0 °C for only 20 min. The actin cytoskeletal rearrangement is followed by activation of Ca^{2+} channels and increase in cytosolic Ca^{2+} levels, which are involved in the acclimatization process. Therefore, for protection of plants from cold stress, cytoskeleton assembly is necessary (Al-fageeh and Smales 2006).

4.3.3 Effect of Reactive Oxygen Species

In stress condition, due to oxidative burst, various reactive oxygen species (ROS) like superoxide, hydrogen peroxide, hydroxyl radicals, peroxy radicals, alkoxy radicals, and singlet oxygen are generated (Bhattacharjee 2005). They can accumulate at toxic level, which causes the cellular damage. However, these ROS also play an important role, i.e., their accumulation activated the defense-signaling pathways and they alleviate the cellular damage. Nitric oxide and hydrogen peroxide are beneficial ROS that are involved in stress response in plants (Li et al. 2018).

4.3.4 Effect on Photosynthesis

The process of photosynthesis is extensively affected by the exposure to low temperature. The interruption in plant growth due to cold stress reduces the energy utilization capacity that causes the photosynthesis feedback inhibition (Theocharis et al. 2012). In winter annuals, several enzymes of the Calvin cycle maintained the photosynthetic activity by increasing their affluence. Xanthophylls are not considered photosynthetic pigments, but they help to protect the photosystems by increasing their abundance at low temperature (Ivanov et al. 2006).

4.3.5 Low Temperature and Phytohormones

Several phytohormones play an important role for plant growth and regulation. Phytohormones, such as abscisic acid, auxin, gibberellic acid, salicylic acid, and ethylene, are related to low-temperature responses positively and negatively (Miura and Furumoto 2013). The level of abscisic acid increases when it responds to cold stress. Auxin and gibberellic acid are involved in the cell elongation in warm conditions; in contrast the cold treatment upregulates the effect of gibberellic acid in the case of *Arabidopsis* (Penfield 2008). During chilling the accumulation of salicylic acid occurs in *Arabidopsis* shoots, wheat, and grape berry (Wan et al. 2009; Kosova et al. 2012). On the other hand, cold tolerance is enhanced by the treatment of salicylic acid in rice, maize, potato, etc. (Tasgin et al. 2006; Hara et al. 2012).

4.3.6 Microbes and Cold Stress

Cold temperature also influences the microorganisms which are useful for plant growth directly and indirectly. Low temperature affects the functional diversity of agriculturally important microorganisms. These include some rhizospheric and endophytic bacteria and symbiotic fungi that play a role to provide resistance to cold stress. They develop the mechanism of triggering osmotic response and novel gene induction (Grover et al. 2011).

Cold stress is involved in poor germination and growth of plants, which result to stunted seedlings, yellowing of leaves, withering, reduction in tillering, pollen sterility, reduction in grain yield, plasma membrane damage, reduction in membrane integrity, alteration of biomembrane's lipid composition, changes in carbohydrate metabolism, boost in radical-scavenging potential, solute leakage, and alteration of photosynthetic machinery. Virtually cold stress affects all aspects of cellular and physiological functions of plants. It adversely affects the growth and development of plants (Yang et al. 2009). Some of the microorganisms also play a significant role for promotion of plant growth, nutrient management, and disease control. The rhizosphere and endorhizosphere of plants are colonized by these microorganisms, and they promote plant growth by using many direct and indirect mechanisms (Saxena et al. 2005). PGPR have the capability to develop tolerance against abiotic stress (Venkateswarlu et al. 2008; Yang et al. 2009). Induced systemic tolerance is a generalized term proposed for PGPR-induced physiochemical change for enhanced abiotic stress tolerance in plants (Grover et al. 2011).

4.4 Role of Cold-Responsive Gene in Conferring Resistance to Plant

Cold acclimatization in plants can be achieved by the activation of cold-responsive genes either by up- or downregulation in their expression (Thomashow 1999). Proteins encoded by these cold-responsive genes are involved in activation and regulation of signaling pathway cascade (Fowler and Thomashow 2002). Expressions of these genes are essential for acclimatization of plants during cold stress and tolerance of chilling injury. It has been reported that stress-regulating pathways occur by the expression of various genes which indicates that a cold-responsive gene can be a part of more than one cold regulon (Kreps et al. 2002; Seki et al. 2003). In complete transcript profiling, the *Arabidopsis* genome is found to have more than 10% of genes responsible for cold acclimatization (Kreps et al. 2002; Seki et al. 2003; Vogel et al. 2005). Products of these genes are involved in protecting cells from cold stress by producing important metabolic proteins such as COR, LEA, etc., and these stress regulation proteins are useful in signal transduction during stress conditions. On the basis of these different functionalities, there is a clear demarcation in these cold-responsive genes which enables them to be categorized; group I includes metabolic regulating proteins such as chaperones, osmotin and those involved in osmolyte biosynthesis which include sugar and proline, lipid transport proteins, enzymes of fatty acid metabolism, water channel protein, LEA proteins, etc. (Kreps et al. 2002; Seki et al. 2003). A research study was conducted to test the effect of overexpression of some stress-inducible genes which encode LEA proteins and enzyme for osmolyte biosynthesis and detoxification. Transgenics developed in this study have enhanced stress tolerance, which provides an evidence of these gene products in stress tolerance in plants (Cushman and Bohnert 2000). Group II comprises of genes that encode transcription factors involved in signal transduction pathway responsible for functioning in stress conditions (Seki et al. 2003). A co-operative, individual, and constitutive stress-inducible gene network is regulated by these transcription factors.

4.4.1 Role of Hydrophilic Peptide Providing Cold Tolerance in Plants

A group of polypeptides encoded by cold-responsive genes have almost similar amino acid sequences and are extremely hydrophilic in nature. Moreover, they have simple amino acid sequences with repeated motifs which remain soluble upon boiling in dilute aqueous buffer. These sequences are generally rich in Ala, Thr, and Lys residues and devoid of Pro, Trp, Cys, and Phe (Hong et al. 1988; Thomashow 1994). These polypeptides are predicted to possess motifs which have the ability to form amphipathic helices possessing strong effect on the intrinsic curvature of monolayers and having the tendency to form hexagonal II phase. Several proteins having hydrophilic nature were reported to provide cold acclimatization to plants, for example, *COR78*, *COR15*, *COR6.6*, and *COR47* (LEA II) of *Arabidopsis*, *HVA1* (LEA III) of *H. vulgare*, and WCS19 of *Triticum aestivum*. *COR15a* gene of *Arabidopsis* encodes a 15-KD polypeptide expressed in response to abiotic stress condition especially during low temperature (Thomashow 1994). Transformed chloroplasts constitutively expressing *COR15a* were 1–2 °C more freezing tolerant in comparison to wild-type plants while protoplasts isolated from the transgenic plant constitutively expressing *COR15a* were 1°C more cold resistant in comparison to wild type (Artus et al. 1996). The underlying mechanism behind cold tolerance of *COR15a* is not completely understood.

Late embryogenesis abundant (LEA) proteins possess a divergent family, which on the basis of some motifs can be divided into three loosely defined groups. Group 1 is found to have 20-amino acid hydrophilic motifs, and group 2 has three distinct motifs (Y, S, and K), while group 3 is found to possess repeating motif of 11 amino acids, particularly, TAQAAKEKAGE (Dure 1993; Tunnacliffe and Wise 2007; Wise and Tunnacliffe 2004). This protein was first identified in cotton seeds; high accumulation was reported in the last stage of seed maturation and fugitive tissues having a water deficiency (Wise and Tunnacliffe 2004). LEA protein is generally associated with abiotic stress tolerance, especially cold stress tolerance, by an inexplicable molecular mechanism. However, ion sequestering, redox balance, buffering of hydrate water, and chaperone activity are some of the mechanisms that had been suggested on the basis of biochemical evidences. Whereas it is generally agreed that LEA proteins are associated with abiotic stress tolerance, particularly with cold stress and dehydration, their molecular functions have been rather mysterious thus far. Based primarily on limited biochemical evidence, several molecular functions have been suggested, such as that of ion sequestration (Arumingtyas et al. 2013), membrane binding and stabilization (Information 1984), effect on redox balance as an antioxidant and buffering of hydrate water (Cammarano et al. 2012), and chaperone activity (Pilon et al. 2013). Several studies had been carried out to generate cold-tolerant plants by overexpressing LEA proteins. Ectopic expression of LEA 25 protein of *Solanum lycopersicum* in *Saccharomyces cerevisiae* has provided high tolerance against salinity and freezing stress (Imai et al. 1996). Genetically engineered *E. coli* expressing PM2, a seed maturation protein belonging to soya bean group 3 LEA proteins, provides resistance to recombinant bacterial cells in low- and high-temperature conditions (Liu et al. 2010). ZmLEA3, a single-copy gene in maize genome, on overexpression is found to confer tolerance to low-temperature stress to transgenic tobacco, yeast (GS115), and *E. coli* (BL21) (Liu et al. 2016). On the contrary, where overexpression of LEA protein enhances cold tolerance, the expression of individual genes encoding LEA protein from spinach (*Spinacia oleracea*) and *Craterostigma plantagineum* in transgenic tobacco resulted in no significant changes in cold and drought stress condition (Iturriaga et al. 1992; Kaye et al. 1998) which indicate that maybe not all LEA proteins have a significant role as cryoprotectant. (Houde et al. 2004; Hundertmark and Hincha 2008). Largely on the basis of expression characteristics to freezing and dehydration, contribution of genes can be speculated in mitigating their potential in providing cold stress tolerance to plants. Some of the genes isolated in the last decade are listed in Table 4.1.

4.4.2 Role of Transcription Factors in Augmenting Cold Tolerance in Plants

Various transcription factors regulate the expression of cold-responsive genes, belonging to two major families, DREB and MYB (Zhang et al. 2011). The general mechanism of transcription factor behind the regulation of cold acclimatization in plants is to bind the cis-acting elements of cold-responsive genes which generally code for proteins and enzymes involved in metabolism of lipids, carbohydrates, antifreezant, antioxidant, molecular chaperones, etc. (Guy 1990; Thomashow 1999). Various C-repeat/dehydration-responsive element-binding factors (CBF/DREB) were identified and have shown a predominant role in cold tolerance (Yamaguchi-Shinozaki and Shinozaki 2006; Chinnusamy et al. 2007). These transcriptions regulate the expression of cold-responsive genes and are themselves induced by low temperature which suggests the presence of transcriptional cascade prominently during cold stress (Jaglo-Ottosen et al. 1998; Liu et al. 1998). Studies had shown both CBF-regulated and CBF-non-regulated activations of cold-responsive genes. In *Arabidopsis* 12% of cold-responsive genes were regulated by CBF transcription factor while 28% of genes were activated without CBF (Fowler and Thomashow 2002) which suggest the existence of CBF/DREB1-dependent and CBF/DREB1-independent pathways.

Activation of CBF/DREB1 gene was induced by the presence of an upstream transcription factor *ICE1* (*inducer of CBF expression 1*) (Chinnusamy et al. 2003). *ICE1* is an MYC-type basic helix-loop-helix transcription factor that binds to cis-acting elements of CBF3 promoter to regulate the expression of genes during cold stress. Hypersensitivity to cold stress was observed in plants having mutant *ICE1 gene, and increased freezing tolerance was observed in plants having overexpressed ICE1 gene, which lays a significant framework of the post-translational modification for activation of cold-responsive genes during cold acclimatization in plants. A*

Table 4.1 List of some transgenic plants developed for testing the efficacy of cold stress-related gene

S. no.	Genes/gene product	Host plant	Performance of transgenic plants	References
1.	*OsDREB*	*Arabidopsis*	Overexpression of target stress-inducible gene with resulting high tolerance to cold stress	Dubouzet et al. (2003)
2.	*CBF*1, *CBF*2, and *CBF*3	*A. thaliana*	Increased freezing tolerance	Zhang et al. (2004)
	LeCBF1			
3.	*OsNAC6*	Rice	Enhanced cold, salt, and drought response	Ohnishi et al. (2005)
4.	*CBF2* and *ZAT 12*	*A. thaliana*	Increased freezing tolerance	Vogel et al. (2005)
5.	*DREB1/CBF*	Rice	Improvement in cold stress tolerance	Ito et al. (2006)
6.	*OsNAC6*	Rice	Transformants show improved tolerance to abiotic stress	Nakashima et al. (2007)
7.	*OsDREB1F*	*A. thaliana* and rice	Enhanced cold stress tolerance in both plants	Wang et al. (2008)
8.	*SCOF-1*	Sweet potato	Low temperature can be efficiently modulated	Kim et al. (2011)
9.	*GmbZIP1*	Wheat	Transformants show highly significant tolerance against cold stress	Gao et al. (2011)
10.	*MbDREB1*	*Malus baccata*	Enhanced cold tolerance via ABA pathway	Yang et al. (2011)
11.	*DaCBF7*	Rice	Enhanced tolerance against cold stress	Byun et al. (2015)
12.	Soybean *DREB1/ CBF*-type	*Arabidopsis*	Transformants show ABA-independent expression and enhanced cold stress tolerance	Kidokoro et al. (2015)
13.	*CaPUB1*	Rice	Enhanced cold stress tolerance and decreased drought stress tolerance	Min et al. (2016)
14.	*TaAREB3*	*Arabidopsis*	Enhanced cold and drought stress tolerance	Wang et al. (2016a, b)
15.	*AsDREB1*	*Adonis amurensis*	Gene is useful for genetic engineering to improve plant stress tolerance	Zong et al. (2016)
16.	*AtP3B*	Sweet potato	Low-temperature tolerance and increased storage ability	Ji et al. (2017)
17.	*IbCBF3*	Sweet potato	Showed highly significant tolerance against low temperature	Jin et al. (2017)
18.	*DaCBF4*	Rice	Enhanced cold stress in cereal plants	Byun et al. (2018)
19.	*OsDIRP1*	Rice	Negative regulator for drought and salt stress and positive for cold	Cui et al. (2018)

group of researchers had shown that MYB-regulated HEPTAHELICAL PROTEIN (HHP) activates the CBF pathway in association with *ICE1* and *ICE2* (Lee and Seo 2015). *MYB*, *ERF*, *bZIP*, *NAC*, *ARF*, and *CCAAT-HAP* are the group of transcription factors associated with this network of DREB/CBF pathway. *AtCBF1* (DREB1B), *AtCBF2* (DREB1C), and *AtCBF3* (DREB1A) genes of *Arabidopsis* (Liu et al. 1998; Stockinger et al. 1997) and *OsMYB2*, *OsMYB511*, *CMYB1*, and *OsMYB48-1*(Yang et al. 2012; Xiong et al. 2014; Huang et al. 2015) are some of the genes reported for cold stress regulation with an unclear molecular mechanism.

The underlying mechanisms of most of these genes are not fully understood; however, some of these genes have been overexpressed to generate stress-tolerant phenotypes in transgenic plants (Zhang et al. 2004). Overexpression of *OsMYB4*, *AtCBF1*, or *AtCBF3* was found to enhance the expression of cold-responsive genes to increase freezing tolerance in *Arabidopsis* and apple plants (Pasquali et al. 2008). Zhao et al. (2018) reported that overexpression of *NtbHLH123, a tobacco transcription factor binds to*-box/E-box motifs in the promoter of the *NtCBF* genes to enhanced cold tolerance in plants.

CBF transcription factors are also negatively regulated in the presence of an upstream transcription factor, MYB15, which is an R2R3-MYB family protein predominantly found in *Arabidopsis*. MYB15 factor is found to be expressed even in the absence of cold stress; knockout studies of MYB15 had shown the enhanced chilling tolerance in plants (Chinnusamy et al. 2007). *MYB15* and *ZAT12* negatively regulate the expression of *CBF*. Further *HOS1* also suppressed the expression of CBF by ubiquitination and proteosomal degradation of *ICE1* (Chinnusamy et al. 2003; Agarwal et al. 2006).

4.4.3 Role of Promoters in Regulating Cold Tolerance in Plants

Expression of a gene is driven by the presence of a promoter upstream of its coding region. Promoters have cis-acting elements, which determine their strength of gene regulation. Dehydration-responsive elements or C-repeats and abscisic acid-responsive binding cis-elements are often found in the promoter region of cold-responsive genes (Yamaguchi-Shinozaki and Shinozaki 2006; Stockinger et al. 1997). Due to adverse environmental conditions, such as cold stress, these transcription factors activate and bind to the promoter region of cold-responsive genes showing evidence of tight regulation of genes in the presence of cold stress. *Fad* gene was found to be expressed in the presence of cold-inducible promoter *COR15A*. Transgenic lines generated from *COR15A-FAD7* had showed enhanced tolerance on exposure to low temperature for up to 44 days (Khodakovskaya et al. 2006).

Promoters of two *Arabidopsis* cold-regulated genes cor15a and cor15b were analyzed in potato and tobacco, and subsequent new sets of synthetic promoters were generated by domain swapping, which had affected the strength of promoters to an extent (Li et al. 2013). Similarly a group of scientists had isolated and characterized three promoters of cold-inducible gene (*Ppbec1*, *Ppxero2*, and *Pptha1*) of peach. To develop a better understanding of cold acclimatization in peach, they had cloned these promoters upstream of *GUS* reporter gene and analyzed their cold inducibility, transiently in peaches and transgenically in *Arabidopsis*. Two promoters Ppbec1 and Ppxero2 had shown positive cold inducibility which suggests a conserved heterologous cold-inducible regulation of these promoters in peach and *Arabidopsis* (Tittarelli et al. 2009). These studies suggest there is a wide scope of promoter engineering for improving the expression of cold-related genes.

4.5 Advancement in Molecular Approaches for Cold Stress

The conventional plant breeding strategies had limited accomplishment in providing cold tolerance to crop plants. The present status of success is few and far between; hence, biotechnology tools and techniques possess a powerful hope for fighting with cold stress in crop plants. The advancement of technology has revealed a variety of cold resistance-imparting genes. Biotechnology tools have been exploited worldwide for modulating the expression of these genes for enhancing cold tolerance in plants. Some biotechnology approaches, including development of transgenics, genome editing, and use of omics approach, have been discussed as follows:

4.5.1 Transgenic Approach

Developing the transgenic plants with ectopic gene expression opens the stimulating opportunities for improving cold acclimatization in plants by up- and downregulating the genes associated with specific traits (Pino et al. 2008). Discovery of genes in association with functional genomics projects had offered countless mechanisms and genes which provide a platform for increasing the resistance capacity of plants in adverse abiotic stress conditions. Easy manipulation can be done in the architecture of these genes to overexpress ectopically in plants in a way in which they do not naturally occur with a vision of trait improvement. Selection of a desired gene for the trait to be enhanced, a suitable promoter for specific or constitutive expression of the vector that serves as a vehicle for gene transfer, a gene for screening positive transformants, and a mode of transformation is one of the essential components for developing transgenics.

Advancements in plant biotechnology had identified several transcription factors and cold-responsive genes which have been genetically engineered for development of cold-resistant transgenic crops. A group of scientists had developed rice transgenic plants by overexpressing *DaCBF4* gene in regulation of constitutively expressed ubiquitin promoter. They had reported that transgenic rice plants expressing *Ubi:DaCBF4* had shown enhanced cold tolerance in comparison to wild-type plants without any growth retardation (Byun et al. 2018). Another group had analyzed the expression of *Arabidopsis* ribosomal P3 (*AtP3B*) gene in transgenic sweet potato in downstream of constitutively expressed CaMV 35S promoter. Based on the *AtP3B* transcript levels, they have selected three lines and shown that these lines have higher photosynthetic efficiency with less membrane permeability and enhanced chilling tolerance in comparison to wild plants (Ji et al. 2017). Transgenic rice plants developed by overexpressing *OsCYP19-4* gene had shown enhanced cold tolerance with increased spike number and grain weight in comparison to wild-type plants (Dae et al. 2016; Yoon et al. 2015). Dong and his coworkers had generated transgenic *Arabidopsis* plants by overexpressing miR397 transcripts in regulation of CaMV35S promoter. These transformed plants have an improved chilling tolerance and acclimatization at 4 °C. In the northern blot analysis, they had found that overexpression of miR397a affects the expression of CBF and COR genes (Dong and Pei 2014). *TaCBF14* and *TaCBF15* genes isolated from winter wheat are analyzed for their chilling tolerance capability in transgenic barley. The analysis had shown a high resistance of transgenic plants to several degrees of low temperature as compared to wild spring barley, and the transgenic leaves have lower ion leakage in cold stress to prevent leaf damage during frost (Soltész et al. 2013). Evidence of enhanced protection of PSII and PSI during cold stress was found in tomato leaves having overexpressed *CBF1* gene in comparison to wild-type plants. Higher content of superoxide dismutase (SOD), higher non-photochemical quenching (NPQ), and lower content of malondialdehyde (MDA) were detected in transgenic tomato which were essential for providing cold acclimatization to these plants (Zhang et al. 2011). Similarly transcription of DREB1A of pearl millet and *DREB2A* of rice had provided enhanced cold stress tolerance in transgenic tobacco and transgenic rice, respectively, in comparison to wild-type plants (Ito et al. 2006; Agarwal and Jha 2010). The potentiality of the associated gene for providing cold stress tolerance was examined by developing several transgenic plants. Some of the transgenic plants that developed an enhanced cold stress tolerance are listed in Table 4.2.

4.5.2 Genome Editing

With the advancement of genome sequencing, enormous possibilities had opened up for improving the quality of any desirable trait. Zinc finger nucleases (ZFNs), transcription factor activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein9 nuclease (Cas9) are the triumvirate of nucleases commonly used in editing the targeted gene of interest (Shen et al. 2017). Targeted genome editing employing CRISPR/Cas9 is widely used as effective technology of the present era for genetic engineering of various plants to increase yield potential under abiotic stress conditions. According to the guidelines of the United States Department of Agriculture

Table 4.2 List of some cold-responsive genes

S. no	Plants	Genes	References
1	*Allium sativum*	*CR174-2*, *CR74-3*, *CR181-1*, *CRM14-2*, *CRM5*, *CR105*, *CRM14-2*, and *CR84-1*	Son et al. (2012)
2	*Prunus dulcis*	*PdCBFs*	Barros et al. (2012)
3	*Lepidium latifolium*	*LlaNAC*	Aslam et al. (2012)
4	*Capsella bursa-pastoris*	*CbCOR15a*	Zhou et al. (2012)
5	*Oryza sativa*	*OsMYB30*	Miura and Furumoto (2013)
6	*Malus sieversii*	*MsDREB2C*	Zhao et al. (2013)
7	*Solanum lycopersicon*	*SlICE1*	Miura and Furumoto (2013)
8	*Triticum aestivum*	*TaICE141*, *TaICE187*	Miura and Furumoto (2013)
9	*Hordeum vulgare*	*HvCBF4*	Miura and Furumoto (2013)
10	*S. habrochaites*	*ShCBF1*	Li et al. (2014)
11	*Brachypodium distachyon*	*BdCBF1*	Ryu et al. (2014)
		BdCBF2	
		BdCBF3	
12	*Capsicum annuum*	*CaNAC2*	Guo et al. (2015)
13	Maize	*ZmSEC14p*	Wang et al. (2016a, b)
14	*Poncirus trifoliata*	*PtrERF109*	Wang et al. (2018a, b)
15	*Nicotiana tabacum*	*NtbHLH123*	Zhao et al. (2018)
16	*Raphanus sativus*	*RsICE1*	Man et al. (2017)
17	*Medicago truncatula*	*MtPP2Cs*	Yang et al. (2018)
18	*Camellia sinensis*	*CsAOX1a*	Ding et al. (2018)
		CsAOX1d	
19	*Manihot esculenta*	*MeTCP4*	Cheng et al. (2019)

(USDA), crop plants produced by CRISPR/Cas9-mediated genome editing are considered as non-GMO crops; hence this technology holds unforeseen possibilities for revolutionizing agriculture to meet the demands of the increasing population (Waltz 2018).

Few studies were reported for increasing the understanding of cold stress tolerance genes in plants; for example, recently the chilling tolerance capabilities of plants were analyzed, using CRISPR-Cas9-mediated genome editing of SlCBF1 gene in tomato (*Solanum lycopersicum*). This group has generated SlCBF1 mutant plants and evaluated their efficiency in comparison to wild-type tomato plants. These mutant SlCBF1 plants have higher electrolyte leakage and malondialdehyde content, lower proline content, antioxidant enzymes, ROS accumulation, and an altered plant hormone level in comparison to wild-type plants which are possible consequences of reduced chilling tolerance in tomato (Li et al. 2018). Similarly the role of rice annexin gene *OsAnn3* was examined by generating *OsAnn3* CRISPR knockout rice plants; mutant T_1 lines were found to be more susceptible in comparison to wild-type plants under cold treatment due to increased relative electrical conductivity (Shen et al. 2017). The multiplex CRISPR/Cas9 technology is becoming predominant for site-directed dissection of genetic code for improvising the qualitative and quantitative traits of various crops to withstand adverse environmental conditions (Brooks et al. 2014).

4.5.3 Omics Approach

In the light of research, various stress-related mechanisms have been predicted with the integrated use of omics approach in the last two decades. The use of omics approach along with bioinformatics tools and techniques helps to understand the genotypic/phenotypic relation in the presence of abiotic stress condition in plants. Functionality of plants depends on four interdependent constituents such as mRNA, gene, protein, and metabolites; the studies of these constituents are referred to as transcriptomics, genomics, proteomics, and metabolomics, respectively (Vogel and Marcotte 2012). Microarray and deep-sequencing technology such as next-generation technology had advanced the hasty accretion of expedient data under various abiotic stress conditions (Molina et al. 2008, 2011). Several studies have reported on the correlation of transcript and protein level emphasizing the prominence of post-transcriptional processes in prediction of protein expression and metabolic studies (Maier et al. 2009; Foss et al. 2011; Ghazalpour et al. 2011; Vogel and Marcotte 2012). Diagrammatic representation of various omics approach is depicted in Fig. 4.2.

Transcriptomics analysis of cold-hardened mutant of *barley albina* and *xantha* revealed the major role of the chloroplast for enhancing cold acclimatization in plants (Svensson et al. 2006). Recently a group of researchers had reported a transcriptomics profile of two winter rapeseed varieties Longyou-7 (cold tolerant) and Lenox (cold sensitive) under 24-h cold stress conditions; they had identified 10,251 and 10,972 differentially expressed genes in Longyou-7 and Lenox, respectively. Their study provides a transcriptomics database for future cold stress-related studies in rapeseed (Ma et al. 2019). Besides upregulated genes, the transcriptomics approach is also useful for identification of downregulated or repressed genes. Transcriptomics analysis of transgenic *Arabidopsis* plants overexpressing *ZAT12* revealed 24 cold standard set of genes, among which 9 are cold-induced and 15 are cold-repressed genes (Vogel et al. 2005).

Similarly proteomics analysis in cold-sensitive (*93–11*) and cold-resistant (*DC907*) hybrid rice cultivars revealed the presence of 366 unique proteins when treating the rice seedlings at 8–10 °C for 24, 72, and 120 h (Wang et al. 2018a, b). Combining both metabolomics approach and molecular techniques will further

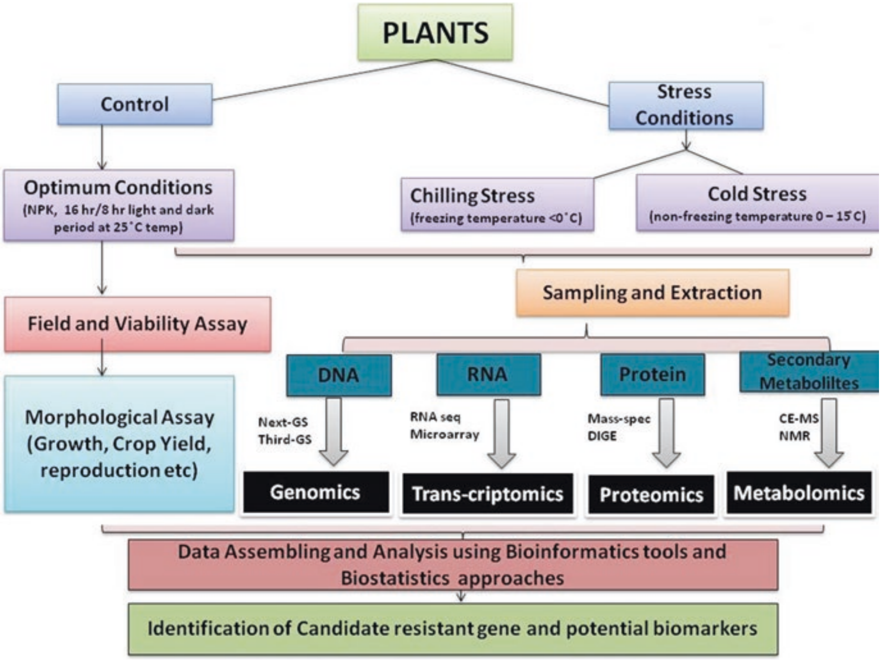

Fig. 4.2 Integrated OMICS approaches for cold stress tolerance in plants. *GS* generation sequencing, *RNA seq* RNA sequencing, *DIGE* difference gel electrophoresis, *CE-MS* capillary electrophoresis-liquid-chromatography mass spectrometry, *NMR* nuclear magnetic resonance

enhance the understanding of cold acclimatization in plants. A non-targeted metabolic fingerprinting approach indicates the occurrence of global reprogramming in *Arabidopsis* under cold stress conditions (Gray and Heath 2005).

The integrated use of these omics approaches improves the understanding of the role of associated genes for the formation of desired proteins essential for precise regulation of metabolic pathways which can be implemented in genetic engineering and biotechnology applications for enlightening stress tolerance in plants.

4.6 Challenges Faced in Harboring Plants from Cold Stress

There is a big gap between areas of plant stress tolerance and acclimatization both in research and field application. Traditional breeding programs for developing cold-resistant varieties gained less success. This is because breeders analyzed their genetic materials under ideal conditions. It is an important issue to be resolved because seed companies are demanding to develop cold-resistant varieties of crops. Producers tend to develop varieties in laboratory conditions while seed companies require varieties which can be grown in field conditions. So, measures taken to improve the tolerance yielded no beneficial consequences (Blumwald et al. 2004).

From an economic point of view, plant breeding companies tend to invest in the improvement of new varieties with high stress tolerance, but there will always be a question raised on whether the investment in the development of these cultivars is worth the effort. There is no advantage in raising cold-tolerant plants until and unless there are methods which will allow the plants to be equally or more productive than non-cold-tolerant plants which grow on uncompromised soil. Apart from improving the tolerance ability of the plants, there are areas which need the attention of researchers like genes undergoing changes when experiencing cold stress. Promoters such as CaMV35S promoter, ubiquitin, and actin are the constitutive promoters used for transgenic insertion (Grover et al. 2003). Studies have shown that extra expression of a stress-induced gene under the regulation of a tissue-specific promoter shows better phenotype than a gene which is expressed constitutively (Zhu et al. 1998; Kasuga et al. 1999).

Though there are many success stories on the cold stress acclimatization of plants such as *Arabidopsis*, rice, and tobacco, researchers need to identify other genetic backgrounds like those of other crops. The genetic environment also plays a vital role in the effectiveness of specific transgene (Kasuga et al. 1999). Position effect is a well-known phenomenon reported in the development of transgenics, which may limit the expression of genes in abiotic stress-engineered plants. Though the work on cold tolerance ability in plants is slow, there are many reasons for optimism like development of genetic markers and construction of associated maps, sequencing of genomes, EST libraries, construction of T-DNA, and other genetic tools which are used in analysis of gene functions. Thus we need to look for the comparative effect, interaction of transgene, and efficiency of these improved varieties in fields (Grover et al. 2003).

Identification and characterization of cold sensor signaling checkpoints, basis of intercellular and intracellular mechanisms, and addressing the counteracting signaling communication during cold stress are some of the deficit areas having a huge potential of research for improvising the crop conditions for increasing the world's economy.

4.7 Conclusion

Plants being sessile in nature need to adapt to changing surroundings rapidly and proficiently to survive under stressful environmental conditions. The flexible nature of higher plants makes them more suitable to withstand adverse conditions, which may be helpful to develop a better understanding on the underlying mechanisms of these responses, in regard to developing robust agricultural crops. Several components which include membrane fluidity, calcium ion influx in cytosol, chaperones, hydrophilic proteins, lipids, protein kinase phytohormones, antifreeze proteins, osmotins, and ion transporters take part in the signaling events of cold stress. Due to upregulation of these components, a signaling cascade is generated which leads to a variety of physiochemical changes necessary for adaptation of plants during cold stress. Engineering cold stress tolerance in plants associated with transcription factors and responsive genes is being explored with a vision of enhancement in cold stress tolerance and productivity of agricultural crops.

With the succeeding era, extraordinary advancements in terms of accuracy and precision are observed in the field of molecular and systematic biology (Sanghera et al. 2011). Both molecular and systematic biology including omics-based approaches complement each other and develop a new insight for better understanding of plant responses under stress conditions (Tuteja et al. 2011). This suggests the robustness in the interconnected pathways of transcription factors and cold-responsive genes, which can be manipulated to attain maximum progress in freezing tolerance. Several problems and challenges had been reported with the available genetic engineering and transgenic approach which opens a wide scope of research in the development of advanced biotechnology tools and techniques for generating precise and efficient cold tolerance in plants.

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Chapter 5
Mechanism of Waterlogging Stress Tolerance in Pigeonpea Plants: Biochemical and Anatomical Adaptation Under Waterlogging

Savita Duhan and Sunita Sheokand

Contents

Abstract Pigeonpea is the most important legume grown in semiarid tropics and generally grown in low-lying areas. In low-lying areas, the chances of waterlogging are maximum. Some pigeonpea varieties are sensitive but some are quite tolerant to waterlogging. The main cause of damage is suffocation which the plant has to face due to waterlogging. Oxygen deficiency causes electrolyte leakage due to which the cell is exposed to the outside environment which can also cause peroxidation of lipid and nucleic acid and ultimately death. So any mechanism that can reinstate oxygen supply to stressed tissue can be a major trait for waterlogging tolerance. Formation of aerenchyma, adventitious roots, and lenticels is helpful in restoring oxygen to waterlogged plants. Besides the formation of these, various types of biochemical changes also occur for waterlogging tolerance in pigeonpea plants. Biochemical changes include increase in reducing sugars, activity of enzymes used in glycolysis and fermentation, and participation of antioxidants. Glycolytic enzymes include alcohol dehydrogenase and sucrose synthase. The antioxidant system includes enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase, catalase peroxidase, ascorbate peroxidase, and

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glutathione peroxidase, while ascorbate and glutathione fall under the category of non-enzymatic antioxidant defense system. We emphasize the attributes responsible for waterlogging tolerance in pigeonpea.

Keywords Waterlogging · Pigeonpea · Antioxidant aerenchyma · Adventitious roots · Sucrose synthase

Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbic acid
ATP	Adenosine triphosphate
CAT	Catalase
DHAR	Dehydroascorbate reductase
GPX	Glutathione peroxidase
GR	Glutathione reductase
GS	Glutathione synthase
GSH	Reduced glutathione
GSNO	*S*-nitrosoglutathione
GSSG	Oxidized glutathione
GST	Glutathione *S*-transferase
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
POX	Peroxidases
ROS	Reactive oxygen species

5.1 Introduction

Cajanus cajan L. Millsp. or pigeonpea is an important pulse crop of South Asia. Exposure to waterlogging during germination and early vegetative growth is the main problem in pigeonpea cultivation. Waterlogging is identified as one of the most severe stresses all around the world (Martínez-Alcántara et al. 2012). Waterlogging in plants is accompanied by closure of stomata which decreases the exchange of gases and also affects passive absorption of water (Aldana et al. 2014). It also causes a decrease in transpiration which is followed by wilting of leaf and early senescence and ultimately leaf fall (Kozlowski and Pallardy1997). Pigeonpea is highly sensitive to waterlogging (Perera et al. 2001; Krishnamurthy et al. 2012).

Sairam et al. (2008) found that waterlogging blocks the uptake of nutrients and water by creating an oxygen-deficient environment around the roots due to which plants wilt even in the presence of excess water. Oxygen deprivation later on causes membrane breakdown (Blokhina et al. 2003). Main consequences of waterlogging in pigeonpea are yellowing and senescence of leaves and decrease in leaf area, dry matter, relative water content and chlorophyll content in leaves, and membrane stability index (Kumutha et al. 2009). Oxygen is the main reason behind life on Earth but it produces ROS when reduced which further disturbs the metabolism of plants (Tripathy and Oelmuller 2012; Mittler 2017). One of the means for production of ROS is reaction of oxygen with chlorophyll molecule which further produces singlet oxygen (Ashraf and Akram 2009). Superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), singlet oxygen, and the hydroxyl radical (OH^-) all fall under the category of reactive oxygen species (ROS). Exposure to these ROS causes damage to proteins, lipids, pigments, DNA, etc. (Ashraf 2012). Production of ROS becomes quite high under stressed condition compared to a normal one. But under stressed condition, plants produce such an amount of ROS which is detrimental for cellular metabolic reactions like photosynthesis by affecting PS II (Ashraf 2009) and Calvin cycle (Yadav and Atri 2017).

Plants develop some effective mechanisms for survival under adverse effects of ROS. One of them is production of antioxidants which are of two types: enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione reductase (GR), whereas ascorbic acid, glutathione, tocopherols, and carotenoids are included in non-enzymatic antioxidants (Gupta et al. 2005). An alteration was noticed in activities of different enzymatic and non-enzymatic antioxidants under waterlogging stress. Pigeonpea plants showed increased activities of various enzymatic antioxidants such as glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) when exposed to waterlogging stress (Duhan et al. 2017a, b). Similarly, increase in the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) was noticed when pigeonpea genotypes were exposed to waterlogging stress (Kumutha et al. 2009).

Carbohydrate deficiency has been found to be behind waterlogging-induced injuries (Akhtar and Nazir 2013). Carbohydrates are quite important as these are an energy source. Reducing sugars like glucose and fructose act as a source for ATP by maintaining glycolysis under hypoxia as oxidative phosphorylation is completely blocked (Kuai et al. 2016; Duhan et al. 2017d). Thus, the plants have to maintain sufficient readily metabolizable sugars under hypoxic or anoxic conditions to survive (Sairamet al. 2009a; Duhan et al. 2017d). Under hypoxic conditions, a higher amount of sugar content (total, reducing, and non-reducing sugar) was observed in roots of comparatively tolerant genotypes than susceptible genotypes of pigeonpea (Hossain and Uddin 2011). Development of gas-filled cavities (aerenchymas) in root cortical tissue also plays a pivotal role in the tolerance of plants against waterlogging. Aerenchyma formation is considered as an adaptive response of plants against flooding stress (Drew et al. 2000; Evans 2004) and shown by several crops like wheat, maize, pigeonpea, and sunflower, These gas-filled cavities commonly known as aerenchymas act as a pathway for the movement of O_2 from the shoots to the roots (Hossain and Uddin 2011). In this chapter we discuss biochemical and anatomical mechanisms of waterlogging stress tolerance in pigeonpea plants.

5.2 Biochemical Adaptation

The main reason behind waterlogging-induced damage is oxygen deficiency which cuts down the respiration chain. On the other hand, ROS are also produced under stress which further causes peroxidation of protein, nucleic acid, and lipids and results in cell membrane disruption. To cope up with this unfavorable situation, the tolerant plant undergoes some biochemical adaptations which include anaerobic respiration, attainment of carbohydrate need for anaerobic respiration, and development of an antioxidative defense system (Fig. 5.1).

5.2.1 Antioxidant Activities

Hypoxic and anoxic conditions cause oxidative stress in plants, which further decreases plant growth by producing reactive oxygen species (ROS) like superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2) (Mittler et al. 2004). Due to oxidative stress, cell membranes get disrupted and DNA proteins get denatured due to peroxidation by ROS (Young and Woodside 2001). To cope up with this unfavorable condition, the plant produces various types of enzymatic and non-enzymatic substances. Enzymatic components include SOD, CAT, APX, POX, GR, MDHAR, and DHAR, while non-enzymatic components include ascorbic acid, glutathione, tocopherols, and carotenoids (Sheokand and Kumari 2015). In this section we will study the role of these enzymatic and non-enzymatic components in providing waterlogging tolerance to pigeonpea plants.

5.2.1.1 Enzymatic Components

Superoxide Dismutase (EC 1.15.1.1)

Under waterlogging stress, SOD enzyme acts as the first line or front line of defense as it dismutates superoxide radical to oxygen and hydrogen peroxide which are comparatively less reactive. SOD activity is supposed to be increased when the plant is subjected to any type of stress (Lascano et al. 2001; Wu et al. 2012). It is also observed that SOD with other antioxidative enzymes plays a major role in providing tolerance to plants against waterlogging stress (Duhan et al. 2017a, b). Three isoforms of SOD are identified in plants depending on the metal that binds to the

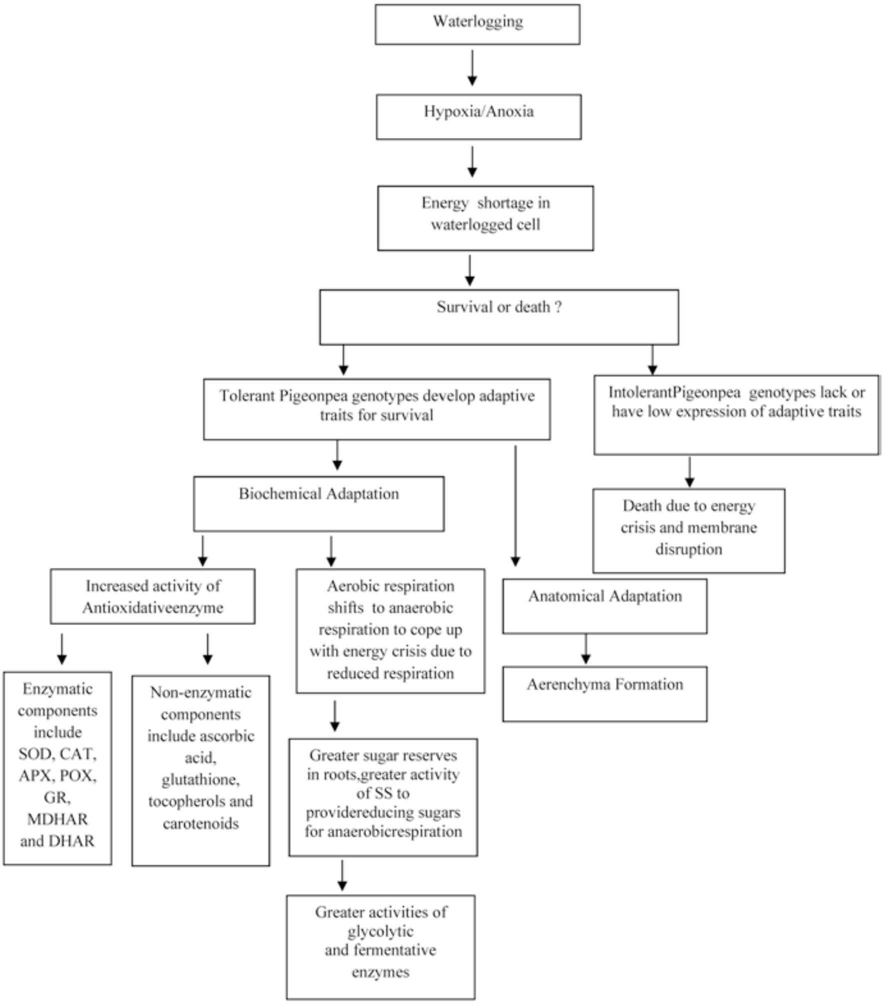

Fig. 5.1 Flow diagram showing morphological and metabolic adaptation for waterlogging tolerance in pigeonpea

catalytic active site. First is manganese SOD (Mn-SOD), which is localized in mitochondria (Kuzniak and Sklodowska 2004) and in peroxisomes (Rodríguez-Serrano et al. 2007) and works in association with CAT, APX, GR, MDHAR, and DHAR in mitochondria and CAT in peroxisomes. The second isoform is copper/zinc SOD (Cu/Zn-SOD), which is present in numerous cell compartments such as chloroplasts (Pillon et al. 2011), mitochondria (Kuzniak and Sklodowska 2004), cytosol (Hurst et al. 2004), and peroxisomes (Corpas et al. 2001) as well as in the apoplast (Ogawa et al. 1996) and works in association with GPX, MDHAR, and DHAR in cytosol; CAT, APX, GR, MDHAR, and DHAR in mitochondria; CAT in peroxisomes; and

APX, GPX, MDHAR, and DHAR in chloroplasts. The third one is iron SOD (Fe-SOD), which is identified as a chloroplastic enzyme (Asada 2006) and works in conjunction with APX, GPX, MDHAR, and DHAR. Two pigeonpea genotypes ICPL-84023 (waterlogging resistant) and MAL-18 (waterlogging sensitive) when subjected to waterlogging showed increase in SOD content with comparatively higher increase in the resistant genotype (ICPL-84023) as compared to the sensitive one (MAL-18) (Bansal and Srivastava 2012). A study was conducted to observe the effect of waterlogging on antioxidant enzymes in two pigeonpea (*Cajanus cajan* L. Halls) genotypes ICP-301 (tolerant) and Pusa 207 (susceptible) and found antioxidant enzymes such as superoxide dismutase increased under waterlogging (Kumutha et al. 2009). Waterlogging-induced increased activity of SOD was also found in pigeonpea genotypes (Sairam et al. 2009b). Duhan et al. (2017a, b) observed an increase in SOD in pigeonpea under waterlogging. From these reports, it is proved that SOD plays an essential role in providing tolerance to plants when subjected to abiotic stress like waterlogging.

Catalase (EC 1.11.1.6)

Reaction of superoxide dismutase (SOD) with superoxide radical ($O_2^{\bullet-}$) produces hydrogen peroxide (H_2O_2) which is also a toxic metabolite (Halliwell 1987). H_2O_2 attacks cell membrane lipids resulting in increased membrane permeability (Sharma et al. 2012). Decrease in catalase enzyme, which is responsible for H_2O_2 breakdown, allows its accumulation (Das and Roychoudhury 2014). Duhan et al. (2017a) observed a 39–80% increase in CAT activity 1 day after removal from 12-day waterlogging treatment in the case of 20-day-old plant leaves. Forty-day-old plants achieved no survival 8 days after removal from 12-day waterlogging treatment (Duhan et al. 2017a, b). An experiment was conducted with two pigeonpea genotypes, ICPL-84023 (tolerant) and ICP-7035 (susceptible), and the effect of 2, 4, and 6 days of waterlogging on catalase enzyme activity was studied, and a significant increase was observed in both genotypes after 2 days of waterlogging (Sairam et al. 2009b). ICP-7035 showed a decrease in CAT activity under 4 and 6 days of waterlogging, while a continuous increase was observed in ICPL-84023 up to the sixth day of waterlogging. Two pigeonpea genotypes ICPL-84023 (waterlogging resistant) and MAL-18 (waterlogging sensitive) also showed increased H_2O_2 content followed by increased activity of catalase in both the genotypes when subjected to waterlogging (Bansal and Srivastava 2012). Kumutha et al. (2009) also studied the consequences of 2, 4, and 6 days of waterlogging on two pigeonpea (*C. cajan* L. Halls) genotypes ICP-301 (tolerant) and Pusa 207 (susceptible) and found decreased oxidative stress and increased catalase activity under 2 days of waterlogging, but later on, under 4 and 6 days of waterlogging, both oxidative stress and catalase activity increased in ICP-301 (tolerant). Pusa 207 showed increased H_2O_2 content and decreased catalase activity which is the main reason behind its sensitivity toward waterlogging.

Ascorbate Peroxidase (EC 1.11.1.11)

Ascorbate peroxidase is a hydrogen peroxide-scavenging enzyme. Catalase performs this function in peroxisomes and mitochondria, whereas in chloroplast and cytoplasm, H_2O_2 is degraded by APX. APX detoxifies the cell by using ascorbic acid and produces water and DHA. Duhan et al. (2017b) studied the effect of 8 days of waterlogging on four genotypes of pigeonpea, i.e., ICPH-2431, UPAS-120, PARAS, and HO933, 1 day after removal from treatment and recorded a 30–64% increase. Increase was minimum in UPAS-120 and maximum in ICPH 2431. The effect of waterlogging on pigeonpea genotypes ICP-301 (tolerant) and Pusa 207 (susceptible) was studied, and a significant increase in APX activity at the second day of waterlogging in both genotypes was observed with a comparative higher increase in ICP-301. Waterlogging for 4 and 6 days decreased APX activity in Pusa 207, whereas ICP-301 showed increased activity of ascorbate peroxidase even under 4 and 6 days of waterlogging (Kumutha et al. 2009). Sairam et al. (2009b) studied the effect of 2, 4, and 6 days of waterlogging on two pigeonpea (*Cajanus cajan* L.) genotypes, ICPL-84023 (tolerant) and ICP-7035 (susceptible), and found increased APX enzyme activity under 2 days of waterlogging. ICP-7035 showed decreased APX activity when subjected to continuous 4 and 6 days of waterlogging; however, ICPL-84023 showed increased APX activity even when subjected to 6 days of continuous waterlogging. Two pigeonpea genotypes, viz., ICPL-84023 and DA-11, were exposed to 4 and 6 days of waterlogging, and both genotypes showed increased APX activity under 4 as well as 6 days of waterlogging. The ICPL-84023 genotype performed better as compared to the DA-11 genotype in terms of APX enzyme activity (Aarzoo et al. 2017).

Peroxidases (EC1.11.1.x)

Peroxidases are a large group of heme-containing proteins, which oxidize H_2O_2 by consuming some phenolic as well as non-phenolic substrates. Vacuolar H_2O_2 is mainly consumed by peroxidases. Duhan et al. (2017b) noticed increased POX activity in 20- and 40-day-old pigeonpea leaves when exposed to 8 days of waterlogging with a comparative higher increase in tolerant genotypes (ICPH-2431 and PARAS) compared to sensitive ones (UPAS-120 and HO933). Forty-day-old plants showed comparatively lower increase. Increase in peroxidase activity was also observed in pigeonpea leaves when exposed to 12-day waterlogging treatment. Increase was more in tolerant genotypes (ICPH-2431 and PARAS) compared to sensitive ones (UPAS-120 and HO933) (Duhan et al. 2017a). Aarzoo et al. (2017) examined the effect of 4 and 6 days of waterlogging on two pigeonpea genotypes, ICPL-84023 and DA-11, and observed increased peroxidase activity in both the genotypes with comparatively higher increase in ICPL-84023.

Glutathione Reductase (EC 1.6.4.2)

Enzyme GR helps in maintaining high cellular GSH/GSSG ratio by catalyzing the reduction of GSSG and producing GSH which acts as a substrate for DHAR enzyme (Noctor and Foyer 1998). GR is one of the most important enzymes in the Foyer-Halliwell-Asada pathway as it produces GSH substrate for DHAR which further produces ascorbate which acts as a substrate for APX and plays a direct role in the scavenging of H_2O_2 (Karuppanapandian et al. 2011). Hence it is clear that GR plays a regulatory role in the Foyer-Halliwell-Asada pathway (Asada 2000). Duhan et al. (2017a) studied the effect of 12 days of waterlogging on 20- and 40-day-old pigeonpea plants and observed significant increase in GR activity. Increase was comparatively higher in tolerant genotypes (ICPH-2431 and PARAS) than in sensitive ones (UPAS-120 and HO933). Twenty-day-old plants showed comparatively higher increase than 40-day-old plants. Duhan et al. (2017b) subjected four pigeonpea genotypes (ICPH-2431, PARAS, UPAS-120, and HO933) to 8 days of waterlogging and observed increased GR activity with maximum in ICPH-2431 and PARAS and minimum in UPAS-120 and HO933.

Dehydroascorbate Reductase (EC 1.8.5.1)

Dehydroascorbate reductase also plays a major role in the Foyer-Halliwell-Asada pathway as it reduces DHA and produces AA which is a substrate for APX and plays an indirect role in H_2O_2 scavenging (Sharma et al. 2012). DHAR is located in chloroplast, mitochondria, and cytoplasm. Increase in DHAR activity was observed when 20- and 40-day-old pigeonpea plants were subjected to 12 days of waterlogging. Increase was comparatively higher in 20-day-old plants. ICPH-2431 and PARAS showed higher tolerance compared to HO933 and UPAS-120 in terms of DHAR activity (Duhan et al. 2017a).

Monodehydroascorbate Reductase (EC 1.6.5.4)

Monodehydroascorbate reductase is also located in mitochondria and peroxisomes, where it plays an indirect role in the scavenging of H_2O_2 (Sharma et al. 2012). It regenerates AA and NADP from MDHA using NADPH. Increased MDHAR activity was observed when 20- and 40-day-old pigeonpea plants were subjected to 12 days of waterlogging. Increase was comparatively higher in 20-day-old plants. ICPH-2431 and PARAS showed higher tolerance compared to HO933 and UPAS-120 in terms of DHAR activity (Duhan et al. 2017a).

5.2.1.2 Non-enzymatic Components

Some types of low-molecular-weight compounds accumulate in chloroplast, cytoplasm, mitochondria, peroxisome, vacuole, and apoplast. These help in detoxification of cells by acting as substrates for various enzymes. The APX-GR system is far efficient in detoxification of H_2O_2 than CAT and POD (Dolatabadian and Jouneghani 2009).

Ascorbate Content

Ascorbic acid is found in various cell organelles, i.e., chlorophyll, cytoplasm, mitochondria, peroxisome, vacuole, and apoplast. It helps in detoxification of cells by scavenging H_2O_2 by APX reaction. It acts as a substrate for APX in its reduced form, i.e., ascorbic acid, and for DHAR in its oxidized form, i.e., DHA. Duhan et al. (2017a) observed a significant increase in ascorbate content in all the four genotypes of pigeonpea plants under 12 days of waterlogging. Increase was comparatively higher in 20-day-old plants. Tolerant genotypes ICPH-2431 and PARAS showed comparatively higher increase as compared to susceptible ones (HO933 and UPAS-120). Duhan et al. (2017b) also studied the effect of 8 days of waterlogging on four pigeonpea genotypes and recorded an increase in ascorbate content in all the four genotypes with maximum in ICPH 2431 followed by PARAS and minimum in UPAS-120 followed by HO933. Twenty-day-old plants performed better in terms of higher ascorbate content.

Glutathione Content

Glutathione is an abundant tripeptide (γ-glutamylcysteineglycine) found in virtually all cellular components such as chloroplasts, mitochondria, ER, vacuoles, and cytosol and performs multiple functions (Sharma et al. 2012). Glutathione plays a very important role in antioxidative defense system as it acts as a substrate for DHAR in its reduced form, i.e., GSH, and for GR in its oxidized form, i.e., GSSG. Anoxia or shortage of oxygen is the main condition that is created under waterlogging stress; plants can decrease their oxygen demands by lowering their respiration rate and try to survive by improving antioxidant protection through the synthesis of phenolic compounds and glutathione (Kurutas 2016). Duhan et al. (2017b) also studied the effect of 8-day waterlogging treatment on four genotypes, i.e., ICPH-2431, PARAS, UPAS-120, and HO933, of pigeonpea and recorded a significant increase in glutathione content in all the four genotypes with maximum in ICPH 2431 and minimum in UPAS-120. Duhan et al. (2017a) also recorded a significant increase in both 20- and 40-day-old pigeonpea plants under 12 days of waterlogging. Increase was comparatively higher in 20-day-old plants. Tolerant genotypes (ICPH-2431, PARAS) compared to sensitive ones (UPAS-120 and HO933) performed better in terms of higher GSH content.

5.2.2 Carbohydrate Metabolism

A major energy source used in plants is carbohydrates, and the decline or increase in carbohydrate level plays a major role in waterlogging tolerance mechanism in plants. Sugars are of two types: one is a non-reducing sugar while the other one is a reducing sugar on the basis of absence and presence of hemiacetal group. Sugars with a hemiacetal group are known as reducing sugars, while those without a hemiacetal group are the non-reducing ones. Non-reducing sugar, i.e., sucrose, is used as a transport and storage molecule due to its low chemical reactive nature, whereas reducing sugars like glucose and fructose are used as substrates in glycolysis. Waterlogging stress shuts off the aerobic pathway due to which energy supply is decreased and the glycolysis pathway remains the only pathway which produces ATP (Atwell et al. 2015). Thus the plant tries to increase the glycolytic cycle in order to complete energy demands which needs easily fermentable sugars, i.e., glucose and fructose, which can be considered as one of the adaptive mechanisms to waterlogging or oxygen-deficient environment (Fig. 5.1) (Setter et al. 1997; Xia and Saglio1992; Kumutha et al. 2008a; Sairam et al. 2009a).

5.2.2.1 Total Sugar

When plants are subjected to waterlogging, there is a significant decrease in chlorophyll content followed by arrested photosynthesis (Duhan et al. 2018) and increase in accumulated starch consumption to maintain respiration which ultimately results in carbohydrate starvation which is one of the possible reasons of hypoxia/anoxia-induced injuries (Schluter and Crawford 2001). Leakage of carbohydrates from tissue due to loss of membrane integrity can be a possible reason for decrease in sugar (Duhan et al. 2018). Kumutha et al. (2008b) studied the effect of waterlogging on four pigeonpea genotypes and observed a continuous decline in total sugar content and maximum decline was observed on the sixth day of treatment. However, tolerant genotypes, i.e., ICPL-84023 and ICP-301, performed better as compared to sensitive genotypes, i.e., ICP-7035 and Pusa 207, as tolerant genotypes maintained higher total sugar content. Duhan et al. (2017d) studied the effect of 12 days of waterlogging on total sugar content of four pigeonpea genotypes (ICPH-2431, PARAS, UPAS-120, HO933) 1, 4, and 8 days after removal from treatment and observed a significant decrease in all the four genotypes with maximum in UPAS-120 followed by HO933 and minimum in ICPH-2431 followed by PARAS. Waterlogging stress was found more deleterious when given at later stages of development, i.e., 40 days after germination. Duhan et al. (2017c) also studied the effect of 8-day waterlogging treatment on sugar content of 20- and 40-day-old pigeonpea plants and recorded a decline in total sugar content in all the four genotypes. Tolerant genotypes (ICPH-2431 and PARAS) performed better in terms of higher total sugar content compared to sensitive ones (UPAS-120 and HO933). Decrease was more in 40-day-old plants compared to 20-day-old ones.

5.2.2.2 Reducing Sugar

Waterlogging restricts the supply of oxygen to the roots hence initially decreasing it, and finally to cope up with this unfavorable condition, aerobic respiration is replaced with an anaerobic one. This causes the energy demands of plants to be greatly restricted as anaerobic respiration produces only 2 ATPs per molecule of glucose instead of 38 produced by the aerobic one (Barickman et al. 2019). Thus, for maintaining the adequate level of ATP, plants speed up anaerobic respiration which needs readily metabolizable sugars, i.e., glucose and fructose (reducing sugars). So the increase in reducing sugars can be an adaptive mechanism for providing tolerance against waterlogging to pigeonpea plants (Sairam et al. 2008; Duhan et al. 2017c, d). Kumutha et al. (2008b) subjected two tolerant (ICPL-84023 and ICP-301) and two susceptible (ICP-7035 and Pusa 207) genotypes to waterlogging and recorded an increase in the content of reducing sugars in ICPL-84023 and ICP-301 and a decrease in ICP-7035 and Pusa 207. Duhan et al. (2017c) also examined the effect of 8-day waterlogging treatment on reducing sugar content of pigeonpea root and leaves and observed that the tolerant genotype (ICPH-2431) showed higher increase in reducing sugar content as compared to the susceptible genotype (UPAS-120). The increase in the reducing sugar content of 20-day-old plant leaves was 12–24% during waterlogging treatment. Forty-day-old plant leaves recorded a comparatively higher increase (16–36%) in reducing sugar content with maximum increase in ICPH-2431 and minimum increase in UPAS-120. Twenty- and forty-day-old plant roots recorded 16–35% and 23–42% increase in reducing sugar under waterlogging treatment. Duhan et al. (2017d) also observed similar results when pigeonpea plants were subjected to 12 days of waterlogging.

5.2.2.3 Non-reducing Sugar

Sucrose is the major product of photosynthesis. It is a non-reducing sugar which is used as a transport and storage molecule in most plants due to its lower chemical reactive nature. As explained earlier, waterlogging limits photosynthesis; thus, plants accomplish their need by using stored sugar which results in decrease in non-reducing sugar content. Kumutha et al. (2008b) studied the effect of waterlogging stress on root carbohydrate levels and metabolism in four pigeonpea genotypes, i.e., tolerant genotypes (ICPL-84023 and ICP-301) and sensitive genotypes (ICP-7035 and Pusa 207). A significant decrease in non-reducing sugar content was found in all the four genotypes. Tolerant genotypes performed better in terms of less decline in non-reducing sugar. Duhan et al. (2017d) subjected four pigeonpea genotypes to 12 days of waterlogging and observed decline in non-reducing sugar content with higher decline in sensitive genotypes (UPAS-120 and HO933) and fewer decline in tolerant ones (ICPH-2431 and PARAS). Duhan et al. (2017c) also observed similar results when pigeonpea plants were exposed to 8-day waterlogging treatment.

5.2.2.4 Sucrose Synthase

Sucrose synthase (SuSy) plays a pivotal role in conversion of sucrose and UDP to UDP-glucose and fructose and helps in maintaining adequate sugar supply during anoxic stress (Barickman et al. 2019). Duhan et al. (2017c) studied the effect of 8 days of waterlogging on sucrose synthase activity of four pigeonpea genotypes, i.e., ICPH 2431, PARAS, UPAS-120, and HO933, and observed a significant increase in all the four genotypes with maximum in ICPH 2431 followed by PARAS and minimum in UPAS-120 followed by HO933. Forty-day-old plants comparatively showed lower increase in sucrose synthase activity. The effect of waterlogging on two tolerant (ICPL-84023 and ICP-301) and two susceptible (ICP-7035 and Pusa 207) pigeonpea genotypes was studied, and a significant increase in all the four genotypes with maximum in tolerant and minimum in sensitive ones was observed (Kumutha et al. 2008b). Duhan et al. (2017d) subjected four pigeonpea genotypes to 12 days of waterlogging and observed an increase in sucrose synthase activity with lower increase in sensitive genotypes (UPAS-120 and HO933) and higher increase in tolerant ones (ICPH-2431 and PARAS). From the above reports, it is clear that under hypoxic condition plants need easily metabolizable sugars to speed up anaerobic respiration and fulfill their energy requirement for survival; increased expression of sucrose synthase enzyme can be an important trait for providing waterlogging tolerance to pigeonpea plants.

5.2.2.5 Alcohol Dehydrogenase

Alcohol dehydrogenase recycles NADH to NAD, which is further used in the glycolytic pathway (Fukao and Bailey-Serres 2004; Keyhani and Keyhani 2004). The glycolytic pathway is the only source when under oxygen-deficient condition. Duhan et al. (2017d) subjected four pigeonpea genotypes to 12 days of waterlogging and observed an increase in alcohol dehydrogenase activity with higher increase in tolerant ones (ICPH-2431 and PARAS) and lower increase in sensitive genotypes (UPAS-120 and HO933). Four pigeonpea (*C. cajan*) genotypes, two of which were tolerant (ICPL-84023 and ICP-301) and two susceptible (ICP-7035 and Pusa 207), were subjected to waterlogging stress and showed an increase in alcohol dehydrogenase (ADH) activity in all the four genotypes with higher increase in ICPL-84023 and ICP-301 than ICP-7035 and Pusa 207. Duhan et al. (2017c) also examined the effect of 8-day waterlogging treatment on alcohol dehydrogenase activity of pigeonpea root and leaves and observed that the tolerant genotype (ICPH 2431) showed higher increase in alcohol dehydrogenase activity than the susceptible genotype (UPAS-120). Forty-day-old plants showed comparatively fewer increase than 12-day-old plants. On the other hand, roots showed higher increase than leaves.

5.3 Anatomical Adaptation

Besides biochemical adaptation, plants also undergo some anatomical adaptations such as aerenchyma formation to survive under stress condition.

5.3.1 Aerenchyma Formation

Waterlogging stress suppresses the root growth in sensitive genotypes. However, the tolerant genotypes undergo normal growth of roots under the stress to some level. Generally the plant shows growth of seminal roots and produces a large number of cavities in the root cortex which are called aerenchymas when subjected to waterlogging (Hossain and Uddin 2011). Continuous gas-filled channels or much enlarged gas spaces are called aerenchymas. Under waterlogged conditions, roots are unable to respire aerobically because these are surrounded with water and cannot take oxygen from the environment so the plant develops cavities in the root cortex which makes oxygen movement from cell to cell easy and through these cavities oxygen from shoots is transferred to roots easily (Nishiuchi et al. 2012). Aerenchyma tissue in roots reinstates the supply of oxygen, and as a result, roots start to respire aerobically. Moreover, through aerenchyma, some oxygen is transferred to the soil around roots which allows the growth of some useful microorganisms which block the entry of poisonous substance to roots (Visser et al. 1997; Colmer 2003). Therefore, aerenchyma formation is necessary for the tolerance to hypoxic or anoxic stress (Fig. 5.1). Formation of aerenchyma as a response to hypoxia is a trait of many plant species (Thomas et al. 2005). Increased ethylene production under waterlogging stimulates aerenchyma formation in roots (Yamauchi et al. 2015). Duhan and Sheokand (2016) observed aerenchyma formation in 20-day-old pigeonpea plant roots 1 and 8 days after removal from 8-day waterlogging treatment and 1 day after removal from 12-day waterlogging treatment. Tolerant genotypes (ICPH 2431 and PARAS) showed more aerenchymas compared to sensitive ones (UPAS-120 and HO933). Similar results were observed in 40-day-old pigeonpea plants 1 day after removal from 8-day waterlogging treatment (Duhan et al. 2017a).

5.4 Conclusion

When pigeonpea plants are experiencing waterlogging stress, a situation of hypoxia or anoxia is created around the roots which is identified as the main cause of damage to plants as it affects the respiration which causes an energy crisis because aerobic respiration is replaced by an anaerobic one which only produces 2 ATPs per glucose molecule instead of 38 ATPs produced by the aerobic one. So, the plants try to complete their energy needs by increasing the rate of anaerobic respiration by increasing the production of readily metabolizable sugars. As sucrose synthase (SuSy) converts sucrose and UDP to UDP-glucose and fructose and produces easily fermentable sugar, the amount of root sugar reserve and activity of sugar-hydrolyzing enzyme, i.e., sucrose synthase, have been identified as important trait for providing waterlogging tolerance to pigeonpea plants. The products of anaerobic respiration, i.e., ethanol and lactate, are also hazardous to plants. But ethanol rather than lactic acid is less hazardous, so increased expression of ADH enzyme is also identified as the second important trait for providing waterlogging tolerance to pigeonpea plants because it favors ethanol production instead of lactate. Besides carbohydrate metabolism, oxidative metabolism is also affected by waterlogging as it produces ROS which cause damage to membrane DNA and nucleic acid; to counter this effect, the plant produces antioxidative enzymes like SOD, CAT, APX, POX, MDHAR, and DHAR and antioxidative metabolites such as ascorbate and glutathione. So, increased expression of antioxidative enzymes and metabolites is also considered as one of the important traits for providing waterlogging tolerance to pigeonpea plants. By developing adaptations like increased anaerobic metabolism, the plant can survive for a limited time period by reinstating the oxygen supply through developing cavities in the root cortex for movement of oxygen from shoots to roots which are known as aerenchymas. Aerenchymas help roots in respiring aerobically and maintaining growth even under hypoxic conditions. Therefore, aerenchyma formation is also considered to be an important morphological adaptation for providing waterlogging tolerance to pigeonpea plants.

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Chapter 6
Mechanisms of Plant Adaptation and Tolerance to Metal/Metalloid Toxicity

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Contents

Abstract Metal/metalloid (HM) toxicity/stress has become a worldwide menace due to the rising accretions in water, soil, and air which lead to detrimental effects in plants. The general consequences of HM toxicity include oxidative injury which causes polypeptide oxidation, lipid peroxidation, enzyme inactivation, DNA mutilation, and/or alteration of other key components of plant cells. To limit the hazardous effects of HMs and their accumulation, plants have evolved detoxification

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instruments to preserve physiological accumulations of essential metal ions and to reduce the concentration of non-essential HMs. Such mechanisms are mainly based on compartmentalization and chelation by phytochelatins, amino acids, organic acids, glutathione, metallothioneins, etc. Also, toxicity induced by HM can be compensated by decreasing the uptake of it by reduction/alteration of membrane-bound transporters. In addition, plants can be conferred tolerance by sophisticated antioxidant defense machinery, glyoxalase system, and signal transduction pathways induced by HM. In the last decade, studies on omics have revealed many of the genes or their products that have been implicated in these mechanisms, offering the tools for the improvement of crops. Hence, it became vital to integrate recent understanding of different pathways involved in physiological and biochemical processes of HM-stimulated stress responses and adaptation and tolerance in plants on the basis of findings of current molecular biology research.

Keywords Adaptation mechanisms · Heavy metal · Oxidative stress · Signal Transduction · Tolerance mechanisms

6.1 Introduction

In the last few decades, due to rapid industrialization and urbanization, enhanced human practices, extensive mining, and day-to-day agricultural activities, there is enhanced contamination of heavy metal(s) (HMs) in the environment, responsible for the disturbances in the biogeochemical cycle, thus affecting all forms of life (Singh et al. 2015; Chandrakar et al. 2016a). The term HM refers to the elements having a specific gravity of more than five, but in plant physiology, this includes a range of metals/metalloids which are toxic to plants (Xalxo and Keshavkant 2019). This includes arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), zinc (Zn), etc. (Hossain et al. 2012). Contamination of HMs in the agricultural soil is a serious problem as they alter the key physiological and metabolic processes of plants (Rughani et al. 2016). In plants, toxicity generated by HMs depends on many factors such as concentration, exposure time, oxidation state of the metals, and species and developmental stage of plants (Singh et al. 2015). Some of the symptoms of HM toxicity in plants are reduced and/or inhibited germination and growth, low biomass accrual, wilting, chlorosis, reduced rate of photosynthesis, imbalance in uptake and transport of water and nutrients, disturbance in the membrane integrity, disruption in the functionalities of proteins and enzymes, oxidative stress, and senescence, which consequently cause death to plants (Chandrakar et al. 2016b; Xalxo and Sahu 2017).

Increased exposures of plants to HMs are associated with the oxidative stress via elevated generation/accumulation of methylglyoxal (MG) and reactive oxygen species (ROS). The major ROS are singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2) that cause perturbations in the homeostasis between the generation and sequestration of ROS in the cellular compartments (Rughani et al. 2015; Chandrakar et al. 2017a). This circumstance is associated with the causation of multiple deteriorative effects such as lipid peroxidation, binding with the sulfhydryl groups, disruption in the membrane structure, ion leakage, redox imbalance, inactivation of the antioxidant defense system, and oxidation of amino acids, proteins, and DNA (Yadu et al. 2016, 2019). It is well established that plants are equipped with various inherent and extrinsic defense strategies for neutralizing/tolerating cytotoxic MG and ROS or detoxification of HMs whenever confronted with the stressful situation (Yadu et al. 2018a). Also, to get rid of the deleterious effects of several abiotic stresses, plants synthesize a number of metabolites as a cellular metal detoxification approach (Chandrakar and Keshavkant 2018a).

To combat with abiotic stress-induced oxidative injury, plant cells acquire a complex system of antioxidant protection mechanism (Fig. 6.1). There are enzymatic and non-enzymatic constituents that defend the stressed cells against unfavorable circumstances (Yadu et al. 2017a). Enzymatic components involve superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione-*S*-transferase (GST), and so on (Hossain et al. 2012), whereas non-enzymatic components are ascorbic acid (AsA), glutathione (GSH), organic acids, amino acids, α-tocopherol, carotenoids, flavonoids, etc. (Chandrakar and Keshavkant 2018a).

Therefore, this review focuses its glance on HM tolerance mechanisms of the plants possessing multitude components for the regulation of HM-induced ROS and MG homeostasis (Fig. 6.1). Key components of this mechanism are extracellular and intracellular chelation and sequestration that can either eliminate the HM or regulate the route of essential metals to their specific target sites in the cell. This indicates mutual pathways that help in decreasing the deleterious effects of excessive presence of HMs. These mechanisms are also associated with the chaperones and signal trans-

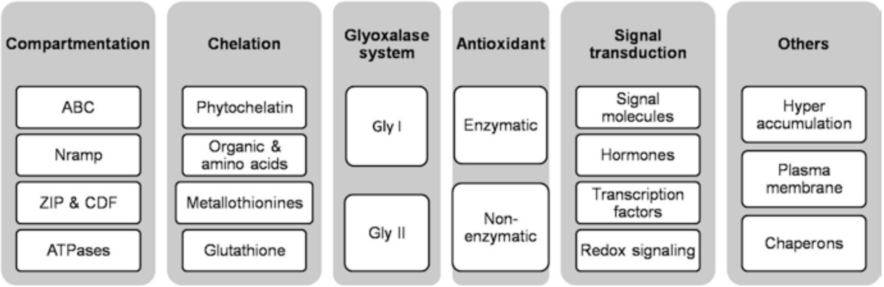

Fig. 6.1 Generalized scheme showing the mechanisms of plant adaptation and tolerance to metal/metalloid toxicity

duction mechanisms prompted by HMs. Nevertheless, there are precise strategies and some of them will be addressed with special focus on the cellular level.

6.2 Tolerance Mechanisms

6.2.1 Compartmentalization by Various Transporters

Transporters play a very significant role in the clearing of HMs by translocating them into the extracellular space or cellular compartments where chelation and sequestration occur. In plant cells, the major transporters involved in HM tolerance are HM ATPases, cation diffusion facilitators, natural resistance-associated macrophage protein (Nramp) transporters, ZRT- and IRT-like protein (ZIP) transporters, and ATP binding cassette (ABC) transporters (Latowski et al. 2018). The P1B-ATPases, also known as HMATPases, are energized by ATP hydrolysis and help in the transportation, compartmentalization, and detoxification of HMs from cytoplasm to plasma membrane or vacuole. Cation diffusion facilitators also called as metal tolerance proteins are involved in maintaining homeostasis of cellular HMs specifically cations like Fe^{2+}, Mn^{2+}, and Zn^{2+} (Podar et al. 2012). Occurrence of HMs in the water and soil increases the gene expression of this transporter. Another transporter, Nramp, is principally found in the plasma membrane of root endodermis and belongs to the extremely conserved group of integral membrane proteins (Latowski et al. 2018). Various cationic HMs such as Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and $Zn^{2+}$are actively translocated inside and/or outside the cells via this transporter, hence helping in the detoxification of these HMs. Transporters of ZIP family can localize the HMs from organelles or from the extracellular spaces into the cytoplasm. ABC transporters, which are another important group of transporters, localized in the tonoplasts of the cells are responsible for the transportation of complex formed by phytochelatins (PCs) and HMs into the vacuoles, where sequestration of HMs takes place (Chandrakar et al. 2016b).

6.2.2 Chelation

6.2.2.1 Extracellular

Mycorrhiza

Mycorrhiza is a symbiotic association of a fungus with a plant, growing on contaminated site, which helps in reducing HM toxicity in host plant. The mechanisms involved in detoxification of HMs by the ectomycorrhizal associations may be either intracellular or extracellular. Extracellular mechanisms include restriction in the entry of HM ions and cell wall binding, whereas intracellular system comprises binding of HMs to non-protein thiols and their transport into intracellular compartments. The mechanism of binding of HMs with the cell wall and their precipitation by the excreted substances is called as biosorption. The cell wall of fungus consists of polymers of chitin, glucan, and galactosamine and a trace of protein. Hence, several potential-binding positions remain available on the fungal cell wall due to the presence of free hydroxyl, carboxyl, phosphate, amino, and mercapto groups. Among different cell wall components, the existence of melanins also magnifies the biosorption of HMs and their binding strength. Fungal cells excrete different organic molecules such as di- and tricarboxylic acids to chelate HMs. Additionally, the fungus also strengthens the antioxidative detoxification systems in counteracting HM-induced accumulation of ROS directly or indirectly. The hyphal sheath of fungus absorbs the HMs and decreases their entry into the apoplast because of fungal exudates binding the HMs, lack of water affinity of the fungal sheath, and surface assimilation with the external mycelium (Hall 2002).

Cell Wall and Root Exudates

In plants, the interface between cell wall and plasma membrane serves as potential site for the accumulation of HMs (Hossain et al. 2012). The cation exchange capacity is determined through the presence of exchange sites in their cell walls. Polygalacturonic acid acts as a cation exchanger and is present in the pectins of cell walls. The HMs make complexes with the carboxylic groups of polygalacturonic acid thus preventing their entry into the cells (Dalvi and Bhalerao 2013). Masion and Bertsch (1997) demonstrated that tolerant plants have higher cation exchange capacity than that of sensitive cultivars; hence they can bind the HMs at the cell wall and restrict their access inside the cell. In the cell wall, the presence of extracellular carbohydrates, pectic sites, and histidyl groups is responsible for the immobilization of HMs hence restricting their uptake by the plants (Dalvi and Bhalerao 2013). It is well known that the chemical properties of the cell walls are responsible for the modification of their capacities to take in HMs. Due to the presence of fewer absorption sites in the cell wall, it has little influence in the detoxification of HMs. The key mechanism of HM tolerance by the cell wall is not yet understood completely.

Plant roots release several diffusates (such as inorganic ions, amino or organic acids, sugars, water, etc.), secretions (like siderophores, mucilage, allelopathic compounds, etc.), and excretions (such as protons, bicarbonates, carbon dioxide, etc.) which are collectively called as root exudates that boost up the plant's tolerance to various HMs. These excretions bind the HMs in the vicinity of the roots that makes the HMs unavailable for their entry into the cells thus decreasing the toxicity (Dalvi and Bhalerao 2013). Also, some of the root exudates increase the pH of the rhizosphere that is favorable for the precipitation of HMs and, hence, restricts their bioavailability.

6.2.2.2 Intracellular

After entering into the cell, HM immediately bounds to an appropriate cellular compound in the cytoplasm. A ubiquitous mechanism for the detoxification of HM is the ligand chelation with the metal ion (Viehweger 2014). This avoids free cellular metal ions thereby helping in the detoxification process. Well-known ligands for metal ions are PCs, organic acids, amino acids, GSH, and metallothioneins (MTs) (Sharma et al. 2012; Sytar et al. 2013).

Phytochelatins

The chelating peptides known as PCs are an important class of HM-binding ligands and are crucial for HM detoxification (Chandrakar et al. 2016b). Their general structures consist of repetitive units of γ-glutamyl cysteine. In the cytosol, syntheses of PCs are rapidly induced upon exposure of HMs, except for Co and Mn. They are synthesized from chain extension of GSH by the enzyme PC synthase (PCS) (Das and Roychoudhury 2014). This is a constitutively expressed cytosolic enzyme whose activity is controlled post-translationally as PC-mediated chelation of HM is required for enzyme activity (Hossain et al. 2012). With the cysteine thiol group, PCs chelate HMs which results in the formation of HM-PC complexes with a molecular weight of 2.5–3.6 kDa which are sequestered into the vacuoles (Cobbett 2000). These complexes are either low-molecular-weight (LMW) or high-molecular-weight (HMW) complexes. The former one serves as transit for transferring HM ions from cell plasma to vacuole via ABC transporters where the extrasulfides are integrated to form the latter complex (Yadav 2010). Apart from this transportation, HMs can also get inside the vacuoles through direct interchange with protons, by proton/cation exchange (CAX) transporters located in the vacuolar membranes (Hall 2002). Hirschi et al. (2000) reported that increased expression of *AtCAX2* gene enhanced the transportation of Cd^{2+} and Mn^{2+} in the tonoplast vesicles of *Nicotiana tabacum* root. Because of the high affinity for metals, PCs are considered to be involved in the regulation and maintenance of essential metals such as Cu and Zn and in the detoxification of HMs (Yadav 2010). The increased transcript levels of *AtPCS1* and *CePCS* genes enhanced the PC level that mediated increased detoxification potential of *N. tabacum* plants under HM stress (Wojas et al. 2010).

Organic Acids and Amino Acids

The presence of sulfur, nitrogen, and oxygen in organic acids and amino acids is responsible for their reactive interactions with HM ions; hence they are important ligands involved in metal chelation. Mechanisms behind the detoxification and tolerance of HMs in the plants by organic acids are categorized as external elimination and internal resistance. In the first category, plant roots excrete organic acids which form stable complexes with HM. This binding changes the mobility and bioavailability of

HMs, thus preventing them from entering the cells and also reducing their accumulation in the roots. In the second mechanism of HM detoxification, chelation of HMs by organic acids takes place in the cytosol (Hossain et al. 2012).

Organic acids such as carboxylic, sulfonic, citric, oxalic, and malic acids have well-known HM-binding capacity and are found to be very crucial for providing resistance to HMs (Hall 2002; Viehweger 2014). Sulfonic acids contain sulfur in the form of SO_3^{3-} that is also present in sulfolipids, glucose-6-sulfonate, and cysteic acid, which are also implicated in HM tolerance (Sytar et al. 2013). Also, malic acid and oxalic acid are important parts in the HM tolerance mechanisms via transporting metal ions through xylem sap and metal ion sequestration in vacuoles (Hall 2002). Citric acid is a well-known ligand for Cd and Al, hence providing tolerance to the plants (Rauser 1999). Also, HM-mediated secretion from the roots contains organic acids that help in the detoxification of HMs (Hall 2002).

Amino acids (histidine, proline, and nicotianamine) are also potential ligands for HMs and are very important in tolerance and detoxification processes. The histidines present in the root exudates formed during abiotic stress play a role in the detoxification of HMs (Hall 2002). Accumulation of aromatic amino acids under oxidative stress is a reflection of the activation of shikimate pathway for the syntheses of phenolic and indolic compounds that play key roles in strengthening the defense system of the plants (Noctor et al. 2015).

Proline is an osmolyte and serves as an effective antioxidant stimulator and can prevent lipid peroxidation reaction (Das and Roychoudhury 2014). It is also involved in stress adaptation, recovery, and signaling processes (Chandrakar et al. 2017b). It serves as a protein and membrane stabilizer and is a source of energy which helps the plants to recover during post-stress conditions (Xalxo and Sahu 2017). Additionally, it also acts as a cell wall plasticizer and prevents the enzymes from denaturation caused by HM chelation with the thiol assemblies of proteins and enzymes (Chandrakar and Keshavkant 2018b). It is a direct scavenger of ROS hence lowering the oxidative injury in plants (Yadu et al. 2016). In the cellular proline-proline cycle, initial abstraction of hydrogen occurs by proline and occupies one •OH, followed by abstraction of hydrogen thereby occupying another •OH and forming pyrroline-5-carboxylate. This pyrroline-5-carboxylate again comes to proline by the action of pyrroline-5-carboxylate reductase (Gill and Tuteja 2010). Furthermore, proline is also involved in the formation of specific PCs for binding of HMs, thus decreasing their accumulation (Chandrakar et al. 2017a). Exogenous application of proline in the growing seedlings showed tolerance against oxidative stress by lowering lipid peroxidation as well as protein oxidation observed after exposure to salt (Agami 2014), Cd (Rasheed et al. 2014), As (Chandrakar et al. 2018), etc.

Glutathione

Another LMW tripeptide and the most abundant chelating agent is GSH (GSH, reduced form, and GSSG, oxidized form). The equilibrium between these two forms is a crucial factor in sustaining the regulation of redox homeostasis in the cell

(Yadu et al. 2018b). Moreover, GSH is necessary for the maintenance of normal reduced cellular state essential for the neutralization of deleterious impacts of HM-induced overproduction of ROS (Yadu et al. 2019). In the reduced state, donation of hydrogen occurs from the cysteine residues of thiol groups of GSH through thiol-disulfide exchange to ROS and proteins which converts the GSH into radical form and binds to the next GSH radical and generates its oxidized form GSSG. A substantial proportion of this GSSG is sequestered in the vacuole, where it escapes the action of GR; hence, its level increases during stress (Noctor et al. 2015). It is found in all the cellular parts including apoplast, cytoplasm, endoplasmic reticulum, mitochondria, chloroplasts, vacuoles, and peroxisomes (Gill and Tuteja 2010). The GSH synthesis reaction is completed in two different ATP-dependent pathways; in the first step, a peptide bond is formed between the carboxyl group and amino group of glutamate and cysteine, respectively, and forms γ-glutamyl cysteine catalyzed by γ-glutamyl cysteine synthetase (GSH1). In the next step, ligation of γ-glutamyl cysteine with a glycine residue takes place by GSH synthetase (GSH2) to form GSH (Yadav 2010).

In plants, the GSH is involved in regulating myriad cellular processes, including developmental processes like cell division and flowering, sequestration of HMs, defense against ROS and MG, syntheses of proteins and nucleic acids, signal transduction, conjugation of metabolites, xenobiotics cleaning, protection of proteins against denaturation, and regulation of expression of stress-responsive genes (Yadav 2010; Hossain et al. 2012). Additionally, it is also a major storage form of and carrier for reduced sulfur. Furthermore, GSH is crucial for plant's antioxidant defense mechanism, stimulating the formation of other potential components like AsA, through the AsA-GSH cycle, and α-tocopherol to protect the cellular membrane assembly (Karuppanapandian et al. 2011; Hossain et al. 2012). Also, GSH serves as a substrate for antioxidant enzymes, POX and GST, which are also responsible for the elimination of ROS, thereby preventing oxidative injury (Sharma et al. 2012). The thiol assembly of GSH makes it appropriate to form complexes with HMs, and formation of these complexes is necessary for inducing PC synthesis (as discussed earlier) that is involved in the sequestration of HM ions (Hall 2002). Also, GSH is involved in oxidative stress signaling via formation of protein disulfides, *S*-glutathionylation, or *S*-nitrosylation. In the apoplast, signaling of Ca^{2+} is also driven by increased level of GSH under stressed conditions (Noctor et al. 2015). In plants, genetic manipulation of GSH-related genes, such as *GSH1*, *GSH2*, and *GR*, has been found to be a potential strategy for enhancing tolerance to HM stress by regulating GSH level.

Metallothioneins

Metallothioneins are ubiquitous LMW (approx. 8 kDa) cysteines containing (20–30%) metal-chelating proteins having highly flexible structures (Sytar et al. 2013). Because of this flexibility, MTs have different coordination geometries and folding for binding of different HM ions (Viehweger 2014). The biosynthesis of

MTs is regulated at the transcriptional stage and can be triggered by various stimuli such as hormones like ethylene and abscisic acid, heat shock, cold and salt stress, cytotoxic agents, wounding and virus infection, sucrose starvation, and HMs (Yan et al. 2017). These sulfur-containing proteins play a key role in essential metal homeostasis and HM detoxification by balancing redox cellular state, sequestration of HMs, metabolism of metallo-drugs, regulation of metalloenzymes and transcription factors (TFs), and responses to stressed environments (Gautam et al. 2012; Ovečka and Takáč 2013). Also, MTs are potential ROS trappers. Due to the presence of large quantities of nucleophilic thiol in their assembly, MTs serve as a suitable nucleophilic "sink" for the entrapment of electrophiles and free radicals such as $O_2^{\bullet -}$ and •OH, leading to their scavenging. Furthermore, MTs are also recycled with GST through thiolate interchange (Sharma et al. 2012).

The MT proteins can be commonly categorized as mammalian class I and plant class II proteins. Plant MT genes are again divided into four classes, out of which type 1 *MT* genes are mainly responsible for HM tolerance in plants (Li et al. 2016). Type 1 MTs can potentially bind with physiological (Cu, Zn, and Se) and xenobiotic (As, Cd, Ag, and Hg) HMs via thiol groups of cysteine molecules in them (Gautam et al. 2012). In addition, MTs exchange Zn with Zn transporter (ZnT1), SOD, chelator ethylenediaminetetraacetic acid, and other Zn proteins. These metal relocation mechanisms should be a keystone for the dual roles of MTs in HM detoxification and donating the essential metals. Overexpression of MTs genes generally confers increased HM tolerance to plants (Ovečka and Takáč 2013). Several studies have showed that in plants, the MT genes can be upregulated by the exposure of HMs, and the upregulation of these genes might have a function in the HM elimination and also in the alleviation of oxidative injury (Gautam et al. 2012; Nguyen et al. 2014). Chen et al. (2014) found that under Cd stress, in the leaves of *Kandelia obovata*, there was an upregulation in the expression of type 2 MT genes.

6.2.3 Hyperaccumulation

Hyperaccumulation is one of the HM tolerance mechanisms under which plants accumulate HM ions at levels 100-fold more than that of non-accumulators (Dalvi and Bhalerao 2013). For hyperaccumulation of HMs, plants should have the capability to solubilize metals available in the soil and should be able to uptake metals via specific ion transporters and detoxify them by the process of chelation, sequestration, and compartmentalization. A plant is categorized as hyperaccumulator of HMs on the basis of the following four criteria: (1) transfer factor or shoot/root quotient, i.e., quantity of HM measured in shoot divided by its quantity in the root must be greater than 1; (2) extraction coefficient, i.e., quantity of HM measured in shoot divided by its total quantity in the growth medium must be greater than 1; (3) greater amount of HMs, i.e., 100 times the amount present in normal or uncontaminated plants; and (4) accrual of metals beyond a threshold value of 1% or 10,000 mg kg^{-1}(Zn and Mn), 0.1% or 1000 mg kg^{-1}(Ni, Co, Cr, Cu, Pb, and Al), and

0.01% or 100 mg kg^{-1} (Cd and Se) of the total dried biomass of plants (Dalvi and Bhalerao 2013).

6.2.4 Plasma Membrane

In root cells, the plasma membrane is the first physiological barrier of plants during uptake of HMs into the symplast. The metal ions serve as a crucial component for biological membrane structures and are also important for maintaining the specific electrochemical potentials in the cell organelles (Ovečka and Takáč 2013). The presence of HMs leads to peroxidation of lipid moieties of plasma membranes, loss of some of the essential ions, alterations in the membrane permeability, and uptake of ions which leads to imbalance in the electrochemical potential of the cells (Liptáková et al. 2013). The impacts of divalent metal cations in unbalancing the potential of the plasma membrane may depend on the concentration, mode of action, plant species, and affinity for various cell wall and membrane components. Metal ions such as Cd and Zn have been reported to induce serious and continuous depolarization in root cells' plasma membrane (Ovečka and Takáč 2013). Different plant species have varied potential for accumulation and sequestration of different HMs.

The active efflux of the HMs lowers their intracellular concentrations to subtoxic levels; hence, it is a commonly applied strategy to achieve tolerance (Reichman 2002). The transporters, namely, P1B-ATPases that are from P-type ATPase group and ABC transporters, are utilized in the form of HM efflux pumps in plants. The two sub-families, namely, MRAP and pleiotropic drug resistance of ABC transporters, are mainly functional in the sequestration of chelated HMs (Dalvi and Bhalerao 2013).

6.2.5 Chaperones

Chaperones are a special type of proteins which are associated with intracellular trafficking and release of HMs to enzymes such as Cu-ATPases and SOD. Heat shock proteins (HSPs) are molecular chaperones involved in normal folding, protection, assembly, translocation, and maintenance of proteins under stressed conditions (Hall 2002). Moreover, HSP70 has essential functions in stabilizing the proteins, in preventing unwanted aggregation of protein, and in assisting refolding of non-native proteins under both normal and stressed situations. The expression of HSP genes increased under high temperatures for providing tolerance to the organisms. The pathway for intracellular Cu trafficking comprised of its transporter, pump, and chaperone; thus, chaperones make a great contribution in the transportation and detoxification of HMs (Robinson and Winge 2010).

In plants, many evidences have been presented for the elevated expression of HSP in the presence of HMs. Goswami et al. (2010) stated that at the mRNA and protein stages, HSP70 gene was overexpressed under As and heat stress. This effect was cumulative and increased with the duration of stress in *Oryza sativa*. Also, Zhang et al. (2017) revealed that HSP70 expression was increased in *Lemna minor* grown in the presence of HM. Elevated levels of HSP70 might cooperate in the re-establishment of the conformation of damaged proteins or to facilitate the degradation of oxidized/denatured proteins.

6.2.6 Crosstalk Between Oxidative Stress and Tolerance

One of the phytotoxic impacts of HMs is the generation of ROS which causes oxidative stress resulting in disturbed cellular redox status. Various distinctive ROS are generated during normal oxidative metabolism in any aerobic life forms, yet these ROS can pose a serious threat when generated in large amounts (Chandrakar et al. 2018). Exposure of HMs induces generation of ROS inside the plants and such generation relies on magnitude of the stress, repeated stress periods, and plant age and species. Due to these ROS, oxidative degradation of polyunsaturated fatty acids (PUFAs) of membrane lipids occurs (Yadu et al. 2017b). However, the generation of ROS is limited under normal conditions, but exposure to HMs accelerates their production. To defend from these harmful oxygen intermediates, plant cells and its compartments have defense systems. The components of plant defense system are grouped as enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants are SOD, CAT, POX, and members of the AsA-GSH cycle like APX, MDHAR, DHAR, and GR, whereas non-enzymatic antioxidants include AsA, GSH, α-tocopherol, carotenoids, and phenolic compounds (details are given in other sections of this chapter).

6.2.7 Glyoxalase System

The glyoxalase system, localized in the cytoplasm and mitochondria of the cells, is one of the crucial components and major pathways engaged in the detoxification of MG and increasing plants' adaptation to oxidative injury (Hasanuzzaman et al. 2017a). The two important enzymes of this system are glyoxalase I (Gly I, lactoyl GSH lyase) and glyoxalase II (Gly II, hydroxyacyl GSH hydrolase). These two enzymes use GSH as a cofactor and convert the MG and other aldehydes into less toxic 2-hydroxyacids (Hossain et al. 2012; Yadu et al. 2018b). Many researchers have reported that enhanced or overexpressed Gly I and/or Gly II reduced the amount of endogenous MG and awarded tolerance to plants against several environmental stresses (Hossain et al. 2012; Hasanuzzaman et al. 2017b; Yadu et al. 2018b).

During severe HM stress, the level of MG gets exaggerated which further results into the oxidative stress inside the plant cells. Several scientists have used a variety of plant regulators to counteract the HM stresses and observed that use of regulators boosted or upregulated the enzymes of the glyoxalase system and guarded against MG or MG-impelled oxidative injury inside the cells (Hossain et al. 2012; Rahman et al. 2016). For instance, Hossain et al. (2012) found that when seedlings of *Cucurbita maxima* were exposed to Cd, it resulted in increased amount of MG and upregulated activity of Gly I enzyme. However, when similar concentration of Cd was applied to the tissues of *Vigna radiata*, it led to a minor increment in Gly I activity with decreased activity of Gly II and high accretion of MG. However, when proline or glycine betaine was applied exogenously, significant lessening of MG amount was detected. Rahman et al. (2016) reported that because of the ineffective activities of two enzymes of the glyoxalase system under Cd stress, the *O. sativa* plants showed higher MG content, while co-exposure to $MnSO_4$ upregulated these two enzymes, thereby diminishing the MG content. Nahar et al. (2016) reported that under Cd stress there was significant accumulation of MG in *V. radiata* seedlings, with more Gly I and less Gly II activities. Conversely, co-application of spermine (0.25 mM) and $CdCl_2$ for 24 h in *V. radiata* tissues lessened the content of MG with a minute elevation in Gly I and significant elevation in Gly II activities. Hence, the above results disclosed that, when plants were exposed to HM stresses, the level of MG got increased in response to that, and consequently the glyoxalase system to their best initially helped in the detoxification of MG; however, it became weak or incapable under intense stress (Hossain et al. 2012; Hasanuzzaman et al. 2017a). However, to overcome these problems, the exogenous application of diverse protectants can perform well, up to an extent, by regulating the increased activities of two enzymes, thus initiating the prospect of upregulating the content of GSH and ratio of GSH/GSSG through the glyoxalase system. High content of GSH further facilitates the syntheses of the PCs, and this leads to the sequestration of the HM-PC complex into the vacuoles (Hasanuzzaman et al. 2017b).

6.2.8 Antioxidative Mechanisms

Plants possess various stratagems to guard themselves against the stressful conditions triggered in response to excessive presence of HMs, for tolerance or detoxification. The foremost step toward dealing with metal detoxification is limiting the intake of metals from soil, thereby inhibiting the entrance in the plant root (Chandrakar et al. 2016b; Xalxo and Keshavkant 2019). Some of the mechanisms, like HM entrapment by mycorrhizal association, compartmentalization/sequestration of metals, etc., are involved to achieve the first step of HM restriction (Jutsz and Gnida 2015). If these mechanisms are unsuccessful, and somehow HMs succeed in getting inside the roots, then at the next stage, the defense system to detoxify HMs gets stimulated including biosyntheses or accumulations of osmolytes and osmoprotectants like proline and intracellular complexation/chelation/compartmentalization of metal ions by liberation of various molecules, viz., organic acids, PCs, polysaccharides, and MTs, and eventually if all these measures fail, then the HM toxicity gets increased in the plants resulting into the activation of antioxidant defense mechanisms that are comprised of enzymatic and non-enzymatic antioxidants (Chandrakar et al. 2016b; Xalxo and Keshavkant 2019).

6.2.8.1 Enzymatic

A diverse range of enzymatic antioxidants comprising SOD, POX, CAT, APX, GST, etc., which may efficiently dismutate $O_2^{\bullet-}$ into H_2O_2 and subsequently into H_2O and O_2, are available in the plants (Chandrakar et al. 2018; Yadu et al. 2018a). The major task of these antioxidants is to quench or eradicate a number of harmful oxygen derivatives and help in the cellular protection from oxidative injury. The quenching mechanism of these enzymes differs on the basis of their site at various cell organelles. So, SODs are a group of metalloenzymes that convert $O_2^{\bullet-}$ into H_2O_2, whereas CAT, POX, and APX are responsible for the breakdown of H_2O_2 into H_2O and O_2. Likewise, GST is another enzymatic antioxidant that is proficient to catalyze the ligation of various electrophilic substrates through GSH (Gill and Tuteja 2010; Chandrakar et al. 2016b; Yadu et al. 2018b).

The indigenous high antioxidant level or upliftment in antioxidant potential might check HM-impelled oxidative damage and can increase tolerance to the oxidative injury established. Dong et al. (2006) examined the toxic impacts of Cd in the activities of antioxidants in *Lycopersicon esculentum* seedlings. Their results revealed that activities of POX and SOD elevated markedly in those which were treated with Cd (1–10 μM), suggesting that oxidative damage was due to Cd treatment. Shi and Zhu (2008) reported remarkable upsurge in ROS contents as well as activities of SOD, CAT, APX, and POX in the leaves of *Cucumis sativus* exposed to Mn. Likewise, de Oliveira et al. (2013) stated that POX and APX activities were elevated in both the leaves and roots of Fe-exposed *Euglena uniflora*. Augmentation in the activities of these antioxidative enzymes clearly suggested that they are involved in the regulation of ROS generation under Fe toxicity. Khatun et al. (2008) reported increased APX, GST, and POX activities in Cu-stressed *Withania somnifera* leaves signifying that these enzymes are crucial in defending the plants exposed to Cu. Similarly, Chen et al. (2015) stated that in Cu-stressed *Bambusa vulgaris* higher activities of SOD and POX help in the adaptation of plants from oxidative injury; however, intense Cu exposure (>25 μM) resulted in huge accumulation of ROS consequently inhibiting enzyme activity.

During higher Hg stress, Anjum et al. (2014) determined increased CAT and APX activities, whereas POX was found to be inhibited in *Juncus maritimus*. Chandrakar et al. (2018) have recently been observing decreases in SOD, CAT, and APX activities under As stress in *Glycine max*. In contrast, elevated CAT activity was noted in As-tolerant *Pteris vittata* as compared to As-sensitive *Pteris ensiformis* and *Nephrolepis exaltata* (Srivastava et al. 2005). Alaraidh et al. (2018) and Xalxo and Keshavkant (2019) have observed that Pb toxicity also raised the activities of SOD and CAT in *Trigonella foenum-graecum* seedlings.

6.2.8.2 Non-enzymatic

Non-enzymatic antioxidants are AsA, GSH, proline, flavonoids, α-tocopherol, alkaloids, carotenoids, etc. that may directly detoxify the ROS (Yadu et al. 2016). Both AsA and GSH are water-soluble non-enzymatic antioxidants involved in the plant's protective system against oxidative damage and are chiefly present in chloroplasts and also in other cell organelles (Noctor and Foyer 1998). Ascorbic acid is one of the most potent antioxidants because it can transfer its outer electrons in a wide range of enzymatic and non-enzymatic reactions. Also, it has the ability to scavenge $^1O_2$, $O_2^{\bullet-}$, and •OH radicals directly and can reduce H_2O_2 to water and oxygen via APX activity (Yadu et al. 2016).

GSH (γ-glutamyl cysteinyl glycine), another non-enzymatic antioxidant and a tripeptide, is abundantly present in plant cells. It is the key component of cellular defensive mechanism against HMs and oxidative stress and plays a vital role in growth and development of plants. Also, it constitutes the basics of AsA/GSH cycle, involved in the scavenging of H_2O_2 (Noctor and Foyer 1998; Gill and Tuteja 2010). Both GSH and its oxidized form GSSG regulate the redox equilibrium in the cellular parts. It also plays an important function in the defensive mechanism by restoring extra powerful water-soluble antioxidant AsA through AsA/GSH cycle (Noctor and Foyer 1998). Also, GSH is considered as an important and predominant non-protein thiol, and the main pool of non-protein reduced S helps in the binding of metal ions. It is also involved in the synthesis of PCs; thereby it is involved in providing tolerance toward HM stress to the plants. Mostly, the toxic HM ions are initially chelated by GSH followed by their transportation to PCs for subsequent sequestration and compartmentalization. However, GSH is considered as the first line of protection against HM toxicity by chelating metals prior to the induced syntheses of PCs attaining effective levels (Bhaduri and Fulekar 2012).

The carotenoids and α-tocopherol are other essential membrane-bound chelators. These are the non-enzymatic antioxidants which are synthesized in harsh environments like HM exposure (Gill and Tuteja 2010). They are also indulged in quenching and sequestering the free radicals (Safafar et al. 2015). Other antioxidants such as flavonoids, tannins, and lignin are the plant's phenolic compounds known to play roles as useful antioxidants that can quench toxic ROS. In one of the experiments, it was demonstrated that in *Raphanus sativus*, the amount of phenolic as well as AsA content was elevated with Cu exposure along with GSH oxidation and lipid peroxidation. Also, the increase in the contents of phenolic compounds was noted in the radicles of *Pinus sylvestris* (Schützendubel et al. 2001) and *Zea mays* (Shemet and Fedenko 2005) under Cd stress. Shi and Zhu (2008) observed that when the leaves of *C. sativus* were treated with excess of Mn, the concentrations of the GSH and AsA increased, providing tolerance toward the oxidative injury induced by Mn stress.

Proline, a vital amino acid, is a non-enzymatic component and well-known protector of proteins and enzymes and also supports membrane assembly. It has been considered as one of the important osmoprotectants and may raise antioxidant defense mechanisms to lessen the deleterious effects of oxidative damage. Islam et al. (2009) observed that in response to Cd stress, proline protected *N. tabacum* against ROS accrual by acting as a plant growth regulator and regulated the osmotic pressure. Likewise, externally applied proline reduced the phytotoxic effects of Se via reducing oxidative injury and also recovered growth of *Phaseolus vulgaris* seedlings (Aggarwal et al. 2011). Hayat et al. (2013) and Chandrakar et al. (2018) revealed that the pretreatment of proline mitigated the adverse impacts of Cd and As in *Cicer arietinum* and *G. max*, respectively, and thereby enhanced the growth and tolerance against toxicity caused by applied stressors.

6.2.9 *Signal Transduction*

6.2.9.1 Signaling Molecules

In plants, HM stress results into modulation of biochemical and molecular mechanisms inside the cells. These amendments are certainly elicited by important networks of signal transduction pathways, operated inside plant cells that are formed by various units of signal transduction molecules. To counteract the excessive HM stress, plants synthesize metal transporters and metal-binding proteins (Peng and Gong 2014).

One of the very crucial and extremely conserved signaling molecules is MAPK which functions well under several stresses like HM, salt, ultraviolet, etc. and during a number of developmental mechanisms (Sinha et al. 2011). The MAPK pathway is comprised of three constituents including MAPKKK-, MAPKK-, and MAPK-mediated phosphorylation reactions (Hamel et al. 2006). These signaling cascades mediate the conduction of signals associated with oxidative stress and thus help in the regulation of various cellular processes (Kreslavski et al. 2012; Baxter et al. 2014). Also, HM-induced ROS and other metal ligands activated the signaling cascades and also severely affected the MAPK signaling cascade (Kreslavski et al. 2012).

In plants, the activation of MAPKs under HM stress like Cd, Cu, and As has been reported by several scientists (Jaspers and Kangasjarvi 2010; Karuppanapandian et al. 2011; Kreslavski et al. 2012; Baxter et al. 2014). Some of the well-considered MAPKs, viz., MPK3 and MPK6, in *Arabidopsis thaliana* were reported to be induced by Cd and Cu, respectively (Pitzschke et al. 2009; Liu et al. 2010; Sethi et al. 2014). Likewise, there was elevation in the transcripts of OsMSRMK2 (OsMPK3 homolog), OsMSRMK3 (OsMPK7 homolog), and OsWJUMK1

(OsMPK20-4 homolog) under Cu and Cd exposures in *O. sativa* (Yeh et al. 2007; Rao et al. 2011).

The ROS molecules produced in response to HMs play a crucial role in activating MAPK signaling. Two chief MAPK cascades (MEKK1-MKK4/5-MPK3/6 and MEKK1-MKK2-MPK4/6) involved in both abiotic and biotic stress signaling are reported to downstream the levels of ROS in *A. thaliana* (Pitzschke et al. 2009; Jalmi and Sinha 2015). In one more study, radicles of *O. sativa* pretreated with GSH under Fe stress rescued the root cells by triggering MAPK signaling, indicating the participation of ROS-mediated MAPK stimulation (Tsai and Huang 2006).

Another crucial signaling molecule is Ca^{2+}, which serves as a well-known secondary messenger and is actively involved in maintaining the cellular metabolism in the plant cells under normal and various abiotic stresses (Baxter et al. 2014). The modulation in the levels of cytosolic unbound Ca^{2+}occurs in response to several signal transmission cascades and stress stimulus triggering complex interactions (Rudd and Franklin-Tong 2001). The highly sensitive Ca^{2+}-sensing protein recognizes the transient increase in its cytosolic concentration and mediates the chemical signal into a biological reaction. Plants possess a variety of Ca^{2+}-recognizing proteins including calcineurin B-like proteins, calmodulins, calmodulin-resembling proteins, and Ca^{2+}-dependent protein kinases. These proteins affectionately chelate with Ca^{2+} and elicit several cascades of signaling pathways (Kreslavski et al. 2012). In order to determine the involvement of Ca^{2+}-sensing and Ca^{2+}-binding proteins in augmentation of tolerance toward various abiotic stresses, several studies in various plants like *C. arietinum*, *A. thaliana*, and *B. juncea* have been carried out (Tripathi et al. 2009; Li et al. 2012; Singh et al. 2018). Further, Ca^{2+} has been reported to maintain the water potential and transpiration rate of the cell; prevent the leakage of nutrients, solute, and ions from the cytoplasm; stabilize membrane moieties; and regulate photosynthesis and cellular metabolism (Singh et al. 2018). A number of studies revealed that externally applied Ca^{2+} mitigated the toxic effects of HM stress via modulating the physiological and biochemical responses. Singh et al. (2017) reported that externally applied Ca^{2+} alleviated the deleterious impacts of As by maintaining the auxin level and activating signal pathways. Fang et al. (2014) demonstrated that pretreatment of Ca^{2+}reduced the harmful effects of Cr by activating the antioxidant enzymes. All these findings indicated the involvement of MAPK and Ca^{2+} in maintaining the cellular homeostasis and ionic balance under HM stress.

6.2.9.2 Plant Hormones

Studies have demonstrated that external supplementation of plant growth hormones (also considered as plant hormones or phytohormones) can enhance tolerance against toxic impacts of HMs. These phytohormones, viz., auxin, cytokinin, ethylene, abscisic acid (ABA), gibberellin, brassinosteroid (BR), salicylic acid (SA), jasmonic acid, etc., act as secondary messengers that play important roles in the growth and development of plants and also in signaling and crosstalk (Bücker-Neto et al. 2017; Sytar et al. 2018). In agronomical crop management practices, these phytohormones have been utilized to alleviate HM-induced oxidative injury (Piotrowska-Niczyporuk et al. 2012; Chandrakar et al. 2016a).

Abscisic acid is one of the crucial and multifunctional phytohormones involved in several stages of a plant's life cycle like development of seed. It provides tolerance to the plants in adverse environmental conditions via its signaling pathways (Bücker-Neto et al. 2017; Sytar et al. 2018). Yeh et al. (2003, 2004) reported that Cd and Cu treatments boosted the MAPK signaling and ABA level in *O. sativa*, thereby providing tolerance toward Cd and Cu. Likewise, in *Empetrum nigrum* and *C. arietinum*, when exposed to Cu and Pb, respectively, increased amount of ABA was detected (Monni et al. 2001; Atici et al. 2005). The three major constituents of ABA signaling cascade, i.e., PYL/PYR/RCAR, PP2C, and SnRK2, are indulged in its regulation. Wang et al. (2014) have identified nine *PYL*, three *PP2C*, and two *SnRK2* genes responsible for ABA signal transference during seed germination in *C. sativus* under Cu and Zn stress.

Auxin (indole-3-acetic acid, IAA) is an essential phytohormone having multiple functions in the growth and development of plants exposed to various abiotic stresses (Sytar et al. 2018). It has been reported that one protein PIN-FORMED1 (PIN1) participates in IAA distribution under HM stress. For instance, distribution of IAA gets altered under boron (B) starvation leading to lesser production of the PIN1 protein which limits the root elongation (Li et al. 2015). It has also been reported that exogenously applied IAA enhanced the growth of *B. juncea* under As stress (Srivastava et al. 2013). Similarly, the exogenous application of various concentrations of L-tryptophan (a precursor of IAA) in the *O. sativa* radicles enhanced the growth and yield under Cd stress in comparison to seedlings raised without IAA precursor in Cd-contaminated pots (Farooq et al.2015).

Brassinosteroids are plant steroids that help in regulation of cellular expansion and elongation, photomorphogenesis, flowering, seed germination, vascular differentiation, formation of stomata, and senescence (Chandrakar et al. 2017a). So far, 60 BRs have been identified and their classification has been done on the basis of the carbon number in their structures. Some of the BRs such as brassinolide, 28-homobrassinolide, and 24-epibrassinolide (24-EBL) have been widely used in studies against a number of abiotic stresses (Rady 2011; Chandrakar et al. 2017b; Sytar et al. 2018). Kanwar et al. (2013) have observed that exogenous application of 24-EBL mitigated Ni-induced toxicity in *B. juncea* by perking up the activities of antioxidant enzymes. Rady (2011) and Chandrakar et al. (2018) have also reported that external application of 24-EBL provided tolerance against Cd and As stress in *P. vulgaris* and *G. max*, respectively.

Ethylene is another vital phytohormone and also known as "triple response" plant hormone (fruit ripening, leaf abscission, and senescence). Various reports have demonstrated the positive effects of ethylene under abiotic stress, and it was also reported by several experiments that in plants production of ethylene gets enhanced when exposed to toxic levels of HMs. Steffens (2014) and Trinh et al. (2014) observed an increase in transcripts of ethylene biosynthesis-related genes (*ACS1*, *ACS2*, *ACO4*, and *ACO5*) by performing transcriptome analyses of roots of *O. sativa* exposed to Cr, suggesting the involvement of ethylene in awarding tolerance toward

Cr toxicity. Similarly, in *A. thaliana*, the biosyntheses of ACC and ethylene are enhanced via increased gene expressions of ACS2 and ACS6 under Cd stress (Schellingen et al. 2014).

Salicylic acid is a well-recognized endogenous natural signaling molecule that provides defense to the plants by regulating various physiological and biochemical processes (Chandrakar et al. 2016a). It was observed that during HM stress, SA reduced the contents of ROS, leakage, and lipid peroxidation reaction, while it elevated the contents of chlorophyll, total lipids, and linolenic acid. Singh et al. (2015) and Chandrakar et al. (2016a) also reported that co-exposure of SA with As effectively reduced the metalloid-exerted oxidative injury via strengthening the antioxidant defense mechanism in *O. sativa* and *G. max*, respectively.

Thus, plants may survive better when exogenously primed with phytohormones (ABA, IAA, ethylene, BRs, and SA) in HM-contaminated areas, resulting in the increased levels of antioxidants and decreased levels of ROS with lipid peroxidation reaction and thereby stimulating the plants' growth.

6.2.9.3 Transcription Factors

Transcription factors (TFs) are one of the essential controllers to study the gene expression involved in various developmental pathways and defensive mechanisms of plants. It has been reported by several studies that upon exposure of HMs, the downstream TF targets get stimulated by the MAPK signaling pathway (Singh et al. 2016; Jalmi et al. 2018). There are various TFs that have been recognized as possible downstream targets of MAPKs, viz., MYB (MYeloBlastosis), ZAT (C_2H_2-type zinc finger TF), WRKY (having a conserved WRKYGQK domain and a zinc finger-like motif), bZIP (basic region leucine ZIPper), activator protein 2 (AP2), and dehydration-responsive element-binding protein (DREB) (Singh et al. 2016; Jalmi et al. 2018).

For instance, Ogawa et al. (2009) have demonstrated that exposure of Cd upregulated the expression of genes of most of the TFs, namely, AP2, DREB, MYB, and WRKY, in *Oryza sativa*. In *A. thaliana*, it was reported that several MYBs like MYB4, MYB28, MYB43, MYB48, MYB72, and MYB124 were extremely activated in response to Cd and Zn stress (van de Mortel et al. 2008). In one more study, it has been observed that MYB2 was inactivated upon Cd exposure in *A. thaliana* by the induction of nitric oxide production that leads to nitrosylation of cysteine residues in the MYB2 TF (Serpa et al. 2007). Moreover, the roles of MYB-TF in the regulation of As-associated transporters genes have been explained by Wang et al. (2017) in *Oryza sativa*. They have found that this TF gets attached with the conserved MYB binding sites in the promoter regions of OsLsi1, OsLsi2, and OsLsi6 that encode the transporters of As thereby affecting the gene expression.

Wei et al. (2008) found that treatment of Cd upregulated the expression of WRKY53 in *Thlaspi caerulescens*. Smeets et al. (2013) have observed that during Cd exposure, expression of *WRKY25* was upregulated (which is a downstream target for MPK4) in *A. thaliana* (Smeets et al. 2013). The induction of bZIP and zinc finger TFs was determined in *A. thaliana* and *B.* juncea, respectively, in response to Cd (Ramos et al. 2007). Nakashima and Yamaguchi-Shinozaki (2006) have observed that under HM stress, the TF-DREB was downregulated in the radicles of *A. thaliana* and suggested that DREB might be involved in adjustment of osmotic balance to decrease the flow of HM-contaminated water, thus providing tolerance to the plants toward the toxic impacts of HM. Plant AP2/ERF-TF is another transporter that is involved in providing defense to the plants against HM stress, such as in *A. thaliana* wherein Cd modulates the transcript levels of this TF's family members, i.e., ERF1 and ERF5 (Herbette et al. 2006). Hence, a deep research is required on the interrelated mechanisms that are indulged in the regulation of expression of these genes, which might be helpful for the production of genetically improved crops to withstand against different HM stresses (Singh et al. 2016).

6.2.9.4 Redox Signaling

One of the crucial parts regulated by the plants is redox signaling. These redox signals are now widely accepted as the key regulators of several metabolic pathways in plants like growth and development and may even perform coordinately with other systems of signal transduction. Along with other mediators like Ca^{2+}, the controlled generation of ROS in plants can also act as "second messenger" under exposure of various abiotic stresses like HMs and in response to hormone signaling (Bhattacharjee 2012; Foyer and Noctor 2013).

Due to the inherent reactivity, some of the ROS such as H_2O_2 and $^1O_2$ act as signaling molecules. Hence, through chemical reactions, ROS can operate proficiently in signaling with specific residues of target proteins leading to the covalent protein modifications. Mostly the amino acids like Cys residue on proteins and thiols (LMW) such as GSH are prime targets of ROS signals (Fischer et al. 2013). Therefore, the generation and accretion of ROS creates a localized oxidative environment inside the cell which in turn helps in signaling by other pathways such as mobilization of Ca^{2+}, protein-protein interactions, thiol-disulfide exchange, and TF-binding properties. Thus, it can be inferred that predetermined redox signaling pathways or cascades may not exist (Foyer and Noctor 2013).

Generation and accrual of various ROS occurs in different compartments of the cells, and each of them has a different capacity for that. Also, the different cellular locations consist of a specific array of antioxidants and redox-sensitive proteins that would be susceptible to redox reactions in a manner leading to modifications in the signaling pathways (Gill and Tuteja 2010). These reduction-oxidation amendments can therefore instigate, influence, or control a wide range of different pathways ranging from Ca^{2+} fluxes to TF-binding properties. In this way, the fate of each redox signaling pathway would be intrinsically determined by its site of production and accumulation and also the composition of redox-sensitive proteins (Foyer and Noctor 2013).

Among the different oxidants, particularly H_2O_2 is considered as one of the crucial components of signal transduction that helps plants in their growth and development.

Ample research has revealed that deleterious impacts caused in response to various abiotic stress conditions like salinity, HMs, cold, and drought in the plants can be reduced by the pretreatment of H_2O_2, also known as "H_2O_2 priming" (Bhattacharjee 2012). For instance, a study showed that pretreatment of *Z. mays* leaves with H_2O_2 lowered the adverse impacts of Cu exposure through maintaining the relative water content, dry mass, soluble proteins, and sugar content (Guzel and Terzi 2013). In another experiment, Yildiz et al. (2013) demonstrated an increase in root-to-shoot translocation by H_2O_2 priming in *B. napus* exposed to Cr. In addition, H_2O_2 enhanced the fresh mass and reduced the lipid peroxidation under Cr stress.

Another redox signaling mechanism includes oxidation of GSH pool in association with the increase in total GSH under abiotic stresses. This GSH is considered as one of the key players in H_2O_2-induced HM tolerance (Chandrakar et al. 2016b). In fact, it was reported in several experiments that H_2O_2 priming elevated the level of GSH in HM-exposed plants than the non-primed ones (Bai et al. 2011; Xu et al. 2011). In plants, increased level of GSH can thereby reduce the negative impacts of oxidative damage. As previously stated, GSH can directly bind the sulfhydryl-loving metal ions (Yadu et al. 2018a). Additionally, it is a chief component of metal-binding PCs (Cuypers et al. 2016). Therefore, for sensing this redox perturbation process of protein glutathionylation is involved, in which with the target proteins GSH forms a disulfide linkage, thereby modifying the enzymatic activities and various TFs. In this way, GSH can protect the structures and functions of proteins toward the detrimental impacts of HMs via playing an important role in redox signaling (Bhattacharjee 2012). Nevertheless, our current knowledge toward the redox signaling in plants is still scanty; further efforts are required to understand the interaction between ROS and signaling molecules involved in various signaling pathways under HM stress condition.

6.3 Transgenic Plants and Heavy Metal Tolerance

The advancement in biotechnology and genetic engineering is regarded as a very convenient and essential tool for identification and better perception of various significant steps at the molecular level for developing resistance in plants against various environmental stresses, particularly HM toxicity (Wani et al. 2018).

Overexpressing the known genes and encoded polypeptides responsible for the HM intake, transference, and compartmentalization may possibly allocate the creation of such plants that can be effectively used in phytoremediation. A number of studies have reported the upregulation in the expression of metal-binding peptides—PCs and MT genes thereby help in the detoxification of HMs (Nguyen et al. 2014; Chandrakar and Keshavkant 2018b). And, PCs are also extensively applied as biomarkers in revealing the presence of HMs in plants. The first and foremost PC genes that were recognized and well isolated were from *A. thaliana* (*phytochelatin synthase* (*AtPCS1*)) and from *Triticum aestivum* (*phytochelatin synthase* (*TaPCS1*)) (Bohra et al. 2015). It has also been observed that the HM accumulation capacity was comparatively more in transgenic *A. thaliana* plants than that of the wild type due to the expression of synthetic PCs (Wani et al. 2018).

Martinez et al. (2006) studied the upregulation of *TaPCS1*gene in *N. tabacum* that gives rise to enhanced accretion of Cd and other HMs. Authors have also recognized that due to the upregulation of gene *TaPCS1*, the transgenic *N. tabacum* plants could accrue 100 times more biomass on contaminated soils in comparison to the hyperaccumulator *T. caerulescens*. Pramanick et al. (2017) observed higher tolerance toward Cd and As in *A. thaliana* due to the overexpression of As-PCS1 (*AsPCS1*) and yeast Cd factor 1 (*YCF1*) (extracted from *Allium sativum* and yeast), which also enhanced its capability to congregate the metals to a considerable level.

In plants, MTs are cysteine-containing, metal-binding proteins that are implicated in evacuating the HM-induced toxicity via cell sequestration, storage of metals, and their transportation in plants (Nguyen et al. 2014; Lionetto et al. 2016). Hou et al. (2017) investigated that expression of MT 1 (*EhMT1*) in *N. tabacum* augmented the tolerance in its transgenic variety toward Cu toxicity and also diminished the production of H_2O_2 and boosted the POX activity in roots, resulting into higher strength of plants to counteract the oxidative injury. In some other studies, expression of *TaMT3* (a type 3 MT) from *Tamarix androssowii*, inserted into *N. tabacum*, led to enhancement in the tolerance against Cd stress through a remarkably raised SOD activity, which elevated the capacity of eradicating the overproduction of ROS in transgenic plant (Ashraf et al. 2017). Soda et al. (2016) reported that expression of *OSMT1e-p*, a type 1 MT acquired from a salt-resistant *O.* sativa genotype, helps in the detoxification of Cu and Zn in transgenic *N. tabacum* plant. The PsMTA1 gene (an MT-like protein) obtained from *Pisum sativum* was produced by *Agrobacterium tumefaciens*-mediated transformation and engineered into *P. salba*. The expression of *PsMTA1* gene in transgenic *P. salba* increased the tolerance against Cu and Zn toxicity by allowing lesser accumulation of ROS in the leaf tissues (Balestrazzi et al. 2009).

6.4 An Integrated Approach

In plants, abiotic stresses including HMs cause perturbations in several physiological and biochemical pathways. Also, HM-induced excessive generation of ROS alters the uptake and assimilation of essential metal ions and other cellular metabolites, hence limiting crop productivity. Also, the HMs accumulate in the grains of crop plants and thus enter into the food chain. Therefore, to protect themselves, plants develop various tolerance mechanisms. In the recent decade, molecular biology research on how plants deal with HMs has become a new approach to make the crops safer by lowering the accumulation of metals. Plants have these defensive trappings under stressed situation through regulation of omics tools, i.e., metabolic, genomic, and proteomic levels. The newly developed gene-editing technology can also help in regulating the expression of endogenous genes. It is important to explore how plants metabolize HMs so that new genes can become available for further improvement.

6.5 Conclusions

Exposure of plants to various HMs triggers the overproduction and accumulation of ROS and MG, which are highly toxic for the cellular macromolecules, and creates oxidative stress inside the cells. Against this backdrop, plant cells have a defense system. There is profound evidence that cells detoxify the deleterious consequences of HMs by several mechanisms such as compartmentalization, chelation, hyperaccumulation, syntheses of various compatible solutes and signaling molecules, and activation of a defense system in plants. Furthermore, it was suggested that PCs and MTs are involved in the chelation and sequestration of HMs; hence, they are promising candidates for successful elimination of toxic metals from the affected areas. Henceforth, development of abiotic stress-tolerant crops via altering the expression of genes of transporters, PCs, MTs, and ROS-scavenging enzymes may be useful. However, the responses exerted by the antioxidant molecules to the HM stress are not always similar and successive but can be varied with dose of HMs, plant species, metal type, and duration of exposure. Even with the accessibility of abundant reports, the basic fundamentals behind HM-induced toxicity and tolerance mechanism(s) at the physiological, biochemical, as well as molecular levels are still dependent on the extent of scientific drive.

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Chapter 7
Arsenic Tolerance Mechanisms in Plants and Potential Role of Arsenic Hyperaccumulating Plants for Phytoremediation of Arsenic-Contaminated Soil

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Abstract Arsenic (As) is a biological non-essential metalloid toxic to both plants and animals. Sources of As toxicity include both geogenic and anthropogenic. Arsenic accumulation in soil leads to deterioration of physiological properties of soil resulting in reduction of soil fertility and crop yield. Arsenic enters into the food chain either by consumption of As-contaminated water or by intake of plants cultivated in As-contaminated soil as plants accumulate As in different edible parts. Arsenate [As (V)] and arsenite [As (III)] are two inorganic forms of arsenic that are available to plants. They are well known for toxicity symptoms in plants with the former being the most toxic form of arsenic. Arsenic toxicity in plants leads to many morphological and physiological changes like reduction in growth, decrease in number of leaves, decrease in germination rate, plasmolysis of the root cells, demodulation, necrosis of leaf tips, leaf wilting, disruption of cellular membrane structure leading to electrolyte leakage, photosynthesis inhibition, and disruption of cellular energy flow. In response to arsenic toxicity, the plants use various detoxification mechanisms, which include non-enzymatic and enzymatic antioxidant defense mechanisms, hyperaccumulation, and phytochelation synthesis for protection of cells and subcellular systems. Hence, it is necessary to understand the bioavailability of As, its assimilation, metabolism, and toxicity in plants for mitigation of As from the contaminated soil and water. Many plant species including halophytes and glycophytes are potential candidates for mitigating toxic effects of As, thus paving the way for detoxification of the As-contaminated soils through phytoextraction, phytostabilization, and phytoexcretion of As. The chapter presents an overview of significant sources of As contamination, As toxicity and bioavailability, potentiality of various plant species to cope up with arsenic toxicity, and their mechanisms of adaptations to heavy metal stress proving their potential role in phytoextraction, phytostabilization, and phytoexcretion of heavy metal-contaminated saline as well as non-saline soils.

Keywords Antioxidants · Glycophytes · Halophytes · Heavy metals · Hyperaccumulation · Translocation factor

Abbreviations

APX	Ascorbate peroxidase
As	Arsenic
ATP	Adenosine triphosphate
BCF	Bioconcentration factor
CAT	Catalase
DMA	Dimethylarsinic acid
GR	Glutathione reductase
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde

MMA	Monomethylarsonic acid
$O_2^{\bullet -}$	Superoxide
OH•	Hydroxyl radicals
ROS	Reactive oxygen species
SOD	Superoxide dismutase
T*f*	Translocation factor
TMAO	Trimethylarsine oxide

7.1 Introduction

Heavy metal toxicity has emerged as a major concern owing to the rapid industrialization and urbanization leading to contamination of soil and water resources. Heavy metal toxicity depends on various factors like dosage, chemical species, and exposure route. Arsenic (As) is a trace metalloid mineral element, found in the redox-active form in nature, and highly toxic to the living organisms. It is one of the most abundant elements in Earth's crust and usually found in trace quantities in soil, air, water, and rock. Arsenic toxicity is a serious global concern due to its carcinogenic activity causing lung, bladder, and skin cancer, neurological disorders, and immunological, reproductive, as well as cardiovascular diseases in animals. In plants, arsenic toxicity leads to reduced growth, photosynthesis, necrosis of the leaf, impeding important plant metabolic activities, and ultimately leading to death of the plant. Also, it is a threat to human beings as it enters the food chain through contamination of waterbodies and consumption of plant having accumulated As. Arsenic mainly exists in organic and inorganic forms. In general, inorganic compounds of As are more toxic than organic form. Inorganic arsenic is generally available as As (V), As (III), As (0), and As (-III) in the environment (Jang et al. 2016). Among these, As (V) and As (III) frequently exist in the terrestrial environment. Arsenate [As (V)] is predominantly present in the aerobic soil, while arsenite [As (III)] have been found in anaerobic soil such as flooded areas and submerged soil. Because of the interconversion nature between these two species, these two forms are biologically important forms of As. However, the interconversion depends on redox conditions, pH, and mineral composition of the environment. Several plant species also contain methylated As compounds such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), and rarely tetramethylarsoniuum. Also, a trivalent form of methylated As species was more efficient to the breakdown of DNA as it has been found to be more toxic than the inorganic arsenic (Zhao et al. 2010).

Various anthropogenic activities like overuse of wood preservatives, As-containing herbicides and pesticides, feed additives, mining, and smelting, paint, dye, soap, and drug industries also use arsenic (Zhao et al. 2010). Soil contamination by heavy metal remains one of the challenging problems. Based on the current scenario, the most widely implemented techniques for decontamination of soil are categorized as physical, chemical, and biological techniques. The physical treatment involves landfilling and capping that does not remove contaminant from soil and soil excavation, while chemical technique includes immobilization, electro-osmosis, and use of strong acids and chelators to wash contaminated soil. However, these methods alter soil properties and may destroy soil biodiversity; also these approaches are impractical and expensive because they require specialist operators and equipment and only appropriate for small areas of land (Padmavathiamma and Li 2007). Hence, it is crucial to develop a suitable low-cost biological technique to decontaminate polluted soil without affecting soil fertility.

Apart from the listed remediation techniques, recently phytoremediation has shown immense potential for remediation of contaminated soil and could provide an appropriate method for heavy metal remediation. Phytoremediation technique uses living plants to reduce, transfer, degrade, stabilize, and remove pollutant from the soil, sediments, air, and water. A sub process of phytoremediation, known as "phytoextraction", is used for removal of toxic metal ions from contaminated soils. Phytoextraction is a secondary tolerance mechanism against excess ions utilizing the salt excretion mechanism in some halophytes through salt glands, bladders and trichomes for maintaining ion homeosatsis in cells. The main focus of phytoremediation technique is to accelerate degradation of organic impurities using plants especially root-associated microbes in rhizosphere and reduce or remove toxic heavy metal from soil and water (Perdiguero and Muñoz-Cánoves 2007). Moreover being toxic, As also interacts with a number of elements such as iron (Fe), zinc (Zn), phosphorus (P), silicon (Si), and sulfur (S) limiting their uptake by plants that are essential to the human health.

Halophytes are referred as plant species that have the capability to survive, grow, and reproduce in high saline conditions. Halophytic species have evolved several mechanisms of adaptation which allows them to survive under high salt concentration, xerothermic environments, and seasonal temperatures and may also confer tolerance to other toxic ions including heavy metals (Flowers et al. 2010). Some physiological and molecular mechanisms of halophytes that contribute to salt tolerance include ion compartmentalization, synthesis of organic osmolytes, robust antioxidative system, and ability to restrict the entry of ions in the transpiration stream (Flowers and Colmer 2008; Manousaki and Kalogerakis 2009). Moreover, similar mechanisms have been reported for heavy metal tolerance in plant species (Panda et al. 2017; Shevyakova et al. 2003). Furthermore, some halophyte plants have been reported to grow in polluted areas and are being extensively promoted for phytoremediation, phytoextraction, or phytostabilization (Manousaki and Kalogerakis 2011; Lutts et al. 2004). Some research studies have revealed that halophytes can tolerate greater heavy metal toxicity than glycophytes and thus are generally studied for phytoextraction and phytostabilization (Manousaki and Kalogerakis 2009). Many plant species have been acknowledged for their potential for heavy metal hyperaccumulation which also includes some halophytes (Wang et al. 2014a). Based on the previous reports, heavy metal hyperaccumulating halophytes yielding high biomass are difficult to obtain because most hyperaccumulators do not produce high biomass. Therefore, halophytes that can accumulate a moderate level of heavy metals and produce excessive biomass may be an effective and convenient approach for selecting halophytes for phytoextraction and phytostabilization. Some halophytes utilize the mechanism of salt avoidance to cope with high level of salinity and secrete excess salt on the surface of their leaves through salt gland and other glandular trichomes (Aslam et al. 2011). Furthermore, several halophytic plant species like *Tamarix aphylla*, *Armeria maritima*, *Avicennia marina*, *T. smyrnensis*, and *Atriplex halimus* whose glandular tissue is not only specific for salt accumulation but can also secret toxic heavy metal such as cadmium (Cd), Zn, and lead (Pb) through trichomes and salt glands, and these methods may constitute for metal toxicity removal (Lokhande and Suprasanna 2012). Some previous studies also suggest that salinity is a key factor for translocation of metal ions from root to other harvestable part of plants providing additional benefit for phytoextraction strategy (Fitzgerald et al. 2003). Previously, it has been shown that a number of halophyte plants can accumulate and tolerate high concentration of toxic ions in the environment, making them a potential candidate for phytoextraction and phytostabilization of toxic ion in the polluted soil. Therefore, halophytes are suggested as perfect candidates for phytoremediation or phytoextraction of heavy metal-contaminated saline soils. According to Lokhande et al. (2011), *Sesuvium portulacastrum*, a mangrove associate halophyte plant that comes under category of "salt-accumulator," is found to be potential arsenic accumulator as observed from its high bioaccumulation factor, arsenic accumulation, and biomass production. Hence, it can be implemented for arsenic removal and land reclamation projects. Vromman et al. (2016) concluded that salinity improves plant resistance to the hazardous contaminant in a xero-halophyte *A. atacamensis* Phil. and can be a fascinating tool for phytomanagement of contaminated arsenic site in a desert area. *T. gallica* L. is mostly found in coastal and desert region, and they are highly resistant under harsh environmental conditions. According to Moreno-Jiménez et al. (2009), *T. gallica* is resistant to arsenic and shows greater arsenic accumulation ability. Table 7.1 enlists important plant species that are used for phytoremediation of arsenate-contaminated soil.

The present chapter deliberates a brief overview of uptake mechanisms of various arsenic species by plant and adaptation strategies of the plant to cope up with high level of arsenate, offering greater potential for phytoremediation research. Furthermore, this chapter will add to the present knowledge of arsenic toxicity in plant and various detoxification or tolerance strategies developed by plants under arsenic stress condition. Moreover, it will highlight the potential mechanisms involved for phytoremediation of arsenic-contaminated saline soil emphasizing the need of developing remediation technologies for resolving the heavy metal contamination in saline and non-saline soil.

Table 7.1 Arsenic tolerance mechanisms of some important halophytic and glycophytic plant species having significant contributions toward arsenic phytoremediation

No.	Plant types	Plant species	Family	Tolerance mechanisms	References
1	Halophytes	*Arthrocnemum fruticosum*	Amaranthaceae	Accumulation of arsenobetaine, osmoregulation	de Bettencourt et al. (1997)
2		*A. perenne*	Chenopodiaceae		
3		*Aster tripolium*	Asteraceae	Formation of iron plaque	Otte et al. (1991)
4		*Atriplex atacamensis*	Chenopodiaceae	Increase in the endogenous polyamines and interaction between the inorganic As species and the polyamines	Vromman et al. (2011)
5		*A. halimus*	Amaranthaceae	Exclusion of arsenic, endomycorrhizal symbiotic associations	Rabier et al. (2014)
6		*A. nummularia*	Amaranthaceae	Phytostabilization of As	Fernández et al. (2016)
7		*Leersia oryzoides*	Poaceae	Accumulation of arsenic in shoot and root	Ampiah-Bonney et al. (2007)
8		*Cakile maritima*	Brassicaceae	Maintenance of enzymatic and non-enzymatic antioxidants	Demir et al. (2013)
9		*Halimione portulacoides*	Amaranthaceae	Accumulation of arsenobetaine, osmoregulation	de Bettencourt et al. (1997)
10		*Holcus lanatus L.*	Poaceae	Loss of induction of the synthesis of the arsenate (phosphate) carrier	Meharg and Macnair (1992)
11		*Pistacia lentiscus*	Anacardiaceae	Increase in thiol compounds	Moreno-Jiménez et al. (2009)
12		*Salicornia brachiata*	Amaranthaceae	Upregulation of various biochemical pathways like proline biosynthesis and generation of various antioxidative enzymes like catalase and SOD	Sharma et al. (2010)
13		*Sesuvium portulacastrum*	Aizoaceae	Unidentified mechanism	Lokhande et al. (2011)
14		*Spartina densiflora*	Poaceae	Accumulation of arsenic in the roots	Mateos-Naranjo et al. (2012)
15		*S. maritima*	Poaceae	Accumulation of arsenobetaine, osmoregulation	de Bettencourt et al. (1997)

(continued)

Table 7.1 (continued)

No.	Plant types	Plant species	Family	Tolerance mechanisms	References
16		*Suaeda maritima*	Amaranthaceae	Phytochelatin synthesis, induction of antioxidants and osmolytes	Panda et al. (2017)
17		*Tamarix gallica*	Tamaricaceae	Complex formation with the chloride of NaCl salt	Sghaier et al. (2015)
18	Glycophytes	*Arrhenatherum album*	Poaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
19		*Azolla caroliniana*	Salviniaceae	Complex formation with thiol compounds	Zhang et al. (2008)
20		*Azolla filiculoides*	Salviniaceae	Complex formation with thiol compounds	Zhang et al. (2008)
21		*Corrigiola telephiifolia*	Caryophyllaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
22		*Cortaderia atacamensis*	Poaceae	Phytostabilization of As	Fernández et al. (2016)
23		*Cynosurus echinatus*	Poaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
24		*Daucus carota*	Apiaceae	Phytostabilization of As	Fernández et al. (2016)
25		*Digitalis thapsi*	Plantaginaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
26		*Distichlis spicata*	Poaceae	Phytostabilization of As	Fernández et al. (2016)
27		*Eleocharis acicularis*	Cyperaceae	Hyperaccumulation of arsenic	Sakakibara et al. (2011)
28		*Glycine max L.*	Fabaceae	Decrease stomatal conductance and osmotic potential, smaller xylem vessel, abnormal stomata	Vezza et al. (2018)
29		*Holcus mollis*	Poaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
30		*Jasione montana*	Campanulaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
31		*Brassica juncea*	Brassicaceae	Production of antioxidant enzymes, phytochelatin production	Gupta et al. (2009)
32		*Lemna gibba*	Araceae	Biomethylation, assimilation, or compartmentalization	Mkandawire and Dudel (2005)

(continued)

Table 7.1 (continued)

No.	Plant types	Plant species	Family	Tolerance mechanisms	References
33		*Lycium humile*	Solanaceae	Phytostabilization of As	Fernández et al. (2016)
34		*Oryza sativa*	Poaceae	Accumulation of arsenic in the roots	Liu et al. (2007)
35		*Plantago lanceolata*	Plantaginaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
36		*P. alba*	Salicaceae	Root proliferation	Vamerali et al. (2009)
37		*P. nigra*	Salicaceae	Fine root proliferation	Vamerali et al. (2009)
38		*P. tremula*	Salicaceae	Arsenic compartmentation, antioxidant induction (glutathione, SOD, and CAT)	Danh et al. (2014)
39		*Pteris biaurita*	Pteridaceae	Higher activity of antioxidative enzymes and lower thiobarbituric acid-reacting substances	Srivastava et al. (2006)
40		*P. cretica*	Pteridaceae	Phytochelatin production	Zhao et al. (2003)
41		*P. quadriaurita*	Pteridaceae	Higher activity of antioxidative enzymes and lower thiobarbituric acid-reacting substances	Srivastava et al. (2006)
42		*Pteris ryukyuensis*	Pteridaceae		
43		*P. vittata*	Pteridaceae	Phytochelatin production	Dong et al. (2005)
44		*Rumex acetosella*	Polygonaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
45		*Salix integra*	Salicaceae	Phytofiltration through the roots	Chen et al. (2014)
46		*S. matsudana*	Salicaceae		
47		*Schinus molle*	Anacardiaceae	Phytostabilization of As	Fernández et al. (2016)
48		*Sorghum bicolor L.*	Poaceae	Accumulation of arsenic, biomass increase	Muranyi and Kodobocz (2008)
49		*S. sudanense*	Poaceae		
50		*Spirodela polyrhiza L.*	Araceae	Adsorption of arsenate on Fe plaque and internalization	Rahman et al. (2008)

(continued)

Table 7.1 (continued)

No.	Plant types	Plant species	Family	Tolerance mechanisms	References
51		*Tessaria absinthioides*	Asteraceae	Phytostabilization of As	Fernández et al. (2016)
52		*Thymus zygis*	Lamiaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
53		*Trisetum ovatum*	Poaceae	Accumulation of arsenic in the roots and aboveground parts	García-Salgado et al. (2012)
54		*T. vulgare*	Poaceae	Induction of antioxidants	Danh et al. (2014)

7.2 Arsenic Bioavailability, Toxicity, and Source of Arsenic Contamination

Arsenic is distributed ubiquitously in nature and is considered as one of the major toxic metalloids for plants, animals, and human beings (Chandrakar et al. 2016). Many factors that include chemical and physical characteristics of soil, bioaccumulation kinetics, environmental conditions, and rhizosphere soil modifications govern the bioavailability of As to the plants. In nature, four oxidation states of As exist, namely, As (-III), As (0), As (III), and As (V). Predominantly As (V) and As (III) are respectively present in the aerobic and anaerobic conditions. The following order (AsIII > As(V) > MMA(V) > DMA(V) has been reported for the uptake of As by plants (Raab et al. 2007). However, during translocation of As from root to other parts of the plant follows the reverse order. Out of the two inorganic forms As (V) and As (III), arsenite is considered as more toxic than other forms present due to its higher solubility, mobility, and cytotoxic nature. Biotic and abiotic factors like microorganism present in the soil and changes in the pH and redox potential have been stated to play pivotal role in interconversion of As (V) and As (III) (Zhao et al. 2010; Nearing et al. 2014). As (V) has been reported to have low bioavailability (<1 μM) than As (III) in soil solution because of its high affinity for oxides. Due to which As (V) rapidly co-precipitate with FeIII/FeII ions under anoxic conditions resulting in arsenate immobilization and prevention of As (V) reduction to As(III) (Farooq et al. 2016). Some factors that accelerate As mobilization include the content of dissolved organic carbon (DOC) and radial oxygen loss in the root. A positive correlation between the two has been reported which increases the bioavailability of arsenic in the soil (Williams et al. 2009). This suggests that bioavailability of As in the soil is a function of organic matter breakdown for releasing the adsorbed As. Arsenic bioavailability is also affected significantly by the loss of radial oxygen in the root where the oxygen is diffused into the rhizosphere with the help of root aerenchyma, resulting in formation of Fe plaque on the root surface due the oxidation of Fe^{2+} to Fe^{3+} (Pan et al. 2014). As toxicity in human beings and animals can occur either by consumption of arsenic-contaminated water or through food chain contamination and sometimes by both ways (Mishra et al. 2008). As toxicity is mainly caused due to its structural analogy with phosphate, thus replacing the former in biomolecules after converting to As (III) by interaction with the thiol groups of proteins. As toxicity severely affects roots of the plant as roots serve as the first tissue to be in contact with As. As hampers root proliferation and extension. Alongside this, when translocated to the shoot, As have been reported to constrain growth of the plant by arresting accumulation of biomass and slowing down shoot expansion. It also results in reduction of reproductive capacity of the plant leading to low yield and less production of fruit (Garg and Singla 2011). Under high concentration, As have been reported to hinder metabolic processes of the plants that are critical for its survival, thus ultimately leading to death of the plant. As a countermeasure, most of the plants avoid translocation of As from root to shoot and retain As in the root as much possible. The main sources of arsenic include uses of arsenate in various pesticides, insecticides, and herbicides, wood preservatives, irrigation with As-contaminated water, mining activities, and weathering and mineralization of Earth's crust (Chandrakar et al. 2016). Figure 7.1 represents schematic overview of different sources of arsenic.

7.3 Arsenic Uptake and Translocation in Plants

To obtain essential micronutrients, which are present at low ppm level, plants have developed highly precise and more efficient mechanisms. These mechanisms include production of chelating agents by the plant roots, changes in the pH, and

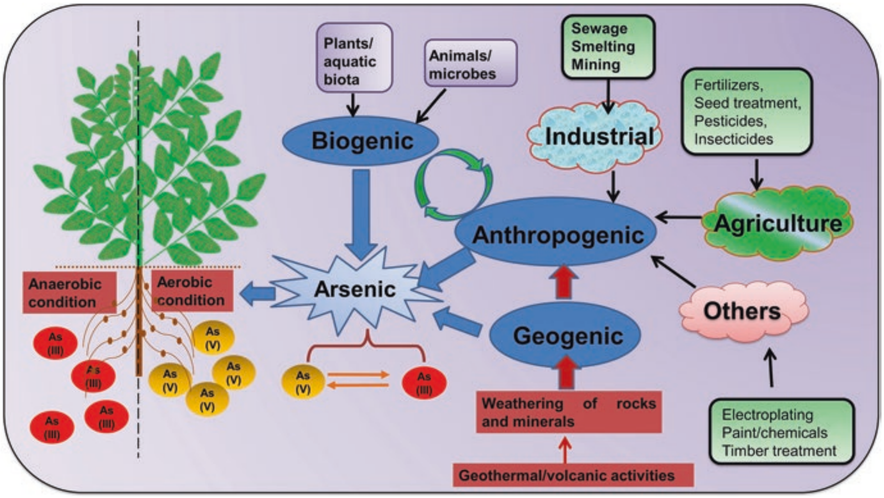

Fig. 7.1 Schematic overview of different sources of arsenic in the environment. *As (V)* arsenate, *As (III)* arsenite

redox reaction which solubilize the microelements for natural uptake. Processes similar to uptake of mineral ions are used by plants to absorb, translocate, and store toxic heavy metals which are similar in properties to the essential mineral ions. Plants which can grow under high content of toxic metals and accumulate high amount of metal or metalloids in their tissue through root are known as hyperaccumulators having shoot-root metal concentration ratio more than one. As compared to non-hyperaccumulating plant species, hyperaccumulating plant roots absorb metal or metalloid at a higher rate from soil and translocate rapidly to aboveground biomass. Plants take up and translocate various arsenic species via different transporters and distinct pathways. The common form of arsenic uptake by plants from soil includes arsenate, arsenite, and methylated arsenate (MMA and DMA). A few plant species so far have been known as arsenic hyperaccumulators. The Chinese brake fern *Pteris vittata* L. has been identified as a first arsenic accumulator (Ma et al. 2001) and has shown great potential for arsenic accumulation, thereby providing opportunity for removal of arsenic from polluted soil through phytoextraction (Ma et al. 2001). Moreover, *Pityrogramma calomelanos* a single, non-*Pteris* fern accumulates 8350 mg As kg^{-1} DW (Francesconi et al. 2002). Figure 7.2 represents the mobilization, uptake, and translocation of different arsenate forms throughout different parts of the plant.

7.3.1 Arsenate (As (V)) Uptake and Translocation in Plants

Arsenate or arsenic acid dissociates as oxyanions $H_2AsO_4^-$ or $HAsO_4^{2-}$ under pH range 4–8. As demonstrated by electrophysiological and physiological studies, the transport pathway for As (V) and phosphate are similar in higher plants. However, arsenate affinity decides the functionality of different phosphate transporters. Some phosphate transporters have been reported in plants. The phosphate transporter 1 family (Pht 1) have been reported for their dominant expression in the roots for phosphate uptake (Bucher 2007). Among them, phosphate transporters Pht1;1 and Pht 1;4 have been documented for their key role in acquisition of phosphate in *Arabidopsis thaliana* from environment (Shin et al. 2004). *A. thaliana* double mutant *Pht1;1*Δ*4*Δ, when compared to wild type, was resistant to arsenate which shows that phosphate transporters Pht1;1 and Pht 1;4 facilitate arsenate uptake. The shoot biomass increased up to 55% than the wild type of another mutant *apht1;1–5* under arsenate exposure, and an increased number of lateral roots also have been stated in the mutant under As treatment (Nagarajan et al. 2011). Besides this, overexpression of the phosphate transporters in *Arabidopsis* mutant *A. thaliana AtPht1* has demonstrated greater accumulation of arsenate (LeBlanc et al. 2013). Many WRKY transcription factors (WRKY6 and WRKY45) which are known to be involved in arsenate influx and also regulate the uptake of arsenate and mediating arsenate defense in the plants (Castrillo et al. 2013; Wang et al. 2014b). Similarly, in *Oryza sativa*, a phosphate transporter Ospht1;8 which expresses in both shoot and root tissues individually have been documented for regulatory role in influx of

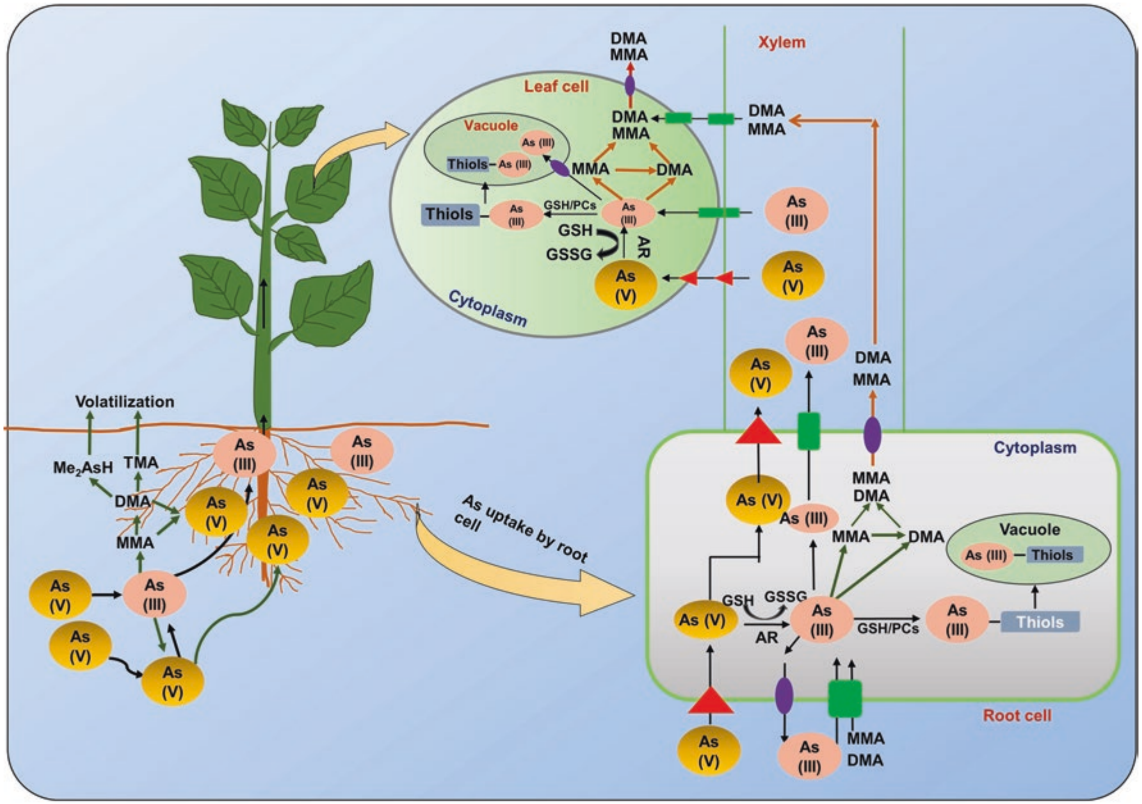

Fig. 7.2 Arsenic uptake, translocation, and tolerance mechanisms in plants. *As (V)* arsenate, *As (III)* arsenite, *AR* arsenate reductase, *DMA* dimethylarsinic acid, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *MMA* monomethylarsonic acid, *TMA* trimethylarsine

As due to its affinity for both arsenate and inorganic phosphate (Wu et al. 2011). However, the uptake of As and its translocation in the shoot have been reported under overexpression of Ospht1;8 in rice (Jia et al. 2013).

7.3.2 Uptake and Translocation of Arsenite

Arsenite is highly toxic and is formed by reduction of arsenate in the cell. Due to its high affinity for thiols, arsenite forms a complex with thiol compounds mostly phytochelatins. Arsenite-phytochelatin is the main storage form in roots and transported into vacuoles through ABCC-type proteins (Song et al. 2010). It is a common mechanism in plants for detoxification of arsenite and cope up with other heavy metal toxicity (Li et al. 2014). In plants, some aquaglyceroporin, a subfamily of aquaporin superfamily, a transmembrane channel protein which allows the entry of water, small neutral molecules, and metalloids including arsenite have been reported (Mukhopadhyay et al. 2014). The aquaglyceroporin proteins act as bidirectional pumps that can efflux and/or influx metal and metalloids from most of the plants (Isayenkov and Maathuis 2008). Evidence from different studies suggests that some plant aquaporin channels can facilitate the influx of arsenite (Bienert et al. 2008; Ma et al. 2008). Arsenite or arsenous acid mostly remains undissociated at normal $pH < 8$ condition. Hence, plant roots take up arsenite in the form of neutral molecule of $As(OH)_3$. It has also been identified that Lsi1 (silicic acid NIP transporter) remains undissociated in rice (Ma et al. 2008). However, arsenite is structurally similar to silicic acid. Therefore, silicic acid transporters are reported to transport arsenite into the root cell. In agreement with this, *lsi1* mutant of rice has been reported to reduce 60% arsenite uptake in comparison to the wild type of rice root (Zhao et al. 2009). Rice and *Arabidopsis* genome contains 9–13 NIP (nodulin 26-like intrinsic protein) genes. Few genes of NIP in rice are largely expressed in shoot and inflorescence tissue, but their role in arsenite accumulation toward grains remains unknown (Sakurai et al. 2005). Several studies reported that some transporters such as NIP5;1 and NIP 6;1, in *A. thaliana* and *Lotus japonicus*, respectively, and NIP2;1 and NIP3;2 present in *O. sativa* are known to transport arsenite across the cell membrane (Bienert et al. 2008). Additionally, in *A. thaliana*, NIP1;2 and NIP3;1 also perform the function of arsenite uptake (Xu et al. 2015). Besides this, PIP (plasma membrane intrinsic protein) has also been reported for transport of arsenite and has role in tolerance mechanism against arsenic stress. For example, Mosa et al. (2012) have reported enhanced arsenic tolerance in *Arabidopsis* due to overexpression of PIP transporters like OsPIP2;4, OsPIP2;6, and OsPIP2;7 that execute efflux of toxic arsenite into the external growth medium from the root cell, and they also suggested that there is a possibility that PIP proteins play role in export of excess arsenite into the apoplasm of the shoot from the cytoplasm.

7.3.3 Uptake and Translocation of Methylated Arsenic

Methylated arsenic species which includes MMA (monomethylarsonic acid), DMA (dimethylarsinic acid), TMAO (trimethylarsine oxide), and rarely tetramethylarsoniuum are found mostly in paddy soil which may have been derived from inorganic arsenic by some microbial and algal biomethylation or past application of arsenic-based pesticides/herbicides. Both MMA and DMA (called as cacodylic acid) are widely used as herbicides for weed control or as a cotton defoliant. After reduction of As (V) to As (III), further methylation results in formation of pentavalent MMA (V) by *S*-adenosyl methyltransferase which is further reduced to trivalent MMA (III) by reductase combining with glutathione (GSH). Continuous reaction of reduction and methylation produced di- and trimethylated arsenic compounds. However, the formed di- and trimethylated arsenic species are unstable as they are intermediates formed in the arsenic methylation pathway (Gong et al. 2001). Simple diffusion of methylated As species across the lipid bilayer of plasma membrane is slow for their uptake into root cell due to big molecular size. Zhao et al. (2010) reported inorganic arsenic and DMA as main arsenic species in the rice grain. The relative concentration of DMA in rice grain may vary from 10 to 90% of the total arsenic depending on the several factors such as rice cultivars, water management, and soil structure in which rice was grown. Previous studies suggested that several plants species can take up both MMA and DMA by root cells and translocate to shoot tissue. According to Li et al. (2009), OsLsi1 is essential for the uptake of methylated As species including MMA and DMA by rice roots. Moreover, the rice mutant *lis1*, which lacks OsLsi1 and is defective in silicon/arsenite transporter, reduce the uptake capacity of both MMA (V) and DMA (V) species by 80% and 50%, respectively, as compared to wild-type rice. Additionally, Rahman et al. (2011) stated MMA, DMA, and glycerol shared the same route, for transportation through aquaporins.

7.4 Arsenic Toxicity and Plant Metabolism

The toxicity of arsenic is dependent on the frequency and route of intake, rate of intake, bioavailability, oxidation form of As, and exposure time. Minor exposure of arsenic can affect internal organs like lung, liver, kidney, and urinary bladder and cause cancer in humans. Methylated arsenic species can enter into human body and have been stated to disturb DNA transcription and metabolism. Besides this, methylated arsenic species also interfere with different enzymatic reactions. Arsenic uptake in the plants adversely affects its metabolism leading to several structural and functional disorders. The highly toxic trivalent arsenic affects the structure and catalytic function of many enzymes due to its higher affinity toward sulfhydryl groups of the enzymes (Garg and Singla 2011). As (III) can similarly inhibit various metabolic processes by interacting with thiol (-SH) group of enzymes and tissue proteins, inhibit cellular function, and cause death of plants (Garg and Singla 2011).

However, arsenic can strictly constrain the growth of plant by reducing biomass accumulation and reduce crop yield by restricting plant reproductive ability through losses in fertility.

7.4.1 Effect of Arsenic on Uptake and Translocation of Essential Mineral Nutrients

Exposure of the plants to As-contaminated groundwater may not only lead to accumulation of arsenic in different parts of the plant but also has been reported to interfere with the uptake and translocation of different mineral nutrients (Dwivedi et al. 2010a, b). Owing to the fact that As is a structural analogue of phosphate, As competitively restricts phosphate for binding to the phosphate transporters, thereby hampering the phosphate metabolism as reported earlier that phosphate concentration decreased in rice root under high As concentration due to competitive inhibition of phosphate uptake by the roots (Dwivedi et al. 2010b). Similarly, reduction in the level of trace mineral elements Zn, Ni, and selenium has also been stated (Dwivedi et al. 2010b; Williams et al. 2009); however, a positive correlation of Fe and As has been reported (Dwivedi et al. 2010b). Furthermore, Dwivedi et al. (2010a) have reported that higher concentration of As hampered the uptake of mineral ions (Fe, Cu, Mn, and Zn) leading to reduced photosynthetic activity and growth. Similarly, altered mineral ion (nitrogen, phosphorus, magnesium, sodium, sulfur, and zinc) balance in *Pisum sativum* has been reported under As toxicity (Päivöke and Simola 2001). Likewise, reduction in mineral ions such as K, Ca, Mn, Fe, Zn, and Cu has been reported in *Helianthus annuus* (Gunes et al. 2010). However, in halophyte *Suaeda maritima*, no significant effect on Na^+ and K^+ uptake has been reported under As stress, suggesting that halophytes possess some capability to maintain ion homeostasis even under heavy metal stress like As (Panda et al. 2017).

7.4.2 Growth and Photosynthesis

High As concentration has been stated to disturb the normal metabolism of the plant resulting in reduced biomass, low fruit production and yield, stunted growth, loss of fertility, and inhibition of root expansion and proliferation (Abbas et al. 2018). Arsenate exposure has been reported to decrease plant height, total biomass, photosynthetic pigments, and leaf area along with reduced photosynthetic rate and stomatal conductance in *Brassica napus* and *B. juncea* (Niazi et al. 2017). Likewise, reduction in growth was documented for *H. annuus* (Gunes et al. 2010) under arsenic stress. Arsenic has been reported to negatively affect photosynthesis rate in plants by affecting the light harvesting apparatus and reduction of chlorophyll concentration or may affect downstream photosynthesis complexes suppressing the process of photosynthesis (Abbas et al. 2018). Decrease in chlorophyll content has been documented in *Trifolium pratense* (Hasanuzzaman et al. 2017). Arsenic toxicity can affect the photosynthesis process either at the photochemical stage or by hindering the biochemical reaction of the photosynthesis or by affecting both. The arsenic toxicity varies between different arsenic species. Pentavalent arsenic affects the process of oxidative phosphorylation by substituting phosphate in ATP synthesis and deactivates internal energy storage. As (V) also directly disturbs photosynthetic apparatus, energy production, machinery of cell division, and redox status of plant system. Arsenic accumulation can hinder the biosynthesis of chlorophyll through the generation of Fe shortage. In addition, arsenic disturbs the heat dissipation and photobiochemical efficiency and causes changes in chlorophyll fluorescence emission and affects the gas exchanges (Rahman et al. 2007). Arsenic also disturbs membrane structure and permeability of chloroplast, in addition to electron transport chain ultimately disrupting the structural and functional integrity of photosystem II (Santos et al. 2014). The carbon fixation in the process of photosynthesis is also affected by arsenic stress. Proteomic studies of rice leaf reported a decrease in content of RuBisCO large subunit under As stress suggesting interference of As with carbon fixation process of the plant (Ahsan et al. 2010). Similarly, inhibition of photosynthetic carbon fixation has been reported in pea under As treatment (Marques and Anderson 1986). Moreover, an increase in the RuBisCO small subunit transcripts has been reported for *Arabidopsis* under arsenic treatment due to decreased RuBisCO activity (Abercrombie et al. 2008). It is likely that under arsenic toxicity, CO_2 fixation is reduced due to decrease in the available carbon for the plant to carry out photosynthesis.

7.4.3 Nitrogen Metabolism

A greater fraction of the nitrogen present in the biological system is contributed by the biological nitrogen fixation carried out in the root nodules of legumes. As reported earlier, symbiotic nitrogen fixation is sensitive to arsenic toxicity demonstrated by a decrease in root nodule formation in alfalfa when exposed to As-contaminated soil by destroying the nitrogen-fixing symbiosis of the plant (Carrasco et al. 2005). Besides this, As toxicity also implicated increased root necrosis and root hair damage (Pajuelo et al. 2008). Furthermore, it has been reported that As (III) interferes with the expression of genes involved in development of early nodulation (Lafuente et al. 2010). These studies have suggested that arsenic contamination interferes with the legume-rhizobium symbioses, thereby reducing the nitrogen fixation in the ecosystem. Besides this, nitrogen assimilation has also been reported to be disrupted by As contamination of the soil (Foyer et al. 2011). Apart from the leguminous plants, the source of nitrogen for the non-leguminous plants is the inorganic nitrogen forms [ammonium (NH^{+4}) or the nitrate (NO^{-3})]. The assimilation of inorganic nitrogen (NH^{+4}) occurs in the form of glutamate predominately by the action of glutamine and glutamate synthase enzymes, which allows the transfer of inorganic nitrogen form NH^{+4} to organic pool (Foyer et al. 2011). With this background knowledge, many previous transcriptomic studies have stated a decrease in the transcripts of NH^{+4} transporter and NO^{-3} transporters when exposed to As treatment (Norton et al. 2008; Chakrabarty et al. 2009). Alongside, a decrease in nitrite reductase, nitrate content have also been pen downed upon exposure of plants to As toxicity which hampers the NH^{+4} supplies to GSH, ultimately leading to reduced nitrogen assimilation (Ahsan et al. 2008). This implicates that As affects the nitrogen assimilation pathway in two ways: first by interfering with the supply of inorganic nitrogen and second, by reducing the activity of enzymes of the nitrogen assimilation pathway. Furthermore, amino acid pool changes under As stress in plants which may be either due to the effect of As on amino acid synthesis, protein metabolism, or both (Dwivedi et al. 2010a; Pavlík et al. 2010). Decrease in total protein content under As stress has been reported in *Pteris ensiformis* and *P. vittata* (Singh et al. 2006). Moreover, dramatic effect on content of RuBisCO has been reported in plants when exposed to As due to its higher potentiality for nitrogen storage in the form of amino acids (Ahsan et al. 2008; Bona et al. 2010). Another explanation for the decrease in the amino acid pool under higher As concentration has been stated as first As induces protein degradation, which is secondarily accompanied with a decrease in carbohydrate content. Under low carbohydrate levels, protein degradation act as a reservatory for supplying respiratory carbon, thereby hindering the amino acid biosynthesis and leading to changes in the amino acid pool under As stress (Araújo et al. 2011). Genetic studies have revealed that As causes change in expression of genes involved in transportation of nitrogen. It has been reported that in rice seedlings exposed to As, downregulation of amino acid transporters take place in root of rice (Norton et al. 2008; Chakrabarty et al. 2009).

7.4.4 Arsenic Stress and Oxidative Stress in Plants

Production of reactive oxygen species (ROS) is a well-known mechanism induced by arsenic toxicity in plants (Chandrakar et al. 2016). It induces ROS either by interconversion of As (V) to As (III) or by inactivating the antioxidants by binding to their thiol group resulting in an array of metabolic perturbations in plant. There are mainly two ways of ROS production by arsenic. First arsenic inhibits important antioxidative enzymes and causes electron leakage during the reduction of As (V) to As (III). It has been reported that oxygen is used as a final electron acceptor during interconversion of arsenate to arsenite that occurs via cytochrome/cytochrome oxidase pathway in mitochondria and chloroplast. The oxygen reacts with the cytochrome oxidase generating superoxide radicals leading to increased oxidizability of the root and production of high amount of hydrogen peroxide (Sharma 2012). Second, the way of ROS production is methylation process which happens after As (V) is converted to As (III). The methylation of As produces mono- and dimethylarsonic acid and tri- and tetramethylarsonium oxides. These methylated arsenic forms were more reactive toward oxygen, increasing ROS generation (Abbas et al. 2018). ROS like $O_2^{\bullet-}$, H_2O_2, and OH• are strong oxidizing agent, causing disruption of lipid bilayer, increasing electrolyte leakage, and damaging DNA and proteins of the plant cells oxidatively which afterward cause death of the cells. Plants imposed to arsenic stress cause electrolyte leakage due to cellular membrane damage which leads to increased MDA content, a product of lipid peroxidation which indicates oxidative stress under arsenic toxicity. It also disturbs the electron transport process in thylakoid membrane, photosynthesis, various metabolic processes, and membrane permeability which causes leaf necrosis and chlorosis (Danh et al. 2014).

7.5 Arsenic Tolerance Mechanisms in Plants

As a response to arsenic stress, plants have evolved different detoxification strategies to not only maintain the cellular concentration of free metalloids but also repair the damage caused by free radicals due to oxidative stress. The important detoxification mechanisms modulated by the plants due to arsenic stress include enzymatic scavenging of ROS produced by As stress, hyperaccumulation, non-enzymatic antioxidant defense mechanisms, and phytochelation of arsenic to protect cells and subcellular system.

7.5.1 ROS Homeostasis and Antioxidative Defense System

Plants exposed to arsenic stress employ various antioxidative enzymes and non-enzymatic antioxidant compounds to mitigate the negative effects of oxidative stress caused by arsenic. Non-enzymatic antioxidant compounds include GSH, α-tocopherol, ascorbate, β-carotenoids, and polyphenols, and antioxidative enzyme defense system consists of enzymes such as SOD, APX, GR, and CAT. Changes in the activity of antioxidants and antioxidative enzymes are common indicators of oxidative stress. Glutathione which is involved in detoxification of cellular toxicants is considered as the most vital cellular antioxidants, and it directly functions as scavenger of free radicals. Glutathione also plays key role in phytochelatin synthesis which generally acts as heavy metal-binding peptide in plants. Superoxide dismutase neutralizes the $O_2^{\bullet-}$ into O_2 and H_2O_2 playing significant part in mitigating the effect of oxidative stress in plant cell. A positive correlation between SOD stimulation and reduction in lipid peroxidation under arsenic stress has been documented in mung bean (Singh et al. 2007) and *Holcus lanatus* (Hartley-Whitaker et al. 2001). An increase in the activity of various SOD isozymes in rice seedlings and red clover has been reported (Mascher et al. 2002; Shri et al. 2009). Requejo and Tena (2005) have reported high response of Cu/Zn SOD isozyme to arsenic in maize root for maintenance of cellular homeostasis. Abercrombie et al. (2008) reported suppression of FeSOD and induction of SOD chaperone and Cu/Zn SOD in chloroplast of

A. thaliana. Singh et al. (2007) reported decrease in CAT content in mung bean. Similarly, changes in GSH levels have been reported under heavy metal stress (Sarma 2011). Halophytes have been reported with high heavy metal tolerance capacity, and this may be associated with the characteristics of salt resistance. However, synthesis of various enzymatic and non-enzymatic antioxidant compounds in halophytes can also improve heavy metal resistance against oxidative stress as they have more efficient antioxidant systems than those of glycophytes. Previously, it has been reported that halophytes have high levels of SOD, POX, and CAT activities and less damage to membrane caused by ROS due to heavy metal stress. However, according to Sharma et al. (2010), an increase in activity of SOD and CAT was found in halophyte *Salicornia brachiata* in response to arsenic stress.

7.5.2 Phytochelatin Synthesis

To withstand arsenic stress, the plants modulate several pathways to not only maintain the cellular concentration of free metalloids but also repair any damage caused by free radicals. Chelation of heavy metals by ligand and compartmentalization of ligand-heavy metal complex are the common mechanisms adopted by the plants for detoxification of heavy metals (Garg and Singla 2011). To cope up with the arsenic stress, plants synthesized metal-binding cysteine-rich, thiol-reactive peptide called phytochelatins (PCs), known to play a significant role in removal of many thiol-reactive toxic metals and metalloids. Phytochelatins are a set of novel peptides, which were first isolated from the higher plant suspension culture after exposure of cadmium. The enzyme phytochelatin synthase synthesizes phytochelatins from GSH (Lokhande et al. 2011). Previously PCs have been reported for their essential role in metal ion homeostasis (Ghosh et al. 2016). The formation of As (III) by enzymatic and non-enzymatic reduction of As (V) is favored by GSH, and further As (III) is complexed with GSH and phytochelatins for the production of As(III)-PC complex for sequestration of As(III) into the vacoules. Arsenic directly interfere with the plant metabolic processes that occur in cytoplasm and therefore, its detoxification is crucial. Arsenic detoxification can be done by complexations and/or vacuole sequestration (Zhao et al. 2003). In arsenic stress condition, reduced arsenite bound to a sulfhydryl group (-SH) of phytochelatins to form As-PC complexes. These PC-As complexes are sequestrated in vacuoles where pH condition (pH 5.5) supports high molecular weight PC-As complexes. Thus, the formation of PC-As complex leads to decrease free concentration of arsenic in cytosol and thereby reducing arsenic toxicity. Moreover, for arsenic detoxification, non-hyperaccumulating plants mainly favor formation of PC-As complex and sequestration of As (III)-PC complex in the root vacuoles (Tripathi et al. 2012).

7.5.3 Hyperaccumulation of Arsenic

Metal hyperaccumulation is a complex and very uncommon mechanism in plants which can be described as plants with low biomass having high bioconcentration factor. Hyperaccumulation of metal can not reflect only by the metal concentration in aerial biomass of plant but also the metal concentration from the soil. However, evaluation of hyperaccumulator capacity of a plant is carried out by calculating bioconcentration factor (BCF) or bioaccumulation and translocation factor (T*f*). Bioaccumulation or BCF is used to determine the efficiency of plant for removal of the metals from the soil and their translocation from root to shoot which can be calculated by the ratio of metal concentration in plant biomass to that of the nutrient medium (Tu and Ma 2002). Hence, BCF and T*f* for arsenic hyperaccumulator should be greater than 1, and total arsenic accumulation should be 1000 mg kg^{-1} As per plant biomass. Even though some plants can grow in extreme metal concentration and accumulate high arsenic in their aboveground biomass, but if their BCF and T*f* are less than 1, they cannot be called as arsenic hyperaccumulator. Hyperaccumulator must have developed a mechanism to protect themselves from metal toxicity through which they either exclude or form complex and inactivate heavy metals. Phytoextraction is a green in situ technology that completely depends on the hyperaccumulator plant species grown on metalliferous soils and their ability to extract and translocate toxic heavy metals to aerial parts. Metallophytes or metal hyperaccumulators are ideal for phytoextraction due to some unique physiological, biochemical, and genetic characteristics. According to Sheoran et al. (2011), approximately 45 plant families from 400 plant species have been reported for hyperaccumulation of heavy metals. Moreover, the phenomenon of hyperaccumulation can be natural or chemically induced hyperaccumulation. The natural hyperaccumulator can solubilize soil metals, absorb them efficiently, and translocate them to shoot from root and store in aboveground parts in nonphytotoxic form. However, some metals like gold and lead are highly immobile in soil; hence their extraction and translocation can be difficult for natural hyperaccumulator; therefore, the mobility of such metals can be induced by several chemicals such as fertilizing salt, acidifying agents, and various chelating agents which increase bioavailability of metal in soil and made easy uptake for plants (Sheoran et al. 2011). Hyperaccumulator discriminates from non-hyperaccumulator taxa by three ways: enhance rate of heavy metal uptake, rapid root-shoot translocation, and detoxification of heavy metals in leaves. The process of hyperaccumulation includes boosted As (V) uptake, decreased As (III)-thiol complexation, and reduction of outflux of As (III) to the external medium, resulting in increased translocation of As (III) in xylem and vascular sequestration of As (III). However, the degree of heavy metal hyperaccumulation is dependent on various plant species. *P. vittata* L., the first plant species identified to hyperaccumulate arsenic, was reported 3 years ago by Ma et al. (2001). It can accumulate up to 21 g kg^{-1} of arsenic in its fronds after growing in soil containing 0.5 g kg^{-1} arsenic for 6 weeks. Furthermore, several halophytes such as *Suaeda maritima*, *Sesuvium portulacastrum*, and *T. gallica* have also been reported for their hyperaccumulation properties.

7.6 Conclusion and Future Perspectives

As discussed above in the chapter, exposure to arsenic negatively affects the plant metabolism. Arsenic toxicity influences various biochemical and molecular processes of the plants leading to reduction in growth, biomass accumulation, and photosynthetic efficiency. Also, it inhibits different metabolic pathways either indirectly by interfering with essential enzyme activities or affects directly as competitive inhibitor of phosphate. Likewise, arsenic toxicity causes damage to biomolecules like proteins and lipids. Arsenic uptake by plant species is an important phytoremediation approach which seems to be a very effective way to remediate arsenic contamination from the saline as well as non-saline soil. Some adaptive mechanisms of different plant species such as exclusion, exudation, sequestration, and metabolic adjustment provide strong support to survive under extreme arsenic toxic environmental conditions. Use of phytoremediation techniques is an emerging area of interest for detoxification of arsenic-contaminated saline soils. Although phytoremediation of arsenic-contaminated soil is considered as a good alternative technology, it has few limitations. Therefore, prolong research require to overcome the limitation to apply this technology effectively. Further studies also require to understand the relationship between arsenic and salt tolerance in halophytic species for exploring their potential for remediation of contaminated soils in diverse environment.

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Chapter 8
Adaptive Strategies of Plants Under Adverse Environment: Mitigating Effects of Antioxidant System

Dragana Jakovljević and Milan Stanković

Contents

Abstract Variation in plant adaptive strategies to the diversity and variability of the environmental factors is the key to plant developmental success. Climate change phenomenon may be considered as one of the important factors of the adverse environment since it may lead to visible changes in rainfall and temperature in the global as well as regional aspects. Under the conditions of such an unfavorable environment, plants increase the production of reactive forms of oxygen which further trigger disequilibrium between their production and removal. To control the production of reactive oxygen species (ROS), higher plants possess the ROS detoxification system which includes enzymatic and non-enzymatic antioxidant components that remove ROS and protect plant cells from oxidative damage. This chapter provides main information on ROS generation, redox balance, and plant protection in the view of ecophysiological adaptations to the adverse environment with a special focus on the antioxidant defense system.

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Abbreviations

$^1O_2$	Singlet oxygen
AOX	Alternative oxidase
A-POX	Ascorbate peroxidase
AsA	Ascorbic acid
CAT	Catalase
Cu/Zn-SOD	Copper/zinc superoxide dismutase
ETC	Electron transport chain
Fe-SOD	Iron superoxide dismutase
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
Mn-SOD	Manganese superoxide dismutase
$O_2^{\bullet -}$	Superoxide anion radical
$OH\bullet$	Hydroxyl radical
PAL	Phenylalanine ammonium lyase enzyme
PSI	Photosystem I
PSII	Photosystem II
ROS	Reactive oxygen species
SOD	Superoxide dismutase

8.1 Introduction

In their surrounding environment, plants are in constant relation with the different environmental factors which interact in a complex spatial and temporal variability. Influences from the environment can be classified according to their origin on abiotic factors (physical and chemical influences) and biotic factors (influences of other living organisms including human activities named anthropogenic factor). As a part of adaptive strategies to the diversity and variability of the environmental factors, plant respond to morphological, anatomical, physiological, and phenological levels, and the adaptations, which result from this process, represent biological responses in order to survive and optimize the development of populations. Variation in adaptive strategies and the ability of a plant to dynamically shift the response to the environment in optimal conditions, as well as under modified conditions, is the key to plant developmental success. The adverse environment may have local or global origin and does not necessarily lead to stress (Schulze et al. 2009; Chelli-Chaabouni 2014; Gray and Brady 2016).

In recent years, climate change phenomenon may be considered as one of the important environmental factors since it may lead to visible changes in rainfall and temperature in the global as well as regional aspects (Valizadeh et al. 2014). Therefore, climate change may be considered as one of the important factors of the adverse environment with the global origin from the aspect of living organisms and their ecology. Main influences related to this phenomenon include several changes on the abiotic environment at the global level such as climate warming associated with temperature and drought stress, as well as elevated atmospheric CO_2 and O_3 concentrations, and a wide spectrum of plant responses to these stresses are in general morphological, biochemical, and physiological responses (Ceulemans and Mousseau 1994; Veteli et al. 2002; Hatfield and Prueger 2015; Kyoro et al. 2012; Fahad et al. 2017; Holopainen et al. 2018; Hatfield and Dold 2019).

The objective of this chapter is to present the main effect of the adverse environment on plants in terms of plant ecophysiological responses in order to mitigate these effects. Having in mind that climate change leads to specific adverse environmental conditions, which is associated with the generation of reactive forms of oxygen and activation of plant defense systems, plant antioxidant system including enzymatic and non-enzymatic components was reviewed.

8.2 Reactive Oxygen Species and Antioxidant System in Plants

Climate change leads to variation in frequency, duration, and intensity of main climate ecological parameters like precipitation, temperature, and drought which further leads to better tolerance of changes in the frequency of extreme weather events in species with increased adaptive plasticity (Cassia et al. 2018). Under the conditions of such an unfavorable environment, plants increase the production of reactive oxygen species (ROS), particularly singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•). Further detoxification of ROS is essential for the protection of the main structures of plant cells against the harmful effect of these products (Mittler 2002; Caverzan et al. 2012). In order to control the ROS production, in higher plants is active the ROS detoxification systems. This system include enzymatic and non-enzymatic antioxidant components that help in scavenging of ROS and protect plant cells from oxidative damage (Foyer and Noctor 2005; Das et al. 2015). Enzymatic antioxidants are presented in particularly all subcellular compartments and include superoxide dismutase (SOD), catalase (CAT), and several classes of peroxidases, while non-enzymatic antioxidants include ascorbate (AsA), glutathione (GSH), carotenoids, phenolic compounds, flavonoids, etc. (Mittler 2002; Caverzan et al. 2012; Gengmao et al. 2015).

The antioxidant defense system does not completely remove ROS since the coordination of the production and the elimination of ROS allow adequate signaling of the defense system during stress, including enzyme activation and gene expression. In addition to the production during coordinated metabolic pathways, ROS are produced in higher concentrations because of abiotic and biotic stress factors in different cellular compartments (including mitochondria, chloroplasts, peroxisome, cell wall, apoplast, endoplasmic reticulum, and plasma membrane). In this way, the equilibrium between producing and removing reactive oxygen species is endangered by numerous factors of stress such as ultraviolet radiation, extreme temperatures, nutrient deprivation, pathogen attack, drought, etc. This disturbance of the balance causes oxidative stress, i.e., a condition that follows a significant imbalance between ROS production and antioxidant defense (Foyer and Noctor 2003; Asada 2006; Gill and Tuteja 2010; Foyer and Shigeoka 2011).

Hot and dry areas have severe negative effects on plant production. The rise in temperature and the decrease in rainfall together with the heat, drought, coldness, or flood will affect plant production (Valizadeh et al. 2014). Since warming is commonly accompanied by drought, plant growth and development are limited directly or indirectly through the heat stress or water shortage. Furthermore, under the condition of individual heat or drought stress, a small amount of H_2O_2 and $O_2^{\bullet -}$ were detected, whereas when plants are exposed to combined stress, ROS levels increased substantially (Li et al. 2014). Accumulation of ROS under individual and combined stress treatments is presented in Fig. 8.1.

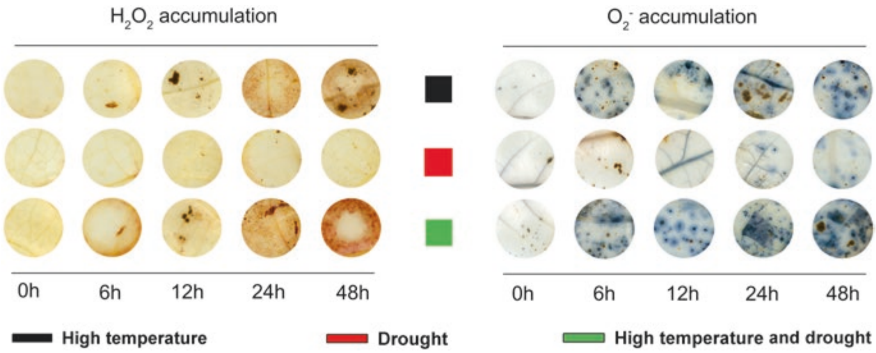

Fig. 8.1 Accumulation and in situ detection of reactive oxygen species levels (H_2O_2 and $O_2^{\bullet -}$) in the leaf of *Populus yunnanensis* after exposure to heat, drought, or their combinations at different times (According to Li et al. 2014)

8.2.1 Enzymatic Components of Antioxidant System

Harmful effects of ROS can be seen through the disruption of photosynthetic pigments and proteins, dysfunction of cellular metabolism, and protein oxidation and lipid peroxidation which ultimately lead to oxidative stress (Girotto et al. 2016). It has been well documented that the activities of antioxidants correlate with the tolerance ability of the plant (Gill and Tuteja 2010; Hussain et al. 2016). The ability of plants to minimize oxidative stress depends mainly on the effectiveness of enzymatic and non-enzymatic antioxidants. However, different plant species, including different genotypes of the same species, are characterized with highly variable antioxidant capacity (Mir et al. 2015; Kalisz et al. 2016; Jakovljević et al. 2019). The adaptable and flexible antioxidant system which can control the optimum level of ROS is the result of differences in subcellular localization and biochemical properties of antioxidant enzymes (Vranová et al. 2002).

8.2.1.1 Superoxide Dismutase

Superoxide dismutase (SOD) is one of the most effective metalloenzymes whose basic role is to prevent the formation of OH• through the Haber-Weiss reaction. Two molecules of $O_2^{\bullet-}$ are involved in the process of dismutation of this reactive oxygen species catalyzed by SOD. One molecule is reduced to H_2O_2, and the second molecule is oxidized to O_2. Based on the bonded metal ion, SODs are classified into three isozymes: copper/zinc superoxide dismutase (Cu/Zn-SOD) localized in the cytosol, peroxisomes, and chloroplasts; manganese superoxide dismutase (Mn-SOD) in mitochondria; and iron superoxide dismutase (Fe-SOD) in chloroplasts. The specific activity of individual forms is related to stress factors and plays a pivotal role in surviving adverse conditions (Mittler 2002; Gill and Tuteja 2010; Das and Roychoudhury 2014). The protective role of SOD was noted in the case of oxidative stress induced by different factors (Rai et al. 2004; Wongsheree et al. 2009; Landi et al. 2013; Jakovljević et al. 2017a; Jakovljević et al. 2017b). Since climate change scenario includes drought and increase in temperature and it is known that SOD is a stable and functional enzyme under the wide temperature ranges, SOD engineering may lead to plant improved performance under conditions of oxidative stress, as well as to the development of crop tolerance to drought, high temperature, and other climate change-related stresses (Kumar 2016).

8.2.1.2 Catalase

Catalases (CAT) are tetrameric heme proteins involved in the degradation of H_2O_2 into water and oxygen. They are characterized by high affinity for H_2O_2 and the highest rate in H_2O_2 removal. Although CAT is predominantly active in peroxisomes, this enzyme can also be active in other cellular compartments, whereby the isoforms of this enzyme are independently regulated. Angiosperms have three CAT genes: *CAT1* expressed in pollen and seeds and localized in cytosol and peroxisomes; *CAT2* expressed in seeds, photosynthetic tissues, and roots and localized in cytosol and peroxisomes; and *CAT3* expressed in vascular tissues and leaves and localized in the mitochondria (Gill and Tuteja 2010; Das and Roychoudhury 2014). Previous investigations of CAT regulation mechanisms showed that mechanisms of plant adaptions to temperature stress could be positively correlated with CAT responses in different plants including maize, cucumber, olive, banana, and broccoli (Scandalios et al. 2000; Gao et al. 2009; Cansev et al. 2011; Lin et al. 2010; Figueroa-Yáñez et al. 2012).

8.2.1.3 Ascorbate Peroxidase

Peroxidases utilizing H_2O_2 or organic hydroperoxides to oxidize various substrates. In their active sites, these enzymes can contain a heme cofactor (ascorbate peroxidases or guaiacol peroxidases) or redox-active cysteine or selenocysteine residues (non-heme peroxidases). The non-heme peroxidases comprise thiol peroxidases, such as peroxiredoxins (Prxs) and glutathione peroxidases (GPXs) (Koua et al. 2008; Bela et al. 2015).

Ascorbate peroxidases (A-POX) belong to the class I heme-containing intracellular peroxidases. The main function of these oxidoreductases is the removal of H_2O_2 via ascorbate-glutathione pathway (with ascorbic acid (AsA) as the donor of the electron) in which protection against oxidative stress is provided by ascorbate-glutathione through the series of coupled reactions in photosynthetic tissues, in mitochondria and peroxisomes (Reddy and Raghavendra 2006). There are several isoforms of A-POX in plant cells classified according to their subcellular localization and the presence of various amino acids. They can be localized in the cytosol (cA-POX), chloroplast stroma (sA-POX), and chloroplast thylakoids (tA-POX), as a membrane-bound isoform in glyoxysomes and peroxisomes (mA-POX), and in mitochondria (mitA-POX) (Shigeoka et al. 2002; Caverzan et al. 2012). Due to a higher affinity to H_2O_2 than catalase and other peroxidases, it is considered that A-POX has a crucial role in the removal of this reactive species, but the form of activity is conditioned by the taxonomic affiliation of the plant organism and factors leading to oxidative stress (Gill and Tuteja 2010; Das and Roychoudhury 2014).

A high level of AsA is required to maintain the antioxidant system since A-POXs are unstable in the absence of AsA (Shigeoka et al. 2002). In response to environmental stress, A-POX activity increases together with SOD, CAT, and GPX and with higher activities of A-POX in tolerant compared to sensitive plants (Shigeoka et al. 2002; Caverzan et al. 2012).

8.2.1.4 Glutathione Peroxidase

The multiple isoenzymes with specific subcellular locations are included in the glutathione peroxidase (GPX) family. These isoenzymes display different tissue-specific expression patterns and environment-related responses (Bela et al. 2015). The GPX enzyme family comprises proteins phylogenetically related to non-heme thiol peroxidases that catalyze the reduction of H_2O_2 or organic hydroperoxides to H_2O or the respective alcohols using glutathione (GSH) as a reducing agent (Depège et al. 2000; Bela et al. 2018). However, detailed functional analysis of individual GPX isoenzymes and identification of GPX interaction patterns is still required (Bela et al. 2015). In general, GPXs play a complex role in plants during their whole life. However, thiol-dependent activities of GPX isoenzymes indicate that they may be involved in the maintenance of thiol/disulfide or NADPH/NAD+ balance and therefore in the regulation of the cellular redox homeostasis (Jung et al. 2002; Rodriguez Milla et al. 2003). Briefly, in stress responses, they maintain H_2O_2 homeostasis through the elimination of H_2O_2 and organic hydroperoxides; participate in protein complexes related to stress defense; perform redox modification of nuclear proteins with putative signaling function; and contribute to different signaling pathways (Bela et al. 2015).

8.2.1.5 Class III Peroxidases

Class III peroxidases include hem enzymes with isoforms that may vary in more than 50% of the amino acid sequences. These oxidoreductases catalyze the oxidation of various groups of organic components, using the aromatic compounds as donors and H_2O_2 as the final acceptor of the electrons (e.g., guaiacol peroxidase (G-POX) using guaiacol while pyrogallol peroxidase (P-POX) using pyrogallol as a donor). In addition to protecting against oxidative damage by removing H_2O_2, these enzymes are involved in many metabolic processes such as auxin catabolism, lignin biosynthesis, defense against predators, etc. They are localized intracellularly (in cytosol and vacuoles) and in the cell wall and extracellular space. As the main functions of peroxidases are linked to the cell wall, the isoforms of these enzymes represent a significant regulatory factor of cell elongation and growth. Like other enzymatic components of the defense system, the activity of these enzymes is spatially and temporally different and is also conditioned by external factors (Quiroga et al. 2000; Gill and Tuteja 2010; Kukavica et al. 2013; Das and Roychoudhury 2014).

Among enzymes that participate in scavenging of ROS and which activity can improve tolerance to major abiotic stresses, glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) are significant components of plant integrative physiological responses to prevent oxidative damage.

8.2.2 Non-enzymatic Components of Antioxidant System

In addition to enzymatic antioxidant protection, the process of removing the ROS is also performed through non-enzymatic components of the plant defense system. The non-enzymatic components of protection from oxidative stress include carotenoids, ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, and different compounds generally named secondary metabolites.

8.2.2.1 Carotenoids

Carotenoids are isoprenoid compounds whose unique chemical structure determines biological roles and activities. Most carotenoids are a skeleton of 40 carbon atoms (with conjugated double bonds) with cyclic end groups which can be substituted by functional groups containing oxygen. The basic functions of these pigments are the absorption of light and further transfer to chlorophylls, as well as the protection of plant cells by removing free radicals (antioxidant function). The conjugated double bonds determine the ability of carotenoids to absorb light and affect their antioxidant activity. The antioxidative activity of carotenoids in terms of ROS is based on their ability to neutralize $^1O_2$ and OH•. The synthesis of these plant pigments is induced by various stressors including salt, radiation, and lack of nutrients (Stahl and Sies 2003; Polesskaya et al. 2004, 2006; McElroy and Kopsell 2009; Gill and Tuteja 2010; Nisar et al. 2015; Hou et al. 2016).

8.2.2.2 Ascorbic Acid

Ascorbic acid (AsA, vitamin C) is one of the universal non-enzymatic antioxidants and a key substrate for the detoxification of ROS (Foyer and Noctor 2011), and it is possible that apoplastic AsA contents could be vital for environmental stress (Akram et al. 2017). Because of the regenerative nature, this molecule is one of the most powerful antioxidant molecules which together with vitamin E plays a pivotal role in ROS removal either directly or through the enzymatic catalysis (Noctor and Foyer 1998; Akram et al. 2017). The specific nature of AsA and involvement in the ascorbate-glutathione cycle and ascorbate peroxidase-glutathione reductase system allow this molecule to be part of the enzymatic as well as non-enzymatic antioxidant defense system, and this makes AsA a fragile substrate in plant development as well as stress responses (Akram et al. 2017). According to Athar et al. (2008), application of AsA improves both the growth and activities of antioxidant enzymes. Potentially, in plant genetic engineering, as a regulator, AsA could efficiently increase plant stress tolerance (Akram et al. 2017).

8.2.2.3 Vitamin E

α-Tocopherol (vitamin E) is synthesized in plastids. This lipophilic antioxidant is located in membranes of the cells (particularly in the thylakoid membranes of the chloroplasts), and its function is determined by the structure and location. Due to their structure, these non-enzymatic antioxidants are capable to interact with the polyunsaturated acyl groups and protect polyunsaturated fatty acids from lipid peroxidation by quenching the ROS through the donation of a single electron and formation of stabilized tocopheroxyl radical (Wang and Quinn 2000; Yamaguchi et al. 2001; Jaleel et al. 2009). Oxidative stress and generated ROS activate the synthesis of α-tocopherol, and increased level of α-tocopherol has been reported in response to the different abiotic stresses including drought and water stress (Bartoli et al. 1999; Sandorf and Hollander-Czytko 2002; Munne-Bosch and Algere 2003).

8.2.2.4 Glutathione

Glutathione (c-glutamyl-cysteinyl-glycine) is considered as a strong non-enzymatic antioxidant detected in different cell compartments including vacuoles, chloroplasts, mitochondria, endoplasmic reticulum, and cytosol. This small intracellular thiol molecule with the highest concentration in chloroplasts occurs predominantly in the reduced form (GSH), whereas the ratio of reduced (GSH) to oxidized (GSSG) form is important in redox signaling pathway (Jaleel et al. 2009; Hasanuzzaman et al. 2017). GSH content varies due to the intensity of stress, and an increase in a GSH content can be related to its ability to scavenge singlet oxygen, hydroxyl radicals, as well as peroxides. Furthermore, GSH is part of the ascorbate-glutathione pathway in chloroplasts (Pei et al. 2000; Jaleel et al. 2009). One of the main roles of GSH includes improving plant tolerance to different abiotic stresses (see Hasanuzzaman et al. 2017).

8.2.2.5 Secondary Metabolites

The diverse range of compounds produced by plants can be generally classified into primary or secondary metabolites. The primary metabolites are molecules essential for growth and development, while secondary metabolites allow plants to adapt to adverse environmental conditions (Caretto et al. 2015; Yoshikawa et al. 2018). Inappropriate water supply and abnormal temperatures negatively affect plant growth and development mainly through the physical damages, biochemical changes, and physiological disruptions (Fahad et al. 2017). When environmental conditions are adequate, plant growth and photosynthesis will be favored, but when the availability of essential nutrients is limited, the growth allocation will decrease, while the production of secondary metabolites increases (Jakovljević et al. 2017b, 2019). Secondary metabolites are among the most important non-enzymatic components of the plant defense system since they play an important role in responding to the abiotic and biotic stress factors. Polyphenols (phenolic compounds) represent one of the three main groups of secondary metabolites and can be primarily classified according to their chemical structures (Larbat et al. 2014; Yoshikawa et al. 2018). Among the secondary metabolites, several thousand molecules are characterized by a polyphenolic structure, i.e., an aromatic ring with several hydroxyl groups. Most of the plant phenolic compounds are biosynthesized through the phenylpropanoid pathway which starts with amino acid phenylalanine. Phenylpropanoid metabolism is a necessary metabolic pathway since plants through this pathway synthesize the structural components necessary for vascular integrity and structural support. Additionally, for plants, this is the starting place to produce many important metabolites involved in the defense of abiotic and biotic environmental factors. The phenylalanine ammonium lyase (PAL) enzyme catalyzes the first step in phenylpropanoid metabolism in which phenylalanine (the product of the shikimate pathway) undergoes deamination to yield trans-cinnamic acid and ammonia (Fraser and Chapple 2011). The expression of the genes responsible for the activity of the PAL enzyme is spatial and time-controlled, and the differences in activity can also be attributed to biotic and abiotic stress factors. Despite the insufficient clarification of the genetic mechanism underlying this mechanism of regulation of PAL gene expression, a versatile and adaptable control of the diversion of the products of the shikimate pathway and phenylpropanoid metabolism to the synthesis of secondary metabolites is possible only with the adequate development of the photosynthesis process (Vogt 2010; Fraser and Chapple 2011). Although PAL cannot be considered as a part of the antioxidant system in plants, compounds produced regarding the PAL activity significantly contribute to the plant's antioxidant capacity. A variety of phenylpropanoids based on the general phenylpropanoid pathway and the secondary metabolites synthesized by the activity of PAL enzyme are presented in Fig. 8.2.

In this way, there are about 8000 aromatic metabolites that are classified into different subclasses (or classes) including coumarins, flavonoids, anthocyanins, stilbenes, phenolic acids, and others. PAL activity is induced during growth and development, but also with many external factors such as pathogen attack, UV radiation, lack of nutrients, and other processes in which aromatic components protect the plant organism (Jakovljević et al. 2017a; Baque et al. 2010). Understanding mechanisms that control the nature and quantity of phenolic compounds synthesized under different conditions remains a priority in researches (Lattanzio et al. 2009; Zhang and Liu 2015).

Secondary metabolites play a significant role in the process of plant adaptation to an unfavorable environment. Their quantitative and qualitative composition in a particular plant part or tissue, within a specific period of the plant's life cycle, is consistent with the biotic and abiotic factors of the environment to which the plant is exposed (Edreva et al. 2008; Oh et al. 2009; Vander Wall and Jenkins 2011). The drought stress is accompanied by an increased synthesis of secondary metabolites involved in the process of adaptation to water loss and alleviation of the dehydration consequences (Marchese et al. 2010; Khan et al. 2010). Exposure of *Lactuca sativa* L. to water deficiency under laboratory conditions led to an increased concentration

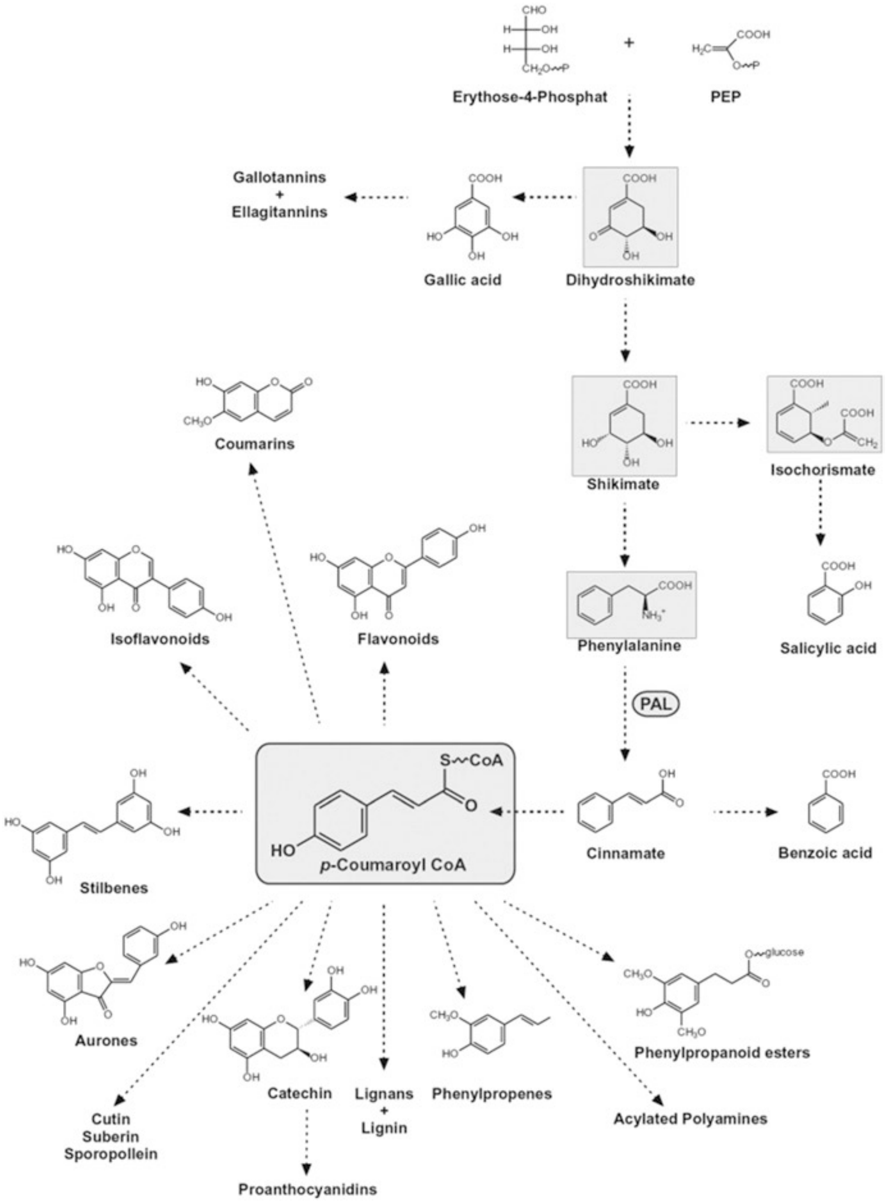

Fig. 8.2 Diversification of plant phenylpropanoids based on the general phenylpropanoid pathway and the secondary metabolites synthesized by the activity of PAL enzyme (According to Vogt 2010)

of enzymes involved in the process of biosynthesis of phenolic compounds (Oh et al. 2009). It was found that the water deficit in the *Ctenanthe setosa* (Rosc.) Eichler results in the increased concentration of phenolic acids, which is associated with increasing the resistance of plants to the disturbed water regime (Ayaz et al. 1999). In the process of increased synthesis of secondary metabolites in plants, there is no intensification of the synthesis of all types of secondary metabolites, but only those that enable the maintenance of plant cell and tissue homeostasis (Szabó et al. 2008). In arid habitats, in addition to stress caused by drought, plants are also exposed to temperature stress. The results of numerous studies of temperature stress and related plant adaptation mechanisms showed that stress caused by increased temperature led to synthesis of some flavonoids and phenylpropanoids, as indicated by the increased activity of the PAL enzyme involved in the biosynthesis process of these biomolecules (Rivero et al. 2001; Wahid et al. 2007). In low-temperature-exposed plants, phenolic compounds are responsible for the plant adaptation, and these metabolites were found in large quantities in plants inhabited by cold regions (Pennycooke et al. 2005; Koç et al. 2010). In the case of both water and temperature stresses, it has been established that only the metabolites that protect cellular structures are increased (Fletcher et al. 2010). Radiation is a known abiotic factor that has been associated with the synthesis and accumulation of secondary metabolites (Shohael et al. 2006). An increase in radiation intensity causes an increase in total phenolic content (Bakhshi and Arakawa 2006; Ghasemzadeh et al. 2010). In general, polyphenols are considered as a significant protector against the negative impact of ultraviolet radiation (Dixon and Paiva 1995; Meijkamp et al. 1999).

Phenolic compounds act as an antioxidant due to their hydroxyl and carboxyl groups which donate a hydrogen atom or an electron to free radicals. Furthermore, metal ions in phenolic structure decompose lipid hydroperoxide (LOOH). Therefore, the antioxidant activity of phenolic compounds is related to the structure of molecules as well as the number and position of the hydroxyl group (Milić et al. 1998; Stanković et al. 2012). Several studies have shown that flavonoids are capable to scavenge various forms of ROS (Michalak 2006).

8.3 Earliest Protection from Oxidative Damage

The germination and early growth of seedlings can be disrupted or inhibited under unfavorable conditions (Jakovljević et al. 2017b). Therefore, the prevention of environmental stress during the growing season and maintenance of suitable redox status of plant cells are of great importance (Balabusta et al. 2016). The regeneration from seeds can be precluded or delayed due to alterations in temperature and water supply caused by the global climate changes (Walck et al. 2011). Hence, the fate of plant populations and communities are affected by the factors that influence the ability of seeds to develop into adult plants (Mondoni et al. 2012). As a storage product, different compounds including carbohydrates, starch, proteins, and lipids are stored in seeds, and the content of these compounds depends on the characteristics of the plant itself as well as the environment conditions. The imbibition and further development of seed is a dynamic process coordinated by different structural components of the seed, and early phases in germination processes are crucial for seed viability (Sreenivasulu 2017; Gupta et al. 2017). Early reactivation of the mitochondrial electron transport chain leads to an increase in the production of ROS so that the dual function of ROS (both as signaling and harmful molecules) is present throughout the whole life cycle of the plant. The duration of imbibition and further germination depend on the plant species and environmental factors in which the seed was exposed to contact with water (Van Dongen et al. 2011; Rosental et al. 2014; Kumar et al. 2015; Macovei et al. 2017). In hydrated seeds, all metabolically active compartments may become sources of ROS such as glyoxysomes (through the catabolism of lipids), peroxisomes (through the catabolism of purines), mitochondria (through the respiratory activity), plasma membranes (through the NADPH oxidase), as well as chloroplasts by electron transfer (Gomes and Garcia 2013). Therefore, complete germination requires protection from reactive oxygen species produced during this process and activation of the antioxidant system (Espanany et al. 2016). The duration of the germination process (from the moment of passive imbibition to the degradation of the stored products and the occurrence of radicle) is genetically conditioned but also depends largely on the environmental factors. Radicle protrusion and hypocotyl elongation are among the most important phases of plant life; however, they are also periods in which the plant is most vulnerable to changes in the environment. Although desiccated seeds are mainly resistant to stressful environmental conditions, germinated seeds and seedlings are not and require defense strategies to deal with abiotic and biotic stress to ensure survival. The response to such conditions depends on the type and intensity of stress, but also the plant species and genotype (Macovei et al. 2017). The generation of ROS under the conditions of excessive heat, UV, or hypoxia stresses would prevent radicle emergence with consequent detrimental effects (Bailly et al. 2008). Reactive oxygen species tend to chemically react with nucleic acids and affect the genetic code of the embryo (Kranner and Colville 2011). Increased activities of GR, SOD, and CAT have been demonstrated during dehydration in sensitive as well as tolerant plants; however, activities of these enzymes are sustained upon rehydration only in the case of tolerant ones (Farrant et al. 2004). The importance of associated activities of SOD and CAT in maintaining the oxidative status of germinated seeds together with the pivotal role of A-POX was confirmed (Pergo and Ishii-Iwamoto 2011; Gomes et al. 2012).

8.4 Plant Growth and Development Under Adverse Environment: ROS Generation and Redox Balance

Plant productivity depends on the absorption and conversion of solar energy in the process of photosynthesis as well as the subsequent incorporation of reduced carbon into organic compounds during plant growth and development (Attaran et al. 2014).

Further fundamental processes during which plants assimilate carbon and nitrogen into the components necessary for growth and development are conditioned by a selective particle of organic metabolites between organelles, cells, tissues, and organs. The diversity of the produced metabolites, as well as the metabolic pathways, is wide and partitioned between organelles, cells, tissues, and organs (Tegeder and Weber 2006). Although during the plant growing seasons ROS are permanently generated even under normal growth conditions, their concentration increased significantly in response to abiotic stresses. Among the most important abiotic stresses related to climate change at the molecular level is the increase of ROS inside the cells (Farnese et al. 2016). ROS are generated in the chloroplasts, mitochondria, peroxisomes, plasma membrane, and apoplast, and it was proposed that each stress (individual or in different combinations) produces its own “ROS signature” (Farnese et al. 2016; Choudhury et al. 2017; Cassia et al. 2018). Figure 8.2 simplifies the participation of main organelles in the generation of ROS inside the cell together with activities of pivotal enzymatic components.

As a dynamic and flexible process that takes place in chloroplasts, photosynthesis generates oxygen through the activity of photosystems resulting in the formation of ROS. In this way, photosynthesis (particularly parts that include photosystem I and photosystem II — PSI and PSII) makes chloroplasts the main sources of ROS (Gill and Tuteja 2010; Foyer and Shigeoka 2011). One of the main characteristics of the PSII is that it is easily subjected to light damage. Insufficient dissipation of light energy during photosynthesis can lead to the formation of triplet chlorophylls. Triplet chlorophylls can easily react with triplet oxygen ($^3O_2$), thus resulting in the formation of singlet oxygen ($^1O_2$) in the reaction center of this photosystem. This damage and further regeneration occur even in the case of low-intensity light. The limitation of electron transport function occurs only if the damage to the reaction center is more intense than the possible renewal, whereby the photoinhibition of photosynthesis has extremely harmful effects on the PSI, as well as the entire photosynthetic apparatus (Gill and Tuteja 2010; Foyer and Shigeoka 2011).

The effective protection of the photosynthetic electron transport chain (ETC) involves the reduction of the production of singlet oxygen in PSII, but also the removal of superoxide anion radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) originating from PSI. Proper functioning of the cyclic transport of electrons is considered one of the ways of protection against reactive oxygen species since the energy balance of chloroplast is maintained by the production of ATP that is favorable in relation to NADPH. The superoxide anion radical is converted by the superoxide dismutase into H_2O_2 and O_2, and consequently, H_2O_2 is reduced to H_2O and ascorbate by ascorbate peroxidase (Asada 1999, 2006; Foyer and Shigeoka 2011).

Although the formation of H_2O_2 in chloroplasts is significantly lower than in the glyoxylate cycle, this form of ROS is a significant inhibitor of the photosynthetic process because even at low concentrations, it can inhibit the fixation of CO_2 up to 50%. Therefore, the balance between production and elimination of ROS in chloroplasts is a strictly controlled process. The enhanced chloroplast antioxidant protection has proven to be one of the best systems for protecting plants from abiotic stress since, with more efficient removal of ROS, the photosynthetic process is less sensitive to environmental changes (Foyer and Noctor 2003; Asada 2006; Gill and Tuteja 2010; Foyer and Shigeoka 2011). Under the conditions of drought-induced stress, the activity of RuBisCo may be reduced, and due to altered electron transport, ROS can be generated. Stressful conditions caused by high temperature (usually 10–15 °C above an optimum temperature) can lethally affect leaf photosynthesis (Wang et al. 2018). Under such unfavorable conditions, functional decrease in photosynthetic light reaction and high electron leakage from the thylakoid membrane consequently lead to the higher concentration of ROS (Hasanuzzaman et al. 2013; Cassia et al. 2018). Furthermore, the modified balance between hydrogen peroxide and superoxide under heat-induced stress could be due to degradation of Cu/Zn SOD localized in chloroplasts, as well as induction of CAT activity (Sainz et al. 2010; Wang et al. 2018). Higher activities of antioxidant enzymes (particularly SOD, CAT, POXs, and GR) were detected in heat-tolerant plants in comparison with heat-sensitive plants which may lead to the conclusion that enzymatic antioxidant defense system could have a pivotal role against damages caused by long-term heat stress (Du et al. 2013). Under photooxidative stress, AsA is a significant part of the plant cell protection against photooxidative stress since this non-enzymatic compound maintains the membrane-bound antioxidants. AsA is also a cofactor in the xanthophyll cycle, and this abundant metabolite is always associated with the glutathione cycle (Davey et al. 2002; Conklin 2001). Among the non-enzymatic chloroplast protectors, carotenoids are well known by their ability to quench the $^1O_2$ under light excess, while flavonoids are known to protect photosynthetic apparatus from UV radiation. According to Agati et al. (2012, 2013), flavonoids possess multiple functions in photoprotection by acting as UV-B screening compounds and as free radical scavengers. It was demonstrated that higher doses of UV lead to the generation of $^1O_2$ and $O_2^{\bullet-}$ in irradiated leaves, and increased leaf flavonoid production in response to UV-B radiation is primarily considered as a physical defense mechanism (Barta et al. 2004; Dobrikova and Apostolova 2015). Quercetin is one of the most common natural flavonoids which interacts with the ATP complex as well as with the components of the electron transport pathway in thylakoid membranes, inhibits the electron transport, and suppresses lipid peroxidation during strong illumination (Dobrikova and Apostolova 2015).

Despite continuous chloroplast production, ROS are inevitable products from other cellular compartments with high metabolic activity and electron flow. In addition to chloroplast and under the presence of light, peroxisomes are the main organelles that generate ROS, while in the absence of light, significant amount of the total oxygen is translated into ROS through the respiratory electron transport chain (ETC) of the mitochondria (Fig. 8.3). Besides the fact that mitochondrial production of ROS is minor in comparison to chloroplasts and peroxisomes, at the cellular level, impact of mitochondrial ROS needs to be clarified (Morgan et al. 2008). ETC contains a series of membrane protein complexes that harbors electrons with enough free energy to directly reduce O_2 (Rhoads et al. 2006). Activities of complexes I and II result in $O_2^{\bullet-}$ formation which is further subjected to dismutation using SOD to H_2O_2 (Rodríguez-Serrano et al. 2009). H_2O_2 can react with reduced Fe_2^+ and Cu_2^+, resulting in a highly reactive and extremely toxic hydroxyl radical (OH•), which can

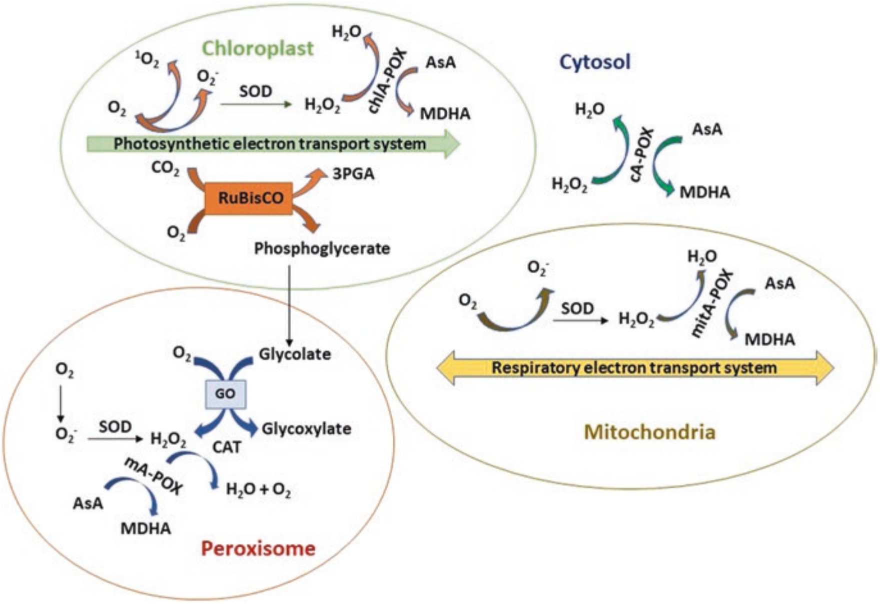

Fig. 8.3 Production and elimination of reactive oxygen species in different subcellular compartments. *$_1O^2$* singlet oxygen, *$O_2^{\bullet-}$* superoxide anion radical, *H_2O_2* hydrogen peroxide, *SOD* superoxide dismutase, *3PGA* 3-phosphoglycerate, *RuBisCo* ribulose 1,5-bisphosphate carboxylase/oxygenase, *chlA-POX* chloroplastic ascorbate peroxidase, *mAPX* peroxisomal ascorbate peroxidase, *cAPX* cytosolic ascorbate peroxidase, *mitAPX* mitochondrial ascorbate peroxidase, *AsA* ascorbic acid, *MDHA* monodehydroascorbate, *GO* glycolate oxidase, *CAT* catalase

lead to the formation of cytotoxic lipid aldehydes, alkenes, and hydroxy alkenes including malondialdehyde (MDA), and in the reaction with nucleic acids, proteins, and lipids, these products cause damage. With the energy dissipation system, plant cells can control the generation of reactive oxygen species and therefore have an extremely important role in plant adaptation to the conditions of oxidative stress on the cellular level (Gill and Tuteja 2010). By efficient energy dissipation mechanisms, mitochondria play an important role in the avoidance of ROS production during drought stress which enables plants to adapt to water shortage without extensive damage (Méller 2001). Generally, enzymes mitochondrial alternative oxidase (AOX) and Mn-SOD are very crucial to counteract oxidative stress in the mitochondria (Das and Roychoudhury 2014). In the mitochondria, the alternative oxidase (AOX) pathway is an alternative to the cytochrome pathway, and it diverts electrons flowing through the electron transport chain to produce water by the reduction of O_2 (Vanlerberghe and McIntosh 1997; Maxwell et al. 1999). According to Cruz de Carvalho (2008), three active energy-dissipating systems have been shown to coexist in Mediterranean plants well adapted to drought, including AOX. Moreover, *Arabidopsis* lacking a functional AOX is sensitive to drought stress and has altered transcription profiles of different components of the antioxidant machinery (Ho et al. 2008; Das and Roychoudhury 2014). In addition to AOX, plant mitochondria possess a highly conserved Mn-SOD, and the H_2O_2 produced as a catalytic by-product of the Mn-SOD is further reduced to water by several peroxidases including A-POX and GPX (Morgan et al. 2008). Furthermore, Mn-SOD may be the crucial difference between sensitive and tolerant plants (Mittova et al. 2003).

When it comes to peroxisomes (Fig. 8.3), contribution in maintaining cellular homeostasis is mainly due to CAT which depletes the peroxisomal H_2O_2 generated through photorespiratory glycolate oxidase pathway (Das et al. 2015). The CAT-lacking plants are more sensitive to diseases since leaves show bleaching due to H_2O_2 accumulation in peroxisomes (Chamnongpol et al. 1998). Additionally, peroxisomal CAT protecting AsA and GSH pools from oxidation (Das et al. 2015).

ROS is also generated by the cell wall oxidases and polyamine oxidases which generally generate H_2O_2. During the stress, the cell wall can generate $O_2^{\bullet-}$, OH•, H_2O_2, and $^1O_2$. Furthermore, the production of reactive oxygen species was also recorded by the endoplasmic reticulum (Das and Roychoudhury 2014). Various components of enzymatic and non-enzymatic origin play a significant role in ROS elimination in vacuolar compartments including class III peroxidases together with different secondary metabolites that serve as peroxidase substrates (Idänheimo et al. 2014; Das et al. 2015).

8.5 Conclusion

The maintenance of appropriate redox status of plant cells and prevention of environmental stress during the growing season are a key factor for plant adaptations in their adverse surrounding environment. With more efficient removal of reactive oxygen species, plants are less sensitive to environmental changes, and integrative physiological responses of enzymatic and non-enzymatic components of the antioxidant system are essential in avoidance of oxidative damage. Since climate change leads to the multidimensional and simultaneous variation of main environmental factors, particularly drought and increase in temperature, engineering with plants that shows functionality and stability over wide ranges of abiotic factors may have implications in improving plant performance under climate change-related stresses.

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Chapter 9
Biochemical and Molecular Mechanisms of Abiotic Stress Tolerance

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Contents

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Abstract Changes in the climate have dramatically increased the incidence of abiotic stress in plants, thus limiting their optimum growth, production, and metabolism. Plants have numerous adaptive tolerance or resistant mechanisms to acclimatize with the changes in the environment like drought, salinity, heat, flood, cold/freeze, ultraviolet (UV), and heavy metal stress. Plant adaptation to stress is strictly attributed to a paragon of biochemical and molecular crossroads. Moreover, it mainly relies on molecular stress signaling network involving stress perception, signal transduction, modulation of stress-related gene expression, and change in the metabolite profile. Adaptive stress tolerance mechanisms involve adjustment of hormonal balance, synthesis of stress proteins, activation of antioxidant defense mechanism, reconfiguration of the metabolite accumulation, and restructuring of cellular membrane. Apart from innate adaptive responses, several advance strategies including breeding and bioengineering are being used to combat abiotic stresses in the plants. In the given chapter, plant molecular and biochemical responses have been reviewed and discussed with respect to various crop plants to impart better understanding of tolerance mechanisms under abiotic stress.

Keywords Abiotic stress · Adaptation · Metabolism · Signaling molecules · Tolerance

Abbreviations

ABA	Abscisic acid
ABRE	ABA-responsive elements
ADC	Arginine decarboxylase
AP2	Apetala 2
APX	Ascorbate peroxidase
AREB/ABFs	ABRE-binding protein/ABRE-binding factors
Arg	Arginine
AsA	Ascorbic acid
AsA-GSH cycle	Ascorbate-glutathione cycle
BADH	Betaine aldehyde dehydrogenase
bHLH	Basic helix-loop-helix
bZIP	Basic leucine zipper
C2H2 ZF	Cys2Hizinc fingers
Ca^{2+}	Calcium ions
CaMs	Calmodulins
CAT	Catalase
CBF	C-repeat/DRE-binding factor
CBL	Calcineurin B-like proteins
CcaMKs	Calmodulin-dependent protein kinases
CDPKs	Ca^{2+}-dependent protein kinases

CMO	Choline monooxygenase
COR	Cold responsive
Cys	Cysteine
dcSAM	Decarboxylated *S*-adenosylmethionine
DHAR	Dehydroascorbate reductase
DRE/CRT	Dehydration-responsive element/C-repeat
DREB 2	DRE-/CRT-binding protein 2
ER	Endoplasmic reticulum
ET	Ethylene
GB	Glycine betaine
GHR	Guard cell hydrogen peroxidase resistant
gi-3	Gigantea
GLR	Glutamate-like receptor
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GRF7	Growth-regulating factor 7
GSH	Glutathione
GSTs	Glutathione *S*-transferases
H_2O_2	Hydrogen peroxide
ICE1	Inducer of CBF expression 1
JA	Jasmonic acid
M6PR	Mannose-6P reductase
MAPKs	Mitogen-activated protein kinases
MDA	Malonyldialdehyde
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
mtlD	Mannitol-1P dehydrogenase
MYB	Myeloblastosis oncogene regulon
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
$O_2^{\bullet-}$	Superoxide radical
OAT	Orn-δ-aminotransferase
ODC	Ornithine decarboxylase
OH^-	Hydroxyl radical
Orn	Ornithine
OSCA1	Reduced hyperosmolality-induced calcium increase 1
OST1	SnRK2 open stomata 1
P5CDH	*P5C* dehydrogenase
P5CR	Pyrroline-5-carboxylate reductase
P5CS	Pyrroline-5-carboxylate synthase
PA	Polyamines
PDH	Proline dehydrogenase
PEG	Polyethylene glycol
POD	Peroxidases
PP2Cs	Protein phosphatase 2Cs
Put	Putrescine

RbohF	Respiratory burst oxidase protein
RLKs	Receptor-like kinases
ROS	Reactive oxygen species
R-SOH	Sulfenic form
SA	Salicylic acid
SnRK2	SNF1-related kinase 2
SOD	Superoxide dismutase
SOS	Salt overly sensitive
Spd	Triaminespermidine
SPDS	Spd synthase
Spm	Tetraminespermine
SPMS	Spm synthase
TFs	Transcription factors
UV	Ultraviolet

9.1 Introduction

Plants are confined to their habitats and are continuously threatened by the environmental stresses (Lichtenthaler 1998). They have plasticity in their physiology, development, and cellular metabolism to ensure their survival despite the prevailing fluctuations in their surroundings (Mickelbart et al. 2015). Unfavorable environmental conditions for plants include both biotic (insect, parasites, bacteria, fungus, and virus) and abiotic factors (salinity, flooding, drought, freeze, heavy metals, cold, and heat). Drought, salinity (ion toxicity), temperature variations, ultraviolet (UV), and heavy metal stress are the abiotic plant stresses that have dramatically threatened the food security by limiting the productivity in agriculture (Zhu 2016).

Plants tolerate or avoid stress through adaption and acclimation mechanisms. They respond to these stresses in precise ways by tailoring their biochemical, physiological, and molecular responses (Mittler 2006). Plants usually deal with stresses in four phases: (1) response phase, (2) restitution phase, (3) end phase, and (4) regeneration phase. In response phase, plant senses the stress that causes disturbance in plant homeostasis. Stage of resistance is actually the restitution phase that includes repair and adaptation processes. In the case of long-term stress, plant enters end phase that involves overcharge of the adaptation ability and leads to chronic illness and death. If a plant overcomes the stress, it enters regeneration phase that comprises of partial or full recovery of normal homeostasis (Lichtenthaler 1998).

Stresses mainly induce burst of reactive oxygen species (ROS) and activate the stress sensors to initiate stress-related responses. Plant stress tolerance is a complex trait encompassing various biochemical and molecular mechanisms (Muchate et al. 2016). At molecular level, plants regulate the expression of stress-responsive genes that directly protect them from abiotic stresses. There is a broad range of stress-responsive genes. These genes have been divided into two groups based on their functions (Onaga and Wydra 2016). One group contains regulatory genes that encode protein kinases, RLKs, ribosomal protein kinases, MAPKs, proteinases of signal transduction (phospholipases and phosphoesterases), and transcription factors (TFs). The other group includes biosynthetic and structural genes that encode proteins embedded in membrane, enzymes of ROS scavenging (superoxide dismutase, SOD; catalase, CAT; glutathione, GSH; dehydroascorbate reductase, DHAR; ascorbate peroxidase, APX; guaiacol peroxidase, GPX; glutathione *S*-transferase, GST; glutathione reductase, GR; and quinine reductases, QR), proteins for protecting other macromolecules (anti-freezing proteins, mRNA-binding proteins, chaperons, and LEA proteins), and enzymes for biosynthesis of osmolytes (Onaga and Wydra 2016; Anjum et al. 2011). Plants' response to stress involves change in photosynthesis, alternation in ion levels, change in membrane fluidity, accumulation of osmolytes, synthesis of plant secondary metabolites, phytochelation, activation of ROS scavenging machinery, and modulation of metabolism and morphology. These stress responses are precisely coordinated by pant hormones (Onaga and Wydra 2016).

Primary abiotic stress (drought, salinity, heat and cold stress) induce secondary stresses including oxidative stress and osmotic stress. The plants sense variability in their homeostatic status and activate their stress-related signaling pathways including ROS signaling, calcium-mediated signaling, hormonal signaling, and transcriptional networking. Stress signaling triggers stress-responsive mechanisms. Successful stress response involves tolerance or resistance to abiotic stress with restoration of cellular homeostasis and repair of damaged proteins and membrane. On the other side, inadequate stress response can lead to irreversible cellular damage followed by cell death (Gómez-Cadenas et al. 2015). Various conventional and advance approaches are under consideration to avoid inadequate stress response in agronomical important crop plants, thus avoiding threat to food security.

9.2 Plant Stress Tolerance and Abiotic Stress Signaling

Plant cells have capability to sense stress signals and generate stress response accordingly. Stress signals can either be primary or secondary in nature. For example, plants primarily recognize drought by hyperosmotic stress, while ion toxicity and osmotic stress are the main effects of salinity. Secondary stress signals are more complex and include metabolic dysfunction, cellular destruction, and oxidative stress. Moreover, secondary stress signals mainly arise from primary ones (Zhu 2016). Abiotic stress signals cause the regulation of various phytohormones, ROS production, calcium level, and TFs that in return generate adaptive and acclimation mechanisms for stress tolerance in plants (Zhu 2016).

Generally, plant stress signaling initiates with stress signal perception through receptors, receptor-like kinases (RLKs), ion channels, or histidine kinases that are majorly present on the surface of a plant cell (Mahajan and Tuteja 2005; Tuteja 2007). Receptor passes the stress signals through the production or regulation of secondary messenger such as calcium ions (Ca^{2+}), ROS, inositol phosphate, and hormones. Secondary messengers transduce the stress signal through signaling cascade involving Ca^{2+} sensing, protein dephosphorylation/phosphorylation, TF activation/deactivation, and phospholipid metabolism. At the end, signal transduction leads to the regulation of proteins that are involved in the transcription of stress-responsive genes and provides protection to plant cell against oxidative stress. Short-term stress responses further regulate the long-term plant stress tolerance through regulation and synthesis of regulatory molecules in the next round of stress signaling (Muchate et al. 2016). Stress regulatory molecules of stress signaling pathway (hormones, TFs, ROS, Ca^{2+} regulatory genes, and mediators) are the key players of developing stress tolerance in plants (Rao et al. 2006). According to the nature of stress regulatory mediators, abiotic stress signaling has been categorized into signal transduction cascades, calcium-mediated signaling, hormonal signaling, transcriptional networking, and ROS signaling. Overview of stress signaling leading to tolerance against abiotic stresses has been shown in Fig. 9.1.

9.2.1 Abiotic Stress Sensing

Stress signal perception is often thought to take place only on the plant cell surface or membrane like ligand perception, but it is not always true. The stress can be sensed not only at the surface of the plant cell, but it can also take place anywhere in the cell. The stress signals are mainly detected by the change in the cellular components or activity. The changes can either be at physiological, metabolic, or gene expression level which is dependent on stress conditions (Zhu 2016).

Most of the abiotic stresses generate osmotic stress that can be detected by plasma membrane protein encoded by *OSCA1* (reduced hyperosmolality-induced calcium increase 1) gene. These protein sensors give rise to hyperosmolality-gated calcium-permeable channels and increase the Ca^{2+} level of cells in combination with aequorin and other calcium channels for sensing osmotic stress (Yuan et al. 2014). Plants have been reported with the presence of mechano-sensitive channels including one Piezo homolog and MscS-like proteins (Hedrich 2012). Such receptors are required for the survival of plants during hypo-osmotic stress as they act as sensor to detect stress-induced tension of membrane (Hamilton et al. 2015). Under stress conditions, plants own other receptors including glutamate-like receptor (GLR) and cyclic nucleotide-gated channels that create stress signals using Ca^{2+} as secondary messenger (Swarbreck et al. 2013). Moreover, plants have stress-specific receptors. They have COLD1 (a transmembrane stress sensor) that perceive cold and regulate Ca^{2+} channels for stress sensing (Ma et al. 2015). Cold and heat stress behave quite differently from other stresses. They majorly change the fluidity of cellular membranes that can be sensed by proteins embedded in membrane such as RLKs and transporters. Molecular chaperone, also, aids the plant to sense heat stress through detecting misfolded proteins in the cell (Scharf et al. 2012).

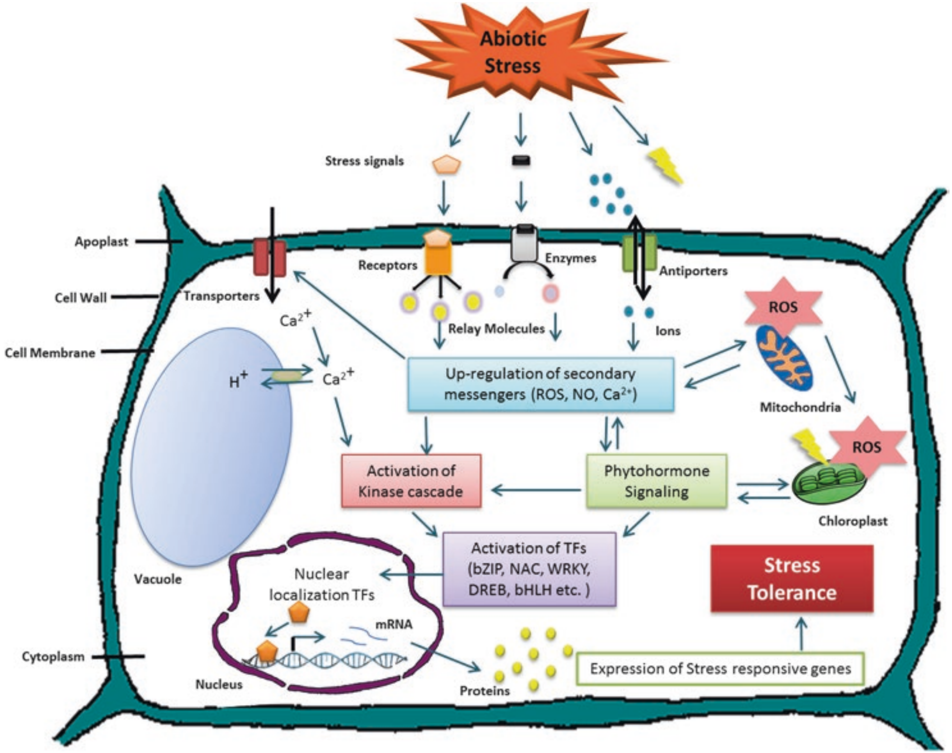

Fig. 9.1 Stress signaling pathways leading to tolerance responses in a plant cell under abiotic stress. Plant cell perceives abiotic stress signals through specific receptors, enzymes, and antiporters on the cell membrane. Plant organelles including mitochondria, cell wall, peroxisome, and chloroplast, also, take part in stress perception and initiate stress signaling by producing reactive oxygen species (ROS). Moreover, relay molecules generated in response of stress signals produce secondary messengers (ROS, nitric oxide (NO), and Ca^{2+}). These secondary messengers along with stress-responsive hormones (especially abscisic acid) activate specific signal transduction pathway that include cascade of kinases that further activate transcription factors (TFs) specific to stress stimuli. Activated TFs translocate in the nucleus and bind to their respective elements to regulate the expression of stress-responsive genes. Most of the abiotic stress-responsive genes encode ROS scavenging enzymes, transporters, long-term stress regulatory genes, and biosynthetic enzymes that are involved in the synthesis of secondary metabolites and osmolytes (Information retrieved from (Zhu 2016; Marco et al. 2015))

Other than proteins embedded in the cellular membrane, plants contain various proteins in their organelles which are, also, involved in stress perception. Stress-associated organelles include mitochondria, chloroplast, nucleus, cell wall, and peroxisome. Stress signals generated by these organelles are used to regulate stress adaptation mechanisms and help the plant to restore cellular homeostasis (Zhu 2016). Abiotic stress causes protein misfolding that develops stress in the endoplasmic reticulum (ER) which is also known as ER stress. The sensing of ER stress leads to enhanced protein folding capacity, suppression of protein translation, and ER-associated protein degradation (Walter and Ron 2011). The metabolic homeostasis of chloroplast can be easily disturbed by stresses. The change in the chloroplast metabolism is detected by nucleus through retrograde signals which help the plant to fine-tune its stress responses according to the availability of energy and compounds. Chloroplast, mitochondria, and peroxisomes are the main sites of the ROS production in the plant cells and are vital to manage ROS-mediated stress responses under various environmental stresses (Mignolet-Spruyt et al. 2016).

9.2.2 Hormonal Signaling Regulating Abiotic Stress Responses

In plants, hormones and their signaling play crucial roles in regulating stress tolerance mechanisms apart from their role in the growth and development. Abscisic acid (ABA) is an endogenous messenger that regulates stress tolerance mechanisms in plants under various abiotic stresses by the accumulation of various stress-related proteins and regulating the opening/closing of stomata (Gómez-Cadenas et al. 2015). Furthermore, ABA aids the seeds to avoid stress conditions and promote germination when favorable conditions for the growth are achieved.

Plants induce the biosynthesis of ABA from β-carotene in response to abiotic stress/stresses. Basically, ABA is a stress hormone that mainly regulates osmotic stress tolerance and water level of plants (Tuteja 2007). ABA mainly regulates stress-responsive gene expression through ABA-independent or ABA-dependent signaling pathway (Yoshida et al. 2014). Further, ABA along with other phytohormones forms an intricate network of hormonal signaling that is essential to fine-tune stress acclimatization (Yamaguchi-Shinozaki and Shinozaki 2006). ABA-generated stress responses often rely on the existence of transcriptional regulatory network and cis-regulating elements such as ABA-responsive elements (ABRE), dehydration-responsive element/C-repeat (DRE/CRT), MYB, and MYC recognition sequences (Uno et al. 2000; Tuteja 2007). ABRE-binding protein/ABRE-binding factors (AREB/ABFs) – a group of basic leucine zipper TFs with ABRE – are central to ABA-dependent signaling, while DRE-/CRT-binding protein 2 (DREB2) along with DRE/CRT element are pivotal for ABA-independent signaling pathway (Yoshida et al. 2014).

In ABA-dependent signaling, AREB/ABF TFs are activated by SNF1-related kinase 2s (SnRK2s) through multiple-site phosphorylation and regulate the proteins that are actively involved in seed germination, stomatal opening/closure, osmotic stress response, and seedling growth (Fujii and Zhu 2009; Fujita et al. 2009). Drought, heat, and high salinity induce DREB2 TFs through ABA-independent signaling (Sakuma et al. 2002, 2006). Under normal growth conditions, the DREB2A are repressed by growth-regulating factor 7 (GRF7) (Kim et al. 2012b). Moreover, the expression of DREB2A is tightly controlled by DRIPs and other ubiquitin E3 ligases. Upon abiotic stress, ABA accumulation activates DREB2 TFs through inhibiting their degradation and initiates osmotic stress-responsive genes (Cheng et al. 2012). There is no distinct line that separate ABA-dependent from ABA-independent pathway because of various convergent points of crosstalk. Calcium and SnRK2s mostly mediate such crosstalk in abiotic stress signaling (Tuteja 2007). Moreover, DREB2A has also been found regulated by AREB/ABFs; thus it has been associated with ABA-dependent and ABA-independent pathway crosstalk (Yoshida et al. 2014).

In plants, ABA is the main stress hormone that confers abiotic stress tolerance, but other phytohormones, especially jasmonic acid (JA), ethylene (ET), and salicylic acid (SA), either directly or indirectly, have shown their significant roles in abiotic stress responses and signaling (Sánchez-Vallet et al. 2012). Jasmonic acid signaling interacts with ABA signaling through MYC TFs, while ERF1 is the convergent point of JA/ET signaling (Lorenzo and Solano 2005). Ethylene signaling also interacts with ABA signaling at many points. Such intricate hormonal interactions are essential for plant to maintain balance in stress response reactions both at cellular and whole plant levels (Hirayama and Shinozaki 2010).

9.2.3 Role of Signal Transduction Cascades Under Abiotic Stresses

Protein kinases are another of chapter of abiotic stress signaling in the plants and play an integral role to link stress-adaptive plant responses such as osmotic homeostasis, ion regulation, and ROS regulation to cellular metabolic signaling (Golldack et al. 2014). In plants, a group of SNF1-related kinase (SnRKs) has been reported with its roles in abiotic stress adaptation (Halford et al. 2003). SnRKs, also, interact with ABA signaling (via NAC TFs) and ROS signaling pathway by regulating ionic homeostasis and oxidative stress, respectively (Diédhiou et al. 2008; Kim et al. 2012a). Furthermore, SnRKs intermingle with SOS pathway via salt overly sensitive 3 (SOS3)-like calcium-binding proteins and regulate Na^+/H^+ antiporters that are embedded in plasma membrane (Du et al. 2011). SnRK2 open stomata 1 (*OST1*) are the ABA-responsive SnRKs and modulate the production of H_2O_2 at cellular level through *NADPH oxidases*, thus controlling the stomatal opening/closure (Sirichandra et al. 2009).

Mitogen-activated protein kinases (MAPKs) transduce signals in cascades and provide fine-tuning of cellular and physiological responses to abiotic stresses. Functionally, the cascades of MAPKs are linked together by MAP 3K serine/threonine kinases, MAPK serine/threonine kinases, and MAP 2K dual-specificity kinases (Colcombet and Hirt 2008). *MKK4* via *MPK3* activity has been reported to regulate ROS accumulation, ABA biosynthesis, and osmotic stress responses in *Arabidopsis* (Kim et al. 2011). *MPK6* along with other MAPKs such as *MKK1* and *MKKK20* regulate the oxidative burst and ROS accumulation (Kim et al. 2012a; Xing et al. 2008). Various studies have shown the roles of MAPK cascade in abiotic stress responses by regulating intracellular membrane trafficking, stress-dependent lipid signaling, and stress-adaptive changes in cell membrane (Yu et al. 2010). MAPK networking is actually a signaling hub and links all the signaling, either defense or physiological signaling, in stressed or non-stressed plant (Golldack et al. 2014).

9.2.4 Calcium and ROS-Mediated Abiotic Stress Signaling

Fluctuations in the levels of Ca^{2+} and ROS are among the earliest responses to stress in plants. In abiotic stresses, calcium-mediated signaling is integral to stress perception and mediates coordination in stress tolerance mechanisms modulated by other signaling networks (Wilkins et al. 2016). Ca^{2+} signaling pathway consists of various carriers, channels, pumps, and calcium-binding proteins or calcium sensors (Ca^{2+}-dependent protein kinases (CDPKs), calcineurin B-like proteins (CBL), calmodulin-dependent protein kinases (CCaMKs), and calmodulins (CaMs)) and maintains cellular homeostasis of plants cells (Dodd et al. 2010; Kudla et al. 2010). Ca^{2+} signaling is a robust signaling pathway that is used by plant to bring coordination in its molecular and biochemical processes under both stress and normal conditions (Nookaraju et al. 2012). Fluctuation in the Ca^{2+} is the starting point of calcium signaling and activates the CBL- and CDPK-mediated signal transduction pathway in the plants under abiotic stress. Plants accumulate Ca^{2+} that generate stress tolerance in plants (Hasanuzzaman et al. 2019). After the stress, plant restores its Ca^{2+} level through initiating various cytosolic Ca^{2+} buffering processes (Bose et al. 2011). The specificity in the stress responses in calcium-mediated signaling is achieved by binding of Ca^{2+} to specific TFs. These TFs regulate the crosstalk of calcium-mediated signaling with other signaling pathways and control oxidative stress within a stressed plant (Zeng et al. 2015; Reddy et al. 2011).

In abiotic stress signaling, the importance of ROS as signaling molecule cannot be neglected as ROS is the controller of stress responses, systemic signaling, and programmed cell death and pathogen defense-related responses (Mittler 2002). ROS signaling basically revolves around maintaining the balance between the production of ROS and antioxidants. The shift in the homeostasis between the ROS production and ROS scavenging decides the specificity of stress responses. ROS signaling is vital to regulate programmed cell death and stress responses and tolerance in plants under abiotic stresses (Noctor et al. 2018). The antioxidant components of ROS signaling (CAT, GSH, MDHAR, peroxidases (POD), and thiols) act as modulators of plant stress responses through the crosstalk among hormonal signaling, metabolic signaling, and developmental signaling (Noctor et al. 2018; Datta et al. 2015). In plants, sequential oxidative bursts in apoplast are important for systemic stress responses that can lead to stress adaptation and acclimation. Moreover, reciprocal interactions of ROS signaling and calcium signaling cause the transmission of stress-related signals from cell to cell for systemic acquired immunity against abiotic stresses (Gilroy et al. 2016). Indeed, ROS signaling is fundamental to a plant to maintain homeostasis in its physiology and morphology under stress conditions.

9.2.5 Abiotic Stress Signaling and Transcription Factors

Transcription factors are the DNA-binding proteins that control the transcription of genes. When plant suffers from stress, these regulatory proteins confer tolerance and initiate defense mechanism through regulating expression of stress-responsive genes in a sequence-specific manner (Lindemose et al. 2013). There exists a large diversity in the TFs that is essential for creating specific stress responses. bZIP (basic leucine zipper), AP2/ERF (APETALA 2/ethylene-responsive element binding factor), MYB (myeloblastosis oncogene regulon), WRKY, C2H2 ZF (Cys2Hizinc fingers), NAC, and bHLH (basic helix-loop-helix) are among the prominent TFs that coordinate stress-tolerant responses in plant under abiotic stress (Hasanuzzaman et al. 2019; Lindemose et al. 2013). bZIP TFs along with ABRE, DREBs, and AP2/ERF play vital roles in ABA-dependent signaling and create stress responses in plants affected by drought, low temperature, and salinity (Mizoi et al. 2012; Gao et al. 2011). On the other hand, DREB together with DRE/CRT elements regulate defense responses to salinity, heat, cold, and drought through ABA-independent signaling pathway (Yoshida et al. 2014).

C2H2 ZF TFs are the stress-responsive TFs that inhibit the expression of auxin-responsive genes of growth under stress conditions and ABA-dependent signaling under unstressed conditions (Jiang et al. 2008; Kodaira et al. 2011). Their overexpression has been found to be associated with enhanced stress tolerance in plants under salinity, heat, and drought stress (Huang et al. 2009). MYBs antagonistically work within JA signaling and ABA signaling, and their roles have been reported in response to osmotic stress, ABA, and cold stress (Jung et al. 2010; Seo et al. 2009). WRKY TFs are mainly associated with biotic stress, but their roles have been found to be linked with ABA-dependent signaling pathway that is essential for plants to develop tolerance and acclimation against abiotic stress in plants (Jiang and Deyholos 2009). NAC TFs form an intricate signaling network during stress either biotic or abiotic and confer stress tolerance and adaption in plants (Nuruzzaman et al. 2013). ABA-independent and ABA-dependent mediated stress responses are positively regulated by bHLH (Bailey et al. 2003). Along with MYC, bHLH provides the platform for crosstalk among stress signaling and metabolic signaling

(Kazan and Manners 2013). Precisely, regulation of transcriptional networking is pivotal for a plant to generate physiological, biochemical, and molecular stress responses to deal with abiotic stresses.

9.2.6 Importance of Crosstalk Between Signaling Pathways

The tolerance or susceptibility to abiotic stress is much dependent on the coordination among plant stress signaling especially at signal transduction level (Tuteja 2007). Abiotic stress signaling is highly dependent upon ABA and SnRKs in plants. Under abiotic stress, plants lessen their energy supply by constraining photosynthesis and increase SNF1/AMPK-related kinases to mediate abiotic stress signaling for conferring stress tolerance. Some of the SnRKs participate in the ion homeostasis, whereas others regulate ABA signaling and osmotic stress. SnRKs, also, include CPKs that are involved in Ca^{2+}-mediated signaling and are significant for stress adaptations (Hrabak et al. 2003). MAPKs are the part of every type of stress in plants, animals, protozoa, etc. Secondary messengers including Ca^{2+}, NO, ROS, and lipid molecules are vital for signal transduction and generation of stress responses in plants under abiotic stress. These secondary messengers along with specific TFs provide various convergent points of crosstalk between cellular and stressed signaling pathways. Moreover, crosstalk of hormonal, abiotic stress, growth, and development signaling is vital to provide coordination among growth and development in plants growing under abiotic stresses (Zhu 2016). Precisely, the nature and amount of the stress responses is highly determined by the metabolic status of the plant, the harshness of the encountered stress, and the fine-tuning of its signaling pathways (Tuteja 2007).

9.3 Abiotic Stress Tolerance and ROS Regulation

Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$), and hydroxyl radical (OH•) are actively involved in cellular metabolism and regulation of cellular processes including cell differentiation, proliferation, signaling reactions, cell cycle, and cell death (Mittler 2017; Gilroy et al. 2016). In plants, ROS is generated either as a by-product of metabolic processes or by the activity of peroxidases and oxidases in response to either biotic or abiotic stressors such as drought, salinity, mechanical stress, light alteration, and temperature variations (Schopfer et al. 2001; Bolwell et al. 2002). The oxidative burst (rapidly increased ROS) occurs in chloroplast, peroxisomes, endoplasmic reticulum (ER), mitochondria, and apoplast after exposure to any stress (Corpas et al. 2015). The environmental stressors alter the photorespiration rate and modulate the redox status in peroxisomes, chloroplast, and mitochondria through a decline in stomatal conductance (Chaouch et al. 2010; Kangasjärvi et al. 2012).

The stress perception induces the activation of membrane-localized K^+ efflux and Ca^{2+} influx channels leading to the modifications in downstream targets such as nucleic acid, proteins, and lipids (Mittler 2017). Cysteine (Cys) and methionine residues of proteins are prone to modifications during redox signaling; therefore low concentrations of H_2O_2 lead to the generation of sulfenic form (R-SOH) of thiolate anion protein by the oxidation of Cys residues (Jacques et al. 2013). The sulfenic form interacts with thiolates and facilitates the formation of disulfide bonds. However, the increased H_2O_2 concentration causes the generation of irreversible sulfonic (R-SO3H) or sulfenic (R-SO2H) species by the oxidation of thiolates that are involved in hormonal signaling leading to cell death (Sevilla et al. 2015). Lipid peroxidation due to ROS accumulation generates oxylipins such as phytoprostanes and hydroxyl fatty acids and interplays with plant growth, immunity, and other stress-associated responses (Eckardt 2008). The *Arabidopsis* mutants depicted increased $^1O_2$ under photo-oxidative stress that lead to increased lipid peroxidation proceeding to cell death owing to loss of photosystem II (Das and Roychoudhury 2014; Triantaphylides et al. 2008). The lipid peroxidation products such as alkenals and malonyldialdehyde (MDA) alter membrane fluidity and permeability leading to leakiness (Ayala et al. 2014). This leakiness of membrane allows the passage of substances that may cause permanent DNA damage through deoxyribose oxidation. Moreover, mitochondrial and chloroplast DNA and histones are more prone to damage and degradation than nuclear DNA as ROS is generated as a consequence of photorespiratory reactions and photosynthesis (Sharma et al. 2012). The altered redox homeostasis due to abiotic stressors instigates the signaling cascades that lead to acclimation responses to various abiotic stressors (Wrzaczek et al. 2013).

Plants possess two arms of antioxidant machinery, the enzymatic and non-enzymatic components, to mediate redox signaling and ROS homeostasis and generate acclimation responses to abiotic stressors. The generation of $^1O_2$ and $O_2^{\bullet-}$ from overloaded photosystem I instigates the catalytic activity of SOD, which converts these free radicals to H_2O_2. If not reduced, these toxic $O_2^{\bullet-}$ react with metallic ions such as Cu^{2+} and Fe^{3+} through Haber-Weiss reaction to generate OH•. During the first step of reaction, metal ion is reduced by $O_2^{\bullet-}$. The reduced metallic ions then react with H_2O_2 and generate OH• (Fenton reaction) (Puthur 2016). The OH^- radicals are responsible for lipid peroxidation and nucleic acid damage. Therefore OH^- radical is scavenged by the activity of POD like CAT, APX, and GPX not only in chloroplast but also in cytosol (Poiroux-Gonord et al. 2013). CAT is unique in aspect that it directly converts H_2O_2 to H_2O and O_2 without any reduction, whereas GPX catalyzes H_2O_2 through reduction of guaiacol (Lata et al. 2011; Puthur 2016). APX (located in thylakoid membranes) scavenges H_2O_2 through oxidation of ascorbic acid (AsA) and glutathione (GSH). The reduced AsA and GSH are regenerated through ascorbate-glutathione cycle (Halliwell-Asada pathway) that includes consecutive oxidation and reduction of AsA, NADP, and GSH by the action of APX, DHAR, MDHAR, and GR (Puthur 2016). APX oxidizes ascorbic acid/ascorbate to monodehydroascorbate (MDHA) which is subsequently reduced to ascorbate by the action of ferredoxin in photosystem I. On other hand, MDHA is reduced to ascorbate and glutathione in stroma by the activity of DHAR that dismutates MDHA to

AsA and DHA. Moreover MDHA can be reduced to ascorbate in cytosol, stroma, and chloroplast by NADPH-mediated MDHAR activity (Puthur 2016). Glutathione *S*-transferases (GSTs) are actively involved in plant growth and development. They are localized in subcellular compartments and confer the antioxidant ability by catalyzing the GSH and phytotoxin electrophilic sites (Dixon et al. 2010; Gong et al. 2005).

9.3.1 Enzymatic Antioxidant Machinery

The redox machinery plays vital role in developing tolerance to abiotic stresses. ROS has diverse role in plant growth, development, and senescence that are associated with plant reproduction and survival. Extension of lifespan in mutant *Caenorhabditis elegans* has been observed as a stress tolerance mechanism to confer oxidative damage generated during environmental stress (Martin et al. 1996; Murakami and Johnson 1996). The gigantea (gi-3) *Arabidopsis* mutant was observed to be resistant to paraquat-induced oxidative stress and have reduced growth and delayed flowering. Tolerance to paraquat is known to alter flowering time and plant growth. Moreover, the mutant depicted pleiotropic effects including delayed leaf senescence and flowering, tolerance to H_2O_2, and altered starch metabolism, phytochrome b signaling, and circadian rhythm (Huq et al. 2000; Kurepa et al. 1998). The loss-of-function gi-3 mutation leads to oxidative stress tolerance through constitutive expression of SOD and APX (Cao et al. 2006). Presence of SOD residues in chloroplast (Fe-SOD and Cu/Zn-SOD), cytosol (Cu/Zn-SOD), mitochondria (Mn-SOD), and peroxisomes (Cu/Zn-SOD) decreases the probability of OH^- formation during Haber-Weiss reaction conferring protection against oxidative stress generated as a consequence of abiotic factors (Gill et al. 2015; Gupta et al. 1993; Mittler 2002). Targeted antioxidant system downregulation provided insight to tolerance mechanisms against abiotic stressors. CAT and APX are known for H_2O_2 scavenging, thereby preventing damage to nucleic acids. The simultaneous knock down CAT and APX modulated and induced mechanisms that confer nucleic acid damage (Vanderauwera et al. 2011). However, the expression and activity of CATs are localized and depict tissue-specific responses under drought, cold, UV radiation, and heat stress (Mhamdi et al. 2010b). The enzymes of ascorbate-glutathione cycle are partially responsible for the CAT-dependent mechanisms as it has dependence on H_2O_2-dependent altered glutathione status (Han et al. 2013; Mhamdi et al. 2010a). Localization of APX generally determines its response to abiotic stress. However, the co-localization and overexpression of APX and MDHAR in transgenic tobacco ensure the stress tolerance to osmotic, ozone, and salt stress (Eltayeb et al. 2007). DHAR, the AsA-regenerating enzyme, has isoforms localized in cytosol, mitochondria, chloroplast, and peroxisomes (Khan and Khan 2017). Its overexpression is attributed to tolerance against heavy metal and salt stress (Kim et al. 2014; Yin et al. 2010). The last enzyme of AsA-GSH cycle, GR and its isoforms, is usually present in chloroplast, but under stress conditions, their activity has been observed in cytosol and mitochondria (Khan and Khan 2017). The overexpression is associated with increased stress tolerance as it is extremely important for H_2O_2 detoxification during AsA-GSH cycle (Caverzan et al. 2012; Gill et al. 2015; Reumann and Corpas 2010). Other than AsA-GSH cycle enzymes, GPXs and GSTs are actively involved in ROS detoxification. GPXs vary in localization and substrate specificities; thus they are actively involved in redox mechanisms and are known to link glutathione-thioredoxin-based system (Bela et al. 2015). Enhanced tolerance to chilling and salt stress has been observed in transgenic tobacco due to overexpression of GPX (Yoshimura et al. 2004). Notably, localization of GPX in chloroplast elicited a crosstalk between immune responses and photo-oxidative stress in *Arabidopsis* (Chang et al. 2009).

9.3.2 Non-enzymatic Antioxidant Machinery

The non-enzymatic components are low molecular antioxidants that mediate ROS detoxification and retrograde signaling. AsA and GSH are most abundant and swiftly react with H_2O_2 under the influence of peroxidases after which they are regenerated by reductases (Noctor et al. 2018). Mostly, AsA is present in cytoplasm, chloroplast, ER, nuclei, mitochondria, and vacuoles. Under abiotic stress conditions such as excessive light, ozone intensity, UV radiation, and heavy metals, the concentration of AsA dramatically increases in process to confer the oxidative stress generated as a consequence (Zechmann 2011). Similarly, GSH is also rigorously involved in ROS scavenging mostly through AsA-GSH cycle. Elevated GSH content imparts tolerance to plants against high/low temperature variation, drought, heavy metals, and salinity (Cheng et al. 2015; Hasanuzzaman et al. 2017). Tocopherols (e.g., α-tocopherol or vitamin E) elicit antioxidant activity through quenching $^1O_2$. Thus, alteration in tocopherol concentration leads to tolerance against high light and osmotic stress through hindering lipid peroxidation and photosystem II degradation (Czarnocka and Karpiński 2018; Havaux et al. 2005; Krieger-Liszkay et al. 2008; Traber and Stevens 2011). Carotenoids and flavonoids impart color to plant and are involved in the photosynthetic machinery of the plant. During abiotic stress, the cellular concentration of carotenoids and flavonoids increase and assist in scavenging of $^1O_2$, thus harnessing the ability of plant to develop stress tolerance (Agati et al. 2007; Nisar et al. 2015). Proline is considered as a powerful osmoprotectant with ROS scavenging ability. The increased concentrations of proline are known to confer tolerance against drought, low temperature, and salinity stress through blockade of lipid peroxidation (Kaur and Asthir 2015).

9.4 Acclimation and Adaptation Mechanisms

Abiotic stresses put increase in the demands of plants that lead to disruption of functions which is then followed either by death or stress tolerance (Larcher 1987). Plants go through various stages of stress tolerance upon stress perception. At first, plants decline their physiological functions (such as decline in the transport of metabolites, uptake of ions, translocation of ions, and photosynthesis). Next, they activate their stress tolerance mechanisms that include short-term acclimation mechanisms (including change in metabolic fluxes and activation of repair mechanisms) and long-term morphological and metabolic adaptation. Tolerant plants set new optimum stage for their growth and development, while senescence and acute damage occur in plants with no or low level of tolerance (Lichtenthaler 1998).

Plant adaptation to abiotic stress involves multitude of biochemical and molecular stress responses that allow them to survive and reproduce (Kissoudis et al. 2014). Plants upregulate the expression of respiratory burst oxidase homolog D (Rboh) that generate ROS wave as a first systemic response to abiotic stress. The wave of ROS further coordinates with calcium-mediated signaling and phytohormones to signal the plant stress regulators for the activation of responses to stress tolerance. Furthermore, the production of H_2O_2 is encouraged via SOC activity. H_2O_2 diffuses between various cellular compartments and activates the differential expression of hundreds of stress-responsive genes (Miller et al. 2009). Plant acclimation to stress involves change in photosynthesis, alternation in ion levels, change in membrane fluidity, accumulation of osmolytes, synthesis of plant secondary metabolites, phytochelation, activation of ROS scavenging machinery, and modulation of metabolism and morphology (Onaga and Wydra 2016).

9.4.1 Accumulation of Osmolytes and Maintenance of Osmotic Equilibrium

Stressed plants avoid the damage to essential biomolecules through accumulating the small molecules called osmolytes (also known as osmoprotectants) that mainly include sugars, proteins, nucleic acids, and amino acids (Balfagón et al. 2019; Hirayama and Shinozaki 2010). The accumulation of osmolytes is controlled by the intricate cellular processes that are involved in water flux and osmotic adjustment during abiotic stress conditions (Golldack et al. 2014). Soluble sugars due to their availability and biochemical properties are critical to stress perception, and the regulation of their status in a cell is important to determine survival capacity of plants under stress. The regulation of soluble sugars is the basic mechanism that is involved in multiple stress tolerance (Puniran-Hartley et al. 2014). The levels of soluble sugars in a plant cell are associated with starch synthesis/breakdown that is in turn controlled by circadian clock (Graf et al. 2010). Soluble sugars bring coordination in stress responses in plants by regulating energy-sensing SnRK1 and activate stress tolerance mechanisms by controlling osmotic potential, scavenging ROS, hydrating membranes, and protecting photosynthetic apparatus against heat, freezing, drought, and chilling stress (Tarkowski and Van den Ende 2015). Moreover, soluble sugars synergistically interact with phenylpropanoid pathway and scavenge ROS to protect plants from oxidative stress. Soluble sugar-mediated stress responses are modulated by ABA-ethylene dynamics (Thomashow 2010). Soluble sugars along with ABA cause the accumulation of DELLA proteins. These proteins through C-repeat-binding factor 1 (CBF1) activate stress tolerance in plants affected by freezing (Tarkowski and Van den Ende 2015). The accumulation of maltose and raffinose have also been observed as response to stress (Tarkowski and Van den Ende 2015).

Various amino acids have been found to be associated with abiotic stress tolerance. The accumulation of g-aminobutyric acid (GABA) is related to water retention and stomatal closure and confers tolerance against stress (Mekonnen et al. 2016). Proline is an amino acid that has been associated with stress tolerance in the plants under cold, drought, salinity, and heavy metal stresses (Szabados and Savoure 2010). Proline is a compatible osmolyte that protects the cellular machinery from oxidative damage and maintains homeostasis of photosynthesis (Signorelli 2016). Proline metabolism plays its role as redox shuttle that transfers electron to mitochondria from chloroplast/cytosol, thus protecting the cell from ROS (Sharma et al. 2011). On the other hand, plant cell enhanced the activity of proline dehydrogenase that in return initiates ROS-dependent autophagy to enhance viability of stresses in plant cells (Zabirnyk et al. 2009). Amino acid-derived compounds (betaine, glycine, and polyamines), also, contribute to abiotic stress tolerance in various plants (Chen and Murata 2011; Signorelli et al. 2019). The accumulation of these osmolytes in the cytosol is an essential stress response to adjust osmotic equilibrium in the plants under abiotic stress (Signorelli et al. 2019).

9.4.2 Regulation of Photosynthesis and Cellular Energy Supply

Plants reprogram their primary metabolism and bring balance between energy supply and energy consumption to produce stress response (Golldack et al. 2014). Abiotic stresses via ROS confer grave damage to photosynthetic machinery especially to photosystem II (PSII). Plants partially or completely shut down their photosystem through ROS signaling and inhibition of PSII repair; such stress response is known as photoinhibition (Gururani et al. 2015). Moreover, PSII is a rate-limiting step that converts light energy into ATP and NADPH and readjustment of plant growth under stress. Plants modulate their bioenergetics as a stress response. They use pyrophosphate as energy donor rather than ATP and bypass glycolytic reaction. They initiate additional pathways for electron transport chains and reclaim these pathways after stress removal (Dobrota 2006).

9.4.3 Regulation of ROS Production and Accumulation

Abiotic stresses induce the ROS production that must be controlled by plant to stand the adverse effects of stress. Plants avoid oxidative stress resulted by ROS through either controlling ROS production or ROS scavenging. ROS production can be controlled by three stress-responsive mechanisms that include (1) regulation of CAM and C4 metabolism (physiological adaptation); (2) hiding of stomata in specified structures, leaf movement, leaf curling, and development of refracting epidermis (anatomical adaptation); and (3) rearrangement of photosynthetic apparatus or suppression of photosynthesis (molecular mechanisms) (Mittler 2002, 2017).

Plant further activates ROS scavenging mechanisms as stress response to avoid damage to essential macromolecules present in the cell. ROS scavenging mechanisms are divided into enzymatic and non-enzymatic processes. Plant induces the production of CAT, APX, and SOD to scavenge ROS as stress response to oxidative stress (Noctor et al. 2018). The accumulation of osmoprotectants (proline, betaine, soluble sugar, etc.), AsA, GSH, tocopherol, flavonoid, and carotenoid is the non-enzymatic process to balance ROS in a stressed plant (Noctor et al. 2018; Mittler 2002).

9.4.4 Cellular Transport Homeostasis

The maintenance of cellular transport and ion homeostasis is important for the plant adaptation to abiotic stress. Plant restores ion homeostasis by maintaining membrane potential, modulating the levels of specific osmolytes, and changing cell volume and activities of various enzymes (Conde et al. 2011). Cellular ion homeostasis under abiotic stress basically involves net ion flux that depend upon the activity and expression of antiporters or ion transporters present in the plasma membrane and vacuole membrane (Brini and Masmoudi 2012). Plants employ two transport mechanisms (primary and secondary transport) to regulate ion flux. Primary transport is mediated by H^+-ATPases, while secondary transport involves channels and co-transporters that maintain high K^+/Na^+ ratio in the cytosol which is essential for the ion homeostasis (Conde et al. 2011).

9.4.5 Increase in the Mechanical Strength

Cell wall of plants consists of various stress-responsive elements including peroxidases, pectin, phenolics, extensins, Ca^{2+}, and other proteins. Plant adjusts to the environmental changes by the virtue of cell wall-modifying enzymes. Moreover, accumulation of ROS has been observed in the plant cell wall in response to osmotic stress, salinity, and drought. The accumulation of ROS causes stiffness in the cell wall during stress by forming crosslinking of glycoproteins and phenolics. Remolding of cell wall and loss of Ca^{2+} have, also, been observed under abiotic stress conditions (Tenhaken 2015). Plants alter the architecture of cell wall through increase in xyloglucan endotransglucosylase/hydrolase (XTH), expansin activity, and deposition of hemicellulose and lignin to thicken the cell wall (Le Gall et al. 2015). Rice under flooding stress accelerates the shoot growth due to the increased accumulation of expansin in the cell wall. The increased activity of cell wall-modifying proteins allows the cell to expand and tolerate the stress (Sasidharan et al. 2011). However, in drought stress, pectin accumulation and modification is most observable. The drought-tolerant plant cultivars possess arabinose-rich polymer as pectin side chains. These side chains protect the plant from desiccation and water loss (Moore et al. 2014). Plants respond to temperature stress by accumulating pectin, cellulose, and hemicellulose in their cell wall. The cold-resistant plants reduce the xylose and glucose concentration and accumulation leading to increase in hemicellulose accumulation that protects the plant from ice crystal formation (Le Gall et al. 2015).

9.4.6 Alteration in the Fluidity of Cellular Membrane

Alterations in ambient conditions have direct impact on membrane fluidity. The exposure of high and low temperature induces changes in plasma membrane and cytoskeleton of the cell. The heat and cold stress alter Ca channels and associated proteins that act as sensor for downstream signaling and lead to the activation of MAPKs, CPKs, and CIPKs and production of effector proteins such as COR and HSPs. Production of effector proteins produces thermotolerance in the plants (Sangwan et al. 2002). Change in membrane fluidity is mediated by alteration in unsaturated fatty acid levels. The cold-tolerant plants possess fewer amounts of saturated fatty acids and have depositions of linolenic acid, trienoic fatty acid, and hexadecatrienoic acid in plasma membrane and chloroplast membrane (Murata et al. 1992). However, the levels of unsaturated fatty acids such as trienoic fatty acid were lower in response to heat stress (Hou et al. 2016). The modulation of unsaturated lipids present in plasma membrane confers heat and cold stress tolerance. Transgenic plants with overexpressing, co-expressing, or suppressing genes encoding fatty acid desaturase have ability to tolerate abiotic stress (Marco et al. 2015).

9.4.7 Modulation of Autophagy

Autophagy is a process that is used by plants to promote stress tolerance. Basically, autophagy recycles and remobilizes nutrients under both normal and stressed conditions. Stress conditions yield damaged biomolecules and organelles due to overproduction of ROS and reactive nitrogen species (RNS). The damaged cellular components need to be removed through active autophagy process. Failure of the removal of impaired cellular components can potentially reduce plant survival and can lead to hypersensitivity to stress conditions (Signorelli et al. 2019).

Autophagy removes the aggregated and misfolded proteins from a stressed plant cell by the help of heat shock proteins (HSPs). The expression of HSPs is regulated by Hsfs (heat stress transcription factor). In the plants, HSPs are divided into five classes (HSP 100, HSP 90, HSP 60, HSP 70, and small HSPs). HSPs are the stress-responsive proteins and confer stress tolerance especially in plants under heat stress. HSPs include molecular chaperones that help to degrade the misfolded and aggregated proteins produced in a stressed plant and confer tolerance to abiotic stress (Al-Whaibi 2011).

9.4.8 Stomata Opening/Closure

Stomatal movement (opening/closing) is a key stress response to plant abiotic stresses. The stomatal movement involves intricate regulatory network and ion channels in the guard cells. Anion channels of guard cells are significant for stomatal closure, while K^+ channels regulate stomatal opening. Under stress conditions, plants mostly close their stomata to prevent water loss by regulating the activity and regulation of ion channels present on the surface of guard cells (Saito and Uozumi 2019).

9.4.9 Induction of Accumulation of Plant Secondary Metabolites

Plants produce secondary metabolites (e.g., isoflavonoids, flavonoids, terpenoids, and nitrogen-containing metabolites) that ensure the plant survival under abiotic stress. The biosynthesis of these metabolites is regulated by alterations in phenylpropanoid pathway. Phenolic compounds including lignin precursors and flavonoids accumulate in the cell wall of plants and scavenge the ROS to deal oxidative stress generated by abiotic stress (Bartwal et al. 2013).

9.4.10 Phytochelation

Phytochelation is plant stress response to heavy metal stress. This process is carried out by small metal-binding peptides known as phytochelatins (PCs). These peptides are synthesized by *phytochelatin synthase* that require activation by heavy metals or metalloids including arsenic, zinc, cadmium, lead, mercury etc. PCs take part in cellular homeostasis and trafficking of heavy metals within a plant cell (Schat et al. 2002). Phytochelation is a ubiquitous mechanism for heavy metal detoxification that includes the aspects PC compartmentalization, stress gene regulation, sulfur assimilation, and biosynthesis of sulfide and GSH (Cobbett 2000).

9.4.11 Plant Growth and Development

Stress conditions alter the primary metabolism that can potentially restrict cell proliferation thus limiting the growth and development of plant. Plants have evolved various stress regulatory mechanisms to create balance between growth and stress adaptation. Transcriptional responses involving ERFs and DELLA proteins have been found to promote cell survival. These factors cause the accumulation of secondary metabolites that promote ROS scavenging to delay cell death, thus enhancing plant growth (Bechtold and Field 2018). Moreover, plants regulate their growth regulators, i.e., hormones. ABA along with ethylene and JA is a stress regulator and confers stress tolerance by crosstalking with other hormones. Depending upon stress, plants regulate the hormonal balance and regulate their growth and development under abiotic stresses (Peleg and Blumwald 2011).

9.4.12 Evolutionary Adaptations

The whole body of plant is raised from few meristematic stem cells that transfer the information obtained from the environment to gametes. Abiotic stress causes epigenetic gene regulation including DNA methylation and chromatin remolding for long-term gene expression. Plants lock the special adaptive responses to stress in their genome by chromatin remolding and DNA methylation for their future generation. Such responses are basically required for growth under prevailing abiotic stresses (Hirayama and Shinozaki 2010).

9.5 Approaches to Enhance Abiotic Stress Tolerance

Improving plant tolerance to abiotic stressors is critical for sustainable agriculture. Environmental stressors including drought, salinity, light and temperature variation, etc. hamper plant growth, development, and productivity. Plants have evolved various mechanisms to confer abiotic stress through three stress-induced responses: (1) expression of genes encoding structural function, (2) expression of proteins with undetermined functions, and (3) regulatory gene or protein expression (Bhatnagar-Mathur et al. 2008; Wani et al. 2016). Dissecting and modulating plant responses through which plants confer stress tolerance can provide better understanding for development of stress-tolerant crops (Zandalinas et al. 2018). However, to enhance the ability of plant to resist stress, three possible strategies have been devised: (1) stress escape strategy that includes alteration in life cycle of plant, (2) avoidance strategy that comprises of modifications in the architecture of plant organs and tissues, and (3) tolerance strategy that implies the alteration in stress resistance mechanisms that enhance cell and tissue viability (Mizoi and Yamaguchi-Shinozaki 2013). Mostly, studies pertaining to plant stress tolerance and acclimation have focused on the third strategy. This has increased the basic understanding of the molecular mechanisms involved along with the development of stress tolerance paradigms leading to generation of transgenic plants and new plant breeds tolerant to stress (summarized in Tables 9.1 and 9.2).

In order to combat abiotic stressors that cause reduction in productivity and yield of plant, development of stress-tolerant cultivars is the need of the hour. Recently drought-tolerant safflower, chickpea, peanut, maize, barley, and wheat have been developed. In order to develop a stress-tolerant cultivar, three crucial elements, duration, time, and intensity of stress, must be verified. Using conventional breeding methods such as pedigree method, selection and introduction, modified bulk pedigree method, shuttle breeding, mutation breeding, diallel selective mating system, backcross method, and recurrent selection method, various salt and drought stress-resistant varieties have been produced (Meena et al. 2016). The primary focus of conventional breeding was to increase yield in any case and limited the scope of plant breeding as focus was on a single trait that could only be produced through crossing of closely related plants thus was prone to environmental interactions (Cooper et al. 1999). This has led to the intervention of non-conventional breeding approaches like somaclonal approaches, F1 anther culture derivatives, and marker-assisted selection (MAS). MAS has been the most exploited and focused breeding approach as heritability of gene or trait is certain even under harsh circumstances (Meena et al. 2016). In this aspect, the most famous is QTL-assisted MAS that has led to the development of salt-resistant rice cultivar (Ashraf and Foolad 2013). However, there is still possibility of undesirable trait transfer with QTL, thus limiting the pros of MAS.

The limitations of breeding approaches have led to the innovation of approach of developing stress-tolerant varieties through genetic engineering. Adaptation to abiotic stress includes a cascade of physiological processes comprising of stress-protective gene and protein expression, biosynthesis of secondary metabolites, and induction of intricate cellular signaling cascades. In response to abiotic stress, osmoprotectants including amino acids such as proline, amines like polyamines (PA) and glycine betaine (GB), and sugars such as mannitol and trehalose start to accumulate in the cells. The overexpression and accumulation of these low molecular weight water-soluble chaperones maintain the osmotic balance in a cell and act as ROS scavengers (Bhatnagar-Mathur et al. 2008; Chen and Murata 2002). Proline is known to be a molecular chaperone, a metal chelator, and a potential antioxidant that plays active role in stress tolerance signaling. It is synthesized through two mechanisms in cytosol and plastids either by conversion of glutamate to proline catalyzed by pyrroline-5-carboxylate synthase (P5CS) and pyrroline-5-carboxylate

Table 9.1 Stress signaling molecules that are targeted by genetic engineering for developing abiotic stress-tolerant transgenic plants

Genes targeted in genetic engineering	Gene family	Transgenic plants developed	Targeted stress	Tolerance mechanism	References
Transcription factors					
DREB1/2 A, *TaDREB*	DREB	*Arabidopsis*, tobacco, peanut, wheat, barley, rice	Drought, salt, and freezing stress	Regulate the transpiration and the accumulation of protein (LEA/COR/DHN), sugars, and chlorophyll in leaves	Bhatnagar-Mathur et al. (2007); Gao et al. (2009); Ito et al. (2006); Kasuga et al. (1999, 2004); Morran et al. (2011)
OsWRKY45	WRKY	*Arabidopsis*	Drought	Induce the expression of stress-responsive genes	Qiu and Yu (2009)
OsbZIP23	ABF/AREB	*Arabidopsis*, rice	Salinity and drought	Induce the expression of stress-responsive genes	Kim et al. (2004); Xiang et al. (2007)
TaPIMP1	MYB	*Arabidopsis*, tobacco	Salt, cold, and drought stress	Induce the expression of stress-responsive genes	AbuQamar et al. (2009); Dai et al. (2007)
GmERF3	ERF	Tobacco	Salt and drought stress	Proline accumulation	Zhang et al. (2009)
SNAC2, *TaNAC69*, *GmNAC11*, *AhNAC2*	NAC	*Arabidopsis*, tobacco, wheat, rice	Drought, salt, and cold stress	Induce the expression of stress-responsive genes; improve seed germination and plant growth and development	Hu et al. (2008); Liu et al. (2011a); Liu et al. (2011b); Xue et al. (2011)
Antioxidant enzymes					
APX	APX	*Arabidopsis*, tobacco	Salinity and drought	ROS scavenging	Badawi et al. (2004); Lu et al. (2007)
CAT	CAT	Tobacco, rice	Salinity and cold stress	ROS scavenging, induce resistance in chloroplast machinery and protect photosystem	Al-Taweel et al. (2007); Zhao and Zhang (2006)

(continued)

Table 9.1 (continued)

Genes targeted in genetic engineering	Gene family	Transgenic plants developed	Targeted stress	Tolerance mechanism	References
ZnSOD, *CuSOD*, *MnSOD*	SOD	*Arabidopsis*, pepper plant, tobacco, potato, rice	Salt, drought, and cold stress	ROS scavenging	Chatzidimitriadou et al. (2009); Gupta et al. (1993); Prashanth et al. (2008); Wang et al. (2004); Waterer et al. (2010)
Signaling molecules					
HVA1, *CuCOR19*, *JcLEA*, *COR5a*, *RAB18*, *LTI30*, *COR47*, *LTI29* *WCOR410*	LEA	*Arabidopsis*, rice, wheat, mulberry, tobacco, strawberry	Drought, salt, and cold/ freeze stress	Improve biomass production and water uptake efficiency, lead to early seed germination and better growth and development under stress	Bahieldin et al. (2005); Checker et al. (2012); Hara et al. (2003); Houde et al. (2004); Liang et al. (2013); Rohila et al. (2002)
NPK1, *OsMKK6*, *MAPKKK*, *MAP3Kδ4*	MAPK	*Arabidopsis*, tobacco, rice, maize	Salinity, drought, heat, and cold stress	Induced protection of photosystems and enhanced photosynthesis	Kumar and Sinha (2013); Lu et al. (2013); Shitamichi et al. (2013); Shou et al. (2004)
OsCDPK13, *AtCPK23*	CPK	*Arabidopsis*, rice	Salinity, drought, and cold stress	Activates antioxidant system, lead to improved recovery after stress	Asano et al. (2012); Ma and Wu (2007)
AtCBL5, *SOS1*, *SOS2*, *SOS3*	CBL	*Arabidopsis*, tobacco, rice	Salinity, drought, and cold stress	ROS scavenging, induce resistance in chloroplast machinery, protect photosystem	Li et al. (2013)
OsCIPK03, *OsCIPK12*, *OsCIPK15*, *MdCIPK6L*, *TaCIPK29*	CIPK	*Arabidopsis*, tobacco, rice	Drought, salt, and cold/ freeze stress	Improve growth and seed germination under stress	Deng et al. (2013); Wang et al. (2012); Xiang et al. (2007)
SAPK4, *SRK2C*	SnRK2	*Arabidopsis*, rice	Salt stress	Improve growth and seed germination under stress	Diédhiou et al. (2008); Umezawa et al. (2004)
NDPK2	NDPK2	*Arabidopsis*	Salt and cold stress	Regulation of ROS accumulation	Moon et al. (2003)
GHSP26, *sHSP17.7*, *HSP101*	HSPs	*Arabidopsis*, rice, cotton	Salt, drought, and heat stress	Protection against sudden temperature shift	Al-Whaibi (2011); Maqbool et al. (2010); Sato and Yokoya (2008); Sun et al. (2001)

reductase (P5CR) or through transamination of ornithine (Orn) to P5C by the action of Orn-δ-aminotransferase (OAT). On the other hand, proline degradation occurs in mitochondria and includes the activity of proline dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) (Delauney and Verma 1993; Verbruggen and Hermans 2008). Although the glutamate pathway is more prominent for proline biosynthesis during osmotic stress, the Orn biosynthetic pathway was also enhanced in *Arabidopsis* (Verbruggen and Hermans 2008). Overproduction of proline biosynthesis genes has been explored as an option for developing stress resistance in transgenic plants (Kavi Kishor and Sreenivasulu 2014). In plant, GB, a quaternary amine, is produced and accumulated in response to stress through catalysis of choline by choline monooxygenase (CMO) and betaine-aldehyde dehydrogenase (BADH). The overexpression and accumulation of GB is associated with salinity stress tolerance (Chen and Murata 2008). The ability of GB to impart stress tolerance has been mimicked in plants that under normal circumstances (stress or non-stressed) do not accumulate GB. The transgenic plants are produced as a consequence of engineering GB biosynthesis and exhibit tolerance to various abiotic stresses (Hussain Wani et al. 2013). The transgenic tobacco with transformed BADH gene depicted enhanced GB accumulation that leads to heat stress tolerance (Yang et al. 2007). The analysis revealed lesser accumulation of ROS in the plant. Another study revealed increased photosystem II activity and tolerance to salt stress in *CodA* gene transgenic *Arabidopsis* (Hayashi et al. 1998). The *betA* gene transformed maize plants had increased tolerance to osmotic stress (Quan et al. 2004). Other than GB, engineering of aliphatic amine-containing molecules such as PAs has been an effective approach as well. Plants synthesize different forms of PA such as diamine putrescine (Put.) either through decarboxylation of Orn catalyzed by ornithine decarboxylase (ODC) or by decarboxylation of arginine (Arg) by the action of arginine decarboxylase (ADC). Spd synthase (SPDS) and Spm synthase (SPMS) use decarboxylated *S*-adenosylmethionine (dcSAM) as aminopropyl donor and result in the formation of triaminespermidine (Spd) and tetraminespermine (Spm) (Marco et al. 2015). Modulation in PA biosynthetic machinery resulted in better stress tolerance. The overexpression of *ADC*, *ODC*, *SAMDC*, *SPDS*, and *ACCs* in transgenic plants results in increased PA levels in cells and cellular compartments and exerts protection against abiotic stress (Marco et al. 2011). The capability of PA to bind with anions and cations contributes to the antioxidative property, thus mediating oxidative stress generated as a consequence of environmental stress (Groppa and Benavides 2008). The SPDS transgenic *Arabidopsis* constitutively overexpressed Spd, Spm, and Put that further conferred tolerance to chilling, freezing, and salt stress. Moreover, their overexpression resulted in the activation of antioxidant machinery, thus mediating the oxidative stress (Kasukabe et al. 2004). The ACC-modulated antisense tobacco corroborated with tolerance against various abiotic stressors (Wi and Park 2002). Sugars and sugar alcohols such as mannitol and trehalose, also, act as potent osmoprotectants; thus they have been exploited as transgenic approach to confer stress. Accumulation of mannitol and trehalose have protective role against environmental stressors. *Mannitol-1P dehydrogenase* (*mtlD*) (a mannitol biosynthetic gene) has been overexpressed in tobacco, wheat, eggplant,

Table 9.2 Stress-responsive genes that are targeted by genetic engineering for developing abiotic stress-tolerant transgenic plants

Gene encoding osmolyte	Engineered plants	Targeted stress	Tolerance mechanism	References
P5CS	*Arabidopsis*, rice, wheat, tobacco, carrot, petunia, olive, sugarcane, chickpea, potato	Drought, salt, and cold/ freeze stress	Increased root biomass, proline accumulation that leads to the activation of ROS scavenging enzymes, CAT, and APX	Behelgardy et al. (2012); Ghanti et al. (2011); Han and Hwang (2003); Hmida-Sayari et al. (2005); Karthikeyan et al. (2011); Kishor et al. (1995); Konstantinova et al. (2002); Molinari et al. (2007); Nanjo et al. (1999); Razavizadeh and Ehsanpour (2009); Sawahel and Hassan (2002); Su and Wu (2004); Vendruscolo et al. (2007); Yamada et al. (2005); Yamchi et al. (2007); Zhu et al. (1998)
P5CS1	*Arabidopsis*	Salt and drought stress	Low thermotolerance but increase proline accumulation	Chen et al. (2010)
P5CS2	Tobacco, rice	Salinity, drought, and cold stress	Proline accumulation	Chen et al. (2008); Hur et al. (2004)
P5CR	*Arabidopsis*, tobacco, soybean	Salinity, drought, and heat stress	Alter amino acid concentration and enhance root growth	De Ronde et al. (2004); Kocsy et al. (2005); Ma et al. (2008); Simon-Sarkadi et al. (2005); Szoke et al. (1992)
OAT	*Arabidopsis*, rice	Drought and salt stress	Enhance proline accumulation and increase yield	Roosens et al. (2002); Wu et al. (2003); Yu et al. (2010)
ProDH	*Arabidopsis*, rice, tobacco	Salt, drought, cold, and heat stress	Increase proline accumulation	Kavi Kishor and Sreenivasulu (2014); Kolodyazhnaya et al. (2007); Nanjo et al. (1999)
BADH	Potato, carrot, tobacco	Drought, salinity, and heat stress	Increase glycine betaine accumulation and improve growth	Hasthanasombut et al. (2010); Kumar et al. (2004); Yang et al. (2007); Zhang et al. (2011)

(continued)

Table 9.2 (continued)

Gene encoding osmolyte	Engineered plants	Targeted stress	Tolerance mechanism	References
CodA	*Arabidopsis*, mustard, tomato, potato, rice	Salt, drought, and cold stress	Increase glycine betaine accumulation, activate antioxidant machinery, and enhance reproduction and growth	Ahmad et al. (2008); Goel et al. (2011); Huang et al. (2000); Kathuria et al. (2009); Su et al. (2006); Wang et al. (2010)
betA	Tobacco, cotton, rice,	Salt and drought stress	Enhance plant growth and development under stress	Halford et al. (2003); Holmström et al. (2000); Lv et al. (2007); Takabe et al. (2006)
ADC	*Arabidopsis*, rice	Salt and drought stress	Overproduce of Put, Spd, and Spm	Alcázar et al. (2010); Altabella et al. (2009); Capell et al. (2004); Roy and Wu (2001)
ODC	Tobacco	Salt stress	Involve in constitutive overexpression of Put	Kumria and Rajam (2002)
ACC	Tobacco	Salt stress	Overexpression of Spd and Put	Wi and Park (2002)
SPDS	*Arabidopsis*, pear, sweet potato	Salt, cold/ freeze, and heavy metal stress	Involve in constitutive overexpression of Spd and Spm	Kangasjärvi et al. (2012); Kasukabe et al. (2004); Kasukabe et al. (2006); Wen et al. (2010)
SAMDC	*Arabidopsis*, tomato, tobacco, rice	Salt and heat stress	Involve in constitutive overexpression of Put, Spm, and Spd	Cheng et al. (2009); Marco et al. (2015); Roy and Wu (2002); Waie and Rajam (2003); Wi et al. (2006)
mtlD	Tobacco, eggplant, wheat, rice, poplar	Salt, chilling, and drought stress	Mannitol accumulation	Abebe et al. (2003); Chan et al. (2011); Hu et al. (2005); Karakas et al. (1997); Prabhavathi et al. (2002); Pujni et al. (2007)
M6PR	*Arabidopsis*	Salt stress	Induce antioxidant system	Chan et al. (2011)
mtlD/ glucitol-6-phosphate dehydrogenase	Loblolly pine, tomato	Salt stress	Induce antioxidant system	Gupta and Rajam (2013); Tang et al. (2005)

(continued)

Table 9.2 (continued)

Gene encoding osmolyte	Engineered plants	Targeted stress	Tolerance mechanism	References
TPS 1/2	*Arabidopsis*, tomato, tobacco	Drought, heat, salt, and cold stress	Avoid dehydration and scavenge ROS s	Cortina and Culiáñez-Macià (2005); Karim et al. (2007); Miranda et al. (2007)
Ots A/B	Rice, tobacco, potato	Drought, salt, and cold stress	ROS scavenging	Garg et al. (2002); Goddijn et al. (1997); Pilon-Smits et al. (1998)
DHAR	*Arabidopsis*	Salt stress	ROS scavenging	Ushimaru et al. (2006)
GPX + GST	*Arabidopsis*, tobacco	Salt and chilling stress	Scavenge ROS, improves seed germination and seedling growth	Qi et al. (2010); Roxas et al. (1997)

rice, and poplar leading to increased mannitol accumulation, thus inducing tolerance to abiotic stressors (Pujni et al. 2007; Prabhavathi et al. 2002; Karakas et al. 1997; Hu et al. 2005; Abebe et al. 2003). Simultaneous expression of *glucitol-6-phosphate dehydrogenase* enhanced salt stress tolerance in loblolly pine (Tang et al. 2005). Genetic engineering of *mannose-6P reductase* (*M6PR*) in *Arabidopsis* resulted in better tolerance to salinity along with induction of antioxidant system to confer oxidative stress (Chan et al. 2011). Stress tolerance through modulation of *trehalose phosphate synthase* (TPS) and *trehalose phosphate phosphatase* (TPP), that catalyze glucose to trehalose, has been achieved in various plants (Redillas et al. 2012). Modulation of Ots leads to aberrant growth pattern, stunted growth, and drought tolerance in transgenic tobacco (Karim et al. 2007).

Proteins such as HSP, LEA, and kinases, transcription factors like DREB, enzymes of antioxidant machinery, membrane transporters, and hormones play active role in stress signaling. Modulation of such molecules and their biosynthesis has known to impart stress tolerance. Glycerol-3-phosphate acyltransferase (GPAT) overexpressing transgenic tobacco had increased level of plasticidal cis-unsaturated phosphatidylglycerol that leads to improved photosynthesis and cold stress tolerance (Murata et al. 1992). However, the overexpression of group 3 and group 5 LEA proteins *OsLEA3* and dehydrins confer freezing, salt, and drought stress in transgenic lines of various plants (Liang et al. 2013; Houde et al. 2004). Activation of stress-responsive genes and proteins is strictly regulated by transcription factors. Ectopic expression of transcription factors has been attributed to enhanced stress tolerance. *Arabidopsis* DREB1/CBF genes possess stress tolerance mechanisms; thus their engineering in transgenic plants and lines has been promising (Kasuga et al. 1999; Agarwal et al. 2013). Although DREB1/CBF genes confer stress tolerance, as a consequence of abnormal gene expression, dwarf phenotypes of transgenic lines were produced. The inoculation of a stress-induced promoter resolved the issue in peanut, tobacco, and *Arabidopsis* (Kasuga et al. 1999, 2004; Bhatnagar-Mathur et al. 2007; Agarwal et al. 2013). Moreover, the upregulation of CBFs has been associated with induction of ABA-responsive genes and ABA accumulation (Lee et al. 2003). Other than transcription factors, the over- or under-expression of SOD, APX, CAT, GST, and GPX is responsible for induction or hampering of antioxidant machinery. Plants overexpressing SOD and APX depicted increased tolerance to drought stress, whereas the plants overexpressing GPX, GST, and DHAR depicted increased salt tolerance. The co-expression of GST and CAT induces salinity tolerance through managing oxidative stress in transgenic rice (Zhao and Zhang 2006). Furthermore, the simultaneous expression of APX, DHAR, and Cu/Zn SOD leads to protection of photosystems and tolerance to oxidative and salt stress (Lee et al. 2007).

Other than genetic engineering and breeding approaches in order to combat abiotic stress, application of phytohormones has been a new approach to confer stress. Studies have been conducted to decipher the role of hormonal treatment to achieve abiotic stress tolerance. Stressors mostly affect the plant at reproductive stage and may elicit permanent damage leading to yield loss (Zinn et al. 2010). GA and ABA mimic cold-induced signaling and produce male sterility effect; thus recently a photoperiod-sensitive rice variety with genetic male sterility has been developed using this approach (Sharma 2014). The application of ABA, SA, and JA instigated a cascade of phosphoproteins that led to cold stress tolerance (Kolaksazov et al. 2013). Recently, SA-treated common bean seeds depicted better germination, growth, and development under chilling stress conditions through activation of antioxidant enzymes (Gharib and Hegazi 2010). Variation in the hormonal concentrations has led to discovery of various proteins and carriers, e.g., identification of auxin efflux carrier, *OsPIN3t*, that has the ability to confer drought stress in rice (Zhang et al. 2012). Moreover, phytohormone levels mitigate various abiotic stresses through hormone-mediated signaling that leads to increase in growth and productivity of the plant (Wani et al. 2016).

9.6 Conclusion

Abiotic stresses (heat, cold/freeze, salinity, drought, UV, and heavy metal stress) are unavoidable and ubiquitous to the plants. Stress-responsive biochemical and molecular mechanisms have been evolved by plants to deal with the adversities caused by abiotic stresses. Stress adaptation and acclimation involve specific stress regulatory mechanisms that modulate gene expression, physiology, and morphology (Sun and Zhou 2018; Muchate et al. 2016). Plant activates signal transduction pathway upon stress perception and modulate stress tolerance responses (Sun and Zhou 2018). Stresses induce cellular and metabolic dysfunction and cause the accumulation of phytohormones such as ABA that in turn trigger stress-adaptive response (Zhu 2016). Based on alteration in environmental and physiological conditions, plants modify the stages of ABA and lead to production and storage of lipids and proteins, physiological alterations such as stomatal closure, leaf senescence, seed dormancy, and delayed germination (Vishwakarma et al. 2017; Tuteja 2007). The binding of

START domain receptor, PYLs (PYR/PYL/RCAR), to ABA inhibits the action of PP2Cs and releases SnRK2 from the association leading to the production and accumulation of downstream effector molecules (Park et al. 2009; Ma et al. 2015; Fujii and Zhu 2009). Mostly, ABA signaling that happen at plasma membrane is C2 domain protein such as SLAC1 mediated that act as substrate for SnRK2. This leads to the activation of MAPK signaling cascade and phosphorylation of NADPH oxidase and respiratory burst oxidase protein (RbohF), thus contributing to the subsequent release of O_2 and H_2O_2 (Sirichandra et al. 2009; Rodriguez et al. 2014; Geiger et al. 2009). Another plasma membrane protein, guard cell hydrogen peroxidase resistant (GHR) 1, is crucial for SLAC1 activation and regulation of ROS production (Hua et al. 2012; Grondin et al. 2015). Furthermore, H_2O_2, under the influence of GHR, has been observed to modulate calcium signaling that is crucial for the regulation of stomatal closure (Hua et al. 2012). The antioxidant system swiftly detects the presence of $^1O_2$ and instigates SOD that mends the ROS dysregulation by producing H_2O_2 as a consequence. Further activation of CAT and enzyme of AsA-GSH cycle catalyzes H_2O_2 releasing water and a molecule of O_2, thus restoring ROS homeostasis (Puthur 2016).

Notably, modulation, overexpression, or accumulation of TFs, signaling molecules, effector proteins, and osmolytes leads to better stress tolerance. Thus, SnRK2 mutant *Arabidopsis* depicted better growth and development under osmotic, salinity, and drought stress (Fujii et al. 2011). However, the ectopic expression of TFs, signaling molecules, and effector proteins produces undesirable traits such as overexpression of *DREB1* that led to dwarf transgenic lines (Agarwal et al. 2013). In order to counteract such unwanted characters, further exploration and understanding of the mechanisms is indispensable.

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Chapter 10
Use of Biostimulants in Conferring Tolerance to Environmental Stress

Pooja and Renu Munjal

Contents

Abstract Plants being sessile are continuously exposed to various adverse environmental conditions which include water-deficient and extreme temperatures, which limit crop production. The Intergovernmental Panel on Climate Change (IPCC) has recommended that global warming increases the numbers of various natural disasters and agriculture is particularly prone to the influence of such events. As these stresses cause such a massive yield reduction, it is important to alleviate the effect of these stresses to maintain or increase the production under unfavorable environmental conditions. The use of biostimulants has become a common trend that provides benefits like the stimulation of plant growth and protection against various stresses. A biostimulant is defined as an organic material and/or microorganism that, when applied to enhance uptake of nutrients, stimulates growth and boosts stress tolerance or quality of the crop. Biostimulants in the future might make agriculture more sustainable, resilient. Literature suggests an obvious role of biostimulants in protection against various environmental stresses. In this chapter, a broad overview

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of a diversified number of biostimulants, their protective effects against environmental stresses, has been focused. However, it is also revealed the urgent need to deal with the accurate mechanisms of these biostimulants which are responsible for their stress tolerance effects.

Keywords Environmental stresses · Biostimulants · Abiotic stress tolerance

10.1 Introduction

Plants are sessile and sensitive organisms that come across a variety of environmental stresses like heavy metal toxicity, water-deficient conditions, and temperature stress including both high and low throughout their life cycle resulting in a decline of crop yield. Abiotic stresses causes loss of about 70% of potential yield (Yamauchi 2018) which stipulates that crop production is only 20% of its potential yield. Thus, if crop is set free from these stresses by even 10% of its potential yield. then the net production of the crop would increase by an average of 50% (Fig. 10.1). Crop yield under environmental stress has been improved by the application of different agrochemicals (pesticides, herbicides, and fertilizers), irrigation optimization, and the use of various plant growth-promoting chemicals.

In addition to these conventional approaches, biostimulants are products that have pragmatic effects and that are being used with the goal of enhancing crop productivity and enhancing stress tolerance and to repair the damage caused by different environmental stresses. According to the latest definition given by Oosten et al. (2017), biostimulants, which are also called agricultural biostimulants, are a diverse group of substances that could be added to the plant or plant environment and provide tolerance to abiotic and biotic stress tolerance (Oosten et al. 2017).

Biostimulants differ from fertilizers because they exert effects on the metabolism of plant, and nutrient concentration of biostimulants is negligible as compared with fertilizers. Biostimulants are also not considered as nutrients, but they facilitate the

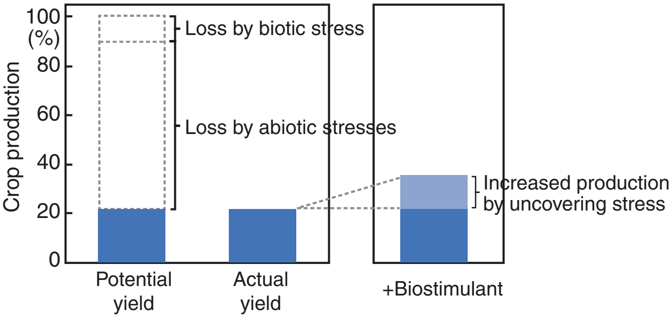

Fig. 10.1 Concept of yield increase

uptake of other nutrients present in the environment of the plant. Legally, biostimulants can contain trace amounts of naturally occurring plant growth regulator, but their biological action should be different from growth regulators. Biostimulants do not directly act on pests and thus do not come under the regulatory framework for pesticides and could be used in biotic stress.

Biostimulants are applied with the standard dose fertilization and which directly operate on the physiology and metabolism of plants (Nardi et al. 2009). They enhance nutrient use efficiency and product quality of plants (Heckman 1994). Biostimulants also modify root confirmation and accelerate the root development process (Petrozza et al. 2013). Various biostimulants have been reported to stimulate the growth of plants by various mechanisms like enhancing metabolism and increasing rate of germination, photosynthetic rate, and nutrient use efficiency. Biostimulants may also alleviate the negative effects of various environmental stresses. Biostimulants can be applied to both soils and to leaves as a foliar spray but can only exert action if they get to penetrate the tissue of a plant. Various factors like leaf permeability, leaf cuticle, and chemical structure of compounds affect the penetration of biostimulant in the tissue. The absorbability of biostimulants also depends on the field conditions of plants to which it is exposed.

10.2 Classification of Biostimulants

For the first time, scientist Filatov in 1951categorized biostimulants into four groups. In 2004 Ikrina and Kolbin precise nine natural raw material categories that are used to derive biostimulants; then Kauffman et al. (2007) grouped biostimulants broadly into three categories which include humic substances, products containing hormone, and products that have amino acid. In 2012 Du Jardin also developed a classification system consisting of eight categories. In his list, he excluded microorganisms to avoid disagreement with an existing categorization of microorganisms which is used as biopesticides, but later in 2015, he reduced his eight-category classification into a list of seven categories which includes beneficial bacteria and fungus (Jardin 2015). Torre et al. (2016) classified biostimulants into five groups which include seaweed extracts, hydrolyzed proteins, humic substances, inorganic acids, amino acids, and microorganisms. Biostimulants are generally classified based on chemical nature, but they should be grouped on the basic mode of action and physiological process which are induced by them because biostimulant composition is only partially known and isolation of single component from the biostimulant is difficult. The action of biostimulants or their physiological response can be identified using molecular biology techniques. There should be a close relationship between the gene activated and the corresponding physiological process to understand the effects of biostimulant on plant behavior (Table 10.1).

Table 10.1 Classification of biostimulants as proposed by different scientists (Yakhin et al. 2017)

Filatov (1951)	Ikrina and Kolbin (2004)	Kauffman et al. (2007)	Jardin (2012)	Jardin (2015)	Torre et al. (2016)
Carboxylic fatty acids (oxalic acid and succinic acid)	Microorganisms (bacteria, fungi)	Humic substances	Humic substances	Humic and fulvic acids	Humic substances
Carboxylic fatty hydroxy acids (malic and tartaric acids)	Plant materials (land, freshwater, and marine)	Hormone-containing products (seaweed extracts)	Complex organic materials	Protein hydrolysates and other N-containing compounds	Seaweed extracts
Unsaturated fatty acids and aromatic and phenolic acids (cinnamic and hydroxycinnamic acids, coumarin)	Sea shellfish, animals, bees	Amino acid-containing products	Beneficial chemical elements	Seaweed extracts and botanicals	Hydrolyzed proteins and amino acids
Phenolic aromatic acids containing several benzene rings linked via carbon atoms (humic acids)	Humate- and humus-containing substances		Inorganic salts (such as phosphite)	Chitosan and other biopolymers	Inorganic salts
	Vegetable oils		Seaweed extracts	Inorganic compounds	Microorganisms
	Natural minerals		Chitin and chitosan derivatives	Beneficial fungi	
	Water (activated, degassed, thermal)		Antitranspirants	Beneficial bacteria	
	Resins		Free amino acids and other N-containing substances		
	Other raw materials (oil and petroleum fractions, shale substance)				

10.2.1 Features of Biostimulants

All biostimulants have few common features regardless of their chemical composition and application:

- *Nature of biostimulants*: Biostimulants can be composed of single compounds like glycine betaine or compound group having single origin like seaweed extract. In the case of microorganism, inoculants could be of single strain like *Bacillus subtilis* in mixture of microorganism which has synergistic effects.
- *The diverse physiological functions*: All biostimulants have diverse physiological functioning which may finally convert into financial benefits by saving money on agrochemicals, good quality of the product, improved ecosystem services, etc.
- *Convergent effects*: All biostimulants converge to at least one or more than one positive function like enhance nutrition use efficiency, stress tolerance, and crop traits related to the quality of the crop.

10.3 Mode of Action

Mode of action and mode of the mechanism are commonly used for growth regulators, pesticides, and biostimulants. Mode of action is "a specified effect on the regulatory and biochemical process," and mode of mechanism results in stimulation of a physiological process or the downregulate signaling without understanding the biochemical and molecular basis of mode of action. Some biostimulants have neither specified mode of action nor their mode of action is known. This could be known because of the diverse nature of raw materials which are used for producing these materials which make it hard to recognize the specific components which are responsible for a particular specific effect and mode of action which are involved in inducing that response. Thus, more focus should be given for the recognition of the "mechanisms of action" involved rather than a mode of action. Modes/mechanisms of biostimulant action can be systematized at different levels which include

- Penetration, translocation, and transformation of biostimulants
- Expression of a gene, plant signaling, and regulation of hormone status
- Integrated effect on whole plant and metabolism

10.3.1 Penetration, Translocation, and Transformation

Biostimulants should have good solubility for sufficient penetration into plants. If the biostimulants do not have suitable solvent, then surfactants or any other adhesive could be used in place (Pecha et al. 2012). The penetration of biostimulants in the treated plants is studied by two methods which are radioactive probe and mathematical modeling. Radiolabel probe allows detection of penetrated substance and path followed by biostimulant inside the plant, whereas mathematical modeling is mainly based on "mechanism of diffusion" which allow the assessment of time required for absorption of minimum amount of biostimulant and describes the process of its transport from point of penetration to main plant part where it exerts its effect (Kolomazník et al. 2012).

10.3.2 Expression of Gene, Signaling, and Regulation of Hormone Status

A wide number of molecular biology tools are been used to study the expression of genes regulated by biostimulators. The bioactive compounds of some biostimulants display signaling activity. Various amino acids (Arbona et al. 2013) and peptides act as signaling molecules in the signaling pathway which regulate plant growth and development. Peptide signaling is mainly important in various physiological processes. Some proteins contain peptide sites, "cryptides" or "crypteins," in their sequence, which can have biological activity of their own, and it is different from the biological activities of their precursor. Recently it has been demonstrated that cryptides can activate the defense system of plants. Some externally applied amino acids may also involve in different biological processes by acting as a signaling molecule and some by influencing hormone action. Biostimulants contain hormone-like substances, or hormone is de novo synthesized from biostimulants which act as precursors or activators of endogenous plant hormone.

10.3.3 Integrated Effects on Whole Plant and Metabolism

Biostimulants get better productivity of plants by increasing the assimilation of various nutrients resulting in improved photosynthesis, stress response, and altered process of senescence. Biostimulants are also known to increase the level of proteins, carbohydrates, phenolic compound, and pigment levels. They are linked with a decline in reactive oxygen species and activation of antioxidants of a plant which ultimately results in stress tolerance.

10.4 Abiotic Stress Tolerance

10.4.1 Algal Extracts

Biostimulants like seaweed extracts (SWE) are gaining important commercial formulations that are used as plant growth-stimulating activities and a method forgotten to have better tolerance to various abiotic stress. Seaweeds include red, green, and brown algae which account for 10% marine productivity. A considerable amount of seaweeds (15 million ty^1) is used as a biostimulant in agriculture (FAO 2006). Algal extract marks different plant parts and different processes of plants (Fig. 10.2). Burchett and others in 1998 reported that the application of seaweed extract in *Hordeum vulgare* results in improved winter hardiness and increased frost resistance. In Kentucky bluegrass, the algal extract was used as biostimulants to alleviate salinity stress (Nabati et al. 1994). Seasol commercial formulation of *Ascophyllum* enhances freezing tolerance in grapes by a reduction in leaf osmotic potential (Wilson et al. 2006). Foliar application of isopropanol from marine microalgae to grape plants improved drought tolerance as a result of greater-than-before leaf water potential and stomatal conductance (Mancuso et al. 2006). SWE on *Agrostis stolonifera* L. plant gets better tolerance to high-temperature stress (Zhang and Ervin 2008). Rayirath et al. (2009) tested SWE in *Arabidopsis thaliana* for freezing tolerance and found that SWE component ethyl acetate enhanced freezing tolerance. A liquid extract from alga *Ulva lactuca* applied to wheat under saline condition restored growth (Nabti et al. 2010). SWE stimulates germination and growth of tomato plant seedling under salinity (Alalwani et al. 2012). *U. lactuca* also reestablished the leaf area and pigment content of *glycine betaine* under salt stress conditions (Ramarajan et al. 2013). *Sargassum vulgare* extract improved the germination of two cultivars of bean under salt stress (Latique et al. 2014). *A. nodosum* demonstrated increased relative water status and fresh and dry weight in spinach under water stress (Xu and Leskovar 2015). The physiological effects of seaweeds under the drought in wheat were evaluated in the presence as well as in the absence of different SWE, namely, *S. latifolium* and *U. lactuca*, and their mixture (Kasim et al. 2015). *Spiraea nipponica* and *Pittosporum eugenioides* plants treated with SWE *A. nodosum* give a higher proline content, flavonoid content, and phenolic content and improved physiology under mild drought stress conditions (Elansary et al. 2016). Seaweed extract rich in zinc and manganese enhanced cold tolerance. It may supply plants with essential micronutrients that act as cofactors in different antioxidative enzymes (Bradacova et al. 2016).

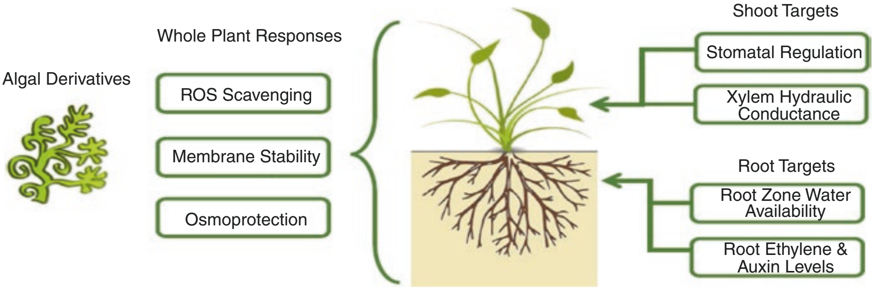

Fig. 10.2 Mechanism of action for algal extracts

10.4.2 Humic Substances

Humic substances (HS) are organic matter formed from the decomposition of leftover of plants, animals, and microorganisms (Fig. 10.3). HS is a group of heterogeneous substances categorized on the basis of molecular weight and solubility. They are of three types: (1) humic acids have high molecular weight and soluble in basic media, (2) fulvic acids have low molecular weight and are soluble in the alkali and acid media, and (3) humins are not extractable from the soil.

10.4.2.1 Humic Acids

Humic acids (HAs) are a polymer of organic compounds produced from the decomposition of organic substances (Sharif et al. 2002). HA stimulates growth by improving nutrient uptake, cell elongation, accumulation of nutrients, and chlorophyll biosynthesis. Combined application of humic acid and seaweed extract enhanced mass of root by 21–68%, tocopherol by 110%, and zeatin riboside by 38%. Both compounds were also found to exert the same effect in tall fescue and creeping bentgrass (Zhang and Ervin 2004); also Zhang and Ervin (2004) showed that in bentgrass, they found that these extracts when combined with cytokinins enhanced drought tolerance. Bell pepper treated with humic acid in combination with phosphorous resulted in reduced sodium content and elevated other elements in roots and shoots (Çimrin et al. 2015). Aydin et al. (2012) found that in high-salinity condition, application of humic acids results to enhanced level of amino acid proline and reduced leakage of a membrane which are considered as indicators for better adaptation to salinity situation. Humic acid made from vermicompost was tested in rice seedling for its effect on growth and physiology under drought, and it was found that it has a major role in the antioxidant defense system (García et al. 2012). Moghaddam and Soleimani (2012) tested humic acid to alleviate salt stress and

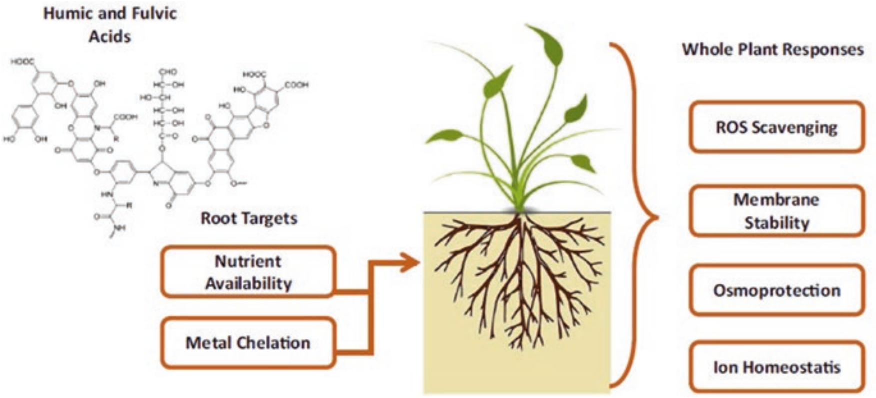

Fig. 10.3 Mechanism of action for humic substances

found that it increases the shoot growth and decreases levels of abscisic acid (ABA) and proline content. Applications of humic acid in chrysanthemum mitigate the negative effects caused by salt stress (Mazhar et al. 2012).

10.4.2.2 Fulvic Acids

Fulvic acids are low-molecular-weight humic substances that are soluble in both alkali and acid media. Fulvic acids have high total acidity, larger numbers of carboxyl groups, and higher adsorption and cation exchange capacities than humic acid (Bocanegra et al. 2006). There are only a few reports regarding the role of humic acid in the amelioration of environmental stresses. Xudan (1986) tested the effects of fulvic acids in wheat under two conditions, pot and field conditions, by applying them as foliar sprays for drought tolerance and found that it partially alleviated grain yield. Harper et al. (1995) found that fulvic acids eliminate the negative effects of aluminum. Anjum et al. (2011a) found that maize plants when treated with fulvic acid under drought conditions exhibited increased plant growth; also Anjum et al. (2011b) showed that the application of fulvic acids increased leaf area, plant dry weight, chlorophyll content, and yield of maize under drought stress. Shahid et al. (2012) investigated fulvic acid effect on lead toxicity and found that it can be used to eliminate the toxic effects.

10.4.3 Carbohydrates, Proteins, Amino Acids, and Lipids

Carbohydrates, proteins, amino acids, and lipids may enhance tolerance to stress through different mechanisms (Fig. 10.4). Megafol (Valagro, Atessa, Chieti, Italy) is a biostimulant available in the market made from vitamins, amino acids, proteins, and betaines of plant and algal extracts. Megafol application on tomato plants under water stress results in high relative water content and fresh weight and upregulated drought-responsive genes like *RAB18* and *RD29B* (Petrozza et al. 2014). Protein hydrolysates are a mixture of peptides and amino acids from animal or plant origin or individual amino acids. The application of protein to lettuce plants (*Lactuca sativa*) subjected to cold resulted in high fresh weights and improved stomatal conductance. Botta (2013) found that hydrolyzed amino acids provide high-temperature tolerance in perennial ryegrass (*Lolium perenne* L.) by improving photosynthetic efficiency. Seeds priming of melatonin improve chilling tolerance; also Kołodziejczyk et al. (2016) reported that corn seeds pretreated with melatonin confirm enhanced tolerance to chilling stress. Exogenously applied proline precursor glutamate and/or ornithine can improve tolerance to salinity (Da Rocha et al. 2012). The non-proteinous amino acids like beta-aminobutyric acid (BABA) and gamma-aminobutyric acid (GABA) provide resistance to abiotic and biotic stresses in plants (Zimmerli et al. 2008).

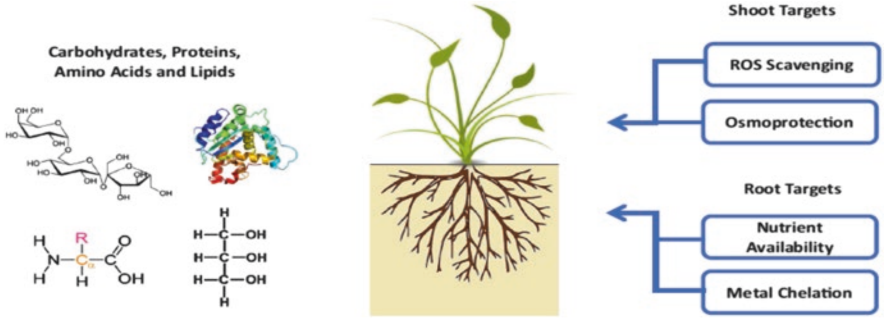

Fig. 10.4 Mechanism of action for Carbohydrates, proteins, amino acids, and lipids

10.4.4 Microorganism

The understanding of relationships between plants and microorganisms in abiotic stress is undeveloped. Some of the targets of microorganisms that increase abiotic stress tolerance have been identified (Fig. 10.5; Ali et al. 2011). Thermotolerant *Pseudomonas putida* strain AKMP7 was found to induce thermotolerance by increasing seed size, root length, shoot length, and biomass (Ali et al. 2009). *Pantoea dispersa* enhances uptake of nutrients and growth of plant due to the production of auxin and solubilization of phosphorus in wheat under cold stress (Selvakumar et al. 2008). Tomato plants were treated with inoculums of *Pseudomonas vancouverensis*, *P. frederiksbergensis*, and *Flavobacterium glaciei* showed significantly less electrolyte leakage and ROS activity (Subramanian et al. 2016). Maize seedling treated with *Azospirillum brasilense* was found to mitigate drought stress under greenhouse condition. Casanovas et al. (2002) similarly found that the same strain resulted in increased yield by 12% in wheat. Grains of a plant treated with *Azospirillum* under drought conditions had increased Mg, K, and Ca contents in comparison with uninoculated plants (Creus et al. 2004). Strains of *P. putida* and *B. megaterium* which produce auxin resulted in high water content and shoot and root biomass of white clover under drought conditions (Marulanda et al. 2009). Mycorrhizal symbioses also take part in confirming abiotic stress tolerance. Plant growth-promoting rhizobacteria (PGPR) strains of *B. subtilis*, *B. atrophaeus*, *B. sphaericus*, and *Staphylococcus kloosii* are reported to have high chlorophyll content, nutrient content, and yield in the strawberry plant under high soil salinity (Karlidag et al. 2013). Maize inoculated with *Azotobacter* strains has general affirmative effects under saline condition by facilitating uptake of K^+ and exclusion of Na^+ (Rojas-Tapias et al. 2012); similarly, wheat inoculation of *Azotobacter* strains enhanced biomass, nitrogen content, and grain yield under salt stress (Chaudhary et al. 2013). Other strains of *Azotobacter* like *A. chrococcum* has been shown to increase yields of pea, potato, rice, wheat, and cotton in saline arid soils. Arbuscular mycorrhizal fungi (AMF) was related to increased salt stress roots, independent of plant nutritional (P) status (Feng et al. 2002).

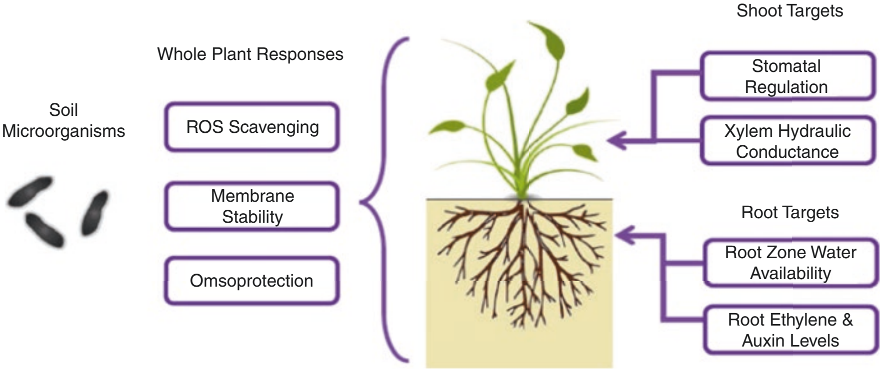

Fig. 10.5 Mode of mechanism for microorganisms

10.5 Conclusion and Future Perspective

Plants being sessile are continuously exposed to a wide variety of hostile environmental conditions which are major limiting factors in crop production. As these environmental stresses cause a massive reduction in yield, it is important to alleviate the ill effect of these stresses to maintain optimum yield in such conditions. Recently the application of biostimulants in agriculture has become a trend. Biostimulant treatments to crops have the potential to overcome the yield barrier imported by environmental stresses and to realize maximum potential yield without using much fertilizers and pesticides. In the future, the main concern should be given for better understanding of the mechanism of biostimulants, and more studies on the transcriptomic and proteomic effects of biostimulants should be carried out as it will help elucidate how specific it is to elicit plant growth, nutrient uptake, and stress tolerance responses.

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Chapter 11

Use of Phytohormones in Conferring Tolerance to Environmental Stress

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Abstract Due to sessile nature of plants, they face a variety of biotic and abiotic stresses during their life cycle. These stresses are responsible for disturbed cellular processes that adversely affect their growth and yield. To cope with these stresses, plants have developed various physiological mechanisms at cellular level that result in a change in morphology and help them to tolerate these environmental changes and respond to these changes with an optimal response. These responses of plants toward environmental stresses are both dynamic and complex that help them complete their life cycle rapidly under these stressful environmental conditions. The defense response of the plants to these stresses starts through variations in different molecular events with the involvement of different signaling molecules including the phytohormones. Phytohormones are small low-molecular-weight endogenous molecules that play important roles in different defense responses of plants against biotic as well as abiotic stresses. Along with their role in defense signaling, they also regulate various physiological, growth, and developmental processes in plants. These phytohormones include the auxins, cytokinins (CKs), gibberellins (GAs), ethylene (ET), jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA), and brassinosteroids (BRs) tocotrienols, triacontanols, and polyamines which are important ones that have ability to help plants to respond to different environmental stresses through their specific signaling properties. These signaling defense responses are the results of the interaction of various genes, helping the phytohormones to be involved in almost all cellular metabolic processes due to their specific modulations in the activities of these genes. All these phytohormones have their specific roles, e.g., auxin is involved in the regulation of differentiation and plant growth, cytokinin is responsible for cell division, gibberellin is responsible for stem elongation, seed germination, dormancy, senescence, and flower development, ethylene is involved in fruit ripening, and ABA has stress tolerance ability. The stress tolerance mechanism of plant is much complex that also has the involvement of other phytohormones, including the brassinosteroids (BRs), jasmonic acid (JA), salicylic acid (SA), polyamines (spermine, spermidine, putrescine, and thermospermine), tocotrienols, triacontanols (TRAI) as newly discovered ones. All the phytohormones help plants to survive under adverse environmental conditions with their specific roles in various growth, developmental, and physiological processes either through their endogenous accumulation or by exogenous application (foliar spray or seed priming) where the optimal concentration for the stress response is not sufficient. The exogenous use of these phytohormones has been increasing in crop plants with their economic value for obtaining the desired characters along with better production. The information given in the chapter will be helpful for plant growers and researchers to understand the mechanism of action of these phytohormones for better growth and production under changing environmental conditions.

Keywords Phytohormones · Abiotic stresses · Biotic stresses · Foliar spray · Seed priming · Endogenous increment

11.1 Introduction

Plants are affected by various adverse environmental challenges during their life cycle that not only threaten their survival but also hamper their growth and final production, ultimately leading to the reduction in the final yield due to poor nutrition. It was reported that abiotic stresses account for more than 50% reduction in crop yields. It is estimated that yield losses in agricultural crops due to different abiotic stresses include 17% due to drought, 20% due to salinity, 40% due to high temperature, 15% due to low temperature, and 8% due to other environmental factors. Among them, drought is considered the most devastating at present and in the future as expected (Iqbal and Ashraf 2013). Along with water stress, salinization is another feature that inhibits water uptake by plants due to low soil osmotic potential and also causes ionic imbalance leading to ionic toxicity and osmotic stress. However, the damaging effect of each abiotic factor depends on the intensity and quantity of that abiotic stress factor that disturbs the normal physiological activity of plants. Climate change increased the proportion of agricultural land facing abiotic stresses which is the major cause of the inability to meet the food demands of the ever-increasing population. The abiotic stresses severely affected the process of photosynthesis through degradation of de novo chlorophyll synthesis and the reduction in electron transport chain that leads to photooxidation, ultimately hampering the efficiency of photosystem-II. The levels of deleterious and damaging effects of abiotic stresses are incredibly increased at genetic, morphological, physiological, and ecological events by hindering every stage of plant life from seed germination to others by disturbing DNA, RNA levels, and activity of protein synthesis and seedling growth through cell division, cell enlargement, and cell differentiation under stressful environments, and the first and foremost effect is on the levels of signaling molecules including the phytohormones (Duque et al. 2013).

To tolerate these adverse environmental conditions, plants have developed specific mechanisms including plant biochemical adaptation for survival under stressful conditions, but it is a problem for agronomic crops (Agami and Mohamed 2013). Most importantly, plants compete with stressful environmental conditions due to the presence of several classes of small signaling molecules known as plant hormones which are critical for regulating and coordinating plant growth and development through controlling the altered metabolic activities (Gangwar et al. 2014). With regard to plant stress tolerance, the signaling molecules including plant hormones play a significant role for tolerance against adverse conditions. Phytohormones are the most important signaling molecules found in small quantities in cells for modulating physiological and molecular responses by acting either at their site of synthesis or transportation to different plant parts to initiate responses against adverse environmental conditions and genetically programmed developmental changes (Guo et al. 2018). Phytohormones regulate the every aspect of plant life cycle and play major roles in confirming plant tolerance to different ever-changing environments, by mediating growth, development, and nutrient allocation (Pavlovic et al. 2018). Plant hormones/phytohormones or plant growth regulators play key roles in changing the gene expressions through preventing the degradation of transcriptional regulators. Phytohormones are well known and of great importance due to their multifunctional roles in plants' responses to adapt and to ameliorate the damaging effects of abiotic stress in crop plants. The abiotic stressful environmental factors such as drought, salinity, extremes of temperature, and others cause both altered levels of phytohormones and widespread crop losses at the physiological, biochemical, and molecular levels throughout the world. Phytohormones include the classical well-known auxins, cytokinins, gibberellins, ethylene, abscisic acid, polyamines, and new members including jasmonic acid, salicylic acid, tocotrienols, and triacontanols which confer abiotic stress tolerance for the survival of sessile organisms at all organizations (Saedipour 2016). Especially in higher plants where a long distance is manifested among different plant parts for the regulation of metabolism, growth, and morphogenesis, chemical signals play a significant vital role. German botanist Julius von Sachs reported that these chemical messengers ensure growth and development of different plant organs by stimulating external factors responsible for the distribution of these substances in plants (Sergiev et al. 2018). Commonly, these substances are known as plant hormones or phytohormones which are naturally occurring organic compounds of low molecular weight and are produced by plants at very low concentrations (10^{-4} M) that promote, inhibit, or change all cellular, morphological, and physiological processes (Zhang et al. 2015). The mechanism of action of plant hormones is associated with the direct and specific molecular interactions, which initiate a series of biochemical and physiological responses. Phytohormones perform their action by forming the hormone-receptor complexes when it binds to specific protein receptor, and this complex is responsible for the liberation of secondary messengers that move to the nucleus and cause gene expression. Deoxyribonucleic acid (DNA) in the nucleus transcribed to messenger RNA (mRNA) which ultimately induces the synthesis of enzymes that act on polysaccharide linkages, mediating cell growth (Thomas and Jiménez 2005; Kumar et al. 2016).

Phytohormones are primarily linked with the tolerance of plants to different abiotic stresses and play important roles in mediating plant responses to various abiotic stresses which influence phytohormone homeostasis by altering their metabolism. Phytohormones are involved in many morphogenesis processes like the formation of leaves and flowers, elongation of stems, development and ripening of fruits, etc. produced naturally by plants. Under abiotic stresses, well-known phytohormones that play significant role include the essential hormones such as auxins, cytokinins, and GA that have enhanced rate of biosynthesis, metabolism, and signaling for protecting the plants against various abiotic stresses. Their increased biosynthesis is not measured at every time in all plants because their decreased biosynthesis has also been found. In high yielding crop varieties, to maintain their required levels for normal cellular functions, their exogenous use through different modes is also in progress that is found most effective as depicted in a plethora of literature (Khan et al. 2012b; Kumar et al. 2016).

Especially in agriculture for agronomic crops, phytohormones are being used to improve the growth and yield of plants. Auxins are being used especially to promote the formation of apical meristems as well as root differentiation in developing young plants. The application of gibberellic acid enhanced the growth, physio-biochemical parameters, proline and glycine betaine content, activities of antioxidant enzymes, photosynthesis, and plant water relations in linseed *Linum usitatissimum* L. under salt stress (Shi et al. 2014). The essential hormones including ABA must be produced in roots under changing environment and must regulate the plant growth especially the shoot responses to cope with adverse environmental conditions. In some cases, cytokinin levels at short term increased surprisingly in plants when encountering severe abiotic stresses (Batool et al. 2016). Cytokinins are essential plant hormones which significantly improved shoot meristem and leaf formation, cell division, chloroplast biogenesis, photosynthetic activities, and fresh biomass productions. ABA is also known as the primary plant stress hormone, aside from its involvement in stomatal regulation and root hydraulic conductivity that may induce responses leading to enhanced abiotic stress tolerance in plants (Batool et al. 2016).

Each specific phytohormone plays a specific role in counteracting the adverse environmental conditions by controlling the specific metabolic activities that are plant type/species specific at specific growth stage. For example, auxin is important due to its role in favoring the growth differentiation, cell wall extensibility, etc. (Januskaitiene and Kacienė 2017). GAs have specific role in protein biosynthesis by controlling amino acid metabolism under stressful conditions. The other specific plant hormone cytokinin is specifically important for cell division and roles in biosynthesis of photosynthetic pigments, thus playing a role in plant photosynthetic efficiency (George et al. 2008).

11.2 Roles of Phytohormones in Abiotic Stress Tolerance

11.2.1 Auxins

Auxin, meaning “to increase” or “to grow,” was first discovered in 1926 by Charles Darwin in the coleoptile tips of canary grass (*Phalaris canariensis*) seedlings in finding the mechanism of phototropism. It was reported that indole-3-acetic acid (IAA) was a principal auxin in higher plants because of its most simple structure and physiological roles that are effective at the concentrations of 0.02–0.2 mg/L. Auxin bioassay depicts that IAA primarily synthesizes in shoot apical meristems, young leaves, developing fruits and seeds as well as in rapidly dividing and growing tissues of shoots. Amino acid tryptophan is a probable precursor of auxin biosynthesis; therefore, plants convert tryptophan to IAA through several pathways such as indole-3-pyruvic acid (IPA) pathway, tryptamine (TAM) pathways, and indole-3-acetonitrile (IAN) pathway. IAA is also synthesized from indole or from indole-3-glycerol phosphate in a tryptophan-independent pathway (Mano and Nemoto 2012; Agami and Mohamed 2013). In plants, IAA is the biologically active phytohormone, but the majority of auxins found in cells are in covalently bound state with both high- and low-molecular-weight compounds. The distribution of auxin in the cell is regulated by pH. Metabolically, auxin is accumulated in the alkaline portions of the cell especially in cytosol and chloroplast. It is the only growth hormone that depicts unidirectional or polar transport from the apical ends of shoots to the basal root ends. In 1960, a chemiosmotic model has been purposed to explain the polar transport of IAA. According to which the polar transport is energy dependent and gravity independent. However, IAA also transports non-polarly in the phloem that is from mature leaves to the rest plant parts. Auxin imparting their important roles in several developmental and physiological processes such as growth enhancement in stems and coleoptiles as well as inhibiting growth in roots acidifies the cell wall and promotes the cell extension, enhancing the extensibility of cell wall through turgor pressure (Tiryaki 2009; Ryu and Cho 2015).

There are two major physiological effects of auxin, namely, phototropism and gravitropism. Phototropism is mediated by the lateral redistribution of auxin, while statoliths and secondary messengers (PH and calcium) serve as gravity sensors in shoots and roots in gravitrops. The phenomena of apical dominance, leaf abscission, vascular differentiation, floral bud development, and fruit development are regulated by IAA and considered as the developmental effects of auxin. The mechanism of signal transduction of auxin is complex and multistep in which ABP1 functions as auxin receptor, while calcium and PH act as signaling intermediates, whereas the auxin responsive domains are composite structures (Zhao 2010; Rozov et al. 2013).

11.2.1.1 Auxin’s Role Under Different Abiotic Stresses

The regulatory effects of plant hormones under stressful environmental conditions through its internal biosynthesis as well as its use externally through different modes are dependent on the type of plant species and its cellular levels. The improvement in stress tolerance through endogenous increments in the levels of auxin has been found in a number of plants under various abiotic stresses, and the stress tolerance was based on the improvement in various physiological parameters. For example, in *Brassica rapa* grown under salt stress, the improvement in growth was associated with better photosynthetic rate and protein accumulation and decreased Na^{2+} and K^+ ratio at 5.5 mg/g cellular levels of auxin (Pavlovic et al. 2018), while in *Gracilaria corticata*, a 2.5 mg/L level of auxin was effective for maintaining better leaf photosynthetic pigments and growth, when grown under salt stress (Anuraj et al. 2017). Similarly, 2, 60, and 200 ppm cellular levels of auxin were found effective for better growth of *Triticum aestivum* (Abdalla and El-Khoshiban 2007), *Poncirus trifoliata* (Liu et al. 2018a, b, c, d), and *Lolium perenne* (Zhang et al. 2015), respectively, when grown under water-deficit conditions. Endogenous increment of auxin at cellular levels, i.e., 140 ng/g, 20 mg/g, and 20 mg/g, has been found effective for better growth, which was associated with its better LRWC and ion accumulation in *Arabidopsis thaliana* (Fattorini et al. 2017), *T. aestivum* (Bucker-Neto et al. 2017), and *Oryza sativa* (Farooq et al. 2015), respectively, when grown under heavy metal stress. An endogenous increase of auxin up to 10 mg/g in roots of salt-stressed *Agrostis stolonifera* plants was reported by Krishnan and Merewitz (2015), and this concentration was effective for the improvement in cell division, total soluble protein, LRWC, as well as ion accumulation. Not only the endogenous increments but also the exogenous application of auxin has been reported for stress tolerance induction in various crop plants, but the effective level was species specific or dependent on the type of stress (Abu-Muriefah 2017; Gangwar et al. 2014; Saedipour 2016; Pavlovic et al. 2018; Sergiev et al. 2018). For example, seed priming of wheat with auxin at 200 ppm was found effective in reducing the adverse effects of drought stress in terms of growth and yield associated with improved physiological parameters (Guo et al. 2018). In another study conducted by Shi et al. (2014), it was reported that exogenous application of auxin through seed priming at 10 mmol improved the rate of photosynthesis, stem diameter, nutrient uptake, and phenolic content in drought-stressed *A. thaliana* plants. Foliar application of auxin at 20 mmol was found helpful in improving the photosynthetic pigments such as chl *a* and chl *b*) as well as photosynthetic efficiency of *Hordeum vulgare* plants grown under water stress as reported by Januskaitiene and Kacienė (2017). In *Glycine max* L. exogenous application of auxin through seed priming (10–100 μmol) was found helpful in alleviating the adverse effects of heavy metal stress with an improvement in seed germination, antioxidative defense mechanism, photosynthetic activity, as well as the chlorophyll biosynthesis (Abu-Muriefah 2017). A significant reduction in the harmful effects of heavy metal stress in *B. campestris* plants was reported through foliar spray of auxin. This resulted in enhanced seed yield, nutrient quality, seed germination index, and other biochemical attributes (Hotumalani et al. 2016). Furthermore, 25 ppm concentration of auxin was found helpful in reducing the negative effects of salt stress in *Helianthus annuus* L. associated with increased biomass production, membrane stability, plastid pigment content, as well as the activities of different antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) when applied foliarly (Zayed et al. 2017). Auxin-treated plants with 30 mg/L concentration improved the growth, leaf relative water content (LRWC), total dry mass, and total chlorophyll and carotenoid contents which resulted in more resistance in pea plants against salt stress in comparison with non-treated plants (Husen et al. 2016). The effect of seed priming on germination yield, nutritional quality as well as physiological parameters of *Cicer arietinum* and *T. aestivum* L. plants grown under salt stress was reported (Javid et al. 2011a, b; El-Awadi et al. 2018). They reported that an increase was found in seed germination, yield, nutritional quality and seed oil, carbohydrate, phenolic content, and the antioxidative defense mechanism in plants grown from seeds treated with auxin. In another study conducted by Jung et al. (2011) on salt-stressed *A. thaliana* plants to find out the effects of foliarly applied auxin on its growth, biosynthesis of photosynthetic pigments as well as the seed yield, it was found that 20 μmol concentration of auxin as foliar spray was found most effective in improving the above mentioned attributes by alleviating the adverse effects of salt stress (Table 11.1).

Table 11.1 Roles of auxins under different abiotic stresses

Stress type	Plant species	Endogenous increment/ exogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt and water stress	*Triticum aestivum* L.	Exogenous (seed priming)	200 ppm	Improved growth, physiological and yield attributes	Soil culture	Guo et al. (2018)
Salt stress	*Cicer arietinum*	Exogenous (seed priming)	10–30 mg/L	Increased yield nutritional value, seed oil, carbohydrate, phenolic content, and antioxidant activity	Soil culture	El-Awadi et al. (2018)
Salt stress	*Brassica rapa*	Endogenous increase	5.5 mg/g	Improved growth, photosynthetic rate, elevated proline content and the Na^+/K^+ ratio	Soil culture	Pavlovic et al. (2018)
Heavy metal stress	*Zea mays* L.	Exogenous (foliar application)	0–25 mg/L	Improved activities of CAT, POD, and SOD, growth, gas exchange characteristics, and accumulation of photosynthetic pigments	Soil culture	Habiba et al. (2018)
Water stress	*Poncirus trifoliata* L.	Endogenous increase	60 ng/g (in roots)	Improved growth, physio-biochemical attributes, and plant water relation	Soil culture	Liu et al. (2018a, b, c, d)
Heat stress	*Pisum sativum* L.	Exogenous (foliar application)	1 mmol	Improved activities of antioxidant enzymes and nonenzymatic compounds, LRWC, phenolics, and tocopherol contents	Hydroponic culture	Sergiev et al. (2018)
Heavy metal stress	*Vicia faba*	Exogenous (foliar application)	200 ppm	Improved growth, soluble sugars, soluble protein, and ion accumulation in different parts of bean seedlings	Soil culture	El-Samad et al. (2018)
Salt stress	*Helianthus annuus* L.	Exogenous (foliar application)	25 ppm	Increased biomass, membrane stability, plastid pigment content, and activities of antioxidant enzymes	Hydroponic culture	Zayed et al. (2017)
Heavy metal stress	*Sorghum bicolor*	Exogenous (foliar application)	0.5 mg/L	Improved growth and maximal photochemical efficiency	Soil culture	Zhan et al. (2017)
Salt stress	*Gracilaria corticata*	Endogenous increase	2.5 mg/L	Improved growth, photosynthetic pigment content, accumulation of total carotenoid, and better induction of lateral buds	Soil culture	Anuraj et al. (2017)
Water stress	*Hordeum vulgare*	Exogenous (foliar application)	20 mmol	Improved contents of photosynthetic pigments (*a* and *b*) and efficiency of photosynthesis	Sand culture	Januskaitiene and Kacienė (2017)

Heavy metal stress	*Vicia faba*	Exogenous (seed priming)	0.1–0.5 mmol	Improved growth, accumulation of photosynthetic pigments, total soluble carbohydrates and protein contents	clay-sandy soil culture	Kasim et al. (2017)
Heavy metal stress	*Arabidopsis thaliana*	Endogenous increase	140 ng/g in hypocotyls	Improved growth and physiological activities	Soil culture	Fattorini et al. (2017)
Heavy metal stress	*Triticum aestivum* L.	Endogenous increase	20 mg/g	Better stomatal conductance, water relation, accumulation of total soluble sugars, seed yield and nutritional quality	Soil culture	Bucker-Neto et al. (2017)
Water stress	*Triticum aestivum* L.	Endogenous increase	25.9 µg/mL	Improved growth, photosynthetic pigments accumulation, and biochemical attributes	Soil culture	Raheem et al. (2018)
Salt stress	*Glycine max* L.	Exogenous (foliar application)	20 mg/g	Increased growth, accumulation of photosynthetic pigments, and photosynthetic rate	Soil culture	Shuai et al. (2017)
Water stress	*Jatropha curcas*	Endogenous increase	50 ppm	Improved photosynthetic rate, number, and diameter of flowers, leaf protein contents as well as content of proline	Soil culture	Sun et al. (2017)
Heavy metal stress	*Glycine max* L.	Exogenous (seed priming)	10–100 µmol	Improved seed germination, activities of antioxidant enzymes, photosynthetic efficiency, and chlorophyll biosynthesis	Soil culture	Abu-Muriefah (2017)
Salt stress	*Oryza sativa*	Exogenous (seed priming)	30 mmol	Improved growth, physiological and biochemical parameters	Soil culture	Batool et al. (2016)
Salt stress	*Pisum sativum* L.	Exogenous (foliar application)	30 mg/L	Improved growth, LRWC, total dry mass, total chlorophyll and carotenoid contents	Soil culture	Husen et al. (2016)
Water stress	*Phaseolus vulgaris* L.	Exogenous (seed priming)	0.5 mmol/L	Increased number of grains per pod, activities of antioxidant enzymes, and biological yield	Soil culture	Mohtashami et al. (2016)
Salt stress	*Oryza sativa*	Exogenous (foliar application)	15 mg/L	Improved oxidative defense mechanism, ion accumulation, total soluble sugars, and total soluble proteins	Soil culture	Saedipour (2016)
Heavy metal stress	*Brassica campestris*	Exogenous (foliar application)	2.5 and 5 µmol	Enhanced seed yield and nutrient quality, seed germination index, and other biochemical attributes	Soil culture	Hotumalani et al. (2016)
Heavy metal stress	*Oryza sativa*	Endogenous increase	20 mg/g	Increased plant height, number of tillers, number of panicles, the 1000 grain weight, and paddy yield	Soil culture	Farooq et al. (2015)

(continued)

Table 11.1 (continued)

Stress type	Plant species	Endogenous increment/ exogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Lolium perenne*	Endogenous increase	200 ppm	Increased LRWC, total soluble sugars, root fresh weight, and ion accumulation	Soil culture	Zhang et al. (2015)
Heavy metal stress	*Solanum melongena* L.	Exogenous (foliar application)	10 μmol	Increased pigment contents, photosynthetic activity, activities of antioxidant enzymes, protein and proline contents	Sand culture	Singh and Prasad (2015)
Salt stress	*Agrostis stolonifera*	Endogenous increase	10 mg/g (in roots)	Improved cell division, accumulation of total soluble proteins, LRWC, ion accumulation	Sand culture	Krishnan and Merewitz (2015)
Water stress	*Zea mays* L.	Exogenous (seed priming)	0.0005 mg/L	Increased seedlings dry biomass, seed vigor, germination rate, soluble protein content, and activities of antioxidant enzymes	Soil culture	Yuan et al. (2014)
Water stress	*Vicia faba*	Exogenous (foliar application)	0–200 ppm	Increased growth, photosynthetic pigment contents as well as nutrients uptake	Soil culture	Zade et al. (2014)
Water stress	*Arabidopsis thaliana*	Exogenous (seed priming)	10 mmol	Improved the rate of photosynthesis, stem diameter, nutrient uptake and phenolic content	Soil culture	Shi et al. (2014)
Heavy metal stress	*Triticum aestivum* L.	Exogenous (foliar application)	40 mmol/L	Improved activation of antioxidant enzymes	Soil culture	Gangwar et al. (2014)
Heavy metal stress	*Oryza sativa*	Exogenous (foliar application)	10^{-3} M	Improved biomass production, seed yield, and nutritional quality	Soil culture	Ali et al. (2013a, b)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	5.7 mg/L	Increased growth, physio-biochemical attributes as well as seed germination	Soil culture	Abdoli et al. (2013)
Heavy metal stress	*Triticum aestivum* L.	Exogenous (seed priming)	500 μmol	Improved growth, pigment content, LRWC, activities of SOD, CAT, and POD as well as leaf structure	Soil culture	Agami and Mohamed (2013)
Water stress	*Zea mays* L.	Exogenous (foliar application)	40 mg/L	Enhanced seed yield, oil percentage, chlorophyll content, LRWC, and grain zinc content	Soil culture	Moghadam et al. (2013)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	0.0009.84 mol/L	Improved yield, net CO_2 assimilation rate, protein and phenolics contents	Soil culture	Iqbal and Ashraf (2013)

Water stress	*Zea mays* L.	Exogenous (foliar application)	10 mg/L	Enhanced diameter of stem and root, cell expansion, LRWC, and nutrient uptake	Soil culture	Nabizadeh et al. (2012)
Salt stress	*Arabidopsis thaliana*	Exogenous (foliar application)	20 µmol	Improved growth, photosynthetic pigments accumulation, and seed yield	Soil culture	Park et al. (2011)
Water stress	*Glycine max*	Exogenous (foliar application)	10 mg/L	Increased content of total free amino acid, growth and yield components	Soil culture	Zhang et al. (2011)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	20 ng/g	Improved seed germination, seed yields, and nutritional quality	Soil culture	Javid et al. (2011a, b)
Salt stress	*Nicotiana tobacum* L.	Endogenous increase		Improved activities of antioxidant enzymes	Soil culture	Zamanzedeh and Ehsanpour (2011)
Salt stress	*Arabidopsis thaliana*	Exogenous (foliar application)	300 ppm	Improved rate of photosynthesis, chlorophyll contents, LRWC, and growth	Soil culture	Jung et al. (2011)
Salt stress	*Oryza sativa*	Exogenous (foliar application)	0.00005 M	Improved yield, growth, physio-biochemical attributes as well as nutritional quality	Soil culture	Javid et al. (2011a, b)
Salt and drought stress	*Sorghum bicolor*	Exogenous (seed priming)	10 µmol	Improved nutritional quality, seed germination potential, seed yield, and total soluble proteins	Sand culture	Wang et al. (2010a, b)
Salt stress	*Tropaeolum majus* L.	Exogenous (foliar application)	10^{-6} M	Increased accumulation of chlorophyll and carotenoids contents, leaf photosynthetic rate, and enzyme activities	Soil culture	Karatas et al. (2010)
Heavy metal stress	*Helianthus annuus* L.	Exogenous (foliar application)	0, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} M	Improved growth, root volume, and root surface area	Hydroponic culture	Fassler et al. (2010)
Water stress	*Zea mays* L.	Exogenous (foliar application)	1 mg/L	Increased total soluble sugars, nutrient uptake, reducing sugars, and the activities of antioxidant enzymes	Soil culture	Vasconcelos et al. (2009)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	1–2 mg/L	Increased germination percentage, seedling biomass, and hypocotyl lengths	Soil culture	Akbari et al. (2007)
Water stress	*Carica papaya* L.	Exogenous (foliar application)	80 ng/g	Increased LRWC, stomatal conductance, photosynthetic rate, transpiration rate, biomass	Soil culture	Mahouachi et al. (2007)
Water stress	*Triticum aestivum* L.	Endogenous increase	2 mg/L	Improved activities of both hydrolytic and oxidative enzymes in shoots and roots as well as the biosynthesis of photosynthetic pigments	Soil culture	Abdalla and El-Khoshiban (2007)

11.2.2 Gibberellic Acid (GA)

In the 1930s, Japanese scientists in Tokyo University isolated the impure crystals of cultured fungus which they termed *gibberellin A* and *B* termed after *Gibberella fujikuroi*, the name of the fungus. Gibberellin has vital roles in growth and development by imparting its significant roles in enhancing the stem growth in dwarf and rosette plants, regulating the transition from juvenile to adult phases, stimulating floral initiation and sex determination as well as promoting fruit set and seed germination as well. Foliar spray of gibberellins is useful to manage fruit production in fruit crops, to malt barley, and to increase sugar yield in sugarcane as well as is useful for crop breeding. Gibberellins constitute a large family of diterpene acids, synthesized by the terpenoid pathway in three stages, regulated by a large variety of enzymes and genes. Three phases are involved in the production of terpenoid precursors, i.e., ent-kaurene in plastids, oxidation reactions on the endoplasmic reticulum to form GA12 and GA53, and formation of all other gibberellins from GA12 or GA53 in the cytosol as last step of biosynthesis. The biosynthesis of GA usually takes place in the apical tissues that are tightly bound to environmental factors such as light, temperature, etc. Studies revealed that gibberellin biosynthesis may also be induced by auxin in higher plants (Shomeili et al. 2011; Hedden and Sponsel 2015). Gibberellin is the essential biological active growth hormone in the form of GA_1 that is closely associated with the character of tallness in higher plants as well as the covalently bound form with sugars. In comparison with others, gibberellins have unique character of regulating their own metabolism through stimulating and inhibiting the transcription factors (Halo et al. 2015).

Gibberellins also play their vital roles in various physiological mechanisms, including stimulating cell elongation and cell division, increasing cell wall extensibility without acidification, and regulating the transcription of cell cycle kinases in intercalary meristems. The crop plants are genetically supposed to retain GA_1, RGA, and DELLA repressor protein which are the negative regulators of growth. Gibberellins cause the degradation of these repressors. Signal transduction pathway of gibberellins primarily was discovered in the aleurone layer of cereal grains, regulated by signaling intermediates such as cGMP, Ca^{2+}, and protein kinases. The pathway involves the gibberellin-induced α-amylase production in aleurone layers, which leads to enhanced transcription of α-amylase mRNA, and ultimately GA-MYB transcription factor regulates α-amylase gene expression (Hedden and Sponsel 2015).

11.2.2.1 Roles of GA Under Different Abiotic Stresses

The improvement in stress tolerance through endogenous increment in cellular levels of GA has been found in numerous plants under various abiotic stresses, and the stress tolerance mechanism was based on its role in the improvement of various physiological parameters. In *A. thaliana* Leitao and Enguita 2016) and *G. max*

(Sofy 2015), the cellular levels of 1.95 ng/mL and 35–45-folds were found effective in the improvement of growth and photosynthetic pigment accumulation, nutrient uptake, TSP content, the antioxidative defense mechanism, and improved phenolics content when grown under salt stress. Similarly, under water stress, 100 μmol and 2–7 mg/g levels of GA were found effective for better growth, transpiration rate, epidermal cell expansion, carotenoids, and the accumulation photosynthetic pigments of *Solanum lycopersicum* (Nir et al. 2014) and *Populus alba* (Zawaski and Busov 2014), respectively. In *Phragmites australis* grown under heavy metal stress, the improvement in growth, secondary metabolites, chlorophyll pigment biosynthesis, and ion accumulation was found at 20 ppm level of GA (Hipol et al. 2015). Along with endogenous accumulation of GA, the exogenous application of GA was also found to play a significant role in improving various growths and physiological and yield processes and also associated with the stress tolerance induction in various crop plants (Khavari-Nejad et al. 2013; Colebrook et al. 2014; Halo et al. 2015; Leitao and Enguita 2016; Mustafavi et al. 2016). For example, in *Capsicum annuum* L. plants, foliar spray with 0 and 250 mg/L was found helpful in alleviating the negative effects of salinity by improving the growth, production, fruit quality, and POD activity (AlTaey 2017). Similarly, Halo et al. (2015) reported that an increase was found in biomass production with reduction in lipid peroxidation and increased glutathione content in salt-stressed *S. lycopersicum* plants when supplied with 10 and 100 μmol of GA as foliar spray. In another study, conducted on *Lycopersicon esculentum*, to find the effects of foliarly applied GA on growth, chlorophyll biosynthesis, soluble proteins and insoluble sugar contents under heavy metal stresses, it was found that foliarly-applied different levels (0, 5, and 10 μmol) of GA were found effective in positively stimulating the growth, chlorophyll biosynthesis, soluble proteins, and insoluble sugar contents (Khavari-Nejad et al. 2013). Another experiment was carried out in soil culture to identify the effect of seed priming with GA on germination, growth, and antioxidant defense system in *Sorghum bicolor* plants grown under high temperature and heavy metal stresses. An improvement in these studied attributes was reported in plants that were raised from seeds treated with 288.7 μmol of GA (Nimir et al. 2015). Similarly, the seed priming with 20 ppm of GA has been found associated with the reduction in the harmful effects of heavy metal stress on secondary products along with plant growth in *P. australis* plants grown in soil culture (Hipol et al. 2015) (Table 11.2).

11.2.3 Cytokinins (CKs)

In 1974, Lethem discovered the most abundant natural cytokinin *zeatin* in coconut milk at the University of Wisconsin. The major role of cytokinin is the induction of cell division in mature and differentiated tissues in meristems to regulate growth, development, and metabolism. Cytokinin occurs in both free and bound forms in plants, but the earlier one is the biologically active form. The biosynthesis of free cytokinin is also associated with pathogenic bacteria, insects, and nematodes

Table 11.2 Roles of GAs under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Zea mays* L.	Exogenous (foliar application)	10^{-2} M	Promoted growth, ion uptake and transport, and the nutrient utilization	Sand culture	AlBalawi and Roushdy (2018)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	100 ppm	Improvement in growth, photosynthetic pigments biosynthesis, and better nutritional quality	Sand culture	Chauhan et al. (2018)
Salt stress	*Zea mays* L.	Exogenous (seed priming)	150 ppm	Increased growth, number of flowers, chlorophyll biosynthesis, and seed yield	Soil culture	Mohamed and Mahmoud (2018)
Salt stress	*Zea mays* L.	Exogenous (foliar application)	60 g/ha	Increased all growth and yield parameters	Soil culture	Rahim et al. (2018)
Salt stress	*Moringa oleifera*	Exogenous (foliar application)	10, 25, 50, and 75 mg/L	Enhanced biosynthesis of photosynthetic pigments such as chlorophyll, carotenoids, and xanthophyll	Sand culture	Elayaraja et al. (2017)
Heavy metal stress	*Glycine max* L.	Exogenous (seed priming)	10 and 100 μmol	Improved seedling growth, photosynthetic rate, seed germination, and yield parameters	Soil culture	Abu-Muriefah (2017)
Salt stress	*Glycine max* L.	Exogenous (foliar application)	0–2 mg/mL	Improved plant growth and yield by modulations in physiological changes	Soil culture	Kim et al. (2017)
Heavy metal stress	*Solanum lycopersicum*	Exogenous (seed priming)	7–10 mg/mL	Improved physiological efficiency	NB media	Kang et al. (2017)
Salt stress	*Capsicum annuum* L.	Exogenous (foliar application)	10 ppm	Increased ion uptake, soluble protein, accumulation of organic solutes	hydroponic culture	Uzal and Yasar (2017)
Salt stress	*Capsicum annuum* L.	Exogenous (foliar application)	0 and 250 mg/L	Improved growth, crop production, fruit quality, and POD activity	Soil culture	AlTaey (2017)
Salt stress	*Oryza sativa* L.	Exogenous (foliar application)	5–20 mmol	Better maintenance of nutrient homeostasis, growth, yield, and productivity	Soil culture	Rahman et al. (2017)
Salt stress	*Olea europaea* L.	Exogenous (foliar application)	0, 10 and 100 mg/L	Increased uptake of K and biomass production	Sand culture	Shekafandeh et al. (2017)

Salt stress	*Glycine max* L.	Endogenous increase	35–45-folds and cultivar specific	Improved growth, photosynthetic pigment contents and nutrients	Soil culture	Sofy (2015)
Water stress	*Oryza sativa* L.	Exogenous (foliar application)	5 μmol	Improved plant growth, stomatal conductance, grain yield, and nutritional quality	Soil culture	Plaza-Wüthrich et al. (2016)
Salt stress	*Arabidopsis thaliana*	Endogenous increase	1.95 ng/mL	Improved seed germination and seed vigor	Soil culture	Leitao and Enguita (2016)
Water stress	*Zea mays* L.	Exogenous (foliar application)	100 and 50 ppm	Improved cellular homeostasis, growth, and biosynthesis of photosynthetic pigments	Soil culture	Mustafavi et al. (2016)
High temp. and salt stress	*Sorghum bicolor* L.	Exogenous (seed priming)	288.7 μmol	Improved germination, growth, and antioxidant defense system	Soil culture	Nimir et al. (2015)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	200 mg/L	Enhanced pigments biosynthesis, increased content of reducing sugars and protein, activities of POD and CAT	Soil culture	Rahdari and Hoseini (2015)
Salt stress	*Solanum lycopersicum*	Exogenous (foliar application)	10 and 100 μmol	Increased biomass production, reduced lipid peroxidation, and increased glutathione content	Soil culture	Halo et al. (2015)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	6 μmol	Increased nutrient uptake, growth biomass, and yield	Soil culture	Chauhan and Kumar (2015)
Salt stress	*Mentha piperita* L.	Exogenous (foliar application)	50 mg/L	Improved TSP, soluble sugar contents, and antioxidant enzyme system	Soil culture	Soleymani and Taheri (2015)
Heavy metal stress	*Vigna radiate* L.	Exogenous (foliar application)	10^{-4} M	Increased growth and yield and decreased Ni uptake in roots and shoot	Soil culture	Ali et al. (2015a, b)
Heavy metal stress	*Phragmites australis*	Endogenous increase	20 ppm	Decreased the harmful effects of heavy metal stress on secondary products and plant growth	Soil culture	Hipol et al. (2015)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	600 mg/L	Improvement in biochemical and physiological attributes	Soil culture	Al-Mashhadani et al. (2015)
Heavy metal stress	*Cajanus cajan* L.	Exogenous (seed priming)	10 and 100 μmol	Enhanced seed germination and seed vigor as well as yield parameters	Soil culture	Sneideris et al. (2015)

(continued)

Table 11.2 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Medicago sativa*	Exogenous (seed priming)	0, 3, 5 and 8 mmol	Increased activities of antioxidant enzymes and decreased the membrane damages	Petri dishes	Younesi and Moradi (2014)
Water stress	*Ocimum basilicum*	Exogenous (seed priming)	200 ppm	Improved seed germination and emergence, seedling growth, and seed yield	Soil culture	Bagheri et al. (2014)
Water stress	*Solanum lycopersicum*	Endogenous increase	100 μmol	Improved leaf water status, transpiration rate, stomatal conductance, and epidermal cell expansion	Soil culture	Nir et al. (2014)
Salt stress	*Medicago sativa*	Exogenous (seed priming)	0, 3, 5 and 8 mmol	Higher level (8 mM) increased the photosynthetic pigment contents, growth, and reduced membrane damages	Soil culture	Younesi and Moradi (2014)
Salt stress	*Satureja hortensis* L.	Exogenous (foliar application)	0 and 4 mmol	Increased transpiration rate, LRWC, chl. *b*, total chl, and membrane stability	Soil culture	Nikee et al. (2014)
Cold, drought, and salt	*Arabidopsis thaliana*	Endogenous increase	36–37-fold increase	Improved stress tolerance	Soil culture	Colebrook et al. (2014)
Water stress	*Populus alba*	Endogenous increase	2–7 mg/g	Increased pigment biosynthesis such as chlorophyll and carotenoids, improved growth and physiological mechanisms		Zawaski and Busov (2014)
Salt stress	*Glycine max* L.	Endogenous increase	5 and 10 mmol	Improvement in growth parameters, chlorophyll biosynthesis, and nutrient accumulation	Soil culture	Hamayun et al. (2014)
Salt and water stress	*Brassica napus*	Exogenous (foliar application)	100 ppm	Increased rate of seed germination, root, stem, and leaf growth, flowering, and fruit and seed growth	Soil culture	Kurepin et al. (2013)
Salt stress	*Trigonella foenum graecum* L.	Exogenous (foliar application)	0 and 2 mmol	Increased the LRWC and seed yield	Soil culture	Alizadeh et al. (2013)
Heavy metal stress	*Lycopersicon esculentum*	Exogenous (foliar application)	0, 5 and 10 μmol	Improvement in growth, chlorophyll biosynthesis, soluble proteins, and insoluble sugar contents	Sand culture	Khavari-Nejad et al. (2013)

Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	100 mg/L	Improved growth, soluble protein contents, and seed yield	Soil culture	Shaddad et al. (2013)
Salt stress	*Phaseolus vulgaris* L.	Exogenous (seed priming)	0.05 mmol	Improved protein biosynthesis and activity of antioxidant enzymes such as guaiacol peroxidase	Soil culture	Saeidi-Sar et al. (2013)
Salt stress	*Abelmoschus esculentus*	Exogenous (foliar application)	10^{-2} M	Enhanced photosynthetic rate, number, and diameter of flowers, protein, and nutrient uptake	Sand culture	Mary and Merina (2012)
Salt stress	*Lactuca sativa* L.	Exogenous (seed priming)	0, 3, 4.5 and 6 mmol	Enhanced seed germination rate, growth, and antioxidant defense mechanism	Soil culture	Hela et al. (2012)
Salt stress	*Saccharum officinarum*	Exogenous (foliar application)	100 ppm	Enhanced nutrient uptake, as well as the morpho-physiological parameters	Hydroponic culture	Shomeili et al. (2011)
Heavy metal stress	*Triticum aestivum* L.	Exogenous (foliar application)	10^{-5} M	Enhanced cell division, respiration, photosynthetic efficiency	Soil culture	Falkowska et al. (2011)
Salt stress	*Glycine max* L.	Endogenous increase	0–2.5 mmol	Increased growth and yield	Sand culture	Hamayun et al. (2010)
Salt stress	*Amaranthus cadaous*	Exogenous (foliar application)	0.01, 0.1, and 0.3 mmol	Improved α- and β-amylase activity and seed germination	Soil culture	Bialecka and Kepczynski (2009)
Salt stress	*Brassica juncea* L.	Exogenous (foliar application)	10^{-5} M	Improved growth and physio-biochemical attributes	Sand culture	Siddiqui et al. (2008)
Water stress	*Cajanus cajan*	Exogenous (foliar application)	153 µg	Enhanced hypocotyls elongation, leaf expansion, and fruit developments under stressed conditions	Soil culture	Chudasama and Thaker (2007)
Heavy metal stress	*Cucumis sativus* L.	Exogenous (foliar application)	400 ppm	Partially counteracted the inhibitory effects of heavy metals on flowering	Soil culture	Khan and Chaudhry (2006)
Heavy metal stress	*Vicia faba* L.	Exogenous (foliar application)	200 mg/L	Stimulated mitotic activity and biochemical constituents of the seeds as well as counteracted the adverse effects of heavy metals on metabolic mechanisms	Soil culture	Mansour and Kamel (2005)

(continued)

Table 11.2 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Nicotiana tabacum*	Exogenous (foliar application)	10 and 50 µmol	Increased biosynthesis of photosynthetic pigments as well as amino acid content and mediated lignin biosynthesis	Soil culture	Biemelt et al. (2004)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	100 mg/L	Improved photosynthetic capacity and ion accumulation in different plant parts	Sand culture	Ashraf et al. (2002)
Water stress	*Gossypium hirsutum* L.	Exogenous (foliar application)	50 µmol	Improved photosynthetic rate, transpiration rate, stomatal conductance, carboxylation efficiency, and water potential	Soil culture	Kumar et al. (2001)
Salt stress	*Cicer arietinum* L.	Exogenous (foliar application)	20 mg/L	Improved dry and fresh biomass but no effect on a number of branches	Petri dishes	Iqbal et al. (2001)

associated with higher plants. For example, crown gall cells proliferate in culture without the addition of any hormones to the culture medium; these cells have genes for cytokinin biosynthesis. In plants, cytokinin biosynthesis is catalyzed by IPT. Root apical meristems are the major sites of the synthesis of free cytokinins in whole plants (Kieber 2002; Ha et al. 2012). Cytokinins transport from roots to shoots through the xylem and are rapidly metabolized by plant tissues when the signals were responsible to regulate their transport from shoot apical meristem to roots. Cytokinins also impart their significant biological roles by regulating cell division in shoots and roots, where specific components of the cell cycle lead to differentiation and senescence. The phenomena of apical dominance and lateral bud growth have also the involvement of cytokinin. However, the overproductions of cytokinin are toxic for plants that results in tumor formation. Auxin/cytokinin ratios regulate the morphogenesis in cultured tissues, whereas high auxin/cytokinin ratios stimulate the formation of roots, and low auxin/cytokinin ratios led to the formation of shoots. It also delays leaf senescence and promotes the movement of nutrients, chloroplast development, and cell expansion in leaves and cotyledons.

Diverse effects of cytokinin on plant growth are significantly related to bacterial two-component receptors, where rapid increase in the expression of response regulator genes also the fusion of cytokinin. Cytokinin signaling cascade mediated by histidine phosphotransferases and cytokinin-induced phosphorylation activates the transcription factor and controls the development at the cellular and molecular levels (Kieber 2002; Ha et al. 2012; Kieber and Schaller 2018).

11.2.3.1 Roles of CKs Under Different Abiotic Stresses

Studies revealed that CK has multiple roles in plant development by playing its role as an essential plant hormone by regulating its specific roles especially the chlorophyll biosynthesis and translocation of amino acids along with gibberellic acid and auxin. However, under stressful environmental conditions, its roles are extensively studied that not only control its own metabolism but also trigger the synthesis or accumulation of other plant hormones that alternatively control many other metabolic activities for the induction of stress tolerance (Keshishian et al. 2018). It has been found that under multiple abiotic stresses, its concentration increases tremendously, but it is plant type or cultivar specific (Moura et al. 2017). Though under stressful environmental conditions, it plays its role for the induction of stress tolerance, it not happens in all plant species. So, its exogenous use is being employed through different modes for induction of stress tolerance (Moura et al. 2017). The endogenous increments in cellular levels of CK was found responsible for the improvement in stress tolerance in various plant species under different abiotic stresses, and the stress tolerance was based on the improvement in various physiological parameters (Merewitz et al. 2011). In *A. stolonifera* (Merewitz et al. 2011), *A. thaliana* (Nguyen et al. 2016), and *Helianthus annuus* (Nguyen et al. 2016), 0–5 mmol, 0–10 μmol, and 15 mg/L cellular levels of CK, respectively, were found effective in the improvement of plant growth, content of amino acids, antioxidative defense mechanism, carbohydrate accumulation, biosynthesis of organic acids, and photosynthetic pigments when grown under soil water stress. Similarly, under salt stress, 10^{-4} M, 3, 5, and 7 nmol, and 10 and 100 ng/g levels of CK were found effective for better growth, improved physiological processes and chlorophyll content, and better antioxidative defense mechanism of *G. max* (Moura et al. 2017), *T. aestivum* (Nishiyama et al. 2012), and *A. thaliana* (Wang et al. 2015), respectively. In *A. thaliana* grown under salt stress, the improved accumulation of amino acids, proteins, and activities of enzymes and of photosynthetic pigments was recorded at 0–4 mg/g levels of CK (Zwack et al. 2016). Not only the endogenous increments but also the exogenous application of CK has also been reported effective for stress tolerance induction in various crop plants, but the effective level was species specific or dependent on the type of stress (Younesi and Moradi 2014; Hamayun et al. 2014; Soleymani and Taheri 2015; Ali et al. 2015a, b; Sofy 2015). For example, the exogenous application of cytokinin as seed priming has been found to be involved in improving the capacity of various seed germination processes in *Sorghum bicolor* L. (Ismail 2003), *Lotus corniculatus* L. (Nikolic et al. 2007), and *C. arietinum* L. (Tyagi and Kumar 2016) plants grown under salt-stressed conditions. Germination is an important process that is associated with better growth and production of plants. Many studies have been reported regarding the involvement of exogenously applied cytokinin in triggering seed germination along with growth and biomass production in a variety of plant species. For example, in *S. lycopersicum* (Kumar et al. 2016), *Zea mays* (Davani et al. 2017), and *O. sativa* (Rajakumar 2013) plants, 0.1–1.0 mmol, 100 mg/L, and 205.4 mg/g of cytokinin were associated with the improvement in the growth and biomass production when applied foliarly. As heath of a plant is determined from its biomass production, this results in various changes in the final production, and cytokinin has also been involved in improving the final yield of plants. For example, Iqbal and Ashraf (2006) reported an increase in growth and final yield of salt-stressed wheat plants when its seeds were grown after primed with 100, 150, and 200 mg/L of cytokinin (Table 11.3).

11.2.4 Abscisic Acid

Abscisic acid, a ubiquitous stress plant hormone, has been found in every cell of vascular plants containing chloroplast and other plastids and in every plant part from the root cap to the apical bud. A variety of physical, biological, and chemical bioassays have been used for ABA, including inhibition of coleoptile growth, seed germination, and/or GA-induced α-amylase synthesis. However, physical bioassays are much more reliable than biological ones, where ABA is measured with the help of gas spectrometer filled with mass spectrometer. ABA bioassay depicts the biological as well as the physiological activity of ABA that totally depends on its structure. Studies revealed that in higher plants, ABA is synthesized through the terpenoid pathway, where the carotenoid is a probable precursor of ABA biosynthesis; therefore, 6-hydroxymethyl serves as an intermediate in the conversion of carotenoids to ABA (Fernando and Schroeder 2016; Ratnayaka et al. 2018).

Table 11.3 Roles of CKs under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective conc.	Improvement/amelioration	Growth medium	References
Water and heat stress	*Triticum aestivum* L.	Exogenous (foliar application)	10 ppm	Increased photosynthetic pigments contents, chlorophyll stability index, LRWC, and growth	Soil culture	Kumari et al. (2018)
Water stress	*Arabidopsis thaliana*	Endogenous increase	20 μmol	Increased fresh and dry biomass production, LRWC, and total soluble sugars	Sand culture	Prerostova et al. (2018)
Salt stress	*Solanum lycopersicum* L.	Exogenous (foliar application)	5 μmol	Increased of photosynthetic pigments as well as amino acid content	Soil culture	Keshishian et al. (2018)
Salt stress	*Zea mays* L.	Exogenous (foliar application)	0, 75 and 100 mg/L	Increased plant height, stem diameter, ear length, row number per ear, and the biological yield	Soil culture	Davani et al. (2017)
Water stress	*Triticum aestivum* L.	Endogenous increase	20 and 70 ng/g	Activated transcription of defense-related genes in roots and leaves	Soil culture	Paul et al. (2017)
Salt stress	*Glycine max* L.	Endogenous increase	10^{-4} M	Delay of oxidative damage and increased growth	Soil culture	Moura et al. (2017)
Water stress	*Agrostis stolonifera* L.	Exogenous (foliar application)	0, 10 and 100 μmol	Improve drought stress through improved antioxidant metabolism	Sand and soil	Chang et al. (2016)
Water stress	*Arabidopsis thaliana*	Endogenous increase	0–10 μmol	Increased plant growth, the content of amino acids as well as antioxidative enzymatic activities	Soil culture	Nguyen et al. (2016)
Salt stress	*Arabidopsis thaliana*	Endogenous increase	0–4 mg/g	Increased accumulation of amino acids, proteins, activities of enzymes and of photosynthetic pigments	Soil culture	Zwack et al. (2016)
Water stress	*Hordeum vulgare*	Endogenous increase	300 μmol	Increased biosynthesis of chlorophyll, photosynthetic rate, and lignin production	Soil culture	Vojta et al. (2016)
Salt stress	*Solanum lycopersicum* L.	Exogenous (foliar application)	25 mg/L	Improved growth, physio-biochemical mechanism, and seed yield	Soil culture	Basra and Lovatt (2016)
Salt stress	*Cicer arietinum* L.	Exogenous (seed priming)	10^{-5} M	Enhanced seed germination, growth, and the levels of total N and of enzyme activities such as amylases, proteases, and phosphatases	Soil culture	Tyagi and Kumar (2016)

(continued)

Table 11.3 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective conc.	Improvement/amelioration	Growth medium	References
Heat stress	*Oryza sativa* L.	Exogenous (foliar application)	5–20 mmol	Improved nutritional quality of grains, % age of carbohydrates, proteins, macronutrients, micronutrients, total flavonoids, antioxidant activity, total amino acids, and content of essential amino acids in yielded grain	Soil culture	Wu et al. (2016a, b)
Water stress	*Solanum lycopersicum* L.	Exogenous (foliar application)	100 μL/L	Enhanced transpiration rate, stomatal activity, plant water status, and all biochemical attributes	Soil culture	Farber et al. (2016)
Water stress	*Tectona grandis* L.	Exogenous (seed priming)	0.08, 0.22, 0.35, 0.80, 2.20, or 3.50 μmol	Improved seed germination and growth, chlorophyll biosynthesis, and soluble proteins	Soil culture	Akram and Aftab (2015)
Salt stress	*Arabidopsis thaliana*	Endogenous increase	100 ppm	Increased accumulation of total free amino acid, growth, and seed yield	Soil culture	Zwack and Rashotte (2015)
Salt stress	*Arabidopsis thaliana*	Endogenous increase	10 and 100 ng/g	Increased activities of antioxidant enzymes and content of nonenzymatic antioxidative defense mechanism	Soil culture	Wang et al. (2015)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	69% increase	Improved growth, biochemical and physiological attributes, and photosynthetic rate	Soil culture	Mohammadi et al. (2015)
Salt stress	*Solanum lycopersicum* L.	Endogenous increase	0–500 μmol	Increased biosynthesis of photosynthetic pigments, flavonoids, and total soluble sugars	Soil culture	Zizkova et al. (2015)
Water stress	*Helianthus annuus* L.	Endogenous increase	15 mg/L	Positive influences on activities of enzymatic antioxidant	Soil culture	Rabert et al. (2014)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	150 μL	Increase in leaf diameter, achene weight, oil, and total yield	Soil culture	Sarafraz-Ardakani et al. (2014)
Water stress	*Zea mays* L.	Exogenous (foliar application)	50, 100, and 150 mg/L	Improved growth, yield, cob, and seed-bearing capacity	Sandy clay loam soil culture	Akter et al. (2014)

Water stress	*Oryza sativa* L.	Endogenous increase	Tenfold increase	Improved growth and development, crop seed yield	Soil culture	Murai (2014)
Light stress	*Arabidopsis thaliana*	Endogenous increase	150 ppm	Increased content of soluble protein in light-sensitive genotype and content of organic solutes	Soil culture	Cortleven et al. (2014)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	40 µmol	Improved LRWC, photosynthetic pigment accumulation, and nutrient uptake	Soil culture	Dwivedi et al. (2014)
Salt stress	*Oryza sativa* L.	Exogenous (foliar application)	205.4 mg/g	Increased growth, photosynthetic pigment contents as well as nutrients uptake	Soil culture	Rajakumar (2013)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	370 µmol/mol	Maintained the better level of Rubisco as a result normal photosynthetic or gas exchange capacity	Clay-sand soil culture	Gutierrez et al. (2013)
Water stress	*Oryza sativa* L.	Exogenous (foliar application)	0–5 mmol	Increased free amino acids accumulation, sucrose phosphate synthase activity, and net photosynthesis	Sand culture	Reguera et al. (2013)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	0, 200, 400 and 600 mg/L	Stimulated the production of photosynthetic pigments, biosynthesis of total carbohydrates, and polysaccharides	Soil culture	Sadak et al. (2013)
Heavy metal stress	*Glycine max* L.	Exogenous (foliar application)	0.7 mol/L	Reduced lipid peroxidation	Soil culture	Hashem (2013)
Salt stress	*Triticum aestivum* L.	Endogenous increase	3, 5, and 7 mmol	Increased photosynthesis, growth, and physiological processes	Petri dishes	Nishiyama et al. (2012)
Water stress	*Medicago sativa* L.	Endogenous increase	0 and 3 mmol	Improved the tolerance of alfalfa without affecting alfalfa nodulation or nitrogen fixation	Soil culture	Xu et al. (2012)
Water stress	*Glycine max* L.	Endogenous increase	0, 3, and 9 mmol	Improved all parameters that were decreased due to drought stress	Soil culture	Le et al. (2012)
Water stress	*Trifolium pratense* L.	Endogenous increase	0.5 and 1.0 mmol	Enhanced photosynthetic rate, number and diameter of flowers, cellular protein contents as well as contents of proline and protein	Soil culture	Neuberg et al. (2011)
Water stress	*Agrostis stolonifera* L	Endogenous increase	0–5 mmol	Maintained better accumulation of several metabolites, particularly amino acids, carbohydrates, and organic acids	Soil culture	Merewitz et al. (2011)

(continued)

Table 11.3 (continued)

Stress type	Plant species	Exogenous/endogenous use	Effective conc.	Improvement/amelioration	Growth medium	References
Water stress	*Oryza sativa* L.	Exogenous (foliar application)	0–550 mol/L	Increased accumulation of total free amino acid, growth parameters, and yield components	Soil culture	Peleg et al. (2011)
Water stress	*Helianthus annuus* L.	Exogenous (foliar application)	100 and 200 ppm	Increased content of nonessential amino acids such as aspartate, glutamic acid, tyrosine, and proline, but serine and alanine remain unaffected	Soil culture	Yassen et al. (2011)
Water stress	*Nicotiana tabacum* L.	Endogenous increase	0, 50, and 200 mol/g	Improved stomatal opening and faster transpiration	Soil culture	Vysotskaya et al. (2010)
Salt stress	*Solanum lycopersicum* L.	Endogenous increase	0–600 mol/g	Modified both hormonal and ionic status in shoot and improved growth and yield	Soil culture	Ghanem et al. (2010)
Water stress	*Arabidopsis thaliana*	Endogenous increase	2.5 mmol	Increased biosynthesis of photosynthetic pigments as well as amino acid content	Soil culture	Rivero et al. (2010)
Water stress	*Bryum argenteum*	Exogenous (foliar application)	0.001–10 μmol	Increased biosynthesis of chlorophyll and fresh biomass	Soil culture	Sabovljevic et al. (2010)
Water stress	*Zea mays* L.	Endogenous increase	1 mg/L	Activation of antioxidant system	Soil culture	Vasconcelos et al. (2009)
Water stress	*Arabidopsis thaliana*	Endogenous increase	300 mg/L	Better maintenance of physical association among functioning of chloroplasts, peroxisomes, and mitochondria and increased CO_2 compensation point, indicating the cytokinin-mediated occurrence of photorespiration in *Arabidopsis*	Soil culture	Rivero et al. (2009)
Water stress	*Agrostis capillaris* L.	Endogenous increase	10 mg/L	Improved growth and yield	Sand culture	Dacosta and Huang (2007)
Salt stress	*Lotus corniculatus* L.	Exogenous (seed priming)	500 mg/L	Increased percentage of seed germination, seed vigor, and nutritional quality	Petri dish experiment	Nikolic et al. (2007)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	100, 150, and 200 mg/L	Maintained better hormonal balance for adequate physiological adaptation for better growth and grain yield	Soil culture	Iqbal and Ashraf (2006)

Salt stress	*Arabidopsis thaliana*	Endogenous increase	0.01 and 1.92 µg/g	Higher level increased the photosynthetic pigment contents, seed yield, and growth	Soil culture	Riefler et al. (2006)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	100 µmol	Increased antioxidative activity of enzymes, transpiration rate, and stomatal conductance	Water culture	Veselova et al. (2003)
Water stress	*Agrostis stolonifera* L.	Endogenous increase	20–36 ppm	Improved turf quality, pigmentation, and number of seedlings, reduced oxidative damages	Sand culture	Wang et al. (2003)
Salt stress	*Sorghum bicolor* L.	Exogenous (seed priming)	50–300 ppm	Enhanced seed germination capacity, the root and shoot lengths, the production of fresh and dry matter, and LRWC	Soil culture	Ismail (2003)

ABA biosynthesis and their concentrations greatly vary dramatically in specific tissues during development or in response to changing environmental conditions. Free ABA is the biologically active form in plant tissues. The major cause of the inactivation or degradation of free ABA is oxidation or conjugation. ABA transports through the vascular tissues after biosynthesis and has been imparting significant physiological and developmental roles, especially in seed development, which are as follows. ABA content of seeds usually fluctuates during embryogenesis, is very low in early embryogenesis, reaches a maximum at about the middle of embryogenesis, and then gradually falls to low levels as the seed reaches maturity. Enhancing the desiccation tolerance in the developing seed is considered as another important property of ABA. ABA enhances the capability of developing seeds to accumulate and store large quantities of proteins during the process of embryogenesis. Similarly, under unfavorable conditions, mature seeds become dormant and fail to germinate due to specific protein biosynthesis and then control the state of dormancy in mature seeds. On the other hand, various external factors such as chilling and light release the seed from embryo dormancy, and thus the typically dormant seeds respond and start to germinate (Oritani and Kiyota 2003; Lim et al. 2015).

Under drought conditions, ABA synthesizes in the roots and is exported to the shoots and causes the stomatal closure. Another important feature of ABA had recorded to overcome abiotic stresses, i.e., it controls the growth rate of different plant parts and increases the growth rate of roots while inhibiting the shoot growth rate. ABA signaling cascade mediated by protein kinases, phosphatases, phospholipid metabolism, and cytosolic Ca^{2+} raises cytosolic PH as well as degrades the polarity of membranes that resulted from the activation of slow anion channels at the cellular and molecular levels (Lim et al. 2015).

11.2.4.1 Roles of ABA Under Different Abiotic Stress

The reports depict that ABA is a well-known stress hormone that acts as a signaling molecule and has a significant role in different abiotic stress tolerance of crop plant by imparting its significant role in the improvement of different parameters through different ways. Normally, the stress tolerance in plants is taken on the basis of improvement in growth in combination with different physio-biochemical attributes. ABA-based improvements in stress tolerance are strictly genetically controlled that is specific to plant type or stress type. The improvement in stress tolerance through its endogenous increment at cellular levels has been reported in detail at genetic levels in a number of plants under various abiotic stresses, and the stress tolerance was based on the improvement in various physiological and biochemical parameters in comparison with the alterations in the levels of other essential hormones (Table 11.4). Under salt stress, 50-folds, 2.8 nmol, and 27–50-fold increase in the levels of ABA were found effective for better growth, nutrient uptake, antioxidative defense mechanism, leaf expansion, ion homeostasis, and metabolism of *S. lycopersicum* (Gharsallah et al. 2016) and *T. aestivum* (Liu et al. 2017) respectively. Similarly, in *T. aestivum* (Bhagi et al. 2013) and *Physcomitrella patens*

Table 11.4 Roles of ABA under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Triticum aestivum* L	Exogenous (foliar application)	20 μmol	Increased biomass, ascorbate to dehydroascorbate ratio, antioxidant enzyme activities, and decreased H_2O_2 and MDA contents	Petri dish culture	Kaur and Zhawar (2018)
Salt stress	*Sesbania cannabina*	Exogenous (foliar application)	10 μmol	Improved antioxidant defense mechanism	Soil culture	Ren et al. (2018)
Water stress	*Glycine max* L.	Exogenous (seed priming)	100 and 200 μmol	Increased chlorophyll and carotenoid contents, stomatal regulation, and leaf photosynthetic rate	Soil culture	Ratnayaka et al. (2018)
Water stress	*Physcomitrella patens*	Endogenous increase	10 nmol/g	Enhanced the activities of POX, CAT and SOD, epidermal cell expansion, and transpiration	Soil culture	Vujicic et al. (2017)
Salt stress	*Triticum aestivum* L	Exogenous (seed priming)	1 mmol	Improved antioxidant defense systems to protect photosynthetic electron transport and carbon assimilation		Wang et al. (2017)
Salt stress	*Oryza sativa* L.	Exogenous (foliar application)	0.05 M	Maintained higher levels of chlorophyll and proline contents as well as CAT and APX activities	Soil culture	Kibria et al. (2017)
Water and salt stresses	*Solanum lycopersicum* L.	Endogenous increase	100 μmol	Improved seed germination, physiological parameters, growth, and yield attributes	Soil culture	Ijaz et al. (2017)
Salt and water stresses	*Arabidopsis thaliana*	Endogenous increase	150 mmol	Positively regulated the physiological responses through stomatal aperture and improved desiccation and salinity tolerance	Soil culture	Fernando and Schroeder (2016)
Salt stress	*Solanum lycopersicum* L.	Endogenous increase	2.8 mmol	Improved growth, photosynthetic pigment contents as well as nutrient uptake by decreasing salt concentration	Hydroponic culture	Gharsallah et al. (2016)
Water stress	*Pyropia orbicularis*	Exogenous (foliar application)	100 or 200 μmol	Improved plant water status, osmotic adjustments, antioxidant enzymatic activities, oxidation of biomolecules, and cellular viability	Soil culture	Guajardo et al. (2016)
Salt stress	*Lycium barbarum* L.	Exogenous (foliar application)	100 mg/mL	Improved photosynthesis, hormonal regulation, and osmotic adjustment	Soil culture	Liu et al. (2016a)

(continued)

Table 11.4 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Hordeum vulgare*	Exogenous (foliar application)	100 μmol	Enhanced the content of free polysomes, membrane-bound polysomes, cytoskeleton-bound polysomes, and cytomatrix-bound polysomes	Soil culture	Szypulska and Weidner (2016)
Water stress	*Cucumis sativus*	Endogenous increase	300 ppm	Increased in LRWC, leaf fresh, and dry biomasses and other growth parameters	Soil culture	Liu et al. (2016a, b, c)
Water stress	*Triticum aestivum* L.	Exogenous (seed priming)	10, 20, 25, and 30 μmol	Improved physiological responses such as LRWC, chlorophyll stability index, coefficient of relative inhibition and proline accumulation, and germination index	Petri dish culture	Razzaq et al. (2016)
Heavy metal stress	*Pyropia orbicularis*	Exogenous (foliar application)	0 and 10 μmol	Higher level (10 μmol) increased growth, photosynthetic pigment content, and seed yield	Sand culture	Shi et al. (2015)
Water and salt stresses	*Triticum aestivum* L	Exogenous (foliar application)	30 μmol	Improved activities of antioxidant enzymes (POD and CAT), total protein contents, and electrophoretic protein profile	Soil culture	Khan and Farzana (2014)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	30 μmol	Ameliorated the adverse effects of drought stress on CAT activity and total protein contents and improved antioxidant defense mechanism	Petri dish culture	Naz and Khan (2014)
Salt stress	*Arabidopsis thaliana*	Exogenous (foliar application)	50 μmol	Enhanced root-specific activation of the carotenoid pathway, flavonoids, and anthocyanin contents	Soil culture	Ruiz-Sola et al. (2014)
Salt stress	*Brassica napus* L.	Exogenous (foliar application)	100 μmol	Improved activities of both antioxidant defense enzymes and glyoxalase enzymes coffering salt stress tolerance	Soil culture	Hasanuzzaman et al. (2014)
Water stress	*Triticum aestivum* L.	Endogenous increase	50 mmol	Increased dry biomass, ascorbate to dehydroascorbate ratio, and antioxidant enzyme activities and decreased MDA contents	Hydroponic culture	Bhagi et al. (2013)
Salt stress	*Oryza sativa* L.	Exogenous (seed priming)	10^{-5} M	Increased growth and dry biomass, net assimilation rate, and stomatal conductance as well as reduced Na^+ concentrations in the leaf blades and sheaths	Soil culture	Gurmani et al. (2013)

Salt stress	*Fragaria ananassa*	Exogenous (foliar application)	0, 5, 10, 20, and 40 μmol	Enhanced root and shoot fresh and dry masses, activation of antioxidant defense mechanism, growth, and yield maintenance	Soil culture	Jamalian et al. (2013)
Water stress	*Oryza sativa* L.	Exogenous (foliar application)	100 μmol	Enhanced rate of photosynthesis, enzymatic and nonenzymatic antioxidant defense mechanism	Soil culture	Shi et al. (2012)
Water stress	*Calendula officinalis* L.	Exogenous (foliar application)	33 mg/L	Enhanced POD activity, content of carotenoids, and improved nutritional quality and ion homeostasis	Soil culture	Sedghi et al. (2012)
Water and salt stresses	*Agrostis stolonifera*	Exogenous (foliar application)	4 mmol	Improved physiological parameters such as turf quality, LRWC, and decreased membrane lipid peroxidation	Soil culture	Yang et al. (2012)
Salt stress	*Vitis vinifera*	Exogenous (foliar application)	5.5 mg/g	Increased total grapevine mass, with improved carbon allocation, glucose and fructose levels in berries and roots, net carbon fixation, and carbohydrates	Soil culture	Moreno et al. (2011)
Water stress	*Oryza sativa* L.	Exogenous (foliar application)	47 mmol	Mitigated the harmful effects on leaf photosynthesis through regulating stomatal opening and mesophyll area and improved the antioxidant defense mechanism	Soil culture	Chaves et al. (2009)
Water and salt stresses	*Zea mays* L.	Endogenous increase	0.1 mmol	Increased content of hydrogen peroxide, glutathione, polyethylene glycol, and lipid peroxidase	Soil culture	Kellos (2008)
Water stress	*Triticum aestivum* L	Exogenous (foliar application)	30 μmol	Improved growth, photosynthetic pigment contents as well as nutrients uptake	Soil culture	Sabeva and Nedeva (2008)
Water stress	*Helianthus annuus* L.	Exogenous (foliar application)	40 mg/L	Increased growth, nutritional quality, various yield parameters such as achene weight, achene diameter, and the number of achenes/capitulum	Soil culture	Rauf and Sadaqat (2007)
Water and salt stresses	*Lycopersicon esculentum*	Exogenous (foliar application)	10, 50, 100, 250, 500, 750, and 1000 μmol	Decreased harmful effects of salt and drought stress on secondary products and growth	Soil culture	Achuo et al. (2006)

(continued)

Table 11.4 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Citrus reticulata*	Exogenous (foliar application)	10 µmol	Enhanced leaf Cl^- concentration, ethylene production, and leaf abscission and CO_2 assimilation	Soil culture	Arbona et al. (2006)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	0.5 mmol	Increased activities of antioxidant enzymes, chlorophyll and carotenoid contents, and LRWC and improved membrane stability index, leaf area, and total biomass	Sand culture	Agarwal et al. (2005)
Salt stress	*Citrus reticulata*	Exogenous (foliar application)	10 µmol	Increased leaf diameter, other growth parameters, achene weight, oil, and yield	Sand culture	Mengual et al. (2003)
Water stress	*Arabidopsis thaliana*	Exogenous (foliar application)	100 µmol	Protection of cellular activities through the activation of enzymatic and nonenzymatic antioxidant defense mechanism	Soil culture	Xiong et al. (2002)

(Vujicic et al. 2017), the cellular levels of 50 mmol and 10 nmol/g, respectively, were found effective in the improvement of growth, antioxidative defense mechanism, epidermal cell expansion, transpiration rate, TSP, nutritional quality, and seed yield when grown under water stress. Kao (2014) reported that an endogenous increase in ABA level (24 mmol) was found in *O. sativa* plants grown under water, heavy metal, and salt stress, and this level was found effective in improving the osmoregulation, osmolyte accumulation, and ion accumulation with improved stress tolerance. Huge literature is available about the roles of ABA in abiotic stress tolerance in a variety of plant species through its exogenous application either as foliar spray or seed priming (Hasanuzzaman et al. 2014; Fernando and Schroeder 2016; Gharsallah et al. 2016; Guajardo et al. 2016). For example, exogenous application of 100 or 200 μmol of ABA (as foliar spray) was found effective for the induction of water stress tolerance in *Pyropia orbicularis* that was associated with improved plant water status, osmotic adjustments, antioxidant enzymatic activities, oxidation of biomolecules, and cellular viability (Guajardo et al. 2016). Similarly, fertigation of drought stressed *P. orbicularis* and *O. sativa* plants with ABA at 100 or 200 μmol and 100 μmol, respectively, improved the plant water status, osmotic adjustments, photosynthetic efficiency, antioxidant enzymatic activities, oxidation of biomolecules, and cellular viability (Shi et al. 2012; Guajardo et al. 2016). Furthermore, seed priming of *T. aestivum* and *O. sativa* with 1 mmol and 10^{-5} M, respectively, was found helpful in mitigating the adverse effects of salt stress by improving the growth, biomass production, and net photosynthetic rate antioxidative defense systems to protect photosynthetic electron transport and carbon assimilation with a decreased Na^+ accumulation in leaf blades and sheath (Gurmani et al. 2013; Wang et al. 2017).

11.2.5 Ethylene

Ethylene was first identified by Gane (1934) as a natural product of plant metabolism in different crop plants. In higher plants, all the plant parts are supposed to produce ethylene, although the rate of production depends on the type of tissue, the stage of development, and the environmental condition. However, the biologically active sites for the synthesis of ethylene are meristematic regions and nodal regions of young developing leaves. Amino acid methionine is a probable precursor of ethylene biosynthesis, and 1-aminocyclopropane-1-carboxylic acid (ACC) serves as an intermediate in the conversion of methionine to ethylene. The effective concentrations of ethylene for biological response usually extend the storage life of the fruit. The internal ethylene concentration in a ripe apple has been reported to be as high as 2500 μL/L. The increased regulation of ethylene biosynthesis is largely associated with increasing physiological activity of ethylene. Developmental state; environmental conditions such as flooding, chilling, temperature, or drought stresses; other plant hormone such as auxin; as well as physical and chemical injury stimulate the ethylene biosynthesis in plants. As fruits mature, the rate of ACC and ethylene biosynthesis increases ethylene biosynthesis in the abscission zone, and this process is mainly regulated by auxin (Iqbal et al. 2017a, b; Egea et al. 2018; Kim et al. 2018).

Inhibitors of ethylene synthesis as well as ethylene action had been reported to exist in plants to regulate the physiological activities of ethylene. Ethylene imparts vital and significant roles in numerous developmental and physiological responses such as it promotes the ripening of fruits and rate of leaf senescence, transports ACC from roots to shoots resulting in leaf epinasty, induces lateral cell expansion, produces hooks in dark grown seedlings, and causes the breakdown of seed and bud dormancy. Ethylene is also capable of inducing adventitious root formation in leaves, stems, flower stems, and even in roots and inducing flowering in pineapple and its relatives; therefore, it is also responsible for fruit synchronization. It was reported that bacterial two-component system, histidine kinases are closely related with ethylene receptor (Tamimi and Timko 2003; Iqbal et al. 2017a, b).

11.2.5.1 Ethylene Roles Under Abiotic Stresses

Ethylene, a natural gaseous plant hormone, has significant effects in various plant growth processes especially in fruit ripening and leaf senescence in combination with other plant hormones. Its importance has been studied, and its roles in stress tolerance have also been studied extensively through its exogenous application, but the effective cellular concentrations are plant type and stress type specific. The improvement in stress tolerance through endogenous increment of ethylene at cellular levels has been found in a number of plants under various abiotic stresses, and the stress tolerance was based on the improvement in various physiological and biochemical parameters (Table 11.5). In *G. max* (Arraes et al. 2015), *S. pennellii* (Egea et al. 2018), and *T. aestivum* (Habben et al. 2014), cellular levels of ethylene 2.5 mmol, 200 nmol/L, and 40–45 mg/g were effective in improving the growth and seed yield that was associated with their better photosynthetic rate, antioxidant defense mechanism, phenolics contents, and leaf chlorophyll pigment accumulation when grown under drought stress. Similarly, under salt stress, 35 mg/g, 200 ppb, and 0.8 ng/g levels of ethylene were found effective for better growth, nutrient uptake, and biosynthesis of photosynthetic pigments of *T. vulgaris* (Cordovilla et al. 2014), *Z. mays* (Hurst et al. 2004), and *A. thaliana* (Jiang et al. 2013), respectively. In *A. thaliana*, endogenous concentration of ethylene was increased due to different abiotic stresses that improved the plant growth, biosynthesis of photosynthetic pigments as well as the nutrients uptake and also found helpful in decreasing the Cd uptake (Zelicourt et al. 2018). Endogenous levels of ethylene 50 nmol/L and 200 ppm in *A. thaliana* (Mahdaviani et al. 2010) and *O. sativa* (Steffens 2014), respectively, have been found effective for better growth associated with their improved LRWC, TSP, TSS, and ion accumulation when grown under heavy metal stress. Furthermore, imposition of water stress increased the cellular levels of endogenous ethylene (1.5–5 mmol) in *Hevea brasiliensis* plants that resulted in better growth and yield associated with an improvement in different biochemical

Table 11.5 Roles of ethylene under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Glycine max* L.	Exogenous (foliar application)	100 μmol	Improved the efficiency of photosynthesis, protein content, and glutathione s-transferase activity	Soil culture	Kim et al. (2018)
Water stress	*Solanum pennellii*	Endogenous increase	200 nmol/L	Improved relative water contents, chlorophyll contents, total soluble sugars, phenolics, and photosynthetic pigments	Soil culture	Egea et al. (2018)
Salt stress	*Oryza sativa* L.	Endogenous increase	0.05 and 0.01 nmol/L	Increased the activities of antioxidant enzymes, accumulation of chlorophyll, ion accumulation	Petri plate experiment	Lee and Yoon (2018)
Heavy metal, water and salt s	*Arabidopsis thaliana*	Endogenous increase	4 mg/L	Increased all growth, biosynthesis of photosynthetic pigment contents as well as nutrient uptake decreasing by concentration of Cd	Soil culture	Zelicourt et al. (2018)
Water stress	*Thymus vulgaris*	Exogenous (foliar application)	75% increase	Increased photosynthetic pigments biosynthesis and photosynthetic efficiency	Soil culture	Hassan et al. (2018)
Water stress	*Saccharum officinarum*	Exogenous (seed priming)	100 ppm	Enhanced pectinase activity, growth, and yield attributes	Soil culture	Praharaj et al. (2018)
Water stress	*Poa pratensis*	Exogenous (seed priming)	150 mg/L	Increased the activities of antioxidant enzymes, LRWC, proline content, and soluble protein content	Soil culture	Zhang et al. (2018)
Water stress	*Zea mays* L.	Exogenous (foliar application)	1 mmol	Increased the accumulation of proline, activities of SOD, POD, and CAT, water status, and membrane stability	Soil culture	Yu et al. (2017)
Water stress	*Brassica napus*	Exogenous (foliar application)	200 μL/L	Increased proline and glutathione contents, activity of antioxidant enzymes and proline	Soil culture	Iqbal et al. (2017a, b)
Salt stress	*Olea europaea* L.	Exogenous (foliar application)	20 mmol	Enhanced pectinase activity, growth, and yield attributes	Soil culture	Goldental-Cohen et al. (2017)
Water stress	*Pyrus pyrifolia*	Exogenous (foliar application)	6.25, 12.5 and 25 mg/L	Improved source-sink strength associated with leaf photosynthesis, specific rate of accumulation in fruit, and strengthened cell expansion	Soil culture	Ma et al. (2016)

(continued)

Table 11.5 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Helianthus annuus* L.	Exogenous (foliar application)	0.75 mmol	Improved thickness of primary and secondary structures in the stem lodging zone, diameter of the stem lodging zone, sclerenchyma packages area, secondary xylem tissue area and yield	Sand culture	Mangieri et al. (2016)
Water stress	*Litchi chinensis*	Exogenous (foliar application)	100 ppm	Increased flowering, yield, shoot and root length, and other biochemical attributes	Soil culture	Cronje et al. (2014)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	3000 L/ha	Improved accumulation of chlorophyll content, chlorophyll fluorescence, number of spikes, spike productivity, and plant water relations	Soil culture	Baranyiova and Klem (2016)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	0.1 μmol	Increased shoot relative growth rate and physiological and various biochemical attributes	Soil culture	Valluru et al. (2016)
Water stress	*Hevea brasiliensis*	Endogenous increase	1.5 and 5 mmol	Improvement in biochemical parameters, such as low sucrose and high inorganic phosphorus contents as well as yield attributes	Soil culture	Putranto et al. (2015)
Water stress	*Glycine max* L.	Endogenous increase	2.5 mmol	Increased the activities of antioxidant enzymes and seed yield	Soil culture	Arraes et al. (2015)
Heavy metal stress	*Arabidopsis thaliana*	Endogenous increase	5 mmol	Increased photosynthetic pigment content	Soil culture	Schellingen et al. (2014)
Heavy metal stress	*Oryza sativa* L.	Endogenous increase	200 ppm	Enhanced photosynthetic rate, the number and diameter of flowers, soluble protein contents as well as contents of proline and protein	Soil culture	Steffens (2014)
Water stress	*Zea mays* L.	Exogenous (foliar application)	20 mmol	Mitigated the adverse effects of drought stress on maize seedlings by increasing the accumulation of photosynthetic pigments and photosynthetic efficiency	Soil culture	Chandiposha and Chivende (2014)
Salt stress	*Thymus vulgaris*	Endogenous increase	35 mg/g	Increased the carbon and nitrogen contents in roots and production of essential oils	Soil culture	Cordovilla et al. (2014)

Water stress	*Triticum aestivum* L.	Endogenous increase	40 and 45 mg/g	Improved growth and yield such as the number of spikes, grain yield, and 1000 grain weight	Sand culture	Habben et al. (2014)
Salt stress	*Helianthus annuus* L.	Endogenous increase	30 mmol	Improved seed germination, grain yield, the number of achenes, and various yield attributes	Soil culture	Luan et al. (2014)
Salt stress	*Arabidopsis thaliana*	Endogenous increase	0.8 ng/g	Increased activities of antioxidant enzymes and chlorophyll contents	Soil culture	Jiang et al. (2013)
Salt stress	*Spinacia oleracea* L.	Exogenous (foliar application)	10 mmol	Increased POD and CAT activities, photosynthetic efficiency, and proline content	Soil culture	Ozturk and Demir (2003)
Water stress	*Pachystroma longifolium*	Exogenous (foliar application)	600 mg/L	Increased root volume and biomass, the number of leaves, seedling height, and photosynthetic efficiency	Soil culture	Dranski et al. (2013)
Water stress	*Acacia senegal*	Exogenous (seed priming)	100 mg/L	Enhanced photosynthetic efficiency, photosynthetic pigments accumulation, and water use efficiency	Soil culture	Abib et al. (2013)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	400 mg/L	Improved grain yield per spike, 1000 grain weight, total biomass, grain yield, and harvest index	Soil culture	Akram et al. (2011)
Water stress	*Prunus dulcis*	Exogenous (foliar application)	75, 150, and 300 mg/L	Improved pistil length, nut quality, harvest date, and gum exudation	Soil culture	Grijalva-Contreras et al. (2011)
Heavy metal stress	*Arabidopsis thaliana*	Endogenous increase	50 nmol/L	Improved leaf area index, crop growth rate, plant height, relative water content, and ion accumulation	Soil culture	Mahdaviani et al. (2010)
Water stress	*Brassica napus*	Exogenous (foliar application)	100–400 μL	Improved leaf area, relative growth rate, photosynthesis, leaf nitrate reductase activity, and photosynthesis	Soil culture	Khan et al. (2008)
Water stress	*Medicago sativa*	Exogenous (foliar application)	0.15 and 0.35 mmol	Increased seed yield in terms of the number of racemes per shoot and the number of pods per raceme	Soil culture	Wenhua et al. (2008)
Water stress	*Zea mays* L.	Exogenous (foliar application)	0, 0.56, and 0.84 kg/ha	Improved leaf area index, crop growth rate, plant height, grain yield, and LRWC	Soil culture	Shekoofa and Emam (2006)

(continued)

Table 11.5 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	0, 150, and 300 g/ha	Increased growth, yield, photosynthetic efficiency, photosynthetic pigments accumulation, and water use efficiency	Soil culture	Al-Tabbal et al. (2006)
Water stress	*Saccharum officinarum*	Exogenous (foliar application)	400 mg/L	Improved root activity, differentiation of chloroplast, total photosynthetic area, leaf chlorophyll content, and activities of antioxidant enzymes	Soil culture	Li (2004)
Salt stress	*Zea mays* L.	Endogenous increase	200 ppb	Enhanced leaf photosynthetic activity, activities of antioxidant enzymes, photosynthetic pigments biosynthesis, and total soluble proteins	Sand culture	Hurst et al. (2004)

parameters, such as low sucrose and high inorganic phosphorous contents (Putranto et al. 2015). The involvement of exogenous ethylene in abiotic stress tolerance has also been reported in a number of studies that is plant species, stress type, and growth stage specific (Khan et al. 2008; Akram et al. 2011; Abib et al. 2013; Baranyiova and Klem 2016; Iqbal et al. 2017a, b). For example, exogenous application of ethylene at 0, 150, and 300 g/ha increased the growth, yield, photosynthetic efficiency, photosynthetic pigments accumulation, and water use efficiency in *T. aestivum* plants grown under water stress (Al-Tabbal et al. 2006). Goldental-Cohen et al. (2017) examined the effects of foliarly applied ethylene on different parameters of salt-stressed *Olea europaea* plants. They reported that an increase was found in pectinase activity, growth, and yield attributes in plants supplied with 20 mmol of ethylene in comparison to control ones. Treatment with 150 mg/L ethylene (as seed priming) in *Poa pratensis* plants resulted in enhanced activities of antioxidant enzymes, improved LRWC, and increased accumulation of proline and soluble proteins when grown under water shortage (Zhang et al. 2018). Biosynthesis of photosynthetic pigments and photosynthetic efficiency of water-stressed *Z. mays* plants also increased by the exogenous application (foliar spray) of ethylene when applied as foliar spray at 20 mmol, and it also helped in mitigating the adverse effects of drought stress (Chandiposha and Chivende 2014).

11.2.6 Polyamines

The presence of polyamines (crystals) was first discovered by Leeuwenhoek in 1678. These crystals were isolated by Nicolas Vauquelin in 1791, and he called these crystals as phosphate salt of inorganic cation due to their insoluble nature in water as well as ethanol. Later on, many studies have been made studying the nature of these crystals, but they remained unidentified until a century later. In 1853, a paper was published by Charcot about the crystalline nature of spermine phosphates. In 1865, Boettcher called these substances as apermartine that were made up of protein. In 1888, Landenburg and Abel called these crystals as spermine, and finally, in 1926, Otto Rosenheim explained the chemical nature of spermine and identified it as spermidine. Spermidine was also isolated from animal organs, microorganisms, and plants that later on resulted in universal polyamines (Mendez 2017). Polyamines (PAs) are the ubiquitous natural compounds with aliphatic nitrogenous structure that play a significant role in many growth and developmental processes and also act as plants growth regulator to trigger growth in various biotic and abiotic stresses. Many studies reported their role in flower initiation, bud differentiation, embryo development, and plant senescence (Chen et al. 2018a, b). It is found at all organizations of organism as in eukaryotic and prokaryotic cells, even in plant RNA viruses and tumors. It exists in various forms especially in higher plants such as putrescine, spermidine, spermine, cadaverine, and thermospermine (Kim et al. 2014; Nahar et al. 2016; Liu et al. 2017). The biosynthesis of main polyamines such as spermidine, spermine, thermospermine, and putrescine is as follows: spermidine is synthesized by catalyzing putrescine with the help of the enzyme spermidine synthase by using aminopropyl group from decarboxylated *S*-adenosyl-L-methionine (SAM). Spermidine is further catalyzed into spermine and thermospermine by the action of enzyme spermine synthase and thermospermine synthase, respectively (Takano et al. 2012).

11.2.6.1 Polyamines' Roles Under Different Abiotic Stress

Most of the literature depicts that the endogenous accumulation of PA at cellular levels helps in improving the abiotic stress tolerance in almost all types of plant species, and the stress tolerance was based on the improvement in various physiological parameters such as contents of total chlorophyll, carotenoids, total phenolic, soluble sugars, proteins, proline, and some antioxidant enzymes with an accumulation of MDA. The higher accumulation of polyamines in different plants species under different abiotic stresses explains its roles in ameliorating the adverse effects of these stresses (Table 11.6). In *Lupinus luteus* and *Theobroma cacao*, the 10–60 mg/g, 0–35 mg/g, and 37 nmol cellular levels of PA, respectively, were found effective in the improvement of growth, accumulation of N-containing compounds, amino acids, and vitamins when grown under drought stress (Juzoń et al. 2017; Niether et al. 2017). An endogenous increase of 10–60 mg/g in *O. sativa* L. was reported by Do et al. (2013) when grown under water stress, and this endogenous increase was responsible for the improvement of growth, in terms of dry biomass production and photosynthetic quantum yield. An improvement in growth, rate of photosynthesis, and contents of photosynthetic pigments, proline, total soluble sugars, and proteins in water-stressed *O. sativa* L. plants was reported through endogenous accumulation of polyamines (0–300 ng/g) as reported by Yang et al. 2007. Similarly, under salt stress, 1, 2, and 3 nmol, 0–80 nmol/g, and 0–140 mg/g levels of PA were found effective for better growth, antioxidative defense mechanism, transpiration rate, and better accumulation of photosynthetic pigments in *T. aestivum* (Liu et al. 2016a, b, c), *P. vulgaris* (Mejia et al. 2003a, b), and *O. sativa* (Yamamoto et al. 2011), respectively. Not only the endogenous increments but also the exogenous application of PA has been reported for stress tolerance induction in various crop plants, but the effective level was species specific or dependent on the type of stress (Al-Kandari et al. 2009; Ahmad et al. 2013; Barzegar et al. 2016; Montilla-Bascon et al. 2017; Akter et al. 2019). In *Gossypium barbadense* L. plants, exogenous application of polyamines significantly affected its growth and yield when grown under salt stress, and it improved the growth and fruiting braches per plant by decreasing the adverse effects of salinity when applied at concentrations of 0, 1, and 2 ppm (Ahmed et al. 2013). Exogenous application of polyamines as foliar spay (20 and 60 μmol) also improved the growth, seed quality as well as productivity of *H. vulgare* L. plants grown under water deficiency (Montilla-Bascon et al. 2017). Similarly, in drought-stressed *V. faba*, an improvement in the nutritional quality of yielded grains was found in terms of % of contents of carbohydrates, proteins, macro- and micronutrients, total flavonoids, total amino acids, essential

Table 11.6 Roles of polyamines under different abiotic stresses

Type of stress	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Zea mays* L.	Exogenous (foliar application)	20 μmol	Increased biosynthesis of photosynthetic pigments, LRWC, and amino acid accumulation and improved activities of antioxidant enzymes	Soil culture	Akter et al. (2019)
Water stress	*Vigna radiata*	Exogenous (foliar application)	0 and 0.1 mmol	Improved activities of antioxidant enzymes, membrane stability index, N, P, Ca^{2+}, Mg^{2+}, and grain yield	Soil culture	Ghassemi-Golezani and Farhangi-Abriz (2018)
Salt stress	*Zea mays* L.	Exogenous (foliar application)	0.5, 1.0, and 2.0 mmol	Improved accumulation of reducing and nonreducing sugars, TSP, accumulation of photosynthetic pigments. and total carbohydrates	Soil culture	Gul et al. (2018)
Water stress	*Vicia faba*	Exogenous (foliar application)	0.5, 1.0, and 1.5 μmol	Improved the nutritional quality of yielded grains in terms of % of carbohydrates, proteins, macronutrients, micronutrients, total flavonoids, antioxidant activity, total amino acids, and essential amino acids of grain	Soil culture	Hendawey et al. (2018)
Water stress	*Trigonella foenum-graecum* L.	Exogenous (foliar application)	100 mg/L	Enhanced accumulation of photosynthetic pigments, carbohydrate, total soluble sugars, and crude protein	Sand culture	Ahmed et al. (2018)
Water stress	*Hordeum vulgare* L.	Exogenous (foliar application)	20 and 60 μmol	Improvement in growth, seed quality, and productivity	Soil culture	Montilla-Bascon et al. (2017)
Water stress	*Lupinus luteus* L.	Endogenous increase	0–35 mg/g	Increased accumulation of nitrogen containing compounds, flavonoids, and amino acids	Soil culture	Juzoń et al. (2017)
Water stress	*Theobroma cacao* L.	Endogenous increase	37 mmol	Improved LRWC, accumulation of proteins, amino acids, and vitamins	Sand culture	Niether et al. (2017)

(continued)

Table 11.6 (continued)

Type of stress	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Zoysia japonica*	Exogenous (seed priming)	0.15, 0.30, 0.45, and 0.60 mmol	Role as an external signaling molecule to modulate root proliferation, growth, and branching	Soil culture	Li et al. (2016a, b)
Salt stress	*Triticum aestivum* L.	Endogenous increase	1, 2, and 3 mmol	Improved antioxidative defense mechanism, increased activities of antioxidant enzymes, and transpiration rate	Soil culture	Liu et al. (2016a, b, c)
Water stress	*Abelmoschus esculentus* L.	Exogenous (foliar application)	0, 0.5, 1, and 1.5 mmol	Improved fruit yield, LRWC, vitamin C content, and water use efficiency	Sand culture	Barzegar et al. (2016)
Water stress	*Agrostis stolonifera*	Exogenous (foliar application)	500 and 750 mmol	Increased photochemical efficiency, higher quantum yield, less electrolyte leakage, and lipid peroxidation in terms of MDA	Soil culture	Shukla et al. (2015)
Water stress	*Valeriana officinalis* L.	Exogenous (foliar application)	1 mmol	Improved LRWC, biosynthesis of chlorophyll a and b, quantity and quality of yield, and concentration of essential oils	Sand culture	Mustafavi et al. (2015)
Water stress	*Trifolium pratense*	Endogenous increase	20 μmol	Improved activities of enzymes and content of nonenzymatic antioxidants	Petri dish culture	Li et al. (2015)
Water stress	*Cerasus humilis*	Exogenous (foliar application)	0.2 mmol	Improved activities of antioxidant enzymes, photosynthetic parameters, and LRWC	Soil culture	Yin et al. (2014)
Water stress	*Oryza sativa* L.	Endogenous increase	10–60 mg/g	Improved growth, dry biomass production, and photosynthetic quantum yield	Soil culture	Do et al. (2013)
Salt stress	*Gossypium barbadense* L.	Exogenous (foliar application)	0, 1, and 2 ppm	Improved growth and fruiting branches per plant	Soil culture	Ahmed et al. (2013)
Water stress	*Capsicum annuum* L.	Exogenous (seed priming)	25, 50, 75, and 100 mmol	Improved growth and biomass production and seedling vigor index	Soil culture	Khan et al. (2012a)

Water stress	*Zea mays* L.	Exogenous (foliar application)	1 mmol	Improved nutritional values of the grains in terms of % carbohydrates, proteins, macronutrients, micronutrients, total flavonoids, antioxidant activity, total amino acids, and essential amino acids of grain	Soil culture	An et al. (2012)
Salt stress	*Oryza sativa* L.	Endogenous increase	0–140 mg/g	Improved physio- and biochemical responses	Soil culture	Yamamoto et al. (2011)
Water stress	*Oryza sativa* L.	Exogenous (seed priming)	10 μmol	Improved net photosynthesis, water use efficiency, leaf water status, production of free proline, anthocyanins, and soluble phenolics	Soil culture	Farooq et al. (2009)
Water and salt stress	*Conocarpus lancifolius*	Exogenous (foliar application)	1.5 and 4.5 mmol	Increased accumulation of total free amino acids, plant growth, and seed yield	Soil culture	Al-Kandari et al. (2009)
Water stress	*Oryza sativa* L.	Endogenous increase	0–300 ng/g	Improved growth, rate of photosynthesis, increased accumulation of photosynthetic pigments, proline, total soluble proteins, and total soluble sugar	Soil culture	Yang et al. (2007)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	2.5 and 5.0 mmol	Increased accumulation of total free amino acid, plant growth, and seed yield	Soil culture	Iqbal et al. (2006a, b)
Water stress	*Oryza sativa* L.	Endogenous increase	0–600 nmol/g	Activated the transcription of defense-related genes in roots and leaves. Induced blast resistance in rice	Soil culture	Capell et al. (2004)
Salt stress	*Phaseolus vulgaris*	Endogenous increase	0–80 nmol/g	Improvement in growth and physio- and biochemical attributes	Soil culture	Mejia et al. (2003a, b)
Water stress	*Vitis vinifera* L.	Endogenous increase	25–100 nmol/g	Improved accumulation of phenolics, ion accumulation, nutritional quality, growth, and fruit yield	Soil culture	Miklós and Sarjala (2002)

amino acids, and the antioxidant activity of grains when foliarly supplied with 0.5, 1.0, and 1.5 μmol of levels of polyamines (Hendawey et al. 2018). Exogenously applied polyamines as foliar spray (0, 0.5, 1, and 1.5 mmol) significantly improved the fruit yield, LRWC, vitamin C content, and the water use efficiency of *Abelmoschus esculentus* L. plants grown under water stress. Similarly, seed priming of *T. aestivum* and *Conocarpus lancifolius* with polyamines increased the accumulation of total free amino acid, plant growth, and seed yield by reducing the negative effects of salinity at a concentration of 2.5 and 5.0 mmol and 1.5 and 4.5 mmol, respectively (Iqbal et al. 2006a, b; Barzegar et al. 2016). Seed priming of *C. annum* L. seeds with different levels of polyamines (25, 50, 75, and 100 mmol) improved its growth, biomass production, and seedling vigor index by counteracting the adverse effects of water stress.

Putrescine, Spermine, and Spermidine

Among different polyamines, spermine (Spm), spermidine (Spd), and putrescine (Put) are the most important ones due to their necessary roles in different growth processes of prokaryotes and eukaryotes. They are present either free or in conjugated form with some other organic molecules such as proteins, phenolic acids, or nucleic acids. They are also involved in stimulation of DNA replication, transcription, and translation. They act as antiaging, antifungal, and antiviral agents as well as protect plants from different biotic and abiotic stresses. In plants, the first step in the biosynthesis of these major polyamines is catalyzed either with the help of the enzyme arginine decarboxylase or ornithine decarboxylase that converts arginine or ornithine, respectively, into Put. Then, Put is further converted into Spd and Spm by catalyzation, and this step is catalyzed with the enzyme Spd and Spm synthase, respectively, by transformation of aminopropyl from decarboxylated *S*-adenosylmethionine (dSAM). The function and activity of these biosynthetic enzymes are dependent on the type of tissues and growth stage (Igarashi and Kashiwagi 2010; Fazilati and Forghani 2015). These are widely distributed in almost all types of plants species, especially in actively proliferating cells where they are present in much higher levels, and also play a key role in various cellular processes, viz., translation, transcription, as well as activities of various enzymes (Takahashi and Kakehi 2009). Most of the studies confirm their involvement in cell division, formation of root, fruit ripening and its development, as well as biotic and abiotic tress tolerance. Spermidine is a polyamine that is also found in ribosomes and thus stimulates many metabolic processes as well. It is also a precursor of many polyamines such as spermine and thermospermine. A number of biological processes are synchronized by Spm such as maintenance of the membrane potential and by its pH and volume. Put is also a precursor for biosynthesis of other polyamines such as pyridine and nicotine when its level exceeds the threshold level in response to different stresses (Capell et al. 2004). It also plays a significant role in abiotic stress tolerance either applied exogenously or by endogenous accumulation (Urano et al. 2005). Spermine was first identified as crystalline structure in human semen by Antonie van Leeuwenhoek in 1678. In 1888, Ladenburg and Abel (German chemists) first used the term spermine. Ornithine is used as a precursor for its biosynthesis. It has been distributed in a number of organisms and tissues, where it acts as an essential regulator of growth and also helps in maintaining the physiological pH. It is also associated with nucleic acid and helps in stabilization of its helical structure especially in viruses.

Roles of Putrescine, Spermine, and Spermidine Under Different Abiotic Stresses

Polyamines are well known through their specific role in different stresses, but individually, putrescine also has its specific roles in stress tolerance through controlling different metabolic activities. The improvement in stress tolerance through endogenous increment of Put at cellular levels has been found in a number of plants under various abiotic stresses, but the stress tolerance mechanism was based on the improvements in various physiological parameters (Tables 11.7, 11.8, and 11.9). In *A. thaliana* grown under water stress, the improvement in chlorophyll pigment biosynthesis, antioxidant defense mechanism, and ion accumulation was recorded at 200 mg/g level of Put (Wu et al. 2016a, b). Similarly, in *Camellia sinensis*, 5 mmol level of Put was effective for maintaining better leaf photosynthetic pigments, growth, LRWC, and total soluble sugars under salt stress (Xiong et al. 2018). It has been found that exogenous application of these newly discovered polyamines can also serve as growth regulators and have been found involved in alleviating the harmful effects of different abiotic stresses, when applied as foliar spray or seed priming (Ahmed et al. 2013; Ghosh and Adak 2016; Barzegar et al. 2018). For example, priming *Z. mays* L. seeds with 0.1, 0.01, and 0.001 mmol levels of Put showed an improvement in seed germination and seedling emergence, and this improvement was more pronounced in plants raised from seeds treated with different levels of Put as compared to non-treated seeds (Hussain et al. 2013). Foliarly applied different levels of Put were found helpful in mitigating the adverse effects of salt, water, and cold stress in *Punica granatum* L. (Amri et al. 2011), *A. esculentus* L. (Barzegar et al. 2018), and *T. aestivum* L. (Cakmak and Atici 2009) plants, respectively, by improving the growth, yield, and different physio-biochemical attributes. Similarly, antioxidative defense mechanism in various drought-stressed plants such as *T. aestivum* L. (Hassan and Bano 2016), *Carthamus tinctorius* (Khosrowshahi et al. 2018), and *G. barbadense* L. (Shallan et al. 2012) was also improved by treating these plants with different levels of Put as foliar spray. An improvement in the accumulation of photosynthetic improvement (Chl *a*, Chl, *b* as well as total Chl) was recorded in *P. sativum* (Hussein et al. 2006), *Phoenix dactylifera* (Naser et al. 2016), and *Citrus karna* (Sharma et al. 2011) plants when sprayed with different levels of Put along with improved tolerance improving salt stress. Khalil et al. (2009) reported that exogenous application of Put as foliar spray (0. 1.25, 2.5 mmol) was found helpful in mitigating the harmful effects of heat stress by improving the nucleic acid contents and protein electrophoretic pattern in

Table 11.7 Roles of spermine under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
water stress	*Lactuca sativa*	Exogenous (foliar application)	0.1 mmol/L	Improved growth and physio-biochemical mechanism	Petri plate experiment	Liu et al. (2018a)
Heat stress	*Oryza sativa* L.	Exogenous (foliar application)	1 mmol/L	Improved activities of SOD and POD, photosynthetic electron transport rate, stomatal conductance, and photochemical reactions of light energy	Soil culture	Tang et al. (2018a, b)
water stress	*Eremochloa ophiuroides*	Exogenous (foliar application)	1–7 mmol	Improved LRWC, net photosynthetic rate, stomatal conductance, reduced ion leakage, and improved enzymatic and nonenzymatic antioxidative mechanism	Sand culture	Liu et al. (2017)
Salt stress	*Oryza sativa* L.	Exogenous (seed priming)	2.5 mmol	Increased activities of antioxidant enzymes, anthocyanin, tocopherol, phenolics, and photosynthetic pigments	Petri plate experiment	Paul and Roychoudhury (2016)
Salt stress	*Triticum aestivum* L.	Exogenous (seed priming)	0.3 mmol	Improved photosynthetic efficiency, photosynthetic pigment accumulation, nutrient uptake, and ion accumulation as well as total soluble sugar	Soil culture	Aldesuquy et al. (2014)
Heat and water stress	*Poncirus trifoliata* L.	Exogenous (seed priming)	1 mmol/L	Counteracted the adverse effects of abiotic stresses on orange seedlings, increased activities of antioxidant enzymes, and other biochemical attributes	Petri plate experiment	Fu et al. (2014)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	25 and 35 mg/g	Increased biomass, photosynthetic pigments accumulation, tocopherols, and total soluble sugar content	Soil culture	Abdel-Fattah et al. (2013)
Water stress	*Cymodocea nodosa*	Endogenous increase	10^{-6} M	Improved photochemical efficiency, antioxidative defense mechanism, content of total soluble sugars, phenolics, and tocopherols	Sand culture	Elso et al. (2012)

Chilling stress	*Zea mays* L.	Exogenous (seed priming)	30, 60 and 90 mg/L	Improved growth, seed germination, LRWC, chlorophyll content, and photosynthetic efficiency	Soil culture	Saeidnejad et al. (2012)
Salt stress	*Vigna sinensis*	Exogenous (seed priming)	0.3 mmol	Improved leaf chlorophyll contents, photosynthetic efficiency, growth, and yield	Soil culture	Alsokari (2011)
Salt stress	*Helianthus annuus* L.	Exogenous (seed priming)	10 mg/L	Improved growth, yield, yield components, oil yield, and levels of K^+, Ca^{2+}, P and Zn as well as K/Na ratio	Soil culture	Sakr (2010)
Water stress	*Citrus reticulata*	Exogenous (seed priming)	1 mmol	Increased activities of POD and SOD, leaf photosynthetic efficiency, and chlorophyll contents	Soil culture	Shi et al. (2010)
Water stress	*Theobroma cacao*	Endogenous increase	50, 100 and 150 nmol/g	Improved stomatal conductance, photosynthesis, PS-II efficiency, leaf water potential, and altered emission of blue-green fluorescence	Petri plate experiment	Bae et al. (2008)

Table 11.8 Roles of spermidine under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Malus domestica*	Exogenous (foliar application)	0.25 mmol	Improved growth, fruit yield, and physio-biochemical parameters	Soil culture	Sayyad-Amin et al. (2018)
Cold stress	*Eremochloa ophiuroides*	Exogenous (foliar application)	0.1 mmol/L	Increased antioxidant enzyme activities, maximum photochemical efficiency, PS-II efficiency, photochemical quenching efficiency, and net photosynthetic rate	Soil culture	Chen et al. (2018a, b)
Water stress	*Zea mays* L.	Exogenous (foliar application)	0.1 mmol/L	Improved growth, photosynthesis, chlorophyll content, and osmotic adjustment	Soil culture	Li et al. (2018)
Salt stress	*Citrus aurantifolia* L.	Exogenous (foliar application)	0.5 mmol	Increased leaf $Ca^{2+,}$ Mg^{2+}, and K^+ contents, LRWC, chlorophyll fluorescence, antioxidant enzyme activities, leaf photosynthetic rate, intercellular CO_2 concentration, stomatal conductance, and transpiration rate	Soil culture	Khoshbakht et al. (2018)
Water stress	*Zea mays* L.	Exogenous (foliar application)	0.1 mmol	Improved growth, photosynthetic pigment content, photosynthetic rate, and photochemical quenching	Soil culture	Li et al. (2018)
Water stress	*Eleusine coracana* L.	Exogenous (foliar application)	0.2 mmol	Improved biosynthesis of chlorophyll, LRWC, protein content, and growth	Soil culture	Satish et al. (2018)
Water stress	*Phyllostachys edulis*	Exogenous (foliar application)	0.01, 0.1, and 1.0 nmol/g	Increased accumulation of chlorophyll content, LRWC, and activities of antioxidant enzymes	Soil culture	Shao et al. (2018)
Heat stress	*Oryza sativa* L.	Exogenous (foliar application)	1 mmol/L	Improved the activities of SOD and POD, photosynthetic and transpiration rate, and stomatal conductance	Soil culture	Tang et al. (2018a, b)

Water stress	*Phaseolus vulgaris* L.	Exogenous (foliar application)	5 mmol	Improved LRWC, chlorophyll content, stomatal conductance, intercellular CO_2 concentration, transpiration rate, maximal quantum yield of PS-II, net photosynthetic rate, and grain yield	Soil culture	Torabian et al. (2018)
Salt stress	*Solanum lycopersicum* L.	Exogenous (seed priming)	200 μmol	Improved growth and biosynthesis of photosynthetic pigments	Petri dish experiment	Siddiqui et al. (2017)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	0.3 and 0.5 mmol	Improved LRWC, photosynthetic rate and activities of antioxidant enzymes	Soil culture	Szalai et al. (2017)
Water stress	*Solanum melongena* L.	Exogenous (foliar application)	40 and 60 nmol/g	Improved accumulation of amino acids, total soluble sugars, proteins, ion accumulation, and seed nutritional quality	Soil culture	Peng et al. (2016)
Heat stress	*Lactuca sativa* L.	Exogenous (seed priming)	0, 0.01, 0.05, 0.1, 0.15, and 0.2 mmol	Improved photochemical quenching efficiency of PS-II, CO_2 assimilation efficiency, and stomatal regulation	Soil culture	Sun et al. (2016)
Heat stress	*Pisum sativum* L.	Exogenous (foliar application)	1 mmol	Increased activities of CAT, SOD, and POD, leaf pigment accumulation, and maintained better photosynthetic rate	Hydroponic culture	Todorova et al. (2016)
Water stress	*Trifolium repens*	Exogenous (foliar application)	0.05 mmol	Improved LRWC, antioxidative defense mechanism, proline metabolism, reduced leaf senescence, and maintenance of growth	Sand culture	Li et al. (2016a, b)
Water stress	*Oryza sativa* L.	Exogenous (foliar application)	0.5 mmol	Maintained better antioxidant enzyme activities, growth, and physio-biochemical attributes	Soil culture	Liu et al. (2015)
Water stress	*Trifolium pratense*	Exogenous (foliar application)	20 μmol	Increased activities of SOD, CAT, peroxidase POD, and APX, photosynthesis, and LRWC	Sand culture	Peng et al. (2016)
Water stress	*Trifolium repens*	Exogenous (foliar application)	0.05 mmol	Improved activities of antioxidant enzyme, accumulation of organic solutes such as soluble sugars, reducing sugars, betaine, and free proline	Soil culture	Li et al. (2014a, b)

(continued)

Table 11.8 (continued)

Stress type	Plant species	Exogenous/endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Solanum lycopersicum* L.	Exogenous (foliar application)	0 and 0.25 mmol	Increased photosynthetic rate and morphological parameters, maximum efficiency of PS-II, quantum yield of linear electron flux, and coefficient of photochemical quenching	Soil culture	Hu et al. (2014)
Salt stress	*Cucumis sativus* L.	Endogenous increase	0.06 and 0.3 μmol	Improved photosynthetic pigments accumulation, protein, and enzyme activities	Soil culture	Radhakrishnan and Lee (2014)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	0.5 nmol	Improved accumulation of total phenolics, photosynthetic pigments, and protein contents	Petri dish experiment	Rahdari and Hoseini (2013)
Salt stress	*Cucumis sativus* L.	Exogenous (foliar application)	0 and 1 mmol	Improved accumulation of chlorophyll, maximum quantum efficiency, and net photosynthetic rate	Sand culture	Shu et al. (2012)
Salt stress	*Nelumbo nucifera*	Exogenous (foliar application)	1, 1.5, 2, 3, and 4 mmol/dm^3	Increased accumulation of anthocyanin content in petals, LRWC, content of photosynthetic pigments, and physiological parameters	Soil culture	Rubinowska et al. (2012)
Salt stress	*Citrus aurantifolia* L.	Exogenous (foliar application)	0.1 or 0.5 mmol	Improved growth, leaf chlorophyll content, chlorophyll fluorescence yield, and net photosynthetic rate	Soil culture	Anjum (2011)
Water stress	*Citrus aurantifolia* L.	Exogenous (foliar application)	0, 1, and 2 mmol	Improved plant biomass and yield	Soil culture	Amri and Shahsavar (2010)
Water stress	*Arabidopsis thaliana*	Endogenous increase	150 nmol/g	Increased chlorophyll contents, photosynthetic rate, and ion accumulation	Soil culture	Kasukabe et al. (2004)

Table 11.9 Roles of putrescine under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Abelmoschus esculentus* L.	Exogenous (foliar application)	0, 0.5, 1, and 2 mmol	Increased growth and yield attributes, tocopherol, total soluble sugars, and total soluble proteins	Soil culture	Barzegar et al. (2018)
Salt stress	*Camellia sinensis*	Endogenous increase	5 mmol	Improved ion accumulation, activities of antioxidant enzymes, photosynthetic pigments, and photosynthetic efficiency	Soil culture	Xiong et al. (2018)
Water stress	*Carthamus tinctorius*	Exogenous (foliar application)	0, 40 and 60 μmol	Improved LRWC, photosynthetic pigments, soluble protein, APX, CAT, POX, and SOD activities, proline, and soluble sugar contents but decreased electrolyte leakage	Soil culture	Khosrowshahi et al. (2018)
Water stress	*Thymus vulgaris* L.	Exogenous (foliar application)	0, 20, and 40 mg/L	Improved plant growth and biomass accumulation, water status, photosynthetic pigment content, free proline and soluble protein contents, activities of antioxidant enzymes with improved essential oil content	Soil culture	Mohammadi et al. (2018)
Water stress	*Citrus limon*	Exogenous (foliar application)	0, 50, 100 and 150 ppm	Improved activities of antioxidant enzymes, plant height, stem diameter, leaf number, area, fresh and dry weight, LRWC and leaf carbohydrates and protein contents	Soil culture	Shaimaa et al. (2018)
Salt stress	*Pyrus communis*	Exogenous (foliar application)	0.5, 1, and 2 mmol	Increased total soluble sugars, titratable acidity, flavor index, vitamin C, total phenol (TP), and total antioxidant activity of pear seedlings under salinity	Soil culture	Hosseini et al. (2017)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	150 ppm	Increased number of grains per spike, 1000 grain weight, total chlorophyll contents, and LRWC	Soil culture	Karimi (2016)
Salt stress	*Oryza sativa* L.	Exogenous (foliar application)	2 mmol	Improved antioxidant defense system, photosynthetic efficiency, and yield attributes	Soil culture	Ghosh and Adak (2016)

(continued)

Table 11.9 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*T. aestivum* L.	Exogenous (foliar application)	0.24 mg/L	Increase accumulation of K^+, Ca^{2+}, and Mg^{2+}, activities of antioxidant enzymes, and photosynthetic efficiency	Soil culture	Hassan and Bano (2016)
Salt stress	*Phoenix dactylifera* L.	Exogenous (foliar application)	2.5 mmol	Improved fruit quality, better biosynthesis of photosynthetic pigments, activities of antioxidant enzymes, and organic solutes	Soil culture	Naser et al. (2016)
Water stress	*Arabidopsis thaliana*	Endogenous increase	200 mg/g	Improved antioxidant defense mechanism	Soil culture	Wu et al. (2016a, b)
Cold stress	*Foeniculum vulgare*	Exogenous (seed priming)	10 and 20 ppm	Improved leaf chlorophyll contents and total soluble sugars	Soil culture	Mustafavi et al. (2015)
Water stress	*Zea mays* L.	Exogenous (foliar application)	1 and 2 mmol	Improved accumulation of photosynthetic pigments, leaf photosynthetic efficiency, 1000 grain weight, number of seed rows, ear length, and number of grain per ear	Soil culture	Zarghami et al. (2014)
Water stress	*Trachyspermum ammi*	Exogenous (foliar application)	1 ppm	Improved biosynthesis of chlorophyll b and total carotenoids, increased root length, root fresh weight, soluble sugars, and total soluble phenols	Soil culture	Zeid et al. (2014)
Salt stress	*Glycine max* L.	Exogenous (seed priming)	10 mmol/L	Improved activities of antioxidant enzymes, increased biomass production and seed yield	Soil culture	Zhang et al. (2014)
Water stress	*Zea mays* L.	Exogenous (seed priming)	0.1, 0.01, and 0.001 mmol	Increased seedling emergence, seedling vigor, leaf area, biomass, and water relation	Soil culture	Hussain et al. (2013)
Salt stress	*Gossypium barbadense* L.	Exogenous (foliar application)	0, 1, and 2 ppm	Increased morphological characters, e.g., plant height, number of leaves per plant, leaf area per plant, fruiting branches per plant, shoot fresh, and dry weight	Soil culture	Ahmed et al. (2013)
Water stress	*T. aestivum* L.	Exogenous (foliar application)	0.1 mmol	Improved photosynthetic efficiency, LRWC, and leaf chlorophyll content	Soil culture	Gupta et al. (2012)

Heat stress	*T. aestivum* L.	Exogenous (foliar application)	10 mmol	Increased content of ascorbate and tocopherol in grains, root and shoot lengths, and activities of antioxidant enzymes	Soil culture	Asthir et al. (2012)
Water stress	*Gossypium barbadense* L.	Exogenous (foliar application)	200, 400, and 600 ppm	Increased pigment content, total soluble sugars, proline content, total free amino acids, total phenols, total soluble proteins, total antioxidant capacity, and antioxidant enzyme activities	Soil culture	Shallan et al. (2012)
Water stress	*Lycopersicon esculentum*	Exogenous (foliar application)	1 mmol	Improved protein content, photosynthetic efficiency, LRWC, and ion accumulation	Soil culture	Slathia et al. (2012)
Salt stress	*Citrus karna*	Exogenous (foliar application)	0 and 50 mg/L	Improved LRWC, photosynthetic rate, and photosynthetic pigment accumulation	Soil culture	Sharma et al. (2011)
Salt stress	*Punica granatum* L.	Exogenous (foliar application)	0, 1, and 2 mmol	Improved length of stem, the length and number of the internode, and the leaf surface and biochemical attributes	Sand culture	Amri et al. (2011)
Water stress	*T. aestivum* L.	Exogenous (foliar application)	1.25 and 2.5 mmol	Increased accumulation of carbohydrate and protein in grains and better leaf total soluble sugars and photosynthetic pigments	Soil culture	Mostafa et al. (2010)
Salt stress	*Oryza sativa* L.	Exogenous (seed priming)	1 mmol	Improvement in physiological and biochemical attributes	Soil culture	Quinet et al. (2010)
Cold stress	*T. aestivum* L.	Exogenous (foliar application)	0.1, 1, and 10 mmol	Increased activities of apoplastic CAT, POD, and SOD, growth, and yield parameters	Soil culture	Cakmak and Atici (2009)
Heat stress	*T. aestivum* L.	Exogenous (foliar application)	0, 1.25, and 2.5 mmol	Improved enzyme activities, nucleic acid contents, and protein electrophoretic pattern	Soil culture	Khalil et al. (2009)
Salt stress	*Vicia faba*	Exogenous (foliar application)	0 and 1 mmol	Increased K^+ content, roots and shoots growth, and relative water content	Soil culture	Suleiman (2008)
Salt stress	*Pisum sativum* L.	Exogenous (foliar application)	25 and 50 mg/L	Improved plant height, number of leaves and branches as well as fresh and dry biomass of leaves, content of photosynthetic pigments	Soil culture	Hussein et al. (2006)
Salt stress	*Brassica juncea*	Exogenous (seed priming)	1 mmol	Better ion accumulation and improved activities of antioxidant enzymes	Soil culture	Lakra et al. (2006)

(continued)

Table 11.9 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Medicago sativa* L.	Exogenous (seed priming)	0.01 mmol	Improved accumulation of total soluble reducing and nonreducing sugars, proteins, activities of α- and β-amylases, and protease	Soil culture	Zeid and Shedeed (2006)
Cold stress	*Cicer arietinum* L.	Exogenous (foliar application)	1, 5, 10, and 15 mmol	Increased seed yield per plant, seed number per 100 pods, and individual seed weight	Soil culture	Nayyar (2005)
Salt stress	*B. juncea*	Exogenous (foliar application)	0.1 mmol/L	Improved enzymatic activities, total soluble proteins, total soluble sugars, and phenolics	Soil culture	Verma and Mishra (2005)

T. aestivum L. Among polyamines, spermine is another member that has great contribution in various cellular metabolic activities along with its role in plant stress tolerance. It has been found that the endogenous increment in cellular levels of Spm is responsible for the improvement in stress tolerance in a large number of plant species under different abiotic stresses, and the stress tolerance was associated with the improvement in various physiological parameters. For example, in *Theobroma cacao* (Bae et al. 2008) and *Cymodocea nodosa* (Elso et al. 2012), 50, 100, and 150 nmol/g and 10^{-6} M of Spm were found effective for better photochemical efficiency of PS II, in maintaining better LRWC, TSP, and phenolics content when grown under soil water-deficit conditions. Spm has also been found effective for stress tolerance induction in various crop plants, but the effective level was species specific and stress type specific (Liu et al. 2018a, b, c, d). For example, in *T. aestivum* L. plants, foliarly applied different levels of Spm (25 and 35 mg/g) were effective in reducing the adverse effects of salt stress that was associated with increased growth, biomass production, accumulation of photosynthetic pigments, and content of tocopherols as well as total soluble sugars (Abdel-Fattah et al. 2013). Another study that was conducted by Liu et al. (2017) found out the effects of foliarly applied Spm on LRWC, photosynthetic pigments, and activities of enzymatic and the non-enzymatic antioxidants in *Eremochloa ophiuroides* plants when grown under soil water-deficit conditions. They reported that foliarly applied different levels of Spm (1–7 mmol) were found effective in improving all the abovementioned attributes. Changes in different growth and physio-biochemical attributes in water-stressed *Lactuca sativa* plants supplied with Spm as foliar spray were reported by Liu et al. (2018a). An increase was found in these attributes in plants sprayed with 0.1 mmol/L level of Spm as compared with non-treated plants. Similarly, seed priming with different levels of Spm (30, 60, and 90 mg/L) showed an increase in its germination, growth, as well as photosynthetic activities in *Z. mays* plants grown under low-temperature stress. Seed priming of Spm was also found helpful in enhancing the growth, yield, and its components as well as the acquisition of different nutrients such as $Ca^{2+,}$ P, Zn, and K^+/Na^+ ratio in *H. annus* plants grown under salt stress (Sakr 2010). It was reported that this improvement also has a significant role in plant stress tolerance, but it is solely dependent on its cellular level. The endogenous increment in cellular levels of Spd was found responsible for the improvement in stress tolerance in a variety of plants grown under different abiotic stresses. In *A. thaliana* grown under water stress, the improvement in chlorophyll pigment biosynthesis and ion accumulation was reported at 150 nmol/g level of Spd (Kasukabe et al. 2004). Similarly, in *Cucumis sativus*, 0.06 and 0.3 μmol levels of improving were found effective for maintaining better leaf photosynthetic pigments, protein levels, enzyme activities, and growth under salt stress (Radhakrishnan and Lee 2014). Not only the endogenous increments but also the exogenous application of improving has been found effective for stress tolerance induction in various crop plants, but the effective level was species specific and varied with the type of stress (Sayyad-Amin et al. 2018). For example, exogenously applied of Spd as foliar spray was found effective in improving the growth, biomass production, and yield-related parameters in *Malus domestica* (Sayyad-Amin et al. 2018) and *C. aurantifolia* plants (Amri and Shahsavar 2010) when grown under soil water-deficit conditions. Similarly, 0 and 0.25 mmol levels of Spd as foliar spray improved the antioxidant enzyme activities, maximum photochemical efficiency, PS-II efficiency, photochemical quenching efficiency, and net photosynthetic rate in *S. lycopersicum* L. subjected to salt stress (Hu et al. 2014). Li et al. (2018) reported an improved growth, associated with better photosynthesis and chlorophyll content and cellular osmotic adjustment in water-stressed *Z. mays* plants when supplied foliarly with 0.1 mmol/L of Spd. The effects of exogenous application of Spd as a foliar spray on nutrient question, water relation as well as improvement in photosynthetic efficiency of *C. aurantifolia* plants when grown under soil water stress condition were reported by Khoshbakht et al. (2018). They found that an improvement was recorded in all these parameters by applying when supplied with 0.5 mmol level of Spd both under control and stressful conditions. Furthermore, *L. sativa* seeds treated with Spd showed an improvement in photochemical quenching efficiency of PS-II, CO_2 assimilation, and stomatal regulation when subjected to heat stress (Sun et al. 2016).

11.2.7 Salicylic Acid (SA)

SA, an important plant hormone phenolic in nature, is synthesized in different parts of plants in variable amount. Large amount of SA is found in the leaves and bark of willow trees that has a wide use in pain relief medicines from ancient times. In 1828, Buchner, a German scientist, purified SA derivative named “salicin” from willow bark. After 10 years of the discovery in 1838, Raffaele Piria converted salicin into aromatic compound named salicylic acid. It was chemically synthesized by Kolbe–Schmitt reaction in 1859, with some side effects with a bitter taste that limited its long-term use as a medicine. Afterward in 1897, Felix Hoffmann synthesized acetyl salicylic acid with reduced side effects, and he called it aspirin. It has been found to be involved in a variety of functions and serve as plant growth regulator. Salicylic acid also a potential signaling molecule found largely in plants. However, in plants SA is synthesized from phenylalanine by two different pathways such as shikimate-phenylpropanoid and isochorismate pathway, catalyzed by phenylalanine ammonia lyase (Chen et al. 2009). Chorismate (shikimic acid pathway derivative) is a probable precursor in both these pathways. In the first pathway, phenylalanine ammonia lyase (PAL) converts isochorismate into cinnamic acid which is then converted into ortho-courmarate or benzoic acid by hydroxylation or oxidation, respectively. In the final step, ortho-courmarate oxidizes, while benzoic acid hydroxylates to yield SA (Metraux 2002). The second pathway requires isochorismate synthase (ICS) to catalyze the chorismate into isochorismate, which is then converted to SA when metabolized with the enzyme isochorismate pyruvate lyase (IPL) (Serino et al. 1995). Salicylic acid bioassay depicted that it occurs in both free and bound forms in plants, but the biologically active form of SA is the free form. The effective concentrations of SA in plants are usually 10–100 μM that improve the plant water relations and other morphological attributes. It was reported that free SA is the biologically active form that is capable of generating a wide range of metabolic responses and usually metabolizes and transforms into several conjugates such as SA O-β-glucoside (SAG) that transports from the cytosol into the vacuole to minimize the toxicity level in plants through its conversion back to SA. In 1979, the roles of SA in plant defense response were reported by White (1979). He reported that tobacco plants treated with aspirin increased their resistance against tobacco mosaic virus. SA also plays significant physiological, biological, and developmental roles in growth regulation, seed germination, leaf senescence, fruit yield, flowering time, photosynthesis, chlorophyll biosynthesis and proline accumulation, stomatal conductance, nutrient uptake, and plant water relations, plays role in enhancing the activities of antioxidant enzymes and glyoxalase pathway enzymes, as well as improves the mechanisms of plant related to the improvement of tolerance to abiotic stresses (Vlot et al. 2009). Several studies revealed that SA imparts its vital roles in modulating plant response to various abiotic stresses such as drought, salinity, temperature, and heavy metal stress (Vlot et al. 2009; Khan et al. 2015a, b).

11.2.7.1 Roles of SA Under Different Abiotic Stresses

Along with other plant hormones, SA is also found to be important for the improvement of stress tolerance in plants in parallel with its roles in animals. Salicylic acid-induced improvements in plant stress tolerance have been recorded at its higher cellular levels (Table 11.10). The stress tolerance was based on its role in the improvement in various physio-biochemical parameters. For example, in *P. tremula*, 7 and 8 mg/g cellular levels of SA were found effective for better plant growth, for improved content of amino acids, and for better stomatal conductance when grown in saline soil (Xue et al. 2013). Not only the endogenous increments but also the exogenous application of SA has been found effective for stress tolerance induction in various crop plants, but the effective level was species specific or dependent on the type of stress (Abbadi et al. 2015; Bahari et al. 2015; Aldesuquy and Ghanem 2015; Abbaspour and Babaee 2017; Anaya et al. 2017). It was reported that foliarly applied different levels of SA were associated with better seed germination, plant growth, and improved antioxidant capacity and yield increments in *C. arietinum* plants when grown under water-deficit conditions (Boukraa et al. 2017). In *Salvia nemorosa* (Habibi 2017), *H. vulgare* (Hafez and Seleiman 2017), and *C. arietinum* (Hayat et al. 2012a, b), foliar spray of SA improved growth in terms of biomass production as well as final yield when applied at 50 μmol, 0.2 mmol, 10–4, 10–5, and 10–6 mol, respectively, and helped the plants to survive better under limited water supply. Similarly, exogenously applied different levels of SA have also been found involved in enhancing the photosynthetic efficiency of *H. vulgare* (Hafez and Seleiman 2017), *C. arietinum* (Bhutali and Lal 2017), and *O. basilicum* (Karalija and Parić 2018) plants both under normal and stressful environmental conditions. Changes in the activities of different antioxidant enzymes and content of

Table 11.10 Roles of SA under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Hordeum vulgare* L.	Exogenous (seed priming)	0.5 mmol	Increased plant growth, content of amino acids, as well as the enzymatic antioxidant activities	Soil culture	Torun (2019)
Cold stress	*Phaseolus vulgaris* L.	Exogenous (foliar application)	0 and 3 mmol	Increased growth, photosynthetic pigment, as well as nutrient uptake	Soil culture	Soliman et al. (2018)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	0.25 and 0.5 mmol	Improved growth, carotenoid and soluble protein content, physiological and biochemical parameters	Soil culture	Suhaib et al. (2018)
Water stress	*Ocimum basilicum* L.	Exogenous (foliar application)	1 mmol	Improvement in physio-biochemical parameters such as leaf photosynthetic pigments content, total phenolics, flavonoids, flavanols, and protein contents and POD activity	Soil culture	Paric and Karalija (2018)
Water stress	*Lactuca sativa*	Exogenous (foliar application)	1 and 3 mmol	Increased plant height, plant biomass, LRWC, rate of transpiration, and evaporation	Soil culture	Bankole et al. (2018)
Salt stress	*T. aestivum* L.	Exogenous (foliar application)	100 μmol	Improved germination parameters such as germination %, vigor index and mean germination time, seedling height, total chlorophyll, and total carbohydrate contents	Petri dish culture	Alamri et al. (2018)
Water stress	*Scutellaria baicalensis*	Exogenous (foliar application)	70, 140, and 280 μmol	Increased the plant growth, the content of amino acids, as well as antioxidant enzymatic activities	Soil culture	Su et al. (2018)
Water stress	*Carthamus tinctorius*	Exogenous (seed priming)	0, 400, 800, 1200, 1600, 2000, and 2400 μmol	Enhanced photosynthetic rate, chlorophyll biosynthesis, LRWC, and carboxylation efficiency	Soil culture	Mohammadi et al. (2017)
Water stress	*Sesamum indicum* L.	Exogenous (foliar application)	0 and 0.6 mmol	Improved photosynthetic rate, stomatal conductance, leaf area index, total chlorophyll content, quantum efficiency of PS-II, plant dry matter, and seed yield	Soil culture	Najafabadi and Ehsanzadeh (2017a, b)
Water stress	*Vitis vinifera* L.	Exogenous (foliar application)	0, 1, and 2 mmol	Enhanced carotenoid contents and activities of CAT, APX, and GPX	Soil culture	Abbaspour and Babaee (2017)

Water stress	*T. aestivum* L.	Exogenous (seed priming)	20 mmol	Reduced lipid peroxidation and improved antioxidant defense system and total soluble proteins	Soil culture	Aldesuquy and Ghanem (2015)
Salt stress	*Vicia faba* L.	Exogenous (seed priming)	0, 0.5, and 1.0 mmol	Increased the leaf chlorophyll and carotenoid contents, total phenolic content, soluble sugar, TSP, and proline	Soil culture	Anaya et al. (2017)
Water stress	*Cicer arietinum* L.	Exogenous (foliar application)	0.5, 1.0, 1.5, and 2.0 mmol	Increased leaf chlorophyll and carotenoid contents, LRWC, harvest index, activities of antioxidant enzymes, seed yield, and biomass	Soil culture	Bhutali and Lal (2017)
Water stress	*C. arietinum* L.	Exogenous (foliar application)	0.05 and 0.5 mmol	Improved the seed germination, plant growth, antioxidant capacity, and yield attributes	Soil culture	Boukraa et al. (2017)
Salt stress	*Rosmarinus officinalis* L.	Exogenous (foliar application)	0, 100, 200, and 300 ppm	Increased leaf total phenolics, chlorophyll, carbohydrates, and proline contents with a decline in Na^+ and Cl^-	Soil culture	El-Esawi et al. (2017)
Water stress	*Salvia nemorosa*	Exogenous (foliar application)	50 μmol	Improved plant growth rate, LRWC, quantum yield of PS-II through the improvement of reaction centers	Soil culture	Habibi (2017)
Water stress	*H. vulgare* L.	Exogenous (foliar application)	0.2 mmol/L	Improved growth, LRWC, leaf area, chlorophyll content, seed yield, and antioxidant enzymes activity	Soil culture	Hafez and Seleiman (2017)
Water stress	*O. basilicum* L.	Exogenous (foliar application)	0, 0.01, 0.1, and 1.0 mmol	Improved growth, photosynthetic pigments, protein, proline, total carbohydrates, and secondary metabolite contents as well as POD activity	Soil culture	Karalija and Parić (2018)
Water stress	*T. aestivum* L.	Exogenous (foliar application)	1.44 and 2.88 mmol	Improved physiological, yield, and growth attributes	Soil culture	Kareem et al. (2017)
Water stress	*T. aestivum* L.	Exogenous (seed priming)	0.2 mmol	Improved cell division, leaf size, stem elongation and root proliferation, and disturbed stomatal oscillations, and plant water and nutrient relations	Soil culture	Nikju (2017)
Heat stress	*Solanum lycopersicum*	Exogenous (foliar application)	0 and 2 mmol	Higher level increased photosynthetic pigments, growth, and seed yield	Soil culture	Shaheen et al. (2014)
Water stress	*H. vulgare* L.	Exogenous (seed priming)	2, 4, and 6 mmol	Improved stress tolerance	Soil culture	Shahnawaz and Sanadhya (2017)

(continued)

Table 11.10 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Pisum sativum* L.	Exogenous (seed priming)	1 mmol	Improved antioxidant defense system, growth rate, dry biomass, photosynthetic pigments, and alteration in nutritional contents	Petri-dishes culture	Ahmad et al. (2017a)
Water stress	*P. sativum* L.	Exogenous (foliar application)	100 ppm	Improved growth, biosynthesis of photosynthetic pigments, yield parameters, and carbohydrates	Soil culture	El-Samad et al. (2018)
Water stress	*Sesamum indicum* L.	Exogenous (foliar application)	0 and 0.6 mmol	Improved leaf water potential, LRWC, seeds/capsule, capsules/plant, 1000 seed weight and seed yield, and oil content	Soil culture	Najafabadi and Ehsanzadeh (2017a, b)
Water stress	*T. aestivum* L.	Exogenous (foliar application)	500 μmol	Improved plant height, grain yield, chlorophyll content, LRWC, and activities of SOD, POD, and CAT	Soil culture	Yavas and Unay (2016)
Water stress	*Coriandrum sativum* L.	Exogenous (foliar application)	0 and 1 mmol	Improved leaf area, chlorophyll content, fresh weight of root and leaf, and grain yield	Soil culture	Yeganehpoor et al. (2016)
Water stress	*Lippia citriodora* L.	Exogenous (foliar application)	300 mg/L	Increased contents of sugar, proline, and the activity of antioxidant enzymes such as SOD, CAT, and POD	Soil culture	Dianat et al. (2016)
Salt stress	*Olea europaea*	Exogenous (foliar application)	0.25 mmol	Increased chlorophyll index, growth photosynthesis, stomatal conductance, and transpiration rate	Hydroponic culture	Aliniaeifard et al. (2016)
Water stress	*Thymus daenensis*	Exogenous (foliar application)	1 and 2 mmol	Mitigated the adverse effects of drought stress through increasing the activities of antioxidant enzymes	Soil culture	Bahari et al. (2015)
Water stress	*T. aestivum* L.	Exogenous (foliar application)	0.1 mmol	Improved photosynthesis and leaf antioxidant defense mechanism	Soil culture	Abbadi et al. (2015)
Water stress	*Brassica napus* L.	Exogenous (foliar application)	0.1 mmol	Ameliorated the adverse effects of drought stress on growth, antioxidant capacity, and reduced lipid peroxidation	Soil culture	Habibi (2015)
Water stress	*T. aestivum* L.	Exogenous (foliar application)	100 ppm	Increased membrane stability, carbohydrate, soluble protein, proline, activities of antioxidant enzymes, plant height, yield attributes, LRWC, and electrolyte leakage	Soil culture	Sofy (2015)

Salt stress	*S. lycopersicum*	Exogenous (foliar application)	10^{-4} and 10^{-7} M	Increased amino acid contents such as aspartate, glycine betaine, proline, and glutamine	Soil culture	Tari et al. (2015)
Water stress	*Sorghum bicolor* L.	Exogenous (foliar application)	1 mmol	Improved root length, shoot length, fresh and dry biomasses, plant leaf area, and photosynthetic pigment	Soil culture	Arivalagan and Somasundaram (2015)
Water stress	*Fragaria ananassa*	Exogenous (foliar application)	0 and 0.1 mmol	Improved leaf area and biomass production, LRWC, stomatal conductance, yield, and fruit weight	Soil culture	Ghaderi et al. (2015)
Water stress	Lolium perenne	Exogenous (foliar application)	0.0, 0.75, and 1.5 mmol	Increased content of chlorophyll a and b, proline accumulation, antioxidant enzyme activity, and reduced electrolyte leakage	Soil culture	Hosseini et al. (2015)
Salt stress	*B. juncea* L.	Exogenous (foliar application)	0.5 mmol	Increase in leaf sulfur content, photosynthesis, growth, and activities of antioxidant enzymes	Soil culture	Nazar et al. (2015a, b)
Water stress	*Helianthus annuus* L.	Exogenous (foliar application)	0, 0.375, 0.75, and 1.5 mmol	Increased protein synthesis, total soluble sugars, and tocopherol	Soil culture	Zaidi et al. (2015)
Water stress	*Foeniculum vulgare*	Exogenous (foliar application)	0, 0.5, and 1.0 mmol	Improved root length, root dry mass, root length and density, shoot dry mass, and grain yield and components	Soil culture	Askari and Ehsanzadeh (2015)
Salt stress	*Moringa oleifera*	Exogenous (seed priming)	1 mmol	Improved growth, number of green pods, and dry seed yields	Soil culture	Rady et al. (2015)
Water stress	*Plantago ovate*	Exogenous (foliar application)	0, 0.01, 0.5, and 1.0 mmol	Improved growth yield and yield components	Soil culture	Shekofteh et al. (2015)
Salt stress	*Torreya grandis*	Exogenous (foliar application)	0.5 mmol	Increased leaf chlorophyll content, RWC, CO_2 assimilation rates, proline content, and the activities of SOD, CAT, and POD	Soil culture	Li et al. (2014a, b)
Water stress	*V. vinifera* L.	Exogenous (foliar application)	0, 1, and 2 mmol	Improved leaf area, LRWC, transpiration rate, stomatal conductance, and net photosynthetic rate	Soil culture	LovinBabaei et al. (2014)
Salt stress	*Trigonella foenum-graecum* L.	Exogenous (foliar application)	0 and 100 mg/L	Improved biomass production, photosynthesis, and leaf chlorophyll contents	Sand culture	Babar et al. (2014)
Water stress	*H. vulgare* L.	Exogenous (foliar application)	50 μmol	Increased biomasses, leaf photosynthetic pigments, K^+ contents, proline, and soluble carbohydrates	Soil culture	Fayez and Bazaid (2014)

(continued)

Table 11.10 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*T. aestivum* L.	Exogenous (foliar application)	2.5 mmol	Improved chlorophyll content, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature, total soluble sugars, and proline	Soil culture	Lakzayi et al. (2014)
Water stress	*Matricaria chamomilla* L.	Exogenous (foliar application)	0.0 and 0.5 mmol	Increment in terpenoid and flavonoid contents, membrane integrity, antioxidant activity, proline and soluble sugars, and reduced electrolyte leakage	Soil culture	Nazarli et al. (2014)
Water stress	*Capsicum annuum*	Exogenous (seed priming)	0.25, 0.50, 1.00, and 2.00 mmol	Increased content of soluble protein and tocopherols, nutrient uptake, and content of organic solutes	Soil culture	Prabha and Kumar (2014)
Water stress	*Nigella sativa*	Exogenous (foliar application)	0, 5, and 10 μmol	Improved photosynthetic pigments biosynthesis, contents of polyphenol, anthocyanin, flavonoids, water soluble sugar and proteins, and activities of phenylalanine ammonia-lyase and lipoxygenase	Hydroponic culture	Kabiri et al. (2014)
Water stress	*O. basilicum* L.	Exogenous (foliar application)	0.0, 0.75, and 1.5 mmol	Improvement in morphological, physiological, and biochemical attributes such as plant height, fresh and dry biomasses, LRWC, and chlorophyll content	Sand culture	Kordi et al. (2013)
Water stress	*T. aestivum* L.	Exogenous (seed priming)	0.5 and 1 mmol	Increased activities of the SOD, CAT, and glutathione peroxidase, improved growth and grain yield	Soil culture	Sajedi and Boojar (2013)
Water stress	*B. juncea* L.	Exogenous (foliar application)	50 μmol	Improved the LRWC, chlorophyll content, activities of antioxidant enzymes, and proline content	Soil culture	Alam et al. (2013)
Water stress	*Zea mays* L.	Exogenous (seed priming)	1 μmol	Increased the activities of antioxidant enzymes, H_2O_2, and content of osmoprotectants	Sand culture	Demiralay et al. (2013)
Salt stress	*Z. mays* L.	Exogenous (foliar application)	200 ppm	Improved growth fresh and dry biomass, leaf area, chlorophylls *a* and *b*, total chlorophyll content, and chlorophyll stability index	Soil culture	Ismail (2013)

Heavy metal	*T. aestivum* L.	Exogenous (foliar application)	0.5 mmol	Improved photosynthetic pigments and better maintenance of nutrients homeostasis in wheat seedlings under heavy metal stress	Soil culture	Siddiqui et al. (2013)
Salt stress	*Populus tremula*	Endogenous increase	7 and 8 mg/g	Increased plant growth and the content of amino acids as well as enzymatic activities	Soil culture	Xue et al. (2013)
Water stress	*Z. mays* L.	Exogenous (foliar application)	0, 0.5, 1.0, and 1.5 mmol	Improved kernel yield, rows per ear, kernel number per row, cob diameter, and ear length	Soil culture	Zamaninejad et al. (2013)
Water stress	*T. aestivum* L.	Exogenous (seed priming)	1 mmol	Increased carotenoid content, antioxidant enzymes activities, growth, content of photosynthetic pigments, carbohydrates, nitrogenous constituents, and minerals	Soil culture	Abdelkader et al. (2012)
Water stress	*Z. mays* L.	Exogenous (foliar application)	100, 150, and 200 ppm	Improved LRWC leaf membrane stability index, chlorophyll biosynthesis, and potassium accumulation	Soil culture	Rao et al. (2012)
Water stress	*T. aestivum* L.	Exogenous (foliar application)	0, 0.7, 1.2, and 2,7 nmol	Improved grain yield, number of spikes, number of grain per spike, biological yield, and harvest index	Soil culture	Sharafizad et al. (2012)
Water stress	*H. vulgare* L.	Exogenous (foliar application)	500 μmol	Increased activities of antioxidant defense system, leaf photosynthesis, and yield under drought stress	Soil culture	Habibi (2012)
Water stress	*C. arietinum* L.	Exogenous (foliar application)	10^{-4}, 10^{-5}, and 10^{-6} mol/L	Increased growth, dry biomasses, and activities of antioxidant enzymes	Soil culture	Hayat et al. (2012a, b)
Water stress	*Plectranthus tenuiflorus*	Exogenous (foliar application)	0.05 and 0.5 mmol	Improved dry biomass, relative growth rate, photosynthetic pigments, and essential oil content	Soil culture	Jalal et al. (2012)
Salt stress	*H. annuus* L.	Exogenous (foliar application)	100, 200, and 300 mg/L	Improved growth, photosynthetic pigments (chlorophyll *a* and *b*), water relation, and accumulation of proline and minerals	Soil culture	Noreen et al. (2012)
Water stress	*Z. mays* L.	Exogenous (foliar application)	10^{-6} M	Increased antioxidant defense mechanism, prevented water loss, and delayed leaf rolling	Soil culture	Kadioglu et al. (2011)
Water stress	*Satureja hortensis*	Exogenous (seed priming)	0, 1, and 3 mM	Increased content of photosynthetic pigments as well as content of amino acid content	Soil culture	Yazdanpanah et al. (2011)
Salt stress	*Z. mays* L.	Exogenous (foliar application)	250 mg/L	Increased content of photosynthetic pigments and amino acids	Soil culture	Sakr (2010)

(continued)

Table 11.10 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Vigna radiate*	Exogenous (seed priming)	10^{-5} M	Increased the activities of antioxidant enzymes, proline, membrane stability index, photosynthetic rate, and chlorophyll contents	Soil culture	Hayat et al. (2012a, b)
Heat stress	*V. vinifera* L.	Exogenous (foliar application)	0.2 and 0.4 mmol	Improved photosynthetic efficiency PS-II electron transport, energy dissipation, and Rubisco activation state	Soil culture	Wang et al. (2010a, b)
Water stress	*Arabidopsis thaliana*	Exogenous (foliar application)	200–1200 ng/g	Increased amino acid contents such as aspartate, glycine betaine, proline, and glutamine	Soil culture	Bechtold et al. (2010)
Salt stress	*Helianthus annuus* L.	Exogenous (foliar application)	0, 100, 200, and 300 mg/L	Enhanced activities of antioxidant enzymes such as SOD, CAT, and POD, photosynthetic activity, and growth	Soil culture	Noreen et al. (2009)
Heavy metal	*Z. mays* L.	Exogenous (seed priming)	500 μmol	Increased activities of APX and SOD, improvement in net photosynthesis, seed nutritional quality, and seed yield	Soil culture	Krantev et al. (2008)
Salt stress	*H. annuus* L.	Exogenous (foliar application)	0, 100, 200, and 300 mg/L	Improvement in growth, photosynthetic pigments biosynthesis, and other physiological parameters	Soil culture	Noreen and Ashraf (2008)
Water stress	*T. aestivum* L.	Exogenous (seed priming)	0, 5 and 10 mg/L	Increased stomatal conductance, transpiration rate, N and P contents of shoot and root	Petri dish culture	Waseem et al. (2006)

non-enzymatic antioxidants of water-stressed *Vitis vinifera* and *B. juncea* plants found by the foliar application of SA. An improvement was recorded in leaf carotenoid content, LRWC, leaf chlorophyll content, as well as the activities of CAT, APX, and GPX in plants supplied with SA as compared to non-treated ones (Abbaspour and Babaee 2017; Alam et al. 2013). Along with the foliar spray, seed priming with different levels of SA was also found helpful in improving the different growth, physio-biochemical, and yield attributes in a variety of crop plants such as *T. aestivum* (Sajedi and Boojar 2013), *V. faba* (Anaya et al. 2017), and *H. vulgare* (Torun 2019).

11.2.8 Jasmonic Acid (JA)

Jasmonic acid, a naturally occurring well-known plant growth regulator, derived from the oxidation of fatty acids, plays an active role to modify and maintain the growth and development of higher plants in response to environmental conditions. It has been proposed that JA and its derivatives are capable of to switch on a variety of genes which can regulate the biosynthesis of vegetative storage proteins, phosphatases, lipoxygenase, ethylene-forming enzymes, large subunit of ribulose bisphosphate carboxylase, proteinase inhibitor II, thionin (an antifungal protein), osmotin antifungal protein, chalcone synthase, phenylalanine ammonia lyase, and hydroxymethylglutaryl CoA reductase as well as tuber formation in potato. The levels of JA fluctuate greatly in different organs of the same plant depending upon the function of tissue type, developmental stage, and external conditions (Avanci et al. 2010). The meristematic tissues, flowers, and reproductive tissues are recognized as with the highest levels of JA, while roots and mature leaves are with the lowest levels of JA (Tavallali and Karimi 2019). The effective concentrations of JA were less than 10 μM recorded in different plant parts. However, the concentrations above 50 μM were found extremely toxic for plant organs to perform their vital functions (Yastreb et al. 2018).

In 1983, JA biosynthesis in plants was first described by Zimmerman in *V. faba* who reported that JA is a signaling molecule, produced from the oxidation of linolenic acid by the action of lipoxygenase. JA can impart its significant morphological and biochemical roles in the improvement of tolerance to different abiotic stresses by inhibiting the stomatal opening and improvement in cell division, plant growth, and photosynthetic activities. It was reported that JA biosynthesis is very complicated and that it is a rapid procedure, catalyzed by a variety of enzymes such as LOX, AOS, and AOC that are found abundantly in leaves and become active in response to external stimulus (Wasternack and Hause 2013; Wasternack and Song 2016). Jasmonic acid is also found to be involved in the formation of a variety of metabolites through a series of modifications in plants. It was reported that signaling cascade of JA involves the repressor, operator, and promoter resulting in the activation of JA genes responsible for metabolite formation. Jasmonic acid has been found playing various physiological roles in plants. It is involved in the regulation process of seed germination through increasing the α-amylase activity as well as in inhibiting the production of ethylene in mature seeds. It is involved in the development of germinated seeds and a number of flowers per plant at maturity. Studies illustrated that free JA is also involved in the root growth inhibition, lateral root and adventitious root formation, and tuber formation in potato and regulates the trichome development. Jasmonic acid also play its roles in increasing some secondary metabolite such as increased accumulation of total phenolic and flavonoids controlled regulates the photosynthetic rate, abscission and senescence (Avanci et al. 2010; Wasternack and Hause 2013).

11.2.8.1 Jasmonic Acid Roles Under Different Abiotic Stress

Jasmonic acid is a hormone of importance due to its specific role in controlling different abiotic stresses, but it also plays a significant role in the induction of abiotic stresses by acting as a signaling molecule and by controlling cellular metabolic activities. It improves the plant stress tolerance through its endogenous increment based on the improvement in various physiological parameters such as plant water relations and fresh biomass production (Attaran et al. 2014; Fugate et al. 2018). In *B. napus* grown under heavy metal stress in soil, the improvement in growth, glyoxalase system, chlorophyll pigment biosynthesis, and ascorbate glutathione was recorded at 10 and 100 μmol levels of JA (Shahid et al. 2015). Similarly, in *A. thaliana*, 0–24 mg/g level of JA was effective for better leaf photosynthetic pigments, cellular osmoregulation, and ion accumulation when grown under soil water stress (Attaran et al. 2014). In *B. napus* and *Ipomoea batatas*, 0, 6, 9, and 12 M and 10 nmol levels, respectively, were found effective for improving the antioxidant defense mechanism and in maintaining the proper LRWC and TSP contents grown in saline soil (Kaur and Sirhindi 2015; Zhang et al. 2017). Kim et al. (2009) reported that an endogenous increase (20 mmol) in JA level was found in *O. sativa* plants grown under different abiotic stresses (water, salt, and heavy metal stress) that were associated with enhanced seed germination, growth, and biosynthesis of photosynthetic pigments. It also plays a significant role in triggering a number of physiological, biochemical, and morphological processes (Abdelgawad et al. 2014; Alam et al. 2014; Hassini et al. 2017; Mahmud et al. 2017; Ahmadi et al. 2018; Luo et al. 2018; Abdi et al. 2019). For example, treatment with 50 μmol level of JA for *Z. mays* seeds was found effective to increase the accumulation of pigments, total carbohydrates, total soluble sugars, polysaccharides, as well as contents of free amino acids, proline, and TSP and with an improvement in plant growth parameters such as plant height and fresh and dry biomasses with a mitigation in adverse effects of drought stress (Abdelgawad et al. 2014). Seed priming with different levels of JA also plays a significant role in tolerating different abiotic stresses such as drought, salt, and heavy metal stress in *Z. mays*, *Matricaria chamomilla*, and *G. max* plants, respectively. It is also involved positively in stimulating germination, growth, and different physio-biochemical processes (Mir et al. 2018; Salimi et al. 2016; Sirhindi et al.

2016). Not only the exogenous application (as seed priming) of JA but its foliar spray is also found responsible for the induction of tolerance to abiotic stresses (Mir et al. 2018; Abdi et al. 2019). Exogenous application of JA as foliar spray was associated with increased content of flavonoid and total phenolic as well as the antioxidant capacity in *Mentha piperita* plants subjected to drought stress (Abdi et al. 2019). Another study was conducted on *B. napus* plants grown in Petri dishes under PEG-induced water stress to find out the effect of foliarly applied JA on seedling biomass production, leaf chlorophyll content, LRWC, and proline. An increase was found in all the abovementioned parameters by foliarly applied 0.5 mmol of JA that were reduced by severe effects of drought stress (Alam et al. 2014). An improvement in leaf photosynthetic rate, activities of APX, SOD, and POX, root and shoot dry mass accumulation, as well as cell membrane integrity were also found to be increased in salt-stressed *Prunus dulcis* plants treated with foliarly applied 0, 0.025, 0.050, and 0.075 mmol levels of JA (Tavallali and Karimi 2019) (Table 11.11).

11.2.9 Triacontanol

Triacontanol (TRIA) is a natural growth-regulating hormone that is found especially in epicuticular waxes and positively influences many physiological and biochemical processes in plants such as germination, growth, photosynthesis, synthesis of protein, water and nutrient uptake, activities of various enzymes, free amino acids, reducing sugars, and soluble protein contents. It has been reported as an important component of essential oil in various crops and can also be used to increase the crop production (Naeem et al. 2012). A number of studies have been reported about its roles in improving the genetic potential of plant tress tolerance (Naeem et al. 2012). It has been reported that exogenously applied TRIA positively stimulated the growth and photosynthetic efficiency through improvement in gas exchange attributes of the seedlings of various crops such as wheat (Ries 1991), maize (Ries 1991), and rice (Kumaravelu et al. 2000). It can be synthesized by a number of methods. In earlier studies, it was synthesized by reducing the esters of n-tricontanic acid with the help of sodium alcohol or aluminum hydride. Primarily, it was firstly synthesized by Bleyberg and Ulrich (1931) from ethyl behenate by repeated synthesis of malonic ester. Later on, its synthesis was reported by the methods of Robinson (1934), Jones (1947), and Oura et al. (1956), but these methods were not suitable for obtaining sufficient quantity of TRIA because most of these methods produced the unstable organometallic reagents and gave poor yield. However, the easiest and appropriate method for obtaining the sufficient quantity of TRAI was its synthesis from stearic acid by two consecutive additions of enamine intermediates (Rama Rao et al. 2006). It has been widely distributed in epicuticular waxes of many plant species such as Californian *Croton californicus*, *Vaccinium ashei*, *Copernicia cerifera*, *P. multiflorus*, *Trifolium repens*, *Medicago sativa*, as well as in *Jatropha curcas* (Naeem et al. 2012). Moreover, it also enhances the biotic and abiotic stress tolerance mechanism in many plant species by stimulating

Table 11.11 Roles of jasmonic acid under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Mentha piperita* L.	Exogenous (foliar application)	50 μmol	Increased content of flavonoid and total phenolic as well as antioxidant capacity	Soil culture	Abdi et al. (2019)
Salt stress	*Prunus dulcis*	Exogenous (foliar application)	0, 0.025, 0.050, and 0.075 mmol	Increased photosynthetic rate, activities of APX, SOD, and POX, root and shoot dry mass accumulation, as well as cell membrane integrity	Soil culture	Tavallali and Karimi (2019)
Salt stress	*Brassica napus* L.	Exogenous (foliar application)	0 and 100 μmol	Improvement in CO_2 compensation point, respiration rate, soluble sugar content, MDA and proline contents, proline content, and activities of antioxidant	Sand culture	Ahmadi et al. (2018)
Water stress	*B. oleracea* L.	Exogenous (foliar application)	0.5 μmol/L	Reduced the bitterness while increasing the sweetness and content of sulforaphane, increased antioxidant activity	Soil culture	Luo et al. (2018)
Heavy metals	*Zea mays* L.	Exogenous (Seed priming)	10 and 100 μmol	Increased activities of antioxidant and glyoxalase systems, ascorbate glutathione cycle, and growth	Sand culture	Mir et al. (2018)
Salt stress	*Ocimum basilicum* L.	Exogenous (foliar application)	0 and 0.5 mmol	Increased accumulation of essential oil, antioxidant potential, metabolism, and ion accumulation	Soil culture	Talebi et al. (2018)
Salt stress	*Arabidopsis thaliana*	Exogenous (foliar application)	50 μmol	Enhanced activities of SOD, CAT, and POD as well as the contents of proline and anthocyanins	Soil culture	Yastreb et al. (2018)
Water stress	*Beta vulgaris* L.	Exogenous (foliar application)	0, 0.01, 0.1, 1, or 10 μmol	Improved LRWC, biomass, net photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency, and PS-II quantum efficiency	Soil culture	Fugate et al. (2018)

Heavy metal stress	*B. napus* L.	Exogenous (foliar application)	25 μmol/L	Improved gas exchange mechanism and accumulation of photosynthetic pigments, activities of antioxidant enzymes, and nutrient uptake	Soil culture	Ali et al. (2018)
Water stress	*Gossypium hirsutum*	Exogenous (foliar application)	0, 25, 50, and 100 mg/L	Increased boll number per plant, 1000 seed weight, seed cotton yield, lint yield and lint percentage, the content of proline and soluble sugars	Soil culture	Yosefi et al. (2018)
Salt stress	*Limonium bicolor*	Exogenous (foliar application)	0.03 mmol	Improved physiological parameters relating to ion contents, reduced plasma membrane permeability, photosynthetic parameters, salt gland density, and salt secretion	Soil culture	Yuan et al. (2019)
Salt stress	*Vigna unguiculata*	Exogenous (seed priming)	0, 25, and 50 μmol	Increased proline and total soluble sugars contents, chlorophyll value, stomatal conductance, net photosynthetic rate, total soluble proteins, LRWC	Soil culture	Sadeghipour (2017)
Water stress	*Glycine max* L.	Exogenous (foliar application)	10 mg/g	Increased growth, content of photosynthetic pigments, LRWC, and oil content	Soil culture	Mohamed and Latif (2017)
Salt stress	*Ipomoea batatas*	Endogenous increase	10 mg/g	Improved activities of antioxidant enzymes and content of nonenzymatic antioxidative compounds, improved stomatal conduction and TSP	Soil culture	Zhang et al. (2017)
Salt stress	*Oryza sativa* L.	Exogenous (foliar application)	10–20 μmol	Increased plant growth, K^+ concentration, POD, and APX activities and chlorophyll content	Hydroponic culture	Mahmud et al. (2017)
Salt stress	*Moringa oleifera*	Exogenous (foliar application)	0.2 and 0.3 μmol	Improved activities of antioxidant enzymes, levels of peroxidase and polyphenol oxidase isozymes, amino acid contents, and ion accumulation	Sand culture	Hamideldin et al. (2017)

(continued)

Table 11.11 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*Oryza sativa* L.	Exogenous (seed priming)	100 µmol	Increased activities of antioxidant enzymes CAT, POD, and SOD	Soil culture	Samota et al. (2017)
Salt stress	*B. oleracea* L.	Exogenous (foliar application)	25 µmol/L	Improved seed germination, mineral nutrient accumulation, glucosinolates, flavonoids, and phenolic contents	Soil culture	Hassini et al. (2017)
Water, salt, and heavy metal	*G. max* L.	Exogenous (foliar application)	20 µmol	Improvement in morphological parameters, photosynthetic pigments accumulation, soluble protein, amino acids, glycolipids, and phospholipid contents	Soil culture	Mohamed et al. (2016)
Water stress	*Satureja hortensis*	Exogenous (foliar application)	0, 75, 150, and 225 µmol	Improved growth as well as LRWC, proline accumulation, antioxidant activity, and essential oil percentage and yield	Soil culture	Miranshahi and Sayyari (2016)
Salt stress	*G. max* L.	Exogenous (foliar application)	0.5 mmol	Increased accumulation of sulfur containing amino acids such as methionine and aromatic amino acids such as phenylalanine and tyrosine	Soil culture	Farhangi-Abriz and Ghassemi-Golezani (2016)
Salt stress	*O. sativa* L.	Exogenous (seed priming)	10 and 20 µmol	Increased shoot and root length, fresh and dry weight, and seed germination	Soil culture	Mahmud et al. (2016)
Heavy metal	*B. napus* L.	Endogenous increase	200 µmol	Improved ROS scavenging through enhanced antioxidant defense system, accumulation of secondary metabolite	Soil culture	Shahid et al. (2015)
Salt stress	*Solanum lycopersicum* L.	Exogenous (foliar application)	0.0, 10, 20, 30, 40, 50, and 60 µmol	Improved physio- and biochemical mechanism	Sand culture	Manan et al. (2016)

Salt stress	*Matricaria chamomilla* L.	Exogenous (seed priming)	75 µmol	Improved activities of CAT, POD, and APX, leaf photosynthetic rate concomitantly with membrane stability index, K^+ as well as free proline content	Soil culture	Salimi et al. (2016)
Heavy metal	*G. max* L.	Exogenous (seed priming)	1 nmol	Increased accumulation of proline, GB, total protein, and total soluble sugars, osmolytes, activity of antioxidant enzymes	Petri-dishes culture	Sirhindi et al. (2016)
Heavy metal stress	*Cajanus cajan*	Exogenous (seed priming)	1 µmol, 1 nmol, and 1 pmol	Improved accumulation of photosynthetic pigments, osmolytes, antioxidants protein, sugars, and vitamins contents	Petri dishes culture	Sirhindi et al. (2015a, b)
Salt stress	*B. napus* L.	Exogenous (seed priming)	0, 6, 9, and 12 M	Improved proline content with reduced electrolyte leakage and lipid peroxidation, improved content of protein	Soil culture	Kaur and Sirhindi (2015)
Salt stress	*A. thaliana*	Exogenous (foliar application)	10^{-7} M	Increased activities of Cu/Zn, SOD, CAT, and POD, leaf chlorophyll and photosynthetic pigments accumulation	Hydroponic culture	Yastreb et al. (2015)
Water stress	*Hordeum vulgar* L.	Exogenous (foliar application)	100 ppm	Improved stomatal conductivity, proline content, and LRWC	Soil culture	Pazirandeh et al. (2015)
Salt stress	*Carthamus tinctorius*	Exogenous (foliar application)	0.5 mmol	Promoted growth and development, improvement in physiological parameters, leaf chlorophyll content index, PS-II efficiency, LRWC, leaf area index, and grain yield	Perlite culture	Ghassemi-Golezani and Hosseinzadeh-Mahootchi (2015)
Water stress	*Z. mays* L.	Exogenous (seed priming)	50 µmol	Increased accumulation of pigments, total carbohydrates, total soluble sugars, polysaccharides, as well as free amino acids, proline and TSP, and plant growth parameters such as plant height and fresh and dry biomasses	Soil culture	Abdelgawad et al. (2014)

(continued)

Table 11.11 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*B. napus* L.	Exogenous (foliar application)	0.5 mmol	Improved seedling biomass production, leaf chlorophyll content, LRWC, proline, oxidized ascorbate, and glutathione	Petri dish culture	Alam et al. (2014)
Water stress	*A. thaliana*	Endogenous increase	0–24 mg/g	Improved photosynthetic rate, ion accumulation, and osmoregulation	Soil culture	Attaran et al. (2014)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	0 and 0.25 μmol	Improved plant water status and antioxidant capacity, photosynthesis, and dark respiration	Soil culture	MA et al. (2014)
Water stress	*H. vulgare* L.	Exogenous (foliar application)	100 μmol	Improved dry weight, leaf surface, total protein content, H_2O_2, ascorbate, and activities of POD, CAT, and APX	Soil culture	Pazirandeh et al. (2013)
Heavy metal	*Cajanus cajan* L.	Exogenous (seed priming)	1 μmol, 1 nmol, and 1 pmol	Enhanced activities of SOD and POD, chlorophyll, and carotenoid accumulation	Soil culture	Poonam et al. (2013)
Salt stress	*O. basilicum* L.	Exogenous (seed priming)	0, 0.1 and 0.01 μmol	Increased germination percentage and yield attributes	Soil culture	Enteshari and Jafari (2013)
Water stress	*B. oleracea*	Exogenous (foliar application)	10 μmol	Increased accumulation of chlorophyll pigment and net photosynthetic rate, activation of enzymatic and nonenzymatic antioxidant as well as proline and soluble sugar accumulation	Soil culture	Wu et al. (2012a, b)
Water and salt	*Haematococcus pluvialis*	Exogenous (foliar application)	500 μmol	Increased activities of SOD and APX, accumulation of primary and secondary carotenoids	Soil culture	Raman and Ravi (2011)
Water stress	*G. max* L.	Exogenous (foliar application)	50 μmol	Enhanced growth, improvement in leaf gas exchange attributes as well as the photosynthetic pigment contents	Soil culture	Anjum et al. (2011a, b)

Water stress	*O. basilicum* L.	Exogenous (foliar application	0, 2.4, and 4.8 mmol	Increased plant height, number of inflorescence branches, leaf area, dry weight of leaves and stems, LRWC, and chlorophyll content	Soil culture	Sorial et al. (2010)
Salt stress	*G. max* L.	Endogenous increase	0–250 ng/g	Improved plant length, biomass production, leaf chlorophyll contents, number of pods, 100 seed weight, growth, and yield components	Perlite culture	Hamayun et al. (2010)
Water, salt and heavy metal	*O. sativa* L.	Endogenous increase	20 mmol	Improved growth, photosynthetic pigments accumulation, and seed germination	Soil culture	Kim et al. (2009)
Salt stress	*G. max* L.	Exogenous (foliar application)	20 and 30 μmol	Improved plant growth, chlorophyll content, leaf photosynthetic rate, leaf transpiration rate, and proline content	Perlite culture	Yoon et al. (2009)
Light stress	*A. thaliana*	Exogenous (foliar application)	1 μmol	Improved the growth of cotyledons, rate of transpiration, ion accumulation, and photosynthetic pigment accumulation	Petri-dishes culture	Golovatskaya and Karnachuk (2008)
Water stress	*Lactuca sativa* L.	Exogenous (foliar application)	0.1, 0.25, and 0.5 mmol	Increased total phenolic content, content of carotenoids, and antioxidant capacity	Soil culture	Kim et al. (2007)
Salt stress	*G. max* L	Exogenous (foliar application)	1 μmol	Enhanced accumulation of nontoxic metabolites such as sugars, free proline, and proteins as well as N, P, and K^+ as a protective adaptation	Soil culture	Sheteawi (2007)
Water stress	*Z. mays* L.	Exogenous (foliar application)	0, 5, 10, 20, 50, and 100 μmol	Increased activities of glutathione reductase, guaiacol peroxidase, and ascorbate peroxidase in roots and shoots	Petri-dishes culture	Norastehnia and Nojavan-Asghari (2006)
Water stress	*Rubus idaeus* L.	Exogenous (foliar application)	0.01 and 0.1 mmol	Increased the content of flavonoids and the antioxidative capacities of fruits, fresh weight of fruit, higher anthocyanins, and total phenolics content	Soil culture	Wang and Zheng (2005)

various growth and metabolic processes such as in *H. annus* (Aziz and Shahbaz 2015), *O. sativa* (Chen et al. 2002), and *Z. mays* (Ali et al. 2017). It is also evident that the application of TRAI to rooting medium or foliar application improved the growth and yield of many agronomic, horticultural, medicinal, and aromatic crops, both under normal and adverse environmental conditions. It is biologically active at a very low concentration (concentration) because plants become sensitive at its extremely low doses (Naeem et al. 2012).

11.2.9.1 Triacontanol Roles Under Different Abiotic Stresses

Triacontanol is a new entry in plant hormone category, but it has a vast role in plant metabolism. The improvement in stress tolerance through its endogenous increment in cellular levels of triacontanol has been found in a number of plants species under various abiotic stresses, and the stress tolerance was based on the improvement in various physiological and biochemical parameters (Table 11.12). In *Juniperus procera* grown under water-stressed conditions, the improvement in growth was associated with increased content of free amino acids, accumulation of free proline, better LRWC, and ion accumulation at 200 ppm level of TRIA (Kibatu et al. 2014). Similarly, in *G. max*, 10 mmol level of triacontanol was effective for maintaining better growth, physio-biochemical attributes, accumulation of leaf photosynthetic pigments, and accumulation of total soluble proteins under soil salt-stressed conditions (Krishnan and Kumari 2008). In parallel with endogenous accumulation, TRAI was also found effective in improving the abiotic stress tolerance in many plant species when applied exogenously, either through seed priming or as foliar spray (Khanam and Mohammad 2018; Ali et al. 2017; Li et al. 2016a, b; Aziz and Shahbaz 2015; Perveen et al. 2014). It was reported by Ali et al. (2017) that foliarly applied TRAI (0.1 mg/L) improved the activities of antioxidant enzymes, ion accumulation, and seed nutritional quality traits in *Z. mays* both under normal and water-deficit conditions. Perveen et al. (2012) reported an increase in growth, LRWC, membrane permeability, accumulation of total free amino acids, free proline, GB, and total phenolic contents in wheat plants when its seeds were primed with 0, 10, and 20 μmol of TRAI. The exogenous application of all these levels of TRAI was found helpful for the induction of salt tolerance, but the most effective level was the 10 μmol. Aziz and Shahbaz (2015) investigated the effects of exogenous application of TRAI on the activities of POD, SOD, and GR enzymes, accumulation of free proline and GB, and the total soluble protein contents in sunflower plants grown under salinity stress. They found that foliarly applied all levels of TRAI were found effective in alleviating the negative effects of salt stress by improving the enzymatic and nonenzymatic antioxidative defense mechanism. Li et al. (2016a, b) reported the TRAI-induced (foliar spray) improvement in leaf chlorophyll and sucrose contents and activities of antioxidant enzyme, glutathione, and ascorbate redox states in water-stressed *O. sativa* plants. Furthermore, the foliar spray of TRAI was also found effective in improving various growth and physio-biochemical processes and played a significant role in alleviating the adverse effects of various biotic and

Table 11.12 Roles of triacontanols under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Mentha piperita* L.	Exogenous (foliar application)	10^{-6} M	Improved growth, photosynthesis, carbonic anhydrase activity, NPK content, peltate glandular trichome density, essential oil, menthol content, and yield	Soil culture	Khanam and Mohammad (2018)
Water stress	*Pyrus malus*	Exogenous (foliar application)	10 ppm	Improved ion accumulation, nutrient uptake, photosynthetic efficiency, and fruit yield	Soil culture	Mohd. Zubair et al. (2018)
Water stress	*Zea mays* L.	Exogenous (foliar application)	0.1 mg/L	Improved activities of antioxidant enzymes, ion accumulation, and seed quality traits	Soil culture	Ali et al. (2017)
Water stress	*Oryza sativa* L.	Exogenous (foliar application)	0, 1, 5, and 10 μmol	Enhanced chlorophyll and sucrose contents, activities of antioxidant enzyme, glutathione, and ascorbate redox states	Soil culture	Li et al. (2016a, b)
Water stress	*Z. mays* L.	Exogenous (foliar application)	5 μmol	Increased activities of CAT and POD, the contents of total phenolics, total soluble proteins, GB, and free proline	Soil culture	Perveen et al. (2016)
Salt stress	*Momordica charantia* L.	Exogenous (foliar application)	5 and 10 ppm	Improvement in studied yield attributes	Soil culture	Sureshkumar et al. (2016)
Salt stress	*Helianthus annus* L.	Exogenous (foliar application)	10 mmol	Enhanced activities of POD, SOD, and GR enzymes, free proline and GB contents, and total soluble protein contents	Sand culture	Aziz and Shahbaz (2015)
Water stress	*Theobroma cacao* L.	Exogenous (foliar application)	0, 0.1, 0.5, 1, and 2 mL/L	Improved growth and physiological and yield attributes	Soil culture	Sitinjak and Pandiangan (2015)
Water stress	*Juniperus procera*	Endogenous increase	200 ppm	Improvement in growth and physio-biochemical attributes	Soil culture	Kibatu et al. (2014)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	0, 10, and 20 μmol	Improved growth, yield, activities of antioxidant enzymes, chlorophyll contents, and leaf water relations	Sand culture	Perveen et al. (2014)

(continued)

Table 11.12 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*H. annus* L.	Exogenous (foliar application)	100 µmol	Improved biomass production, net CO_2 assimilation rate, transpiration rate, water use efficiency, and non-photochemical quenching efficiency	Sand culture	Aziz et al. (2013)
Salt stress	*T. aestivum* L.	Exogenous (foliar application)	0, 10, and 20 µmol	Improved growth, net photosynthetic rate, transpiration rate, chlorophyll contents, electron transport rate, membrane permeability, and stomatal conductance	Soil culture	Perveen et al. (2013)
Salt stress	*T. aestivum* L.	Exogenous (seed priming)	0, 10, and 20 µmol	Increased growth, LRWC, membrane permeability, total free amino acids, free proline, GB, and total phenolic contents	Sand culture	Perveen et al. (2012)
Salt stress	*Arachis hypogaea* L.	Exogenous (foliar application)	2 and 3 mg/L	Increased content of chlorophyll, total soluble sugars, total soluble proteins, and activities of antioxidant enzymes	Soil culture	Verma et al. (2011a, b, c)
Water stress	*Lupinus luteus* L.	Exogenous (foliar application)	0.5 and 1 mg/L	Increased lateral shoot blooming, photosynthetic rate, chlorophyll contents, and LRWC	Soil culture	Borowska and Prusiński (2012)
Salt stress	*Glycine max* L.	Endogenous increase	10 mmol	Improved growth, physio-biochemical parameters such as content of chlorophyll pigments, nucleic acids, total soluble sugars, and proteins	Soil culture	Krishnan and Kumari (2008)
Salt stress	*O. sativa* L.	Exogenous (foliar application)	10 µg/L	Improved plant biomass, protein, and chlorophyll content, leaf photosynthetic rate as well as ion accumulation	Soil culture	Chen et al. (2002)

abiotic stresses in a variety of plant species such as sunflower (Aziz et al. 2013), coca (Sitinjak and Pandiangan 2015), and maize (Perveen et al. 2016).

11.2.10 Tocotrienol

Tocotrienols were first introduced by Evans and Bishop in 1922, when they confirmed the role of tocotrienols in human health and nutrition. Pennock and Whittle discovered tocotrienols for the first time in 1964 by explaining their isolation from rubber, but their biological significance was described by Qureshi and Elson in the early 1980s due to the significant role of tocotrienols in reducing the cholesterol levels in humans. Afterward, in 1990s, their anticancer properties were reported by Watson et al. (2008). Tocotrienols are naturally occurring compound/members of the vitamin E family present in a variety of vegetable oils, barley, wheat, as well as various types of grains and nuts. The best source of tocotrienols is vegetable oil, especially rice bran oil and palm oil that have large quantity of tocotrienols, while it is also found in seed oil of maize, grape fruit, olive oil, sunflower oil, and hazelnut. Usually, they are not found in green parts of plants, but only a small amount of α-tocotrienol have been found in leaves of some plants (Dunphy et al. 1965). Comparatively, more tocotrienols are found in monocots, while only a small quantity is in dicots. Tocotrienols exist in various forms such as alpha- (α), beta- (β), gamma- (γ), and delta-tocotrienols (δ). They have similar structural properties as tocopherol but differ in the degree of saturation and content of isoprenoid side chain. They have excellent antioxidant properties that help in preventing the lipid peroxidation in plants and act as neuroprotective, anticancer, and cholesterol-lowering agents in humans (Sen et al. 2006; Seppanen et al. 2010; Ahsan et al. 2015). They can also be used in various kinds of nutritional supplements, foods, as well as antiaging tablets and are available in the market as a source of vitamin E after their extraction from rice, palm, or annatto. A very few studies were reported regarding their role in stress tolerance mechanism by promoting various growth, metabolic, and physiological processes in plants, either by exogenous application or endogenous accumulation (Saini et al. 2012; Ali and Woodman 2015). Different methods have been employed by researchers for the extraction, analysis, identification, and quantification of these vitamin E compounds such as by saponification, high-performance liquid chromatography (HPLC), and thin layer and paper chromatography (Ahsan et al. 2015). Tocotrienols follow the same steps of biosynthesis as for tocopherols but differ only in one step. Its biosynthesis starts with the catalysis of geranylgeranyl diphosphate into 2-methyl-6-geranylgeranyl-1,4-benzoquinol with the help of the enzyme homogentisate geranylgeranyl transferase (HGGT). In the next step, 2-methyl-6-geranylgeranyl-1,4-benzoquinol is further divided into two steps, producing γ-tocotrienol and δ-tocotrienol with the help of enzymes γ-tocotrienol catalase and δ-tocotrienol catalase, respectively. In the next step, γ-tocotrienol is catalyzed by the enzyme γ-tocotrienol methyltransferase to give rise to α-tocotrienol, while δ-tocotrienol yields α-tocotrienol when being catalyzed by the enzyme δ-tocotrienol methyltransferase.

11.2.10.1 Tocotrienol Roles Under Different Abiotic Stresses

Tocotrienol is a newly discovered plant hormone, but it has multiple and important roles in plant growth, metabolism, as well as stress tolerance. The ability of plants to tolerate stressful environmental conditions depends upon the endogenous accumulation as well as the exogenous application of these plant hormones. Their role in improvement in stress tolerance is through their endogenous increment at cellular levels as has been found in a number of plants under various abiotic stresses, and their role in stress tolerance was based on the improvement in various physiological parameters (Table 11.13). In *Elaeis guineensis*, Ali and Woodman (2015) reported an increase in seed germination, mineral nutrients, glucosinolates, flavonoids and phenolic compounds, and ion accumulation by endogenous accumulation of 10^{-3} mg/mL levels of tocotrienol, and this endogenous increase was found helpful in increasing the tolerance of *E. guineensis* plants against salt stress. Similarly, in *Nicotiana tabacum*, 300 and 350 μg/g levels of tocotrienol were found effective for improvement in plant growth, K^+ concentration, POD and APX activities, and biosynthesis of chlorophyll under soil water stress (Matringe et al. 2008). Not only the endogenous increments but also the exogenous application of tocotrienols has been found effective in stress tolerance induction in various crop plants, but the effective level was species specific or dependent on the type of stress (Gan et al. 2017a, b; Saini et al. 2012). For example, exogenous application of tocotrienols as foliar spray at concentration of 50 mg/g was found effective in increasing the sweetness and sulforaphane content, with increased antioxidant activity in *E. guineensis* plants when grown water stress (Gan et al. 2017a, b). Effect of foliarly applied tocotrienol on physiological and biochemical attributes of salt stressed *A. thaliana* has been explained by Saini et al. (2012), where foliarly applied 55 ng/g of tocotrienol was found effective in improving the salt resistance.

11.2.11 Brassinosteroids

Brassinosteroids (BRs) are types of steroidal plant hormones that are discovered recently in plants and regulate specific growth and metabolic activities. They trigger many plant physiological processes such as stem elongation, seed germination, immunity, as well as many developmental and reproductive processes. They have specific steriodol structure similar to animals as they contain approximately 70 derivatives of polyhydroxylated sterol. They also have some applications in animal hormones and act as antiproliferative, antiangiogenic, antibacterial, antiviral, and anticancer agents and also have many other medicinal properties. They were first discovered at the beginning of 1970 by Mitchell et al. (1970, 1971). They isolated the extract of *B. napus* flowers called brassin, initiating the stem elongation and cell division when applied at internodes of bean plants. The first discovered BR was named as "brassinolide" that was isolated from 40 kg pollens of brassica rapa (Oklestkova et al. 2015). They all have 5a-cholestane in their structure, but many alterations has been found in their

Table 11.13 Roles of tocotrienols under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Elaeis guineensis*	Endogenous increase	10^{-3} mg/mL	Enhanced seed germination, in mineral nutrients, glucosinolates, flavonoids, and phenolic compounds under salt stress	Soil culture	Ali and woodman (2015)
Water stress	*E. guineensis*	Exogenous (foliar application)	50 mg/g	Alleviated the bitterness while increasing the sweetness and sulforaphane content, increased antioxidant activity under drought stress	Sand culture	Gan et al. (2017a, b)
Salt stress	*Arabidopsis thaliana*	Exogenous (foliar application)	55 ng/g	Ameliorated the deleterious effects of salinity on tomato plants by inducing physiological and biochemical resistance	Soil culture	Saini et al. (2012)
Water stress	*Nicotiana tabacum*	Endogenous increase	300 and 350 μg/g	Increased plant growth, K^+ ion concentration, POD, and APX activities and chlorophyll content	Soil culture	Matringe et al. (2008)

skeleton that corresponds to their specific functions (Bajguz and Tretyn 2003). They are ubiquitous throughout the plant kingdom, i.e., they include 53 angiosperm, 3 algae, 1 pteridophyte, and 1 bryophyte (Bajguz 2011), and found mostly in plant organs such as roots, stems, leaves, seeds, pollen, flowers, and grains as well as in insect and crown galls (Bajguz 2011). The cellular sites for synthesis of BR have not been demonstrated clearly. Campesterol is the main precursor for BR biosynthesis, and its biosynthetic pathway was explained by many Japanese scientists and later on was shown through the synthesis of BR in *A. thaliana*, tomatoes, and peas (Fujioka and Sakurai 1997). The signal transduction genes for its biosynthesis are expressed in a wide range of plant organs. The sites for BR synthesis in plants have not been experimentally demonstrated. One well-supported hypothesis is that all tissues produce BRs, since BR biosynthetic and signal transduction genes are expressed in a wide range of plant organs, and this was also supported by short distance activity of the hormones (Li and Chory 1997; Clouse and Sasse 1998).

11.2.11.1 Brassinosteroid Roles Under Stress

BRs have multiferous roles in plants under different biotic and abiotic stresses through its endogenous increased biosynthesis or as exogenous use in plants deficit to its biosynthesis. The endogenous increments at cellular levels of brassinosteroids are responsible for the improvement in stress tolerance in plants grown under various abiotic stresses, and the stress tolerance was based on the improvement in various physiological parameters such as the concentration of total chlorophyll, carotenoids, and total phenolic contents (Table 11.14). In *O. sativa*, *S. tuberosum*, and *Arachis hypogaea*, the 10^{-5} M, 0.1 and 0.01 μg/L, and 200 ppm cellular levels of brassinosteroids, respectively, were found effective in the improvement of growth, through the accumulation of N-containing compounds, such as amino acids and vitamins grown under soil salt-stressed conditions (Sharma et al. 2017; Hu et al. 2016; Trivedi and Illa 2016). An endogenous increase of 50 mg/g in *A. thaliana* was reported for better growth under heat stress, and this increase was found helpful in improving the contents of photosynthetic pigments, flavonoids, and total soluble sugars and also reduced the negative effects of heat stress (Thussagunpanit et al. 2015). Similarly, under drought stress, 80 and 15 mg/g levels of brassinosteroids were found effective for better growth, antioxidative defense mechanism, transpiration rate, and the accumulation photosynthetic pigments of *A. thaliana* (Fàbregas et al. 2018a, b) and *Z. mays* (Tumova et al. 2018), respectively. An endogenous increase of 50 ng/g of brassinosteroids was reported in *A. thaliana* plants grown under water stress, and this endogenous level was found helpful in improving the free amino acids, sucrose phosphate synthase activity, and photosynthesis under drought stress (Han et al. 2017). It is reported that not only the endogenous increments but also the exogenous application of brassinosteroids has been found helpful for the induction of stress tolerance in various crop plants, but the effective level was species specific or dependent on the type of stress (Behnamnia et al. 2009; El-Mashad and Mohamed 2012; Efimova et al. 2014; Fariduddin et al. 2014; Kaur et al. 2018;

Table 11.14 Roles of brassinosteroids under different abiotic stresses

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Salt stress	*Brassica juncea*	Exogenous (foliar application)	0, 10^{-6}, 10^{-9}, and 10^{-12} M	Improved activities of POD, CAT, and APX, total soluble sugar and protein content, LRWC	Soil culture	Kaur et al. (2018)
Salt stress	*Eucalyptus urophylla*	Exogenous (foliar application)	0 and 50 nmol	Improved salt stress resistance of bent grass especially through antioxidant metabolism	Soil culture	Oliveria et al. (2018)
Heavy metal	*Raphanus sativus*	Exogenous (foliar application)	10^{-7} M	Significantly increased plant growth, the content of amino acids as well as enzymatic activities	Soil culture	Sharma et al. (2018)
Salt stress	*Solanum lycopersicon* L.	Exogenous (seed priming)	10^{-7} M	Increased total free amino acid concentration, growth parameters, and yield components	Soil culture	Ahmed et al. (2018)
Water stress	*Arabidopsis thaliana*	Endogenous increase	80 ng/g	Maintained the level of Rubisco contents under drought stress as a result normal photosynthetic or gas exchange capacity	Sand culture	Fàbregas et al. (2018a, b)
Salt stress	*Zea mays* L.	Endogenous increase	15 mg/g	Counteracted the adverse effects of salt stress in barley through accumulating organic osmolytes and enhancing antioxidant defense systems, maintaining of high K+/Na+ ratio inside the cytosol	Soil culture	Tumova et al. (2018)
Water stress	*A. thaliana*	Endogenous increase	50 ng/g	Increased free amino acids, sucrose phosphate synthase activity, and photosynthesis under drought stress	Soil culture	Han et al. (2017)
Water stress	*Triticum aestivum* L.	Exogenous (foliar application)	0.1 mg/L	Improved the tolerance of alfalfa to severe drought stress without affecting alfalfa nodulation or nitrogen fixation	Petri-dish culture	Zhao et al. (2017)
Salt stress	*Zea mays* L.	Exogenous (seed priming)	10^{-10}, 10^{-8}, and 10^{-6} M	Counteracted the harmful effects of cadmium-induced lipid peroxidation in the soybean seedlings	Soil culture	Rattan et al. (2017)
Salt stress	*Oryza sativa* L.	Endogenous increase	10^{-5} M	Resulted in effective roles in the delay of oxidative damage and increased growth parameters	Soil culture	Sharma et al. (2017)

(continued)

Table 11.14 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Heat stress	*Abelmoschus esculentus* L.	Exogenous (foliar application)	0, 0.25, 0.5, 0.75, 1, 1.25, and 1.5 μmol	Maintained the appropriate level of growth, metabolism, and final yields of plants under adverse harmful stress conditions	Soil culture	Khan et al. (2017)
Salt stress	*Cucumis sativus* L.	Exogenous (foliar application)	1, 3, and 5 μmol	Improvement in desiccation tolerance increase in concentration of antioxidant enzymes and also improved the nonenzymatic defense mechanism	Sand culture	Ahmad et al. (2017b)
Salt stress	*Capsicum annuum* L.	Exogenous (foliar application)	2 μmol	Improved the growth, biochemical, and physiological attributes of wheat especially the photosynthetic rate of wheat seedlings	Soil culture	Hegazi et al. (2017)
Salt stress	*Lycopersicon esculentum*	Exogenous (foliar application)	0.25 and 0.5 ppm	Increased photosynthetic contents of plant as well as amino acid content	Sand culture	Irfan et al. (2017)
Salt stress	*Brassica juncea* L.	Exogenous (foliar application)	10^{-8} M	Increase in leaf diameter, achene weight, oil, and yield	Soil culture	Wani et al. (2017)
Salt stress	*S. tuberosum*	Endogenous increase	0.1 and 0.01 μg/L	Maintained a physical association among chloroplasts, peroxisomes, and mitochondria and increased the CO_2 compensation point, indicating the cytokinin-mediated occurrence of photorespiration in *Arabidopsis*	Soil culture	Hu et al. (2016)
Salt stress	*Arachis hypogaea* L.	Endogenous increase	200 ppm	Increased the plant height, stem diameter, ear length, row number per ear, and biological yield	Soil culture	Trivedi and Illa (2016)
Water stress	*Pisum sativum*	Exogenous (seed priming)	0.25 mg/L	Increased chlorophyll contents and ultimately the plant fresh biomass under drought stress	Soil culture	Janeczko et al. (2015)
Heat stress	*A. thaliana*	Endogenous increase	50 mg/g	Increased the content of photosynthetic pigments, flavonoids, and total soluble sugars	Soil culture	Thussagunpanit et al. (2015)
Salt stress	*Lolium perenne* L.	Exogenous (foliar application)	0, 0.1, 10, and 1000 nmol	Increase in photosynthesis, growth improvement, and physiological process	Soil culture	Sun et al. (2015)

Salt stress	*S. tuberosum* L.	Exogenous (foliar application)	40 ng/g	Ameliorated the adverse effects of salt stress by its influence on enzymatic antioxidant activities in sunflower seedlings	Soil culture	Upadhyaya et al. (2015)
Water stress	*Panicum miliaceum* L.	Exogenous (seed priming)	20 nmol	Reduced the adverse effects of drought stress in maize seedling through the activation of antioxidant system	Soil culture	Vayner et al. (2014)
Salt stress	*B. napus* L.	Exogenous (foliar application)	10^{-10} M	Improved fresh biomass production, accumulation of photosynthetic pigments, total soluble sugar, and pigment content	Soil culture	Efimova et al. (2014)
Salt stress	*C. sativus* L.	Exogenous (foliar application)	10^{-8} M	Improved LRWC, nutrient uptake, activities of POD, CAT, and APX, osmoregulation and tocopherol contents	Soil culture	Fariduddin et al. (2014)
Water stress	*Helianthus annuus* L.	Exogenous (foliar application)	0.01 and 1 μmol	Improved biomass productions, photosynthetic rate, relative water content, and soluble proteins under abiotic stress	Soil culture	Filova (2014)
Salt stress	*Zea mays* L.	Exogenous (seed priming)	10 μmol	Improves the turf quality, pigmentation, and the number of seedlings, also lowered the rate of oxidative damages due to salt stress	Soil culture	Agami (2013)
Salt stress	*Vigna sinensis*	Exogenous (foliar application)	0.05 ppm	Improvement in contents of ascorbic acid, tocopherol, and glutathione as well as activities of antioxidant enzymes	Soil culture	El-Mashad and Mohamed (2012)
Salt stress	*Sorghum bicolor*	Exogenous (foliar application)	2 and 3 μmol	Modified both shoot hormonal and ionic status, thus ameliorating salinity-induced decreases in growth and yield in tomato seedlings	Soil culture	Vardhini (2012)
Salt stress	*Lactuca sativa* L.	Exogenous (foliar application)	0, 1, 2, and 3 mmol	Improving the growth, yield attributes, the cob and seed-bearing capacity of maize seedling under drought stress	Sand culture	Ekinci et al. (2012)
Heavy metal	*R. sativus* L.	Exogenous (foliar application)	10^{-9} M	Improved seed germination and growth, chlorophyll contents, and soluble proteins contents	Soil culture	Choudhary et al. (2012)

(continued)

Table 11.14 (continued)

Stress type	Plant species	Exogenous/ endogenous use	Effective concentration	Improvement/amelioration	Growth medium	References
Water stress	*A. thaliana*	Endogenous increase	100 ppm	Increased the content of soluble protein in light sensitive genotype and also increased the concentration of organic solutes resulting in tolerance to stress	Soil culture	Chung et al. (2014)
Heavy metal	*S. lycopersicon* L.	Exogenous (foliar application)	10^{-8} M	Promoting the expansion growth of the cotyledons and antioxidant defense system under light stress in cucumber	Soil culture	Hayat et al. (2012a, b)
Salt stress	*L. esculentum*	Exogenous (foliar application)	10^{-10}, 10^{-8}, 10^{-5}, and 10^{-3} M	Maintained the accumulation of several metabolites, particularly amino acids, carbohydrates, and organic acids that are mainly involved in the drought tolerance by osmotic adjustment	Soil culture	Slathia et al. (2012)
Salt stress	*Arachis hypogaea* L.	Exogenous (foliar application)	2 ml/L	Stimulated the production of photosynthetic pigments, total carbohydrates, and polysaccharides contents	Soil culture	Verma et al. (2011a, b, c)
Salt stress	*Triticum aestivum* L.	Exogenous (foliar application)	50, 100, and 200 mg/L	Improved all parameters that were decreased due to drought stress such as growth and yield	Soil culture	Eleiwa et al. (2011)
Water stress	*S. lycopersicon* L.	Exogenous (foliar application)	1 μmol	Increased chlorophyll content, photosynthetic rate, and lignin production in barley seedlings	Soil culture	Yuan et al. (2010)
Water stress	*L. esculentum*	Exogenous (foliar application)	0.01 and 1 μmol	Improved the protein content, SOD, POD, and APX activities and transpiration rate	Soil culture	Behnamnia et al. (2009)

Khan et al. 2017). For example, exogenous application of brassinosteroids as foliar spray improved the photosynthesis, growth, and various physiological processes in *L. perenne* L. (Sun et al. 2015). Another study conducted by Wani et al. (2017) reported that foliar application of brassinosteroids at a concentration of 40 ng/g was found helpful in increasing the leaf diameter, achene weight, oil, and yield of *B. juncea* L. plants grown under salt stress. Seed priming of *Panicum miliaceum* L. with 20 nmol level of brassinosteroids reduced the adverse effects of drought stress in maize seedling through the improvement of antioxidant system (Vayner et al. 2014). The turf quality, pigmentation, and number of seedlings of *Z. mays* L. increased when its seeds were grown after priming with 10 μmol concentration of brassinosteroids. It also lowered the oxidative damages caused by salt stress (Agami 2013). An improvement in seed germination and seedling growth, chlorophyll contents, and soluble proteins of the *Raphanus sativus* L. was reported by Choudhary et al. (2012), when supplied foliarly by 10^{-9} M concentration of brassinosteroids. It also decreased the adverse effects of heavy metal stress in *R. sativus* L.

11.3 Conclusions

The communication in plants among different plant parts for proper organization and structural modification under adverse environmental conditions is a complex mechanism that involves the contribution of different signaling molecules such as phytohormones as discussed in this chapter. Although a significant work has been done and there is progress regarding the role of phytohormones in plant sciences, still further work is needed to understand the exact mechanism of action for stress tolerance under stressful environmental conditions. Understanding the combined action of different phytohormones in this regard entails fruitful debate. The list of phytohormones is increasing with the new discoveries that made this understanding more complex. The knowledge of molecular biology has made this understanding more easy and speedy. Furthermore, the changing environmental conditions have made the study of plant responses more complex due to changed behavior of plants that is a matter of significance in the present scenario. Though lots of work have been reported in this regard, lots of missing gaps are still present in understanding the plant behavior under such adverse environmental conditions. The information given in this chapter will be helpful in understanding this up to some extent for further working on this topic for the researchers.

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Chapter 12
Proline and Abiotic Stresses: Responses and Adaptation

Mohamed Magdy F. Mansour and Karima Hamid A. Salama

Contents

Abstract Abiotic stresses are major threats influencing crop growth and production globally. Abiotic stresses can cause several cellular dysfunctions, and some of them are beneficial and required for stress tolerance enhancement. Compatible solute accumulation is one of these changes, and among them proline (Pro) overproduction is a physiological response commonly found in plants exposed to various abiotic stresses. Pro overproduction has been proposed to correlate with stress resistance in many plants. Pro implication in stress tolerance mechanism is supported by the observations that exogenous supply and genetic manipulation of metabolic pathways associated with Pro biosynthesis have been beneficial in enhancing stress tolerance in many plant species under different stresses. Pro improves stress tolerance via acting as a stress-related signal influencing adaptive responses, osmotic

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adjustment mediator, and molecular chaperone to stabilize subcellular structures, scavenging reactive oxygen species (ROS), acting as a metal chelator, serving as a nitrogen/carbon source for cells under stress conditions and after stress relief, maintaining cytoplasmic pH and hence alleviating its acidosis, triggering gene expression, and buffering cellular redox potential. Pro actual role in conferring stress tolerance is, however, still a matter of debate because there are several inconsistencies among published data. Also, Pro biosynthesis is dependent on a high diversity of regulation mechanisms, and even several of them are still largely obscure, which might exacerbate these discrepancies. Furthermore, the broad natural variation in Pro overproduction and its true role in metabolism of plants under stress conditions necessitate further research for better understanding of the reported variations. Despite the Pro beneficial roles observed under stress conditions, we believe that the often proposed relationship between Pro production and stress resistance may not be universal.

Keywords Abiotic stresses · Biosynthesis · Exogenous application · Functions · Proline · ROS · Signaling · Transgenic plants

Abbreviations

ABA	Abscisic acid
DFR	Drought freezing responsive gene
EC	Electrical conductivity
GSA	Glutamate semialdehyde
GSH	Reduced glutathione
GSSG	Oxidized glutathione
OAT	Ornithine-δ-aminotransferase
P5C	Pyrroline-5-carboxylate
P5CDH?	P5C dehydrogenase
P5CR	P5C-pyrroline-5-carboxylate reductase
P5CS	P5C synthatase
PDH	Proline dehydrogenase
PERKs	Proline-rich extensin-like receptor kinases
Pro	Proline
ProT	Proline transporter
PRP	Proline-rich proteins
ROS	Reactive oxygen species

12.1 Introduction

Plants under their natural conditions are regularly subjected to environmental deviations from their optimal conditions, thus severely affecting plant performance. These environmental threats disturb physiology, biochemistry, and genetics of cells and are termed stresses. Environmental stresses are biotic and abiotic; the first ascribed to injury occurs to a creature by other organisms, such as viruses, parasites, bacteria, and useful and injurious insects. On the contrary, abiotic stress is induced by nonliving factors or stressors, such as heat stress, low-temperature stress (chilling and freezing stresses), high salinity, and water deficit or drought stress that may cause harm to an organism. Other abiotic stresses are less common (e.g., flooding, heavy metals, wind, mineral deficiency, etc.) but can be equally as lethal. Plants in their response to abiotic stresses depend on the degree of stress, and hence, their sensitivity is classified into stress-sensitive and stress-resistant ones.

A brief definition for each abiotic stress has been presented here. In response to the temperature, each plant species has a specific range characterized by a minimum, maximum, and optimum temperature (Hatfield and Prueger 2015). The authors also indicate that hazardous temperatures influencing plant performance are species dependent. In general, 1–4 °C above the optimal temperature appropriate for plant growth is considered mild heat stress, while more than 4 °C above the ideal one is intense heat stress (Hatfield and Prueger 2015). Chilling stress usually takes place when temperatures are below 10–15 °C (usually between 2 and 10 °C), whereas freezing stress prevails when temperatures are below 0 °C and water in the plant tissues converts to ice (Rasheed et al. 2010). Water deficit means a shortage in the amount of water needed to maintain either natural vegetation or agricultural crops. When soil solution or irrigation water contains excessive soluble salts that inhibit plant growth or cause plant death, this is a salt stress (Mansour et al. 1993; Mansour and Stadelmann 1994). These abiotic stresses hence cause a severe threat to plant agriculture worldwide.

Abiotic stresses can bring about several cellular dysfunctions such as membrane injury, reactive oxygen species (ROS) production, altered gene expression, protein denaturation, and accumulation of toxic products (Kaur and Asthir 2015). Some of these stress-induced alterations are, however, needed for plant improved tolerance, e.g., compatible solute accumulation, antioxidant defense, membrane lipids, and protein modulations (Ashraf and Foolad 2007; Salama et al. 2011; Salama and Mansour 2015; Mansour and Ali 2017a, b). One of the compatible solutes that accumulate in plants subjected to different abiotic stresses is Pro. Pro accumulates in various plant tissues not only under abiotic stress but also during normal growth conditions (Kishor et al. 2015). Evidence indicates that Pro does not disturb intracellular metabolism even at high concentrations (Kaur and Asthir 2015; Nahar et al. 2016; Mansour and Ali 2017a), although there are controversial data on this issue (Mansour 1998; Jain et al. 2001; Dawood et al. 2014; Mansour and Ali 2017a and references therein). Many plant species elevate the level of free Pro under different biotic and abiotic stresses, and Pro is ascribed as a signal/regulatory osmolyte that activates multiple physiological, biochemical, or molecular responses (Mansour and Salama 2004; Tatar et al. 2013; Mari et al. 2018; Nounjan et al. 2018). Despite Pro numerous roles in plant normal growth and development, Pro is most renowned for its functions under stressed conditions. Pro is a multifunctional amino acid possessing beneficial diverse roles under stress conditions. These roles may include being a molecular chaperone for the stabilization of cellular macromolecules, scavenging ROS, contributing to osmotic adjustment, buffering redox for reductants, being nitrogen and carbon reserves used after stress relief for growth, maintaining cytoplasmic pH and alleviating its acidosis, and being a metal chelator and source of energy (Kaur and Asthir 2015; Nahar et al. 2016; Suprasanna et al. 2016; Mansour and Ali 2017a). Pro acts mainly as an osmolyte involved in the osmotic adjustment that plays a crucial role in cellular turgor maintenance under stress conditions. Pro is also suggested to be sequester in the cytosol to balance the vacuolar lower osmotic potential and thus importantly participate in osmotic adjustment and continuous water absorption under stresses (Ashraf and Foolad 2007; Borgo et al. 2015). However, Pro production at low concentrations that are not sufficient to contribute to osmotic adjustment has been hypothesized to serve several protective functions under abiotic stresses (Mansour 1998; Ashraf and Foolad 2007; Nahar et al. 2016; Mansour and Ali 2017a).

Evidence supporting Pro involvement in stress resistance is confirmed by the fact that Pro exogenous application has been demonstrated to induce plant stress tolerance in many species (Roy et al. 1993; Cuin and Shabala 2007; Kaushal et al. 2011; Rasheed et al. 2010; Gavelienė et al. 2014). The physiological role of Pro in stress adaptation is also documented by engineering synthesizing and catabolizing pathway genes that result in overexpressing and suppressing Pro production and consequently affect plant responses to stresses (Wang et al. 2014; Kaur and Asthir 2015; Yang et al. 2016; Ren et al. 2018). Despite all this confirming evidence underpinning Pro beneficial roles in various plants under different stress conditions, Pro accumulation and correlation with plant adaptation to stresses have been argued (Chen et al. 2007; Widodo et al. 2009; Signorelli et al. 2014; Signorelli 2016; Kim et al. 2016; Mansour and Ali 2017a). Different lines of evidence have been provided by the previous authors to justify their argument. In order to asses Pro implications in plant responses and adaptation to different stress conditions, the correlation of Pro accumulation with stress tolerance, diverse roles of Pro, exogenous supplication of Pro, and engineering Pro synthesizing enzymes in plants subjected to numerous abiotic stresses have been reviewed. As Pro biosynthesis, signaling, and transport are crucial agents in Pro-induced stress tolerance under different stress conditions, these issues are also discussed.

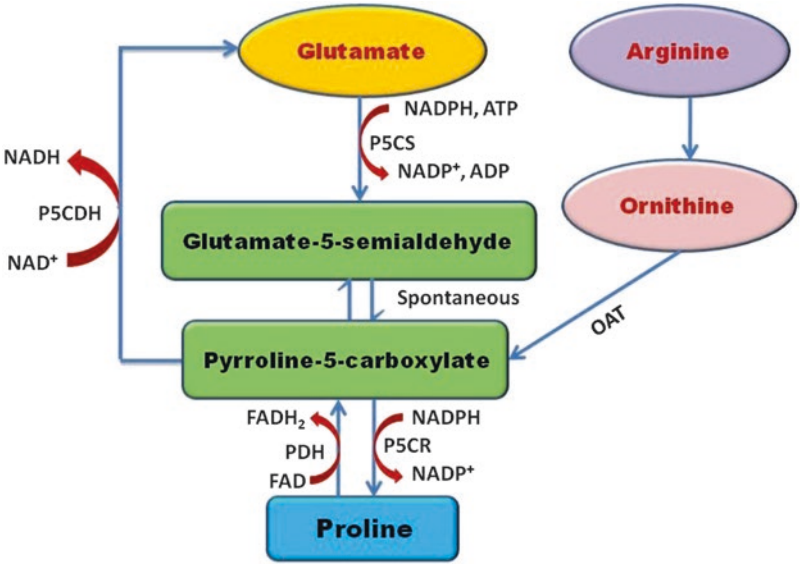

Fig. 12.1 Simplified glutamate and ornithine pathways for Pro biosynthesis where pyrroline-5-carboxylate synthatase (P5CS), pyrroline-5-carboxylate reductase (P5CR), and ornithine-delta-aminotransferase (OAT) are implicated in the cytoplasm or chloroplast. Pro catabolism involves Pro dehydrogenase (PDH) and pyrroline-5-carboxylate dehydrogenase (P5CDH) in the mitochondria

12.2 Pro Biosynthesis, Transport, and Signaling

12.2.1 Pro Biosynthesis and Degradation

Briefly, Pro is metabolized from glutamic acid through pyrroline-5-carboxylate (P5C). P5C synthatase (P5CS) reduces ɣ-glutamate phosphate into glutamate semialdehyde (GSA) which spontaneously cyclizes to P5C (Fig. 12.1; Kaur and Asthir 2015). Reduction of P5C is then occurred by pyrroline-5-caboxylate reductase (P5CR) to Pro. Pro metabolism thus involving these two enzymes (P5CS, P5CR) occurs in the cytoplasm and chloroplast. Evidence indicates that P5CS is the key enzyme which is encoded by two paralog genes, *P5CS1* and *P5CS2*, while the second enzyme P5CR is encoded by only one gene in the majority of plant species (Ren et al. 2018). Another pathway for Pro biosynthesis is ornithine pathway where transamination of ornithine to P5C is performed by ornithine-δ-aminotransferase (OAT) via an intermediate pyrroline-2-carboxylate (Fig. 12.1; Kaur and Asthir 2015). In contrast, Pro catabolism pathway occurs in the mitochondria where Pro is oxidized into glutamate by Pro dehydrogenase (PDH), which is also referred to as Pro oxidase, and P5C dehydrogenase (P5CDH) (Fig. 12.1; Mansour and Ali 2017a). PDH is the key enzyme in Pro catabolism and is encoded by two nonredundant isoforms, *PHD1* and *PHD2*, whereas only one *P5CDH* gene is known to encode P5CDH (Ren et al. 2018). A novel pathway regulating Pro metabolism under drought stress and leading to drought tolerance is reported by Fu et al. (2018), which comprises the histone methylase CAU1 and the transcription factor ANAC055, i.e., *CAU1-ANAC055* pathway. The ANAC055 which is encoded by *ANAC055* acts as a downstream component of *CAU1*, where water deficit stress suppresses the levels of *CAU1 RNA* and CAU1 protein leading to declined H4R3sme2 methylation of chromatin in the *ANAC055* promoter. The following elevation in *ANAC055* expression resulted in the induced expression of its genetically downstream gene *P5CS1* which results in Pro overproduction and drought tolerance. In addition to Pro biosynthesis and catabolism that regulate Pro build-up in plants, other factors like Pro transport, transcription factors, endogenous ABA, cellular redox status, and ROS play prime role in Pro accumulation under abiotic stresses (Lehmann et al. 2010; Kaur and Asthir 2015; Mansour and Ali 2017a; Zaratti and Forlani 2017).

12.2.2 Pro Transport

Pro transport between cells and intracellular compartments also most likely plays a role in Pro accumulation and regulation of its cellular level under normal and stress conditions. For example, Kishor et al. (2015) report that Pro transporters play a vital role in Pro homeostasis both under stress and normal conditions as well as in plant growth and development by supplying Pro as a source of nitrogen and energy. Three transporters of Pro (ProT1, ProT2, and AAP6) have been identified in *Arabidopsis thaliana* (Kaur and Asthir 2015). Moreover, the three Pro transporters were also identified in *Arabidopsis* and shown to be plasma membrane localized (Rentsch et al. 1996), confirming contribution in intercellular Pro transport. These Pro transporters are expressed during stressful conditions as reported by Rentsch et al. (1996) who found that *AtProT2* expression is greatly enhanced in *Arabidopsis* under salt and drought. The result highlights the role of Pro transporters in stress response and tolerance. These authors also showed that when *Arabidopsis* plants are exposed to salinity, ProT1 is highly expressed in roots, stems, and flowers. Also, Lehmann et al. (2010) have reported that Pro transporters are isolated from several plant species and their transcript levels increased under stress conditions, suggestive of their role in response to stresses. These authors also indicate that *atprot2* mutants were resistant to Pro levels showing toxicity, while lines overexpressing *AtProT1* and *AtProT2* were sensitive to supplied Pro, confirming their role in Pro uptake and transport. Despite NaCl-induced Pro biosynthesis in salt-tolerant grass (*Distichlis spicata*), cell suspensions was retarded in growth medium containing 5 mM Pro (Heyser et al. 1989). This work also revealed that the cells took up exogenous Pro which is indicative of Pro transport from external milieu to the cells. Supportive to Pro transport in the plant is the finding that shoot-supplied Pro enhances root Pro accumulation and root elongation under water deficit (Sharma et al. 2011). The result clearly suggests Pro transport from shoots to roots. Additionally, evidence for the presence of Pro transporters and their participation in Pro transport is the data that a rise in Pro transport occurs before its biosynthesis in barley because *HvProT* which encodes a Pro transporter is quickly enhanced in the root tissue 30 min after salt treatment (Ueda et al. 2001). Further, the important role of Pro transporters in modulating its endogenous level is provided by the finding that altered expression of barley Pro transporter (HvProT) resulted in the biomass reduction and decreased Pro accumulation in barley leaves (Ueda et al. 2008). Reduced growth of HvProT transformed barley plants was put back by the external addition of Pro, suggesting that growth reduction was brought about by endogenous Pro deficiency. Also, Pro overproduction in pollen enhanced by salt stress in tomato was not related to enhanced expression of *P5CS*, implying that other possibilities like Pro transport and ornithine pathway might be correlated with alterations in endogenous Pro levels (Fujita et al. 1998).

Pro is synthesized in the cytoplasm and chloroplasts, and to show its functions, it needs to be transported and accumulated over long and short distances which also suggest necessity of Pro transporters. As Pro synthesis occurs in the chloroplast and cytosol but its degradation takes place in the mitochondria, this also necessitates a need for intracellular movement of Pro. The existence of both a mitochondrial and chloroplast Pro transporters has been indicated by Kaur and Asthir (2015). Betaine/proline transporters (BvBet/ProT1) have been reported to be localized in the phloem and xylem parenchyma cells of sugar beet (Yamada et al. 2011), indicating a role in intercellular transport of Pro. As these transporters are expressed in response to stress conditions, salinity has been illustrated to induce Pro transporter in barley root tip (Ueda et al. 2001) pointing to a role in response to salinity. This transporter is similarly overexpressed in *Arabidopsis* root tip resulting in elevated Pro level of the root apex and induced growth under non-saline condition (Ueda et al. 2008). Long-distance Pro transport is important as well to move Pro from the photosynthetic tissue to non-photosynthetic tissue in order to participate in osmotic adjustment or in maintenance purposes. The finding that maize root elongation zone accumulates much more Pro than the root tip at low water potential (Kaur and Asthir 2015) strongly supports the export of Pro under stress. The observed differential expression patterns of these transporters in different plant organs are also suggestive of divergent transport of such molecules (Kishor et al. 2015). Besides Pro, they transport other solutes like glycinebetaine and γ-aminobutyric acid. For example, Pro transporters from tomato have been shown to transport glycinebetaine in addition to Pro (Slama et al. 2015). However, barley HvProT1 is found to recognize Pro but not glycinebetaine (Ueda et al. 2001).

12.2.3 Pro Stress Signaling

One aspect of the Pro participation in signaling is the Pro-rich proteins of the cell walls that are involved in the structural integrity of plant cells and play a major role in cell-cell communications and integrations (Kishor et al. 2015). The authors also show that Pro insufficiency influences the metabolism of cell wall matrix proteins. Cell wall protein transcripts are influenced under heat, drought, and salt stress conditions (Covarrubias et al. 1995), an obvious indication of their role in signaling. Also, Pro-rich extensin-like receptor kinases (PERKs) have been shown to participate in cell wall signaling. For instance, Bai et al. (2009) illustrated the requirement of the gene *PERK4* (coding for a protein kinase located in the plasma membrane) for the influx of Ca^{2+} which is ABA-dependent in seeds and roots of *Arabidopsis*, suggestive of PERK4 involvement in ABA perception and interaction with wall polymers. This finding suggests Pro-rich proteins' implication in cell wall signal transduction events. Moreover, transcription upregulation of both genes encoding antioxidant enzymes and those of *P5CS* and *P5CR* in rice varieties in response to high salinity are also signs for signaling function of Pro (Nahar et al. 2016). Pro exhibits also its protective role via cross-talks with other signaling pathways like ABA, ROS, and polyamines (Roychoudhury et al. 2015) pointing to Pro participation in signaling pathways and hence its involvement as a component of the abiotic stress response. Also, it is early reported by Hare and Cress (1997) that Pro intercellular and intracellular transport may make it serve as a metabolic signal, and hence Pro can be ascribed as a signaling molecule. It is clear that Pro via acting as a signal/regulatory compound is capable of activating numerous physiological, biochemical, or molecular responses under a wide array of environmental stresses.

The signaling mechanisms thereby environmental stress induces Pro biosynthesis in plants include several molecules such as ABA, calcium, and phospholipase C (Liang et al. 2013). For instance, Pro metabolism is required for ABA-mediated growth protection in plants under water deficit (Sharma et al. 2005). Furthermore, H_2O_2-mediated regulation of Pro biosynthesis and hence accumulation has been found in coleoptiles and radicles of maize seedlings, which was suggested to be through upregulation of P5CS and downregulation of PDH activity (Yang et al. 2009). It is worth mentioning that during Pro biosynthesis, the product P5C may lead to ROS production, which at high concentration induce a programmed cell death: electrons produced during Pro biosynthesis go to electron transport chain through PDH and then form superoxide anion and then H_2O_2 (Liang et al. 2013). Similarly, Kishor et al. (2015) report that in *p5cdh* mutants, PDH activity can result in P5C, hence altering cellular redox homeostasis which elevates ROS content. Pro effects on cellular processes may be therefore due, in part, to the numerous signaling roles of ROS. In support to that, it has been shown that ROS can act as important signaling molecules that trigger adaptive responses under different stresses (Liang et al. 2013). That is, low ROS generation leads to protective effects and involvement in signaling function, whereas high ROS production results in cell death. Consistent with the above, elevated ROS generation in response to exogenous Pro application most probably contributes to Pro deleterious effects resulting from high levels of Pro supplication to plants (Szabados and Savoure 2010), while maintaining a lowest level of ROS in cells is essential for cellular proliferation, physiological function, viability, and adaptation to stresses (Mittler 2017). It is clear that Pro modulates cellular functions under abiotic stress through its metabolism-mediated ROS generation that contribute in signaling pathways.

Table 12.1 Accumulation of Pro and its effects in different plant species under various abiotic stresses

Plant species	Stress	Pro accumulation and effect	Reference
Triticum aestivum	Salinity	High (osmotic adjustment)	Rady et al. (2019)
Oryza sativa	Salinity	High (osmotic adjustment)	Nounjan et al. (2018)
Pelargonium hortorum	Salinity	High (osmotic adjustment)	Bres et al. (2016)
T. aestivum	Salinity	High (greater in sensitive cultivar than tolerant ones)	Tavakoli et al. (2016)
Fragaria chiloensis	Drought	High (kept increasing in heat-sensitive cultivars, but decreased in heat-tolerant ones by rehydration)	Gulen et al. (2018)
Hordeum vulgare	Drought	High (high Pro was not correlated with drought tolerance)	Dbira et al. (2018)
O. sativa	Heat	High (although it further increased in heat-sensitive cultivar, it decreased in heat-tolerant ones)	Sánchez-Reinoso et al. (2014)
T. aestivum	Heat	Decline (Pro was not correlated with heat tolerance rather antioxidant enzymes)	Kumar et al. (2012)
Anoectochilus	Heat	High (osmotic adjustment and correlated heat resistance)	Mei et al. (2018)
O. sativa	Heat	High (Pro accumulation correlated with thermotolerance)	Ali et al. (2016)
T. aestivum	Heat	High (osmotic adjustment and Pro production correlated with heat resistance)	Khan et al. (2015)
Cucumis sativus	Heat	High (osmotic adjustment)	Ding et al. (2016)
Sorghum bicolor	Heat	High (osmotic adjustment)	Gosavi et al. (2014)
Prunus persica	Heat	High (growth resumption)	Shin et al. (2016)
Vigna aconitifolia	Heat	Varies with genotypes and not associated with heat tolerance	Harsh et al. (2016)
O. sativa	Chilling	High (greater in cold-sensitive genotype than tolerant one)	Aghaee et al. (2011)
Glycine max	Chilling	High (osmoprotectant)	Borowski and Michaáek (2014)
Cicer arietinum	Chilling	High (osmoprotectant, Pro is higher in chilling-resistant cultivars)	Tatar et al. (2013)
Arabidopsis thaliana	Chilling	High (protective function)	Khavari-Nejada et al. (2013)
Capsicum annuum	Chilling	High (more in cold-tolerant genotype, correlation between cold tolerance and increase of Pro)	KOÇ et al. (2010)
S. bicolor	Chilling	High (osmoprotectant, Pro is higher in chilling-tolerant cultivar)	Vera-Hernández et al. (2018)

(continued)

Table 12.1 (continued)

Plant species	Stress	Pro accumulation and effect	Reference
Juglans regia	Freezing	High (protective function, Pro concentration was reliable marker for cold hardiness)	Aslamarz et al. (2011)
A. thaliana	Freezing and drought	High (Pro was associated with stress tolerance)	Ren et al. (2018)
Vitis vinifera	Freezing	High (osmotic adjustment, Pro correlated with freeing tolerance)	Ershadi et al. (2016)

12.3 Pro Accumulation and Abiotic Stress Tolerance

Many investigations have showed that Pro accumulates in higher plant species in response to various abiotic stresses, leading to suggest Pro overproduction functions and correlation with stress tolerance (Table 12.1; Yadegari et al. 2007; Hossein and Parvane 2013; Shin et al. 2016; Bres et al. 2016; Mari et al. 2018; Nounjan et al. 2018). Different evidence reports that Pro accumulation could be due to hydrolysis of proteins, decreased degradation, de novo synthesis, or lower utilization. Pro correlation with stress resistance has been based on the findings that Pro accumulation in most studies has been reported to be frequently greater in stress-resistant species/genotypes under different stresses implying its implication as a stress mediator enhancing plant tolerance (Benitez et al. 2016; Ahmed et al. 2017; Torre-González et al. 2018; Akbari et al. 2018; Mei et al. 2018). For instance, higher Pro content was found in rice salt-tolerant lines than in sensitive one under NaCl treatment, which mediates high osmotic adjustment and hence salt tolerance (Nounjan et al. 2018). Also, among 32 species of *Anoectochilus* genus, high-temperature resistance was correlated with high Pro accumulation (Mei et al. 2018). Pro accumulation was also higher in groundnut salt-tolerant cell lines than in sensitive ones, and Pro was the primary solute contributing to osmotic adjustment under salt stress (Jain et al. 2001). Similarly, Pro overproduction was elevated with increased salt stress and was higher in salt-tolerant pistachio genotypes relative to sensitive ones (Akbari et al. 2018). Salt stress increased Pro level by 30% in pelargonium leaves, which was responsible for amelioration of the salt stress adverse effects (Bres et al. 2016). High salinity consistently caused higher Pro accumulation in tomato and lentil genotypes, more so in tolerant ones (Torre-González et al. 2018; Gaafar and Seyam 2018). In agreement, when ten sugar beet genotypes grown under high salinity, more tolerant genotypes performed well at EC 8 dSm^{-1}, their better performance was associated with production of leaf Pro and accumulation of less Na^+, more K^+, and high K^+/Na^+ ratio (Mari et al. 2018). More Pro accumulation has been suggested in this research to provide a better protection mechanism against oxidative damage under salt stress and not related to osmotic adjustment. In another study, increase in Pro level and antioxidant enzyme activities in the calluses of pumpkin genotypes was associated with a tolerance to chilling stress (Kusvuran et al. 2013). More evidence supporting the Pro accumulation role in improving plant tolerance to abiotic stresses comes from the study by Ahmed et al. (2017) where water and temperature stress increased

Pro accumulation which regulates leaf water potential and consequently improved the grain yield of wheat, the work highlighting the role of Pro as an osmotic adjustment agent. In the previous field study, Pro level was correlated with genotype tolerance to drought and temperature stress implying Pro role in inducing tolerance to drought and heat stress. In addition, growing the halophyte *Cakile maritima* under salt stress led to Pro accumulation which contributed to osmotic adjustment (36%) particularly in roots (Hmidi et al. 2018). The work also showed that OAT activity mainly serves to Pro synthesis in various organs of *C. maritima* under salt stress. Conversely, Pro amount in wheat seedlings cannot be considered either a symptom of the injury induced by various stresses (salt, water, heat) or a measure of the stress resistance in a study by Song et al. (2005). The study also demonstrated that acquiring cross-tolerance that leads to negative stress effect minimization does not directly correlate to Pro content and activities of P5CS, OAT, and PDH contribute to Pro biosynthesis. These findings lead the authors to propose that the change in Pro content acts most likely as a signaling component regulating gene expression and thus adjusts metabolic process. Also, Pro production in six rice genotypes differing in salt tolerance was insufficient to ensure osmotic adjustment, and therefore other protective mechanisms have been proposed to explain its beneficial effect in response to high salinity (Bertazzini et al. 2018). Similarly, Pro contributed only 0.3–2.0% of the total solute content in three halophytes and even less (0.04%) in *Arabidopsis*; rather, K^+ was the major osmolyte which confers 70–90% of the solute potential of the plants (Hajiboland et al. 2018). It is clear that Pro is not involved in osmotic adjustment and may have other protective or signaling functions in these three halophytes. This raises the question of Pro mode of action in protecting cells against stress conditions that clearly depends upon the accumulation processes.

Although the majority of studies relating Pro accumulation with stress tolerance are carried out under salt and drought stresses, positive correlation between Pro accumulation and improved chilling tolerance has been illustrated in various crops (Table 12.1). For example, cold-resistant almond and sorghum had greater Pro than the sensitive ones, which was correlated with cold resistance (Hossein and Parvane 2013; Vera-Hernández et al. 2018). Similarly, the elevation in Pro content of chilling-sensitive chickpea cultivars was lower than in chilling-resistant cultivars under chilling stress (Tatar et al. 2013), suggestive of Pro correlation with chilling tolerance. Pro accumulation in the leaves of soybean cold-acclimated seedlings consistently was also higher than non-acclimated seedlings, and even acclimated plants recovered faster than non-acclimated ones (Yadegari et al. 2007). In addition, Pro concentration in the leaves was higher in cold-tolerant pepper genotype relative to sensitive one under chilling stress; however, Pro level in the stem showed the opposite trend (Koç et al. 2010). The study reports also a correlation between the increase in Pro concentration and pepper cold tolerance. Also, Pro accumulation under cold stress was greater in cold-resistant Persian walnut genotypes than sensitive ones, and Pro concentration has been shown as a reliable marker for cold hardiness in this investigation (Aslamarz et al. 2011). In the same trend, Pro accumulation in chickpea leaves exposed to cold stress was higher in cold-resistant cultivar compared with sensitive one (Saghfi and Eivazi 2014). Furthermore, greater accumulation of free Pro under cold stress was reported in *Avena nuda*, a cold-tolerant plant species, which was partially accounted for the higher cold tolerance of the species (Liu et al. 2013). Amelioration of chilling stress by arginine in tomato fruits was also associated with increased Pro level (Zhang et al. 2013). Moreover, accumulation of Pro was involved in the caffeic acid-mitigated chilling stress in cucumber seedlings (Wan et al. 2015). Higher Pro generation in winter wheat cultivars than in spring ones is also observed under chilling stress, which is interpreted as Pro playing a key role in combating cold stress (Javadian et al. 2010). Furthermore, frost tolerance was significantly correlated with Pro content in winter wheat (Pecta and Terbea 1995). Similarly, high freezing tolerance of yeast cells was also correlated with higher levels of Pro, which acts as a cryoprotectant defending yeast cells from damage by oxidative stress (Terao et al. 2003). Based on the previous discussion, it is obvious that Pro generates in many plant species under different environmental stresses, and its overproduction is related with stress tolerance.

Other evidence relating higher Pro levels with stress tolerance under abiotic stresses is further reviewed in this section. For instance, higher Pro accumulation which resulted from increased activity of P5CS was observed in tolerant sorghum genotypes compared with susceptible ones in response to heat stress (Gosavi et al. 2014). Pro accumulation and antioxidant defense systems were also associated with thermotolerance in four rice cultivars (Ali et al. 2016). In addition, the increase in Pro content was similarly greater for the rice-tolerant genotype relative to sensitive one under salt and cold stresses (Benitez et al. 2016). In this investigation, the levels of transcripts of biosynthesis genes were more correlated with the Pro content in tolerant genotypes under high salinity, while at cold stress, this correlation in the sensitive genotype was higher. The work further revealed that both glutamate and ornithine pathways contributed to Pro accumulation in tolerant genotype under both stresses. The results also support the known controversy that Pro functions and production might vary for different stresses. Other researchers also indicate Pro differential accumulation as well as Pro metabolic enzyme regulation might be varying under various abiotic stresses (Wanner and Junttila 1999; Lv et al. 2011; Aghaee et al. 2011; Shin et al. 2016). For example, improved freezing tolerance in *Arabidopsis* leaves by a combination of cold and light was associated with soluble sugars and Pro accumulation (Wanner and Junttila 1999). The work indicates, however, that Pro begins to accumulate after approximately 24 h where plants have already obtained remarkably improved freezing tolerance. The authors hence suggest that Pro accumulation most likely has no role in improving freezing tolerance and it may play a role in longer-term adjustments to low-temperature-induced drought stress. In a study carried out by Khan et al. (2015), Pro was remarkably increased in heat-tolerant wheat cultivars and therefore Pro and membrane stability index were recommended by the authors as selection parameters in the breeding program for the development of heat-tolerant wheat cultivars. In agreement, heat shock elevated Pro accumulation in cucumber seedlings, and Pro involvement in osmotic adjustment was suggested as a potential resistance mechanism of plants exposed to heat stress (Ding et al. 2016). Furthermore, the capacity of sorghum genotypes to survive heat stress was strongly correlated with Pro overproduction

(Gosavi et al. 2014). Moreover, although total free amino acids were decreased, a significant rise in the Pro content in maize cultivars was also caused by heat stress, more so in tolerant cultivar (Hussain et al. 2016). Salicylic acid-induced freezing tolerance in spinach leaves was also associated with elevated Pro levels as well as other seven metabolites (Min et al. 2018). Pro accumulation in *Kosteletzkya virginica* seedlings treated with 300 mM NaCl was owing to the upregulated expression of *KvP5CS* (the key gene for Pro biosynthesis) and low expression of *KvPDH*, the key gene for Pro catabolism (Wang et al. 2015). In agreement, Pro accumulation in Jerusalem artichoke plantlets under salinity stress is primarily caused by activation of Pro synthesis via the glutamate pathway, increased HtP5CS2 enzyme activities and transcription while repressed HtOAT, and depression of its degradation via declining HtPDH activities and transcription (Huang et al. 2013). In addition, Li et al. (2013) show that hydrogen sulfide induced-heat stress tolerance in maize is correlated with Pro accumulation through higher P5CS activity, lower lipid degradation, and lower PDH activity. In contrast, varied responses in heat stress with reference to Pro accumulation have been reported. For instance, heat stress showed no Pro accumulation in tobacco and *Arabidopsis* plants (Dobra et al. 2010; Lv et al. 2011). The study of Lv et al. (2011) indicated that enhanced Pro production in chickpea rendered plants more sensitive to heat stress, possibly by induced ROS biosynthesis via the Pro-P5C cycle. To conclude, large uncertainties still exist regarding the Pro role in plant stress tolerance, which is further detailed below.

It is noteworthy that Pro accumulation is not necessarily to be associated with stress resistance (Table 12.1). For example, Sayyari et al. (2013) showed that salicylic acid amelioration of the injury caused by chilling stress in watermelon seedlings was via maintaining membrane integrity and inhibiting Pro accumulation. Also, Pro overproduction was not an indicator of cold hardiness in citrus cultivars (Yelenosky 1979). Further, Pro accumulation in cold-sensitive rice genotype was higher than cold-tolerant one in response to chilling stress (Aghaee et al. 2011). Similarly, exposure of mung bean seedlings to chilling stress decreased endogenous Pro level (Posmyk and Janas 2007). In this work, seeds hydroprimed with Pro barely improved seed germination and seedling growth of mung bean under chilling stress. Under high salinity, Furtana et al. (2013) report that no clear positive correlation has been observed between high salinity and the major osmolytes, including Pro, in three endemic *Limonium* species. Additionally, in salt-tolerant *Sarcocornia fruticosa* and *Inula crithmoides*, there were no significant seasonal variations of major osmolytes including Pro, suggestive of their constitutive tolerance mechanism which is relatively independent of external conditions (Vicente et al. 2016). Pro levels were also not high in these two species to play a role in osmotic adjustment. Tavakoli et al. (2016) similarly demonstrated that salt-sensitive wheat cultivar accumulated more Pro than salt-tolerant ones, although the growth and yield in salt-tolerant ones were greater. The research also showed that the tolerant cultivars had lower Na^+ content, higher K^+ level, and K^+/Na^+ ratio relative to the sensitive one. These results are suggestive of high Pro content correlation with salt sensitivity and even put the correlation with stress adaptation in doubt. Though diluted seawater significantly increased faba bean Pro and the activities of enzymatic antioxidants, foliar application of 25 mM Pro showed no significant changes in Pro concentrations and decreased enzymatic antioxidant activities compared with their controls (Dawood et al. 2014), indicative of other mechanisms possibly involved in mitigation of the adverse salt effects rather than Pro. The work also indicated that higher Pro concentration (50 mM) was toxic confirming the contention that Pro effects are dependent on the supplied concentrations and species. Moreover, Çelik and Ünsal (2013) demonstrate that salt tolerance is independent of Pro accumulation in soybean salt-tolerant mutant, although the *GmP5CS* expression level was increased in this mutant. In another study using two salt-tolerant monocots (*Juncus acutus* and *J. maritimus*) and two halophytic dicots (*Plantago crassifolia* and *Inula crithmoides*), a great inconsistency was observed in osmolyte contents of plants grown in natural field conditions and those grown in artificial conditions (Vicente et al. 2016). These observations lead the authors to argue that it is not plausible to compare such data sets. The authors present several reasons for their argument: “differences in plant developmental stages under both experimental conditions, and limited space with homogeneous salinity for roots grown in controlled potted plants relative to roots grown in the field where they can explore a more heterogeneous and remarkably greater soil volume and also encounter more than one environmental factor.” These conditions result in comparing samples from young plants with those from fully grown individuals, and diverse degree of stress could be considerably found between plants growing in natural field conditions and those subjected to controlled stress treatments. It is not plausible, therefore, to generalize the results ascribed Pro overproduction and roles in stress adaptation carried out in the growth chambers for agricultural practices until they are tested in natural field conditions. We therefore recommend to draw a correct final conclusion concerning the Pro roles and accumulation in plant tolerance to different environmental stresses to subject the data obtained from controlled conditions to experiments carried out under natural field environment.

Furthermore, despite some stress tolerance stimulants showed beneficial effect under different stress conditions, Pro levels have been decreased in various plant species pointing to absence of Pro involvement in stress resistance enhancement. For instance, although salicylic acid treatment induced drought tolerance, Pro content of lemongrass varieties was decreased (Idrees et al. 2010). Also, salicylic acid application decreased Pro production in shoots and roots of tomato plants under high levels of salinity (Shahba et al. 2010), although salicylic alleviated the stress injurious impacts. Consistently, Pro content was unaffected in maize cultivars under salt stress (Perveen et al. 2018). In the study of Perveen et al. (2018) even despite foliar application of sulfur-containing compounds improved salt tolerance, Pro accumulation decreased in both maize cultivars. Although biostimulants enhanced maize performance and increased total free amino acids under salt stress, Pro was not increased and thus plays no role in elevated maize salt tolerance (Abdel Latef et al. 2019). Harsh et al. (2016) showed that Pro accumulation in 37 genotypes of moth bean (*Vigna aconitifolia*), contrasting in heat tolerance, had no association with heat tolerance. Furthermore, Pro generation under heat stress in winter-acclimated shoots of *Prunus persica* was correlated with growth resumption and not high temperature-induced Pro accumulation (Shin et al. 2016). The study also suggests that the ornithine pathway, rather than glutamate pathway, could be the primary Pro metabolic pathway both during growth resumption and heat stress in peach. The results highlight the known controversy for contribution of ornithine pathway to Pro biosynthesis and accumulation under various abiotic stresses. Also, Suprasanna et al. (2016) report that Pro content remained unchanged or does not seem to accumulate; instead, carbohydrate, K^+, or glycinebetaine accumulates under different stresses. Although Pro production was greater in salt-sensitive wheat cultivar, salt-tolerant cultivars performed well and showed higher level of K^+ and K^+/Na^+ ratio and lower level of Na^+ under saline conditions (Tavakoli et al. 2016). This work also illustrated that salt-tolerant cultivars had higher Pro degradation in the fourth leaf which was proposed by the author as a source of energy or nitrogen provision to increase yield production of these cultivars in response to high salinity. Similarly, the results of Dbira et al. (2018) put doubt on using Pro as a marker of drought tolerance in nine barley genotypes. The work clearly reports that higher Pro content in water-stressed plants has no a major role in the better barley response to drought, as the most tolerant genotypes had lower content of Pro. In accordance, although Pro content increased in three rice cultivars as the temperature increases, Pro content decreased in heat-tolerant cultivars whereas further increased in heat-sensitive one (Sánchez-Reinoso et al. 2014), suggesting that absence of Pro role at prolonged stress periods and Pro might correlate with stress sensitivity. Also, Pro level remarkably declined at different stages of growth in response to heat stress and hence was not correlated with heat tolerance in wheat cultivar; rather, antioxidant enzymes were (Kumar et al. 2012). In the same trend, Pro is considered as a symptom of injury in tobacco varieties under saline conditions (Çelik and Atak 2012) as sensitive variety showed higher Pro content. Consistently, salt-sensitive rice genotypes were reported to produce more Pro than tolerant genotypes under salt stress (Demiral and Turkan 2005; Çelik et al. 2019). In agreement, less salt-tolerant cultivated barley (*Hordeum vulgare*) accumulated remarkably more Pro than halophile wild barley (*H. maritimum*) in response to high salinity (Ferchichi et al. 2019). Furthermore, Pro content was increased in four strawberry cultivars, contrasting in heat tolerance, by drought application and kept increasing linearly in heat-sensitive cultivars while it was decreased in heat-tolerant cultivars (Gulen et al. 2018). These conflicting reports concerning the Pro accumulation, functions, and implication in plant stress resistance under various stress conditions put the generally accepted Pro roles in question, not to be conclusive and arguable as well.

12.4 Pro Functions in Abiotic Stress Tolerance

Pro quantity is one of the crucial mechanisms for plant adaptation to abiotic stresses. The mechanisms of how Pro protects plants against stress, however, are not fully understood. Table 12.2 summarizes Pro functions showed in response to its exogenous application in different plant species under various abiotic stresses. Pro

Table 12.2 Exogenous supplied Pro affecting stress tolerance of various plant species in response to abiotic stresses

Species	Pro concentration, duration, and stress imposed	Effects	Reference
Saccharum officinarum	20 mM, 8 h, heat stress	Reduced ROS generation; increased K^+ and Ca^{2+} levels and Pro and soluble sugar accumulation. Enhanced heat tolerance	Rasheed et al. (2010)
Zea mays	30, 60 mM, 24 h, drought stress	Enhanced growth, stomatal conductance, level of photosynthetic pigments, and in turn photosynthetic rate. Improved drought tolerance	Ali et al. (2007)
Beta vulgaris	5, 10 mM, 16–18 days, drought stress	Upregulated enzymatic antioxidants, increased Pro content, downregulated H_2O_2 and lipid peroxidation, and maintained membrane integrity	Ghaffari et al. (2019)
Z. mays	30 mM, salt stress	Increased antioxidant defense systems and K^+ and decreased Na^+, Cl^-, Pro, and P5C. Pro is used as a nitrogen source and improved salt tolerance	de Freitas et al. (2018)
Triticum aestivum	12 mM, 6 h, salt stress	Increased Pro level, biomass, yield, and antioxidant enzyme activities. Induced salt tolerance	Rady et al. (2019)
Pisum sativum	4 mM, five times with 10-day intervals, drought stress	Enhanced antioxidant defense systems, and synthesis and transport of metabolites from source to sink. Improved drought tolerance	Osman (2015)
Cicer arietinum	10 µM, 24 h, heat stress	Reduced cellular injury; protected enzymes involved in carbon and oxidative metabolism. Imparted heat tolerance	Kaushal et al. (2011)
Brassica napus	1 mM, 96 h, freezing stress	Increased Pro and ethylene levels. Enhanced freezing tolerance	Gavelienė et al. (2014)
Arabidopsis thaliana	5 mM, 1 h, oxidative stress	Pro ROS scavenging as it reduces their levels and reduced ROS-induced K^+ efflux. Mitigated oxidative stress	Cuin and Shabala (2007)
Oryza sativa	5, 10 mM, 30 days, salt stress	Increased plant height, root number, root nitrate, and activities of nitrate reductase and glutamine synthetase. Mitigated salt adverse effects	Teh et al. (2016)
Vicia faba	25, 50 mM, three times with 7-day intervals, seawater stress	Increased growth, photosynthetic pigments, mineral content, K/Na ratio, and soluble carbohydrates Decreased Na, Cl, phenols, free amino acids, Pro, and antioxidant enzyme activities. Mitigated salt-induced damaging effects. 50 mM was toxic	Dawood et al. (2014)

(continued)

Table 12.2 (continued)

Species	Pro concentration, duration, and stress imposed	Effects	Reference
Arachis hypogea	50, 100 mM, 15 days, salt stress	Alleviated the salt stress-induced decline in fresh weight; reduced oxidative damage of the membrane lipids. Pro high concentration was toxic	Jain et al. (2001)
Vigna radiata	5 mM, 6 h, chilling stress	Improved seed germination and growth, protected against lipid peroxidation, stabilized membranes, and mitigated chilling stress adverse effects	Posmyk and Janas (2007)
O. sativa	20, 30, 40, 50 mM, 2.5, 24, 48, 72, 96, 120 h, salt stress	Induced germination, growth, and K^+/Na^+ ratio and alleviated the salt effects	Roy et al. (1993)
Saccharum officinarum	20 mM, 24 and 72 h, salt stress	Increased Pro content, activities of antioxidant enzymes, and reduction in Na^+ and maintained membrane integrity in salt-tolerant genotype	Medeiros et al. (2015)
B. napus	5, 10, 15, 20 mM, 1, 3, 5, 7 days, cold stress	Increased endogenous Pro, protected membranes, and improved shoot cold tolerance	Jonytienė et al. (2012)
Larix leptoeuropaea *Picea sitchensis* *Quercus robur*	1, 10, 100 mM, 14 days, cold and salt stresses	Increased endogenous Pro, stimulated growth, maintained internal K^+ content, and protected against the three stresses	Gleeson et al. (2004)
Solanum melongena	10, 20 mM, 15 days, salt stress	Counteracted the NaCl-induced decrease in fresh mass of both cultivars. Improved water use efficiency in one cultivar only. Pro, however, had no role in mitigating NaCl impacts on other growth and physiological responses of both cultivars	Shahbaz et al. (2013)
Zea mays	10, 20 mg L^{-1}, six times during the flowering stage, seawater stress	Increased endogenous Pro, total soluble proteins, and glutamine synthetase activities and decreased Pro oxidase and oxidoreductase activities. High concentration was toxic	Kahlaoui et al. (2018)
Lupinus termis	3, 6, 9 mM, twice: 35, 50 days after sowing, salt stress	Increased growth, endogenous Pro, photosynthetic pigments, total soluble sugars, anatomical traits, and yield. Pro at 6 mM was more effective	Rady et al. (2016)

functions mainly ascribed to its role as osmolyte contributing to osmotic adjustment under stresses, although other studies indicate that Pro accumulation in a range of plants is not sufficiently high to have a significant participation in osmotic adjustment of the cells, and hence other protective functions have been proposed (Mansour and Salama 2004; Ashraf and Foolad 2007; Liang et al. 2013; Mansour and Ali 2017a). It is noteworthy that Pro at such low concentrations improves plant adaption

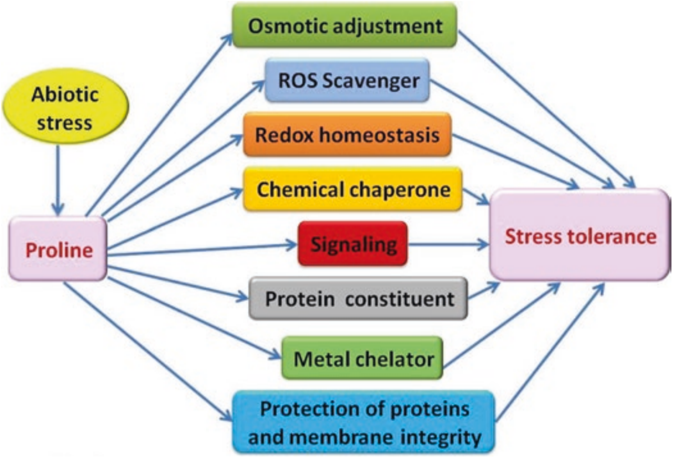

Fig. 12.2 Pro physiological functions that may protect plants from stress-induced adverse effects and hence improved stress tolerance

in a range of different environmental stresses. In addition to Pro role as an osmotic agent, Pro protective mechanisms have also been proposed to involve protein and membrane stabilization, antioxidant activity and thus has direct scavenging of ROS, detoxification system activation, protein precursor, intracellular redox homeostasis (e.g., ratio of $NADP^+$/NADPH and GSH/GSSG), metal chelator, energy source for the stress recovery, and cellular signaling promoted by Pro metabolism (Fig. 12.2). Assessment of Pro functions under stress therefore has been extensively reviewed (Mansour and Ali 2017a). These potential mechanisms thereby Pro counteracts stress injurious effects are discussed below.

12.4.1 Osmotic Adjustment

Pro acts as an osmolyte to essentially facilitate osmotic adjustment which in turn maintains continuous water absorption and hence enables the plant cells to avoid dehydration that may be caused by different stresses like salinity, drought, heat, and cold (Jain et al. 2001; Bres et al. 2016; Hmidi et al. 2018; Nounjan et al. 2018). Accumulation of Pro under stress declines the cellular solute potential and thus restores water potential gradient between the soil and plant to ensure water uptake. The essential role of osmotic adjustment comes from the fact that osmotic adjustment is critical for maintaining cell turgor which is required for plant growth and productivity under stress conditions. For example, Nounjan et al. (2018) demonstrate that higher Pro content in salt-tolerant rice cultivar is involved in osmotic adjustment. Also, accumulation of Pro was the primary cytoplasmic osmoticum responsible for the decline in the solute potential in groundnut salt-tolerant cell lines under high salinity (Jain et al. 2001), confirming Pro involvement in the osmotic adjustment. Similarly, Pro overproduction essentially participates in pelargonium osmotic adjustment in response to saline conditions (Bres et al. 2016). Pro usually overproduces in the chloroplast stroma and in the cytosol which is balanced by ions and other solutes in the cell vacuole (Verslues and Sharma 2010). In support, elevated Pro content in tobacco leaves does not disturb the chloroplast and mitochondria ultrastructure under normal and water deficit conditions (Borgo et al. 2015). These authors show, however, that high Pro production induced by water deficit in transgenic tobacco plants had no association with osmotic adjustment; rather, it acts as a repository of energy and nitrogen which are put to use under stress conditions. A finding similarly supports Pro role as a protectant and not included in osmotic adjustment is the work of Ain-Lhout et al. (2001). When *Halimium halimifolium* and *Pistacia lentiscus* are subjected to both natural and artificial water stress, *H. halimifolium* accumulated Pro only upon severe dehydration, while Pro accumulation in *P. lentiscus* showed gradual increase under either drought or cold stress (Ain-Lhout et al. 2001). These results indicate that in *P. lentiscus*, Pro likely has a role in stress tolerance as having a protective function rather than an osmotic agent and under severe abiotic stress. It appears that although Pro major contribution has been shown to correlate with osmotic adjustment under a variety of stresses, the role that does not hold true in several studies.

12.4.2 ROS Scavenging

Abiotic stresses have been reported to induce oxidative stress, and minimizing the ROS level is crucial to protect the cells from their damaging effects at high concentrations. The equilibrium between ROS production and scavenging is necessary to establish a low content of ROS that has beneficial effects. Studies have demonstrated that Pro generation in plant species in response to a variety of abiotic stresses is positively associated with oxidative stress tolerance (Ashraf and Foolad 2007; Nounjan et al. 2018). Pro has been also found to play a vital role in ROS scavenging via acting as a direct scavenger since Pro has the ability to directly react with ROS or via inducing the activities and transcription of antioxidant enzymes under stress conditions (Smirnoff and Cumbes 1989; Liang et al. 2013; Mansour and Ali 2017a). Consistent with that, *Arabidopsis p5cs1* knockout mutants showed Pro synthesis reduction, increased sensitivity to salt stress, and exhibited ROS accumulation (Székely et al. 2008), suggesting that Pro either protects the detoxification enzymes or enhances their activities during stress. In addition, the antioxidant enzyme activities were drastically greater in the presence of Pro and thus may protect the *Pancratium maritimum* plants against oxidative damage induced by NaCl stress (Khedr et al. 2003), suggesting antioxidant enzyme protection by Pro. Also, Pro application mitigated the deleterious effects of salinity on olive plants by plant water status maintenance and better photosynthetic activity, elevated soluble sugars, and enhanced both enzymatic and non-enzymatic antioxidants (Ahmad et al. 2010).

The role of Pro as a ROS scavenger therefore makes Pro a potent protectant of different plant species against abiotic stress injurious impacts and hence greatly contributes to stress tolerance. External supplication of Pro enhanced the activities of antioxidant enzymes in a wide range of plant species under stresses, which is believed to contribute to ROS scavenging and increased stress tolerance (Gavelienė et al. 2014; Osman 2015; Teh et al. 2016; Rady et al. 2019). Similarly, overexpression of Pro biosynthetic genes in transgenic plant species induced different abiotic stress tolerance via inducing antioxidant defense systems that detoxify ROS (Kumar et al. 2010; Wang et al. 2014; Yang et al. 2016; Ren et al. 2018). Moreover, protective effects of Pro have been proposed in rice genotypes under salt stress: ROS scavenger and thus reduces lipid peroxidation, and acting as molecular chaperone because it protects protein integrity and enzyme activities (Bertazzini et al. 2018). Pro overproduction in response to environmental stresses might therefore overcome the injurious impacts of oxidative stress either by direct scavenging of ROS or by enhancing antioxidant defense systems.

12.4.3 Redox Buffering

Pro metabolism can affect the intracellular redox status through proline-P5C cycle which can maintain proper $NADP^+$/NADPH ratios in the cytosol (Liang et al. 2013). Pro metabolism produces $NADP^+$, whereas Pro degradation generates NADPH. Pro synthesis and catabolism thus mediate a balance of intracellular redox homeostasis and energy production via maintaining the ratio of $NADP^+$/NADPH and GSH/GSSG relevant for cellular metabolic processes. It is evident that redox cycling is one of plant antioxidant defense systems under stresses (Phang et al. 2012; Liang et al. 2013). For instance, Phang et al. (2012) provide a detailed review on how proline-P5C cycle regulates redox homeostasis (Fig. 12.3). In brief, $NADP^+$ produced by Pro metabolism may result in pentose phosphate pathway promotion, which supports energy production and biosynthesis of cellular key molecules. In chloroplasts, $NADP^+$ generated from Pro metabolism may refill depleted $NADP^+$ pools resulting from stress-induced Calvin cycle inhibition. Sufficient levels of $NADP^+$ are important to ensure electron transfer to the electron transport chain, hence minimizing ROS generation during stress. NADPH is used for glutathione (GSH) and thioredoxin antioxidant systems and also for reductive biosynthesis pathways (Fig. 12.3). Consistently, increased biosynthesis of Pro in response to drought or salt stress has been suggested as a mechanism to relieve cytoplasmic acidosis and keep $NADP^+$/NADPH ratio well suited with cellular metabolism (Hare and Cress 1997; Nahar et al. 2016). Hare and Cress (1997) propose that the cycle of Pro metabolism in the cytosol and Pro oxidation in the mitochondria can supply a redox buffer system that facilitates specific metabolic pathways during and after stresses, which may be more important than Pro accumulation. Moreover, Hare and

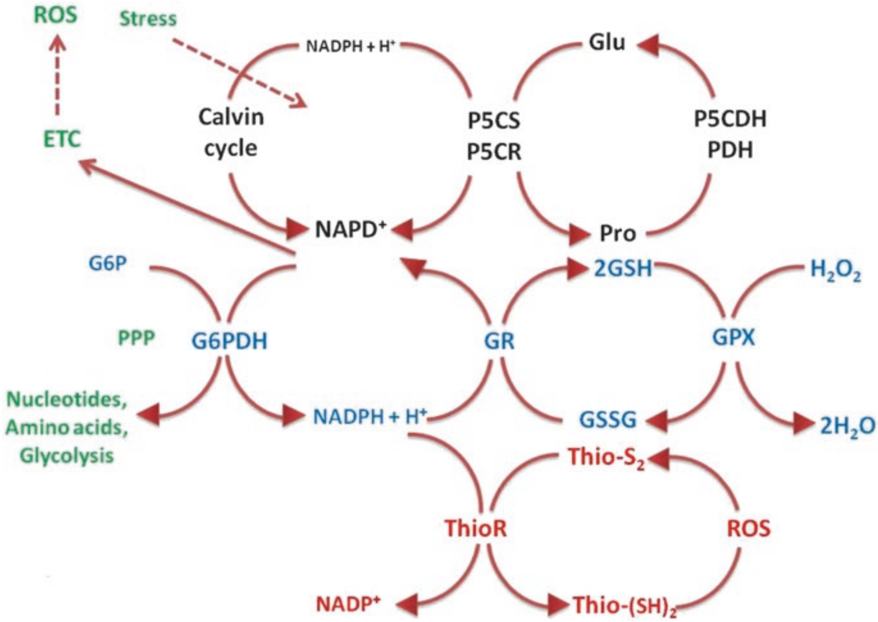

Fig. 12.3 Pro biosynthesis regulates redox homeostasis and energy production through NADP$^+$/NADP. *ETC* electron transport chain, *Glu* glutamate, *GR* glutathione reductase, *G6PDH* glucose-6-phosphate dehydrogenase, *G6P* glucose-6-phosphate, *GPX* glutathione peroxidase, *PPP* pentose phosphate pathway, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *ThioR* thioredoxin reductase, *Thio-S_2* oxidized thioredoxin, *Thio-$(SH)_2$* reduced thioredoxin. Dashed arrows indicate inhibition. (Adapted from Liang et al. (2013) with modifications)

Cress (1997) indicate that oxidation of NADPH was increased by Pro metabolic cycling in soybean nodule and thus stimulates oxidative pentose phosphate pathway, which enhances nucleotide biosynthesis during stress recovery. These authors also report that NADP$^+$/NADPH ratio has been declined under different stress conditions due to Calvin cycle inhibition. Preserving sufficient levels of NADP$^+$ is therefore crucial for electron transfer. Otherwise, $^1O_2$ will be generated under high light conditions, but high light also enhances *P5CS* expression which increases Pro biosynthesis and NADP$^+$ and ultimately lowers $^1O_2$ accumulation in the chloroplasts (Székely et al. 2008). In addition, Liang et al. (2013) indicate that increased Pro synthesis by manipulating its metabolic enzyme expression affects NADP$^+$ levels in plants and hence buffering intracellular redox potential. Furthermore, enhanced salt tolerance by high Pro was correlated with maintaining the glutathione redox state (Hoque et al. 2007), supporting Pro acting as a redox buffer. It is clear that Pro metabolic cycling participates in redox buffering by modulating the NADP$^+$/NADPH ratio and GSH/GSSG ratio in plants.

12.4.4 Metal Chelator

Chelation of metals is another proposed mechanism thereby Pro can protect cells against stresses. That is, Pro can protect cellular structures such as enzymes from heavy metals' inhibitory effect by forming complexes between Pro and metals (Aslam et al. 2017). Pro-metal complexes that bind heavy metals like zinc, cadmium, and copper with Pro are demonstrated under heavy metal stress (Liang et al. 2013). It is known that heavy metal stress induces ROS generation attacking cellular macromolecules (Liang et al. 2013). Therefore, chelating heavy metals with Pro diminishes their toxicities as enzyme inhibitors and oxidizing agents. Enhanced Pro production was observed in response to heavy metals like Zn and Cu in *Triticum aestivum* (Bassi and Sharma 1993) which suggests a possible role of Pro in chelating these metals. Metal-tolerant genotypes have been also shown to accumulate high Pro levels, relative to sensitive ones, under heavy metal stress which may similarly suggest a Pro role in metal chelation (Aslam et al. 2017).

12.4.5 Chemical Chaperone

One of the functions that Pro may achieve to prevent plants from being damaged by stress is to act as a molecular chaperone. When Pro acts as a molecular chaperone, it maintains and stabilizes the molecular structure of membranes and proteins, thereby protecting them and in turn cells from stress injurious effects. For instance, high Pro levels improve salt tolerance of the halophyte *P. maritimum* by enzyme stabilization and detoxification and maintaining protein turnover machinery (Khedr et al. 2003). Pro efficiently and directly stabilizes the plasma membranes under saline conditions through direct binding to the plasma membranes in such a way resulting in maintaining their integrity (Mansour 1998). In this work, maintenance of the plasma membrane integrity by Pro was indirectly probed by measuring its permeability under saline and non-saline conditions. Pro has been also shown to protect the plasma membranes by enhancing antioxidant enzyme activities which lower membrane lipids' and proteins' oxidation triggered by stress-induced oxidative injury (Mansour 2014; Mansour et al. 2015). Due to its chaperone properties, Pro can protect and induce antioxidant defense systems which work against oxidative stress and in turn enhances and stabilizes redox enzymes, photosynthetic apparatus, and subsequently stress tolerance enhancement (Ashraf and Foolad 2007; Hoque et al. 2007; Liang et al. 2013). Another mechanism by which Pro stabilizes protein structures is by burying the peptide backbone and maintaining protein folding (Liang et al. 2013). Moreover, when the cellular water content decreases in response to various stresses, Pro can act as a water substitute to stabilize cellular structure through hydrophilic interactions and hydrogen bonding. Thus, it forms a hydration shell around delicate proteins and averts their deterioration under stressful conditions (Liang et al. 2013). The above mentioned evidence are different facets thereby Pro can perform molecular chaperone and protect cellular macromolecules and consequently contributes to improving plant stress tolerance.

12.4.6 Signaling Molecule

Evidence indicates that Pro production could be also part of the stress signal regulating adaptive processes. Pro may act as a component of signal transduction cascades that influence redox homeostasis and stress responsive genes, thereby improving the tolerance to stresses (Szabados and Savoure 2010). These authors also report that when Pro acts as a signal molecule, it can modulate mitochondrial function and influences cell proliferation and death. Liang et al. (2013) also provide evidence that Pro metabolism promotes Pro as a cellular signaling molecule and as a metabolite greatly affecting plant growth, development, and flowering. In addition, Hare and Cress (1997) proposed that Pro most likely acts as a regulatory signal besides its role as an osmolyte. It can be thus speculated that Pro metabolism is modulated under stress by several and complex regulatory pathways leading to Pro functioning as a stress signal and/or a component of signal transduction cascades and hence can profoundly influence cell survival and death in plants.

At the same time, Pro metabolism can also influence signaling pathways by inducing ROS production in the mitochondria via electron transport chain (Zhang and Becker 2015; Liang et al. 2013). ROS are known as important physiological signaling molecules that trigger adaptive and survival responses. As Pro metabolism leads to increased internal ROS, many Pro effects on cellular processes may be due, in part, to the numerous signaling roles of ROS (Zhang and Becker 2015; Liang et al. 2013). Phang et al. (2012) consistently provide evidence for Pro metabolic signaling via ROS. For example, increased mitochondrial ROS in *p5cdh* mutant of *Arabidopsis* plants in response to Pro application was suggested to be due to enhanced proline-P5C cycling (Miller et al. 2009). It is also reported that induced ROS accumulation resulted from Pro application contributing to Pro toxicity that is often observed in plants treated with high levels of Pro (Liang et al. 2013). On the other hand, P5CDH is considered as an important regulator of ROS generation via regulating flow through the proline-P5C cycle to prevent mitochondrial ROS production. More research is needed, however, to further elaborate the molecular basis of Pro signaling.

12.4.7 Source of Energy, Carbon, and Nitrogen

Evidence indicates that Pro may have a substantial role in plants as a valuable source of energy and nitrogen/carbon either in stress acclimation or in recovery after stress relief (Kaur and Asthir 2015). As Pro is a good way to store carbon and nitrogen, rapid oxidation of Pro after stress is an excellent way to supply nitrogen and carbon reserves for growth after stress relief (Hare and Cress 1997; Kaur and Asthir 2015). The authors also report that oxidation of one molecule of Pro can provide 30 ATP equivalents, indicating that Pro is an excellent means to store energy. Additionally, Amini et al. (2015) provide evidence that Pro piles up in numerous plants under different environmental stresses and acts as a major repository of nitrogen and energy, which can be consumed under stress conditions. Also, Pro metabolism and catabolism participate in maintaining the cellular redox balance which is required for efficient plant flowering and seeds. The Pro impact is through transporting carbon, nitrogen, and reducing agents to flowers and seeds (Amini et al. 2015), highlighting the essential role of Pro to provide plants with energy during the reproductive stage. In addition, Pro and P5C contents in leaves drop dramatically in response to Pro foliar and saline treatment suggesting Pro employment in the reduction of salt effects by providing an additional nitrogen source to maize plants (de Freitas et al. 2018). Pro is also a key constituent of many cell wall proteins (extensins, arabinogalactan proteins, and hydroxyproline- and proline-rich proteins) that play crucial roles in cell wall signal transduction pathways, plant growth and development, as well as stress tolerance (Kishor et al. 2015).

12.5 Exogenous Pro Application Mediates Abiotic Stress Tolerance

Pro has been supplied to various plant species to assess its capability to overcome the deleterious impacts of environmental challenges. A large body of data show that Pro exogenous application improved performance and stress tolerance of numerous plant species when subjected to various abiotic stresses (Table 12.2). For example, pretreatment of sugarcane buds with Pro improved heat resistance of the buds, which was related to reduced reactive oxygen species generation, improved K^+ and Ca^{2+} level, and increased Pro and soluble sugar accumulation (Rasheed et al. 2010). Consistently, foliar Pro supplication improved maize salt tolerance via decreased Na^+ and Cl^- and increased K^+, enzymatic, and non-enzymatic antioxidants (de Freitas et al. 2018). Interestingly, although the Pro level used in this work was relatively high (30 mM), the Pro and P5C contents in leaves decline dramatically after 14 days of salt and Pro treatments, suggestive of Pro and P5C contribution as a nitrogen supply which was confirmed by increasing nitrogen containing compound levels. These nitrogen containing compounds appear to be involved in osmotic adjustment under salt stress. Moreover, foliar application of Pro to sugar beet plants exposed to drought stress upregulated antioxidant enzyme activities and increased Pro production leading to reduced H_2O_2 and lipid peroxidation, which results in protection of the cell membranes (Ghaffari et al. 2019). Exogenous Pro application also significantly increased its level in cold-acclimated and non-acclimated rapeseed seedlings, more so in cold-acclimated seedlings under freezing stress (Gavelienė et al. 2014). The Pro increase is proposed by the authors as an indicator of freezing tolerance. In addition, Pro exogenously applied to the roots of *Arabidopsis* reduced both ROS level and ROS-induced K^+ efflux (Cuin and Shabala 2007). When Pro was externally supplied to tobacco suspension cultures, antioxidative enzyme activities were enhanced under salt stress (Hoque et al. 2007). The study also revealed that higher supplied Pro concentrations were not beneficial. Both studies confirm the Pro potential role to scavenge ROS, but when applied at relevant concentrations. Roy et al. (1993) similarly reported that Pro at low concentrations mitigated the deleterious impacts of high salinity in rice seedlings, whereas higher concentrations were toxic and reduced seedling growth. Pro treatment enhanced leaf water relations, photosynthetic activity, and antioxidant system in olive tree under high salinity (Ben Ahmed et al. 2010). Further support is the results of Nounjan et al. (2012) who indicate that external supply of Pro increased its accumulation, lowered Na^+/K^+ ratio, and enhanced P5CS activity and P5CR transcript levels. The study revealed also that Pro decreased the antioxidant enzyme activities which suggest that ROS induced by salt stress possibly does not reach an injurious extent and hence may contribute to signaling cascades and thus have a role in improving salt tolerance. Further, chilling stress adverse effects were alleviated in mung bean (*V. radiata*) by Pro hydropriming (Posmyk and Janas 2007). Exogenous Pro similarly improves seed germination and growth, protects seedlings against lipid peroxidation, stabilizes membranes, and hence mitigated chilling stress deleterious effects. Moreover, Teh et al. (2016) found that supplementation of Pro in the growth medium of two rice cultivars resulted in increased plant height, number of roots, root nitrate, and activities of nitrate reductase and glutamine synthetase, and therefore, the ability of Pro to alleviate the salt-induced deleterious effects was correlated to changes in nitrogen assimilation activities. Based on the above, it is obvious that Pro application at relevant concentrations showed many induced beneficial effects under stress conditions.

Several studies indicate that Pro supplication counteracts not only the adverse effects of water and salt stresses but also those noted under other stresses. Pro protection and stabilization of metabolic enzymes have been reported by Dionisio-Sese et al. (1999) as stabilization of RuBisCO activity against heat stress inactivation was shown in two rice cultivars by Pro application. Consistently, exogenous application of Pro to heat-stressed (45/40 °C) chickpea enhanced Pro accumulation and less membrane damage, elevated chlorophyll and water contents, enhanced levels of enzymatic and non-enzymatic antioxidants, and improved growth at this stressful temperature (Kaushal et al. 2011). The results clearly show that Pro protects carbon and antioxidative metabolism vital enzymes and has an ameliorative effect against high temperature. Nagesh and Devaraj (2008) similarly found a rise in Pro level in French bean growing even at 46–48 °C. Moreover, Pro applied to bread wheat (6 mM) and to lentil (2 mM) reduced drought deleterious effects, improved growth and cell turgor, and reduced membrane damage on both species; which is due to increased internal Pro accumulation and enhanced antioxidant enzyme activities (Bekka et al. 2018). Furthermore, exogenous application of Pro increased its endogenous content, protected membranes, and improved shoot cold tolerance in winter rapeseed plants (Jonytienė et al. 2012). In accordance, when Pro was added to embryogenic cultures of different forest species, protection of these species against cold, freezing, and salt stresses was reported (Gleeson et al. 2004). External Pro supplication in this research increased endogenous Pro which stimulated growth and maintained internal cellular K^+ under stress conditions. Further evidence supporting the ability of exogenous Pro in alleviating the damaging impacts of environmental stresses is the report that external applied Pro also induced Pro accumulation, *P5CS* and *P5CR* transcripts, as well as transcription of genes encoding several antioxidant enzymes in various plants (Nahar et al. 2016). In the same trend, exogenous application of Pro enhanced drought tolerance of maize through enhanced growth, stomatal conductance, photosynthetic pigment contents, and in turn photosynthetic rate when grown under drought stress (Ali et al. 2007). Also, external application of Pro increased its endogenous content in sugarcane genotypes under NaCl stress, more so in tolerant one (Medeiros et al. 2015). The work showed also a greater elevation in antioxidant enzyme activities, Na^+ reduction, and maintained membrane integrity in salt-tolerant genotype relative to salt-sensitive one in response to Pro and salt treatment. In addition, Pro pretreatment contributes to protecting wheat seedlings against NaCl stress by enhancing the activities of antioxidant enzymes and hence mitigating the oxidative stress induced by salt stress (Rady et al. 2019).

Other studies, however, indicate that optimal Pro concentration used for external application under various abiotic stresses shows broad variations and is dependent on species and genotype. This needs to be taken into account when applying this approach that seeks to improve stress tolerance. In one work, Pro applied at 20 or 30 mM alleviated salt-induced deleterious effects in rice seedlings, whereas at 40 or 50 mM, Pro showed toxic effects and reduced seedling growth (Roy et al. 2014). Similarly, Pro low concentration (50 mM) added to the culture medium effectively ameliorated the decline in fresh mass and reduced membrane oxidative damage induced by salt stress in groundnut, whereas high concentration (100 mM) was toxic (Jain et al. 2001). In addition, Pro application mitigated salt-induced damaging effects in *Vicia faba* by increasing growth, photosynthetic pigments, N, P, K, Ca^{2+}, K^+/Na^+ ratio, and soluble carbohydrates and decreased Na^+ and Cl^- content (Dawood et al. 2014). It is noteworthy that Pro external supply under seawater in this work showed non-significant changes in its endogenous level and decreased the activities of antioxidant enzymes, pointing to a wide range of variable effects of Pro in response to stress conditions. Moreover, when Pro is applied in high concentration (50 mM), it was toxic to *V. faba* plants. In a field experiment recently conducted by Kahlaoui et al. (2018), foliar application of Pro strongly reduced the damaging effects of seawater treatment and improved salt tolerance of two tomato cultivars. It is important to note that Pro accumulation was a major factor participating in the induced *V. faba* salt resistance and also lower Pro concentration (10 mg L^{-1}) was only effective, whereas higher concentration (20 mg L^{-1}) was not efficient. In agreement, among foliar application of three levels of Pro (3, 6, 9 mM), 6 mM Pro showed the greatest effect in enhancing salt resistance of lupine cultivars under saline conditions (Rady et al. 2016). When two eggplant cultivars are subjected to exogenous Pro (10, 20 mM), applied Pro alleviated the deleterious effects of high salinity on shoot fresh mass of both cultivars and improved water use efficiency in one cultivar only (Shahbaz et al. 2013). The research also showed that Pro was not efficient in counteracting the adverse impacts of salinity on the growth, net CO_2 assimilation rate, photosystem II efficiency, and shoot and root K^+ and Ca^{2+} of eggplant cultivars under saline and natural conditions. The study argues against the proposed common Pro roles in countering the abiotic stress undesirable impacts. Unfortunately, endogenous Pro levels were not determined in the previous study. In another study, foliar application of Pro (4 mM) to pea plants decreased the level of internal Pro but enhanced antioxidant defense systems and accumulation of other solutes under drought stress (Osman 2015). The decrease in endogenous Pro in response to Pro application was interpreted by the author as that Pro overproduction is a symptom of stress rather than an indicator of tolerance. In agreement, although Pro foliar application improved maize salt tolerance, plants which received Pro exhibited a decreased P5CS activity and an increased PDH activity in leaves under saline conditions, which was suggested as an adaptive response to prevent a possible toxic effect of high Pro level (de Freitas et al. 2018). Because other findings demonstrate increased P5CS activity and decreased PDH activity in response to Pro and salt treatment, it is obvious that when Pro is treated to various plants, it shows different metabolic changes pointing to the complexity of the response to Pro exogenous treatment. The study of de Freitas et al. (2018) also illustrated decreased Pro accumulation and its participation as a source of energy under high salinity. These results support the notion that Pro likely act as a regulatory signal which might be crucial than Pro accumulation, and also further research is needed to unmask the exact role of Pro and its metabolism in plants under stress conditions. It is important to mention that in the experiments of Pro exogenous application, Pro transporters should be available to participate in its uptake. For instance, plasma membrane-localized amino acid permease has been reported to mediate Pro uptake from exogenous source and increases its accumulation and salt tolerance (Wang et al. 2017). Based on the above discussion, it appears that when Pro is supplied at relevant concentrations, it counterbalances the stress detrimental impacts and enhances plant tolerance to different stress conditions. However, the optimum Pro level that is effective and Pro actual role in mitigating the stress adverse effects show great variations among plant species.

12.6 Genetic Manipulations of Pro Biosynthesis Modulated Abiotic Stress Tolerance

Transformation of Pro biosynthetic genes has been adapted in higher plants to determine whether induced Pro overproduction is able to enhance plant tolerance under different stresses (Table 12.3). Genetic manipulations of Pro biosynthesis were mainly carried out under drought stress and salt stress. Supporting the importance of genetic manipulation approach in this issue, transcriptome studies in different plants indicate the crucial role of various genes involved in biosynthetic pathways

Table 12.3 Overexpression of Pro biosynthetic genes in transgenic plant species induced different abiotic stress tolerance

Plant species	Transgene	Stress enhanced tolerance	Reference
Oryza sativa	*Osa-miR319b*	Cold stress	Wang et al. (2014)
O. sativa	*OsPRP3*	Cold stress	Kishor et al. (2015)
Petunia hybrida	*AtP5CS*	Water stress	Yamada et al. (2005)
Nicotiana tabacum	*OsP5CS1* and *OsP5CS2*	Salt and drought stresses	Zhang et al. (2014)
O. sativa subsp. *indica*	*P5CSF129A*	Salt stress	Kumar et al. (2010)
Daucus carota	*P5CS*	Salt stress	Han and Hwang (2003)
Cajanus cajan	*P5CSF129A*	Salt stress	Surekha et al. (2014)
Arabidopsis thaliana	Antisense *ProDH* cDNA	Freezing and salt stress	Nanjo et al. (1999)
N. tabacum	*P5CS*	Freezing stress	Parvanova et al. (2004)
Glycine max	*P5CS*	Drought and heat stresses	Kocsy et al. (2005)
G. max	*P5CR*	Drought and heat stresses	De Ronde et al. (2004)
Triticum aestivum	*P5CS*	Salt stress	Sawahel and Hassan (2002)
N. tabacum	*P5CS*	Drought stress	Zarei et al. (2012)
N. tabacum	*P5CS*	Water stress	Kishor et al. (1995)
Saccharum officinarum	*P5CS*	Water stress	Molinari et al. (2007)
Citrus sinensis	*P5CSF129A*	Drought stress	Molinari et al. (2004)
C. paradisi	*P5CSF129A*	Drought stress	de Campos et al. (2011)
Phaseolus vulgaris	*P5CS1*and *P5CS2*	Salinity stress	Chen et al. (2013)
Solanum tuberosum	*P5CS*	Salinity stress	Hmida-Sayari et al. (2005)
Panicum virgatum	*P5CS*	Salt stress	Guan et al. (2018)
T. aestivum	*P5CS*	Drought stress	Tuteja et al. (2011)
A.s thaliana	*P5CS*	Freezing stress	Xin and Browse (1998)

of osmoprotectants including Pro (Liu et al. 2017). Using these genes, several plant species have been engineered to improve their tolerance to a wide range of abiotic stresses (Tuteja et al. 2011; Zhang et al. 2014). Table 12.3 presents evidence that overexpression of Pro biosynthetic genes in transgenic plant species induced different abiotic stress tolerance. For instance, overexpression of *V. aconitifolia* Δ1-pyrroline-5-carboxylate synthetase (*P5CS*) cDNA in *T. aestivum* plants increased Pro overproduction under drought stress (Tuteja et al. 2011). The improved drought tolerance in transgenic plants was mainly owing to Pro participation in protection against oxidative stress and not brought about by Pro role in osmotic adjustment. Overexpressing *P5CSF129A* gene in *indica* rice enhanced also Pro accumulation and salt stress tolerance of the transgenic plants (Kumar et al. 2010).

Transgenic plants of *Petunia hybrida* were also developed by transformation of *AtP5CS* gene from *A. thaliana* or *OsP5CS* gene from *Oryza sativa*, which enhanced drought tolerance of the transgenic plants (Yamada et al. 2005). The transgenic plants in this work accumulate higher Pro and showed improved growth compared with wild plants. Also, overexpression of *Arabidopsis* OAT has been shown to enhance Pro levels and increase the salt and drought tolerance of rice (Roosens et al. 2002). When *OsP5CS1* and *OsP5CS2* genes were co-expressed in tobacco, it also increased Pro production, reduced oxidative damage, and enhanced salt and drought tolerance (Zhang et al. 2014). Similar Pro generation was also found in transgenic carrot seedlings overexpressing *P5CS* gene, which contributes to salt tolerance (Han and Hwang 2003). In support to the involvement of Pro metabolism genetic engineering in inducing stress resistance is the finding that antisense suppression of Pro breakdown enhanced freezing and salinity tolerance in *A. thaliana* (Nanjo et al. 1999). In the same trend, overexpression of *P5CS* gene conferred drought and salt tolerance in transgenic petunias (Yamada et al. 2005). Further, overexpression of *PuP5CS* in switchgrass significantly increases Pro content, salt tolerance, and growth under salt stress (Guan et al. 2018). In this study, Pro content was three- to fourfold higher than control plants, and thus, Pro acts as a protectant against adverse effects of salinity. Also, Pro content was positively correlated with decreased membrane damage and lipid peroxidation and increased K^+/Na^+ ratio and relative water content. In the transformation of pigeon pea (*Cajanus cajan*) with the mutagenized version of P5CS (*P5CSF129A*) from *V. aconitifolia*, the transgenic plants had higher Pro content and improved salt tolerance (Surekha et al. 2014). In addition, when Li and Han (2012) introduced the *OjERF* gene into tobacco, the transgenic tobacco plant showed drought stress tolerance through upregulating Pro and antioxidant metabolism. It is clear from these findings that increased Pro biosynthesis in various transgenic plants plays an important role in combating the stress injurious effects and hence induced tolerance to drought and salt stresses.

Moreover, other studies which dealt with transgenic plants overexpressing Pro synthetic enzymes and their behavior in response to salt and water deficit stresses are reviewed below. Wheat plants transformed with *P5CS* gene showed higher Pro accumulation and salt tolerance (Sawahel and Hassan 2002). When *P5CS* gene was overexpressed in tobacco plants, it also resulted in higher Pro levels and increased antioxidant enzyme activities which eventually enhanced drought tolerance (Zarei et al. 2012). Enhanced heat and drought tolerance was also reported in transgenic soybean plants containing an *Arabidopsis P5CR* gene (De Ronde et al. 2004). Further, overexpression of *P5CS* in tobacco resulted in Pro accumulation which improved flower development and root biomass in transformed plants under drought stress conditions (Kishor et al. 1995). Consistently, transgenic sugarcane transformed with a heterologous *P5CS* gene overproduced high Pro content, but no osmotic adjustment was observed in transformed plants during water deficit stress (Molinari et al. 2007). The study revealed that the potentiality of transformed plants to tolerate water deficit stress was due to Pro crucial contribution in antioxidative defense system rather than as an osmotic adjustment mediator. In contrast, transgenic orange plants were able to withstand water deficit stress because of the fivefold increase in Pro content and in turn its involvement in osmotic adjustment (Molinari et al. 2004). In another study, when transgenic 'Swingle' citrumelo root stocks transformed with the *P5CSF129A* gene, the transgenic plants coped with drought stress because high Pro production behaved not only as osmotic adjustment agent but also as a contributor to gas exchange machinery, ROS scavenger, and modulator of antioxidant enzyme activities (de Campos et al. 2011). Moreover, when *Phaseolus vulgaris P5CS* (*PvP5CS1* and *PvP5CS2*) cDNA was introduced into transgenic *Arabidopsis*, it caused Pro overproduction and enhanced salt tolerance (Chen et al. 2013). The work also showed overexpression of *PvP5CS2* accumulated more Pro, biomass production, and efficient membrane protection than expression of *PvP5CS1* in transgenic lines under salt stress implying stronger capacity of PvP5CS2 protein to improve tolerance to saline conditions. In accordance, transgenic potato plants overexpressing *P5CS* gene showed significant increase in Pro production, much less altered tuber yield, and thus improved tolerance to salinity and drought stresses (Hmida-Sayari et al. 2005). Interestingly, overexpression of *FcWRKY40* (transcription factor) enhanced salt tolerance in transgenic tobacco and lemon by regulating genes involved in ion homeostasis and Pro biosynthesis, *SOS2* and *P5CS1*, respectively (Dai et al. 2018). The transgenic plants had enhanced Na^+ exclusion and Pro synthesis and hence alleviated salt-associated damage by maintaining ion homeostasis and osmotic adjustment. Based on the above, overexpressing Pro biosynthetic enzymes induced cellular accumulation of Pro which has been shown to counteract the injurious effects of abiotic stresses and hence improved stress tolerance, although Pro actual role in conferring stress tolerance is still contradictory.

Although engineered plants which overexpressed Pro biosynthetic genes were carried out mainly with drought and salt stress, other investigations have indicated similarly induced Pro accumulation under heat and cold stresses. For instance, overexpression of *Osa-miR319b* led to an enhanced rice tolerance to cold stress by higher Pro content in the transgenic plants (Wang et al. 2014). Calcium treatment which enhanced chilling stress in *Jatropha curcas* occurred via enhanced Pro accumulation, proposing Pro as a key factor in imparting chilling tolerance (Yang et al. 2016). Improved chilling stress in this work was a combined result of both Pro biosynthesis activation via the glutamate pathway and concurrent inhibition of Pro degradation pathway, suggestive of improving chilling stress tolerance by enhancement of Pro accumulation through overexpressing Pro biosynthesis enzymes. Also, transformation of *Oryza sativa PRP3* (*OsPRP3*, Pro-rich proteins) exhibited a cold tolerance enhancement relative to the wild-type plants (Kishor et al. 2015). The authors indicated that *OsPRP3* improves cell wall integrity in the cold-tolerant transgenic plants. Furthermore, when several tobacco lines transformed with the *P5CS* gene, the transgenic plants accumulate Pro which mitigates freezing-induced oxidative damage and thus improved freezing tolerance (Parvanova et al. 2004). Moreover, transgenic soybean plants overexpressing the *P5CS* gene had higher Pro content, lower H_2O_2 accumulation, and ROS detoxification under drought and heat stresses (Kocsy et al. 2005). *Arabidopsis* plants with mutations at *eskimo1* (*esk1*) locus accumulate high Pro and are constitutively freezing tolerant, which is a result of enhanced *P5CS* and decreased *PDH* expressions (Xin and Browse 1998). It is important to mention that transgenic studies not only elevated Pro production but also uncover some of the molecular mechanisms of Pro homeostasis under abiotic stresses. For example, DFR1 as a mitochondrial protein inhibits Pro degradation in *Arabidopsis* and has been found to be sharply activated by cold and drought stresses (Ren et al. 2018). The protein DFR1 is encoded by a gene *DFR1* (*Drought Freezing Responsive gene 1*). The study of Ren et al. (2018) also reports that *dfr1* knockdown mutants were more sensitive to freezing and drought stresses, whereas overexpressing DFR1 in *Arabidopsis* induced adaptation for both stresses, which was positively associated with generation of high Pro levels. It is obvious that upregulation or downregulation of Pro anabolic or catabolic enzymes efficiently increases Pro accumulation which ameliorates the adverse effects of heat stress as well as cold stress.

Conversely, other research demonstrated that transgenic plants overexpressing Pro biosynthetic genes exhibited no significant correlation with Pro accumulation under heat stress. For instance, transgenic *Arabidopsis* plants overexpressing *AtP5CS1* resulted in Pro accumulation under heat stress, which was inhibitory to transgenic seedlings (Lv et al. 2011). The study revealed that decreased thermotolerance by Pro accumulation was due to increased ROS production questioning the role of Pro in ROS scavenging. Also, marginal increase in Pro content was observed in chickpea (*Cicer arietinum*) compared with the control under heat stress (Chakraborty and Tongden 2005). In the same trend, Pro accumulation was not observed in *Arabidopsis* in response to heat stress, and even high temperature intensified the damage impact of supplied Pro (Rizhsky et al. 2004). Further, transgenic tobacco plants constitutively overexpressing a modified gene for the Pro biosynthetic enzyme P5CSF129A resulted in Pro accumulation relative to wild plants under drought and heat stress, but a decisive contribution of elevated Pro to drought or heat stress tolerance of tobacco was not proved in this study (Pospisilova et al. 2011). In addition, the study of Harsh et al. (2016) reports that increased level of Pro in moth bean (*V. aconitifolia*) could not be associated with heat stress tolerance. The question therefore raised here is whether Pro production is beneficial to all stresses or it is important to certain stresses and species/genotypes and under certain conditions.

12.7 Conclusion and Perspectives

Based on the above discussion, it is clearly evident that accumulation of Pro is associated with tolerance to diverse abiotic stresses. The indispensable roles of Pro elevation have been assumed as an osmotic agent imparting cellular turgor, an antioxidative defense molecule minimizing ROS to a tolerable level, a metal chelator reducing their toxic impact, a molecular chaperone stabilizing cellular macromolecules, and a signaling molecule. Additionally, Pro beneficial roles in enhancing plant stress tolerance have been confirmed by Pro exogenous application experiments as well as those overexpressing Pro metabolic genes. Both strategies showed accumulation of higher levels of Pro and improved adaptation to a wide array of environmental stresses. There are, however, conflicting reports among published data concerning the functions of Pro in enhancing stress resistance. Additionally, although the sharp increase in free Pro in stressed plants has been considered as a mechanism conferring stress tolerance, Pro generation has also been reported as a symptom of injury rather than an indicator of stress tolerance in several studies. Further, the role of Pro in improving stress tolerance is greatly varied in different plant species/genotypes. Also, the broadly approved hypothesis that Pro is an innocent compatible osmolyte even when it accumulates at excessive levels does not hold true in several investigations. These discrepancies are a result of the fact that the Pro effects on plants vary with stress duration, stress degree, Pro concentration, species/cultivar, plant growth stage, as well as other environmental factors. There are therefore some remarks to be considered in future research in order to clearly and factually unmask the Pro involvement in plant performance under stress conditions and thus in enhancing stress tolerance. First, plants exhibit species-specific differences in Pro accumulation and response to exogenous application; future studies therefore should focus on the determination of the relevant and effective Pro concentrations used for exogenous application experiments, number of applications, relevant Pro amount accumulated during stress, and the most responsive growth stage for a given species before recommending Pro for practical uses in agriculture. Secondly, responses to abiotic stresses, including osmolyte accumulation, have been frequently carried out under controlled conditions, and thus the physiological relevance of these studies should be confirmed in natural field conditions because of the large disagreement between both types of experiments. These studies are extremely important to factually assess the relative importance of Pro accumulation to plant stress tolerance mechanism. Third, there are still gaps in our knowledge about the molecular mechanisms that regulate Pro biosynthesis and also how Pro acts within the metabolism of stressed plants; both issues play a vital role in the plant response to abiotic stresses and remain an open question. Further research is therefore needed for better understanding of the usefulness and feasibility of the proposed mechanisms for Pro involvement in stress tolerance.

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Chapter 13
RETRACTED CHAPTER: Physiological Role of Gamma Aminobutyric Acid (GABA) in Salt Stress Tolerance

Syed Uzma Jalil and Mohammad Israil Ansari

Contents

Abstract High salt concentrations in soils have been reported to be harmful to several plants which thereby play a limiting role in the crop productivity. The effect of salt stress involves modifications in different metabolic and physiological processes. Salt stress inhibits plant growth, inhibits photosynthesis, alters metabolism of reactive oxygen species, and causes cell death that finally obstructs the production of crops. Plants synthesize various compounds that involve in signaling and

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play an important role in stress tolerance. Gamma-aminobutyric acid (GABA) is a four-carbon, ubiquitous, nonprotein amino acid. The synthesis of GABA occurs in the cytosol by an irreversible alpha-decarboxylation of L-glutamate by L-glutamate decarboxylase (GAD). During salt stress condition, the accumulation of GABA increases that helps in stabilizing the intracellular pH of cell and carbon/nitrogen metabolism for the Krebs cycle. Exogenic GABA treatment enhanced plant growth and improved stress tolerance via scavenging free radicals, regulating enzyme activities, and stabilizing plants against increased salt stresses. In this chapter, we have tried to highlight the physiological and metabolic role of GABA in relation to salt stress tolerance.

Keywords Salt stress · GABA · Stress tolerance · Free radicals · Crop productivity

13.1 Introduction

Salt stress conditions have become a foremost problem constraining the developmental mechanism of several plants. It has been accounted that 7% of the total global land is adversely influenced by salt stress (Munns 2002). The deleterious impacts of salt stress on plants are associated with different morphological, physiological, and biochemical processes (Ashraf 1993). For instance, plant roots can be adversely influenced by increased amount of sodium and chlorine ions. The overaccumulation of sodium and chlorine ions in plants leads to ion toxicity and furthermore diminishes the retention of other ions prompting negative effects on plant development and metabolic disruption during salt stress. Furthermore, salt stress seriously decreases the capacity of plants taking up water bringing about drought condition (Al-Karaki 1997; Bybordi et al. 2010a, b; Woodrow et al. 2016). Those negative impacts specifically or indirectly causes generation of reactive oxygen species (ROS) that results in oxidative harm in plants during high salt condition (Liu et al. 2007). It is notable that salt stress contrarily influences every single vegetative phase of plants, particularly seed germination (Cuartero et al. 2006). Earlier studies have demonstrated that excessive salts basically decreased the germination rate, shoot length, and root length of various plants (Barbagallo et al. 2010; Zhang et al. 2007; Dash et al. 2007). Recently, nano-biotechnology is also receiving considerable attraction of researchers for abiotic stresses to achieve an improved agricultural production throughout the world (Jalil and Ansari 2019).

The γ-aminobutyric acid (GABA) is nonprotein, ubiquitous, and one of the special interests of molecule that is found in all organisms (Shelp et al. 1999). GABA involved in carbon/nitrogen metabolisms is essentially required for plant development (Ansari et al. 2014). The accumulation of GABA in plants is rapidly increased due to different biotic and abiotic stress conditions such as low temperature, γ radiation, low pH, hypoxia, and darkness including leaf senescence process (Lane and Stiller 1970; Wallace et al. 1984; Ansari and Chen 2009; Shelp et al. 2012). GABA in plants acts as an active osmolyte devoid of harmful effects under the salt-induced desiccation and had the free radicals scavenging activity in stress conditions (Carillo 2018). It has been found that during environmental stress conditions, GABA shunt pathway can potentially involve in the regulation of GABA metabolism as shown in Fig. 13.1 (Al-Quraan and Al-Share 2016; Jalil et al. 2016, 2017).

Moreover, the application of GABA encourages plant development, antioxidant metabolism, and expression of antioxidant genes in dose-dependent manner, thus reducing stress-induced oxidative harm in plants (Shi et al. 2010; Vijayakumari et al. 2016; Li et al. 2017). Application of GABA likewise directs the osmoregulation in plants adding to the upgrade of stress resistance (Yu et al. 2014; Vijayakumari and Puthur 2015). More prominently, exogenous GABA successfully hindered the generation of free radicals and decreased oxidative harm through controlling the expression of important genes of free radical generation and genes encoding antioxidant enzyme in salt stress (Shi et al. 2010). The treatment of GABA on seeds also improved seed germination rate and diminished the salt-induced damage during seed germination of *Triticum aestivum* and *Zea mays* (Luo et al. 2011).

The exogenous application of GABA improves the tolerance in several plants against abiotic stresses, but diminutive data are accessible in relation to GABA-regulated salts tolerance. This chapter exposes the GABA-regulated salt tolerance related to antioxidative defense system, osmoregulation, and relevant gene expression during salt stress.

13.2 Salt Stress Damage to Plants

The prolonged exposure of salt stress shows common symptoms of damage such as growth inhibition, senescence, and death. Growth inhibition is the key damage that prompts different side effects in spite of the fact that programmed cell death occurs under extreme salt conditions. Salt stress instigates the production of abscisic acid (ABA) that close stomata while transferred to guard cells, which decreases the photosynthesis and photoinhibition and causes oxidative stress (Assmann 2003; Christmann et al. 2007). A quick influence of osmotic stress on plant development is its hindrance of cell structure directly or indirectly by ABA. Intemperate Na^+ ions on the root disturb K^+ sustenance of plant. Due to the comparative synthetic characteristic of Na^+ and K^+ ions, Na^+ ions suppress the absorption K^+ by the root (Christmann et al. 2007). Plant utilizes slow and high-affinity approach for K^+ absorption. Na^+ ions have an even more negative impact on the low-affinity approach that has a low K^+/Na^+ selectivity (Cheng et al. 2018). Under high NaCl condition, it is fundamental for plants to activate the particular high-affinity K^+ absorption mechanism to sustain sufficient K^+ nutrition. Potassium is the most plentiful cell cation that involves in retaining cell turgidity, membrane stability, and enzyme activities, and its deficiency certainly prompts to growth inhibition. Under salt stress, Na^+ enters into the cytoplasm that hinders various enzyme activities. This hindrance is also dependent on how much K^+ is available: a high Na^+/K^+ proportion is the most injurious (Jouyban 2012).

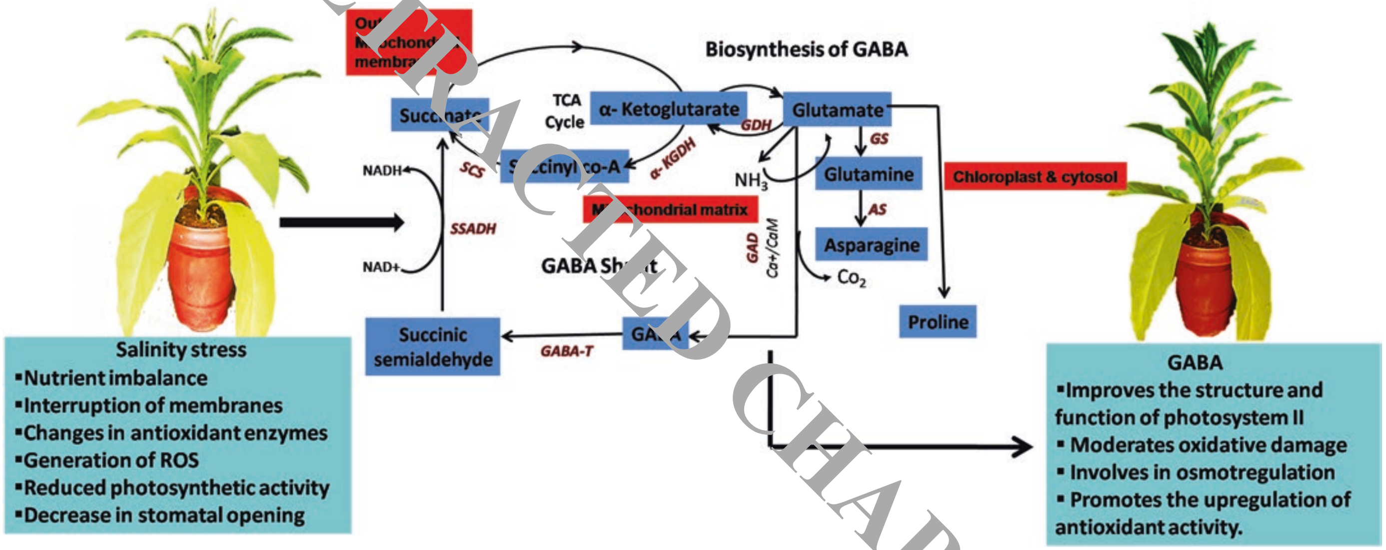

Fig. 13.1 The accumulation of GABA in plants is rapidly increased in salt stress. GABA in plants acts as an active osmolyte that reduces harmful effects due to the salt-induced damage and has the free radicals scavenging activity under stress conditions

13.3 Salt Stress Effects on Nutrient Absorption

Salt stress causes nutrient deficiencies in the cell that decrease plant development by influencing the availability, transportation, and distribution of nutrients. Conversely, salts condition differentially effect the mineral nourishment of plants and causes nutrient imbalances, due to the rivalry of Na^+ and Cl^- with supplements, for example, K^+, Ca^{2+}, and NO_3^-. In salty environments, plant development decreases due to ion toxicities and ionic uneven characters following up on biophysical as well as metabolic segments of plant development (Grattan and Grieves 1999). Elevated NaCl level accounted for prompt increments in Na^+ and Cl^- and in addition diminishes the nitrogen, phosphorus, calcium, potassium, and magnesium contents in *Foeniculum vulgare* (Abd El-Wahab 2006); Ajwain (Ashraf and Orooj 2006); *Mentha piperita, Aloysia citrodora* (Tabatabaie and Nazari 2007), Chamomile (Baghalian et al. 2008), and *Achillea* (Abd EL-Azim and Ahmed 2009).

13.4 Metabolic Changes Under Salt Stress

Salt stress inspired a transformation from C_3 into the CAM (crassulacean acid metabolism) system in *Mesembryanthemum crystallinum* (Ishimaru 1999). A portion of the enzyme mechanism for CAM metabolism is actuated by a very short period of salt stress condition. The primary preferred standpoint of the CAM metabolism is elevated moisture usage efficiency due to the stomata possibly open around at dark hours while minimum water is evaporated. A metabolic change in plants is an aggregation of low molecular weight organic solutes during extreme salt conditions. These solutes incorporate direct polyols (glycerol, mannitol, or sorbitol), cyclic polyols (inositol or pinitol and other mono-and dimethylated inositol subsidiaries), amino acids (glutamate or proline) and betaines (glycine betaine or alanine betaine) (Ishimaru 1999). Plants that regularly encounter nitrogen confinement may aggregate dimethylsulfonium propionate that proportionate to the nitrogen-containing betaines. The organic solutes at increased concentration are not destructive cell assemblies. Consequently, these are regularly suggested as good osmolytes (Jouyban 2012).

Compatible solutes at large amount positively involve in osmoregulation. This is particularly valid in halophytes, which frequently accumulate osmolytes in the range of 0.5–4.0 mol L^{-1} in the cells (Al Hassan et al. 2017). The increased concentrations of osmolytes exist in cytoplasm, to equilibrium the increased concentration of salts exterior to the cell also hindering the high concentrations of Na^+ and Cl^- ions in the vacuole. Not all the organic solutes are destructive but some have a defensive impact against harm by toxic ions or lack of hydration. Genes for proline and polyol biosynthesis were overexpressed in transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* (Lilius et al. 1996; Hayashi et al. 1997; Jouyban 2012). The genetically engineered plants constitutively producing osmolytes execute superior in comparison to control plants in salt stress. The defensive impact can't be completely clarified by the osmotic change hypothesis because the transgenic plants just deliver a few mmol L^{-1} a greater amount of the osmolytes, that was too low for osmoregulation. It recommends that the low concentration of osmolytes can secure plants by scavenging ROS induced by salt stress. Significance of antioxidants in salt stress resistance is additionally bolstered by trials appearing progressive outcome of genetically engineered development of ROS scavenging enzymes on plant execution during high salt condition.

13.5 Biosynthesis and Metabolism of GABA in Plants

GABA is one of the special interest molecules among stress responsive metabolites and synthesized in prokaryotes, yeast, animals, and plants (Shelp et al. 1999). GABA is involved as a neurotransmitter in nervous system of mammals and its metabolization is done in cytosol (Breitbreuz and Shelp 1995; Kinnersley and Turano 2000; Reimer et al. 2001; Bouche and Fromm 2004). GABA is highly water-soluble and presents in both bound and unbound forms in almost all plant tissues (Satyanarayan and Nair 1990). Inactive concentration of unbound GABA normally presents in the concentration of 0.03–2.00 µmol g^{-1} FW in plant cells (Shelp et al. 1999), whereas bound forms present in 6.6% of the total dry weight of root nodules in legumes (Satyanarayan and Nair 1990). GABA is involved in many mechanisms of plants such as metabolic activities and signaling. The accumulation of GABA in plants is rapidly increased due to different stress conditions (Kinnersley and Turano 2000; Lane and Stiller 1970; Wallace et al. 1984; Bown and Shelp 1997). GABA metabolism was generally upregulated under NaCl-induced stress in *Arabidopsis thaliana* (Renault et al. 2010). These stresses initiate signal transduction pathway, which increases cytosolic calmodulin-dependent activity of the GAD. However, it appears that GABA is important for vegetative development.

GABA metabolism occurs in two compartments of the cell: synthesis of GABA takes place in the cytosol whereas degradation of GABA occurs in the mitochondria. GABA is an intermediate product in the synthesis of succinate from L-glutamate via a set of reactions collectively called GABA shunt pathway (Fig. 13.1). The three reactions of the pathway are as follows.

In the first reaction, glutamate is irreversibly decarboxylated by enzyme glutamate decarboxylase to form GABA. In the second step, reversible transamination of GABA takes place by enzyme GABA-transaminase that utilizes α-ketoglutarate for transferring of amino group in mammalian cells, while GABA-transaminase uses pyruvate to form succinic semialdehyde in plant. The third reaction starts with the irreversible oxidation of succinic semialdehyde by succinic semialdehyde dehydrogenase to form succinate that enters into Krebs cycle (Bown and Shelp 1997). Glutamate decarboxylase enzyme exists in cytosol (Breitbreuz and Shelp 1995) and precisely for L-glutamate, retains an auto inhibitory calmodulin-binding domain, and has an optimum pH of 5.8. GABA-transaminase and succinic semialdehyde are present in mitochondria (Breitbreuz and Shelp 1995) with optimum pH of 8 to 10 and 9, respectively. Synthesis of GABA occurs in the cytosol by glutamate decarboxylase and is transferred to the mitochondrion for consequent conversion into succinate by GABA-transaminase and succinic semialdehyde dehydrogenase. GABA shunt components and GABA performs various functions, depending upon species to species on the basis of the compartment in the cell where they are active.

13.6 Mechanism and Function of GABA in Plants in Salt Stress

Studies in plants stressed principally on the mechanism of GABA metabolism, generally in the perspective of stress effect, till some finding were reported to indicate a signaling function of GABA in plants in salt stress condition.

13.6.1 GABA Promotes Photosynthesis and Chlorophyll Fluorescence in Salt Stress

Photosynthesis is an integral mechanism used by plants to transform light energy into chemical energy that can be released as organic complexes via CO_2 fixation (Omoto et al. 2012). Salt stress obstructs absorption ability of plants, causes deficiency of nutrients, and declines chlorophyll content, therefore hindering photosynthesis activity (Buttery and Buzzell 1997). NaCl hinders photosynthetic activity by decreasing CO_2 fixation, stomatal conductance, and transpiration. A sensible clarification is the photosynthetic activity experienced harm from salt stress, bringing about the decrease of CO_2 assimilation. PSII is deliberated as the essential location of the photosynthetic system damage in stress condition (Sharkey and Zhang 2010). Damage to PSII can prompt an adjustment in chlorophyll fluorescence. In this manner, chlorophyll fluorescence utilized as an influential process to evaluate modifications in the purpose of PSII and to study the essential photosynthetic methods in stressful environment (Hajiboland et al. 2010). Maximal fluorescence (Fm), most extreme quantum proficiency of PSII photochemistry (Fv/Fm), and electron transport rate diminished and minimal fluorescence (F0) expanded in maize seedlings and coastal plant species during salt stress (Wang et al. 2017; Naumann et al. 2007).

Extreme salt condition harms the oxygen-advancing system and disturbs the electron transport and photochemical efficiency (Steponkus and Lanphear 1967). Declined PSII activity in salt stress is viewed as the after effect of reduced excitation energy getting PSII reaction centers and modifications in protein-pigment complexes of thylakoids membrane (Misra et al. 2001). Few studies revealed that plants that are exposed with GABA + NaCl showed higher net photosynthetic rate and stomatal conductance than those that are treated only with NaCl (Jiang et al. 2006) and that exogenous GABA improved transpiration rate and intercellular CO_2 concentration in under different level of salt stress (Li et al. 2015). The GABA + NaCl plants had higher Fv/Fm and Fm during extreme salt conditions in comparison to NaCl-treated plants (Karaba et al. 2007). Moreover, exogenous GABA recovers the assembly and role of photosystem II in muskmelon seedlings under salt stress (Xiang et al. 2016). In addition, chlorophyll fluorescence improved in GABA + NaCl-treated plants as compared to only NaCl-treated plants. Exogenous GABA can decrease the accumulation of toxic constituents (Cao et al. 2012), sustain cell morphology (Almeselmani et al. 2009), and enhance the cell function under salt stress. These defensive impacts can defend photosystem II from salt stress-induced damage and increase chlorophyll fluorescence parameters. Exogenous GABA can facilitate the salt stress-induced damage and escalated leaf relative water content. Furthermore, due to the enhancement of cell function, net photosynthetic rate was increased. This impact enhanced the use of CO_2 and diminished intercellular CO_2 concentration in maize seedling leaves (Wang et al. 2017).

13.6.2 GABA Moderates Oxidative Damage in Plants Under Salt Stress

Salt stress may transport calcium ions from plasma membrane binding sites, prompting membrane leakiness that elevates the transudation of ions, commencing an expansion in leaf relative conductivity (Pitann et al. 2013). Toxicity of NaCl in plant damages cell organelles, decreases cellular reduction ability, and degrades chlorophyll prior to plant demise (Davenport et al. 2005; Quintero et al. 2007). The halotolerant cyanobacterium *Aphanothece halophytica* revealed high amount of GABA under salt stress proposing the prospective of genetic manipulation of the genes involved in GABA synthesis to increase GABA content (Boonburapong et al. 2016). Exogenic treatment of GABA can control the osmotic equilibrium in plant for improving the resistance against adverse environmental conditions (Vijayakumari and Puthur 2016; Yu et al. 2014; Malekzadeh et al. 2014). Seeds treated with GABA improved seed germination rate and decreased the salt-induced damage of *Triticum aestivum* and *Z. mays* (Luo et al. 2011). It has been observed that the exogenous GABA enhanced the shoot length, root length, and fresh weight of *Z. mays* L. seedling in salt stress (Wang et al. 2017). Some studies show that salt stress escalates the membrane damage and declines the relative leaf water in NaCl-treated *Z. mays* seedlings (Wang et al. 2017).

Additionally, the treatment of GABA refurbished the membrane permeability, leaf pigment, and role of mitochondria during high temperature or hypoxia stress. (Nayyar et al. 2014). Succinic semialdehyde dehydrogenase (SSADH) mutants of *Arabidopsis thaliana* are oversensitive to stress conditions because of defective scavenging ability for hydrogen peroxide. In the final reaction, the GABA shunt pathway provided succinate and NADH to electron transport chain. Thus, it was proposed that the degradation of GABA could restrict the generation of reactive oxygen species during oxidative stress that limits some of the enzymes of the TCA cycle. It has been reported that GABA shunt genes knocked out mutants of Yeast and showed more sensitivity to hydrogen peroxide (Coleman et al. 2001; Bouché et al. 2003).The mechanism of refurbished these functions under salt-stressed cells by the application of GABA is still need to be investigate and might be related to the decrease in oxidative damage to the cell structure.

13.6.3 GABA Involve in Osmotic Regulation Via Accumulation of the Osmolytes Under Salt Stress

Osmotic regulation is a strategic acclimatization of plants cells to limit the impacts of salt-actuated drought stress. GABA can work as an osmolyte or can encourage the production of osmolytes during drought condition (Shelp et al. 1999). Proline synthesis escalates in *Z. mays* under salt stress (Kaya et al. 2010). Additionally, it has been observed an increase of osmolytes (proline and glycine betaine) in *Z. mays* seedlings during salt stress (Mansour et al. 2005). Furthermore, it has been reported that the proline and soluble sugar considerably escalates in 150 mM NaCl-treated *Z. mays* seedlings but significantly diminishes in 300 mM NaCl-treated seedlings (Wang et al. 2017). The impact of salt stress causes osmotic stress at the primary level and ion toxicity at the secondary level (Fortmeier and Schubert 1995). It has been suggested that the GABA plays a key role as an effective osmolyte at cellular level in plants without harmful impact during the dehydration stress induced by high salt conditions (Carillo 2018).

Exogenous treatment of GABA augmented the proline and soluble sugar accumulation in NaCl-treated (150 mM and 300 mM) *Z. mays* plants. NaCl-treated plants (150 mM) regulate GABA and accumulates high proline and soluble sugar as compared to NaCl-treated plants (300 mM) (Wang et al. 2017). These findings show that application of GABA enhanced osmolytes level under adverse conditions and developed the capability of plants to mitigate with abiotic stresses (Chen et al. 2007). The Krebs cycle is the fundamental method for aerobic organisms to get energy and a distinctive metabolic pathway of carbohydrates, fatty acids, and proteins oxidation. Succinate and α-ketoglutarate are essential transitional products of the Krebs cycle and synthesized via oxidation of GABA. GABA also transported via proline transporters from AtProT2 of *Arabidopsis thaliana* and LeProT1 of tomato. During water or salt stress, AtProT2 is highly triggered. Proline-GABA transporters also play protective role during osmotic stress by transporting organic osmolytes (Breitkreuz et al. 1999; Schwacke et al. 1999). Enhancement of proline and soluble sugar level by exogenous GABA may be indirectly related with its involvement in the Krebs cycle.

13.6.4 GABA Promotes the Upregulation of Antioxidant Activity

The ROS generated by salt stress causes membrane damage and lipid peroxidation by extreme escalation of malonaldehyde (MDA) content (Mittler et al. 2004). MDA accumulation is viewed as a marker of plant oxidative stress (Taulavuori et al. 2001). The antioxidant system, including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD), is involved in ROS regulation (Qiu et al. 2013). The activity of antioxidant enzymes such as SOD, POD, CAT, and APX increased in maize seedlings in salt stress (Wang et al. 2017). Few studies also observed that CAT activity reduced whereas SOD activity enhanced under salt stress (Zhu et al. 2004; Li et al. 2010). Furthermore, MDA and superoxide anion ($O_2^{\bullet-}$) enhanced directly proportional to salt stress. The activity of antioxidant enzymes increased to decrease damage from oxidative stress in NaCl-treated plants (Wang et al. 2017). Maize plants confronting salt stress utilize an assortment of adaptive systems at the molecular level to oppose the negative impacts of salt stress. It shows that the upregulation and downregulation of various gene transcriptions are essential (Zörb et al. 2004). Transcriptomic analysis revealed that the antioxidant defense system plays significant role in *Medicago sativa* L. in response to salt stress by analyzing the expression pattern of ROS-detoxification-related genes and their physiological changes (Luo et al. 2019).

The antioxidant genes transcripts are prompted to defend cells from oxidative damage during salt stress. CAT activity escalates because of the stimulation of mRNA due to increased free radical levels during salt stress in *Z. mays* (Menezes-Benavente et al. 2004). The gene expressions of superoxide dismutase, ascorbate peroxidase, and peroxidase elevated but that of catalase declined during salt stress in *Solanum lycopersicum* plants. The gene expressions of ascorbate peroxidase and peroxidases escalated in NaCl (200 mM) but were lesser than in NaCl-treated plants (100 mM) (Zhang et al. 2015). High NaCl concentrations can inhibit gene expression, ensuing in a decrease in antioxidant enzyme activities. Exogenous GABA reduced MDA and $O_2^{\bullet-}$ level in NaCl-treated (150 mM and 300 mM) maize seedlings (Wang et al. 2017). The seedlings that treated with GABA + NaCl showed increased antioxidant enzyme activity as compared to NaCl-treated plants (Wang et al. 2017). Moreover, some investigations shows that MDA content was lesser in the GABA + NaCl-treated plants in comparison to the NaCl-treated plants that constantly had high antioxidant enzymes activities (Li et al. 2015). Further, it has been observed that an appropriate amount of GABA application can stimulate plant development, antioxidant regulation, and transcription levels of antioxidant genes, thus lessening oxidative destruction by salt stress in plant (Li et al. 2017).

GABA reveals the key function of ROS scavenges activity in plants (Carillo 2018). It has been showed that exogenic GABA can considerably increase the various antioxidant enzyme activities in *Oryza sativa* (Nayyar et al. 2014), *Piper nigrum* seedlings (Vijayakumari and Puthur 2015), and *Lolium perenne* (Krishnan et al. 2013). Application of 1–2 mmol/L GABA on *Z. mays* seeds reduced the impact of high NaCl level on seed germination by increasing the antioxidant enzyme activities (Tian et al. 2014). Exogenous GABA also decreases the low light stress damage by regulating the antioxidant activity (Li et al. 2017). Furthermore, GABA treated enhanced antioxidant enzymes activities in *Solanum lycopersicum* seedlings in relation with decreases in the ROS generation and oxidative damage during NaCl stress (Luo et al. 2011). Additionally, application of GABA efficiently repressed the accumulation of hydrogen peroxide free radicals and decreased the oxidative damage by regulation of the gene expression of hydrogen peroxide synthesis and antioxidant enzyme in *Caragana intermedia* roots, proposing that GABA acts as a signaling ligand to regulate the gene expression under salt stress (Shi et al. 2010). Further, exogenous GABA application induced the accretion of endogenous GABA, proline, and other compounds, thus increasing the antioxidant activity in germinated hulless barley in salt stress (Maa et al. 2018).The mechanism that allows GABA to prompt the upregulation of antioxidant enzymes needs to be studied. Exogenous GABA application alleviated oxidative stress in roots of *Hordeum vulgare* seedlings under aluminum and proton stress (Song et al. 2010) and their study revealed that antioxidant enzymes (SOD, CAT, and POD) activities unregulated in response to declined MDA and ROS level after the application of GABA (Beuve et al. 2004).

13.7 Conclusions

Salt stress restricts the growth and production of crops and becomes the severe problem for the agronomists. GABA is involved in various mechanisms of plants such as metabolic activities and signaling during stress conditions. It has been considered that during environmental stress conditions, GABA shunt pathway can potentially involve in the regulation of GABA metabolism. The accumulation of GABA in plants is rapidly increased due to different biotic and abiotic stress conditions. The application of GABA encourages plant development, antioxidant metabolism, and expression of antioxidant genes in dose-dependent manner and can alleviate the growth restriction and oxidative destruction induced by salt stress and prompts the accretion of endogenous GABA and other osmolytes. GABA alleviates the effect of salt stress on plants that improves crop productivity.

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Chapter 14
Sulfur-Mediated Physiological and Biochemical Alterations to Improve Abiotic Stress Tolerance in Food Crops

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Abstract Sulfur (S) is an important macronutrient that plays a significant role in plant growth and development. In the past few decades, efforts focused on reducing sulfur dioxide emission for environment protection had limited the use of S-based fertilizers in agriculture, thereby causing S deficiency in food crops. It also triggered the susceptibility of crop plants to environmental stresses as S assimilation and synthesis of different S compounds modulate several metabolic processes to induce tolerance against various abiotic stresses. The use of inorganic fertilizers containing S has increased tremendously in recent years due to its significance in enhancing crop yield and quality. Therefore, in this chapter, we discuss recent studies on effects of S fertilizers on growth and yield of major cereals (wheat, maize, rice), legumes (mung bean, chickpea, black gram), and oilseeds (sunflower, brassica, soybean). An overview of current state of knowledge on S-mediated physiological and biochemical alterations in food crops may facilitate in developing appropriate fertilizer management strategies to improve yield and quality under abiotic stress conditions.

Keywords Sulfur · Fertilizer management · Environmental stresses · Food crops

Abbreviations

$(NH4)_2SO_4$	Ammonium sulfate
γGCS	Gamma-glutamylcysteine synthetase
ABA	Abscisic acid
ABA	Abscisic acid
AM	Arbuscular mycorrhizal
APX	Ascorbate peroxidase
As	Arsenic
CAN	Calcium ammonium nitrate
$CaSO_4$	Calcium sulfate
CAT	Catalase
Cd	Cadmium
Cr	Chromium
Cys	Cysteine
DHAR	Dehydroascorbate reductase
$FeSO_4$	Iron sulfate
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GST	Glutathione S-transferases
H_2S	Hydrogen sulfide
HMs	Heavy metals
K_2SO_4	Potassium sulfate
Met	Methionine
NaHS	Sodium hydrogen sulfide

Ni	Nickel
PCs	Phytochelatins
ROS	Reactive oxygen species
S	Sulfur
SOD	Superoxide dismutase
Trx	Thioredoxins
Vit	Vitamins
Zn	Zinc
$ZnSO_4$	Zinc sulfate

14.1 Introduction

Sulfur (S) is recognized as the fourth major nutrient after nitrogen (N), phosphorus (P), and potassium (K). It is an essential nutrient required to improve crop yield and quality due to its key role in protein synthesis (Yi et al. 2010). It is the main constituent of proteins, thioredoxin (Trx), methionine (Met), cysteine (Cys), vitamins (Vit), sulfo-lipids (SL), and Fe-S cluster system that play an important role in the regulation of physiological metabolism of plants (Khan et al. 2013). Increased S demand during metabolic adaptation processes suggests the key role of S-containing compounds (Anjum et al. 2015). Metabolism of S influences the accumulation of osmolytes and osmo-protectants (Gill et al. 2013), whereas S-containing compounds such as glutathione (GSH) interplay with signaling pathways to ensure sufficient production of metabolites for ABA synthesis (Herrmann et al. 2014). Sulfate interacts with ABA and acts as a chemical signal to initiate stomatal closure in leaves under water deficit conditions (Hasanuzzaman et al. 2018). Anti-transparent effect of ABA is increased by the presence of sulfate in stomata of plant leaves (Ernst et al. 2010). Assimilation of S results in the formation of several S-containing defense compounds including GSH and phytochelatins (PCs) involved in plant survival under various abiotic stresses (Honsel et al. 2011) (Fig. 14.1). Moreover, interplay of S with phytohormones helps to regulate crucial metabolic processes in plants (Noctor et al. 2012). S metabolism is directly linked to polyamines and ethylene through salvage pathway involved in plant response to drought stress (Sauter et al. 2013). Deficiency of S markedly affects the yield potential of plants even under well-watered conditions (Rasheed et al. 2004). Low S levels in soil influence the uptake of nutrients and nitrate reductase metabolism in plants (Prosser et al. 2001).

Plants uptake S in metabolically inactive form known as sulfate ($SO4^{-2}$) from soil surface. It is reduced into sulfide (S^{-2}) and assimilated into Cys by the activity of ATP sulfurylase (Herrmann et al. 2014). A variety of S compounds such as GSH, Met, and PCs are synthesized from Cys residues which play an important role in alleviating the drastic effects of environmental stresses like drought (Anjum et al. 2015). Sulfur metabolism induces alterations at metabolic and transcriptional levels

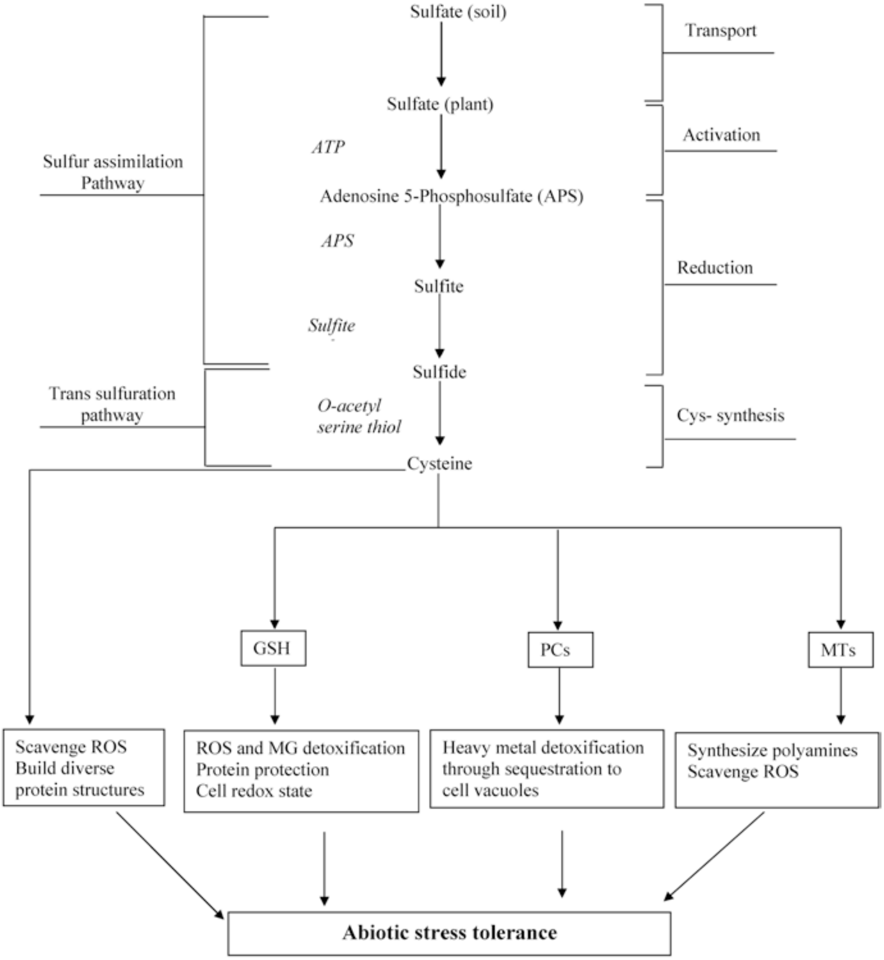

Fig. 14.1 Schematic overview S-mediated physiological and biochemical processes to improve abiotic stress tolerance in crop plants

to activate enzymes and increase root development for maximum uptake of nutrients and water (Ahmad et al. 2016a).

Judicious use of fertilizers and their management is essential to obtain high yield potential in crop plants. Optimum S supplementation significantly influences growth, yield, and quality of plants (Shao et al. 2008). Ahmad et al. (2016b) observed that S supplementation affects N uptake and use efficiency in maize. They applied different levels of S (0, 20, 30, and 40 kg ha^{-1}) in combination with various N application rates (0, 120, and 150 kg ha^{-1}). It was noted that S application significantly increased N uptake in maize at an increasing rate. They recommended S fertilization to improve growth, N use efficiency, and yield of maize. In a greenhouse study, Khan et al. (2015) evaluated the role of S and N fertilization on growth and yield of hybrid sunflower. Although N deficiency delayed all development stages of sunflower, a merked effect of S deficiency was only recorded at floret initiation and anthesis stages. Moreover, S deficiency also reduced Cys and Met by 30% but increased arginine by 34% in achenes. They suggested that timely availability of S is needed to prevent floret abortion in sunflower. Deficiency of S in sunflower at seed filling stage results in kernels with low levels of essential S-containing amino acids. Wani et al. (2001) noted a marked increase in achene yield and quality of sunflower by increasing S doses. A field study on chickpea involving two S sources (gypsum and ammonium sulfate) showed that S supply increased yield by 17% compared to no S application (Islam 2012). It was observed that ammonium sulfate was more efficient than gypsum to improve nutrients uptake and yield in chickpea.

The abovementioned literature highlights the importance of S in plant growth and development. In this chapter, we present a comprehensive overview of S-mediated physiological and biochemical processes involved in improving abiotic stress tolerance in crops (Table 14.1). In addition, comparative effects of different methods of exogenous S supply as well as application of various S sources and their doses are also discussed.

14.2 Role of S in Improving Abiotic Stress Tolerance in Cereals

14.2.1 Wheat

Deficiency of S results in light green to yellow color in young wheat leaves along with stunted growth and spindly stalks. High rainfall and poor drainage during the growth season result in leaching of available S below the root zone. Similarly, fine-textured soils are more likely to be S deficient compared to coarse-textured soil (Shah et al. 2018). Availability of S also facilitates N assimilation in wheat seeds (Tea et al. 2007).

Hydrogen sulfide (H_2S) has emerged as a new stress-signalling molecule with multiple effects on plant metabolism to increase tolerance against various environmental stresses. It is considered an important part of S-induced plant defense mechanisms (Aroca et al. 2018). Application of H_2S donor, i.e., NaHS, was observed to influence ABA metabolism pathway as well as ABA concentration in roots and leaves of wheat seedlings exposed to drought stress (Ma et al. 2016). Moreover, it also upregulated ABA reactivation and catabolism genes in roots. In another study, NaHS application modulated GSH and ascorbate assimilation in wheat leaves under drought stress (Shan et al. 2011). Pretreatment of NaHS markedly decreased malondialdehyde (MDA) content and electrolyte leakage and upregulated dehydroascorbate reductase (DHAR), gamma-glutamylcysteine synthetase (γGCS), glutathione reductase (GR), ascorbate peroxidase (APX), and gamma-glutamylcysteine synthetase (γGCS) compared to no NaHS supply. NaHS-induced upregulation of drought-responsive genes facilitates phytohormones' signal transduction, amino acids'

Table 14.1 Summary of representative reports on the role of S nutrition in improving abiotic stress tolerance in food crops

Sr. #	Crop species	S source and dose	Stage	Stress	Application method	Response	Reference
Legumes							
1	Mungbean	Zinc sulfate (2×10^{-5} M, 3×10^{-5} M, and 4×10^{-5} M)	Before sowing	Salinity stress	Seed priming	Increase in growth and yield	Pandey et al. (2010)
		$ZnSO_4$ (10, 15, and 20 kg ha^{-1})	At sowing	Drought	Soil application	Increase in plant height, yield attributes and protein contents	Usman et al. (2014)
		K_2SO_4 (0, 25, 50, 75, and 100 kg ha^{-1})	Seed bed preparation			Increase in yield	Abbas et al. (2011)
		$FeSO_4$ (5 kg ha^{-1}) and $ZnSO_4$ (10 kg ha^{-1})				Increase in biological yield, grain yield, straw yield, and nodule numbers	Jamal et al. (2018)
		$FeSO_4$	Branching and flowering		Foliar application	Increased growth, yield, and iron and protein contents	Ali et al. (2014)
		Zinc (0 and 0.1%), nitrogen (15 and 25 kg ha^{-1}), and S (0, 20, and 40 kg ha^{-1})	Pre-flowering and pod initiation			Increase in yield and quality of mungbean	Muniswamy et al. (2018)
2	Chickpea	$ZnSO_4$	Before sowing	Salinity stress	Seed priming	Increase in germination and early seedling growth	Seyedi (2011)
		$ZnSO_4$ (0, 20, 50, and 100 mg L^{-1})				Increased dry mass, seed Zn content, and Zn uptake and translocation	Nautiyal and Shukla (2013)
		Gypsum and ammonium sulfate	At sowing	Drought stress	Soil application	17% increase in yield	Islam (2012)
		Zinc sulfate	Pre-flowering	Zn deficiency		Reduces flower abortion, infertility of pollen and ovule	Pathak et al. (2012)
		K_2SO_4 (1.5%) and CAN (2 and 3%)	40 and 60 DAS	Terminal drought	Foliar application	Increase in yield and yield attributes	Mahmood et al. (2017)
3	Black gram	2% DAP, 1% KCL, 1% boron, 1% $MgSO_4$, 1% $ZnSO_4$	30 and 45 DAS		Foliar application	Increase in yield and yield attributes	Maheswari and Karthik (2017)

Cereals							
4	Wheat	Sodium hydrosulfide (NaHS, H_2S donor) (500 µM)	Two leaf seedling stage	Drought stress	(Solution form) Foliar application	Improves physiological characteristics, regulated ABA catabolism, and biomass accumulation	Ma et al. (2016)
		Sodium hydrosulfide (NaHS, H2S donor) 1 mM	Three leaf seedling stages		Pretreatment in solution for 12 h	Regulates the glutathione and ascorbate metabolism	Shan et al. (2011)
		Elemental sulfur (0, 25, 50, and 75 kg ha^{-1})	Before sowing	Salinity stress	Soil application	Increase in K/Ca contents and yield and yield attributes	Ali et al. (2012)
		$CaSO_4$ (0, 50, 100, 150, 200 kg ha^{-1})	Before sowing		Soil application	Positive correlation between K/Ca and increase growth and yield	Arshadullah et al. (2013)
		K_2SO_4 (0. 50, 100, 150, 200 mM) of	Two weeks after sowing (seedling stage)		Fertigation method	Increases nutrient content and biomass accumulation	Kausar et al. (2016)
		Ammonium sulphate $(NH_4)_2SO_4$ (0, 1, 5, and 10 mM)	Two leaf stages	Cadmium (Cd) stress	Fertigation	Increased chlorophyll content, total carbohydrate accumulation, and antioxidant enzyme activity	Gaafar et al. (2012)
		Elemental sulfur + Se (0, 200 mg S kg^{-1})	Before sowing		Soil application	Regulates ethylene formation and proline and GSH metabolism and improves photosynthesis and growth	Khan et al. (2014)
		NaHS (H_2S donor) (0.4, 0.8, 1.2 mM)	Seedling stage	Copper stress	Fertigation	Increased the value of antioxidants, UPSII, Pn, Fv/Fm, qN, and qP in wheat leaves which alleviate Cu stress	Dai et al. (2016)
5	Rice	NaHS (H_2S donor) (0.75 mmol/L)	Before sowing	Drought stress	Seed priming	Improves soluble protein content and lowers osmotic potential, membrane stability, higher germination potential, and germination rate	Liu et al. (2017)

(continued)

Table 14.1 (continued)

Sr. #	Crop species	S source and dose	Stage	Stress	Application method	Response	Reference
		Gypsum (100 soil gypsum requirement)) and elemental sulfur (0, 25, 50, 75, 100, and 125% of SGR)	30 days before sowing and 10 days after transplanting	Salinity stress	Soil application	Maintains oxidative metabolism and mineral homeostasis and improves chemical properties of soil and crop yield	Ahmed et al. (2016)
		NaHS (H_2S donor) (0, 25, 50, 100, and 200 μM)	Twelve-day-old seedling		Pretreated with solution	Regulates minerals homeostases, improves biochemical and physiological processes, and increases growth and production	Mostofa et al. (2015)
		(S° and SO^{-2}_{4}) (0, 30, and 120 mg S kg^{-1})	Before sowing	Arsenic stress	Soil application	Improves tolerance against AS stress	Hu et al. (2007)
		$ZnSO_4$ (control, 13 kg ha^{-1})	Panical initiation, milky stages	Cadmium (Cd) stress	Soil and foliar application	Improves yield and yield attributes in rice	Fahad et al. (2015)
		Elemental sulfur (0, 60, and 120 mg kg^{-1})	Before sowing		Soil application	Decreases Cd toxicity in rice	Fan et al. (2010)
		NaHS (H_2S donor) (100 and 200 μM)	Seedling stage	Mercury (Hg) stress	Pretreated with solution for 24 h	Scavenges peroxy radicals and improves seedling growth	Chen et al. (2017)
6	Maize	(0, 250, 500, 750, and 1000 kg ha^{-1})	At sowing	Salinity stress	Soil application	Enhances yield components and grain production	Manesh et al. (2013)
		K_2SO_4 (0, 20, 40, 60, 80, and 100 mM			Soil application	Improved all germination parameters and decreased time for 50% germination time	Riffat and Ahmad (2016a, b)
		H_2S (0.6 mM NaHS)	Seedling stage		Pretreatment	Reduces electrolyte leakage and malondialdehyde content and increases salt stress tolerance	Shan et al. (2014)

		Elemental sulfur	Before sowing	Zinc stress	Hoagland solution form	Improves leaf chlorophyll contents and physiological characteristics in maize	Xu et al. (2008)
		Elemental S (0, 32, 160, 640 mg kg^{-1})	Before sowing		Soil application	Increases antioxidant enzyme activity and improves tolerance against zinc stress	Cui and Zhao (2011)
					Soil application	Increases activity of CAT and POD and MDA content	Cui and Wang (2006)
		Elemental S (control and 50 mmol kg^{-1} S)	Before sowing	Cadmium (Cd) stress	Side dressing in soil	Increases growth and yield of maize	Sajedi et al. (2010)
		$ZnSO_4$ (0, 25, and 45 kg ha^{-1})	Sowing time	Drought stress	Foliar application	Improves drought tolerance and yield of maize	Shahzad et al. (2017)
		K_2SO_4 (control, 1, 2, and 5%)	(15 and 7 days before pollination)		Foliar application	Increases yield and yield components	Vazin (2012)
		(0, 250, 500, 750, and 1000 kg ha^{-1})			Soil application	Enhances yield components and grain production	Manesh et al. (2013)
		K_2SO_4 (0, 20, 40, 60, 80, and 100 mM	At sowing	Salinity stress	Soil application	Improved all germination parameters and decreased time for 50% germination time	Riffat and Ahmad (2016a, b)
		H_2S (0.6 mM NaHS)	Seedling stage	Salinity stress	Pretreatment	Reduces electrolyte leakage and malondialdehyde content and increases salt stress tolerance	Shan et al. (2014)
		Elemental sulfur	Before sowing	Zinc stress	Hoagland solution form	Improves leaf chlorophyll contents and physiological characteristics in maize	Xu et al. (2008)
		Elemental S (0, 32, 160, 640 mg kg^{-1})	Before sowing	Zinc stress	Soil application	Increases antioxidant enzyme activity and improves tolerance against zinc stress	Cui and Zhao (2011)
		Elemental S (control and 50 mmol kg^{-1} S)	Before sowing	Cadmium (Cd) stress	Soil application	Increases activity of CAT and POD and MDA contents	Cui and Wang (2006)

(continued)

Table 14.1 (continued)

Sr. #	Crop species	S source and dose	Stage	Stress	Application method	Response	Reference
Oilseed crops							
7	Sunflower	$ZnSO_4$ (15, 30, 45, 60, and 75 kg ha^{-1})	At sowing	Zn deficient	Soil application	Increased growth and yield	Cheema et al. (2014)
		$FeSO_4$ (0, 2, and 4 ppm)	Vegetative stage	Drought stress	Foliar application	Increased growth and yield	Ebrahimian and Bybordi (2011)
		$ZnSO_4$ (0, 0.5, and 1%)	Vegetative and reproductive			Increased seed yield	Shahri et al. (2012)
		$FeSO_4$ (0 and 100 mM)	Seedling stage	Salinity stress		Increased net CO_2 assimilation rate, leaf area, shoot dry weight, chlorophyll content, and iron content	Torabian et al. (2017)
8	Brassica	S-deficient and S-sufficient soils	Early growth stage	Cd stress	Soil amendment	Increased activities of glutathione reductase, ascorbate peroxidase, and catalase	Bashir et al. (2015)
		$ZnSO_4$ and $MnSO_4$ (3000 and 4000 mg L^{-1})	Seedling stage	Drought stress	Foliar application	Improved growth attributes and biochemical aspects	Khan et al. (2016)
		Ammonium sulfate (0.4, 0.6, and 2%)	Pod initiation		Foliar application	Increased yield and yield attributes	Khalid et al. (2018)
		Sulfur (40 kg ha^{-1}) and thiourea (500 and 1000 mg L^{-1})	Rosette, bud formation, and flowering stage		Foliar (thiourea) soil application (sulfur)	Increased growth yield and quality	Rehman et al. (2013)

9	Soybean	3% $ZnSO_4$, 3% $FeSO_4$, and 3% $ZnSO_4$ + 3% $FeSO_4$	Before sowing	Drought stress	Seed priming	Increased physiological attributes	Dehnavi and Sheshbahre (2017)
		Mo (400 ppm) and $FeSO_4$ (400 ppm)	Reproductive stage	Drought stress	Foliar application	Improved yield and yield attributes	Heidarzade et al. (2016)
		K_2SO_4 (2.5%)	Early growth stages	Salinity stress	Foliar application	Improved plant growth, flavonoids, total phenols, antioxidant activity, carotenoids, and chlorophyll contents	Adhikari et al. (2019)

metabolism, and translocation of iron from root to shoot to improve drought tolerance in wheat (Liu et al. 2017).

Salinity stress severely hampers wheat growth and productivity. Exposure to salt stress of 6–8 dS m^{-1} causes a marked reduction in wheat yield (Royo and Abió 2003). S-containing compounds play a significant role in enhancing salinity tolerance through upregulation of specific genes and modulating several physiological and biochemical processes (Khan et al. 2014). A negative correlation was observed between sodium (Na) content and S application in wheat exposed to saline-sodic soil (Ali et al. 2012). Increasing S doses (0, 25, 50, and 75 kg ha^{-1}) significantly improved nutrient uptake and yield of wheat resulting in high K and calcium (Ca) content in grains to alleviate salinity/sodicity. In a similar study, Arshadullah et al. (2013) observed a negative correlation between Ca and Na ions in wheat plants treated with different doses of $CaSO_4$ (0, 50, 100, 150, 200 kg ha^{-1}) under saline-sodic soil (ECe = 5.32 dS m^{-1}). In contrast, K and Ca ions were positively correlated resulting in maximum wheat yield by application of 150 kg ha^{-1} $CaSO_4$. Recently, Kausar et al. (2016) reported a marked increase in nutrient uptake and biomass accumulation by treating wheat plants with various K_2SO_4 doses (0.50, 100, 150, 200 mM) under saline conditions.

Heavy metal (HM) contamination of agricultural soils is a major concern for plant scientists due to potential harms on animals and human health. Wheat is a major food crop in most parts of the world, and high concentration of HMs in wheat grains poses serious health risks (Ivezić et al. 2013). Toxic concentrations of cadmium (Cd), nickel (Ni), chromium (Cr), lead (Pb), and zinc (Zn) in wheat flour may cause health disorders like kidney and liver failure. Treatment of seedlings with different S concentrations (0, 1, 5, and 10 mM) was observed to reduce MDA content, improved growth attributes, and increased total carbohydrate accumulation in wheat exposed to Cd toxicity (Gaafar et al. 2012). Khan et al. (2015) evaluated combined effects of S and Se to minimize Cd toxicity in wheat. A significant increase in Cd tolerance was correlated with GSH and proline synthesis due to reduced activity of proline oxidase (PROX) and high glutamyl kinase (GK) activity in leaf tissues. Pretreatment with NaHS (0, 0.4, 0.8, and 1.2 mM) was observed to improve PSII photochemistry, photochemical quenching, and antioxidative activities in wheat seedlings exposed to copper (Cu) stress (Dai et al. 2016). Environmental pollutants like nickel (Ni) interfere with uptake and distribution of mineral nutrients including S to reduce growth and quality of wheat. Matraszek et al. (2016) conducted a series of water culture experiments to evaluate the effects of Ni toxicity on macronutrient composition in wheat. They found that intensive S-SO_4^{2-} nutrition partially improved biomass, enhanced ionic equilibrium, and increased nutrient accumulation in shoots of wheat plants exposed to Ni toxicity.

14.2.2 Maize

Deficiency of S has been more prevalent in maize in recent past due to reduced deposition of atmospheric S, use of crop residues, and no tillage. Low S supply results in yellowing of leaves including interveinal chlorosis. Application of poultry manure may somewhat supplement the soil with organic S however; it needs to undergo mineralization by soil microorganisms to make it available for plants. Microbial inoculation in combination with inorganic S fertilizers has also been found effective in maize. Combined effects of zinc sulfate ($ZnSO_4$) and vesicular arbuscular mycorrhizal (AM) fungus on biomass accumulation and production of maize were investigated by Sajedi et al. (2010). The plants were applied with three different levels of $ZnSO_4$ (0, 25, and 45 kg ha^{-1}). The results showed that maize growth is not significantly affected by AM fungus under well-watered conditions. However, increasing level of $ZnSO_4$ in combination with AM fungus significantly improved maize biomass and yield under water deficit conditions. They concluded that combined application of $ZnSO_4$ (45 kg ha^{-1}) and AM fungus might be utilized as an effective approach to improve growth and yield of maize. A recent report by Shahzad et al. (2017) showed that foliar application of K_2SO_4 reduces pre-anthesis abortion of maize kernel resulting in higher yield under drought stress conditions. In another study, Vazin (2012) found that foliar application of various $ZnSO_4$ doses (0, 0.5%, 1%, and 1.5%) markedly improved yield attributes of maize under water deficit conditions.

High accretion of Na^+ in the leaves is one of the main reasons for susceptibility of maize to salinity stress (Farooq et al. 2015). Accumulation of Na^+ markedly reduces K content in symplast of maize leaves that disturbs stomatal undulations under saline conditions (Jafar et al. 2012). Exposure to salt stress induces high Cys production due to increased activity of O-acetylserine (thiol) lyase (OASTL) leading to better salt tolerance in plants (Fediuc et al. 2005). In a pot study, Riffat and Ahmad (2016a) found a significant effect of S application on biomass accumulation and nutrient content of maize seedlings exposed to salt stress. In another study, they observed a marked increase in germination attributes of different maize cultivars treated with K_2SO_4 under saline conditions (Riffat and Ahmad 2016b). High S supply may cause a reduction in maize yield under salinity stress as reported by Manesh et al. (2013). Maize plants were subjected to saline environment (water salinity 9.79 dSm^{-1} and soil salinity 9.3 dSm^{-1}) and treated with various S doses, i.e., 0, 250, 500, 750, and 1000 kg ha^{-1}. Exogenous S supply up to 750 kg ha^{-1} caused a linear increase in maize yield; however, higher S dose of 1000 kg ha^{-1} markedly reduced the yield. Shan et al. (2014) suggested that application of S sources like H_2S helps to maintain the redox states of ascorbate and GSH to prevent electrolyte leakage that ultimately enhances salt tolerance in maize. Increased GSH content in roots of salt-stressed maize seedlings may be ascribed to high salt tolerance (AbdElgawad et al. 2016), which may also serve as a signal for ABA biosynthesis in shoot (Bittner et al. 2001). Maize seedlings exposed to S deficiency exhibited a marked increase in catalase (CAT) and superoxide dismutase (SOD) activities in leaf sheaths and blades due to increased ROS production under salinity stress (Chorianopoulou et al. 2012).

High concentration of toxic metals in agricultural soils has severely affected yield and nutritive value of maize. Availability of S plays a pivotal role in enhancing tolerance to metal toxicity through regulation of electron transport chain in Fe-S clusters and detoxification of HMs and xenobiotics (Hell and Hillebrand 2001). Efficacy of applied S doses may vary with the soil depth as both sulfate and elemental S exhibited a stronger effect at 0–40 cm soil layer compared to 40–80 cm horizon (Skwierawska et al. 2012). Applied S doses did not significantly affect Cu and Mn content, whereas they increased Cd and reduced Ni content in soils at a depth of 0–40 cm. Xu et al. (2008) observed toxic effects of high Zn content on pigments of maize seedlings. Supplementation of S in nutrient solution mitigated Zn toxicity and reduced the damage to young seedlings. In a similar study, Cui and Zhao (2011) reported positive effect of S application on antioxidative activities of CAT and SOD to alleviate Zn toxicity in maize seedlings. Contrarily, treatment with 50 mmol kg^{-1} S markedly reduced CAT and peroxidase (POX) activities in leaves of maize seedlings exposed to Cd toxicity suggesting that plant response to metal toxicity varies with intensity of stress, metal concentration, and plant species (Cui and Wang 2006).

14.2.3 Rice

Rice (*Oryza sativa* L.) is a staple food of nearly half of the world's population; hence, it is imperative to overcome challenges limiting rice productivity worldwide. S deficiency rarely occurs in irrigated rice and usually affects vegetative stage. It is essential to evaluate the S requirement of rice crop since low S availability results in reduced protein synthesis and causes delayed plant development and maturity (Tsujimoto et al. 2017). In contrast, high S supply may result in reduced nutrient uptake and root respiration due to sulfide toxicity, particularly in low Fe soils. Rice is considered sensitive to drought stress due to presence of shallow root system, little circular wax, and rapid stomatal closure under water deficit conditions (Ji et al. 2012). Liu et al. (2017) evaluated the effect of NaHS on antioxidative mechanism of rice seedlings subjected to PEG-induced oxidative stress. Pretreatment with NaHS markedly enhanced germination rate and prevented the degradation of soluble protein content. In addition, it significantly reduced accumulation of H_2O_2 in seeds and helped to maintain cell membrane stability resulting in slow disintegration and senescence of seedlings.

Among cereals, rice is considered the most sensitive to salinity stress. Exposure to salt concentration of even less than 40 mM may cause a significant loss in rice growth and productivity (Munns and Tester 2008). Apoplastic uptake and distribution of Na^+ cause toxicity which may be prevented by rapid downregulation of OsHKT2;1 in rice roots. An important GSH transporter in rice (OsGT1) is weakly expressed under normal conditions suggesting that it may play a key role in S transport under environmental stresses like salinity (Zhang et al. 2004; Srivalli and Khanna-Chopra 2008). S starvation reduced GSH level by 70% in rice seedlings that ultimately decreased PSII efficiency and the ability of PSI to photoreduce $NADP^+$ by 31 and 61%, respectively. However, no significant effect of S deficiency was observed on metabolites of Calvin or tricarboxylic acid (TCA) cycle (Lunde et al. 2008). Ahmed et al. (2016) found that S fertilization markedly improved rice yield under saline conditions. A positive effect of S application was also noted on soil chemical properties such as such as SAR, pH, and ECe. Maintenance of Na^+/K^+

balance was observed to increase salt tolerance in rice seedlings treated with NaHS (Mostofa et al. 2015). Supplementation of this H_2S donor also helped to maintain oxidative metabolism and mineral homeostasis by inhibiting Na^+ uptake in the seedlings.

Consumption of rice contaminated with toxic metals may cause serious health hazards in humans. Low S availability affects oxidative thiol modifications, thereby increasing HM translocation from root to shoot (Leichert et al. 2008). In young rice seedlings, S application was reported to increase Fe and Mn accumulation in rhizosphere to reduce arsenic (As) toxicity (Wu et al. 2014). S availability positively influences thiol metabolism and glycolytic enzymes to promote amino acid accumulation in leaves of rice seedlings exposed to AS toxicity (Dixit et al. 2015). Fahad et al. (2015) found that application of $ZnSO_4$ in combination with rubber ash alleviated Cd toxicity in different rice cultivars. Foliar application of $ZnSO_4$ at panical initiation and milking stages significantly increased the number of spikelets and panicles as well as spikelet fertility to improve grain yield by 73% under salt stress conditions. In a similar study, Fan et al. (2010) observed significant reduction in Cd accumulation in brown rice by excessive S supply. Pretreatment of rice seedlings with NaHS was reported to reduce mercury (Hg) toxicity in rice seedlings exposed to $HgCl_2$ (100 μM) for 3 days (Chen et al. 2017). The seedlings treated with NaHS exhibited increased expression of prominent thiol-containing compounds (OsMT-1 and NPT) to prevent Hg transport from root to shoot. Moreover, NaHS supplementation helped to scavenge or inhibit H_2O_2 and O_2^- (peroxy radicals) as well as CAT or SOD inhibitors (AT and DDC) even in the presence of Hg.

14.3 Role of S in Improving Abiotic Stress Tolerance in Legumes

14.3.1 Mung Bean

Pulses are part of healthy and balanced human diet and play an *important* role in preventing many acute diseases. They belong to family Leguminosae so they also increase soil C and N, reduce soil erosion, and help to control soil pathogens (Bagayoko et al. 2000; Sainju et al. 2005). Moreover, legumes positively influence growth of the following crops when grown in rotations with cereals. Hence, promoting legumes cultivation in developing countries can be an effective approach to reduce poverty and hunger in poor or developing countries (Abate et al. 2012).

Mung bean is an important pulse crop or food legume that is grown primarily for dry seeds and very occasionally used as a forage (Tomooka 2002). Although mung bean is considered tolerant to limited water supply, low water availability at reproductive and grain filling stages significantly reduces its yield and quality (Ahmad et al. 2015). Exposure to drought stress after 6 weeks of sowing can lead to decline in number of leaves, root nodules, dry matter, and plant height of mung bean (Ranawake et al. 2011). Reduction in plant production under drought stress is related to reduction in absorption, translocation, and redistribution of nutrients (Rouphael et al. 2012). Drought stress increases the concentration of sulfate compared to other ions like phosphate or nitrate, providing evidence that sulfate demand increases under limited water conditions (Ernst et al. 2010). Sulfate interacts with ABA and acts as a chemical signal to initiate stomatal closure in leaves under water deficit conditions. Usman et al. (2014) evaluated the role of $ZnSO_4$ (10, 15, and 20 kg ha^{-1}) on growth and yield of mung bean and reported a significant increase in growth and yield attributes. It was found that soil application of 20 kg ha^{-1} $ZnSO_4$ resulted in maximum growth and yield attributes. However, soil applied $ZnSO_4$ (15 kg ha^{-1}) resulted in maximum plant height. In addition, protein content was found higher in plants supplemented with 10 kg ha^{-1} $ZnSO_4$.

Recently, Jamal et al. (2018) reported that application of $FeSO_4$ at 5 kg ha^{-1} in combination with $ZnSO_4$ at 10 kg ha^{-1} significantly improved the biological yield, grain yield, straw yield, and nodule numbers in mung bean. Similarly, application of K_2SO_4 significantly improved yield and yield attributes in mung bean grown under arid climate (Abbas et al. 2011). Ali et al. (2014) suggested that foliar spray of iron sulfate ($FeSO_4$) at branching and flowering stages can significantly increase the growth and yield attributes of mung bean. In addition, protein and iron contents of mung bean plants supplemented with S were also found higher compared to control plant, i.e., no S supply. Muniswamy et al. (2018) evaluated the role of foliar applied Zn (0 and 0.1%), N (15 and 25 kg ha^{-1}), and S (0, 20, and 40 kg ha^{-1}) on yield and quality of mung bean. They reported that application of 25 kg ha^{-1} N combined with 40 kg ha^{-1} S and 0.1% Zn (at both pre-flowering and pod initiation stages) markedly increased yield and quality of mung bean.

Salinity stress in mung bean leads to significant reduction in yield (Saha et al. 2010) due to poor germination and seedling growth (Promila and Kumar 2000; Misra and Dwivedi 2004). In a pot study, Pandey et al. (2010) found that exogenous $ZnSO_4$ application may reduce the suppressing effects of salt stress on growth of mung bean seedlings. Similarly, Pandey et al. (2010) compared the effects of $ZnSO_4$ seed treatment and foliar spray on mung bean under induced salt stress conditions. They observed that seed priming and foliar spray of $ZnSO_4$ are effective strategies to alleviate the harmful effects of salt stress in mung bean.

Toxic metals negatively affect microbial population in the soil and may affect population size, diversity, and overall activity of soil microbiota (Kelly et al. 2003). Among various HMs, silver (Ag) and Pb were found to exert the most toxic effects on growth of soil microorganisms that delayed seed germination in mung bean (Ashraf and Ali 2015). They found that application of $ZnSO_4$ positively influenced microbial population and helped to increase germination percentage under Ag or Pb toxicity. Positive effects of S supply on microbial population in a mung bean field were also reported by Bahadur and Tiwari (2014). They observed that application of 15 kg ha^{-1} S increased *Rhizobium* and *Azotobacter* population that ultimately increased grain yield in mung bean. Recent reports of Islam et al. (2017) indicate that S supply in combination with boron (B) was more effective than individual application of these nutrients to improve grain yield in mung bean.

14.3.2 Chickpea

Seed proteins in chickpea, like other legumes, are deficient in S-containing compounds such as Cys, Met, and amino acids (Chiaiese et al. 2004). Exogenous S supply significantly improves accumulation of S-enriched seed proteins in legumes including chickpea (Sexton et al. 1998). In a controlled study, Chiaiese et al. (2004) found that addition of a transgene, sunflower seed albumin (SSA), encoding Cys and Met reduced accumulation of S-containing amino acids in chickpea seeds. Excess S supply downregulated SSA expression and improved seed protein composition in chickpea. S deficiency also leads to flower abortion and infertility of pollen and ovule resulting in low yield in chickpea. Combined application of K_2SO_4 and calcium ammonium nitrate (CAN) was observed to ameliorate negative effects of drought stress on yield attributes of chickpea (Mahmood et al. 2017). Application of 1.5% K_2SO_4 + 2 and 3% CAN resulted in maximum increase in yield and yield attributes when applied at 40 and 60 days after sowing.

Chickpea is considered sensitive to salinity as exposure to salt stress results in poor germination and seedling growth (Zawude and Shanko 2017). Seyedi (2011) conducted a laboratory experiment to evaluate the role of S supply in improving salinity tolerance in chickpea. They observed that chickpea seeds primed with $ZnSO_4$ exhibited enhanced germination and seedling establishment under saline conditions. In a similar study, Nautiyal and Shukla (2013) found that S supplementation improved seedling dry mass, seed Zn content, as well as Zn uptake and translocation in salt-stressed chickpea plants raised from $ZnSO_4$-primed seeds. Positive effects of S application were found related with increased carbonic anhydrase and SOD activity in $ZnSO_4$-treated seeds. Islam (2012) conducted a field trial to evaluate the comparative effects of two different S sources, viz., gypsum and $(NH_4)_2SO_4$, on yield of chickpea. They found that exogenous S supply improved yield by 17%; however, $(NH_4)_2SO_4$ was found more effective than gypsum regarding increment in nutrient uptake and yield.

14.3.3 Black Gram

Pulse crops, viz., black gram, green gram, cowpea, and horse gram, exhibited increased yield when supplemented with foliar spray of nutrient solution containing 2% DAP, 1% KCl, 1% boron, 1% $MgSO_4$, and 1% $ZnSO_4$ at 30 and 45 DAS of pulse crop (Maheswari and Karthik 2017). Patel et al. (2018) evaluated the effects of different S sources, viz., elemental S, gypsum, and ammonium sulfate, and S levels, viz., 0, 20, 40, and 60 kg S ha^{-1}, on growth and yield of black gram. Among different sources, gypsum was found superior in terms of growth and yield. Among various levels, 40 kg ha^{-1} gypsum was found more superior compared to others. Srivastava and Shukla (2016) reported an adverse effect of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), and lead (Pb) on growth of black gram. They found that heavy metal toxicity followed the order As > Cr > Cd > Co > Pb in terms of damage caused to black gram plants. Plants grown in As toxic soils failed to survive and wilted within 1 week. A significant loss of pigments and proteins was noted in plants exposed to Cr and Co toxicity compared to control plants. Antioxidant enzymes' activity was found higher in plants grown in Cr and Co toxic soils compared to Cd and Pb plants. It was concluded that black gram is highly sensitive to As followed by Cr and Pb was found least toxic for growth.

14.4 Role of S in Improving Abiotic Stress Tolerance in Oilseeds

14.4.1 Sunflower

Sunflower seeds are used to make high-quality vegetable oil (Okoko et al. 2008). Its yield depends upon the availability of water at different growth stages, and flowering stage is considered as most critical stage regarding water availability. It was reported that application of S at the rate of 25 kg ha^{-1} significantly improved the plant height, head diameter, no. of seeds per achene, biological yield, and achene yield of sunflower plants (Poonia 2000). Wani et al. (2001) suggested that increasing S doses might significantly improve seed yield and quality of sunflower seeds. Recently, Ullah et al. (2019) found a significant increase in sunflower yield by applying S in combination with K. The maximum grain yield and oil quality were recorded in plants supplemented with 60 kg ha^{-1} S + 90 kg ha^{-1} K. Similarly, S fertilization combined with exogenous Zn supply alleviated the adverse effects of drought stress at different growth stages of sunflower (Zafar et al. 2014). Application of S using gypsum at the rate of 60 kg ha^{-1} not only increased the plant height, leaf area index, and dry matter of sunflower but also improved the pH of the soil (Usha Rani et al. 2009). Cheema et al. (2014) investigated the effects of various $ZnSO_4$ concentrations (15, 30, 45, 60, and 75 kg ha^{-1}) on sunflower. They reported that plants supplemented with 45 kg ha^{-1} $ZnSO_4$ resulted in maximum stem diameter, number of achenes per head, achene yield, head diameter, and biological yield. Moreover, harvest index and 1000-achene weight were found maximum in plants supplemented with 60 kg ha^{-1} $ZnSO_4$. A study involving exposure of sunflower to drought stress at vegetative and reproductive stages showed that foliar application of 1% $ZnSO_4$ significantly ameliorated water stress to improve achene yield in sunflower (Shahri et al. 2012). Likewise, foliar application of $FeSO_4$ was found helpful in improving growth and yield of sunflower under normal and drought stress conditions (Ebrahimian and Bybordi 2011).

Nano-particles of $FeSO_4$ were found to significantly reduce salt-induced oxidative stress in sunflower (Torabian et al. 2017). S supplementation markedly reduced Na^+ content and increased net CO_2 assimilation rate, leaf area, shoot dry weight, chlorophyll content, sub-stomatal CO_2 concentration, and Fe content to improve salinity tolerance in sunflower.

14.4.2 *Brassica*

In general, *Brassica* species require S at vegetative stage to synthesize essential proteins for improved oil quality (Blake-Kalff et al. 2001). It is estimated that production of 1 ton rape seed requires about 16 kg S compared to wheat seed that only requires 2–3 kg S (Lee et al. 2016). Moreover, these species contain high amount (20%) of organic S in the form of S-containing metabolites like glucosinolate (Aghajanzadeh et al. 2014). Deficiency of S significantly reduces the incorporation of total amount of S into proteins by 62% (Lee et al. 2013). A study involving two contrasting *Brassica napus* species, viz., Mosa and Saturnin, showed that genotype possessing better sulfur use efficiency exhibited higher tolerance to drought stress (Lee et al. 2014). Dhruw et al. (2017) found that application of S using $ZnSO_4$ (40 kg ha^{-1}) along with basal dose of N-P-K @ 120:60:40 can significantly improve growth, yield, and oil contents of *B. juncea* plants. A field study by Mishra et al. (2010) showed that S fertilization in combination with P resulted in maximum mustard oil production. Khan et al. (2016) compared the effects of $ZnSO_4$ and manganese sulfate ($MnSO_4$) in improving drought tolerance in *B. juncea.* They reported a significant increase in growth attributes, biochemical aspects (chlorophyll and carotenoids), and relative water content of *B. juncea* treated with S under drought stress.

Salt stress tolerance in *Brassica* species is related to modulation in several physiological and biochemical processes (Ashraf and McNeilly 2004). Siddiqui et al. (2012) reported that exogenous S supply in combination with N can alleviate the negative effects of salt stress in *B. juncea.* It was observed that decrease in nitrate reductase activity, N content, growth, and photosynthetic activity under salinity was restored with soil application of N and S at the rate of 100 mg kg^{-1} soil. Moreover, combined application of N and S resulted in better growth and proline accumulation compared to individual application of these nutrients.

Exposure to HMs not only poses serious threat to productivity of food crops but also threatens the safety of human nutrition. S nutrition helps to reduce toxic effects of metal elements in *Brassica* species. For example, Bashir et al. (2015) suggested that S pool is needed to synthesize GSH, PCs, and non-protein thiols to alleviate Cd toxicity in *B juncea*. They found that S-deficient plants exhibited higher oxidative activity under Cd stress. Contrarily, S application markedly enhanced CAT, APX, and GR activities to alleviate Cd-induced oxidative stress in *B. juncea*. Similarly, Zhong et al. (2012) found that S-treated *B. napus* plants exhibited lower As content in roots and grain compared to S-deficient plants. They were of the view phytoremediation capacity of rapeseed could be increased by S fertilization.

14.4.3 Soybean

The production of soybean is threatened by climatic change with more frequent occurrence of drought stress around the globe (Dai 2013; Foyer et al. 2016). Studies conducted under field and controlled conditions have shown that water stress can cause 25–50% yield reductions in soybeans (Frederick et al. 2001; Sadeghipour and Abbasi 2012). It has been well documented that S nutrition plays a significant role against drought stress in crop plants. Its metabolite GSH has been reported to scavenge reactive oxygen species (Astolfi et al. 2012). Exogenous H_2S application increased the biomass and survival of soybean plants grown under terminal drought stress (Zhang et al. 2010). Water deficit conditions drastically decreased the chlorophyll contents and antioxidant activity of soybean seedlings. Foliar application of H_2S significantly improved the antioxidant activity and chlorophyll contents of seedlings. Moreover, delay in the accumulation of hydrogen peroxide, superoxide anion, and malondialdehyde was also observed in plants sprayed with H_2S compared to control. Cigelske (2017) investigated the combined effects of N and S on soybean. It was reported that application of N significantly improved the vigor and yield but decreased the nodulation in soybean plants. However, S application decreased protein content but significantly increased nodulation in rhizosphere. Seed priming with sole and mixed solutions of $ZnSO_4$ and $FeSO_4$ alleviated the detrimental effects of drought stress in soybean at reproductive stage (Dehnavi and Sheshbahre 2017). They found a significant increment in photosynthetic rate, transpiration rate, stomatal conductance, and photosynthetic characteristics of plants raised from nutrient-primed seeds compared to hydropriming. Similarly, Heidarzade et al. (2016) reported that $FeSO_4$ application in combination with molybdenum markedly enhanced the yield attributes of soybean under drought stress. Comparative effects of KCl and K_2SO_4 application on physiological activities of soybean seedlings exposed to salt stress were investigated by Adhikari et al. (2019). They found that application of K_2SO_4 at 2.5% was more effective than KCl to improve growth, flavonoids, total phenols, antioxidant activity, carotenoids, and chlorophyll contents of salt-stressed soybean seedlings.

14.5 Conclusion

Considering the importance of S-containing metabolites in improving abiotic stress tolerance in plants, the application of S-containing fertilizers has become imperative to improve yield and quality of food crops in recent past. Low S availability severely hampers uptake, distribution, and assimilation of plant nutrients such as N, P, K, Zn, B, and Ca. Moreover, plants grown in S-deficient soils exhibit high susceptibility to environmental stresses like drought, salinity, and metal ions. Despite extensive research on S metabolism and assimilation in crop plants in recent years, there are still many questions answered. Future studies focused on increasing our understanding about specific gene expression in response to S application would help us to develop crop species tolerant to a wide array of environmental extremities. Studies triggered to boost our knowledge about interactive pathways of S assimilation and phytohormones regulation are of particular importance in this regard.

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Chapter 15
Magnetic Fields, Temperature, and Exogenous Selenium Effect on Reactive Oxygen Species Metabolism of Plants Under Flooding and Metal Toxicity

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Contents

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Abstract In this chapter the effects of magnetic fields 30 mT, 50 Hz, 30 s (MF), temperature, and exogenous selenium on the plant growth, productivity, and changing in the metabolism of reactive forms of oxygen under stress conditions, induced by flooding and soil pollution with lead, are discussed. It was shown that seed processing with MF caused an enhancing of plant antioxidant potential under root hypoxia, induced by soil flooding. It is also considered the role of temperature in the changing of the oxidative process intensity as well as antioxidant enzyme activities induced by lead in the *Plantago major* L. plants. Increasing of the temperature from 20 to 28 °C resulted in a reduction of lead content in the leaves of plants exposed in the water solution of $Pb(NO_3)_2$, but increased the adverse effects of Pb, especially in the beginning of stress development. It has been also showed that the pros or cons effects of exogenous Se^{6+} on plant resistance to the lead pollution depended on the Se^{6+} concentration in the soil.

Keywords Antioxidant enzymes · Flooding · Lead · Oxidative stress · Photosynthesis · Selenium · Soil hypoxia

Abbreviations

AsP	Ascorbate peroxidase
Chl	Chlorophyll
GPX	Guaiacol peroxidase
GR	Glutathione reductase
MDA	Malondialdehyde
MF	Magnetic fields
P_N	Net CO_2 assimilation rate
ROS	Reactive oxygen species
r_s	Stomata resistance
SOD	Superoxide dismutase
TBARs	Thiobarbituric acid reactive substances

15.1 Introduction

The investigation of the mechanisms of damage and adaptation of plants in conditions of oxygen deficiency is of great scientific and practical interest. Oxygen deficiency in plant tissues often occurs both in the natural conditions of crop growth and in artificial habitat systems and is a consequence of the lack of oxygen in the environment (Grineva 1975; Chirkova 1988; Kennedy et al. 1992). The most common phenomenon causing oxygen deficiency in the root zone of plants is flooding. With excessive soil moisture, the water that fills the pores displaces the air and makes diffusion of gases more difficult. The availability of oxygen in water is decreased as the diffusion coefficient of oxygen in water is significantly lower than in a gaseous environment. During soil water flooding, oxygen disappears within a few hours, while the concentration of carbon dioxide, nitrogen, and hydrogen and the accumulation of poisonous gases—methane, hydrogen sulfide, and sulfur dioxide—increase (Grineva 1975; Chirkova 1988). Thus, flooding is a complex factor. In this case, the plants suffer not only from increasing hypoxia but also from constant contact with water and exposure to the accompanying toxic gases. Reducing of the O_2 content (hypoxia) is observed in the soils during short-term overwatering, when the roots are under water, but the overground parts of plants remain in the atmosphere. The complete absence of the oxygen (anoxia) takes place in soils while prolonged flooding of the root system and even the whole plant. The lack of oxygen affects a lot the intensity and directionality of metabolic reactions and instigates intensification of oxidative processes in plant cells (Balakhnina 2015).

Among the symptoms of hypoxic stress in plants, an increase in stomatal resistance is also considered which is usually associated with water deficiency. The magnitude of stomatal resistance in the leaves of plants depends on the level of abscisic acid, which increases soon from the beginning of flooding. When flooded, within a few hours, a noticeable wilting of the plants is observed (Drew 1997). This appears to be the result of a high resistance to the flow of water from the roots. Withering may be considered as a consequence of the inhibition of respiration and a reduction in ATP synthesis in the roots (Chirkova 1988).

Inhibition of the growth of plant aboveground organs during soil flooding is the next symptom of the root hypoxia and manifests itself soon after the onset of the action of the stress factor. The reason for the suppression of the growth of aboveground organs can be a decrease in the content of water and the main nutrients entering through the roots, inhibition by ethylene, and the accumulation of metabolic toxins (Kozlowski 1984). The rate of CO_2 assimilation and the intensity of photosynthesis in the leaves decrease as a result of the closure of stomata and a number of biochemical plant modifications (Trought and Drew 1980; Jackson and Drew 1984).

The resistance of different plant species depends on their ability to adapt to extreme conditions. The system of plant adaptation to hypoxia includes various levels of coordinated adaptive reactions aimed at providing tissues with oxygen and maintaining homeostasis in an anaerobic environment.

Soil contamination with heavy metals is another negative stress factor of nowadays environment. Lead (Pb) is one of the most widespread heavy metals which is toxic for the majority of plants and has an adverse effect on their morphology and metabolism. Pb causes inhibition of photosynthesis and enzymatic activity, imbalance of water in plant tissues, and changes in membrane permeability (Kosobryukhov et al. 2004; Sharma and Dubey 2005). Concentrations of lead in tissues exceeding 30 ppm are dangerous for plants (Xiong 1997). Pb is generally accumulated in the roots at higher concentrations than in the overground parts of plants (Islam et al. 2008; Sharma and Dubey 2005; Xiong 1997). Nevertheless, some plants, such as field pumpkins, beans, red beets, cabbage, chicory, and parsnips, contained a higher concentration of lead in the leaves than in the roots (Sękara et al. 2005). It was shown (Voskresenskaya et al. 2013) that Pb content in *Plantago major* was approximately equal in the leaves and roots of plants growing on strongly contaminated soils. Soil contamination with lead causes a number of different unfavorable impacts on plant growth and development. Processing of 100 and 1000 μm Pb moss has been shown to result in reduced dry weight and total chlorophyll content, significantly increased production of reactive oxygen species, and lipid peroxidation intensification although concentration of ascorbate and glutathione was still high (Choudhury and Panda 2005). Photosynthesis is very sensitive to Pb treatment. A decrease of the photosynthesis intensity is explained by the decrease in the concentration of plastid pigments (Mishra et al. 2006), inhibition of the electron transfer chain (Gajic et al. 2009), as well as damage of the structure of chloroplasts and changing of stomata state (Ahmad et al. 2008a, b; Islam et al. 2008). A suppression of the rate of photosynthesis in leaves of rice *Oryza sativa* L., processed by lead, was accompanied with a contemporaneous decrease of conductivity and rate of stomata transpiration (Li et al. 2012).

Nonspecific plant reaction to effect of any unfavorable factors is a formation and accumulation of excessive amounts of reactive oxygen species (ROS). According to "a theory of peroxidation," formation of peroxides is one of the constant processes like light, heat, etc., which has a great importance in life cells and to which living organism must adapt in a certain way (Bach 1912). This adaptation to peroxides occurs in such a way that the cell synthesizes or activates enzymes due to which the peroxides can be used and, if it is necessary, neutralized (Bach 1912). This theory was further developed in the works of Michaelis, who believed that the oxidative reactions of organic molecules proceed through successive one-electron stages (Michaelis 1939). The places where the formation of an excessive amount of ROS occurs in plants may be mitochondria and chloroplasts because of a failure in the electron transport chains and decreasing of the $NADPH^+$ content. In this case O_2 turns into an alternative electron acceptor (Egneus et al. 1975).

Excessive production and accumulation of reactive oxygen species such as superoxide anion-radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and others leads to an intensification of the processes of oxidation of cell substances such as lipids, proteins, and pigments and even the destruction of the genetic apparatus (Elstner 1982; Halliwell 1984; Halliwell and Gutteridge 1999; Asada 2006; Balakhnina et al. 2009, 2010, 2012). One of the commonly used indicators of the polyunsaturated fatty acid peroxidation in cell membranes is malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARs) (Balakhnina et al. 2005; Balakhnina and Borkowska 2013; Reddy et al. 2005; Ruley et al. 2004; Verma and Dubey 2003). It was shown that H_2O_2 concentration in the leaves of barley plants under soil overwatering increased (Kalashnikov et al. 1994). Excessive accumulation of ROS results in intensification of the peroxide processes in the plant tissues that causes the serious defects of the cells and in some cases even death of the whole organism (Egneus et al. 1975; Ahmad et al. 2008a, b). In the process of evolution in the cells of aerobes, a system of protection against oxidative damage was formed which consists of low molecular antioxidants (ascorbic acid, carotenoids, glutathione, and so on) and enzymes. The enzymes ascorbate peroxidase (AsP), glutathione reductase (GR), and guaiacol peroxidase (GPX) are the important scavengers, neutralizing H_2O_2 (Mittler 2002; Cakmak and Atici 2009). To date, not so many studies have appeared regarding influence of magnetic fields on biochemical and physiological reactions of plants in optimal growth conditions, and there are practically no studies on their effect on the development of oxidative stress in adverse environmental conditions (Bhardwaj et al. 2012). Soil pollution with heavy metals, including Pb, induces excessive formation and accumulation of ROS that leads to intensification of oxidative reactions, a diminution of saturated fatty acids, and an increase of the unsaturated fatty acid concentrations in the tissues in a number of different plants (Erdei et al. 2002; Geebelen et al. 2002; Girard et al. 2002; Malecka et al. 2001). There are proofs that the mitigation of oxidative damages and enhanced sustainability to affect of unfavorable factors often correlate with the level activity of the system defensive against oxidative destruction (Suzuki et al. 2012). It's known that the intensity of oxidative reactions can be controlled by such enzymes as superoxide dismutase (SOD), peroxidase, and catalase and changing the level of their activities is a reflection of an antioxidant potential of plants (Balakhnina et al. 2005; Balakhnina and Borkowska 2013; Liu et al. 2009; Ruley et al. 2004; Wang et al. 2007). However, at higher doses of stressors, taking into account heavy metals too, inhibition of plant antioxidant potential was shown (Choudhury and Panda 2005; Sharma and Dubey 2005).

15.2 Influence of Magnetic Fields on Growth and Oxidative Metabolism of Plants Under Soil Flooding

In the last decades, the application of magnetic fields (MF) in various fields of science and technology, especially in medicine and agriculture, has been widely used. The effect of magnetic fields on enzymatic and hormonal activity and the metabolism of reactive oxygen species, carbohydrates, protein, and lipids has been shown in the experiments with animals (Sieroń and Cieślar 2003). Treatment of seeds with magnetic fields improved their germination and growth of seedlings and enhanced productivity of a number of valuable agricultural cultures (Moon and Chung 2000; Balouchi and Modarres-Sanavy 2009; Aladjadjiyan 2010; Pietruszewski and Kania 2010) and also increased resistance to certain pathogens (Yinan et al. 2005; De Souza et al. 2006). Stimulating action of MF on plant seed is the result of a range of various events, connected with changing of the enzyme activities, biosynthesis of proteins, and the enhanced content of ascorbic acid which is one of the main low molecular antioxidants (Sidaway and Asprey 1966; Yinan et al. 2005; Rochalska and Grabowska 2007), as well as plant reactions, reflecting the free radicals' metabolism (Murr 1965). Shabrangi and Majd (2009) and Bhardwaj et al. (2012) reported that MF affect the growth and antioxidant potential of plants.

We assume that the pre-sowing seed treatment with MF can lead to increased generation and accumulation of seed ROS, which, in turn, can mobilize the antioxidant system and thus increase the tolerance of seedlings during soil flooding.

In order to find out the possible influence of MF on seed germination, seedling growth, and oxygen metabolism in plants, wheat *Triticum aestivum* L. cv. “Banti” seeds were processed with magnetic fields (+MF) 30 mT, 50 Hz, 30 s. The intensity of oxidative reactions was determined via the concentrations of TBARs. The system defense against oxidative degradation is represented by antioxidant enzymes such as AsP, GR, and GPX (Balakhnina et al. 2015a).

15.2.1 Soil Aeration Conditions

During experiment, oxygen diffusion rate (ODR) in the soil averaged 92 μg^{-2} s^{-1} at the beginning of the experiment and decreased up to 6 μg^{-2} s^{-1} after some hours of soil flooding. At this, redox potential (Eh) of soil was changed from +330 to +70 mV. The ODR meanings that are lower than 35 μg m^{-2} s^{-1} should be considered as a limiting factor for most cultivated plants. These changes on the level of soil oxygen availability affected the common state of plants (Gliński and Stepniewski 1985).

15.2.2 Growth Processes

It was shown that seed processing with magnetic fields (MF 30 mT, 50 Hz, 30 s) had practically no effect on the germination rate of wheat seeds (Fig. 15.1) as well as growth processes, which were determined through the accumulation of biomass of aboveground plant organs and their roots (Fig. 15.2a, b). Under optimal conditions

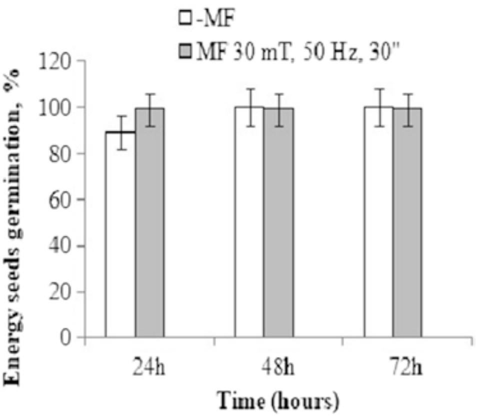
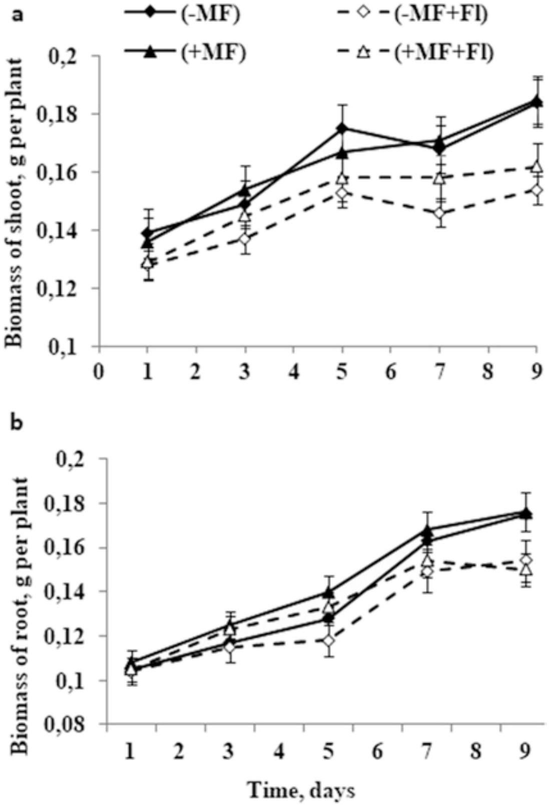


Fig. 15.1 Germination of wheat seeds treated with magnetic fields (MF 30 mT, 50 Hz, 30 s) and untreated ones (−MF). Bars indicate standard deviation ($n = 300$). Adapted from Balakhnina et al. (2015a)

Fig. 15.2 Biomass of shoots (**a**) and roots (**b**) of wheat seedlings grown from seeds treated with magnetic fields (+MF) and untreated ones (−MF) and flooding (+MF +Fl) and (−MF +Fl). Bars indicate standard deviation ($n = 25$). Adapted from Balakhnina et al. (2015a)



of soil moistening, the biomass of the aerial parts of plants grown from seeds treated and not treated with magnetic fields were almost the same both at the beginning and after 9 days of the experiment (Fig. 15.2a). This fact confirms that in this case, the used dose of the acting factor (MF 30 mT, 50 Hz, 30 s) was ineffective for improving plant productivity (Fig. 15.2a, b). A similar reaction to pre-sowing treatment of seeds with magnetic fields was recorded in the roots; the root biomass of (+MF) and (−MF) plants did not differ significantly throughout the experiment (Fig. 15.2b). It was shown that excessive soil moisture suppressed the growth of the biomass of aboveground organs and roots in both plants grown from treated and untreated magnetic fields of seeds (Fig. 15.2a, b). Nine-day soil hypoxia induced by flooding caused a decrease in the increment of biomass of plant shoots (−MF +Fl) and (+MF +Fl) by 19 and 14% and roots by 14 and 17%, respectively, compared with (−MF) and (+MF) plants grown under optimal soil irrigation (Fig. 15.2a, b). The data presented in Fig. 15.3a show that the processing of seeds with magnetic fields had no effect on the growth of shoots and roots in length. Under the influence of soil flooding, the decrease in the increment of shoot length of (−MF +Fl) and (+MF +Fl) seedlings also did not differ significantly, whereas the inhibition of root growth of (+MF +Fl) plants was less pronounced compared to the (−MF +Fl) variant and

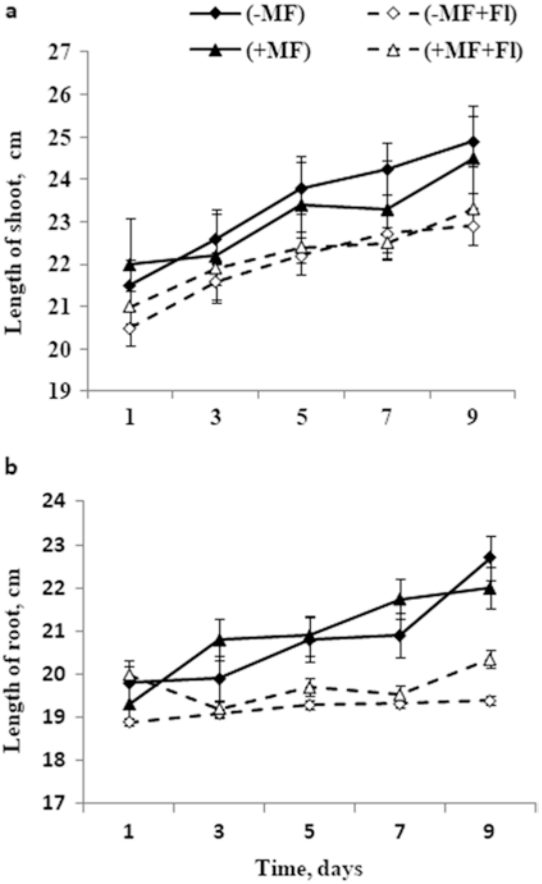


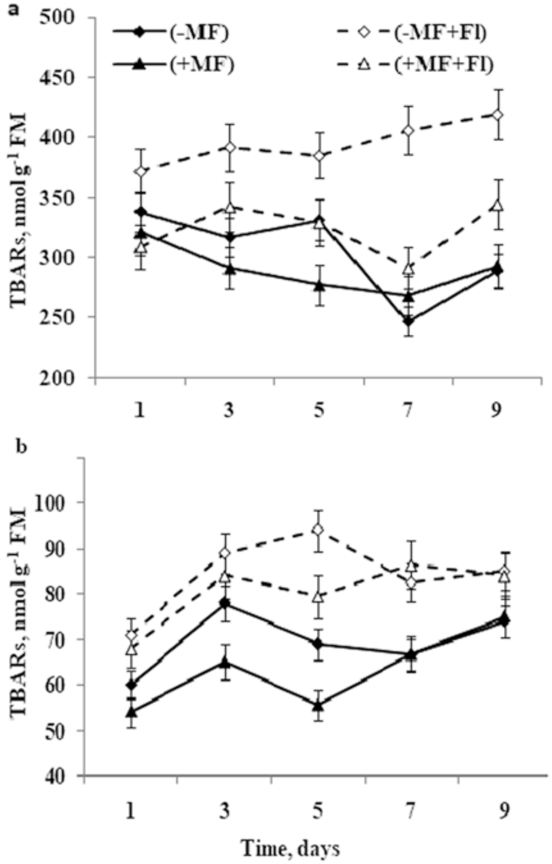
Fig. 15.3 The length of shoots (**a**) and roots (**b**) of wheat seedlings grown from seeds treated with magnetic fields (+MF;) and untreated ones (−MF;) under optimal soil watering and flooding (+MF +Fl) and (−MF +Fl). Bars indicate standard deviation (*n* = 25). Adapted from Balakhnina et al. (2015a)

amounted to 17 and 8%, respectively, relative to the corresponding control (Fig. 15.3b).

15.2.3 Processes of Oxidative Destruction

During 9 days of the investigation, under optimal soil moistening conditions, the TBARs concentrations in the leaves of plants grown from untreated and magnetic field-treated seeds averaged 333 and 284 nmol g^{-1} FM, respectively (Fig. 15.4a). Flooding of the soil led to the intensification of peroxidation processes both in the leaves and in the roots of the experimental plants (Fig. 15.4a, b). Thus, the content of TBARs in the leaves (−MF +Fl) and (+MF +Fl) increased by 21 and 14%, respectively, relative to the non-flooded samples (Fig. 15.4a). During 5 days of the experiment, the intensity of oxidative processes in the roots of magnetic field-treated

Fig. 15.4 The content of the TBARs in the leaves (**a**) and roots (**b**) of wheat seedlings grown from seeds treated with magnetic fields (+MF) and untreated ones (−MF) under optimal soil watering and flooding (+MF +Fl) and (−MF +Fl). Bars indicate standard deviation ($n = 5$). Adapted from Balakhnina et al. (2015a)



plants was far below in comparison with untreated variants. Overwatering and deficit of the oxygen in the soil caused more increment of TBARs concentration in the roots of (−MF +Fl) plants, in comparison to (+MF +Fl) plants.

15.2.4 The AsP, GR, and GPX Activities

AsP activity in the leaves of magnetic field-untreated plants was about 14, 15, and 22 μmol g^{-1} FM min^{-1}, respectively, and it was lower than in magnetic field-treated plants on about 48, 32, and 29%, respectively (Fig. 15.5a). Then, meaning of the AsP activity of plants treated with magnetic fields dropped to the level activity of untreated seedlings. After 1- and 3-day action of flooding, enzyme activity in the leaves of (−MF +Fl) plants was higher on 43 and 20% than in the (−MF) plants, respectively. Soil flooding caused analogical reaction of AsP in the leaves of magnetic field-untreated and magnetic field-treated plants. However, the level of AsP

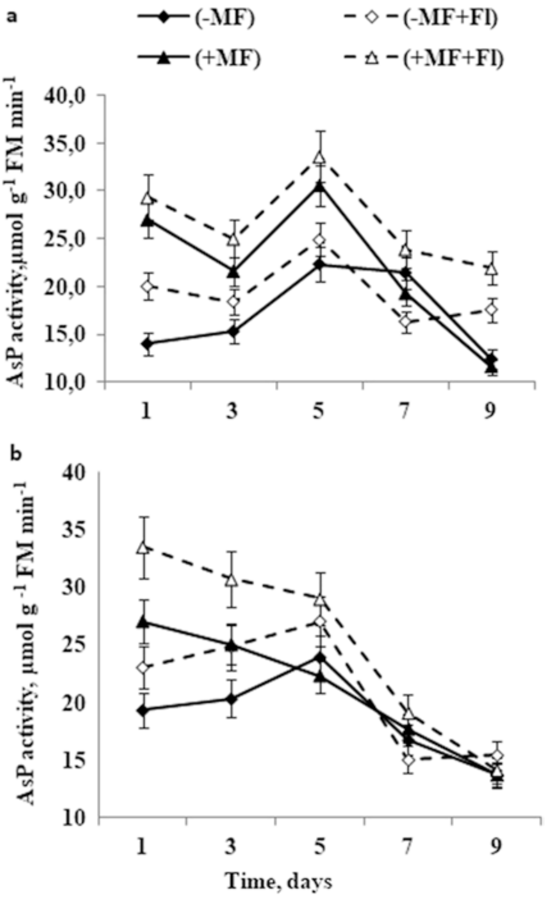


Fig. 15.5 The AsP activity in the leaves (**a**) and roots (**b**) of wheat seedlings grown from seeds treated with magnetic fields (+MF) and untreated ones (−MF) under optimal soil watering and flooding (+MF +Fl) and (−MF +Fl). Bars indicate standard deviation (*n* = 5). Adapted from Balakhnina et al. (2015a)

activity in (+MF +Fl) plants was higher on about 27% than that of (−MF; +Fl) plants during the experiments. AsP activity in the roots of (+MF) plants was higher than in the (−MF) plants by 43 and 23% on the 1st and 3rd day of the experiment, respectively, and decreased in both variants later (Fig. 15.5b). Soil flooding resulted in enzyme activation. However, in comparison with (−MF +Fl) variant, AsP activity in the roots of (+MF +Fl) plants was higher by 46 and 24% on the 1st and the 3rd day as well as surpassed enzyme activity of (−MF) seedlings by 74, 51, and 21% on the 1st, 3rd, and 5th days of the experiment, respectively (Fig. 15.5b).

GR activity of (−MF) seedlings dropped down while experiment in the leaves from 0.86 to 0.51 µmol g^{-1} FM min^{-1} and the roots from 0.36 to 0.23 µmol g^{-1} FM min^{-1}, respectively (Fig. 15.6a, b). It should be noted that only at the beginning of the experiment the enzyme activity in the leaves of (+MF) plants was higher than in (−MF) plants (Fig. 15.6a). Subsequently, steady challenging effect of pre-sowing seed treatment with magnetic field on the GR activity was registered neither in the

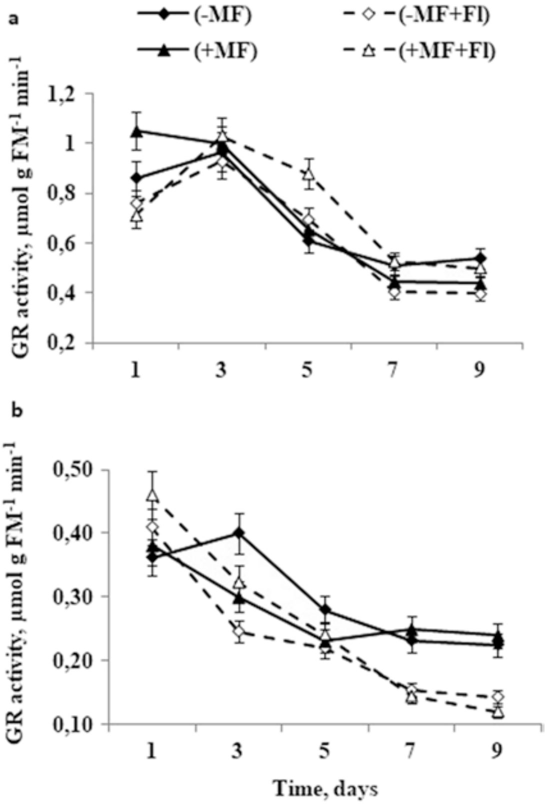


Fig. 15.6 The GR activity in the leaves (**a**) and roots (**b**) of wheat seedlings grown from seeds treated with magnetic fields (+MF) and untreated ones (−MF) under optimal soil watering and flooding (+MF +Fl) and (−MF +Fl). Bars indicate standard deviation (*n* = 5). Adapted from Balakhnina et al. (2015a)

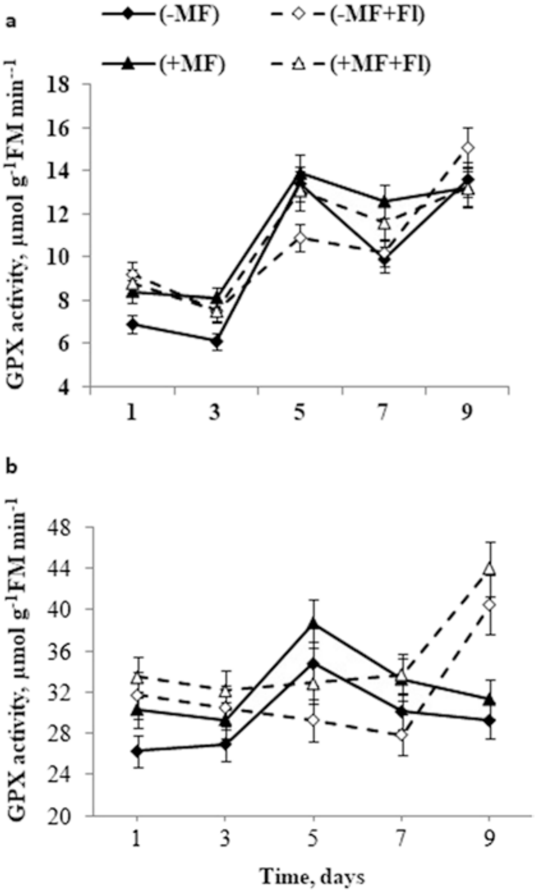
leaves nor roots of experimental plants, as under control soil watering and under flooding (Fig. 15.6a, b).

GPX activity in the leaves of the (+MF) seedlings was significantly higher than the (−MF) ones only on 3rd and 7th day of plant growing. Under soil flooding, GPX activity in the leaves of (−MF +Fl) and (+MF +Fl) plants demonstrated analogical tendency without essential differences, excluding plant responses on the 5th and 9th day of the experiment (Fig. 15.7a). Soil flooding caused an increase of GPX activity in the roots of the (−MF +Fl) and (+MF +Fl) plants by the 9th day of the experiment (Fig. 15.7b).

15.2.5 Discussion

Since the ecological situation on the planet has changed for the worse, many cultivated and wild plants are often subjected to various negative factors. Taking into account impossibility to improve the current situation in a short time, ways, methods, and technologies should be found to increase their survival. So, it was found

Fig. 15.7 The GPX activity in the leaves (**a**) and roots (**b**) of wheat seedlings grown from seed treated with magnetic fields (+MF) and untreated ones (−MF) under optimal soil watering and flooding (+MF;+Fl) and (−MF;+Fl). Bars indicate standard deviation ($n = 5$). Adapted from Balakhnina et al. (2015a)



that the processing of barley seeds with a 4-hydroxyphenethyl alcohol (4-HPEA), a well-known non-purine cytokinin-like nature substance (Balakhnina et al. 2010a), and bioflavonoid dihydroquercetin, which possesses antioxidant properties, led to enhancing of plant resistance to soil hypoxia (Balakhnina et al. 2009). It was reported a positive effect of seed treatment with low-frequency MF on their germination rate (Pietruszewski and Kania 2010). It has been showed that seed processing with MF influences a variety of metabolic processes, in seeds and seedlings (Wadas 1992; Rajendra et al. 2005). Static MF treatment of 200 mT for 1 h demonstrated a significant effect on rate of cucumber seed germination, the water uptake, changing of hydrolytic enzyme activities, generation of reactive oxygen species, and activation of antioxidant enzyme system in germinating seeds (Bhardwaj et al. 2012). However, in the conditions of our experiment was not found any stimulating effect of magnetic fields (MF 30 mT, 50 Hz, 30 s) on the rate of seed germination, wheat growth, and biomass accumulation, under either control watering or soil hypoxia, induced by flooding. In this case, we decided that the plant response was dependent on the dose of applied MF and the kind of plant. Moon and Chung (2000) showed that the rate of tomato seed germination relied on the different intensity and duration of exposure to electric and magnetic fields during pre-sowing seed treatment. The intensification of oxidative processes as a consequence of excess accumulation of ROS was determined by us through the changing of TBARs concentrations in plants. The content of TBARs in the leaves of (+MF) plants was below than in (−MF) plants. At the soil hypoxia, TBARs content in the leaves of (−MF +Fl) plants was higher, in comparison with corresponding control (−MF) and much more higher than that in (+MF +Fl) ones. In the roots of the same experimental plants, reaction was analogical up to the 5th day of the overwatering. After that, TBARs concentrations in roots of (−MF +Fl) and (+MF +Fl) plants were almost the same. The suppression of the intensity of peroxide processes in the leaves and roots of plants (+MF +Fl) may be associated with the activation of antioxidant enzymes, which was recorded at some stage of oxidative stress. Taking into account that the oxidative reactions of plants are non-specific (Asada 2006), in order to understand the effect of seed processing with MF on the metabolism and resistance of seedlings, can draw analogies with the effects of other physical factors. So, pre-sowing seed stratification, laser processing, and UV irradiation accelerated rate of seed germination and improved plant growth and tolerance under unfavorable condition (Einali and Sadeghipour 2007; Sujak et al. 2009; Kacharava et al. 2009). According to Oracz et al. (2009), seedlings grown from stratified seeds contained heightened concentration of H_2O_2 and elevated level of catalase activity. H_2O_2 is a rather stable molecule, compared with other active forms of oxygen, is generated in a plant cell, and, being an uncharged molecule, easily penetrates via plasma membrane, which makes it a predefined one for ROS-mediated signal transmission (Foyer et al. 1997). The antioxidant system defense of the cell, which influences completion or transition of the signal, is one of the important ways to achieve perception of the ROS by the organism (Alscher et al. 1997). He et al. (2009) reported that after seed processing with H_2O_2, the concentration of hydrogen peroxide and the intensity of oxidative processes in seedlings were reduced. At this, increasing of antioxidant capability and improving of membrane stability under unfavorable condition were registered (He et al. 2009). It has been suggested that H_2O_2 can fulfill the role of a signaling molecule, stimulating activity of some antioxidant enzymes in seeds, which keeps in the seedlings and can contribute to easing of the oxidative damage under stress conditions (He et al. 2009). This permits us to suppose that in the conditions of our study, the increasing of antioxidant enzyme activity in seedlings was the result of signaling action of the ROS forced by seed treatment with MF. Activation of the antioxidant system resulted in maintaining the intensity of oxidative reactions in (+MF) seedlings at a lower level compared to (−MF) plants.

15.3 The Role of Temperature in Development of Oxidative Stress Induced by Lead in the Leaves of *Plantago major* L.

When growing plants in the field conditions, they are often subjected to temperature fluctuations both in the atmosphere and in the soil that influences its growth and development (Chaitanya et al. 2001; Nosalewicz et al. 2013). It was demonstrated earlier that dangerously lower or higher temperatures caused irreversible damage on plants (Chaitanya et al. 2001; Gulen and Eris 2004; Wahid et al. 2007). A lot of plants are able to survive changes in temperature in the diapason from 30 to 40 °C. A decline of the protein concentration was found on plants of strawberry subjected to progressive heat stress (from 25 to 45 °C). High-temperature treatment (45 °C without adaptation) caused shock heat stress, which was characterized by increasing of the total peroxidase activity (Gulen and Eris 2004). Heat stress induced or intensified the system defense against oxidative destruction, in particular such enzymes as SOD, catalase, and peroxidase and certain low molecular antioxidant compounds (Chaitanya et al. 2002).

Fluctuations in daytime temperatures from 16 to 30 °C that occur in mid-latitudes in summer, as a rule, are not dangerous for most plants. As for *Plantago major* L. plants, their temperature optimum ranges from 19.0 to 21.2 ± 3 °C during plant ontogenesis (Atkin et al. 2007; Mudrik et al. 2003). Temperature fluctuations in the range from 16 to 30 ° C may not cause an irreversible negative change in plants, but they inevitably affect the intensity of transpiration, nutrient absorption, photosynthesis, and many other metabolic processes, including oxidative stress. Unfortunately, in modern literature there is practically no data on the effect of moderately elevated temperatures on oxidative metabolism caused by heavy metals in plants.

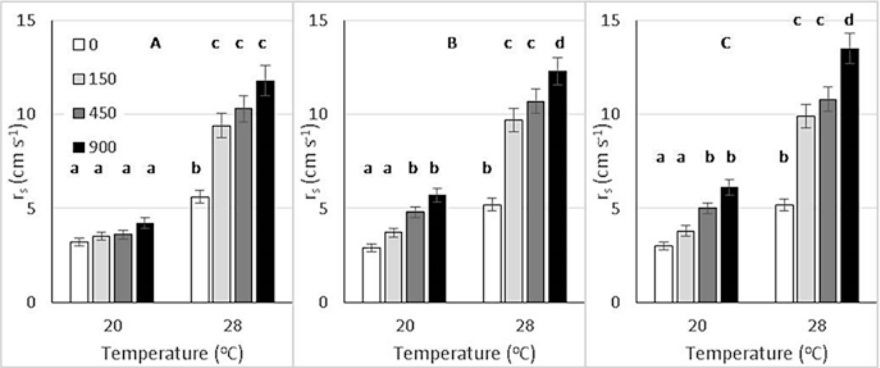


Fig. 15.8 The effect of temperature on the stomata resistance (r_s) in the leaves of *Plantago major* L. plants exposed to the 150, 450, and 900 μM aqueous Pb solutions for 24 (**a**), 48 (**b**), and 72 h (**c**). Adapted from Balakhnina et al. (2016)

Therefore, an experiment where plants of *P. major* L. were exposed to 0, 150, 450, and 900 μM aqueous Pb $(NO_3)_2$ solution at 20 and 28 °C for 2, 24, 48, and 72 h was conducted. The response of the plants was assessed by the content of lead in the leaves, changes in stomatal resistance, and development of oxidative stress (Balakhnina et al. 2016; Fig. 15.8). The intensity of oxidative degradation was evaluated by the content of thiobarbituric acid reactive substances (TBARs) and antioxidant potential through the activity of ascorbate peroxide (AsP) and glutathione reductase (GR) in the leaves.

15.3.1 The Content of Pb in the Leaves of Plantago major L. Plants

The content of Pb in the leaves of untreated *P. major* L. plants averaged about 5.0–5.6 mg kg^{-1} dry mass (Table 15.1). The concentration of this metal in the leaves of plants exposed to Pb depended on its content in the nutrient medium and the duration of exposure. At a temperature of 20 °C, which is most favorable for many metabolic reactions of this plant, the concentration of Pb in leaves of samples exposed in a nutrient medium with lead was higher than at 28 °C (Table 15.1).

15.3.2 Photosynthesis and Stomata Resistance

It was showed (Table 15.2) a decrease in the net CO_2 assimilation rate (P_N) on about 42% as well as stomatal conductance on about 37% without significant changing of chlorophyll content in the leaves of plants at the 20 °C without (0 μM Pb) and with (450 μM Pb) lead processing during 72 h. The stomata resistance values (r_s) of plants kept in the lead-free nutrient medium were higher at 28 °C, by approximately 73–79%, compared with the r_s values recorded at 20 °C (Fig. 15.1). Application of Pb has not led to a significant enhancement in r_s at 20 °C after 24 h exposure.

Table 15.1 Pb concentration in the leaves of *Plantago major* L. plants exposed to water solution of 150, 450, and 900 μM Pb during 72 h at temperatures of 20 and 28 °C

	Pb, mg kg^{-1} dry mass (%)			
	24 h		72 h	
Treatment	20 °C	28 °C	20 °C	28 °C
0 μM Pb	5.6 ± 0.33a (100)	5.4 ± 0.37a (100)	5.2 ± 0.31a (100)	5.0 ± 0.35a (100)
150 μM Pb	17 ± 1.02a (304)	10 ± 0.60b (185)	28 ± 1.96c (538)	15 ± 0.99a (300)
450 μM Pb	26 ± 1.82a (464)	13 ± 0.78b (241)	47 ± 3.10c (904)	22 ± 1.54a (440)
900 μM Pb	39 ± 2.67a (696)	26 ± 1.82b (481)	61 ± 3.66c (1173)	34 ± 2.24a (608)

Different letters indicate significant differences between columns at $p < 0.05$
Adapted from Balakhnina et al. (2016)

Table 15.2 The net CO_2 assimilation rate (P_N), concentration of chlorophyll (a + b), and stomata conductance in the leaves of *Plantago major* L. plants treated with 450 μM Pb for 72 h at temperature of 20 °C

Treatment	P_N, μmol CO_2 (kg^{-1} DM s^{-1})	Chlorophyll (a + b) (mg g^{-1} DM)	Stomata conductance (mmol m^{-2} leaf area s^{-1})
0 μM Pb	131 ± 7a	2.60 ± 0.14a	142 ± 6a
450 μM Pb	93 ± 5b	2.31 ± 0.09a	104 ± 5b

Different letters indicate significant differences between treatments at $p \leq 0.05$
Adapted from Balakhnina et al. (2016)

Maintenance of plants for 48 and 72 h with Pb450 and Pb900 μM led to an increase in r_s that was approximately doubled in the 900 μM Pb variant. After 24 h of plant exposure in nutrient solutions containing Pb150, Pb450, and Pb900 μM at 28 °C, an increase in stomatal resistance was observed. A 72 h incubation with 900 μM Pb increased stomatal resistance by 2.6-fold relative to the lead-free version. The r_s values for plants exposed to 28 °C were higher in all treatments than the r_s values for plants exposed to 20 °C.

15.3.3 Oxidative Processes

Increasing temperature up to 28 °C caused a 26% enhancement of TBARs relative to plants exposed for 2 h at 20 °C in a lead-free growth medium (Table 15.3). Later, after 24 and 72 h of the experiment, the difference in the concentrations of TBARs between the plants maintained at temperatures 20 °C and 28 °C decreased. Pb intensified oxidative reactions in all variants; however, the maximal meanings of TBARs concentration were registered in plants exposed to 900 μM Pb at 28 °C.

15.3.4 AsP, GR, and GPX Activities

AsP activity in the leaves of plants, not stressed by lead, did not change significantly which depends on the temperatures used in the experiment (Table 15.3). Short-term 2 h action of Pb has not enhanced AsP activity neither at the temperature 20 °C nor 28 °C. After 24 h plant treatment with lead (450 μM and 900 μM), the enzyme activity in the leaves increased at both temperatures, but at 28 °C, it was higher at 28 °C. A lower concentration of Pb (150 μM) caused a significant increase in AsP activity ($p < 0.05$) only in plants exposed at 28 °C. As the duration of the experiment increased, the increment in enzyme activity decreased, and after 72 h AsP activity in the leaves of Pb processed plants was lower than that after 24 h treatment.

In contrast to AsP, temperature dependence was observed on another enzyme of the ascorbate-glutathione cycle, glutathione reductase (GR). So, GR activity in the

Table 15.3 Effect of temperatures (20 °C and 28 °C) on the oxidative reactions intensity tested by TBARs content as well as on the antioxidant enzymes AsP and GR activity in the leaves of *Plantago major* L. plants treated with 150, 450, and 900 µM Pb for 2 h, 24 h, and 72 h

	2 h		24 h		72 h	
Treatment	20 °C	28 °C	20 °C	28 °C	20 °C	28 °C
TBARs, nmol g^{-1} FM (%)						
0 µM Pb	31 ± 1.9a (100)	39 ± 2.4b (100)	32 ± 2.2a (100)	35 ± 2.4a (100)	32 ± 2.1 a (100)	33 ± 2.0 a (100)
150 µM Pb	41 ± 2.8a (132)	48 ± 2.9b (123)	39 ± 2.5a (122)	45 ± 2.8ab (129)	38 ± 2.4a (119)	45 ± 2.7ab (136)
450 µM Pb	45 ± 2.8ab (145)	69 ± 4.5c (177)	43 ± 2.3a (134)	49 ± 3.2ab (140)	42 ± 2.3a (131)	50 ± 3.3b (152)
900 µM Pb	51 ± 3.4a (165)	72 ± 4.9c (185)	48 ± 3.1a (150)	58 ± 3.8b (161)	49 ± 3.1a (153)	61 ± 4.0b (185)
AsP activity, µmol g^{-1} FM min^{-1}						
0 µM Pb	17.0 ± 1.1a (100)	20.1 ± 1.3ab (100)	19.0 ± 1.2a (100)	22.0 ± 1.3ab (100)	21.0 ± 1.3ab (100)	23.0 ± 1.5b (100)
150 µM Pb	17.7 ± 1.2a (104)	20.3 ± 1.3ab (101)	22.5 ± 1.5ab (118)	34.3 ± 2.4c (156)	24.0 ± 1.6b (114)	29.0 ± 1.7c (126)
450 µM Pb	18.7 ± 1.2a (110)	20.6 ± 1.4a (102)	25.8 ± 1.5b (136)	34.1 ± 2.0d (155)	27.0 ± 1.7b (129)	30.0 ± 1.9c (130)
900 µM Pb	17.3 ± 1.1a (102)	22.0 ± 1.4a (109)	28.0 ± 1.8b (147)	34.7 ± 2.1c (158)	28.0 ± 1.9b (133)	31.0 ± 1.9b (135)
GR activity, µmol g^{-1} FM min^{-1}						
0 µM Pb	0.40 ± 0.02a (100)	0.56 ± 0.03b (100)	0.46 ± 0.02ab (100)	0.58 ± 0.03b (100)	0.48 ± 0.02ab (100)	0.59 ± 0.03b (100)
150 µM Pb	0.43 ± 0.03a (108)	0.57 ± 0.03b (102)	0.50 ± 0.03ab (109)	0.62 ± 0.04b (107)	0.74 ± 0.05c (154)	0.87 ± 0.06c (147)
450 µM Pb	0.44 ± 0.03a (110)	0.65 ± 0.04b (116)	0.55 ± 0.04ab (120)	0.71 ± 0.04c (122)	0.83 ± 0.05d (173)	0.73 ± 0.04d (124)
900 µM Pb	0.51 ± 0.03a (128)	0.67 ± 0.04b (120)	0.56 ± 0.04a (120)	0.78 ± 0.05c (134)	0.84 ± 0.05c (175)	0.68 ± 0.04b (115)

Different letters indicate significant differences between treatments at $p \leq 0.05$
Adapted from Balakhnina et al. (2016)

leaves of plants without adding Pb at the temperature 28 °C was higher by 23–40% than that at the 20 °C (Table 15.3). Activation of the enzyme in plants, stressed by 450 and 900 µm Pb at 28 ° C, was recorded after 24 h of the experiment. Incubation of plants in nutrient solutions containing lead at all applicable concentrations (150, 450, and 900 µM Pb) at 20 °C led to a significant increase in GR activity after 72 h. Exposition for 72 h at 28 °C in 150 and 450 µM water solutions of Pb caused a significant increase in GR activity, but it was not observed in 900 µM Pb one (Table 15.3).

15.3.5 Discussion

The lead content of about 5 mg/kg dry weight (Table 15.1), which we found in the leaves of *Plantago* plants, corresponds to the normal content (0.1–10 mg kg^{-1} dry weight) of this element in plants grown on soil uncontaminated with lead (Kabata-Pendias and Pendias 1992; Shen and Liu 1998; Voskresenskaya et al. 2013; Yoon et al. 2006). A great increase in the concentration of Pb in the leaves of plants exposed to Pb and the relationship between the accumulation of Pb and the concentration of Pb in the growth medium and the duration of the stressor action, which were observed by us, are consistent with the results of studies by other researchers (Filipović-Trajković et al. 2012). It should be noted that the consumption of Pb by plants from solutions containing lead ions increased with increasing temperature to a certain limit but then decreased; so, in *Cucumis sativus* L. plants, for example, this was observed if the temperature rose above 25 °C (Takeda et al. 2006) and in *Lemna minor* L. if it was higher than 30 °C (Uysal and Taner 2011). We suppose the reduced Pb accumulation in plants at the temperature 28 °C, which we consider as moderately raised temperature for our object, in comparison with 20 °C one, occurred due to an increase in stomatal resistance (Fig. 15.1), a weakening in root pressure, and a corresponding reduction in transpiration and transport of minerals. In our study, the plant reactions to effect of 450 μM Pb during 72 h are suppression of photosynthesis with a contemporaneous diminution of stomata conductance. These data are consistent with the results of a number of investigations demonstrating that Pb-induced inhibition of photosynthesis is accompanied by simultaneous closure of stomata (Ahmad et al. 2008a, b; Islam et al. 2008; Kosobryukhov et al. 2004; Li et al. 2012). A decrease in the concentration of chlorophyll in *Plantago* leaves, as a response to lead treatment, unlike photosynthesis, under the conditions of our experiment was not recorded. Probably, the content of Pb in the nutrient medium and the duration of exposure were not sufficient to cause destruction of the pigment. A significant decrease in the concentration of chlorophyll (more than 50%) occurred in the leaves of cress *Lepidium sativum* (Bafeel 2010) and water hyacinths *Eichhornia crassipes* (Malar et al. 2014) only with severe stress induced by Pb at a concentration of more than 400 ppm for several days.

The higher temperature of incubation (28 °C) amplified stomatal resistance in comparison with that at 20 °C, which, as was shown by Kosobryukhov et al. (2004), is optimal for *Plantago* photosynthesis. The inhibition of photosynthesis and transpiration intensity with increasing temperature from 22 to 32 ° C was noticed by Lipiec et al. (2013). The results of our study showed that Pb caused strengthening in stomata resistance, and this strengthening was significantly higher at the temperature 28 °C in comparison with 20 °C. It is believed that the effect of Pb on stomatal resistance may be related to the effect of Pb on abscisic acid content (Sharma and Dubey 2005). According to the hypothesis (Bazzaz et al. 1974), a decrease in transpiration intensity is a consequence of lead-induced stomatal closure due to inhibition of the energetic system or changes in K^+ transport via membranes.

We admit that a decrease in the rate of transpiration and accumulation of lead in *Plantago* plant leaves, caused by an increase in air temperature of 8 °C, i.e., regulation of stomata resistance, may be considered as the defensive reactions of plants to reduce content of Pb in leaves.

Lead-induced intensification of peroxidation of cell components, tested by TBARs concentration, was strongly dependent on the content of this metal in the nutrient medium. A linear dependence of the intensity of the peroxidation processes, tested by the MDA content, on the lead content in the substrate for plant growth, but independent of the duration of the stressor, was obtained on different plants (Gupta et al. 2009; Reddy et al. 2005). An increment in the concentration of MDA is a reflection of increase in the oxidative destruction of cells, and, obviously, this may be considered as one of the possible ways through which lead toxicity can manifest itself in plant tissues. Concerning the combined effect of air temperature and Pb in the induction of oxidative reactions in Pb-treated plants, data in the literature are practically absent.

It was established (Balakhnina et al. 2016) that increasing of air temperature up to 28 °C led to intensification of peroxide processes in the leaves of Pb-free *Plantago* plants already after the first 2 h of the exposure. The intensity of these processes was lowered, as the incubation time increased, due to the adaptation of plants to specified growth conditions. The maximum concentration of TBARs was found in the leaves of plants experiencing the effect of 900 μM Pb at 28 °C for 2 h, that is, at that moment when the temperature itself led to the greatest increase in the content of TBARs. The reaction of plants to the combination of the effect of Pb and a moderately elevated temperature was less pronounced but did not become weaker with an incubation time exceeding 2 h. In all analyzed time variants, in Pb-treated plants, the content of TBARs was higher at 28 °C compared to 20 °C. AsP activity in the leaves of Pb-non-processed *Plantago* plants, in contrast to the intensity of peroxide processes, was not changed significantly under the influence of the temperatures used in the experiment. Furthermore, shorter, 2 hour lead processing, even at the maximal Pb concentration (900 μM), did not cause significant changes in AsP activity. The other authors showed that the activity of AsP in the leaves of *Sesbania drummondii* kept in nutrition medium with $Pb(NO_3)_2$ (500 mg L^{-1}) remained practically without essential change during the first 2 weeks of the experiment and only after that was enhanced (Ruley et al. 2004). In this investigation, activation of AsP was registered after 24 h of lead processing, and it was stronger, above 50%, at all Pb concentrations at 28 °C, but not so essential at 20 °C, especially at 150 μM Pb concentration. After 72 h of lead-induced stress, the increase in AsP activity was lower than after 24 h treatment; this fact allowed us to suggest that it may be associated with stimulation of other components of the system defensive against oxidative destruction, for instance, the activation of GR, another antioxidant enzyme of the ascorbate-glutathione cycle. Indeed, the greatest increasing of GR activity in the leaves of lead-processing plants was registered after 72 h of the experiment at 20 °C. A significant increase in the activity of antioxidant enzymes—SOD, AsP, guaiacol peroxidase, and catalase—was recorded by Ruley et al. (2004) in the leaves of *S. drummondii* after processing with lead (Pb 500 mg L^{-1}). The authors decided that tolerance of *Sesbania* plants to effect of lead may be explained by the high plant antioxidant capacity. An increase in SOD activity and peroxidase in the leaves of *Allium sativum* L. garlic plant exposed with Pb10^{-3} M was observed (Liu et al. 2009). The activity of SOD and catalase and the ascorbic acid content in *Zea mays* leaves increased linearly with an increase in the Pb content in the seedling growth medium (Gupta et al. 2009).

Thus, it can be said that enzymes of antioxidant nature represent a very important link in the chain of defense mechanisms against oxidative degradation induced by heavy metals and lead, in particular. It should be noted that the negative effects of lead increased with increasing temperature.

15.4 Selenium Effects on Growth and Oxidative Metabolism of Wheat *Triticum aestivum* L. Plants Under Lead-Induced Stress

Among the mineral compounds that positively affect the stability and productivity of plants under stress, silicon (Si) (Balakhnina and Borkowska 2013; Balakhnina et al. 2012a, 2015b) and selenium (Se) (Feng et al. 2013) are especially often noted in the last time because of its possibility to alleviate the negative effects of different stress factors. Selenium is an important trace element which is necessary for humans and animals, while for higher plants the usefulness, but not the need, of the presence of Se in the nutrient medium has been shown (Hartikainen 2005). Moreover, excessive amounts of Se can have a toxic effect on plants, and difference between useful and dangerous concentrations of selenium in the soil is small and depends on the type of plants (Feng et al. 2013; Terry et al. 2000). At present, a fairly extensive material has been accumulated, demonstrating the ability of Se to increase the resistance of plants to cold, drought, salinization, heavy metals, etc. (Terry et al. 2000; Mroczek-Zdyrska and Wyjcik 2011; Filek et al. 2008; Ibrahim 2014; Poldma et al. 2013). It was shown that Se can regulate the ROS content in plants by stimulating superoxide anion radicals' conversion to H_2O_2 through the direct interaction between Se-containing compounds and ROS and by regulating activity of antioxidant enzymes (Feng et al. 2013; Terry et al. 2000). However, the mechanism of these complex processes is still not fully established.

To find out the effectiveness of action of exogenous selenium on growth and on reactive oxygen species metabolism in wheat (*Triticum aestivum* L.), plants were grown on the soil reached with different selenium content with or without lead pollution (Balakhnina and Nadezhkina 2017).

15.4.1 Soil and Plant Pretreatment

Wheat (*Triticum aestivum* L., cv. Triso) seeds after germination were grown in 1 dm^3 pots filled with soil (chernozem, medium thick, medium humus, heavy clayey). The soil characteristics were as follows: the content of humus, 5.17 ± 0.03% (Tyurin's method); mobile forms of nitrogen (N), 106 ± 11 mg/kg of soil (Cornfield's method); phosphorus (P), 80 ± 12 mg/kg of soil (Chirikov's method); potassium (K), 117 ± 8 mg/kg of soil (Chirikov's method); lead, 15.6 mg/kg (as determined in 5 M HNO_3); selenium, 59 ± 3 μg/kg; soil moisture, 20 ± 5%; and pH in H_2O, 6.9 ± 0.03. So, this soil contained enough amount of available nitrogen and medium and elevated content of selenium and phosphorus, respectively. The total amount of Pb without the background content was about 27.6 ± 0.9 mg kg^{-1} soil; i.e., that was lower than the maximum permissible concentration (32 mg kg^{-1}).

Comparative studies of the effect of different concentrations of selenium on the development of oxidative stress, induced by lead in wheat plants, were carried out. The concentrations of selenium used were 0.4 and 0.8 mg/kg of soil—variants (Se 0.4) and (Se 0.8), respectively. Wheat (*Triticum aestivum* L., cv. Triso) plants were exposed to lead at concentrations of 50 and 100 mg/kg, variants (Pb50) and (Pb100), respectively, for 14 days. The response of plants was evaluated by the intensity of growth processes, concentration of plastid pigments, intensity of destructive processes through the concentration of TBARs, and activity of antioxidant enzymes: AsP, GR, and GPX.

15.4.2 Growth of Plants

Growth of wheat (*T. aestivum* L., cv. Triso) plants depended on concentrations of Se introduced into the soil (Table 15.4). Thus, the height and biomass of the shoots of wheat (Se0.4) variant increased by 16 and 25%, respectively, relative to control

Table 15.4 Effect of selenium (Se^{6+}) on growth of aboveground organs, biomass accumulation, and the concentration of chlorophyll (Chl (a + b)) in the leaves of wheat (cv. Triso) seedlings when growing on the soil without and with lead (Pb^{2+}) pollution

Treatment	Height of plant, %	Plant biomass, %	Chl (a + b), %
Control	100	100	100
(Se0.4)	116	125	101
(Se0.8)	68	38	80
(Pb50)	80	79	72
(Pb50 + Se0.4)	104	117	89
(Pb50 + Se0.8)	76	67	44
(Pb100)	64	71	69
(Pb100 + Se0.4)	60	67	47
(Pb100 + Se0.8)	44	38	34

At the beginning of the experiments, the age of plants was 14 days. Adapted from Balakhnina and Nadezhkina (2017) plants. However, with an increase in Se concentration, as in the (Se0.8) variant, a decrease in the above parameters by 32 and 62%, respectively, relative to the control, was recorded. Pb caused plant growth inhibition dependent on its concentration in the soil (Table 15.4). The height and biomass of shoots in plants of (Pb50) variant decreased by 20 and 21% relative to control ones. At the increasing of the stressor strength, like in the (Pb100) variant, the values of these parameters decreased by 36 and 29%, respectively. The response of plants to the combined effect of Se and Pb depended on the concentration of both elements used. The use of smaller selenium concentration weakened the unwished effect of lead. So, in plants of (Pb50 + Se0.4) variant, the height and biomass remained at or above the control values. The introduction of a larger dose of selenium, the (Pb50 + Se0.8) variant, on the contrary, exacerbated the negative effect of the stressor. The synergism of the negative effects of Pb and Se was especially pronounced in plants' growth of the (Pb100 + Se0.8) variant, where the increase in height and the accumulation of biomass of aboveground organs decreased by 56 and 62% relative to the control, respectively, in comparison with the control (Table 15.4). The response of the roots to the action of various concentrations of Pb^{2+} and Se^{6+}, as a whole, was the same as in the aboveground organs but was more pronounced (Table 15.5). However, it was recorded that although the root length (Se0.4) of the plants was almost 20% less than that of the control, their biomass was 15% higher.

15.4.3 Chlorophyll Content

Chlorophyll (Chl) (a + b) concentration in the leaves of control plants was about 2.33 mg/g fresh mass (it was taken as 100%) and did not differ significantly from (Se0.4) variant where Chl (a + b) concentration amounted to 2.35 mg/g fresh mass or 101%, relative to control plants. In plants of (Se0.8) variants content of pigments was 20% less compared control (Table 15.4). The content of Chl (a + b) in the leaves

Table 15.5 Effect of selenium (Se^{6+}) on the length of root and fresh biomass of wheat (cv. Triso) seedlings when growing on the soil without and with lead (Pb^{2+}) pollution

Treatment	Root length, cm, (%)	Root weight, mg FM, (%)
Control	16 ± 0.96a (100)	44 ± 2.64a (100)
(Se0.4)	13 ± 0.85b (81)	51 ± 3.06a (115)
(Se0.8)	11 ± 0.67b, c (69)	31 ± 1.83b, c (70)
(Pb50)	10 ± 0.53c, d (63)	28 ± 1.68b (64)
(Pb50 + Se0.4)	12 ± 0.64b (75)	35 ± 1.81c (82)
(Pb50 + Se0.8)	9 ± 0.55d, e (56)	30 ± 1.45b (68)
(Pb100)	9 ± 0.47d, e (56)	21 ± 1.33d (48)
(Pb100 + Se0.4)	8 ± 0.51e (50)	18 ± 1.26d (41)
(Pb100 + Se0.8)	5 ± 0.28f (31)	11 ± 0.66e (25)

Different letters in columns designate significant differences at $p \leq 0.05$; $n = 18$
Adapted from Balakhnina and Nadezhkina (2017)

of plants, under the influence of lead, decreased dependent on the dose of the stress factor. So, in the (Pb50) and (Pb100) plants, concentration pigment was lower by 28% and 31%, respectively, relative to control variant. The situation was improved by the introduction of low dose of selenium as in the variant (Pb50 + Se0.4), where content of Chl (a + b) increased by 17% compared with (Pb50) plants. In plants (Pb50 + Se0.8), selenium, on the contrary, enhanced the negative effect of lead. The combined action of Pb and Se was especially dangerous for plants at high concentrations of both elements, (Pb100 + Se0.8); there the content of Chl (a + b) lowered by 66%, relative to corresponding control (Table 15.4).

15.4.4 Intensity of Oxidative Processes

The content of TBARs in the leaves of the (Se0.4) variant was 11% lower than in the control plants (Table 15.6). When growing plants on soil with a higher dose of selenium, as in the variant (Se0.8), on the contrary, intensification of peroxide processes was observed; in this case was registered 52% increased TBARs concentration, relative to the control meanings. The introduction of lead into the soil at both concentrations used significantly increased oxidative reactions in plants. Thus, in the leaves of (Pb50) plants, the content of TBARs increased by 44%. The introduction

Table 15.6 Effect of Se^{6+} on the oxidative reactions intensity tested by TBARs content as well as on the antioxidant enzymes AsP, GR, and GPX activity in the leaves of wheat (cv. Triso) plants, grown on soil without and with lead (Pb^{2+}) contamination

Treatment	TBARs, nmol g^{-1} FM, (%)	AsP, μmol g^{-1} FM min^{-1}, (%)	GR, μmol g^{-1} FM min^{-1}, (%)	GPX, μmol g^{-1} FM min^{-1}, (%)
Control	219 ± 13.1a (100)	35 ± 2.1a, c (100)	0.49 ± 0.03a (100)	14.1 ± 0.9a, f (100)
(Se0.4)	196 ± 12.7a, d (89)	49 ± 3.2b (140)	0.51 ± 0.03a (105)	10.4 ± 0.6b (74)
(Se0.8)	332 ± 21.9b, c (152)	43 ± 2.7b, c (123)	0.91 ± 0.05b (186)	30.8 ± 1.6c (220)
(Pb50)	315 ± 20.7b, c (144)	34 ± 1.7a (97)	0.61 ± 0.04a, c (124)	19.9 ± 1.3d, e (142)
(Pb50 + Se0.4)	296 ± 19.7c (127)	41 ± 2.6a, b, c (117)	0.59 ± 0.03a, c (120)	17.3 ± 1.1d, f (123)
(Pb50 + Se0.8)	381 ± 23.4b (174)	47 ± 2.9b (134)	0.58 ± 0.03a, c (118)	27.4 ± 1.4c (194)
(Pb100)	377 ± 24.5b (172)	38 ± 1.9a, c (109)	0.67 ± 0.04c, d (137)	25.3 ± 1.5c, g (179)
(Pb100 + Se0.4)	325 ± 21.1b, c (148)	58 ± 3.7d (165)	0.73 ± 0.05d (149)	22.1 ± 1.4e, g (157)
(Pb100 + Se0.8)	170 ± 11.9d (77)	25 ± 1.3e (71)	0.33 ± 0.07a (67)	12.1 ± 0.7a, b (86)

Different letters in columns designate significant differences at $p \leq 0.05$; $n = 3–4$
Adapted from Balakhnina and Nadezhkina (2017)

of the (Pb50 + Se0.4), Se (0.4 mg/kg soil), variant led to a decrease in the intensity of the processes of oxidative destruction by 17% relative to the level of (Pb50) plants, but by 27% higher than the values of the control samples. In contrast to the action of Se (0.4 mg/kg soil), the introduction of Se (0.8 mg/kg soil), as in the (Pb50 + Se0.8) variant, led to an increase in the content of TBARs by 30% and by 74% in comparison with (Pb50) plants and control, respectively (Table 15.6). When adding lead at the concentration of 100 mg per kg of soil, a 72% increment in the TBAR content in leaves was observed. The introduction of 0.4 mg Se/kg soil reduced effect of lead (Pb100) on the peroxidative processes; the meanings of TBARs content in (Pb100 + Se0.4) plants were significantly less than in the leaves of (Pb100) variant but higher than in the control ones. In the leaves of (Pb100 + Se0.8) plants, a decrease in the content of TBARs was also observed (Table 15.6). The intensity of peroxide processes in the roots was significantly lower than in the leaves (Table 15.7). The plant response to impact of selenium was dependent on the concentration of the element introduced into the soil. In the roots of (Se0.4) plants, the content of TBARs did not change significantly, while in (Se0.8) plants it increased by 26% relative to the control. Lead induced intensification of peroxide processes: an increase of 25% and 45% of the TBAR content in the roots of (Pb50) and (Pb100) was recorded, respectively The combined action of the elements in the variant (Pb50 + Se0.4) was expressed in a decrease in intensities of oxidative degradation by more than 42% relative to the variant (Pb50). In the (Pb50 + Se0.8) plants, alleviating of the negative effect of lead by selenium was less pronounced. It should be

Table 15.7 Effect of Se^{6+} on the oxidative reactions intensity tested by TBARs content as well as on the antioxidant enzymes AsP, GR, and GPX activity in the roots of wheat (cv. Triso) plants, grown on soil without and with lead (Pb^{2+}) contamination

Treatment	TBARs, nmol g^{-1} FM, (%)	AsP, μmol g^{-1} FM min^{-1}, (%)	GR, μmol g^{-1} FM min^{-1}, (%)	GPX, μmol g^{-1} FM min^{-1}, (%)
Control	109 ± 7.1a (100)	34 ± 2.2a (100)	0.36 ± 0.02a,d (100)	39.8 ± 2.2a (100)
(Se0.4)	93 ± 4.3a,d (86)	47 ± 3.1b,c (138)	0.48 ± 0.03b,c (133)	69.4 ± 2.1b (174)
(Se0.8)	137 ± 6.7b, c (126)	42 ± 2.8b (124)	0.43 ± 0.03a,b (119)	69.6 ± 4.4b (175)
(Pb50)	136 ± 7.8b, c (125)	55 ± 3.3c (162)	0.59 ± 0.04 c (164)	23.4 ± 1.6c (59)
(Pb50 + Se0.4)	90 ± 5.4a.d (83)	49 ± 2.8 b, c (144)	0.49 ± 0.02b, c (136)	31.7 ± 2.1a (80)
(Pb50 + Se0.8)	120 ± 7.2a,b (110)	31 ± 2.0a,d (91)	0.37 ± 0.02a (103)	56,6 ± 3.4b,d (142)
(Pb100)	158 ± 9.7c (145)	33 ± 2.1a (97)	0.30 ± 0.02d,e (83)	54.2 ± 3.2d (136)
(Pb100 + Se0.4)	97 ± 6.3a (89)	26 ± 1.6d,e (76)	0.29 ± 0.01e (81)	36.1 ± 1.7a (90)
(Pb100 + Se0.8)	76 ± 4.9d (72)	24 ± 1.4e (71)	0.30 ± 0.02d,e (83)	31.3 ± 3.1a (79)

Different letters in columns designate significant differences at $p \leq 0.05$; $n = 3–4$
Adapted from Balakhnina and Nadezhkina (2017)

noted that in the (Pb100 + Se0.4) and (Pb100 + Se0.8) variants, of selenium caused a decrease in the intensity of peroxide processes, compared with all variants and with (Pb100) plants, including (Table 15.7).

15.4.5 Antioxidant Enzyme Activities

Under the influence of Pb^{2+}, a significant increase in AsP activity was not found. The maximum level of AsP activities has been registered in leaves of (Pb100 + Se0.4) plants. In the leaves of (Pb100 + Se0.8) variant was observed a decrease in enzyme activity almost 30% relative to the control (Table 15.6). AsP activity in the roots of (Se0.4) and (Se0.8) plants was higher by 38% and 24%, in comparison to the corresponding controls (Table 15.7). Under the influence of Pb^{2+}, a significant enhancement of the AsP activity was found in the (Pb50) variant. The combined action of the elements in the variant (Pb50 + Se0.4) caused AsP activation by 44%, and in the variants (Pb100 + Se0.4) and (Pb100 + Se0.8), they decreased by 24% and 29% relative to the control, respectively.

The activity of GR in the leaves of control plants was 0.49 ± 0.03 μmol of NADPH g^{-1} FM min^{-1} (Table 15.6). An increase in this enzyme activity by 86%, relative to the control, was observed in the leaves of the variant (Se0.8). The GR activity in the leaves of (Se0.4) plants slightly differed from the control. In the leaves of (Pb50) and (Pb100) plants, enzyme activation observed was dependent on the concentration of lead in the soil. The combined effect of lead and selenium was accompanied by GR activation by 49% in (Pb100 + Se0.4) and a 33% decrease in the (Pb100 + Se0.8) plants. The activity of GR in the roots of (Se0.4) and (Se0.8) plants was higher than that in the control variants by 33% and 19%, respectively (Table 15.7). Under the influence of lead, the enzyme activity increased in the roots of (Pb50) plants by 64% and was below the control level by 17% in (Pb100) plants. The combined action of the elements resulted in increasing of GR activity by 36% in the variant (Pb50 + Se0.4) and a decrease of almost 20% in (Pb100 + Se0.4) and (Pb100 + Se0.8) plants.

The activity of GPX in the leaves of control plants was 14.1 ± 0.9 μmol g^{-1} FM min^{-1}. In (Se0.4) plants, the enzyme activity declined by 26%, whereas in (Se0.8) plants, it was more than two times higher than the control level (Table 15.6). Under the influence of lead was observed an increase in enzyme activity, which depended on the concentration of the stressor. Thus, in (Pb50) and (Pb100) plants, the increase in GPX activity was 42% and 79%, respectively, relative to the control. Joint action of lead and selenium was accompanied by a significant increase in GPX activity in the leaves of (Pb50 + Se0.4), (Pb50 + Se0.8), and (Pb100 + Se0.4) plants but a decrease in (Pb100 + Se0.8) variants.

In plant roots, unlike leaves, GPX activation was observed in both (Se0.8) and (Se0.4) plants (Table 15.7). The joint effect of Pb and Se was characterized by the rise of enzyme activity in the variant (Pb50 + Se0.4) relative to (Pb50) plants, similarly in the variant (Pb50 + Se0.8) in comparison with (Pb50) and the control plants.

In the variants (Pb100 + Se0.4) and (Pb100 + Se0.8), GPX activity decreased, relative to control, by 10% and 21%, respectively.

15.4.6 Discussion

In connection with the activation of anthropogenic activity, during the past century, soil pollution with heavy metals has become a serious problem for mankind (Sharma and Dubey 2005; Terry et al. 2000). Lead is the most widespread element polluting the environment, very toxic for all living organisms, as well as plants. This study shows the negative actions of lead on growth processes and plant development, previously reported by other researchers (Kosobryukhov et al. 2004; Mishra et al. 2006; Islam et al. 2008; Choudhury and Panda 2005). The toxic effect of lead in wheat plants of (Pb50) and (Pb100) variants is the dose-dependent intensification of peroxide processes, which correlates with inhibition of the growth of roots and overground organs, and a decrease in the content of photosynthetic pigments. One of the reasons for reducing plant growth under stress factors is a violation of the photosynthesis process. Dysfunction of photosynthesis caused by heavy metals leads to excessive accumulation of ROS and the intensification of oxidative stress in cells (Mishra et al. 2006; Islam et al. 2008; Choudhury and Panda 2005). From the other side, it is shown (Kosobryukhov et al. 2004; Mishra et al. 2006; Islam et al. 2008) that under the influence of heavy metals, there is an increase in stomatal resistance of the leaves, a decline in transpiration, and, as a result, a decrease in the rate of water and nutrient uptake from the soil into the plant that ultimately also affects the intensity of growth processes and the biomass accumulation (Islam et al. 2008). Using different plant species as an example, it was shown that the enrichment of the soil with Se-containing substances in low concentrations resulted in the reduction of the negative effects of various abiotic stressors, like salinization, UV radiation, drought, and heavy metals (Terry et al. 2000; Mroczek-Zdyrska and Wyjcik 2011; Filek et al. 2008; Ibrahim 2014; Poldma et al. 2013). However, at higher concentrations, Se can act like prooxidant and cause damage to plants (Feng et al. 2013; Hartikainen 2005). The toxicity of Se at high concentrations is also explained by the fact that selenium replaces sulfur in amino acids, resulting in the formation of nonfunctional proteins and enzymes (Terry et al. 2000). In the conditions of our experiment, the addition of small amounts of selenium ions (Se0.4) to the soil led to an increase in the height and biomass of the aboveground organs of wheat, as well as a weakening of the negative effect of Pb in the variant (Pb50 + Se0.4). The fact that the root length of the (Se0.4) plants was less than the control and the biomass was larger can be explained by the way that the introduction of this concentration (Se^{6+}) into the soil contributed to the formation of a more fibrous root system of plants. The weakening of the blockage of growth of tissues and organs by selenium in the presence of a heavy metal and cadmium, in particular, is also showed in the literature (Filek et al. 2008).

In our experiment, weakening of the negative effect of lead by selenium was found only in the plants of (Pb50 + Se0.4) variant. In (Pb50 + Se0.8), (Pb100 + Se0.4), and especially (Pb100 + Se0.8) plants, on the contrary, a great falling in the concentration of plastid pigments and biomass was registered. Sustainable accumulation of reactive oxygen species in plants with an excess of Se in soil (Filek et al. 2008) may be because of an imbalance between the levels of reduced glutathione (GSH), thiol-SH, ferredoxins, and/or NADPH (Feng et al. 2013). If these substances are not enough to simultaneously satisfy the needs for assimilation of Se and neutralization of ROS, the addition of Se can lead to an excess of ROS generation, intensification of oxidative processes, and braking of plant growth (Feng et al. 2013). Excessive accumulation of ROS is dangerous for plants; on the other hand, they are considered as signals for creating and stimulating a system protection against oxidative degradation (Mittler 2002). Changes in the activity of superoxide dismutase, glutathione reductase, ascorbate peroxidase, and guaiacol peroxidase are often used like indicators of the plant tolerance (Balakhnina 2015; Balakhnina et al. 2005; Balakhnina and Borkowska 2013; Hartikainen et al. 2000). It has been shown that Se ions can improve the resistance of plants to drought, UV radiation, aging, and the actions of higher or lower temperatures by inducing the plant's antioxidant system (Hartikainen et al. 2000; Ibrahim 2014). Confirmation of a decrease in the amount of ROS can be seen when the level of peroxidative processes, enhanced under conditions of oxidative stress development, was inhibited in the presence of Se to values close to control (Proietti et al. 2013). Suppression of the oxidative process intensity of after the introduction of Se into lead-free soil was found only in the roots and leaves of the (Se0.4) variant, although an increment in the AsP, GR, and GPX activity was recorded both in (Se0.4) and (Se0.8) plants. The joint action of Pb^{2+} and Se^{6+} was accompanied by a decrease or retention at a level close to the control, induced by Pb^{2+}, of peroxide processes in variants with a lower dose of stressor: in (Pb50 + Se0.4) plants in the roots and in (Pb50 + Se0.4) and (Pb100 + Se0.4) plants in the leaves. In this case, an increase in the activity of one or more of the analyzed antioxidant enzymes was observed. The dramatic events were observed in the roots and leaves of the wheat (Pb100 + Se0.8) variant. In this case, a decrease in the growth and accumulation of biomass of roots and aerial organs, as well as concentration of photosynthetic pigments and antioxidant enzymes activity in the leaves, was accompanied by a decrease in the content of TBARs. To explain this reaction of plants, one should take into account the fact that the development of deep stress leads to a decrease in the overall level of metabolism. A decrease in the content of cell components, including lipids, proteins, and nucleic acids, which are a substrate of peroxidation processes, indicates the development of the extreme stage of oxidative stress, which passes in the phase of adaptation capacity depletion.

15.5 Conclusions

Thus, soil flooding caused an intensification of peroxidative processes in tissues of the magnetic field (MF)-untreated and MF-treated plants; however, this effect was less strong in the latter. AsP activity in leaves and roots in MF-treated plants was higher than in untreated ones during the first days of the experiment. An increase in AsP activity, observed during soil flooding in all variants, was the highest in the (+MF +Fl) plants. GPX activity in leaves of plants treated with a magnetic field was significantly higher than in untreated ones during certain periods of plant growth. Thus, the MF seed treatment of 30 mT, 50 Hz, 30 s did not stimulate the processes of seed germination and growth of seedlings but contributed to an increase in the antioxidant potential of plants during soil flooding.

The moderately high temperature inhibits the accumulation of lead in *Plantago major* L. leaves because of increased stomatal resistance. The negative effects of lead, especially at the beginning of the experiment, were stronger at the higher temperature (28 °C), which led to an earlier decline in the adaptive potential of plants. *Plantago* plants adapt to Pb contamination in the following order: an increase in AsP activity in the earlier days of stress development is replaced by activation of GR, as the second antioxidant enzyme in the ascorbate-glutathione cycle.

Soil pollution with Pb^{2+} causes a dependence on the concentration of metal in the soil inhibition of the growth of roots and shoots of plants, as well as a decrease in the concentration of Chl (a + b) in the leaves; intensification of peroxide processes as well effects on the level activity of antioxidant enzymes GR and GPX in the leaves and roots of plants.

Selenium has a multidirectional effect at different concentrations of the agent. The introduction of a lower (0.4 mg Se^{6+}/kg of soil) dose of Se^{6+} into the soil without Pb^{2+} leads to stimulation of growth processes, an increase in the activity of AsP in leaves and AsP, GR, and GPX in the roots of plants, and a decrease of TBARs content. The introduction of a larger (0.8 mg Se^{6+}/kg of soil) dose of Se^{6+} into the soil without Pb^{2+} causes a suppression of growth processes and an increase of TBARs concentration, which is accompanied by activation of antioxidant enzymes in the roots and leaves. Applying of a lower (Se0.4) concentration of Se^{6+} in soil contaminated with Pb^{2+} weakens the negative effect of lead on plant growth, whereas the introduction of a higher (Se0.8) concentration of Se^{6+} increases the adverse impact of Pb, especially in the variant with high concentrations of elements (Pb100 + Se0.8) in the soil. Thus, positive or negative the effect of exogenous selenium on the growth and adaptive capacity of plants growing in the soil without and with heavy metal contamination depends on the concentration of Se6+ introduced into the soil.

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Chapter 16
Grafting Plants to Improve Abiotic Stress Tolerance

Maryam Mozafarian Meimandi and Noémi Kappel

Contents

Abstract Because of climate change, primarily water deficit and temperature extremes may negatively affect the plant growth. To develop stress-resistant plants, grafting can be a good solution. The stronger root system due to the rootstock became more resistant against biotic and abiotic stress elements and grafting itself increases the resistance of the whole plant, supported by many physiological changes.

Keywords Vegetable grafting · Biotic stress · Salinity · Drought stress

Abbreviations

ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
K	Potassium
RDI	Regulated deficit irrigation
RSA	Root system architectures
RWC	Relative water content
WUE	Water use efficiency

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16.1 Introduction: The Concept of Grafted Plants

Grafting is a special type of asexual plant propagation, in which a part of a plant (scion) is joined to another plant (rootstock) to grow together and form a new plant. Grafting methods have been used in fruit trees for thousands of years and recently even in vegetables. Seed companies and breeders have introduced different rootstocks with specific characteristics for different situations.

Currently, vegetables (watermelon, melon, cucumber, tomato, pepper, eggplant, and bitter gourd), fruits (citrus, apple, mango, grape, peach, plum, apricot, almond, and cherry), and ornamentals (rose, chrysanthemum, bougainvillea, and bonsai) are grafted in commercial scale for variety of purposes including, improving yield and fruit quality (López-Marín et al. 2017; Kullaj 2018; Arvanitoyannis et al. 2005; Flores et al. 2010), prolong postharvest life (Kullaj 2018), tolerance to low and high temperature (Poudyala et al. 2015; Shibuya et al. 2007; Ntatsi et al. 2013, 2017), tolerance to salinity stress (Santa-Cruz et al. 2002; Albacete et al. 2014; Fernández-García et al. 2004; Fan et al. 2011; Sallaku et al. 2019; Penella et al. 2017), increase drought and flooding resistance (Bhatt et al. 2015; Harbertson et al. 2012), manage soilborne and foliar pathogen (Keatinge et al. 2014; Lee et al. 2010), and root-knot nematodes (Gisbert et al. 2011b).

Different grafting methods can be used depending upon the kind of crops, producers' experiences, and facilities. The most common grafting methods: hole insertion grafting, tongue approach grafting, cleft grafting, splice grafting, pin grafting, tube grafting, side grafting, and bark grafting.

Despite all the advantages some problems of grafting still exist; for instance, labor and technique essential for grafting process (Kumar and Sanket 2017), the price of grafted seedling, and automated grafting machine (Tsaballa et al. 2013).

In the following section, we are focusing on the advantages of grafting in the improvement of crop performance under nonoptimal conditions.

16.2 Advantages of the Use of Grafted Plants

Limitation of arable land and also high market demand for off-season vegetables lead to the cultivation even under unfavorable conditions (Davis et al. 2008) including low and high temperature, salinity, drought, flooding, and diseases.

16.2.1 Improvement of Biotic Stress Tolerance

Nematodes, corky root, fusarium wilt, verticillium wilt, and bacterial wilt (because lack of crop rotation/monoculture) are some of the biotic stress leading to damages in vegetable production. Soilborne diseases can be controlled by crop rotation, solar radiation, different soil disinfection methods like calcium cyanamide, steam, chemical fungicide, biological control, and even by using resistant cultivars (Bletsos and Olympios 2008). Recently, several rootstocks exhibit tolerance to root-knot nematodes (*Meloidogyne incognita*, *M. javanica, and M. aernaria*) and soilborne diseases (*Fusarium*, *Verticillium*, *Phytophthora*, and *Pseudomonas*) (Keatinge et al. 2014). The primary reason for grafting vegetables has been to provide resistance to soilborne diseases (Sakata et al. 2008).

The most common soilborne disease of vegetable crops is fusarium wilt (*Fusarium oxysporum*). The first vegetable grafting recorded in Japan for the prevention of fusarium in watermelon (Kawaide 1985). Bottle gourd and an interspecific hybrid of squash (*Cucurbita maxima* × *Cucurbita moschata*) were identified as fusarium resistance rootstock for *Cucurbitaceae* crops (Davis et al. 2008).

For example, grafting tomato onto different interspecific hybrid rootstocks (i.e., TX301, Multifort, and Aloha) or eggplant rootstocks (*S. integrifolium* and *S. sisymbriifolium*) control nematode (Burelle and Rosskopf 2011; Wang et al. 2008). In eggplant, grafting onto *Solanum* spp. wild relatives *S. torvum* and *S. sisymbriifolium* (Rahman et al. 2002) and onto tomato rootstock cv. Brigeor (Ioannou 2001) protected the plant completely against root-knot nematodes. Other studies by Gisbert et al. (2011a) evaluated yield, quality of eggplant fruits under nematodes infected soil. The grafted eggplant seedling cv. "Black Beauty" onto *S. torvum*, *S. macrocarpon* and crosses between *S. incanum* and *S. aethiopicum* resulted in minimal galling, good compatibility, yield, and quality, especially by *S. incanum* rootstock. Extensive root area, vigor, and genetic resistance lead to nematode tolerance in grafted crops (reviewed by Davis et al. 2008).

Other serious diseases in *Cucurbitaceae* and *Solanaceae* families are phytophthora blight (caused by *Phytophthora capsici*) and *Verticillium dahlia*. Grafting onto verticillium resistant rootstocks delay the expression of disease for 20 days and grafting in combination with other methods cause an effective control (Paplomatas et al. 2002; Ioznnou 2001).

Bacterial wilt (*Ralstonia solanacearum*) results in the death of all crops, especially in a continuous cropping system. Cardoso et al. (2012) reported that rootstock "Hawaii 7996" had good compatibility with tomato cv. Santa Clara, Santa-Cruz Kada and Debora with effective resistance against bacterial wilt. Similar findings were observed by Zhang et al. (2010) and Palada and Wu (2007) who reported that grafting tomato onto LA2701, LA3202, and LA3526; and on eggplant (VI045276) inhibited bacterial wilt disease. The above finding was in agreement with McAvoy et al. (2012) who grafted tomato cv. BHN602 onto Cheong Gang, BHN1054, BHN998, and itself. They confirmed that grafted tomato plants had less bacterial wilt in comparison with self-grafted and non-grafted ones in both greenhouse and open field conditions.

Different mechanisms explained the disease resistance of grafted vegetables: genetic parameters, better uptake of water and nutrients, and shift of microorganism population in the rhizosphere (Cohen et al. 2017).

16.2.2 Improvement of Abiotic Stress Tolerance

Developing tolerant cultivars to abiotic stresses (due to low genetic diversity, the complexity of multigenic traits and complicated genetic control of traits response to stress) has not been successful (Rouphael et al. 2018). In addition, due to rapidly changing abiotic and biotic stresses, plant breeders need new germplasm (King et al. 2010). Vegetable grafting has been considered as a rapid alternative to slow breeding methods for increasing plant resistance against abiotic stresses (Flores et al. 2010; Penella et al. 2017; Schwarz et al. 2010). Several researchers have illustrated that grafting commercial-sensitive cultivars onto tolerant rootstocks can alleviate the deleterious effect of stresses.

16.2.2.1 Temperature Stress

The temperature threshold of most vegetable crops (i.e., pepper, eggplant, cucumber, tomato, and melon) are about 8–12°C (Schwarz et al. 2010). Temperature below or above of the optimum causes stress and changes several morphological, physiological, and biochemical processes in plants and consequently growth and yield reduction occur. The use of cold or heat-tolerant rootstock is an advanced strategy to protect vegetables against suboptimal or supra-optimal temperatures (Ntatsi et al. 2017; Rouphael et al. 2018).

Gao et al. (2009) and Zhou et al. (2007) introduced fig leaf gourd (*Cucurbita ficifolia*) and bur cucumber (*Sicos angulatus* L.) as cold tolerant rootstocks for cucumber. Grafting cucumber onto squash (*Cucurbita moschata* Duch) caused tolerance to low-temperature conditions in comparison with self-grafted plants (Shibuya et al. 2007).

In watermelon, Miguel et al. (2004) used Shintosa-type: an interspecific squash hybrid, (*Cucurbita maxima* Duch×*Cucurbita moschata* Duch) as rootstock. They indicated that grafted plant growth increased under low-temperature conditions. Davis et al. (2008) concluded that the chilling tolerance of the grafted plants can be due to higher antioxidant activities and membrane stability.

According to previous studies by Venema et al. (2008); Keatinge et al. (2014), and Okimura et al. (1986) on cold tolerant rootstocks LA 1777 *(S. habrochaites)*, “KNVF” (the interspecific hybrid of *S. lycopersicum* × *S. habrochaites*) and chill-tolerant lines from backcrossed progeny of *S. habrochaites* LA 1778 × *S. lycopersicum* cv. T5 alleviates suboptimal temperature in tomato.

In comparison with tomato, eggplant is tolerant of high soil temperature, so can be used as rootstock for tomato (Abdelmageed and Gruda 2009). According to Wang et al. (2007) grafting onto heat-tolerant eggplant increased the growth period and fruit yield of tomato at 28 °C.

Wang et al. (2018) conducted an experiment to identify cold resistance apple rootstock (GM256, JM7, M26, M7, SC1, SH1, SH38, SH6, M9, and T337) under different low-temperature stress conditions (0, −15, −20, −25, −30, and −35 °C). They reported that all studied rootstocks can be used for the region where minimum temperature was higher than –20 °C and for the colder areas, GM256, SH38, and SH6 would be better for apple rootstock.

16.2.2.2 Water Stress

Due to global climate change, water limitation is one of the most important issues in arid and semi-arid areas around the world. About 70% of all freshwater consumption is for agriculture. Efficient water use through improving irrigation methods and more efficient cultivars can reduce the demand for water.

A key challenge for water stress mitigation is controlling transpiration through stomata (Marguerit et al. 2012). A previous study by Marguerit et al. (2012) on grapevine revealed that transpiration rate and water deficit resistance are controlled genetically by the rootstock, through different genetic architectures. In cucumber and watermelon, increasing water use efficiency (WUE) is related to higher CO_2 assimilation and lower transpiration when plant is grafted onto luffa (Liu et al. 2016; Rouphael et al. 2008).

Satisha et al. (2007) studied the use of five grape rootstocks (Dogridge, Salt Creek, 1613C, St. George and VC clone) under the condition of deficit irrigation. The authors concluded that Dogridge and Salt Creek rootstocks had better tolerance to water stress due to higher photosynthetic rate, WUE, and lower transpiration rate.

Luffa (*Luffa cylindrica* Roem.) as rootstock increased leaf area and dry weight of cucumber cv. Xiangfei No. 236 and delayed leaf wilting under drought stress conditions (Liu et al. 2016). The same results in tomato were obtained by Sánchez-Rodríguez et al. (2012) who grafted tomato onto drought-tolerant rootstock ("Zarina"). Penella et al. (2014) demonstrated that improving net photosynthesis rate under water-stressed conditions caused increased marketable yield of pepper when grafted onto the rootstocks "Atlante," "PI-15225," and "ECU-973." Similar results were reported by López-Marín et al. (2017) in sweet pepper and by Al-Harbi et al. (2016) in tomato.

One of the tolerance mechanisms is due to the deep root system (root density, length, number of root hair, and surface area) by acquiring nutrients and water stored in deeper soil layers. For instance, López-Marín et al. (2017) and Poudyala et al. (2015) demonstrated that an extensive deep root system of rootstock for watermelon, sweet pepper, and tomato increased drought resistance in the grafted plant compared to non-grafted ones.

Penella et al. (2014) and Rodríguez et al. (2013) concluded that improving grafted plant growth (tomato and pepper) under water stress conditions can be related to increasing nutrient accumulation in plants (through deep and vigorous root system), nitrate reductase activity and NO_3 assimilation. Sánchez-Rodríguez et al. (2014) observed an increased potassium (K) concentration in tomato drought-tolerant rootstock resulted in better osmoregulation.

Regulation of the antioxidant system under soil water restriction improves grafted plant tolerance. For instance, on luffa rootstock grafted cucumber the higher antioxidant activity caused lower stress (Liu et al. 2016).

The ability of tolerant rootstock to improving crop productivity, maintain mean midday CO_{2-} saturated photosynthesis, and water use efficiency with low biomass was observed by several researchers (Holbrook et al. 2002; Cantero-Navarro et al. 2016). Cantero-Navarro et al. (2016) explained that 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration in xylem inhibited plant growth but did not limit fruit yield.

Yıldırım et al. (2018) studied root system architectures (RSA) and transcriptomic regulations of grapevine rootstocks (110R, 5BB, and 41B) under conditions of reduced water availability. They reported that RSA (including root area, length, ramification and number of root tips), relative water content (RWC), total carbohydrate as well as nitrogen content were found to be much higher in 110R relative to other rootstocks during stress conditions. In this tolerant rootstock, totally 2795 differentially expressed transcript were identified at the severe drought with three transcriptomic regulations. Similarly, higher berry yield was observed in grapevine (Albarín Negro) grafted onto 110R and 3309C rootstocks (Loureiro et al. 2016).

Tramontini et al. (2013) tested different combination of grapevine rootstock (140RU, SO4) and scion (Cabernet Sauvignon, Grenache, Merlot, Syrah) response to water stress and concluded that the capacity of water extraction from the soil and the sensitivity of the stomatal control influenced by rootstock and scion, respectively.

Juglans mandshurica Maxim, *J. nigra* L., and *J.s regia* L. cv. Jizhaomian were analyzed as rootstock under drought stress conditions. According to the results *J. mandshurica* Maxim. and *J.s regia* L. cv. Jizhaomian have been recommended as tolerant rootstock for walnut due to larger WUE and growth rate, chlorophyll fluorescence, and gas exchange parameters (Liu et al. 2019).

A similar positive effect of grafting was also recorded by Robles et al. (2017) who grafted Verna lemon onto *Citrus macrophylla* Wester and sour orange (*C. aurantium* L.) under control (100% ET_c) and RDI (25% ET_c) irrigation treatments. They recommended sour orange as rootstock for those regions where the available water resources are limiting. For citrus, several researchers recommended tolerant rootstock against water deficit conditions. Highest fruit yields and good performance were obtained from "Valencia" sweet orange trees grafted onto "Sunki" mandarin×*Poncirus trifoliata* English and 'C-13' "S" citrange, respectively (Fadel et al. 2018). So according to their results, these rootstocks can be used for "Valencia" sweet orange in rainfed agriculture.

Harbertson et al. (2012) compared different grape scion-rootstock and non-grafted plants under deficit irrigation conditions and found that rootstocks had few significant differences in fruit and wine composition.

Increasing abscisic acid closed stomata (in M9 rootstock under short drought condition) and large root system (in MM.111under prolonged drought conditions) of dwarfing rootstock improved drought resistance in apple (Tworkoski et al. 2016). Khadivi-Khub and Anjam (2016) introduced *Prunus scoparia* as a good rootstock for almond under drought stress due to the high fruit yield and dwarfing characterize.

16.2.2.3 Salinity Stress

Salinity is one of the major abiotic stresses limiting all aspects of plant growth and yield depending on the exposure period and salt concentration (Colla et al. 2010). Poor water management, low rainfall, high evaporation, and overfertilization can lead to salt stress (Colla et al. 2010). Low water potential of the root medium, toxic effect of Na^+ and Cl^-, and nutritional imbalance caused the deleterious effect of salinity on plants.

Grafting is a beneficial technique for reducing the deleterious effect of NaCl stress in *Solanaceae* and *Cucurbitaceae* families. Rootstock, scion, and their interactions influence a wide range of physiological and morphological characters (Colla et al. 2010).

Several researchers explained that higher accumulation of proline, sugar, and antioxidants and lower accumulation of Na^+ and Cl^- in leaves of grafted plants lead to increased salt tolerance in plants (Ruiz et al. 2005; López-Gómez et al. 2007; Estan et al. 2005; Fernández-García et al. 2004; Goreta et al. 2008). Moreover, the higher water use efficiency (WUE) indicated higher salt tolerance which may reduce the absorption of ions and can alleviate drought stress induced by salt stress (Colla et al. 2010).

Wild tomato species including *S. pimpinellifolium*, *S. peruvianum*, *S. cheesmaniae*, *S. habrochaites*, *S. chmielewskii,* and *S. pennellii* (Rao et al. 2013; Santa-Cruz et al. 2002) were suggested as salt-resistant rootstock for tomato. A similar positive effect was reported by He et al. (2009) who used Chinese commercial tomato hybrid (Hezuo 903) grafted onto Zhezhen No. 1 as rootstock under salt stress condition; they demonstrated that photosynthesis characteristics improved in grafted plants compared to control and self-grafted ones. In tomato, Fernández-García et al. (2004) designed a greenhouse experiment to compare grafted and non-grafted plant's responses to the different levels of NaCl stress. They concluded that tomato cv. Fanny grafted onto AR-9704 was more tolerant due to low accumulation of Na^+ and Cl^- in shoots.

Liu et al. (2007) and Wei et al. (2007) founded that grafting eggplant cv. Suqiqie onto Torvum vigor (*S. torvum* Swartz) are resistant to saline stress conditions. Grafting eggplant onto wild eggplant rootstock Tuolubamu (*S. torvum*) can alleviate salt injury stress through improving plant growth, proline content, enzyme activity, enhancing photosynthesis, increasing N and P, reduction of Na^+/K^+ value and a balanced absorption of Ca, Mg, Cu, Fe, Zn, and Mn (Qian et al. 2013). Similarly, in watermelon Santa-Cruz et al. (2002) reported that grafting on *C. maxima* Duch and *Lagenaria siceraria* (Molina) Standl rootstocks can improve plant growth under salt stress. Grafting watermelon onto Strongtosa rootstock (*C. maxima* Duch × *C. moschata* Duch) exhibited a lower reduction of shoot weight and leaf area (Goreta et al. 2008). Yan et al. (2018) applied 200 mmol/L NaCl on grafted watermelon onto *C. moschata* (i.e., Quanneng Tiejia; Kaijia No. 1); *L. siceraria* cv. Hanzhen No. 3 and self-grafting. Results indicated that "Kaijia No. 1" as rootstock displayed the greatest tolerance through less accumulation of Na^+, ROS, and exhibition of superior photosynthesis and plant growth relative to other rootstocks.

In another study, Huang et al. (2005) demonstrated that salt-resistant rootstock (bottle gourd) alleviated growth performance and productivity of cucumber relative to non-grafted plants under salt stress conditions.

In addition, rootstocks can improve the tolerance of commercial scion by increasing antioxidant enzyme and hormonal balance in resistant grafting combination (Colla et al. 2010). Liu et al. (2007) concluded that abscisic acid (ABA) and polyamine in grafted plants are higher than in self-grafted ones under NaCl stress conditions.

In a recent study, Miao et al. (2019) grafted cucumber onto bottle gourd by different methods: hole insertion grafting; tongue approach grafting; and spliced grafting and they reported that tongue approach grafting method had higher daily growth and the activity of antioxidant enzyme increased in tongue approach grafting method in the graft union.

Grafting with resistant rootstock can improve plant productivity by increasing water and nutrient uptake due to the rootstock's vigorous root system; production enhancement of endogenous-hormones and improvement of scion vigor under both stress and nonstress conditions (Ruiz et al. 1997; Zijlstra et al. 1994). For instance, transferring cytokinin from root to shoot improved tomato productivity by reducing flower abortion and increasing fruit size under salt stress conditions (Albacete et al. 2014). Similar positive effect was recorded by Penella et al. (2017) who reported that grafting pepper onto different rootstocks (*Capsicum chinense* Jacq., *C. baccatum* L. var. pendulum, and *C. annuum* L. from the COMAV Gene bank at the UPV university) mitigated the deleterious effect of salinity in terms of higher marketable yield in grafted plants (except *C. annuum*) due to high photosynthesis level and lower Na^+ and Cl^- (Penella et al. 2017).

Zhang et al. (2018) investigated the response of grafted mulberry (*Morus alba* L.) and its own root seedlings under NaCl stress conditions. They concluded that the root system of rootstock (Qinglong mulberry) has selective absorption capacity of Na^+ and K^+ which prevented the transportation of Na^+ to aerial parts, also, higher PSII photochemical activity and electron transport rate was observed in grafted plants which reduced ROS and oxidative damage (Zhang et al. 2018).

The use of GF677 and Garnemas as rootstock for sweet almond (cv. Mazzetto) under different salt concentration highlighted on that Mazzetto/Garnem combination had better growth (by maintaining stomatal conductance, protecting chlorophyll, and cytosolic assimilatory enzymes from toxic ions), as well as higher carotenoids/chlorophyll, anthocyanin/chlorophyll, K^+, Ca^{2+}, proline, and soluble sugar concentration (Zarig et al. 2016).

Salinity has a negative effect even on citrus plant morphological, physio-biochemical mechanisms, and mineral content (Balal et al. 2011). In a recent study by Adnan Shahid et al. (2019) who investigated the effect of mandarin grafted plants onto two salt-sensitive (Carrizo citrange and Sanchtoncitrumello) and two salt-tolerant (Rangpur lime and Rubidoux) rootstocks at different NaCl concentrations. Grafting onto salt-resistant rootstock resulted higher plant biomass, chlorophyll, photosynthesis, stomatal conductance, and lower reactive oxygen species compared to grafting onto salt-sensitive rootstock. Simpson et al. (2014) budded Valencia tree onto sour orange and two trifoliate rootstocks: Bitters (C22) and C146 subjected to different salt concentrations and reported that chlorophyll fluorescence, stomatal control, and SPAD decreased with increasing NaCl concentration in grafted plants more than in non-grafted ones. However, grafting onto C22 and C146 showed more tolerance than sour orange. They concluded that both rootstock and scion selection should be considered for saline regions.

Yin et al. (2010) evaluated the tolerance of Chinese apple rootstock to NaCl, and their result showed that plant growth parameters (i.e., RWC, leaf number, plant height) decreased while electron leakage and enzyme activities decreased with increasing salt concentration. Among the studied rootstock, *Malus prunifolia* Borkh cv. dongbei huanghaitang, *M. sieboldii* Rehd, *M. prunifolia* Borkh cv. qiuzi, and *M. xiaojinensis* Cheng et Jia were salt-tolerant rootstocks, which associated with variation of antioxidant enzyme activities and the amount of organic osmotic (Yin et al. 2010).

16.3 Conclusion

Drought, flooding, high or low temperatures, and salinity are the key stress factors with a high impact on plant performance. Plant physiology is heavily influenced by these stress elements and due to these climatic problems severely distress plant development and yield, comprising molecular, biochemical, physiological, and morphological modifications. Therefore, the production of stress-tolerant crop plants has become an important task in current agriculture. There are many useful approaches and using grafted plants are also beneficial. Grafted plants have namely enhanced resistance against different biotic and abiotic stress responses.

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Chapter 17
Role of Molecular Tools and Biotechnology in Climate-Resilient Agriculture

Mohammad Mafakheri and Mojtaba Kordrostami

Contents

Abstract Global climate change is a man-made phenomenon fueled by ever-increasing population on the Earth which has generated a cascade of unprecedented fluctuations in precipitations almost throughout the world with having agriculture in the target as a highly vulnerable as well as a vital sector for humanity. Agriculture in climate change era means to deal with multifaceted scenario owing to the high frequency of multi-stressed environments in which stress-resilient crop cultivars are recommended as a solution with high reliability to facilitate management. By taking into account the acceleration in climate change-driven impacts and vitality of food security, the conventional methods of plant breeding can no longer meet the

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challenges; therefore, it is necessary to devote focus and financial resources to novel advancements in plant breeding. Nowadays, agricultural biotechnology is in its heyday and has significantly contributed to this field specifically in major crops. Biotechnology toolkit equipped with precise, efficient, environmentally friendly, and cost-effective tools has enabled researchers to unmask the genes involved in underlying resistant mechanisms against abiotic and biotic stresses and afterward validate and pyramid them into commercial cultivars with simultaneous durable multi-stress resistance. Further, genome-editing approaches have opened a new window to accurately manipulate plant genomes without off-target introgression. Numerous varieties of major food plants that were cultivated by farmers around the world have developed with the help of new methodologies. However, there is a tough road ahead, and these new tools are promising. Many more researches need to be done; the effects of stresses are intertwined which makes breeding projects challenging. Increasing information on plant genome with the aim of novel tools is the key to develop crop varieties that tolerate stress and still provide a reasonable yield and improve food security ultimately.

Keywords Molecular markers · Abiotic and biotic stress · QTLs · CRISPR/Cas9 system · Biotechnology

17.1 Introduction

Thousands of years ago, humankind initiated the first steps toward agriculture, which is now the largest industry in the world. It begun with collecting food from wild plants and eventually their cultivation which continued by unintentional opting of plants containing their favorable characteristics to cultivate improved food crops, where they started to move exactly against natural selection in which wild plants require to be evolved to protect themselves from herbivores and acquire features that help them survive; however, humans altered the cycle by changing the genetic structure of plant species that resulted in producing particular plants known as “cultivars” with enhanced yield and nutritional quality, resistance to biotic or abiotic stresses, and higher storability and versatility to be cultivated in various ecological conditions. By eventual understanding of cross-pollination structure in plants and the fact that mating plants can be artificially executed and therefore require controllability in their gene exchange, hence the process of improving food crops heightened and has been the cornerstone of agricultural productivity for millenniums (Babinard 2001; Wieczorek and Wright 2012; Zeder 2008). In the late nineteenth century, Gregor Mendel changed the course of plant breeding and revolutionized the foundation of biological knowledge of plant breeding and creation of plant hybrids which announced a novel period in agriculture history and has greatly contributed to increasing crop production. However, developing hybrids caused them a disastrous vulnerability especially against pests and pathogens owing to genetic similarities of the plants (Resende et al. 2011; Wood and Orel 2005).

While conventional methods of agriculture could keep the supply of food products in pace with demand for thousands of years, during the last 150 years, the agriculture section has experienced an ever-increasing demand for agricultural products which ended up with a swift increment in the intensification of agriculture, owing to the population explosion in the early nineteenth century in which human population reached one billion on the Earth for the very first time. Ever since, increasing at an exponential rate closed to seven billion after 2010 that has been projected to cross nine billion in 2050, the pressure on natural resources is dangerously growing mainly in developing countries (Taiz 2013; Zahid et al. 2016). Additionally, unprecedented increase in the world's population has generated catastrophic impacts on environmental resources that is foremost accompanied by global climate change with its multiflorous impacts including 4 °C increscent in global temperature solely in the last quarter of the century, elevated CO_2 and atmospheric O_3, and last but not least inordinate precipitation paradigm with severely prolonged wet or dry seasons that all have initiated a cascade of fluctuations with its paramount detrimental effects on agriculture through shaping a circumstance with multiple environmental stressors (drought, salinity, high temperature, elevated-CO_2, and O_3), which threaten critical staple crops (wheat, rice, maize, soybean, beans, barley, and potato) the most (Dresselhaus and Hückelhoven 2018; Taranto et al. 2018; Varshney et al. 2018), shake up the ecophysiological connections within and between species, and function synergically or antagonistically which might end up with generating irreversible ecological damages such as plant diversity erosion through working in favor of some organisms and against of some others which potentially can lead to outbreak of pests and pathogens (Elad and Pertot 2014; Post 2013; Sinervo et al. 2010). A recent instance could be the ongoing struggle of farmers in Europe (West et al. 2012) and South Asia (Zhang et al. 2011) against the rising infection of wheat farms with Fusarium head blight (FHB) and septoria tritici (Desm.) blotch (STB) diseases or invasive species of weeds (Jain et al. 2014; Lamsal et al. 2018) in farmlands that ultimately cause unexpected crop losses. Even though sometimes abiotic stresses can lower the scale of infections or pests' population, the fact is phytopathogens and pests are thriving and climate change from an overall perspective exacerbates their impacts. Also, stress conditions are multifactorial, and many unknown mechanisms, interactions, and factors have yet to be discovered. At the present day, we are witnessing a noticeably increased depth of gap between supply and demand for food products where conventional methods of agriculture can either no longer keep up with demand or meet the challenges of climate change and difficulty in agriculture production as the prominent issues (Braun et al. 2018; Pretty and Bharucha 2014; Yengoh and Ardö 2014). Food shortage in climate change era, in which aftermaths have manifested in the recent reports of the Food and Agriculture Organization (FAO), exacerbates inadequate food products for 842 million people. Besides, worldwide food supply is projected to have a 70% increase by 2050 with 100% in developing countries (FAO 2013), in addition to an extension in middle-class population which indicates another source of escalation in food demand (Bruinsma 2009; Spiess 2016; Tilman et al. 2011).

Nonetheless, throughout history, more often than not, humankind has successfully addressed the challenges they faced with innovations. Now, in the era of climate change, again technology can assist us to overcome the challenges in this case by developing crops with extraordinary versatility that can cope with difficult growth circumstances through biotechnological techniques. In this climate change scenario, agricultural biotechnology has the capability to serve us as "an element of victory" in dealing with the most potentially devastating challenge of all time. To address and overcome the hunger crisis worldwide effectively, agricultural biotechnology can greatly contribute to fulfill this target through developing resistant and capable crop field varieties to start cultivation and production of food in lands that are already unproductive by existing varieties (i.e., 25 million acres adds to uncultivable lands per year) or enhancing the productivity of farmlands per hectare.

17.2 Biotechnology Applications in Climate-Resilient Agriculture

Given that the climate change majorly targeted the most substantial sector of food production, its impacts become more and more apparent on the daily basis in addition to deterioration of arable lands and vacillation in rainfall paradigms which all set the stage for drought stress, high temperature, and explosion of pest and plant disease, whereas the toolbox of biotechnology enriched with cutting-edge techniques integrated from various disciplines has real potential to master these challenges through developing cultivars of main food plants to tolerate severe climate-driven stresses (Espeland and Kettenring 2018; Tesfahun 2018; Varshney et al. 2018).

The cultivar development process with conventional breeding methods optimistically takes at least 15–20 years (Halterman et al. 2016) and multiple generations to transfer, for example, a specific gene from a newly discovered wild relative to a commercialized improved cultivar. Since the improved cultivars are highly pure and hold various desirable agronomic characteristics, when further genetic work is required to confer a specific feature, genetic engineering can be a substantially reliable method to transfer particular gene or genes from one genome to another in a precise way, while conventional methods jeopardize a years of breeding endeavour by adding unwanted genes to their genomes (Miedaner and Korzun 2012). Of course, considering the limitation of time in figuring out how to tackle the climate change and at the meantime enhance the field crops to continue production in full capacity in this status quo with conventional methods of plant breeding is almost impossible. On a rough estimation, there are 7.2 million stored accessions in 1700 seed banks in the world that certainly can serve researchers in this field with unending untapped variation of crop wild relatives to develop new varieties (Crossa et al. 2017; Venkateswarlu and Shanker 2012; Wambugu et al. 2018), in which an efficient evaluation of this enormous genetic diversity may solely be achieved through new methodologies in biotechnology.

In order to find superior alleles of specific characteristics in plant germplasm applicable in developing varieties with special capability under climate change era, employing one of the most effective and cost-efficient techniques in biotechnology known as high-throughput genotyping and phenotyping is indispensable; the approach serves breeders with highly accurate, conveniently recoverable, and crucial information on suitable alleles or potential lines to develop cultivars for climate-resilient agriculture (Kole 2013; Scheben et al. 2016). The genotyping systems that utilize new methods to detect and concurrently genotype single-nucleotide polymorphisms (SNPs) are already accessible (Patil et al. 2016; Wu et al. 2018). Examples of the commonly used methods for sequencing-based genotyping approaches are genotyping by sequencing (GBS), restriction-site-associated DNA sequencing (RADSeq), double-digest restriction-associated DNA (ddRADSeq), skim-based genotyping by sequencing (GBS), repeat amplification sequencing, exome sequencing, and whole-genome re-sequencing (WGRS) (Goddard and Hayes 2007; Mousavi-Derazmahalleh et al. 2019; Zhang et al. 2017b).

Advancement of biotechnological tools has always provided novel, precise, and optimized methodologies which are relatively new, known as CRISPR/Cas9 system that recently has been introduced in plant breeding, which has manifested a great potential in discovering genes and their impacts and also in meticulous genome editing in plants with high stability and no unwanted introgression (Arora and Narula 2017; Bertier et al. 2018). Its application in plant genome editing is new but has attracted the attention of many researchers; several experiments on creation of CRISPR/Cas systems in either reference plant species such as cottonwood, tobacco, and *Arabidopsis* or in cereal crops and legumes as well as fruit trees, in particular, citrus and apple, to improve their resistance to abiotic stresses have been reported (Fauser et al. 2014; Mushtaq et al. 2018; Osakabe et al. 2016). Of course, it is a really capable system and can help breeders to manipulate the genome to obtain the desired traits without engaging in similar issues as GMO plants, but important prior requirement limited its utilization.

A compilation of cross-field disciplines, namely, next-generation approaches of plant breeding (e.g., genomic selection (GS) and genome editing (GE)) and biotechnological progress, are required for breeders to assess untapped plant germplasms and locate beneficial alleles to create lines and ultimately develop varieties that have high readiness to deal with climate change and its aftermaths.

Plant breeding is an interdisciplinary process of developing and improving plant varieties with desired characteristics to arm crop fields against multi-stress milieus in the changing climate era (Ashraf et al. 2012; Lenaerts et al. 2018). Consequently, owing to the essentiality of using more efficient and time-saving technologies to tackle the negative impacts of climate change on agriculture, in the recent decades, modern biotechnological tools are utilized to fasten the progress of crop development for climate change-resilient agriculture which hereupon have contributed a remarkable and comprehensive understanding of genetic variation in plants through providing a plethora of information (Espeland and Kettenring 2018; Jain et al. 2014; Taranto et al. 2018; Wakelin et al. 2018). Marker-assisted selection is not dependent on the developmental stage; therefore, it can be performed in any stage of plant development, and unfavorable genotype can be identified at the first stage (Arunakumari et al. 2016). Novel markers have been developed with widely distributed polymorphism across genome, i.e., single-nucleotide polymorphisms (SNPs), insertions and deletions (InDels), and simple sequence repeats (SSRs) own an extreme superiority over other molecular markers, tightly linked to the target loci, which make these markers applicable smart toolkits that lead the MAS to be a more capable approach in crop development (Benitez et al. 2010; Hayashi et al. 2006; Scheben et al. 2016). Further, analyzing plant genotypes and wild populations to detect and map potential QTLs that control the expression of genes related to biotic or abiotic resistance has attracted the attention of many researchers which are fundamental knowledge required to execute gene pyramiding of resistance traits, preferably myriad genes, into superior varieties with the aim of the MAS (Boopathi 2012; Elkot et al. 2015). Scientists have been able to observe how closely QTLs are located on the chromosome to the sequence of the target gene or genes by linkage mapping process; hence, those closely linked markers and genes are more likely to transfer to the next generation together (Kordrostami and Rahimi 2015). So, introgression hybridization of effective genes in a reliable, stable, and cost-effective manner is possible through the MAS (Bálint et al. 2007; Nzuki et al. 2017; Paul et al. 2018). As precise, applicable, efficient, and promising as the MAS can get, it still carries some irrefutable drawbacks including introgression of unwanted tightly linked genes to the favorable ones, highly complicated, time-consuming, and not perfectly precise against novel advancement in technology of GE employing sequence-specific nucleases (SSNs) as a capable approach in biological studies. Hitherto, three GE tools were introduced that comprise of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat-associated CRISPR/Cas9 system (Abdallah et al. 2015; Bertier et al. 2018; Noman et al. 2016; Osakabe et al. 2016). Seemingly, owing to lack of complexity, versatility, and high precision, CRISPR/Cas9 system has increasingly been in the interest of scholars to pinpoint the exact site of interest in an extraordinarily efficient, customizable, and precise way and avoid transferring any probable random genes that might destroy the function of the desired gene(s). In addition, genome of plant is manipulated through the import of restriction enzyme to knock out or substitute the gene of interest without introgression of an unrelated gene(s) unlike transgenic methods which can facilitate the authorization of trade of these agricultural products (Fauser et al. 2014; Georges and Ray 2017; Mishra et al. 2018). Although, despite the great potential of the present biotechnology tools, constant upgrade is required.

17.3 Abiotic Stress

17.3.1 *Drought and Salinity*

Salinity and drought are major widespread environmental stresses that are common and simultaneously occur in plants (Kordrostami et al. 2016); at this moment, one-third of arable lands are either arid or semiarid, and by 2050 more than 30% of the arable lands in the world will be salinized and unproductive (FAO 2011). Since the resistance of plants against abiotic stress is involved in multigene mechanisms that are either environment- or species-specific, enhancing genetic gain or developing novel varieties with effective stable resistance is extremely challenging, laborious, and time-consuming, especially with conventional plant breeding approaches; therefore, researches in this area so far have accomplished a lot less than expected (Challa and Neelapu 2018; Essa 2002; Kordrostami et al. 2017; Venkateswarlu and Shanker 2012). However, during recent decades, biotechnological tools have emerged as a powerful modern generation to facilitate plant breeding which heretofore generated a wealth of knowledge on plant genetic diversity and groundbreaking approaches to identify desired genes and translate the outcome data into breeding programs to develop salinity or drought resistance varieties. The MAS has proven its effectiveness in providing proportionally rapid progress to fulfill the goals of breeders (Collins et al. 2018; Mundt 2014; Xu et al. 2017). Nonetheless, the MAS has a body of knowledge and techniques as a prerequisite from which availability of untapped germplasms, mapping QTLs linked to the traits of interest, association mapping (linkage disequilibrium), and selection screening are the most pivotal ones. However, pinpointing the regions on the genome precisely connected to the phenotype, where its expression can confer resistance especially in the case of salinity and drought stress, is still highly controversial and challenging in which replication of the assessment of the identified QTL(s) to prove its capability has been strongly recommended (Hanin et al. 2016; Khan et al. 2016; Takeda and Matsuoka 2008). So far, various attempts have discovered many QTLs related to drought or salinity resistance characteristics in substantially essential food plants. Having said that, the absence of stability in those QTLs, being considerably condition-specific, makes them unreliable, that is, owing to high plasticity (G × E), a particular QTL has the potential to generate beneficial or detrimental cumulative effects (Nogué et al. 2016; Yuan et al. 2018). Thus, a properly designed experimental scenario to detect QTLs associated with salinity and drought stress is fundamentally important. Primarily, experiments on legumes and cereals have been conducted to identify loci and genes responsible to confer salinity or drought resistance; the recent studies by Varshney et al. (2014) reported significant information on chickpea, and they found a unique region on the genome proposed as a "QTL hotspot" containing QTLs associated with drought resistance and root features (Jaganathan et al. 2018; Kale et al. 2015; Thudi et al. 2014). Eventually, through marker-assisted backcrossing (MABC), that region introgressed into superior known varieties of chickpea that markedly enhanced the root system (Abberton et al. 2016). The susceptibility of many crops has ontogeny dependence, in which at the specific stage, i.e., germination, they might show a higher level of sensitivity. In this case, soybean is a good example (Essa 2002), and researchers have found loci related to salinity resistance as a result of QTL mapping assessments (Do et al. 2018; Ha et al. 2013; Hamwieh et al. 2011; Lee et al. 2004). A similar approach has been employed for cereals; a significant study on widely cultivated productive rice cultivar with considerable drought sensitivity known as IR64 in Asia identified some effective QTLs with the aim of MABC approach developed into a more reproductive IR64 under drought stress conditions (Henry et al. 2012; Kumar et al. 2014b). Again, four QTLs associated with canopy size in sorghum through complicated mechanisms that contribute to the inflexibility of ontogeny have been applied in the mapping of several important genomic regions (Morris et al. 2013). Later on, those QTLs are utilized to develop notably compatible varieties of sorghum (Borrell et al. 2014; Sudhakar Reddy et al. 2016). A novel perspective has emerged to prognosticate the in-field functioning of plants under environmental stress through mathematical models which utilize a compilation of environmental and genetic information as well as genetic paradigms. It facilitates the examination of various assumptions on physiological behavior of plants with the aim of a collection of alleles related to a specific effect under diverse conditions and growth phases through conducting QTL analysis individually for each influential factor in the model (Boer et al. 2007; Chapman 2008; Rios-Estepa et al. 2010). A similar methodology was used in corn (Reymond et al. 2003). As mentioned earlier, abiotic stress, especially drought and salinity, involves highly complex, multigene mechanisms that each contribute to various impacts on yield and plants – independently detecting physiological and adaptive reactions against abiotic stress is difficult and requires more effective and precise selection tools, for which new methods with outstanding high efficiency and low cost have been introduced (Arzani and Ashraf 2016; Khan et al. 2016). SNPs have been applied in hybridization-based marker system due to their extreme polymorphism or use of genotyping by sequencing (GB) and DArTseq (diversity array technology sequencing) as effective approaches that are in great demand; they reduce the complication level in the genome by opting proper restriction endonuclease. Briefly, these systems are capable of delivering a manifold of SNPs that can be employed to the marker-trait association at a genomic scale and genomic estimated breeding value (GEBV)-based breeding programs (Nadeem et al. 2018; Paul et al. 2018; Varshney et al. 2014). Further, GS with a holistic perspective employs all accessible markers to cover the entire genome, consequently, with minimum one marker that is associated with each QTL (i.e., LD). This approach effectively assists the selection process of potential genotypes, expedites the gene flow from plant germplasms to superior lines, and shorten the breeding programs. GS needs no prerequisite information on impacts of genes or QTLs the way that MAS does (Challa and Neelapu 2018; Shah et al. 2018). However, the main requirement of GS is a great deal of marker information and still has limited utilization due to its dependence on the accessibility of state-of-the-art genotyping methodologies. It is fairly new to plant breeding since its first application was in animal breeding, but it is recently used to develop maize varieties tolerant to drought stress. Because of distinguished capabilities that GS has, several GS-based breeding programs are already in progress on various crops, i.e., wheat, sugarcane, peanut, apple, and cassava (Hanin et al. 2016; Nepolean et al. 2018; Shi et al. 2017).

While current approaches are significantly promising and have contributed a large body of knowledge to the field of plant breeding, their outcomes come with a level of uncertainty or additive negative impacts on other traits particularly when it comes to abiotic stresses (Khatodia et al. 2016; Mishra et al. 2018). A proportionately novel biotechnological tool for plant breeding, CRISPR/Cas9, an RNA-guided tool, is a targeted precise genome-editing approach that has potential to compensate almost all problems and difficulty of the current toolkit. From the early application of CRISPR/Cas9 system as genome modification tool, it has been employed to develop stress-resilient varieties in many crop fields with groundbreaking outcomes, but there are a couple of reported research to date (Jaganathan et al. 2018; Mushtaq et al. 2018). Meng et al. (2017) reported effective alteration of a gene (*MtPDS*) active in the biosynthetic mechanism of carotenoid in barrelclover (*Medicago truncatula*), a model legume plant, through CRISPR/Cas9 system. A more significant drought resistance potential in a modified line of corn by CRISPR/Cas9 also has been reported (Shi et al. 2017), in which they inducted *ARGOS8* variants with CRISPR/Cas9 that positively influenced grain production under natural drought stress. Prospective CRISPR/Cas9-targeted sites were identified in soybeans by Michno et al. (2015). Effective mutations in genes (*OST2/AHA1*) receptive to drought and salinity were achieved by applying CRISPR/Cas9-mediated mutagenesis (Osakabe and Osakabe 2017). The results mentioned above are proofs of exceptional capability in CRISPR/Cas9 system to finally address the complications that are involved in breeding for abiotic stress-resilient crops with uncomplicated methodology, cost-effectiveness, and simultaneous induction and edition that confer the ability to pyramid genes into a new variety through solely one generation. However, the necessity of extensive information on functionality and regulation of the gene(s) of interest is a potential limiting factor in using the CRISPR/Cas9 system.

17.3.2 High Temperature

There is a shocking estimation on rising temperature globally by 6 °C in the decades ahead which generate ample negative impacts on every biological mechanism as well as global food security, viz., increasing only 1 °C of global temperature averagely leads to considerable lesser yields in major crops by a minimum of 3% in legumes and up to 7% in cereals (Iizumi et al. 2017; Zhao et al. 2017). Plants as assailed organisms have various physiological mechanisms in response to heat stress, but promoting improved food plant varieties tolerant to high temperature is inevitable. However, since abiotic stresses, particularly high temperature, lead to drought and salinity stress, hence breeding varieties that could cope with such multi-stress circumstances is a real challenge; a new technological toolbox might offer assistance to the breeders and researchers in designing multi-stress-resilient crops (Branham et al. 2017; Venkateswarlu and Shanker 2012; Xu et al. 2018). For most of the plants, temperatures over 35 °C are found to be a critical point in the reproductive developmental stage. For example, in rice, temperatures beyond this level negatively impact flowers' fertility and overall fertility rate (Hatfield and Prueger 2015; Xiao et al. 2011). Recently, international breeding programs NICS, RDA (Korea) and CLRRI, IAS (Vietnam) are performed to develop heat-resistant varieties of rice through biotechnological means, from which Lang et al. (2015) employed two previously identified QTLs associated with high-temperature resistance, introgressed into distinguishably potential lines. Cowpea, a highly adaptable legume in marginal lands which are vulnerable environments affected by changes in climate the most, was investigated for QTLs related to heat resistance with SNP markers among inbred lines by Lucas et al. (2013). They identified the loci associated with heat tolerance in IT82E (the desired haploid) which then can be used in the MAS programs pyramiding them efficiently into elite lines to develop heat-resilient crop. In another study on broccoli, a billion dollar annual turnover vegetable in the USA only which is under constant threat due to global warming, Branham et al. (2017) explored the QTLs related to high-temperature resistance using a large number of SNPs by GBS. In this study, they discovered five QTLs associated with heat resistance which employs them into breeding programs that can help to develop heat-tolerant varieties of broccoli. Lately, to prepare the background requirement for the MAS in fine fescue, Xu et al. (2018) conducted a large-scale experiment on five species of fine fescue (*Festuca* spp.) to detect genes associated with high-temperature resistance under an artificial climate control conditions and they found important related genes. The expression of the genes of interest covered photosynthetic, metabolic, regulatory, and heat stress-induced proteins. Using other novel biotechnological tools like CRISPR/Cas9 platform to precisely modify genes at a very effective level with no off-target consequences in developing high-temperature-resilient crops holds promising outcomes in the near future. To the best of our knowledge, published reports in this area are few; Bertier et al. (2018) considerably improved the germination ability in two commercial cultivars (Salinas and Cobham Green) of *Lactuca sativa* under heat stress (37 °C) by 70% through employing CRISPR/Cas9 which knocked down *NCED4*, a gene initiating early stages of abscisic acid biosynthetic pathway. This method conferred a phenotypic selectability on a whole plant scale for pleiotropic impacts on heat-stress resiliency or growth. Our knowledge on new biotechnological advancements increases at a fast rate and in a short run, and major breakthroughs in developing high-temperature-resistant crops to meet the challenges in climate-resilient agriculture strategy will occur.

17.3.3 Elevated CO_2

Global climate change is the aftermath of a multiparameter scenario, from which increased CO_2 injected to the atmosphere is a major one. This notorious greenhouse gas elevated significantly during the last century as a result of anthropological activities such as fossil fuel burning (coal, oil, natural gas) as well as from natural sources (decomposition and oceanic respiration) and which is responsible for initiating a knock-on effect that has rising global temperature as its consequence (Cao et al. 2015; Dong et al. 2018; Solomon et al. 2009). It has been estimated that by the end of the current century, CO_2 inputs will increase by 400% and CO_2 concentration will increase by 100% (Pasricha 2017; Zhai and Zhuang 2012). The influences of elevated $CO_2$are crop-specific, in which C_3 crops may benefit from a CO_2-enriched atmosphere since evolutionary they have the capacity to increase net gain in an atmosphere with elevated CO_2 because the saturation of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) requires a higher level of CO_2. It means that under the future atmospheric conditions, C_3 plants like rice have a higher photosynthesis rate and CO_2 absorption, lower photorespiration, and decrement in stomatal conductance that causes improved water use efficiency (WUE) and a significant overall enhancement in production, whereas C_4 plants that include substantially critical crops (i.e., maize) show no tangible positive reaction to the elevated CO_2 since RuBisCO is already saturated (Kanno et al. 2017; Kant et al. 2012; Raza et al. 2019). So far, several remarkable studies on the model and non-model plants under elevated CO_2 have been reported. Ferris et al. (2002) found putative QTLs related to developmental characteristics of leaf cells and leaf phenology in the lines of model tree poplar (*Populus trichocarpa* T. & G. and *P. deltoides* Marsh) under natural and enriched atmospheric CO_2. Rae et al. (2007) found QTLs associated with biomass production in poplar. Similarly, QTL identification in rice lines under both ambient and artificially elevated CO_2 was attempted, and several QTLs associated with morphological traits were detected. However, results were mostly dispersing and showed line dependency. In another study on 100 barley genotypes under natural (400 ppm) and enriched (700 ppm) concentration of CO_2, Mitterbauer et al. (2015) observed that six genotypes have a significantly higher growth rate; therefore, with the aim of genome-wide association (GWAS) approach and SNP markers, they identified 75 markers tightly linked with numerous morphological features. By considering the restriction impacts of RuBisCO under low CO_2 concentration and non-limited supply in high CO_2, Kanno et al. (2017) used transgenic lines of rice to study the consequences of slight reduction in RuBisCO by *RBCS* genes; they concluded that a small change has positive influences on photosynthesis rate and biomass. Additionally, a higher nitrogen use efficiency (NUE) was observed that is very critical due to the low absorption of N under elevated CO_2 for rice as the main carbohydrate crop. These findings can be further manipulated by precise genome-editing tools to develop resilient varieties for the changing climate. Elevated CO_2 is more of a two-edged sword for plants; surely depending on plant species, it can improve the growth rate, but it may also result in reduction of nutritional quality of crops, especially vegetables (Dietterich et al. 2015; Dong et al. 2018), and in some cases its positive impacts on facilitating the health condition of host plant for pathogens have been reported (Chakraborty et al. 2000; Matsui et al. 1997; Mikkelsen et al. 2015). Even though plant species may manifest positive responsiveness to elevated CO_2 to some extent, there is considerable variation in their reactions, and it should be noted that analyzing physiological mechanisms of plants under an enriched atmosphere needs to consider the simultaneous multi-stress circumstances of plants on field. Comprehensive and integrated approaches in modern biotechnology can only make such breakthrough happen and improve knowledge in this area, and positively improving and developing crops resilient to elevated CO_2 will be possible in the very near future.

17.3.4 Air Pollution

Air pollution is not a novel phenomenon. Ever since mankind found the power of fire, many innovations came afterward that led to construct of civilizations; however, whatever human activities generated after the industrial revolution to this day have injected an unbearable level of pollutants into the atmosphere (Horsman and Wellburn 1977; Varshney et al. 1979). Of course, pollutants in the atmosphere are numerous, but here we focus on two major ones, sulfur dioxide (SO_2) and ozone (O_3). The resources of SO_2 pollution are either anthropogenic or natural. From the early to the mid-twentieth century, industrialization reached its peak in Western Europe and the USA, so air pollutants have increased significantly. Subsequently, mandatory rules for controlling the emission of these gases have led to a significant reduction in these gases globally. Through emerging new industrial powers in East and South Asia, South America, and Eastern Europe as well as constant population growth in developing countries which is accompanied by increase in demand for fossil fuels, ascending the SO_2 atmospheric concentration in those regions has been alerted and has the potential to cause harm to biodiversity in organisms globally (Borland et al. 1999; Dmuchowski et al. 2018; Schmalensee et al. 1998). Another gaseous pollutant is O_3 with a deposit into the atmosphere directly in a small share and primarily through phytochemical reactions with precursors including reactive organic substances and nitrogen oxides which are continuously released into the atmosphere (Ainsworth 2017; Bothe 2011; Myhre et al. 2013).

Plants employ various mechanisms to continue to grow under polluted air (i.e., avoidance and tolerance) that mainly involves enzymatic antioxidants. SO_2 damage symptoms in plants are necrosis, deformation of leaves, halting growth, bleaching vegetative parts, and finally decreasing yield. Further, upper layer of leaves in herbaceous crops often showed injury symptoms, namely, brown dots, lesion, and reduction in growth and yield under elevated O_3, while in legumes and trees (i.e., *Platanus* and Japanese zelkova), symptoms are reddish-brown spots on the leaves (Linzon et al. 1979; Petrov et al. 2015; Takahama et al. 1992). Attempts to develop SO_2-resilient crop varieties mostly have been with the conventional means of plant breeding (Borland et al. 1999; Cuinica et al. 2015), and so far no published report exists on employing genetic engineering to breed novel SO_2-resistant crops despite the fact that food security of millions of people in developing countries is threatened by this pollutant. On the contrary, tropospheric O_3 pollution has received a great deal of attention, and using biotechnological tools to promote O_3 resistance in the recent decade has been common (Ainsworth 2017; Pasricha 2017). Since developing countries that face elevated O_3 in the near future are in Asia and rice is a critical food resource, distinctive attention has been given to developing O_3 resistance crops; elevated ozone affects the grain production and decreases nutritional value (Emberson et al. 2018; Frei 2015). GWAS assessments on rice also revealed the significant diversity in genetic makeup under various doses of O_3 related to different agronomic characteristics (i.e., biomass, grain yield, and straw lignin) (Tsukahara et al. 2015; Ueda et al. 2013; Zhao et al. 2011). Tolerance mechanisms are as complicated as other abiotic stresses; thus, breeding novel varieties with simultaneous multi-stress resistance is extremely challenging, especially by considering the fact that conferring resistance to stress might cause vulnerability to another. It seems that with the emerging biotechnological tools, more research shed light on impacts of air pollution, in particular O_3, on plants and developing new crop cultivar resilience to high O_3 concentrations.

17.3.5 Heavy Metals

Plants strongly rely on soil to fulfill their mineral nutritional needs. The soil minerals originated from constant weathering of the Earth's crust which normally enriches the soil with minerals more or less when sometimes the erosion of rocks containing heavy metals leached to the soil; such places around the world today are not arable (Rajakaruna et al. 2009). By the beginning of the industrial revolution, anthropogenic activities such as mining and processing of heavy metals and their direct or indirect application in industry and agricultural fertilizers, pesticides, herbicides, and fungicides have increasingly added these dangerous heavy metals in soil, and some of these minerals gradually accumulated in soil and water in various locations in the world and reached a toxic concentration where plants are negatively affected by them (Carbonell et al. 1998; Gallagher et al. 2001; Komárek et al. 2010). Many major food plants (i.e., vegetables) are heavy metal (zinc, cadmium or copper) accumulator and grow on soils contaminated with heavy metals that are in our food chain and can generate severe health risks in a large scale (Hu et al. 2008). Therefore, there is an urgent need to address this ever-growing problem. Naturally selected plant species known as "super-phytoremediator" are introduced to the contaminated soils to clean them up by absorbing and aggregating target elements in their vegetative tissues through numerous mechanisms; then, they would be harvested and processed to extract the elements (Chen et al. 2015; Luo et al. 2007). Although phytoremediation approach has proven to be an effective and applicable procedure to remove the heavy metal contaminants from the soil (Carlos and Itziar 2001; Ma et al. 2018), it is not fast and efficient enough according to today's scale of soil contamination. Also, developing food plants that can properly grow in polluted soils without transferring those elements to the edible parts is gaining attention from researchers. Early attempts were made on model plants, for example, *Thlaspi caerulescens*, a metallophyte by nature, can absorb significant concentrations of zinc, cadmium, and nickel. Deniau et al. (2006) explored populations obtained from two locations in Belgium and France contaminated by lead, cadmium, and zinc and used amplified fragment length polymorphism (AFLP) and expressed sequence tags (ESTs) to identify QTLs linked with Zn/Cd tolerance. Two QTLs were mapped for Zn/Cd concentration in the shoot: one QTL was mapped for Cd concentration, and three QTLs were detected for Zn concentration. In *Arabidopsis thaliana*, Chao et al. (2012) conducted a genome-wide association analysis (GWAS) and LD mapping to comprehend mechanisms and regulators involving cadmium accumulation in leaves. By screening a large number of populations of *Arabidopsis*, they found the main variation source, *HMA3*, in which induction in Cd concentration in leaves is linked with low functionality of *HMA3* resulting from alteration in two particular amino acids by an unexplained mutant. Studying the model plants pave the way to reliably understand the underlying genes controlling physiologic responses that leads to Cd or other heavy metal tolerance.

Since the risk of contamination with Cd is prevalent, a large part of literatures in this area is on Cd toxicity. Soybean is a major food plant that can absorb Cd by the root and store it in the vegetative parts. To discover related putative QTLs to Cd accumulation in *Glycine max* grains, Benitez et al. (2010) used a population from a cross between two Harosoy and Fukuyutaka cultivars (with different grain Cd concentrations) for QTL detection using SSR markers. The results indicated a single major-effect QTL (*qcd1*) for grain-Cd accumulation. Similarly, Jegadeesan et al. (2010) using SSR markers identified a major-effect QTL (*qcd1*) related to Cu accumulation in soybean seeds with significant potential for MAS system. Vollmann et al. (2015) selected soybean genotypes containing alleles for reduced Cd accumulation using SSR markers. They identified 19 genotypes that showed significantly low Cd accumulation in comparison with high Cd-accumulator genotypes under field conditions. Wheat (*Triticum aestivum*) as an important carbohydrate source for over half of the Earth's population that is widely cultivated around the world (Yu and Tian 2018) is highly sensitive to high Cu content in the soil that can harm the productivity through lessening flower fertility. Low Cu accumulation or Cu resistance in wheat is a multi-chromosomal characteristic; hence, identifying potential QTLs is laborious. Penner et al. (1995) employed RAPDs to detect QTLs associated with genes regulating Cu absorption in durum wheat (*Triticum turgidum* L. var. *durum*). Bálint et al. (2007) published an inclusive report on the QTLs linked with Cu resistance and trace element (Cu, Fe, Mn, and Zn) content in the aerial parts of wheat under natural and experimental conditions with high Cu concentrations. A single major-effect QTL is located in chromosome 5DL, whereas minor-effect QTLs are found in five other chromosomes. Since the nutritional quality of wheat in terms of micronutrient is not significant (Karkle and Beleia 2010), thus, QTLs related to micronutrients in the vegetative parts can be valuable (Kutman et al. 2011).

Rice is a substantial food plants in Asia that plays the key role in people's diet, extensively cultivated in developing countries in South and East Asia where the urge of industrialization and soil contamination with heavy metals are common owing to the rapid expansion of industries and loss of regulations. Fortunately, genetic engineering techniques are highly capable to develop novel heavy metal-tolerant rice varieties. For example, Ishikawa et al. (2012) exposed rice populations to carbon ion-beam radiation and generated three rice mutants with considerably low Cd content in the grains (35-fold less) compared to the parents. The linked gene to the low Cd absorption was discovered, and the mutants contained various forms of *OsNRAMP5*, where its expression by the mutants impairs carrier proteins that minimize the intake of Cd and consequently the overall reduction in Cd content in the grains and straw. These mutants underwent a successful field test in Cd-polluted soil with no tangible Cd in the grain which makes them perfect for developing Cd-resistant rice varieties through the MAS program. New precision genetic engineering tools will convincingly address the demand for heavy metal-tolerant crop varieties with the minimized health issues (Mishra et al. 2018). Tang et al. (2017) developed low-Cd-content rice lines with knockout *OsNRAMP5* gene by CRISPR/Cas9. Similar to Ishikawa's work, engineered lines showed very low absorption of Cd in the grains (>0.05 mg/kg), while in the wild-type *indica* rice, the Cd content was 5 to 60 times higher. Dealing with heavy metals in the soil due to their high health risk has a long history; in the recent decades, flourishing biotechnological tools and advancement in breeding and engineering crops with exceptional capability to tolerate contaminated soils with heavy metals with very low amount of those hazardous elements in grains have developed, and addressing this almost ubiquitous problem will be effective and leads to the remarkable accomplishments in this field.

17.4 Biotic Stress

17.4.1 Phytopathogens

A significant quantity of the projected climate change-driven vacillations is ambiguous, in particular on phytopathogens (virus, fungi, and bacteria), but evidence indicated that climate change is and will be conducive to plant diseases. Agricultural section will be affected by climate change and plant pathogens multidimensionally including a dramatic increase in crop losses, weakening the validity and effectiveness of current plant disease management schemes, and finally disturbing the dispersion of phytopathogens beyond their ecological barriers (Chakraborty et al. 2000; Sogawa et al. 2003; Stenberg et al. 2015). Visualizing the population growth and climate change which exacerbate the food security challenge, concurringly, increase in plant disease damage will have a devastating impact. Statistics show a vast crop loss caused by plant pathogens up to 20–40% globally (Oerke 2006; Savary et al. 2012); the data is, however, outdated and the percentage might be larger. In the era of changing climate, abiotic stresses are frequent which confers more sensitivity to plants against pathogens (Agrios 2005); additionally, many pathogens are functioning in temperature ranges below their optimal. With the increase in global temperature, the number of plant diseases in warm regions, especially in tropics, exponentially grows (Deutsch et al. 2008). Optimistically, advances in agriculture technology nowadays are promising to assist us to address the prospective challenges efficiently, whereas developing disease-resistance crop varieties is known to be the quintessential way to effectively and economically overcome the plant disease problem. The conventional plant breeding approach has accomplished tremendously to develop varieties resistant to phytopathogens in a long list of crops, bacterial blight (*Xanthomonas citri* pv. malvacearum), Fusarium wilt (*Fusarium oxysporum*), powdery mildew (*Plasmopara viticola*) (Miah et al. 2013), and leaf curl virus (Anjum et al. 2015). The resistance cotton varieties, blast resistance rice (Basmati) (Sreewongchai et al. 2010), Caliverde alfalfa resistance to wilt (Putnam et al. 2001), for fungus disease in ornamental plants, leaf spot infections in rose cultivar resistance to black spot (*Diplocarpon rosae*) (Leus 2017), anthracnose blight (*Sphaceloma rosarum*) (Bagsic et al. 2016), and rust (*Phragmidium* spp.) (Leus and Huylenbroeck 2007) were promoted. Phenotypic selection method has contributed greatly to developing virus-resistant important crops; potato varieties with average stability in resistance to A, M, S, and Y (Foxe 1992; Lecoq et al. 2004); and significantly high permanence to X viruses (Solomon-Blackburn and Barker 2001). In the matter of disease resistance in cereals, varieties are developed containing *Lr34* conferring them simultaneous multiple disease resistance in wheat and barley against wheat rusts (*Puccinia* spp.) and downy mildew (*Blumeria graminis* f. sp. tritici), barley rust (*Puccinia hordei*), and barley powdery mildew (*Blumeria graminis* f. sp. hordei) (Manjunatha et al. 2018). Of course, the conventional breeding approach is used in many other crops or vegetables, flowers, soybean, maize, sugar bit, melons, cucumber, apple, pear, cherry, and fruit trees to develop fungus- or virus-resistant cultivars, and the list goes on. Since for numerous phytopathogens, one or a few genes are in control of resistive responses, known as vertical resistance (Lopes and Boiteux 2012), hence, progress in breeding programs for disease-resistant varieties covers a number of crops as well as pathogens, but it is beyond the scope of this chapter. For further study, readers can see reviews on these matters by Tinline et al. (1989), Bonman et al. (1992), and Elad and Pertot (2014). In conventional plant breeding, selection for pathogen-resistant varieties relies upon specific environmentally dependent phenotypic characteristics without the knowledge of genetic background of how stable or how many associated genes harbored in that line and the existence of pathogens; thus, this method is highly circumstantial and laborious to stack a gene/genes in an elite line because introgression of one gene can destroy years of time consumed on developing a superior line. To overcome the numerous drawbacks of conventional breeding, a selection procedure with the aim of molecular markers, so-called the MAS, can be significantly effective to precisely pyramid gene/genes in an elite line that confers durable resistance to phytopathogens with the least off-target consequences in comparison with traditional breeding. Enriched literatures exist with knowledge on sequence-based genotyping and QTL mapping for qualitative resistance associated with pathogen resistance controlled by multigenes or single gene in various major food plants that are prime resources to discover and pave the way to run the MAS approach on developing novel cultivars with wide-spectrum resistance to phytopathogens.

Since there is a large body of knowledge on using biotechnological techniques to improve plant resistance against various types of pathogens, here we try to categorize and summarize the recently published reports based on the dietary importance and their most threatening pathogens. So far, in these crops, QTL identification and mapping as well as using the MAS system to develop disease-resistant varieties have frequently been reported.

During the last two decades, wheat and barley are crops that the MAS approach has extremely contributed in their resistance improvement against several detrimental widespread fungi diseases controlled by a single heritable gene such as wheat rust (*Puccinia* spp.), downy mildew of wheat (*Blumeria graminis* f. sp. tritici), and head scab of wheat (*Fusarium graminearum*) (Miedaner and Korzun 2012). Those diseases are known to have the most devastating impacts on yield and jeopardize food security of millions of people particularly in developing countries that are most vulnerable to climate change. Wheat leaf rust (leaf, stem, and stripe) known as "polio of agriculture" is greatly capable of the devastation of wheat farms globally which caused an estimated annual $5 billion yield losses in the USA (Schumann and D'Arcy 2006) and $3 billion in Asia (Khan et al. 2013). Host resistance has been considered as the most efficient, cost-effective, and environmentally friendly approach to deal with this disease (Soriano and Royo 2015); of course, many single R-genes have been transferred to elite lines to confer resistance, but the race-specific R-genes mostly broke down in the short run (Periyannan et al. 2017). Hence, utilizing novel approaches including the MAS system can be helpful in pyramiding multiple genes coffering simultaneous durable resistance to several pathogens to a superior line. Therefore, background information to apply such approaches is a prerequisite. Singh et al. (2004) with the aim of STS markers detected two *Lr19* and *Lr24* genes associated with resistance against *Puccinia recondita* f. sp. tritici. Hulled wheat is a well-studied rich source of new genes conferring disease resistance to wheat and barley from which *Yr15* is substantially effective gene against a wide range of various isolates and races of leaf rust (*P. striiformis*) that has been pyramided to domesticated wheat (*T. aestivum* bread wheat and *T. durum* pasta wheat) (Beddow et al. 2015; Gerechter-Amitai et al. 1989; Nevo and Wolfram 2002). Recently, Yaniv et al. (2015) confirmed the location of *Yr15* gene using SSR markers; the outcome is valuable and improves the efficiency and reliability of the MAS system to develop resistant cultivars with significant durability. Most lately, Klymiuk et al. (2018) reported successful cloning of *Yr15* expressing a very specific capable R-gene configuration. While all abovementioned reports can be substantially helpful, providing adult plant resistance (APR) is a prominent approach to develop disease resistance, in the case of yellow rust. Yuan et al. (2018) investigated a wheat traditional variety (Humai 15) in China capable of containing APRs, and one major-effect QTL was detected. The powdery mildew (PM) (*B. graminis* f. sp. tritici) in wheat yearly causes moderate (23%) crop losses based on climatological circumstances, while under the favorable condition, it reaches 50%. Due to changing in climate and rising in overall temperature, the dispersion of wheat powdery mildew may decline in some regions, but the adaptability of this fungus should not be undermined (Matić et al. 2018). Many attempts are reported on pyramiding genes to superior lines through the MAS approach to develop PM-resistant varieties that in some cases turned to be multiresistant. For example, Pietrusińska et al. (2011) transferred two genes, *Pm21* and *Lr41*, conferring resistance against powdery mildew and leaf rust in wheat, respectively, through using molecular markers and the MAS system. Similarly, Elkot et al. (2015) using bridging species, durum wheat, managed to transfer two significantly effective resistance genes (*PmTb7A*.1 and *PmTb7A*.2) against *B. graminis* f. sp. tritici which originated from Einkorn wheat (*T. boeoticum*) into winter wheat. Considering the vast vulnerability of economic cultivars of winter wheat in China to powdery mildew, Xu et al. (2017) employed inbred lines to stack R-gene conferring high PM resistance in different ontology stages through the MAS into two commercially important cultivars. Additionally, exploring wheat has attracted the attention of many researchers. QTL mapping of a recombinant population and identification of two major-effect PM-resistant QTLs (Qu et al. 2018) develop efficient closely linked molecular markers to *Pm4b* gene conferring PM resistance (Wu et al. 2018). Lately, another R-gene locus (*MlLa-H*) conferring race-specific resistance to powdery mildew (*B. graminis* f. sp. hordei) in barley containing four genes was detected through high-resolution mapping by Hoseinzadeh et al. (2019).

Head scab of wheat (*F. graminearum*) is a worldwide increasing threat for wheat and barley production either quantity (i.e., making flowers infertile) or quality (i.e., high concentrations of mycotoxins in grains) due to rising global temperature and more humid conditions. An elevation in its incidence has been reported particularly in North America with over $7 billion estimated crop losses (Gilbert and Tekauz 2000; Nganje et al. 2004) and China (Zhang et al. 2011), which has motivated many researchers to address the problem through developing novel varieties resistant to head scab. Head scab is multigene controlled disease which gained improvement through conventional breeding that is not economically feasible, but using molecular markers to map QTLs related to head scab resistance has generated a wealth of knowledge to employ the MAS approach to stack gene/genes into elite varieties. Yang et al. (2003) used SSR markers detected genes responsible for a large percentage of phenotypic variation in a collection of doubled haploid wheat for host resistance to head scab. Similarly, Jia et al. (2005) reported a durable QTL linked with head scab resistance using SSR markers. Sydenham (2014) developed lines carrying *Fhb1* gene conferring resistant to head scab in spring wheat cultivar, Krokodil, through a two-step MABC. In another study, a resistance local variety in China (Haiyanzhong) and a sensitive one (Wheaton) are crossed, and the selected individuals in the second generation are genotyped using SSR and SNPs. One major-effect and five other minor-effect QTLs were mapped in this study. They suggested that the head scab resistance is regulated by various minor-effect genes (Cai et al. 2016). The resistance source is also found in hexaploid and introgressed successfully to vulnerable durum variety (Giancaspro et al. 2016). There are dozens of reports on detecting QTLs associated with head scab, and a plethora of genes with minor effects are found, whereas only a single gene, *Fhb1*, is repeatedly found in various researches conferring incomplete but still considerable resistance to head scab, and to date several commercial cultivars improved based upon (Anderson 2007; Bai et al. 2018). Many of complicated mechanisms controlled by specific gene expression have been understood using biotechnological methodologies, and all this body of information will enable scholars to gain a holistic view on local resistance varieties and finally effectively address this challenge. Employing new approaches in improving host resistance against head scab such as CRISPR/Cas9 system has significantly helped breeders to further develop more practical head scab-resistant wheat or barley varieties (Jaganathan et al. 2018; Zaman et al. 2018). As an example, an investigation recorded durability and density of mutation in hexaploid bread wheat with the aim of genome-editing approaches, CRISPR/Cas9. Three important genes in wheat with various functionalities were assessed: *TaGW2* (repressor of characteristics of grain), *TaLpx*-1 (lipoxidase, responsible for head scab resistance), and *TaMLO* (amorphic mutation, associated with downy mildew resistance) (Wang et al. 2018). This research manifests the great capability of genome-editing tools to generate new useful genetic variation. Using RNA-guided DNA endonuclease (Cas9), Gardiner and Kazan (2018) generated mutations which through monitoring by phenotypic resistance to fungicides that opted null alleles of *FgOs1* gene osmosensor histidine kinase successfully detected Cas9-mediated mutations. A small variation was found probably due to engagement of a higher repair system. Biotechnological tools, particularly genome editing, hold a promising potential to provide host-resistance mechanisms against head scab.

Of the substantial food plants, rice (*Oryza sativa*), as a major source for over half of current population on the Earth, in particular in Asia, is expected to occupy even more arable lands owing to the exponential growth of population. Understanding of genetic and physiological mechanisms in charge of disease resistance was not improved significantly in comparison to other substantial crops until recent decades; with the emerging biotechnological approaches, now this crop is a model plant (Mishra et al. 2018; Pilet-Nayel et al. 2017). Like any other crops, rice, and some deadly phytopathogens, among them, blast (*Magnaporthe oryzae*), bacterial blight (*Xanthomonas oryzae* pv. Oryzae) (BB), and sheath blight (*Rhizoctonia solani*) (ShB) are known to be the most threatening ones. These fungal diseases, through damaging the vegetative parts, reduce the photosynthetic efficiency, and finally there are up to 30–50% losses in yield and quality of grain. Resistant cultivars have not been significantly effective due to either low durability of resistance in cultivars. The earliest report on investigating blast resistance in rice genetic goes back to the beginning of the twentieth century (Ashkani et al. 2015; Leng et al. 2017; Lin et al. 2018). Thanks to advancement in biotechnology, involved infection as well as resistance mechanisms related to blast, bacterial blight, and sheath blight in rice have been elucidated immensely, durability of resistance in commercial varieties improved significantly, various resistance genes (100) and QTLs (approximately 500) associated with blast resistance have been detected, and more than 20 reports on their cloning and further 39 resistance genes related to BB in rice have been located (Dossa et al. 2015). Here, we mention some of the most important and exemplary studies on using the MAS approach to develop varieties with resistance against these diseases. In an attempt, Singh et al. (2012) employed the MAS system to pyramid multiple resistant genes into a popular Indian economical rice cultivar to fortify its simultaneous host resistance against blast. The ShB and BB utilized a previously resistant cultivar Improved Pusa Basmati 1 containing *Xa13* and *Xa21* genes conferring BB resistance and Tetep known for its high blast and ShB resistance potential bearing *Pi54* gene and *qSBR11*–1 QTL. Using genetic markers (SSRs) for selecting favored progenies, they detected pyramided lines carrying all resistance genes *Xa13* and *Xa21* (BB), *Pi54* (blast), and a major-effect QTL for ShB. Similarly, Arunakumari et al. (2016) applying marker-assisted backcross (MABB) succeeded to stack genes conferring resistant to BB (*xa13* and *Xa21*) in Improved Samba Mahsuri (ISM) and blast (*Pi54* in NLR145) to a recently developed variety of rice "MTU1010." The outcome lines manifested significant host resistance against BB and blast from those donors while carrying high quantity and quality yield of MTU1010. Lately, with the same approach (MABB), in order to enhance the stability of BB resistance of Improved Samba Mahsuri (i.e., carrying *xa13* and *Xa21* genes), Yugander et al. (2018) managed to transfer a BB resistance gene, *Xa38*, from PR 114, a potential line, to ISM with a considerably high 92% RPG. Significant resistance against various strains of BB is observed in the lines from the MAB system with comparable cooking and yield inherited from ISM. Other instances of attempts using the MAS system to improve or develop resistance against BB, blast, and ShB in rice varieties are pyramiding five QTLs (i.e., four for BB and one for blast) to RD6, an elite cultivar of rice (Pinta et al. 2013), using the MAS to stack three blast resistance-related genes (Pi1, Pi2 and Pi33) in rice to a Russian superior rice line to prevent resistance breakdown (Usatov et al. 2016), transferring *Xa21* gene associated with BB resistance to LT2 rice variety with the aid of MABB (Nguyen et al. 2018), and enhancing host resistance against ShB through pyramiding *TAC1* and *qSB*-9 QTLs to near-isogenic lines (NILs) (Zuo et al. 2014). Although the MAS has been confirmed to be significantly suitable to develop resistance against these three diseases, other approaches also have been employed to further unmask the intertwined mechanisms and genes/QTLs that control the resistance of these pathogens and improve the breeding efficiency. The GWAS has frequently been used to detect potential candidate genes regulating blast resistance among nearly one million SNPs (Wang et al. 2014) or identification of 32 QTLs associated with blast resistance (Lin et al. 2018), finding genes responsible for BB resistance in Indian rice germplasm among a large number of SNPs via GWAS (Zhang et al. 2017a), and using SNP polymorphism among various rice varieties and GWAS to detect putative QTLs related to ShB resistance (Chen et al. 2019).

Utilizing more precise, less laborious, and cost-effective systems in breeding for disease resistance, in particular, has been the focus of many scholars. In this case several genetic engineering approaches have variously been applied which include the use of transgenic plants with overexpression of chitinase and glucanase genes that are in charge of cell wall-degrading enzymes and the overexpression of chitinase or glucanase that leads to degradation of chitin in the cell wall of fungus (Ceasar and Ignacimuthu 2012). In the case of sheath blight, insertion of genes *ech42*, *nag70*, and *gluc78* indicated that *ech42* generated a significantly higher resistance in transgenic rice plants (Liu et al. 2004). For the same pathogen, Sridevi et al. (2008) observed that lines with co-expression of genes, rice chitinase (*chi11*), and tobacco b-1,3-glucanase (*glu*) have a considerably higher resistance against sheath blight. Recently, CRISPR/Cas9-mediated mutated lines where a specific gene, *OsERF922*, was targeted were further assessed, and a significant resistance in mutants carrying favorable gene alteration in comparison with wild types was observed. The prerequisite of gaining a comprehensive understanding of phytopathogens, in this case, rice blast, is to finely investigate mechanisms of the pathogen which enables researchers to provide applicable and effective strategies. In this regard, Foster et al. (2018) utilized genetic engineering methodologies, plasmid-free CRISPR/Cas9, since constant expression of Cas9 can be detrimental for the blast fungus (*M. oryzae*).

It can be concluded that biotechnological methodologies are in their zenith, and more advances are underway by which researchers can efficiently run breeding programs to promote rice resistant varieties to prohibit the significant yield losses and also develop crop cultivars to stabilize the production rate of rice as a substantially crucial staple food under climate change era with an ever-growing population on the Earth.

With the drastic change in climate in compilation with a narrow genetic base of improved maize cultivars, the circumstances are in favor of many potentially threatening plant diseases (Mammadov et al. 2015). Gray leaf spot (GLS) is a devastating phytopathogen caused by *Cercospora zeae-maydis* in maize as a multipurpose crop; the pathogen has significant negative impacts on yield up to 60% and complete annihilation of farm in drastic outbreaks. First, symptoms of gray leaf spot are seen on the upper layer of leaves as small, pinpoint lesions limited by yellow halos, here is where correct identification of the disease could be difficult; however, gradually lesions expand parallel with leaf veins up to 2 inches and soon cover the whole leaf. Management strategies in the best case scenario use the resistant cultivars which critically reduces the expenses (Pilet-Nayel et al. 2017; Pozar et al. 2009; Shi et al. 2014). Therefore, detection of genes/QTLs associated with GLS resistance to provide background information and utilize the MAS approach is necessary. Various attempts have reported related QTL identification and validation to GLS resistance. For instance, Veiga et al. (2012) investigated tropical corn germplasm for putative QTLs related to GLS using SSR markers in which they suggested the additive genetic effect of QTLs as a resistance regulator against GLS. Shi et al. (2014) reported a comprehensive study on GLS underlying genetic control in maize; applying GWAS, they pinpointed 51 considerably linked SPNs to GLS resistance as well as three candidate genes apparently engaged in fundamental defense mechanisms. Using GWAS to improve the resolution of population mapping to detect QTLs associated with GLS resistance, Mammadov et al. (2015) located a major-effect QTL and further verified the previously detected QTLs. Similarly, Kuki et al. (2018) performed GWAS on maize tropical populations with SNPs, and seven SNPs closely linked to GLS resistance were identified. The results can have effective applicability in gene stacking to develop GLS resistance maize via the MAS system. Frequent putative QTLs have been identified, and accuracy of their location with GWAS has been improved which makes the prospective MAS projects significantly potential. The other damaging and prevalent phytopathogens in maize are stalk rots mainly caused by *Pythium* and *Fusarium*, but a great diversity in species have been reported worldwide (Wu et al. 2011; Yang et al. 2010). The severity of infection, of course, depends on region or cultivar, but in many cases, 25% of total yield loss was reported

(Lu et al. 1995). By considering a long list of advantages of biotechnological approaches, in particular the MAS system over conventional breeding, many researchers have concentrated on QTL identification to use MAS approach and pyramid resistant gene(s) into superior varieties, among which are Song et al. (2015) who exploited molecular markers and identified two separately inheritable predominant genes, *RpiQI319*-1 and *RpiQI319*-2, in a line Qi319 providing resistance against *Pythium inflatum*. Their co-segregation with several other markers was determined. These results can greatly help to pave the way of employing MAS to develop *Pythium* stalk rot-resistant varieties of maize. In another study on *Fusarium* ear rot (FER) caused by *F. verticillioides*, Chen et al. (2016) used SNP markers to execute GWAS and identify alleles related with FER resistance in rice and ultimately 21 genes mapped in that regard. Validation of GWAS outcomes through linkage mapping of biparental populations indicated 15 FER-related resistant QTLs. This comprehensive study showed that several minor QTLs regulate FER resistant; further, stacking potential alleles integrated with molecular selection has the potential to improve FER resistance. In another economically important fungal species of stalk rot in maize, *Gibberella zeae*, Chen et al. (2017) employed the whole-genome sequencing-based methodology to locate potential loci, and they completely mapped a putative tolerant QTL known as *Rgsr8*.1 and after segregation applied SSR as well as SNP markers; two genes providing *Gibberella zeae* resistance were pinpointed. Their functionality was one receptive to auxin, and another engages in biosynthesis of resistance protein. To improve the accuracy and efficiency of breeding methods, using new technologies such as CRISPR/Cas9 system has been suggested, even though this approach is fairly new to plant breeding, especially maize, but it holds promising future in escalating the process of plant breeding. So far, only a very few studies have considered CRISPR/Cas9, among which are Char et al. (2017) who generated multi-targeted mutants in maize using ISU Maize CRISPR, a system in public sector, employing *Agrobacterium*-mediated CRISPR/Cas9. After evaluating the inheritability, they suggested the unique capability of this platform for the aimed mutation in maize. With the enhancement of the environmental condition to the dispersion of plant pathogens when the climate is rapidly changing, only a robust biotechnological toolkit can assist breeders to cope with fluctuations and new challenges. In the case of maize as a major food plant, developments ahead can be revolutionizing since strong background information is already under construction by researchers.

17.4.2 Pests

Alteration generated by a dramatic climate change during the last decades is hugely and multidimensionally perceived by the agriculture sector through vivid changes in quantity and frequency of outbreak of pests (De Pace et al. 2013). The quantity of loss in yield caused by pests almost averagely reaches 30% globally (Ferry and Gatehouse 2010). In the latest survey on crop loss caused by pests and pathogens, a significant yield loss ranging from 10% to 40% has been indicated, with main concentration on regions with food shortage and rapid population growth (Savary et al. 2019). To tackle the ever-growing threat of pests which is jeopardizing food security worldwide, the host-resistance strategy is the most recommended one. Using conventional perspectives in pest management is a time-consuming, labor-intensive, and costly approach, in this regard, Smigocki et al. (2013) reported the evolution of 574 arthropods as a result of using pesticides and other agrochemicals, in addition to the devastating impact that agrochemicals have on beneficial insects and deterioration in biodiversity. By taking into account the fact that advancements in biotechnology empowered researchers in plant breeding to break the limits and address the challenges properly that otherwise from the traditional plant breeding would be improbable, biotechnological tools have been used on a large scale to speed up the breeding process with great attention to the durability of resistance. The fundamental facets of modern plant breeding are understanding the underlying physiological mechanisms and genetic background of either pests or plants; after that, we can apply effective and precise methodologies to develop resistance (Birkett and Pickett 2014; Lombardo et al. 2016; Yin and Qiu 2019). Reports on characterizing QTLs/genes associated with pest resistance have been tremendous especially in major crops. The MAS utilization to pyramid multiple genes to superior lines to develop crops with simultaneous resistance against pests and diseases is common, from which studies focused on the most damaging herbivore pests such as brown planthopper (BPH; *Nilaparvata lugens*) which is highly destructive for rice production worldwide especially in South and Southeast Asia (Sogawa et al. 2003). Only in China, this pest caused 2.7 million tons of yield loss and a significant portion of spreading other diseases (Reinke et al. 2018). Xu (2013) reported three genes, *Bph14*, *Bph15*, and *Stv-b*^{i}, offering host resistance against BPH and rice stripe virus (RSV), respectively. It was observed that the lines with BPH genes manifested high resistance against BPH and those with RSV genes resisted to RSV. In groundbreaking research, Reinke et al. (2018) managed to stack various resistance R-genes related to three pathogens and one pest to develop a variety of rice resistance to multiple diseases and pests, using the MAS approach. In this way, they transferred genes conferring resistance to BPH (*Bph18*), BB (*Xa40*), blast (*Pib* + *Pik*), and RSV (*qSTV11*^{SG}) from five lines with several resistant genes into a single line with high durability and generating no unfavorable impacts on quality and yield quantity. Many more types of research involved finding suitable markers for BPH resistance (Jairin et al. 2009; Jena et al. 2006; Shabanimofrad et al. 2015). In some cases, resistance to a specific pest results in impairing the yield potential. A number of studies reported on developing resistant varieties to Mediterranean corn borer (*Sesamia nonagrioides*), without dependency on developmental stages of the host plant; however, the gene transfers mainly led to a decrease in yield (Butrón et al. 2012; Ordas et al. 2010). Recently, Samayoa et al. (2019) employed the MAS system, which significantly enhanced the host resistance to this pest with no negative off-target consequences on yield, owing to precise introgression of the desired genes. Hanson et al. (2018) reported several valuable SNPs associated with resistance to soybean aphid (*Aphis glycines*), a considerably destructive pest particularly in the USA, using GWAS on over 2000 accessions of soybean which broadens the information with screening and characterizing potential genetic resources. There were just some examples of researches conducted on developing host resistance in major food crops to the most prevalent and destructive pests through the means of the MAS, but the fact is that to this day, no introduction of commercial varieties of MAS-based breeding programs has been reported, whereas genetic engineering approaches have been considerably more successful in this case, and many commercialized cultivars exist that cover over 185.1 million ha of cultivated lands in 2016 (Brookes and Barfoot 2018) and provide a large proportion of the world's food and fiber; therefore, constant research to improve and stabilize the production is necessary. For example, exploiting *Bt* toxin derived from *Bacillus thuringiensis* expressing crystal toxins (Cry proteins) which is detrimental to pests through disrupting ion channels and causing disintegration of the cell wall and gradually death (Peña-Cardeña et al. 2018). In the recent decade, several resistant cultivars only in corn have been introduced, Agrisure®, Duracade™, and Viptera™, containing *BtCry1Ab* and *Vip3Aa20* genes. These cultivars are obtained through gene pyramiding and crosses between engineered lines with various characteristics (Lombardo et al. 2016). Additionally, owing to the low vulnerability of many pests to *Bt* and the evolution of many others, a novel method was designed to overcome this challenge, which enhanced the chance of molecular interaction in the pest by which *Cry1Ac-RB* gene is gained that confers a significantly strong resistance in engineered rice and corn (Lombardo et al. 2016; Mehlo et al. 2005). Application of CRISPR/Cas9 platform as a robust genome-editing tool to offer stable host resistance to pests has been frequent but still is in experimental stage and majorly on the model insect, *Drosophila*. The researches in this regard mostly involve a novel strategy to have an effective pest management through using Cas9 to generate entirely male populations of pests in *D. melanogaster* which is then applied on *Ceratitis capitata*; so far scholars have introduced applicable alternatives that can be further developed to effectively manage this pest in field. (KaramiNejadRanjbar et al. 2018) or knocking out and modification to figure out the basal mechanisms of the targeted gene (Gratz et al. 2013; Huynh et al. 2018; Xue et al. 2014; Yu et al. 2013). CRISPR/Cas9 as a relatively novel system in plant breeding has greatly contributed many breakthroughs; in the case of insect pest-resistant varieties and amplifying the management of pests in the changing climate, this system will soon provide important findings.

Another promising method to promote pest resistance is RNA interference (RNAi) which inserted dsRNA molecules that prohibit gene expression. Mainly, dsRNA used in developing a genetically modified plant with the capability of self-protection against pests through dsRNA expression. By feeding the pest from the plant, the absorbed dsRNA aims to identify the specific gene in the insect and nullify the messenger RNA which ends up with a decline in its expression, depending on the gene that eventually leads to growth prohibition or death (Gordon and Waterhouse 2007). A large number of researches reported advancement on its application to assist the resistance durability of crops using *Bt* toxin defense system or improve the effect of pesticides (Gatehouse 2008; Price and Gatehouse 2008; Yu et al. 2013; Zhang et al. 2017a). There are some applications of RNAi: the vulnerability of

Western corn rootworm (*Diabrotica virgifera*) was recorded in its consummation of a synthetic diet, and *Snf7* was found to be the highly effective one, and transgenic corn with the ability to express *Snf7* dsRNA was observed to have a significant resistance in its roots against Western corn rootworm. However, *Snf7* impact function is not fast enough; therefore, its application in compilation with *Bt* toxin insecticide, *Cry3B*, with a high reaction potential can magnify their effect (Baum et al. 2007; Bolognesi et al. 2012). An efficient way to use similar approach to circumvent the insect pest resistance mechanisms is to impair the resistant mechanism of the pest by disrupting the specific gene(s) that provide protection to the insect against defense that is generated by the host plant (Bally et al. 2018; Zhang et al. 2015, 2017a). In this case, gossypol, one of the major plant secondary metabolites in cotton, serves as chemical defense mechanisms, but it is not tangibly effective on cotton bollworm (*Helicoverpa armigera*). Inducing the expression of *CYP6AE14* in cotton bollworm under a diet containing gossypol led to a hypothesis that this enzyme is responsible for detoxification of gossypol; hence, a transgenic cotton developed lacking this enzyme, which further test indicated a decline in growth rate of this pest as result of feeding on the transgenic cotton plants in comparison with control plants. However, further investigation showed that the deployed dsRNA acts against the enzyme solely in the gossypol-enriched diet (Mao et al. 2007). In another insect pest, tobacco hornworm *Manduca sexta* (Lepidoptera), either mature insect or larva can resist a high level of nicotine. In larvae, this tolerance protects them from predator spiders. Also, in *M. sexta*, a particular gene is identified to be induced by nicotine, *CYP6B46*; a transgenic of tobacco (*Nicotiana attenuata*) was developed with expression of dsRNA aiming to target *CYP6B46*. The result showed that larvae feeding on transformed plants made them vulnerable to the predator owing to decline in nicotine exhalation by larvae (Kumar et al. 2014a). Despite the fact that it is a markedly potential approach, its applicability has a significant dependency on the level of persistent dsRNA, where the aim of the biotechnological methodologies in this status quo is considerably complicated and laborious and results are controversial.

Many of these sound and highly capable methods are new to the complicated multifactor environment like plant breeding and gene editing; however, due to urgency to improve and develop crop cultivars with climate change-resilient agriculture in perspective, surely this biotechnological toolkit soon goes beyond trial and error and serves breeders to meet the ever-growing demand for agricultural products in addition to constant increasing in outbreak of insect pests.

17.5 Conclusions and Future Prospects

Climate change impacts have become increasingly perceivable in the recent decades which vividly can be observed in the growing percentage of population struggling for their basic diet, enormous shifts and fluctuation in precipitation paradigms, and uncontrollable rise in global temperature and elevated atmospheric CO_2 or O_3. Also, worsening the condition in the farming sector, increment in frequency of abiotic and biotic stresses and disappearance of resistant durability, is another part of these dramatic changes. With all these obstacles in agriculture sector, still food demand must be met when advancement in science reached its climax and agricultural biotechnology is in its heyday; of course, many of its approaches might be new and might not be as practical as they are required nowadays, but probing for better, more resistant, and higher yield crop fields never stops. As always, humans can now address the problems and overcome the challenges; it is in our nature. Further research on the one hand and trying to prioritize the knowledge of biotechnology on the other hand as substantially critical tools to develop climate change-resilient agriculture for politicians and public are two key steps to gain financial resources that considerably facilitate the circumstances for scientists and scholars. Generating new crop cultivars with the capability to tolerate multiple stresses can be achieved with increasing information on their basal physiological and genetic mechanisms, where having more data means higher efficiency in MAS system or CRISPR/Cas9 platform. Numerous accessions in gene banks remained untapped. The application of GWAS with the aim of genotyping by sequence can greatly import novel alleles conferring resistance into breeding programs that can contribute in developing superior lines. In a comparative point of view, assessment plant under field stress would produce more reliable putative genes/QTLs because field condition is more effective in inducting the molecular tolerance mechanisms, whereas simulating similar condition artificially in lab so far have not been considerably successful in generating potential QTLs to pass the field test. Thus, the condition which applies to identify putative genes/QTLs should be more realistic which helps to develop lines with durable resistance. Promoting tolerant crops to biotic or abiotic stresses is complicated and laborious since it involves multiple genes that make their introgression challenging; nonetheless, the assistance of data and precise, inexpensive, and time-saving methodologies enable breeders to obtain the targeted variety with favorable traits in a short period. Ultimately, by glancing through the evolution of biotechnological tools, it can be understood that a brighter future is ahead for agriculture under climate change era.

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Chapter 18
Transcriptomics in Deciphering Stress Tolerance in Plants

Bipul Sarkar, Arijit Ghosh, Indraneel Saha, Arnab Kumar De, and M. K. Adak

Contents

Abstract Abiotic stress responses in plants are the consecutive effects of stress perception, its transduction of signal, induction to gene expression and resultant manifestation of physiological activities. Therefore, gene expression with up- and downregulation is the key to monitor and decipher the stress tolerance in plants. In the analysis of regulatory sequence with the modern state of the art, selecting plant genotypes or trait transfer to other species has been in progress. Transgenic approaches have been successful in manipulating any such trait for responses under stress. The global gene expression through transcriptome studies is another alternative tool in understanding of metabolic reactions supporting stress tolerance. The evaluation of gene transfer and its achievement for stress tolerance is no doubt a time-consuming phenomenon. A single gene transfer through reliable vector is not approach to coordinate the stress responses. However, regulatory binding proteins, which are commonly induced by a number of stressors, are more used to be cloned. So, transcription factors could co-ordinate major stress-responsive genes and their converging tendencies in stress tolerance. Therefore, the agronomically important traits for superior crop genotypes would be more lenient in promoter regulation technology for stress expression. In this review, the combination of regulatory

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mechanism through transcriptomics and selection with molecular marker would be described in specific manner in the realization of stress-resistant genotypes. In addition, the lineage to roles of molecular markers in selection pressure of crops has also been described to support the breeding programme.

Keywords Cis-element · Transcription factors · Promoter binding · Genotype selection · Molecular markers

Abbreviations

ABA	Abscisic acid
CBF	Cold binding factor
CDS	Coding DNA sequence
DREB	Dehydration response element binding factor
DREBP	Dehydration response element binding proteins
GA	Gibberellic acid
PHY A	Phytochrome A

18.1 Introduction

Undoubtedly, environmental fluctuations in the form of different abiotic factors are one of the determinants for the plant sustainability, growth and productivity. With any minimum to moderate changes of the environmental vis-a-vis edaphic factors, plants' growth and their equilibrium with biological and economical yield are perturbed. The molecular, cellular, biochemical and physiological responses in plants to such a fluctuation are ascertained by initially phenotypic and phenological changes. Estimation indicates one prime abiotic detrimental factor like salinity of arable fallows are expected to have a loss of 30% within coming 25 years (Ritzema et al. 2008). The continuous increasing salinity may reach up to 50% by the year 2050. However, plants already have built-in biological strategies to encounter such a change through evolution to adapt these adversities or avoid stressful conditions. Undoubtedly, plant gene and their expression through different modules are the key factors for such adaptation. Genes are present not only to modulate the cellular responses but also to perceive the stress impulses in a co-ordinate way that together imparts the stress tolerance. Therefore, stress tolerance in plants would critically be dissected into two major paths: first, induction of the genes to perceive the environmental stimuli and, second, the downstream reactions as a function of interaction to those stimuli following cell biomolecules resulting in cellular response (Hasegawa et al. 2000). With the advent of technologies, these two different processes could be modulated through transgenic modules in a number of ways that could achieve the alteration of osmoprotectant accumulation, generations of organic moieties in shaping of biomolecules to quench the high redox elements, sequestering or exclusion of unwanted ions through specific paths and revision or moderation of biochemical reactions into favour of tissue to adjust the stress impact.

Therefore, gene manipulation through transform experiment supports strongly the concept of induced stress tolerance in plant breeding. A major class of genes, mostly those that are functional in nature encoding enzyme proteins, are predominantly categorised. In one set, the biosynthetic pathway governing enzymes, particularly those that are rate limiting in nature, are often cloned from tolerance species (Noctor et al. 1998). A number of osmolytes like amino acids, quaternary amines, sugar alcohol and small oligosaccharides are the most common to develop transgenic crops (Kumar et al. 2018). Another class of structural protein like late embryonic abundant proteins, heat shock proteins, carriers or channels on membrane and mechanical cytoskeleton defender proteins contributes to stress tolerance in a different way. Enzymes for detoxification of redox species are another major class of gene expression to focus on transgenesis of crop species with low or moderate stress sensitivity (Bhatnagar-Mathur et al. 2008). Apart from that, a few classes of gene families encode some small molecular weight proteins which are exclusively regulatory in nature for functioning of concerned genes. These regulatory proteins are classically designated as transcription factors with their precise molecular nature and configuration that have been targeted to emphasise on transgenesis of different stress-responsive genes. According to variations of stress factors, those are grouped as dehydration response element binding factor (DREB) (Agarwal et al. 2006), cold binding factor (CBF), APETALA/ethylene response binding protein (AP2/ERBP), N-acetylcysteine (NAC), MTB/NIC, basic leucine zipper (bZIP), helix-loop-helix (HLH) and zinc fingers that are the most important ones for transgenic plant development. In this chapter, the development of transgenic plants under different facets of abiotic stress exposure is described in detail with possible involvement in cellular, biochemical and physiological paths for stress tolerance. Through the use of regulatory proteins, the identifications and its actual molecular mechanisms may support the development of transgenic plants to tackle the inevitable impact of environmental stress and its governance over crop improvement options in unparalleled manner.

18.2 Abiotic Stress Tolerance: Gradual Approach in Techniques Towards Modern State of the Art

Plant abiotic stress, its different facets and corresponding recoveries have been a choice of plant biologist since long ago. Initially, the selection of plant ideotypes with different morphophysiological characteristics supports plant's responses to withstand different stressors. The traditional breeding for a particular environment of few crop species had limited success and scope. Classical breeding programme, either interspecific gene amalgamation or in vitro induced variations, was the only approach to recover from abiotic stress in crop genotypes (Cattivelli et al. 2008). Those were bottlenecked by the complexity of quantitative traits of poor or meagre variations of field attributes, instability of physiological characters, non-reliability of phenotypic traits, etc. Therefore, there is a need to look for alternative or more authentic strategies to achieve stress tolerance in the cultivated crops. Recombinant DNA technology for genetic engineering was the next door to secure a particular gene expression and to contribute to stress tolerance in a more stable manner. In plant breeding, genetic engineering is undoubtedly a landmark of success over the conventional protocols for crop improvement. Simply, the transgenic crops presently cover 170 million ha in 2012 (Atici 2014). Genetic engineering through transgenic approach has contributed to the fastest development in crop science which has been remarked as the breakthrough achievement after the Green Revolution. In the Green Revolution, the cereal crops with dwarf and short stature are now being paid full attention due to their different genes in making high solar energy utilization and corresponding photosynthetic rates based on transcriptome analysis (Wing et al. 2018).

18.3 Transgenic Crops Put Forward the Successful Strategies for Stress Tolerance

Undoubtedly, food security is threateneds for population exploration. This is facilitated by inadequate agricultural lands, crunch of irrigation or water resource, edaphic factors' deficits and extremities, inadequacy of labour, energy, capital, etc. The yield potential of crop species with increased drought and salinized soil is a proposed challenge for different agro-ecological systems. It is well established that crop sustenance over such an adverse condition must be sited on selective genotypes and their adaptability to those conditions. Transgenic approach is such a modern state-of-the-art process where variability in gene expression is exercised by an engineering system to impart more or better tolerance (Davies and Christensen 2019). Therefore, isolation of genes, their extraction and identification through sequencing updates followed by over-expression are the ultimate goal to materialize a suitable way for stress tolerance.

The development of transgenics against diverse array of abiotic stresses, still, meets with more success for two major cases. Drought and salinity are two factors that affect a minimum of 20% of world's cultivated land, of which 40% under irrigation condition has experienced impeded productivities (Hoang et al. 2016). Of those lands under irrigation, at least one-third is very much contaminated with excessive salinity. Therefore, osmotic or water properties and its changes as well as specific ion effects by any metals are the bottlenecks for such a land for crop practice. These are more aggravated by inadequate rainfall. Excess loss of water by evaporation whether it is over burdened with oceanic salts, exhausted of irrigation with brackish water and other unauthenticated agronomic cultures may not be bothered. Those factors increase the salinity of soil affecting the productivity. As estimated by the UN programme, greater than 20% of agricultural fallows and greater than 50% of crop arable lands are affected by salt which increases by almost 10% annually (Flowers 2004). Therefore, crops with its genotypic potential exercise the harshness for adaptation, tolerance or resistance or even may change the life/growth cycle patterns to exercise the avoidance strategies. In support of those many genes and their expression to encounter the stress factors are under precise and stringent control. The promoter-related information was mostly based on interaction with DNA sequence on upstream which either directly induced the over-expression or secondarily becomes the signal preceptor elements for other genes (Pérez-Martín et al. 1994). Genes for adaptation and their eventual resistance may principally be grouped as osmotic adjustment encoding proteins, major anti-oxidative proteins and protein regulation for non-enzymatic antioxidants, cell stabilizing protein for heat stress, phosphorylation-dephosphorylation protein for transcription factors induction. For the transcription factors mostly the protein kinases and cis-element binding factors like DREB1/DREB2, ERFBP/AP2, MYC/NAC are most important. Therefore, mostly the functional and structural proteins, stress perception/signalling residues and gene regulatory factors have been the most predominant in abiotic stress tolerance with its possibility to exercise transgenic plants (Zurbriggen et al. 2010). Different growth regulators mostly abscisic acid, ethylene, jasmonic acid, brassinosteroids are another important facet in stress modulation conferring different modalities of osmotic adjustment, ion sequestering, management of cellular redox and also modification of gene expression (Divi et al. 2010). Regardless the cases, single gene transfer or any sort of modulation of regularity activities through transgenic plants must be optimized with integrity of tissues' native structural-functional synchronization.

18.4 Role of Promoter Deciphering Through Transgenic Model Plants

Transgenic plants, undoubtedly, are significant contributory in anticipation as well as understanding of gene functioning for stress tolerance. This got a dual assurance for up- as well as downregulation of a particular gene and its concomitant reactions on plant growth and development. It is also very much important to regulate the level of expression of a particular gene according to ameliorate the stress effects on the transgenic plants over a control species (Ezaki et al. 2000). The key element of such regulation is the promoter sequence, its variability and acting specifically to some regulatory proteins. For the sequence analysis of promoter, a number of molecular tools have already been recognized which also target the induced local lesions or a particular site of genome which may be under mutation or with variants of alleles. Promoter sequence analysis has other advantages like activation, tagging of some enhancer nucleotides in the gene construct. The whole construct is expected to function in induction of genes on site of integration. The demand of promoter sequence analysis is also aspiratory for inactivation of target genes which is mediated by homologous recombination and its consequent over expression. Promoter by definition are the precise DNA sequence at upstream from site of origin of the coding DNA sequence (CDS) that essentially read out by few proteins to set in transcription. Therefore, promoter sequence and its binding efficiencies are likely most important regulatory strategy for any transgenic plants in stress tolerance (Low et al. 2018). It is also accepted the variation in promoters as a need to monitor the efficiency of transgenes to be expressed both from the fundamental and adaptive research for economically important crops and plant materials within shortest period of time. Regulation for a promoter is also temporal and spatial specific for a gene to express. For most of the cases the RNA polymerase is bound with promoter only when cis-element are acted with transcription factor. Therefore, the differentiation, variation or even discriminations of different genes are fascinated by binding of trans-factors on response element only with stress inducible promoter. With development of plant genomics, a number of promoter sequences which are well characterized and efficient even for different genes also. Still, to compare with housekeeping genes i.e. for constitutive promoter a significant lesser efficiency is recorded for stress inducible promoter. This is also admitted well the stress inducible promoter are only operative when early response genes are activated by constitutive parts (Mizoi et al. 2012). As for example, a common platform of abiotic stress is the water/osmotic stress which is often met with salt, high temperature, excessive cold, metals as well as xenobiotics, high/low irradiance stress. It was reported earlier that few genes are common to express and their alteration under osmotic stress are also common to low temperature stress (Xiong et al. 1999). Dehydration response factor, abscisic acid response factor, ABA binding protein, cold regulated and cold inducible factors, low temperature inducing factors are always homologous on their position as well as expression in many crop species. It is also understood that housekeeping expression of transgene(s) under normal condition are undesired in expression for plants (Volkov et al. 2003). This includes growth anomaly, respiration malfunctioning, altered pigment synthesis, uneven stomatal distribution and regulation etc.

As for example, a significant expression of *Adc* gene is incapable in differentiation in callus under normal condition, however, *otsA* and *otsB* in transgenic species are well satisfactory in water stress tolerance (Feng-Yun and Zhang 2007). This is also featured with an impaired growth of leaves and supressed carbohydrate metabolism of the same transgenic plants but contributory to stress tolerance. Other example signifies of *mltD* and *IMT* in transgenic plants cause delayed floral development, reduced in vigour and sugars compared to control plants (Singhal et al. 2016). In *Arabidopsis,* cold binding factor (CBF) when over expressed in tomato a good tolerance against both cold and oxidative redox the results, however, with minimized and stunted stem. Metabolically plants could retrieve when GA was spread on aerial shoot. This suggest CBF1 through its heterologous position of GA biosynthetic genes but analogous to function. ABA biosynthesis when studied with constitutive promoters with *ABAF3*/*ABAF4* in *Arabidopsis* similar pattern of growth inhibition were the features. In ABA independent pathway dehydration response element binding proteins (DREBP) from rice was transform to *Arabidopsis* under CaMV promoter and resulted well drought tolerance along with good sensitivity to salt and chilling stress (Bajaj et al. 1999). From rice such a factor was cloned under the same promoter and over-expressed in transgenic plants recorded satisfactory responses exclusively under dehydration stress but not in normal condition. The cytokinin biosynthetic genes (*ipt*) was also transformed into transgenic *Arabidopsis* from chilli that resulted more conserve of tissue moisture even under moderate dehydration stress (Thakur et al. 2018). Therefore, promoter coordination to transgenic is more specific according to stress impulse factors and its down-stream perception by cis-element either directly or signal cascade mediated. So, stress inducible promoters much weaker under non-stress condition, still, may have an option in conjugation with desired genes to gain tolerance without any disruption of growth and development. Transcription factors from *Arabidopsis* (*At*-*DREB1A*) when acted with promoter of *At*-rd29A in transgenic rice recorded multiple tolerance without any noticeable hamper in growth (Nakashima and Yamaguchi-Shinozaki 2005). Other crops like peanut, potato also resulted in a compatible manner to over express such a transgene. Dual nature of a promoter in function are not less common in cultivated crops. Composite verities of maize when over expressed under rd29A promoter for *DREB1A* gene in *Arabidopsis*, both water and temperature tolerance were recorded (Shinozaki and Yamaguchi-Shinozaki 2000). Even dehydration and cold response by such a gene construct (DREB1A/CBF3) when expressed in *Arabidopsis* under rd29A, both regulation in water loss coupled with cold sensitivity were results. More so, the delayed wilting percentage occurred on subsequent non-irrigated condition in field (Fig. 18.1).

18.5 Contradiction in Overlapping Promoter Sequences Within Same Gene Family

In promoter sequence, a stretches of DNA sequence either in a single copy or in repetition are common in occurrence. In some cases, few mitochondrial genes are transcribed even by more than one RNA polymerases, however, from same nucleus. The cis-elements recognised by such RNA polymerase are less in knowledge and helps in transcription of such genes. In *A. thaliana* few mitochondrial genes and gene clusters are documented (Unseld et al. 1997). The common technique for such sequence analysis is with 5′ rapid amplification of c-DNA end (RACE) and RNase protection assay. Herein, the transcripts are tagged with in-vitro guanyl residue addition by a 5′ guanyl transferase. Out of 12 genes, a maximum number were reported to be transcribed by more than one promoter. In fact, dicot plant species with example of *Arabidopsis* had been first reported with multiple promoters from

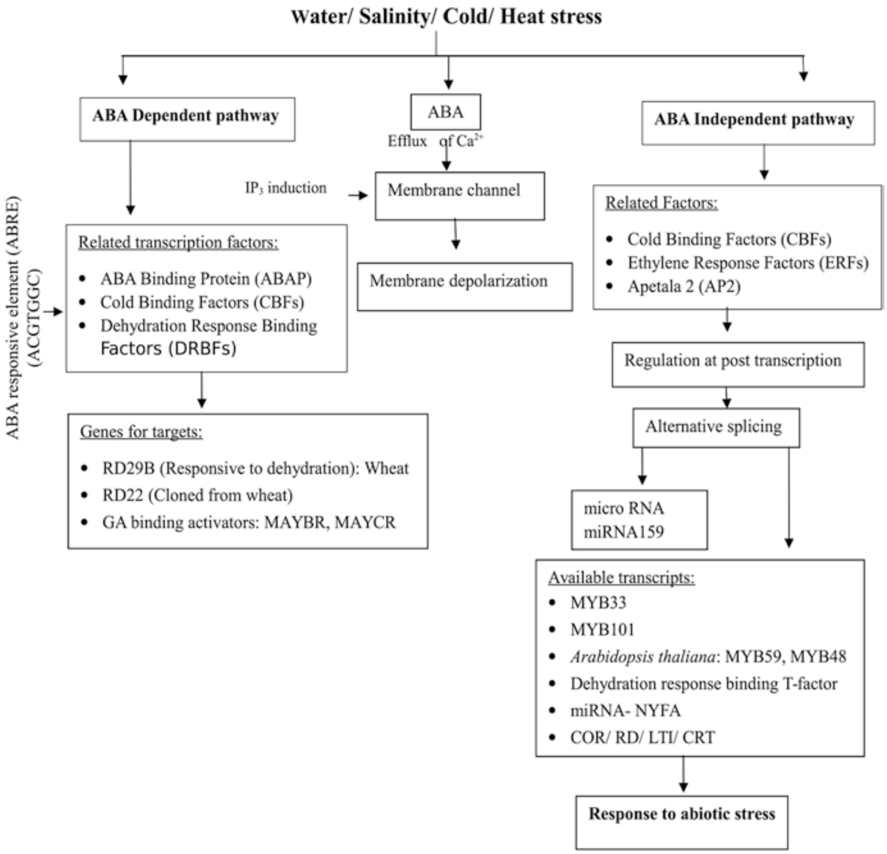

Fig. 18.1 Interact of major stress-responsive promoters to various abiotic stress. Two major pathways: ABA dependant and independent which have different site of signalling by different messengers. Regulation of post transcriptional activities could also coordinate different genes. In few cases miRNAs also are involved for some responses to cold and dehydration

mitochondrial genes even the organ specificity (for leaves and reproductive plant parts). The multiple promoter utilization without any stringent specificity, however, in most of those cases the transcription initiation complex site is reported with a close proximity. There have been prospects for good achievements of plant products via genetic engineering of a key or rate limiting gene in an enzyme mediated biosynthetic pathway. Using the variation in transcriptional by use of cis-element binding affinity to the transcription factors a large quantity of secondary metabolites have been reported in many plant species (Vom Endt et al. 2002). Knowing fully well that transcriptional factors are most conserved with different structural motifs. In RNA polymerase II containing complex transcription regulation pathway a single transcriptional factor can modulate many target genes. Single transcription factors in plant system have some different homologies when bind with cis-element of related genes. The basic similarity of those transcription factors are the features with basic leucine zippers, helix-loop-helix, zinc response element binding factor (CDF1) those can up-regulate a set of cold responsive genes binding with related gene families. This results the freezing tolerance. This may cite the mastery network for a single transcription factor in more complex web developmental events. This is the pattern of gene expression for many target genes in coordinated fashion. In case of *Arabidopsis* gene, the computational analysis or bioinformatics reveals an alternative method for any promoter to finds its efficacy. The far-red light (Pfr) responsive genes as perceived by the signal of phytochrome A (PHY A) would be a model (Castillon et al. 2007). Genes are categorised into functional groups as early and late responsive m-RNA accordingly the time period required for illumination. Using the micro-array information, it was also possible to find the mode of regulation of those transcripts. Therefore, through computational biology any regulatory elements/motifs with their repetition in promoter may vary the levels of expression. In few cases the specific and random binding on cis-elements for transcription factor have revealed several mutations in *Arabidopsis*. Those mutants have anomalous responses to the far-red light treatment which are based on variability of inactive genes to encodes proteins as transcription factors (Talbott et al. 2003). Transcription co-factors are also important in these cases which has been identified by DNA foot printing or gel mobility shift assay. There are some elements found in promoters of few circadian genes (Mockler et al. 2007). These sequences were significantly over represented in the promoter. Therefore, number of repetitive in circadian gene regulation was established. In most of the cases the G-box, CACGTC, A/G rich sequence, flanking sequence as CCACGT were most contributory. There are also sense and anti-sense complementation of sequence matters in promoter efficiency.

18.6 Promoter Homology in Different Species to Find Out Evolution of Crops

In plants, the transcriptional regulation as characterize by environmental factors is facilitated predominantly by molecular genes. Light is the most important signal that through different photobiological reactions can modulate the transcriptional activities (Kami et al. 2010). Phytochrome, the best studied pigments have been precisely governing in flowering, floral bud initiation, after ripening and other floral biology utilize a definite response element as regulator. The most common examples are GATA box, GT-1 sites, G-box, CCAAT box and other basal promoter elements. The over expression of transcription factors is supposed to bind with those elements for utilization of the PHY A regulated genes. Moreover, there are other elements of which sequences individually identified to use in cascades for far-red light responses also. This makes out not only to describe the promoter element for the PHY A regulated genes but also upstream sequence for genes regulated at transcription level by PHY A pathway (Tepperman et al. 2001). Besides classically known an early and late responsive group for their abundance within a stipulated period suggested the involvement of far-red light induction. Regardless of red/far-red light around an upstream sequence of 2 Kb were identified more than 500 of such genes (Chen et al. 2010). There are specific motifs with identified elements as GATA box was more thoroughly characterised. G-box is more recognised in light involving gene regulation. There are some flanking sequences next to core elements was identified as TTATCC/GGATAA, however, fixed for the GATA core elements (Chiang and Marzluf 1994). This is compatible with other sequences of core elements for Rubisco protein. The *RbcS* gene of few plants often made with Box-II sequences as revealed from *Pisum*, *Arabidopsis* and few crops, therefore, the G-sequence may have more variability according to crop species (Brutnell and Langdale 1998). This is also for PHY A repressed genes where more than one trans acting factors found conserved with flanking genes. SORLIPs represents the sequence over represented in light induced promoters are the regulatory sequence of PHY A having the core sequence GCCAC. At both 5′ and 3′ end 1A or G is represented as stable flanking sequence along with SORLIP. Likewise, there are different variation of SORLIPS: SORLIP2 (A-T-AAAACGT), SORLIP-3 (TGTATATAT), SORILP-4 (CTCCTAATT), SORLIP-5 (TTGCATGACT). The consensus sequence for SORLIP-1 is ATTAGTAACC where the most conserved are ATTAGT.

18.7 Conclusion

From the above points on which the discussions were made, it is clearly known that multiple promoter is the basis of genome functioning against environmental fluctuations and its overcome thereby. Plant's genomes with significant variation, particularly, in cellular organelle like mitochondria and chloroplast bear stable number of genes those are arranged either in cluster or dispersed. In mitochondrial genome encoding the respiratory proteins on electron transport chain (ETC) are the common example of those. The *Arabidopsis* would be a model where a frequent presence of multiple promoter are the general features. Two specific RNA polymerases in nucleus are encoded for mitochondrial genes. The cis-element of those genes are mostly conserved and recognised by RNA polymerases. From *A. thaliana* mitochondrial genome of 12 genes and their clusters recorded many transcriptional start sites. Almost 50% of those sequence may possess the multiple promoters. This is the first report from such a dicot plant species. This promoter multiplicity has not any significant differences in plant organs suggesting the non-stringent or relaxed promoter specificity. So, the multiplicity of promoter in such specific organelle may suggest with *A. thaliana* for a comprehensive study of promoter organisation and its distribution. Few other motifs were also found on mitochondrial promoter in other dicot species with their core homologies of up-stream for the coding DNA sequence as good as *A. thaliana*. Therefore, for selection of the plant species to combat environmental stress may be more emphasised on activity of promoter as well as its multiplicity in variations along with molecular markers. There should be also some emphasise on tissue or organ specific promoter multiplicity that may directly or indirectly encodes the stress-responsive genes.

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Chapter 19
Regulatory Role of Transcription Factors in Abiotic Stress Responses in Plants

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Contents

Abstract Unlike animals, plants are sessile and cannot avoid environmental challenges by changing their place. They have to face all these challenges such as drought,

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flooding, salinity, and extreme temperatures. These stresses cause a significant loss in crop productivity and agriculture sustainability. To encounter these abiotic stresses, plants have evolved innate immunity and sophisticated defense system. The expression of stress-responsive genes and the production of stress-related proteins and peptides are key components of their defense system. The expression of such genes is not simple; rather, it involves complex signaling pathways including the regulation of such genes by transcription factors (TFs) which are important players of the regulatory mechanisms enabling plants to tolerate these stresses. There are different families of TFs known so far such as MYB, AVP, MAPK, WRKY, HK, MBF, ERF, etc. In this chapter, we will discuss the role of these transcription factors in regulation of the plant responses to various abiotic stresses. It will be helpful in understanding key regulatory mechanisms of plants' stress responses and their application in enhancing stress tolerance in crop plants for sustainable agriculture.

Keywords Transcription factors · WRKY · MYB · AVP · ERF · MAPK · Abiotic stresses · Stress signaling · Regulation of stress responses · Sustainable agriculture

19.1 Introduction

Plants in the environment face a variety of stresses such as drought, flooding, salinity, extreme temperatures, and malnutrition. These stresses bring significant loss in agriculture productivity all around the world. Sustainable agriculture is very important in meeting the food demand of rapidly increasing population. But these environmental stresses pose a serious threat to agriculture sustainability. There is significant cultivated land area in the world that is affected by salinity, water scarcity, heat waves, and extreme cold. These stresses significantly reduce crop yield and eventually agriculture productivity. The innate immunity of plants initially responds to these stresses and helps the plants to cope with these stresses. Additionally, plants have evolved sophisticated defense system via complex signaling mechanisms involving the regulation of stress-responsive genes and the production of arsenal of stress-related molecules including various proteins and peptides. Transcription factors (TFs) comprise one of the important groups of stress regulatory molecules. The TFs are proteins that bind to specific DNA sequence and control the up- and downregulation of gene expression. There are different families of TFs known so far such as MYB, AVP, MAPK, WRKY, HK, MBF, ERF, etc. The classification of TFs is based upon their structure and binding to DNA motif. The binding of TFs to DNA sequence does not mean that the transcription will proceed further. It also requires further binding of DNA binding accessory elements such as DNA binding proteins, etc. The binding of such type of TFs often activates the transcription in a larger number of processes, but in some cases it halts the transcription, i.e., downregulation. The activation domain is a specific region of DNA binding motif where the TF binds and regulates it. The different types of activation domains have been discovered that are rich in acidic amino acids, proline residues, or glutamine residues. Activation domain may be directly attached to RNA polymerase or indirectly by binding with other proteins to proceed with the transcription. From various studies, it is known that the transcription is mediated by transcription factors that make the basal complex which binds to RNA polymerase. The most famous of these proteins is TATA binding protein (TBP) that is a component of TFIID transcription factor that binds to DNA sequence containing TATA nucleotides in promoter regions.

The regulation of transcription occurs by the binding of cis-element to trans-region of DNA. When the cis- and trans-elements corporate them to hire the transcriptional machinery, then complete productive mechanism occurs. Prokaryotes and eukaryotes have different mechanisms of action for the recruitment of TFs to a particular site of genome. The bacterial genome is small, and TFs tend to identify the extended DNA site easily. In comparison to bacteria, the eukaryotes have large DNA. The TFs' short motifs can repeat in larger genome of eukaryotes. In such genome, TFs may exhibit nonfunctional widespread binding. A process of selective enrichment (Selex) is used to determine the consensus binding sites of TFs in vitro. While the analysis of CRRs series response elements (REs) by ChIP-sequencing method has been followed to do the same in vivo.

Further detail of various families of TFs and their role in regulation of plant responses to abiotic stresses has been discussed in this chapter.

19.2 Transcription Factors (TFs) and Their Families

19.2.1 MYB (Myeloblastosis) TFs

The MYB TFs were first discovered from the avian myeloblastosis virus and named as *v-MYB*. It is the most diverse and largest family of TFs in most eukaryotes (Du et al. 2012). In insects, fungi, and slime molds, these were identified after the avian virus. In plants, the *Zea mays* was the first plant in which the MYB was identified. The role of MYB in *Z. mays* is in anthocyanin biosynthesis (Paz-Ares et al. 1987; Du et al. 2012). In an individual plant species, the 100–250 MYB families have been discovered. The classification of MYB is based on the adjacent repeats. There are seven classes including 1R-MYB and R2R3-MYB. In pearl millet, two classes were identified as major TFs, i.e., 1R-MYB and R2R3-MYB. In *Arabidopsis* plants, there were >100 identified classes of R2R3-MYB TFs involved in plant growth and development. There are two regions of MYB based on the structure: one is N-terminus that is highly conserved DNA binding domain and also called MYB domain, while the other is C-terminus domain (Linger and Price, 2009; Huang et al. 2018).

The MYB domain contains 53 amino acid residues coding a helix-loop-helix. The C-terminus domain is flexible and trans-acting domain which regulates the protein activity (Ding et al. 2009; He et al. 2016b). The expression of the MYB TFs is important in respect to the stress condition such as salinity, drought, heavy metals, flooding, and extreme temperatures. They regulate expression of proteins that ultimately change the plant gene expression that relieves the plant under stressful condition. For example, salt/drought tolerance in *Arabidopsis* is achieved through the downregulation of PP2Cs by *AtMYB20* and *AtMYB15*. The MYB TF*AtMYB96* regulates the balance between the ABA and GA in primary seed dormancy. It also regulates the biosynthesis of wax that helps the plant in drought stress tolerance. In *Arabidopsis*, the stomatal movement is regulated by *AtMYB60*. In addition to this, it also acts as transcriptional repressor and controls the anthocyanin biosynthesis. MYB TFs of rice such as *OsMYB2P-1* and *OsMYB2* are important in respect to various stress conditions (Huang et al. 2018). MBY TFs of wheat such as *TaMYBsm1*, *TaMYB19*, and *TaMYB30-B* play an important role under water deficit condition. Lignin and flavonoid are also regulated by *TaMyb1D* in phenylpropanoid metabolism pathway (Gao et al. 2016a; Wei et al. 2017b). In wheat, *TaMYB30* improves the growth in water deficit condition. Additionally, this gene is also involved in drought tolerance during the germination and seedling growth (Yang et al. 2012; Zhang et al. 2012a).

*19.2.2 AVP (*Arabidopsis *Vacuolar Pyrophosphatase) TFs*

AVP stands for *Arabidopsis* vacuolar H^+ pyrophosphatase (V-H^+-PPase) because it was discovered in the *Arabidopsis*. These TFs are important for sodium exclusion, helping in storing the excess amount of sodium ions in the vacuoles; thus, cells get rid of the harmful effects of sodium ions (Gaxiola et al. 2001). V-H^+-PPase is an electrogenic proton pump. It mediates inorganic pyrophosphate (PPi) hydrolysis. Some conserved motifs have been identified as CS1, CS2, and CS3. For the hydrolysis of substrate, the CS1 motif is important because it carries catalytic sequence DVGADLVGKVE. In the hydrophilic loop, the CS2 motif is located, while the CS3 motif that is exposed to cytosol is found on carboxyl-terminus, and it contains 12 charged residues. It plays a critical role in combination with CS1 and CS2 by catalysis. The V-H^+-PPases contain a single protein having a molecular weight of 75–82 kDa that is intrinsic membrane protein. Types I and II are identified in *Arabidopsis* and are named as *AVP1* and *AVP2*, respectively (Liu et al. 2011).

AVP1 was also identified in rice, tobacco, and cotton (Gao et al. 2006; Zhao et al. 2006; Lv et al. 2008). In tomato plants, leaf water potential, water uptake, and root growth were enhanced due to the expression of AVP1D TF (Park et al. 2005). The AVP1 TF enhanced the tolerance level of transgenic rice to cold stress by increasing cell membrane integrity and proline contents and decreasing the monoaldehydes. Similarly, the overexpression of *AVP* in *Medicago sativa* increased the tolerance to drought and salt stress (Bao et al. 2009). In bent grass (*Agrostis*), the proline contents and biomass production increased under salinity stress by upregulation of *AVP1* (Li et al. 2010).

19.2.3 MAPK (Mitogen-Activated Protein Kinase) TFs

MAPK stands for mitogen-activated protein kinase. It is important protein in respect to signaling in plants, particularly against plant pathogens. It is involved in signal transduction for responses of cell to biotic and abiotic stresses in plants, animals, and yeast. The MAPK cascade is composed of three main protein kinase modules, i.e., MAPKs, MAPK kinases (MAPKKs), and MAPKK kinases (MAPKKKs). These are linked in different ways to downstream and upstream target. When the tyrosine and threonine residues are activated by dual-specificity kinases, MAPKKs are phosphorylated in TXY motif. In plants, these MAPKs are involved in various processes and defensive mechanisms (Zhang et al. 2006).

In tobacco plants, the first MAPK was identified that is involved in signaling pathways of protein kinase (*NtSIPK*) induced by salicylic acid. The activation of protein kinase induced by wounds (*NtWIPK*) is more gradual (Pedley and Martin, 2005). The MAPK and MAPKKK increase the growth and stress tolerance in plants. In drought and cold stress, the accumulation of *MMK4* and *AtMPK3* occurs in alfalfa and *Arabidopsis*, respectively. Under sugar starvation and cold and salinity stress, the regulation of *OsMAPK4* occurs in rice; as a result of this, the MAPK is activated at transcriptional level (Huang et al. 2002).

19.2.4 HK (Histidine Kinase) TFs

HK stands for histidine kinase. The isolation and identification of wheat HK gene led to the discovery and isolation of many HKs from different plants. The product of HK mediates the process of sodium/potassium cation transportation. On the basis of sequence and transportation, HKs have been divided into two classes, i.e., class I and class II. The former mediates more sodium ions, while the latter mediates the Na^+/K^+ cotransport. The *AtHKT1* mutant *Arabidopsis* plants exhibited elevated Na^+ accumulation in leaves under salinity stress which indicated that this HK plays an important role in Na^+ removal from xylem sap. In this way, it protects the plants from the harmful effect of Na^+. In other words, it also regulates the entry of K^+ into xylem vessels by K^+ channels. As a result of this, the ratio of K^+/Na^+ becomes higher (Deinlein et al. 2014).

In *Arabidopsis* genome, eight HKs have been discovered, out of which five are involved in plant hormone regulation. These five HKs include *ERS1*, *AHK2*, *AHK4*, *ETR1*, and *AHK3*. In *Arabidopsis*, *AHK1* is involved in tolerance to osmotic stress, while the mutation in this HK enhanced the sensitivity to osmotic stress. *AHK5* is involved in regulation of stomatal closure in the presence of ROS. HK is also involved in ABA signaling. HK is also produced in response to salinity, drought, and cold stress (Wohlbach et al. 2008; Nishiyama et al. 2013a).

19.2.5 MBF1 (Multiprotein Bridging Factor 1) TFs

MBF1 stands for multiprotein bridging factor 1. It is a highly conserved domain that contains noncoding coactivator. It plays an important role in various physiological and biochemical processes (Liu et al. 2007). *Arabidopsis* was the first in which the MBF1 was identified. It contains three genes *AtMBF1a* and *AtMBF1b* of group I and *AtMBF1c* of group II which encode their relevant proteins. These MBF1 are identified by their amino acid sequences. The group II MBF1 expression gets enhanced under biotic and abiotic stresses. The constitutive expression of *MBF1a* in *Arabidopsis* plants resulted in elevated levels of resistance against fungal disease and salinity tolerance (Tsuda and Yamazaki 2004; Arce et al. 2010).

The upregulation of *MBF1c* enhanced the tolerance to heat stress in soybean (Suzuki et al. 2011). The *CaMBF1* overexpression in *Arabidopsis* plants resulted in reduced tolerance to salinity and cold stress at germination stage (Guo et al. 2004).

19.2.6 ERF (Ethylene Response Factor) TFs

ERF stands for ethylene response factor that is the largest family of TFs in plants and regulates the plant growth and developmental processes. This family of TFs harbors AP2 (APETALA2) domain (Thamilarasan et al. 2014). ERF has highly conserved protein, sequence of which provides GCC box affinity in promoter region of the gene for ethylene response (Klay et al. 2014). The DNA binding domain of AP2-ERF contains 57–70 amino acid residues. It binds to cis-elements at target region in response to expression. Based on sequence similarity and AP2 domain numbers, it is divided into five groups. In AP2 proteins, the two tandem domains are present, while in family RAV B3 domain is present in addition to single AP2 domain. The single domain of AP2 is also present in DREB and ERF. These two ERFs differ from each other by conserved amino acid residues. The ERFs have alanine at position 14 and aspartic acid at position 19, while in DREB there is valine at position 14 and glutamine at position 19 (Kavas et al. 2015). The ERF binds to AGCCGCC, while DREB interacts with core sequence CCGAC (Song et al. 2013).

The AP2 family regulates the development and structure of organs in plants. The subfamily RAV is involved in transduction of plant hormones under abiotic and biotic stresses. ERF and DREB together mediate plant biotic and abiotic stress-responsive signaling (Aukerman and Sakai 2003; Cui et al. 2016). The ERFs have been isolated from many plant species including poplar, wheat, rice, barley, soybean, cucumber, grapes, and *Arabidopsis*. The ERF expression was relatively higher under abiotic stress in *Brassica oleracea*. Similarly, in tomato plants, certain ERFs were upregulated under cold stress and downregulated under salinity stress. The ERFs also regulated salinity stress tolerance in beans (Klay et al. 2014; Thamilarasan et al. 2014).

19.2.7 WRKY (W-, R-, K-, Y-Rich Proteins) TFs

The name "WRKY" comes from protein domains rich in W, R, K, and Y residues. In plants, WRKY TFs comprise a major family. These TFs contain strands of ß sheet (~60 amino acid long) and DNA binding domain (DBD) which contains zinc finger motif (ZFM). There are three groups of WRKY TFs on the basis of DBD and ZFMs. Group I has two DBDs, while the other two groups have only one DBD combined with C2H2 ZFDs. Group I has a, b, c, d, and e subgroups on the basis of variation in amino acid residues (Rushton et al. 2010). Many other domains are also part of WRKY TFs. These include kinase domain, leucine zippers, serine-threonine-rich region, glutamine-rich region, basic nuclear localization domain, TIR-NBS-LRR domain, and proline-rich region (Davletova et al. 2001; Rushton et al. 2010; Chen et al. 2012). The WRKY domain has a patch of seven conserved residues, i.e., WRKYGQK. This hepta-residue domain binds with W-box of the target gene. *OsWRKY13* shows binding with TGCGCTT, a pathogen-responsive element in rice. *HvWRKY46* binds to sugar-responsive elements in barley (Cai et al. 2008). WRKY TFs after binding to W-box activate the process of transcription. WRKY residues may also be replaced by other amino acid sequences such as WVKY, WRRY, WKRY, WKKY, or WSKY (Xie et al. 2005).

WRKY TFs play an important role in inducing tolerance against salinity and drought and other biotic and abiotic stresses (Rushton et al. 2010).

19.3 Plant Abiotic Stresses

The plants being sessile cannot change their place. Therefore, in open environment, they are exposed to various challenges. They have to face two types of stresses, i.e., biotic and abiotic stresses. Abiotic stresses include salinity, water deficit, heavy metals, nutrient deficiency, and extreme temperatures. These stresses, inter-linked with each other or alone, affect plant growth, development, and yield. In response to these stresses, plants try to protect themselves by changing their morphology, anatomy, and physio-biochemical and molecular profile. Due to these stresses, reactive oxygen species are produced in plants which cause metabolic disturbance (Pereira 2016; Zhu 2016).

19.3.1 Drought

Drought is a major abiotic stress in arid and semiarid region. It is a prominent problem of the area with low rainfall and high temperature that increases the evapotranspiration resulting in limited water supply to plants. Under drought stress, the morphological, physiochemical, and molecular changes occur in plants. Among morphological changes, the plant root length and shoot length, number of leaves, leaf area, and stomatal closure are included (Do et al. 2013).

Under drought stress, the rate of photosynthesis gets decreased and premature leaf senescence occurs. The stomata closure occurs under drought stress that reduces the supply of carbon dioxides to plants. The photosynthesis pigments like chlorophyll and its ratio are decreased under drought stress. Drought also decreases the water potential, turgor pressure, and stomatal conductance (Farooq et al. 2009). The production of reactive species (ROS) is a major disadvantage of drought stress. The production of ROS occurs in mitochondria, chloroplast, and peroxisomes. It includes hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxyl radical (OH), and superoxide radical (O_2^-).

It may degrade the proteins and enzymes. In response to production of ROS, the plants produce antioxidants which may be enzymatic or nonenzymatic. Among the enzymatic antioxidants, peroxides, superoxide dismutase (SOD), and catalase (CAT) are important, while non-antioxidants include ascorbic acid, glutathione (GSH), and α-tocopherol (Panda et al. 2013). The molecular events include the expression of different kinds of proteins such as LEA (late embryogenesis abundant), DSP (dentin sialoprotein), RAB, and dehydrin proteins. The drought stress-related genes get positively regulated. The plants under drought stress accumulate low molecular weight proteins/peptides, proline, glycine betaine, sugars, and inorganic minerals such as potassium. In drought stress, the enhanced accumulation of proteins and proline was reported in maize plants (Ashraf 2010; Rashid et al. 2018).

19.3.2 Flooding

Waterlogging is a major abiotic factor that damages many crops, except the rice plants. Floods exert a bad impact on the food supplies and economy of many states. The excess water causes several changes in plant physiology and anatomical structures. Flooding disturbs the gas exchange, soil microflora, and microbiome disturbing the ecosystem. It causes hypoxia and reduction of root and shoot growth. In response to flooding stress, the oxygen level decreased that disturbs the anatomical structure of plant (Bailey-Serres and Colmer 2014).

Under flooding condition, aerenchyma formation is a major adaptation of plant to cope with stress condition. The secondary formation of aerenchyma is absent in woody dicots; that is why these plants are intolerant to flooding condition. The hypocotyl aerenchymatous phellem development increases root aeration in *Melilotus siculus*. An ethylene signaling-related compound 1-methylcyclopropene (1-MCP) plays an important role in aerenchyma formation. The expression of different genes is associated with aerenchyma formation such as cell wall loosening/degradation, calcium signaling, and ROS scavenger genes in maize plants. ROS production and hydrogen peroxide (H_2O_2) promote aerenchyma formation. Some plants of wetland exhibit ventilation of gases in soil faster than diffusion that involves anaerobic decay of organic matter resulting in emission of methane (CH_4). Major proportion of this CH_4 flows through the plants and then escapes to the atmosphere. Loss of O_2 in roots to sediments supports methanotrophy. Balance among methanotrophy, diffusion resistance, and methanogenesis is reflected by net CH_4 efflux (Kozlowski 1997; Jackson and Colmer 2005; Perata et al. 2011).

19.3.3 Salinity

Soil salinity is the most serious abiotic factor that limits crop productivity. The irrigation with saline water is one of the major causes of soil salinity. Worldwide, 45 million ha area of irrigated land is damaged by salt, and 1.5 million ha is declining due to salinity. It affects the seed germination, plant vigor, and crop yield. It affects the plants in various ways such as water stress, nutritional imbalance, ion toxicity, oxidative stress, metabolic disorder, cell division/expansion reduction, and genotoxicity. All these factors combined with each other reduce the crop growth, development, and yield (Hasegawa et al. 2000; Munns and Tester 2008).

Under salinity stress, the plant developmental process, photosynthesis, protein synthesis, and energy and lipid metabolism get disturbed (Parida and Das 2005). Long exposure of salinity stress results in premature senescence of adult leaves, leading to reduction in photosynthetic activity. The excess amount of chloride causes cell swelling and reduction in energy production and interacts with enzymes and reduces their capacity. Ionic stress causes senescence, chlorosis, and necrosis. The high accumulation of sodium ions disturbs the enzyme and protein synthesis (Munns 2002; Cramer et al. 2011).

The ROS such as H_2O_2, OH, and $½O_2$ are produced due to salinity stress. These free radicals damage the membrane and other essential macromolecules. The production of ROS occurs in mitochondria and chloroplast where the photosynthesis and respiration occur; thus, it disturbs the cell metabolism. In response to ROS, plants produce different types of antioxidants that protect them from harmful effect of ROS. These act as scavenger and convert the ROS into useful products (Sairam et al. 2005). The levels of Na^+ and Cl^- ions in tissues of plants growing in saline soil/water are increased that causes disturbance in various metabolic processes. Salt-tolerant plants exhibit Na^+ and Cl^- ion exclusion mechanism (Munns and Tester 2008; Rajendran et al. 2009).

19.3.4 High Temperature

The temperature variation exists in time and space worldwide. The effect of temperature depends on the species and its level of adaptability. The high temperature stress reduces the crop productivity and may cause infertility as reported in cotton (*Gossypium hirsutum* L.), cowpea (*Vigna unguiculata* L. Walp.), pea (*Pisum sativum* L.), peanut (*Arachis hypogaea* L.), and rice (*Oryza sativa* L.) (Angadi et al. 2000). The photosynthesis activity gets decreased under high temperature. It causes injuries in photosynthesis machinery. Under high temperature, metabolic changes occur in plants. The shoot/root elongation, leaf abscission, and leaf/fruit senescence occur under high temperature. It disturbs the cellular signaling, particularly the calcium signaling. High temperature results in degradation of important enzymes. The heat shock proteins (HSPs) are produced under high temperature that protects the plant (Bita and Gerats 2013).

19.3.5 Low Temperature

The cold stress includes both chilling and freezing temperatures, i.e., less than 20°C and 0°C. It results in plant growth reduction and productivity loss. The normal range of temperature varies from species to species. The change in temperature affects the physiological processes depending on the plant optimum temperature. The plants of subtropical and tropical region are affected when they are exposed to low temperature, for example, the rice plants reduce ~30–40% yield under cold stress. Low temperature negatively affects reproductive cycle of plants by floral abscission, pollen tube distortion, pollen sterility, and ovule abortion (Thakur et al. 2010). Under low temperature, ROS are produced. The tolerant plant produces antioxidants that protect them from the harmful effect of the low temperature (Kocsy et al. 2001).

19.3.6 Malnutrition

The world population is increasing rapidly. It is estimated that by the year 2050, world population will be increased to 9.4 billion. In this scenario, a significant pressure is exerted on the increased food production. It is known from previous reports that various biotic stresses result in 28.2%, 37.4%, 31.2%, 40.3%, 26.3%, and 28.8% yield loss of wheat, rice, maize, potatoes, soybeans, and cotton, respectively, while the yield losses caused by abiotic stresses were estimated as 65.8%, 82.1%, 69.3%, and 54.1% of maize, wheat, soybeans, and potatoes, respectively. The plants require 14 essential nutrients for their growth, development, and yield. The nutrient deficiency causes serious diseases in plants; for instance, the phosphorus deficiency results in reduced photosynthesis and respiration (Plaxton 2004). The nitrogen deficiency negatively affects photosynthesis, carbohydrate imbalance in sink and source tissues, and sugar metabolism. The potassium (K) and magnesium (Mg) deficiency in leaves results in sugar accumulation and low root biomass (Hermans et al. 2006). The tolerant plants show different strategies to survive under nutrient deficiency stress.

19.3.7 Heavy Metals

The heavy metals include cadmium (Cd), lead (Pb), nickel (Ni), iron (Fe), cobalt (Co), arsenic (As), zinc (Zn), silver (Ag), and chromium (Cr). These are important pollutants of soil and water (Nagajyoti et al. 2010). These metals get accumulated in cytosol, stroma of chloroplasts, lumen of mitochondria, vacuoles, and other cellular compartments resulting in toxicity. Some of the heavy metals, such as Cu and Zn, are important components of some cellular proteins and are essential for plants for their growth and development, while function of some metals, such as Pb and Cd, is unknown; however, Cd requirement is reported for carbonic anhydrase in marine diatoms (Sharma and Dietz 2006). Heavy metals may result in metabolic/signaling impairment in plants. Tolerant plants can detoxify these metals by various mechanisms. Some plant species accumulate heavy metals in their tissues and act as environment cleaner by phytoremediation.

19.4 Abiotic Stress-Responsive TFs

19.4.1 Drought Responsive TFs

There are many transcription factors reported to play an important role in drought tolerance, for example, wheat Myb1D TFs. The overexpression of this TF in tobacco plants represses some genes involved in metabolism of phenylpropanoid and downregulates the stem lignin and leaf flavonoid accumulation. The transgenic tobacco showed higher chlorophyll and relative water contents, leakage of ions, and reduced malondialdehyde along with hydrogen peroxide contents under drought and oxidative stress (Wei et al. 2017b). *FtMYB10* negatively regulates ABA signaling (Gao et al. 2016b). Its overexpression in *Arabidopsis* plants resulted in decreased expression of some stress-responsive genes such as *RD29B*, *RD22*, *DREB1/CBFs*, and *DRE/CRT* family. In tolerant plants, wax biosynthesis is mediated by *AtMYB96* gene (auxin-ABA cross-talk) under drought stress (Gao et al. 2016b). *MYB15* enhanced the stomata closure and increased the expression of stress-related proteins in *Arabidopsis* under drought stress (Ding et al. 2009). *StMYB1R-1* transgene in potato plants increased the tolerance to drought stress. Some genes were activated in response to drought stress including *RD28*, *AtHB-7*, *ERD1*-like genes, and

ALDH22a. The transgenic plant of these TF factors showed more stomatal closure to drought stress (Shin et al. 2011).

The overexpression of *OsMYB55* in maize plants increased tolerance to drought stress. The transgenic lines of this TFs showed better biomass production and reduced leaf injuries (Casaretto et al. 2016). *TaMYB80* gene enhances drought tolerance in *Arabidopsis* plants (Zhao et al. 2017). *MYB49*-overexpressing tomato plants showed better tolerance toward drought stress by increasing activity of POD, OD, and photosynthesis and by decreasing malondialdehyde (MDA), electrolyte leakage, and the ROS production (Cui et al. 2018). The overexpression of *OsMYB2* gene in rice increased the drought tolerance by increasing the amount of proline, total soluble sugars, and antioxidants and by decreasing the H_2O_2 and MDA accumulation. Furthermore, the transgenic lines of this TF showed higher level of expression of *OsLEA3*, *OsRab16A*, and *OsDREB2A* genes (Yang et al. 2012). The overexpression of *MeMYB1*, *MeMYB2*, *MeMYB4*, and *MeMYB9* of cassava (*Manihot esculenta*) conferred drought tolerance (Li et al. 2017b). There was higher CAT and SOD activity in transgenic plants with reduced H_2O_2, MDA, and ion leakage under drought stress (Wei et al. 2017a; Zhao et al. 2018). MYB60 plays a dual role in *Arabidopsis*, i.e., root growth promotion at early stage of stress by its upregulation and root growth inhibition under severe drought stress by its downregulation (Oh et al. 2011).

The overexpression of *Arabidopsis* vacuolar pyrophosphatase *(AVP1)* gene in tobacco plants enhances the weight and size of shoot and capsules. Its expression enhanced solute accumulation in vacuoles; as a result of this, water retention and turgor of the cell were also enhanced. The transgenic plants showed thick-walled guard cells, fewer vacuoles, and deeper and closed stomata (Arif et al. 2013). *Arabidopsis AVP1* gene confers tolerance against water deficit in sugarcane (Kumar et al. 2014). *AHK1/ATHK1*, *AHK2*, *AHK3*, and *CRE1* were shown to be responsive to water deficit condition in *Arabidopsis* plants (Tran et al. 2007). *OsCDPK7* enhances water deficit tolerance in plants (Knight and Knight 2001). *AtHK1* increased tolerance under drought stress (Wohlbach et al. 2008). *HKs* such as *AHK2, AHK3, and AHK4* and *CRE1* are responsive to drought stress in *Arabidopsis* and regulate ABA signaling (Nishiyama et al. 2013b).

Under drought stress, *MKK1* is activated for phosphorylation of *MPK4*. In water deficit condition, the rice plants gain tolerance by overexpression of DSM1, i.e., a Raf-like *MAPKKK* (Danquah et al. 2014). *MPK2-5*, *MPK2-12*, and *MAPKKK4* are responsive to drought stress in *Arabidopsis* plants. *OsMSRMK2* TF in rice increased the tolerance within 15 min subjected to drought stress. In maize plants, *ZmMPK3* has been identified and showed response to drought. *GhMPK2* and *GhMPK16* are potential activators of transcription factors that increase the tolerance to drought stress (Moustafa et al. 2014). *OsWRKY11* was upregulated in rice under drought stress (Lee et al. 2018). *CiWRKY75–1* and *CiWRKY40–4* overexpression suppressed drought stress and delayed leaf senescence in *Arabidopsis* plants (Wan et al. 2018). The upregulation of *PheWRKY72-2* enhanced drought tolerance by regulating the stomatal closure (Li et al., 2017a). The overexpression of *AtbHLH17* (*AtAIB*) and *AtWRKY28* TFs increased tolerance against drought in *Arabidopsis* plants (Babitha et al. 2013). The overexpression of *ZmWRKY40* in *Arabidopsis* enhanced drought tolerance (Banavath et al. 2018; Wang et al. 2018). TF *GhWRKY17* conferred increased drought tolerance (Yan et al. 2014). The overexpression of wheat TF *WRKY10* in transgenic tobacco increased the tolerance to salinity by increasing the germination rate, survival rate, relative water contents, and root length. In addition to this, the transgenic plants have higher level of proline and soluble sugars and low level of MDA and ROS (Wang et al. 2013). *HaWRKY76* responses to drought and salinity stress (Raineri et al. 2015). The overexpression of *VaWRKY14* increased the tolerance to drought stress in *Arabidopsis* plants (Yang et al. 2017).

19.4.2 Salinity Responsive TFs

The overexpression of *OsMYB91* gene reduces plant growth under normal condition, while it increases proline and RS scavengers under salinity stress. It is also involved in DNA demethylation from promoter region and histone modification in locus (Zhu et al. 2015). The wheat TF *MYB73* exhibited maximum expression with 6 h exposure of plants to 300 mM NaCl. The salt overly sensitive (SOS) transcript accumulation was higher in *AtMYB73* plants when challenged with 300 mM NaCl stress (Kim et al. 2013). *TaMYB73* overexpression in *Arabidopsis* improves salinity tolerance by reducing ionic toxicity. It binds to promoter region of genes responsive to stress such as *AtCBF3* and *AtABF3* (He et al. 2011). The overexpression of *OsMYB2* in rice increased the tolerance to salinity stress by increasing the amount of total soluble sugars, proline, and antioxidants such as CAT, POD, and SOD. The level of expression of *OsLEA3*, *OsRab16A*, and *OsDREB2A* was high in transgenic plant of this TF as compared to wild-type plant (Yang et al. 2012).

TaODORANT1 is R2R3-MYB gene that was isolated from wheat. It is expressed under salinity stress and regulates the lower accumulation of Na^+ in leaves, higher CAT and MDA contents, and lower ion leakage. The overexpression of these TFs regulates several stress-responsive genes (Wei et al. 2017a). The overexpression of *ThMYB13* resulted in lowest accumulation of H_2O_2, O^{2-}, and MDA. It reduces cell death, stabilizes K^+/Na^+ ratio, and lowers electrolyte leakage under salinity stress (Zhang et al. 2018).

IlVP was upregulated by salt stress in *Iris lactea* shoot. The overexpression of *IlVP* enhanced tobacco plant growth under salinity stress. The accumulation of Na^+ and K^+ in stems, roots, and leaves was higher in transgenic plants under salt stress resulting in reduced cell membrane damage and higher leaf relative water content (Meng et al. 2017). Transgenic tomato plants with *Pennisetum glaucum* vacuolar Na^+/H^+ antiporter and *Arabidopsis* H^+-pyrophosphatase showed tolerance to salt stress by ion vacuolar sequestration. In this way, it helps in detoxification of Na^+(Bhaskaran and Savithramma 2011). *AVP1* gene confers tolerance against salinity in sugarcane plants (Kumar et al. 2014).

AHK1/ATHK1, *AHK2*, *AHK3*, and *CRE1* were shown to be salinity stress responsive in *Arabidopsis* (Tran et al. 2007). The overexpression of *AtALDH3* in *Arabidopsis* conferred tolerance against salinity stress (Vinocur and Altman 2005).

AtNHX1 increases tolerance to salt stress. *SERF1* is MAPK enhancer in response to salt stress in rice plants (Deinlein et al. 2014). *HKs* such as *AHK2, AHK3,* and *AHK4* and *CRE1* are responsive to salinity stress in *Arabidopsis* and regulate ABA signaling (Nishiyama et al. 2013b).

The overexpression of *ZmMPK7* in tobacco plants enhanced tolerance against salinity stress and related damages (Danquah et al. 2014).

OsMSRMK2 increases tolerance within 15 min of salinity stress in rice. *MKKK20* is potential activator under salinity tolerance in *Arabidopsis*. This TF also activates *MPK6* and increases the tolerance by osmotic adjustment under salinity stress. MKK4 is induced in cells in seedlings and exhibits osmotic adjustment by activating *MKK3*. *CsNMAPK* induction is reported in response to salinity stress in cucumber roots. Transgenic tobacco seedlings with *CsNMAPK* overexpression showed higher rate of germination (Moustafa et al. 2014).

Transgenic tobacco plants with overexpression of *DgWRKY3* enhanced the salinity tolerance by increasing the proline, POD, SOD, CAT, APX, GSH, and AsA while decreasing the MDA and hydrogen H_2O_2 (Liu et al. 2013). Additionally, it also enhanced expression of genes related to oxidative stress and membrane protection. The overexpression of *GhWRKY41/SpWRKY1* increased the salinity tolerance in tobacco plants by decreasing ROS level and stomatal conductance. *AtWRKY11* and *AtWRKY17* have elevated salinity tolerance in *Arabidopsis* (Ali et al. 2018). *PeWRKY83* overexpression has increased tolerance to salinity by upregulation of certain genes responsive to salt stress such as *AtRD29A*, *AtRD29B*, and *AtABF1* and signaling genes such as *AtABI1* and *AtPP2CA* (Wu et al. 2017).

The expression of *TaWRKY44* tobacco plants enhanced the salinity tolerance by increasing the level of soluble sugars, RWC, SOD, POD, CAT, and proline and lower level of MDA, H_2O_2, and ion leakage (Banavath et al. 2018; Soda et al. 2018; Wang et al. 2015). *SlWRKY3* increased the tolerance level of salinity in transgenic tomato plants by decreasing the sodium accumulation and increasing biomass production (Hichri et al. 2017). *GhWRKY17* increased tolerance of plants to drought stress. It reduces ABA and downregulates its inducible genes such as *DREB, ERD, LEA, NCED*, and *AREB* (Yan et al. 2014). The overexpression of *TaWRKY10* in tobacco increased the tolerance to salinity by increasing the root length, germination rate, RWC, and survival rate (Wang et al. 2013).

JrWRKY2 and *JrWRKY7* are isolated from *Juglans regia*. They form homodimer and interact with each other. This complex enhanced the tolerance to salinity stress by increasing the SOD, POD, GPX, CAT, APX, and MYB proteins and proline and decreasing the MDA and ion leakage (Yang et al. 2017).

19.4.3 Extreme Temperature Responsive TFs

AtMYB15 transcription factor downregulates CBF genes under cold stress (Agarwal et al. 2006). The overexpression of *OsMYB55* in maize increased tolerance to heat stress. The transgenic line of these TFs shows better plant biomass production and reduced leaf injuries (Casaretto et al. 2016). *TaMYB80* enhances the tolerance to heat stress in transgenic *Arabidopsis* (Zhao et al. 2017). The overexpression of *OsMYB2* in rice increased the tolerance to cold stress by increasing the amount of total soluble sugars, proline, and antioxidants such as CAT, POD, and SOD and expression level of *OsLEA3*, *OsRab16A*, and *OsDREB2A* genes while decreasing the accumulation of H_2O_2 and MDA (Yang et al. 2012).

The overexpression of *MeMYB1, MeMYB2, MeMYB4*, and *MeMYB9* of cassava (*Manihot esculenta*) conferred cold tolerance (Li et al. 2017b). *MtVP1* enhances the tolerance to cold stress by increasing anthocyanin synthesis and producing sucrose in potato (Wang et al. 2014).

The expression of *AtPK6, AtPK19, AtMPKK1* (MAPKKK), *AtMPK3* (MAPK), *MMK4*, and *SAMK* gets increased under cold stress in different plants including alfalfa and *Arabidopsis*. The MAPK cascade is activated in *Arabidopsis* plants due to ROS production under cold stress. This cascade contains *AtMPK3, ANP1* (MAPKKK), *AtMPK6*, and nucleoside diphosphate kinase 2. *NPK1* is tobacco homolog of *ANP1*. It plays an important role in signal transduction of auxin and programmed cell death under cold stress. Its overexpression elevates tolerance level of maize plants to freezing stress (Solanke and Sharma 2008).

The heat induces *DREB2A* and *DREB2B* genes in *Arabidopsis*. There is a negative regulatory domain in *DREB2A* gene, deletion of which results in constitutive expression of this gene (DREB2A CA). It enhanced heat stress tolerance when overexpressed in *Arabidopsis* plants (Qu et al. 2013). *OsCDPK7* strengthens plants for extreme temperature and salinity tolerance (Knight and Knight 2001).

HOS1 plays an important role in tolerance to low temperature and vernalization (Zhu 2001). *HKs* such as *AHK2, AHK3,* and *AHK 4* and *CRE1* are responsive to cold stress in *Arabidopsis* and regulate ABA signaling (Nishiyama et al. 2013b).

The first heat shock MAP kinase was identified in alfalfa. *HAMK* acts in heat signaling along with *HSP70* in tobacco plant cells and tomato leaves in calcium-dependent manner. The MAP kinase *MPK6* that is activated by calcium and heat regulates VPE (vacuolar processing enzyme) activity in *Arabidopsis*. *SlMAPK* gets upregulated in tomato under heat stress. *OsMKK6* and *OsMPK3* are produced in rice under chilling stress. *OsMEK1* and *OsMAP1* kinases have a role in signaling under extreme temperatures (Moustafa et al. 2014). The overexpression of *CsWRKY46* enhanced the salinity and extreme temperature stress tolerance by increasing the level of proline and decreasing the amount of MDA and electrolyte leakage (Zhang et al. 2016). *TaWRKY1* is slightly upregulated under high temperature and downregulated under low temperature in *Arabidopsis*. *TaWRKY33* and *WRKY34* TFs enhanced the heat tolerance in *Arabidopsis* (He et al. 2016a; Jiang et al. 2010). *JrWRKY2* and *JrWRKY7* are isolated from *Juglans regia*. They form homodimer and interact with each other. This complex enhanced the tolerance to cold stress by increasing the SOD, POD, GPX, CAT, APX, and MYB proteins and proline and decreasing the MDA and ion leakage (Yang et al. 2017).

19.4.4 Malnutrition Responsive TFs

The zinc is important micronutrient for plants. *bZIP19* and *bZIP23* genes are activated under Zn deficiency (Assunção et al. 2010). *IDEF1* plays an important role under iron deficiency condition, e.g., it increases the expression level of *OsIRO2* gene in rice (Kobayashi et al. 2007). The TF *OsWRKY74* helps in regulation of phosphorus and in coping with iron and nitrogen deficiency (Dai et al. 2015). The TF *OsMYB2P-1* is induced by low or near to depletion of phosphorus in rice plants. The overexpression of this TF upregulates phosphorus-related genes such as *OsIPS1*, UDP-sulfoquinovose synthase, *OsmiR399a*, *OsmiR399j*, and *OsPAP10*, while it downregulates the *OsPHO2* gene in rice plants (Dai et al. 2012).

The TF *OsARF16* is induced in root and shoot under iron-deficient condition (Shen et al. 2015). The iron deficiency stress is suppressed by downregulation of *bHLH104* gene. It is investigated that this TF interacts with other bHLH proteins. The POPEYE (PYE) plays an important role in regulation of iron homeostasis (Zhang et al. 2015). *OsPHR2* is involved in signaling under phosphorus depletion. Its overexpression resulted in root elongation (Zhang et al. 2012b). The overexpression of *ApKUP3* enhances potassium uptake in rice (Song et al. 2014). The overexpression of *ApKUP3* enhanced the root growth and carbon metabolism under low phosphate condition (Li et al. 2011). Under Fe starvation, the *AtNRAMP3* disruption elevates zinc (Zn) and manganese (Mn) accumulation in roots, whereas *AtNRAMP3* overexpression decreases Mn accumulation and downregulates primary Fe uptake transporter IRT1 and the root ferric chelate reductase FRO2 under iron-deficient condition (Thomine et al. 2003).

From above findings, it is clear that transcription factors play a very important role in mediating arsenal of signaling pathways to strengthen plant defense system against a variety of biotic and abiotic stresses.

19.4.5 Heavy Metals Responsive TFs

The overexpression of *TaMYB73* improves growth of *Arabidopsis* under LiCl and KCl stress (He et al. 2011). The overexpression of *RsMYB1* upregulated phytochelatin synthase (PCS) and glutathione S-transferase (GST) genes. It has enhanced the activity of antioxidants (SOD, CAT, and POD) in transgenic petunia plants and increased tolerance to CuSO4, ZnSO4, MnSO4, and $K_2Cr_2O_7$ stress (Ai et al. 2018). *PsnWRKYs* enhances heavy metal stress tolerance (Zhao et al. 2015). *Ta HsfA4a* enhances the tolerance to Cd stress in wheat and rice (Shim et al. 2009). *ART1* is involved in detoxification of aluminum (Yamaji et al. 2009). The overexpression of *ZAT6* significantly enhanced tolerance to Cd stress that was dependent on GSH. *ZAT6* specifically binds to *GSH1* promoter and upregulates *GSH* and *PCS* genes (Chen et al. 2016).

Histidine (*His*) is important for hyperaccumulation of nickel. The overexpression of *NgSAT* confers tolerance to Co, Ni, Cd, and Zn stress in *Noccaea goesingense.* The FER regulatory gene is responsible for uptake of Fe in tomato plants. Its analog FIT confers Fe deficiency tolerance in *Arabidopsis* plants. There are several members of bHLH family such as *AtbHLH38*, *AtbHLH39, AtbHLH100*, and *AtbHLH101* that get upregulated in roots and leaves of *Arabidopsis* under Fe deficiency. *AtbHLH38* or *AtbHLH39* may interact with FIT to form heterodimers for the activation of TFs of ferrous transporters and ferric chelate reductase. These are important genes for Fe uptake regulation under stress condition. IRT1 is one of the most important Fe transporters in *Arabidopsis* that may also transport Mn, Zn, Co, Cd, and Ni.

The AP2/ERF superfamily members ERF1 and ERF5 are induced under Cd stress in *Arabidopsis.* DREB TFs may also have a role in heavy metal stress tolerance in *Arabidopsis*. It is important to acquire deep knowledge of gene expression-related mechanisms so that genetically engineered crops could be designed not only to tolerate heavy metal stress but also for phytoremediation of these contaminants in soil and water (Singh et al. 2016).

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Chapter 20
Molecular Marker Tools for Breeding Program in Crops

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Contents

Abstract Molecular markers play a key role in marker-assisted breeding which is ultimately the new version of conventional breeding. It helps in the development of plants with a new characteristic in a short duration of time which is unknown in the plants. It is also the way for tagging the characteristic present at any location in the whole genome of the species. Various molecular/DNA markers are identified which deals with the sequence changes of the genome. QTL mapping today enhances the knowledge regarding the polygenes controlling important traits of the plants, while association mapping provides the phylogenetic study of the trait. In this regard, molecular marker is greatly used in the study. Here, we summarize the molecular markers for exploring the knowledge about their designs, types, uses, and applications in different aspects of plant breeding. Many markers are described with their procedure involved and new markers, which are extensively used nowadays like dCAPS, CAPS, SNP, etc. Here in this chapter we also cover the protocols to be followed using different types of molecular markers which enhance knowledge about their use.

Keywords Markers · Plant breeding · Marker-assisted selection · Polymorphism

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20.1 Introduction

Molecular breeding is the approach of molecular biology which uses the tools for the breeding program (Stephen and Mumm 2008). Breeding is the method of improving the crop quality and quantity for better use by the process of crossing over the desired plant trait of the same species to the plant recipient lacking the desired trait. When a character is produced by single gene or single allele, it is called a monogenic trait, and, when a characteristic is controlled by two or more genes that are located at different areas on different chromosomes, it is referred to as polygenic traits. Based on this phenomenon, the breeding is vastly analyzed through the mapping of the gene in the whole genome. These traits were analyzed through the visual experience (conventional breeding) or the latest techniques (marker-assisted breeding). Breeding was performed by crossing over (interbreeding) the plants with the desired trait through cross-pollination. Plants are crossed to get the particular genes from a variety to another variety, for example, high-protein soybean is crossed with another soybean species lacking this trait to get hybrid plants. Then, it is backcrossed, again and again, to get the whole genome to recover except high protein content trait. Breeding was performed years ago before by the farmers, but now it becomes a way for the improvement of the resistance power and better performance in the unfavorable condition of the climate. To meet the demand of increasing population of the developing countries, it becomes necessary to adopt such plant breeding approaches which enhance the food production with good quality.

20.1.1 Conventional Breeding

In the past about a thousand years ago, farmers are practicing changing the genetic makeup of the crops they grow. Before the introduction of plant genetics, farmers choose the best plants and seeds by visualizing the appearance of plants and saved those seeds for the next cropping season. Characteristics such as higher yield, pest and disease tolerance, climate-resilient crops, and larger seed size have changed significantly from their wild relatives. For example, the plant breeding paradigm has been found extremely successful on a large scale, such as the expansion of hybrid maize (*Zea mays*), the preface of wheat (*Triticum aestivum*), and rice (*Oryza sativa*) varieties that spawned the Green Revolution (Duvick 2001). In the present generation, the study of breeding has become easier due to the introduction of the techniques and the marker-based breeding methods (Agrawal 1998). To analyze the traits and not just by phenological understanding as in conventional breeding, marker-assisted breeding is important. Marker-assisted breeding is developed for studying phenotypic and genotypic interaction. Mapping of the traits through marker-assisted breeding falls into two categories: first is association mapping and second the QTL mapping. It includes mutation breeding and hybrid seed technology.

20.1.2 Marker-Assisted Breeding

Similar to conventional breeding, marker assisted breeding also involves crossing and development of new varieties by involving transformation of the particular trait from a donor plant to the recipient plant using molecular markers. DNA marker allele linked to the desired trait should be identified for true plant conformation. It does not involves the backcross up to F8 generations or the complete genome recovery except the desired trait. It uses the molecular markers for the recovery percentage to check and gets maximum recovery as early as possible. Through marker-assisted breeding, complete recovery selection is possible to get within three to four backcross generations. Marker-assisted breeding is also useful in association mapping and QTL mapping. When the number of plant representing for the desired study in particular population increases exponentially and the QTL's number increases, the effectiveness of marker-assisted selection decreases (Moreau et al. 1998). According to Ribaut and Betran (1999), basically three QTLs are considered in any marker-assisted breeding to have a feasible result. However, in many research like in tomato, five QTLs are also studied (Lecomte et al. 2004). QTL analyses become possible after the development of SNP marker selection at that time (Kumpatla et al. 2012).

20.1.2.1 Association Mapping

Extensive genome-wide association study (GWAS) utilizes large compact numbers of markers in a population of distinct genotypes that involve a huge number of crossing-over events from the last widespread progenitor, helps in high resolution as compared to the QTL mapping, and helps to forecast or recognize underlying genes (Zhang et al. 2015). It is the phylogenetic study of the trait responsible in various organisms and their association. Phenotypic difference arose due to the genetic variants analyzed through association mapping which provides precise linked alleles in common progenitor from the other species and also assists in trait-linked polymorphism detection in different species from each other. With the help of high-throughput marker like single nucleotide polymorphisms (SNPs), functional variants are identified (Hoeschele 2004). It deals with the non-random association of loci in linkage disequilibrium (association mapping) as compared to the QTL mapping (Ibrokhim and Abdukarimov 2008). It utilizes population based on our study from the germplasm sources or expected populations, whereas, in QTLs, two parents crossed are involved. Association mapping involves the subsequent steps. The first step involves a wide coverage of the genetic diversity sample from the germplasm sources or expected populations from natural collections. The second step involves the analyses or measuring the visualizing characteristics (phenotypic) like crop yield, crop quality, crop tolerance, or crop resistance to particular disease of preferred population in diverse environmental conditions and many replications/experiments. The third step uses molecular marker genotype for mapping population individuals. The fourth step uses the Quantification for the coverage of the LD in the population whole genome based on the molecular marker genotype data. The fifth step seeks evaluation level of genetic differentiation through the structure of the population between the groups in the individuals of the sampled population of the study and coefficient of relatedness (kinship) among the individuals in pairs contained by a sample. Sixth steps based on th results of the fifth step like the structure of population, kinship, relation between phenotypic and genotypic data to reveals the associated marker position nearness to the desired trait study with the progenitor for the phylogenetic study associated with each other (Kraakman et al. 2004, 2006; Hansen et al. 2001; Nordborg et al. 2002, 2005).

20.1.2.2 QTL Mapping

Quantitative trait locus (QTL) is a locus in the whole genomic sequence which relates with the variation in the DNA sequence of a quantitative trait in the phenotype of a population of the species (Miles and Wayne 2008). The characters which are governed by many genes are the study of QTLs. For example in soybean various traits are mapped through QTL mapping (Kumawat et al. 2016). Earlier it was thought that QTL mapping involves the usage of the two parents mapping populations with lower compact marker exposure, but it gave poor resolution in quantitative trait locus identification (Sonah et al. 2013). QTLs study starts with the identification of the trait which is validated through transient expression etc. whereas, other is candidate gene based on the homology with the crops. Several QTLs have been reported and very well characterized for the essential genes, their loci, and also related haplotypes. Legume crops are relatively dissimilar in comparison to the other plant group species in various characteristics like efficiency in nitrogen fixation, high-protein-rich-associated physiology, and production of various secondary metabolites. Also the history shows comparative genomics, and the approaches were not much helpful in genetic study (Koebner and Varshney 2006; Varshney et al. 2015). Soybean being a legume crop is the best model crop for the study for quantitative legume crop characteristics due to close relatedness to the genetic makeup of the legume crop. In different leguminous crops other than the soybean, in silico-based homology study should directly help in gene identification without the use of the QTL mapping revealing gene sequence completely. Molecular marker development for particular characteristics is helpful for the identification of the genes in other legume crops directly (Kang et al. 2015). These are performed in the population type like backcross, double haploid, near isogenic lines, and recombinant inbred lines which all are derived from the crossing of two different parents with donor and recipient parent for the desired trait. Also, a large segregating population is required for QTL study to be performed, and polymorphic markers between the two parents of the segregating population are also to be identified for the QTLs (Collard et al. 2005; Liu 1998; Wu et al. 2007).

20.2 Markers Used for the Breeding Program

Genetic variations in a genome are responsible for identification of the traits linked to a particular characteristic and polymorphism between two species. Characteristics which are transferred through plant breeding from one species to another are traced through the linked markers present in that region. Analysis of the trait/characteristics transferred in plant breeding is the basis of development of the markers. Markers are of different types like molecular markers, morphological markers, cytological markers, and biochemical markers. On the basis of base pairs, changes in the sequence of two dissimilar individuals with comparative nucleotide in that particular site of the DNA sequence reveal the molecular markers. Polymorphism arises due to different sequences in the same site of the two individuals in the base pair study. These changes in nucleotide sequences occur through the various mutations, insertions, deletions, duplications, and translocations. Molecular markers have the advantage of distinguishing polymorphisms which not produce phenotypic variation, highly reproducible and can be applicable to any part of the genome (Mondini et al. 2009). Morphological markers on the other hand can be phenotypically characterize through qualities like plant height, node number, branches, shape of the leaf, colour of the flower, and many more other vital agronomic characters. But morphological markers have been not much effective as compared to other markers due to the influence of environmental changes. These markers are not reliable (Eagles et al. 2001). Cytological markers based on the staining pattern reveal the difference in various changes in chromosome position, chromosome size (short and long arm), order, and shape (Jiang 2013). These variations help in understanding euchromatin and heterochromatin distribution in the chromosomes. Patterns are produced by staining with different stains like Giemsa stain for the production of G bands and also quinacrine hydrochloride for the staining of Q bands in the chromosome study. This helps in the understanding of mutated and normal chromosomes. Biochemical markers are biochemical proteins coded by a gene which influences the function of the trait. This difference in the function encoded by the genes is revealed by the markers to distinguish the individuals from each other (Bayley 1983). These differences in function occur due to the variation in the DNA sequence encoding the gene by the mutation like deletion, duplication, inversion, frame shift, etc. There are certain disadvantages related to using biochemical markers such as environmental effects, less in number and change with plant development stages (Mondini et al. 2009).

20.2.1 Different Types of Molecular Markers

Various markers are known based on their techniques required and application needs. Molecular markers include restriction fragment length polymorphism (RFLP), variable number tandem repeats (VNTR), simple sequence repeat (SSR), derived cleaved amplified polymorphic sequence (dCAPS), cleaved amplified polymorphic sequence (CAPS), random amplification of polymorphic DNA (RAPD), fragment length polymorphism (FLP), single nucleotide polymorphism (SNP) and amplified fragment length polymorphism (AFLP). These markers are advantageous as compared to conventional breeding which is based on the visualization and choice of the particular trait. Markers provide great evidence to support the trait linked to the marker and assessment of the characteristic. The marker should possess certain charters that make the marker good for assessment like it should be polymorphic between two individuals. Here, Fig. 20.1 shows the parental polymorphism between five different individuals plant sample where the same size of bands in each sample indicates the monomorphism and same genomic sequence at that particular place. Whereas, different band sizes show polymorphism between the five individuals and vary genomic sequence in that region.

Experimentally some scientist thought RAPD marker as best due to low cost and reliable result, for example, RAPD marker analysis on 269 landraces of common beans shows the result of grouping is same as earlier based on morphological and ecological (Beebe et al. 2000). Also, genetic diversity of soybean cultivars is detected through RAPD which originated from the USA and China. Similarly, through RAPB markers, the population of maize has been precisely resolute (Zenglu and Nelson 2002; Popia et al. 2000). But the clusters obtained from the morphological and agronomic data in the 42,000 germplasm accessions of sorghum, which were compared with RAPD markers, were found not to be even close (Dahlberg et al. 2002). This reveals that only one marker in RAPD is not suitable for the analysis of genetic diversity. Contrary to this, wild rice species is also successfully characterized through RAPD markers (Farooq et al. 1995). All of these results indicate RAPD marker is conditional and not that much reliable. So other markers were also used for breeding purposes. AFLP markers were used for the study of 14 putative Bermuda grass strains from those cultivated presently in the USA (Anderson et al. 2001). Similarly, 105 bread wheat varieties were characterized with SSR and AFLP markers; SSR marker provides discrimination of all 105 cultivars. AFLP markers help in identifying genetic diversity of wheat in Argentina; also, AFLP and SSR markers are used for genetic diversity in the modern and earlier released wheat cultivars, but no significant results were found. However, by fingerprinting of newly

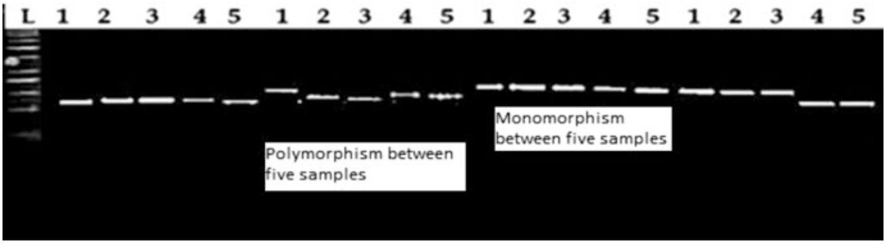

Fig. 20.1 The same band size indicates monomorphic, and different bands' size indicates polymorphic between the parents

developed variety of narrow genetic base is easily identified by SSR and AFLP markers. This shows SSR and AFLP markers are a very reliable for use. RFLP is mostly used for germplasm studies to provide accurate information on genetic diversity (Siedler et al. 1994; Graner et al. 1994). It is predominantly known in important cases where germplasm sources are to be utilized in closely associated pedigree analysis. There are many reasons for the inefficiency in the results found with different markers utilized. One such reason is that RFLPs are dependable markers to be used mainly for the function as exemplified in various crops like in the wheat species (Sasanuma et al. 1996), species of sugarcane crop (Oropeza and Eva 1996), the rice plants (Zheng et al. 1994), species of alfalfa plant crop (Pupilli et al. 2000) and also many more other plant crops, but they are not suitable for inter-specific comparison (Castagna et al. 1997).

20.2.1.1 RFLP

It was first the known marker based on hybridization. In 1975 DNA sequence polymorphism is identified using this marker. RFLP is based on the principle of restriction digestion sites present on the DNA sequences (Fig. 20.2). Due to mutations like deletion, duplication, insertion, the sequence of the DNA changes which ultimately alters the recognition sites present in the unmutated sequence. It causes restriction digestion pattern, and the mutated allele is easily identified through this. RFLP is a co-dominant marker; it shows the presence or absence of the mutation and also indicates the state of allele, i.e., homozygote or heterozygote. It forms two discriminate bands of digestions where mutations in one chromosome from other chromosomes confirm heterozygosity. For most of the plant species the linked trait can be directly understood through RFLP marker (Restriction fragment length polymorphism) and their maps have been constructed for the study (Tanksley et al. 1989). Co-dominant, high reproducibility, medium polymorphic, high DNA require, genomic abundance high, cost also high, sequencing required, no PCR requirement, visualization by radioactivity or chemiluminescent, 10,000 ng DNA. RFLP is beneficial for detecting the larger size of the sample, whereas RAPD is more efficient in smaller sample size (Ragot and Hoisington 1993). Highly abundant in the whole genome, 2–10 μg DNA required for complete restriction. It required high laborious, high technical demand needs.

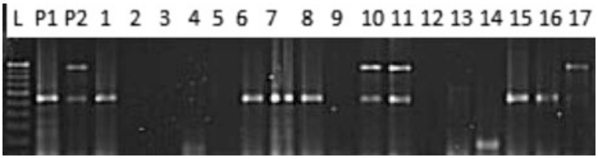

Fig. 20.2 RFLP markers (Kumawat et al. 2016)

Standardized Protocol for RFLP

- Isolation of genomic DNA from the samples
- Restriction and digestion of the genomic DNA samples with the restriction enzymes which is basically isolated from bacterial source
- Separation of a large number of bands on agarose or polyacrylamide gel electrophoresis
- Transferring of isolated bands from the gel to a nitrocellulose or nylon membrane
- Nucleic acid hybridization with the labeled probe
- Autoradiography for the detection of the bands

20.2.1.2 VNTR

VNTR marker was discovered by Jeffreys et al. (1985). It is present in the genome which has short tandem repeats present in clusters and oriented in the same direction. These repeat clusters show variation in length between individuals which is the base of this molecular marker. These variants are responsible for the identification of the allele present from individuals. VNTR markers are dominant showing the presence or absence of the allele. VNTR is analyse by restriction digestion of the repeats blocks and analysis in the gel. VNTRs are generally of two types: microsatellites and minisatellites. Formerly it is a known sequence with less than 5 bp in length but later known as having longer blocks. Nowadays, short tandem repeat (STR) and simple sequence repeat (SSR) terms are used which are more explanatory. VNTRs of 4 bp repeats are mostly analyzed whereas, very short repeats bp blocks are unstable like 2 bp repeats which may vary even from one tissue to another within the same individual. Repeats with 3 bp are found to vary from one generation to another, for example, in Huntington's disease.

Standardized Protocol

- Restriction digestion of repetitive sequence with the restriction enzyme
- Gel electrophoresis for the analysis of fragmented bands

20.2.1.3 SSR

The SSR markers are also co-dominant markers. They are PCR-based markers. These markers are satellite which is found in the whole genome. They are repeats of dinucleotide and trinucleotides. SSR markers are highly polymorphic, reproducible, and easy to detect. But the cost for the detection is comparatively high due to the designing of lots of primers to complete the analysis of the genome or the particular region. It is widely used in every area of molecular breeding and genetics. SSRs are repeats of 1–6 nucleotides. So, it may have one nucleotide repeat, two nucleotide repeats, three nucleotide repeats, and many more (Kalia et al. 2011). SSR markers are highly reproducible and highly polymorphic, and low DNA, medium level of genome abundance, and high cost are required. Their reliability is high, and they have low to medium technical demands.

Standardized Protocol

- Isolation of genomic DNA from samples whose genotypes are to be checked
- Amplification of the sample in PCR using SSR primers with the thermal profile
- Separation of PCR products on MetaPhor agarose gel electrophoresis

20.2.1.4 dCAPS

It is a co-dominant marker. It is based on PCR and restriction digestion. Both mutated and unmutated samples are detected by analysis of dominant and recessive alleles (Fig. 20.3). The recessive allele can be detected using restriction of the amplified product using a restriction enzyme. This mutant allele can be identified using a derived cleaved amplified polymorphic (dCAPS) markers having restriction site for a restriction enzyme. Dominant alleles (unmutated) identify with a single fragment of larger bp, whereas genotypes with recessive allele e2 will produce two fragments of smaller bp.

Protocol

- Isolate genomic DNA from parental genotypes and progenies.
- PCR amplify the target gene fragment using dCAPS primers followed by PCR amplification.
- Check the amplification using agarose gel electrophoresis with a single-band product.
- Clean the PCR product and quantify.
- Perform restriction digestion and analyze for two separate bands (Watanabe et al. 2011).

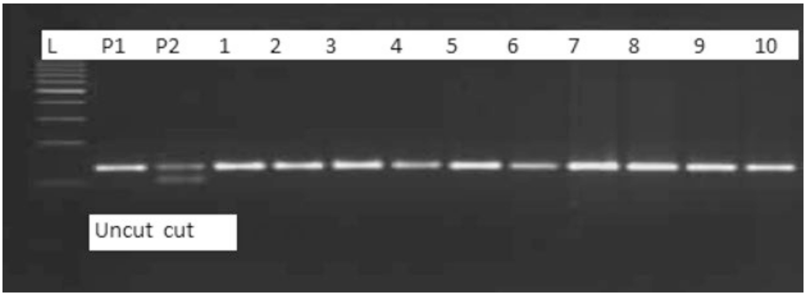

Fig. 20.3 dCAPS markers sowing cut and uncut for E2 gene (Kumawat et al. 2016)

20.2.1.5 CAPS

CAPS marker is basically used for the identification of the recessive allele (Fig. 20.4). PCR amplification of target gene segment amplifies 125 bp DNA fragment which possess restriction site for Pdh1 enzyme. Restriction digestion of PCR amplified DNA results in two fragments of 76 and 49 bp in genotypes with recessive Pdh1. According to studies (Spaniolas et al. 2006; Weiland and Yu 2003; Funatsuki et al. 2014), this marker is co-dominant to discriminate the homozygous and heterozygous alleles and is widely used in genotyping of the population. Also in map-based cloning, it is generally used.

- Isolate DNA samples and amplify the DNA samples.
- Quantify the PCR amplified clean DNA and calculate the concentration of DNA.
- Set up restriction enzyme reaction using the following reaction components.
- After completion of incubation, separate DNA fragments on 3% high-resolution agarose gel.

Take gel photograph. Genotypes with Pdh1 will produce a single fragment of 125 bp, whereas genotypes with recessive gene pdh1 will produce two fragments of 76 and 49 bp fragments.

20.2.1.6 RAPD

It is basically a PCR-based marker. RAPD dominant markers do not discriminate between homozygote and heterozygote. It also does not require prior information about the genomic sequence. Several arbitary primers are required (size basically 8–12 Nucleotide is required) for binding randomly in the genomic sequence of the parental genotypes and progenies. Primer binding depends on the probability of the complementary nt of the genome. Advantages of using RAPDs were able to consecutively detect many polymorphic loci within the whole genome in just one single run (Williams et al. 1990). Dominant, highly reproducibility, highly polymorphic, DNA medium required, genomic abundance is very high, cost less, sequencing information not required just randomly primer bind and amplification occurs, PCR required, visualization on agarose, DNA only 20 ng. It is not a reliable marker but needs low technical and cost demands.

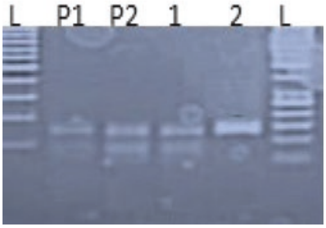

Fig. 20.4 CAPS markers sowing cut and uncut between P1 (uncut), P2 (cut), 1 (same as P2), and 2 (same as P1)

The procedure of the techniques involves the following steps:

- Isolate genomic DNA from parental genotypes and progenies.
- Perform PCR amplification using the short random primers having Nt 8–12 bp.
- Load PCR product on agarose gel electrophoresis and analyze it.

20.2.1.7 FLP

Fragment length polymorphism (FLP) markers have been developed from these large insertion-deletion sites which can be used for the molecular breeding program. Various studies have been done by Watanabe et al. (2009), Liu et al. (2008), and Xu et al. (2013).

Steps involved are the following:

- Isolate genomic DNA from samples.
- PCR amplify the target gene fragment using FLP primers using the thermal profile given below.
- Take out amplified PCR product and separate on agarose.
- Genotypes with the dominant condition will produce a single fragment of 1339 bp (for E3 gene in soybean), whereas genotypes with recessive e3 will produce fragments of either smaller 558 or 275 bp in size (Fig. 20.5).

20.2.1.8 SNP

Sequence-based markers (SNPs) has a single bp change in two individuals in the same region which creates an SNP. It may or may not be changing the recognition sites for restriction enzyme so it involves the sequencing of the region to discriminate the two individuals. Primer designing is based on the single bp change. A single bp change in the sequence of the genome creates SNP. SNPs may be purine: purine changes, i.e., transitions (T/C or A/G), or purine-pyrimidine changes known as transversions (G/C, C/G, A/C, T/A, C/A or G/T). SNPs include a single base pair change, i.e., insertion/deletions (InDel) change the coded mRNA sequence. SNP is the most abundant marker present in the whole genome due to the single base pair change. Frequency of the SNP marker in plants (Xu 2010) ranges between 1 SNP in each 100–300 bp of the sequence. SNP markers are gaining focus in new

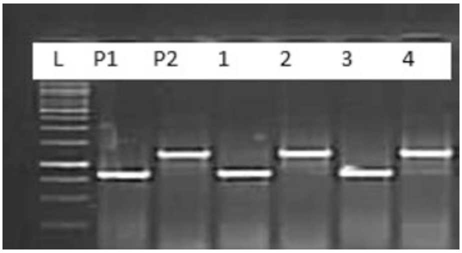

Fig. 20.5 RFLP markers showing E3 gene (Kumawat et al. 2016)

advancement of molecular markers. Co-dominant, highly reproducibility, highly polymorphic, DNA low required around 50 ng, genomic abundance very high, cost variable, sequencing yes, PCR required. Visualization through SNP-VISTA (Mammadov et al. 2012; Ghosh et al. 2002). According to Bhattramakki et al. (2002) and Jones et al. (2007), SNP markers give the highest map resolution as compared to other molecular markers. Wright et al. (2005) discovered the SNP markers experimentally by resequencing UniGene-derived amplicons using Sanger's method. Batley et al. 2003 analysed in-silico SNP discovery through the mining of SNP. EST databases followed by PCR-based validation.

20.2.1.9 AFLP

It involves both restriction and PCR method and produces reproducible results. This method was developed in the early 1990s by Kevgene (1990). Dominant, intermediate reproducibility,very high polymorphic, high DNA required around 500–1000 ng. High cost, no sequencing required, PCR-based, high genomic abundance, visualization on agarose gel needed. AFLP technique described by Zabeau and Vos (1993) is a PCR-based tool used in the DNA fingerprinting. AFLP is a very sensitive method helpful in identification of polymorphism in DNA sequence. Around 500 ng DNA is required, but it is a highly reliable marker with medium cost and technical demands.

Procedure involves the following:
- Genomic DNA is digested with the restriction enzymes.
- Then, adaptor sequence is ligated in half-site restriction.
- Two round PCR amplifications are done with primer extension of 1 bp and then 2 bp extension with primers that have adaptor and specific restriction site sequences.
- Samples are resolved through electrophoresis.

20.3 Applications Based on Molecular Markers

Various molecular markers are helpful for the inheritance analysis of any trait from one generation to the next generation, for the phylogenetic study of any organism. It is also helpful in finding the relationship between the organisms with each other. Genotyping, paternity testing, forensic science, and hereditary disorders can be easily identified using various types of markers. In Agricultural field for the detection of the genes responsible for disease resistance or disease tolerance can be also identified. It is also useful in Genetic mapping, recombination rate study in each generations. These markers are also applicable in micro-organism identifications. Amplified fragment length polymorphic marker, as name indicated, helped simultaneously to identify different polymorphisms within the genome of various species where genomic makeup is unknown of bacteria, fungi, and plants. AFLP technique is one of the tremendously beneficial in the study of taxa and genetic variation in closely associated species of crops, fungi, animals, and bacteria also. It is also a very sensitive and highly reproducible technique for the analysis of the QTLs and linkage map construction. For the construction of linkage map, molecular marker is the best suited tool. Molecular markers are helpful for the analysis of the somaclonal variation, Disease Management and Prediction of Genetic Distance from the particular trait. It is generally the basic tool for the molecular breeding program (Table 20.1).

20.4 Conclusion and Future Perspectives

Our understanding regarding the molecular markers facilitated the complex trait associated with the linked marker. The study of the markers is important in the breeding programme because it is a reliable indicator of particular trait which ultimately associated with beneficial and important for plant development and growth. We have summarized the breeding programs and different types of markers used in plant breeding which helps in better growth of plant species. By using approach of molecular marker, many QTLs are identified. Also vast genomic resources and molecular markers used for the identification of important trait. Marker-assisted breeding gives the opportunity to study and bring changes in the previously untouched areas of plant sciences and also brings together the genetic basis of a broad range of agriculture plant processes. These tools are indication for proper utilization of the plant crop and different species of plants in a beneficial way in the future. Presently, in different labs of the world, studies on these tools are going on. Sequencing tools with low-cost techniques in the future will surely help in the identification of new genes in a short duration of time in many crop species with the high effective way.

Table 20.1 Different molecular markers indicate the important features different from each other

Marker name	Based on	Polymorphic	Sequence information: required or not?	DNA quantity
RFLP	Restriction and hybridization	Co-dominant	Yes	High
VNTR	Restriction	Dominant	Yes	Medium
SSR	PCR	Co-dominant	Yes	Low
dCAPS	Restriction and PCR	Co-dominant	Yes	Low
CAPS	Restriction and PCR	Dominant	Yes	Low
RAPD	PCR	Dominant	No	Medium
FLP	Restriction and PCR	Dominant	Yes	Low
SNP	PCR	Co-dominant	Yes	Low
AFLP	Restriction and PCR	dominant	No	Low

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Chapter 21
Plant-Microbe Interactions in Developing Environmental Stress Resistance in Plants

Palmiro Poltronieri, Ida Barbara Reca, Stefania De Domenico, and Angelo Santino

Contents

Abstract The chapter introduces and discusses general mechanisms of abiotic stress resistance and describes plant-specific mechanisms of sensing and response to fungi. It is not well understood how plants differentiate between pathogens and beneficial bacteria and fungi, such as rhizobia, beneficial bacteria, and fungi that work as biocontrol agents and biostimulants. Plants recognize and interact with the beneficial microbiota, through attenuation of pattern-triggered immunity. Plant secondary metabolites and compounds in root exudates (sugars, metabolites, and proteins) are produced to recruit plant defense-assisting rhizosphere microbes, contributing to defend them from their pathogens. Soil-borne microorganisms, fungi, and bacteria in the rhizosphere play an important role in control of root and foliar pathogens, through induction of systemic resistance in plants. The transfer of specific strains to varieties sensitive to soil-borne bacterium *Ralstonia solanacearum* was shown effective in protecting tomato from the infection. Rhizobacteria and endophytes are able to produce plant hormones and to modulate hormone synthesis and hormone crosstalk in the relief from abiotic stress. Finally, the chapter focuses on Medicago and interplay between production of JA and other oxylipins, in roots

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and leaves, their accumulation, and potentiation under salt stress; lastly a review on the results on gene networks and gene hub regulation transcription, through systems biology and transcriptome studies, on plants under combined abiotic stresses and combined biotic and abiotic stress, is provided. It is envisaged that microorganisms can provide a useful system, alone or in combination with biochar, soil amendment, and nutrient availability, to approach the increasing challenge of abiotic stresses in agriculture, for a next generation of resilient plant crops.

Keywords Drought · Salinity · Heat stress · Hormones · Signaling · Transcription factors · Microbiome · Rhizobacteria · Endophytes · Growth-promoting compounds

21.1 Introduction

In response to water stress, plants retain tissue hydration through changes in organ compartmentalization. Roots tend to sustain their water uptake capacity (as root hydraulic conductivity), made possible through the root xylem potential, −0.6 MPa, to trunk xylem potential, −0.8 MPa, up to the leaf potential, −1.0 MPa, providing the gradient that compels the water to flow to the leaves. The closure of stomata and a reduction of leaf area and size are adaptation measures by which plants reduce water loss by means of osmotic adjustments.

A deep root system protects plant growth and increases plant yield during drought conditions, in particular in varieties with a positive harvest index (high seed number and greater seed size). In case of water stress, plants increase water use efficiency by several means, i.e., through avoidance of dehydration and dehydration tolerance. A reduction in duration of growth (by means of early flowering) is a mechanism that allows the plant to use less water and under certain circumstances may avoid the terminal stress. An early flowering may lead to lower production rates and smaller seeds. Soil NaCl concentrations cause a reduction in water potential, which in turn leads to hyperosmotic and oxidative stress. The accumulation of excess NaCl in the apoplast produces an imbalance in nutrients and in solutes.

During drought and salt stress, plant response includes the synthesis and the accumulation of osmolytes, among which are sugars (trehalose, sorbitol, saccharopine, mannitol, fructans, raffinose, and galactinol), sugar-based alcohols (polyols), amino acids, agmatine, quaternary ammonium salts such as choline, and glycine betaine (Poltronieri et al. 2013). Polyamines (spermidine, putrescine) are regulated in various plant responses, such as during wounding. The plant response to drought includes H_2O_2 production (Hasanuzzaman et al. 2013, 2019). Ureides such as allantoin and allantoate, intermediate products of purine catabolism, activate jasmonic acid (JA) signaling and abscisic acid (ABA) synthesis, through upregulation of expression of stress-induced genes and enhancement of tolerance to abiotic stress in seedlings (Takagi et al. 2016). JA-Ile, the active, conjugated form of JA, induces DELLA RGA-LIKE3 (RGL3) expression through MYC2, by binding to coronatine-insensitive 1 (COI1). Subsequently, RGL3 contributes to enhancing jasmonate (JA)-mediated signals.

The group of genes permissive to abiotic stress includes aquaporins and major intrinsic proteins (MIPs), a family of transporters present in plasma membrane (PIP) and tonoplast (TIP), NOD26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and unclassified X intrinsic proteins (XIPs), that regulate the fluxes of water and other solutes (Bárzana et al. 2014; Quiroga et al. 2017; Rios et al. 2017). Arbuscular mycorrhiza (AM) symbiosis and salicylic acid (SA) improve hydraulic properties of roots, with authors focused on the alteration and amelioration of transfer of water by radial roots and on the role of aquaporins (Quiroga et al. 2018). Water transport in roots is realized through different means: apoplastic transport (through the cell wall continuum) and through cell-to-cell pathways, symplastic (via plasmodesmata) and transcellular (across the cell membranes). During water stress, transpiration is reduced, and water is transported basically by the cell-to-cell pathway through the osmotic gradient between soil and xylem sap. The contribution of these pathways to water uptake and hydraulic conductivity may vary. AM plants increase considerably water circulation in roots through the apoplastic pathway in respect to non-AM plants (Quiroga et al. 2018). Nitric oxide (NO) sustains apoplastic water pathway in roots, with differences in root hydraulic conductivity in AM plants in respect to non-AM plants, possibly mediated by differences in NO content.

Various proteins and enzymes have been identified with a role in drought stress. Among other proteins are the proton antiporter TNHX1, a proton pyrophosphatase TVP1 (Brini et al. 2007), conferring tolerance to salt and drought stresses in *Arabidopsis*, and a series of transcription factors (DREB, NAC, WRKY, AP/ERF, bHLH, bZIP) under the control of germin-like protein (GPL) binding sites in their promoters (Das et al. 2019). The identification of hub genes, regulating these TFs and activating plant adaptation to abiotic stress, would be of great importance for applicative uses.

Dehydrins, being part of the late embryogenesis abundant (LEA/DHN) protein group, and ranging from 17 kDa and 30 kDa, up to 40 kDa in size, also referred as early response to dehydration (ERD), have a role as intracellular chaperones for various proteins. One durum wheat LEA/DHN, named DHN-5, was identified in plants showing resistance to drought and salt stress. *DHN-5* expression in durum wheat transgenic lines decreases the total activity of aspartyl proteases, especially under salt stress conditions (Saibi et al. 2016). LEA/DHN proteins are chaperones supporting the folding of proteins and DNA/RNA under harsh conditions (Kovacs et al. 2008; Hara et al. 2009). Increased expression of DHN-5 protects cell integrity during desiccation and at late embryogenesis stages (Brini et al. 2007). DHN/LEAs do not show a particular three-dimensional structure and maintain a disordered character remarkably stable in macromolecular crowding conditions, such as during dehydration, when globular proteins lose their folded states and tend to collapse (Mouillon et al. 2008). Various LEA/DHN proteins are reported to be activated by abscisic acid (ABA) (Jiménez et al. 2008).

ABA is a plant hormone with roles in senescence, seed dormancy, drought tolerance, and response to stress. ABA is synthesized in the xylem and in leaves and flows in the root cortex across apoplastic barriers. ABA regulates stomatal closure and shoot meristems.

Abscisic acid glucose ester (ABA-GE) is a long-distance stress signal. ABA-GE can be stored in microsomes, where ABA is released by the action of ß-glucosidases, either in the apoplast or in the cytosol. ABA can be produced from root exudates and from ABA-synthesizing microorganisms in soil. ABA-GE occurs in the soil, even at high concentrations. The barrier of Casparian bands (exodermis and endodermis) avoids the ABA-GE entrance. When exodermis is absent (as in Fabaceae and in hydroponic cultures), ABA-GE can enter into the root cortex apoplast from the outside. Apoplastic ß-glucosidases cleave the ester bond releasing free ABA, transferred to the symplast or transported across into the xylem. At the beginning of water stress, the ABA-GE stored in roots is low, and the ABA increase during water stress originates from new synthesis.

Sulfate is a long-distance signal moving through the sap. Sulfate is mobilized by an early over-expressed root sulfate transporter. Sulfate induces ABA biosynthesis in leaves. ABA then is transported to roots via the phloem. In roots, ABA induces water uptake from soil and upregulates the expression of stress-resistant genes.

Subsequently, ABA is transported to leaves through the xylem where it induces stomata closure, reducing the transpiration rate, an activity coordinated together with NO. ABA, sulfate, and ethylene have an additive action on stomatal closure, blocking the transpiration at longer stages of drought. ERECTA, a leucine-rich repeat (LRR) receptor-like kinase, regulating transpiration efficiency, leaf photosynthetic capacity, stomatal density, and patterning, allows plants to maintain biomass production. When stomata are closed, the availability of oxygen for energy synthesis is limited, and CO_2 accumulates. ABA reduces loss of water dependent on transpiration, attenuates stress damage, and upregulates the expression of stress-responsive genes that increase plant salt tolerance (Zhang et al. 2006). Auxins (IAA) may induce reopening of stomata during water stress. Gibberellins (GAs) play a role in modulation of salt stress, through improvement of photosynthetic efficiency, light interception, increase of leaf-area index, and nutrient efficiency (Khan et al. 2007). Exogenous administration of brassinosteroids, such as foliar spray, was shown to improve abiotic stress resistance in wheat seedlings (Dong et al. 2017). Salicylic acid (SA) has an important role to improve plant tolerance to abiotic stress, through the control of plant metabolic processes. Exogenously added SA to stressed plants, either through addition to the nutrient solution, through irrigation, or through spraying, was shown to induce abiotic stress tolerance (Khan et al. 2015; Fatma et al. 2016), mainly through upregulation of osmolytes, detoxification of ROS, protection of photosynthesis systems, and mineral nutrient availability.

Among the executor genes regulated by abiotic stress are E3 ligases that, by directing ubiquitination of specific proteins, target them to the proteasome (Lyzenga and Stone 2012; Chapagain et al. 2017). Among many of these are cullins (CUL). CUL1, through S-phase kinase-associated protein (SKP), binds to F-box proteins, leading to the formation of the Skp, Cullin, F-box containing complex (SCF complex) with E3 ligase activity. DELLA proteins, by inducing ubiquitination of protein targets, regulate hormone signaling (Wild et al. 2012), as in the case of coronatine-insensitive (COI)-SCF complex that targets JAZ repressors for degradation, to activate bHLH transcription factors such as MYC. The DELLA proteins GROWTH REPRESSOR of GA1-3 (RGA1 and RGA2) compete with MYC2 to bind JAZs in the GA-JA crosstalk (Santino et al. 2013).

21.2 Plant Volatile Compounds in Plant Communication

Volatile compounds from plants are a fast means of communication, within plants and between plants and environment. Among plant volatiles are methyl jasmonate (MeJA), a methylated form of jasmonic acid (JA), and the oxylipin pathway products, hexanal, nonenal, and their alcohols. Oxylipin family consists of fatty acid hydroperoxides, hydroxy-, keto-, and oxo-fatty acids, volatiles, aldehydes, divinyl ethers, and green leaf volatiles (GLV) (Poltronieri et al. 2014; Yamauchi et al. 2015). Two branches of the oxylipin synthesis are present in plants, in addition to the JA synthesis pathway. In the first pathway, hydroperoxides are transformed by divinyl ether synthases (DES) into divinyl ethers. In the second branch, short-lived hemiacetals are produced by hydroperoxide lyases (HPL) and give rise to aldehydes and n-fatty acids ($n = 6, 9$) (Poltronieri et al. 2015). These reactive oxylipins are formed during different environmental stresses. Jasmonic acid (JA), as well as its conjugated form (MeJA), is a phytohormone involved in the regulation of interactions between root and bacteria. MeJA accumulated in germinating soybeans and in root tips. Peroxidases and oxylipins are among the bioactive compounds released by roots that exert a selective chemotropism for beneficial microorganisms, able to alter microbiota in the rhizosphere (Fiorentino et al. 2018). The root secretions from tomato were shown to induce formation of biofilms of biocontrol agents on the surface of the plant root (Chen et al. 2013).

Nitric oxide (NO), a gas and a second messenger produced by plants, algae, bacteria, and higher organisms, is involved in cell-to-cell communication. NO, carbon monoxide (CO), and hydrogen sulfide (H_2S) are three volatile gaseous transmitters able to permeate the cell membrane, involved in the regulation of root growth, in bacteria-root communication, and in plant defense toward pathogens. Under drought or salinity, exogenously applied NO increased plant growth (Egbichi et al. 2014) and contributed to the root water content (RWC), stomatal conductance, chlorophyll content, and photosynthetic activity and reduced lipoxygenase activity and membrane ion leakage.

Plant roots are being continually exposed to NO from soil bacteria. NO synthesis occurs during the oxido-reductive steps in the nitrogen cycle. Various factors affect synthesis of NO in the soil, among which are high temperature, availability of oxygen, humidity, soil nitrogen, and pH. These factors influence soil bacteria, able to synthesize NO depending upon the conditions. During nitrogen metabolism, bacteria assimilate nitrate and reduce it to nitrite (NO_2^-) through a two-electron reduction reaction. Nitrite can be reduced to NO. It is known that nitrate reductase (NR) can produce NO non-enzymatically. NR and NOS-oxy in bacteria and rhizobia are involved in the production of NO and signaling between microorganisms and roots and have a role in nodules in sensing abiotic stresses (Poltronieri et al. 2014). NO can inhibit growth of primary roots and increase adventitious lateral root development in tomato induced by auxin.

There are no enzymes in higher plants with similarity to mammalian NO synthases (NOS). Therefore, L-NAME, an inhibitor of NOS used in plant experiments, may affect other enzymes or be active on bacterial NOS-oxy, devoid of the reductase domain.

In legumes, NO-/O_2-binding hemoglobin (hb) proteins, the leghemoglobins, accumulate in symbiosomes (root nodules) of various legumes. In legumes, leghemoglobins (lHbs) are found in symbiotic bacteria organelles. Another type of globins, non-symbiotic *hb* (nsHb), can be expressed in various tissues in plants under abiotic stress. Non-symbiotic hbs are NO-scavenging enzymes in plants, pointing to a role for hemoglobins in protecting from nitrosative stress (Perazzolli et al. 2006; Smagghe et al. 2009). NsHbs function also as O_2 transporters, i.e., bifunctional NO/O_2 transporters, able to modulate levels of ATP and NO.

Nitrogen dioxide (NO_2) can be produced in plants under stress conditions, but little is known about its physiological functions (Mayer et al. 2018). Physiological functions of NO_2 in plant cells have been studied by exogenous NO_2 application through fumigation of *Arabidopsis thaliana*. Fumigated plants produced an immune response protecting them from necrotrophic fungi, such as *Botrytis cinerea*, and hemibiotrophic bacteria, such as *Pseudomonas syringae.* The signaling pathways regulated by SA and JA were required for the NO_2-induced resistance against *B. cinerea*. After an early accumulation of SA following NO_2 application, transient accumulation of oxophytodienoic acid (OPDA) was detected. Fumigated plants produced volatile organic compounds (VOC) monoterpene α-pinene and sesquiterpene junipene.

21.3 Rhizobacteria, Endophytes, and Ectophytes

Plants have symbiotic association with arbuscular mycorrhizal (AM) fungi (Roth et al. 2019). AM interaction is shared by most land plants, Nitrogen-fixing bacteria, actinorhiza, and rhizobia are restricted to species of ten plant families, among them are legumes. Symbiotic fungi belonging to *Glomeromycota* associate with vascular plants (Schüssler et al. 2006). In actinorhiza, symbiotic *Actinobacteria* of the genus *Frankia* associate with plants of *Fagales*, *Cucurbitales*, and *Rosales*. As for legume-rhizobia symbiosis (LRS), rhizobium species associate with legumes. These symbioses are based on intracellular uptake of bacteria and the formation of root nodules.

Rhizobacteria and arbuscular mycorrhiza (AM) such as *Rhizophagus irregularis* drive water transport in roots of flooded tomato plants (Calvo-Polanco et al. 2014).

Plant growth-promoting (PGP) fungi sustain plant growth through nutrient acquisition (Chadha et al. 2015; Igiehon and Babalola 2018), modulation of plant hormone levels, and mitigating the inhibition by plant pathogens (Kandel et al. 2017; Strobel 2018; Khare et al. 2018).

The host plants benefit in a mycorrhizal relationship, by extending its root system, capacity to move water, stress resistance, and nutrient uptake. Most fungi participating in mycorrhizal association are not culturable on media, while cultivable fungi from plant roots are endophytes. Endophytes can live comfortably outside of the host and colonize potential hosts when they sense plant-produced exudates, containing bioactive molecules, sugars, organic acids (malate), amino acids, and phenolics.

Microorganisms within plant tissues and organs are called endophytes or endosymbionts. Endophytes, either bacteria or fungi, together with microbes in the phyllosphere and rhizosphere (plant surfaces), contribute to the plant microbiome (Woźniak et al. 2018; Collinge et al. 2019). Endophytes sporulate outside of the host and can have a mycorrhizal-like behavior and ability to extract and facilitate uptake of inorganic phosphorous (P) (Shehata et al. 2017) and to facilitate extra-radical exploration. The screening of soil bacteria by roots results in a root endophytic microbiome dominated by *Proteobacteria*, and *Actinobacteria*, with a minor contribution of *Bacteroidetes* and *Firmicutes*; *Acidobacteria* and *Gemmatimonadetes* are for the major part absent (Liu et al. 2017; Yamamoto et al. 2018; Latz et al. 2018).

The main difference between endophytes and mycorrhizae is that endophytes do not form any obvious physical interaction structures with the plant. Mycorrhizal fungi are not systemic and are restricted only to roots, and taxonomically they belong to a narrow group of fungi. Mycorrhizal fungi living inside of roots form fungal coils (ericoid and orchid mycorrhizae), while vesicles and arbuscles are not characteristic to endophytes.

Rhizobacteria can also migrate and form mycelia in leaves, such as *Burkholderia phytofirmans* PsJN strain in grapevine (Miotto-Vilanova et al. 2016).

Microorganisms forming endophytic associations can influence the microbiota in the rhizosphere, confer disease protection, improve abiotic stress response, and increase plant growth and yield (Bernardo et al. 2019). The gram-negative bacteria include *Pseudomonas* PS01 conferring salt tolerance in *Arabidopsis* (Chu et al. 2019), *Pseudomonas* spp. (Kim 2012; Bjelić et al. 2018), as well as *Pseudomonas fluorescens* WCS417r, producing plant-stimulating compounds, triggering an induced systemic resistance to pathogens (Pieterse et al. 2002) as well as the "induced systemic tolerance" to salt and drought. *Klebsiella sp*. conferred enhanced tolerance to salinity in *Avena sativa* (Sapre et al. 2018), positively modulating the expression of *rbcL* coding for RuBisCO and *WRKY1*, coding for an important stress-responsive transcription factor (TF).

Many gram-positive bacteria are used in application on plants, such as *Bacillus* spp. (Beauregard et al. 2013; Jiang et al. 2016), as for *Bacillus cereus* AR156 applied to tomato (Xue et al. 2013) and for *Bacillus amyloliquefaciens* FZB42 conferring salinity and drought tolerance to *Arabidopsis* by means of its exopolysaccharides

(Liu et al. 2017; Lu et al. 2018). Among *Actinomycetes,* Hhs.015 is a strain isolated from cucumber roots with beneficial properties (Zhang et al. 2018).

The term "rhizophagy cycle" has been used to describe the passage of nutrients from symbiotic microorganisms to plant roots (White et al. 2018). In this passage, microorganisms alternate from a free-living soil phase to a root endophytic phase. At first, symbiotic microorganisms grow on the rhizoplane in the exudate zone, in close contact with root meristem, after which they enter root tip meristem cells, localizing within the periplasmic spaces, where they lose the cell wall and convert into protoplasts. Reactive oxygen species degrade a certain number of microorganisms, inducing electrolyte leakage and promoting extraction of nutrients. The bacteria that survive in root epidermal cells stimulate root hair elongation allowing bacteria to exit at the hair tip, reconstituting cell walls and cell shapes, while bacteria emerge into the rhizosphere to find nutrients. An opposite cycle, with fungi feeding on dead root cells, has been proposed for *Piriformospora indica* (*P. indica*): at later stages, in stressed plant cells, the fungus secretes hydrolytic enzymes, without a shift to necrotrophy (Lahrmann and Zuccaro 2012).

PGP fungi (Pennisi 2006; White and Torres 2010) and rhizobacteria increase plant productivity and immunity through an improvement of the redox balance. Endophytes stimulate the production of root biomass, neutralize salt-dependent heat efflux, alterate fatty acids, protect from lipid peroxidation, support the activity of antioxidant enzymes, and allow roots to preserve ascorbic acid reduced state during biotic stress response (Baltrushat et al. 2008). Microorganisms promote plant growth-providing components involved in survival to oxidative stress, uptake of nutrients, attachment to roots (pili, hemagglutinin, cellulose production), plant colonization (chemotaxis, flagellar movement, phosphorylation of cellobiose), and defense of plants from pathogens (production of siderophores and antimicrobials, i.e., 2-phenylethanol, 4-hydroxybenzoate, phenyl-lactate) and improve plant physiology due to the synthesis of hormones, such as indole-3-acetic acid, acetoin, and 2,3-butanediol (Taghavi et al. 2010; Lugtenberg et al. 2017).

21.4 *Piriformospora indica*, *Trichoderma* spp., and Ectophytic Fungi

In *P. indica*, the fungal hyphae exudates contain a component inducing root growth (Strehmel et al. 2016; Gill et al. 2016; Xu et al. 2018). *P. indica* colonization of barley roots led to downregulation of ethylene-responsive genes (Schäfer et al. 2009): the inhibition of ethylene signaling stimulates plant growth. In addition, cytokinins, gibberellins, and brassinosteroids are also hormones synthesized or regulated by root endophytes (Schäfer et al. 2009).

In *P. indica*, the fungal hyphae exudates contain a component inducing root growth (Strehmel et al. 2016). It is not clear why there is only one *P. indica* strain possessing plant growth-promoting property. This property may be associated to the presence within *P. indica* of the strain *Rhizobium radiobacter* F4 (Glaeser et al. 2016), a strain close to *Agrobacterium tumefaciens* but devoid of the plasmids conferring pathogenicity. The strain shows plant growth promotion independent of *P. indica* and forms cell aggregates and biofilms at the root surface of maturation zones. Plants colonized by RrF4 strain increase their biomass and immune response regulated by plant jasmonate, enhancing the induced systemic resistance (ISR). As in the case of PGP rhizobacteria, RrF4 colonizes the root outer cortex, the endodermis, and spreads into the stele. The hyphal system of *P. indica* has been shown to transport hormone signals to neighbor plants. Following the infection of *Alternaria brassicae* in *Arabidopsis* leaves and roots, RrF4 stimulates JA accumulation and induces JA-responsive genes and the expression of *NITRATE TRANSPORTER 2.5* (*NRT2.5*) and *REDOX RESPONSIVE TRANSCRIPTION FACTOR1* (*RRTF1*). The systemic JA response in infected plants is transferred to neighboring plants in which abscisic acid (ABA) synthesis is induced with expression of ABA-responsive genes (Vahabi et al. 2018).

In the interaction with plants, *Trichoderma* strains are plant symbionts devoid of virulence (Harman et al. 2004). They are able to penetrate the root epidermis and to grow inter-cellularly in the apoplast of the external layers of cortical root cells, similarly to some plant pathogenic fungi or endophytes; this invasion, however, does not activate a strong plant immune response as in the case of phytopathogens. It is not clear how plants differentiate between pathogens and *Trichoderma*, but the latter interaction ends up with a symbiotic relationship in which *Trichoderma* protect the plant by killing or inhibiting plant pathogens (Matarese et al. 2012; Contreras-Cornejo et al. 2014) and by activating beneficial metabolic changes that modify plant-pathogen interactions as well as plant growth (Shoresh et al. 2010) and abiotic stress tolerance (Mastouri et al. 2010). Plant growth promotion effect by *T. atroviride* on tomato seedlings has been linked to reduced ET synthesis and a lowering of 1-aminocyclopropane-1-carboxylic acid (ACC), caused by degradation of indole-3-acetic acid (IAA) in the rhizosphere and by ACC deaminase (ACCD) present in *Trichoderma* genomes (Druzhinina et al. 2011). *Trichoderma* spp. can produce auxin-like compounds, such as IAA, indole-3-ethanol, indole-3-acetaldehyde, and indole-3-carboxaldehyde (Contreras-Cornejo et al. 2009, 2013). 6-pentyl-α-pyrone (6PP), an auxin inducer (Reithner et al. 2007), stimulates plant growth and root development (plant growth promotion) and can be part of strategies to improve tolerance to biotic or abiotic stresses. Harzianic acid has been described possessing plant growth-promoting activity (Vinale et al. 2013). *Trichoderma virens* (GV41) and *T. harzianum* (T22) have been extensively applied as growth-promoting fungi (Contreras-Cornejo et al. 2016), establishing a symbiosis in roots, that lead to beneficial effect on plant growth and to biotic and abiotic stress resistance (Lombardi et al. 2018). *Trichoderma harzianum* inoculation in rice followed by upregulation of *aquaporin*, *dehydrin*, and *malondialdehyde* genes was shown to mitigate abiotic stresses (Pandey et al. 2016).

Ectomycorrhizal fungi such as *Pisolithus tinctorius* (*P. arenarius*) promoted drought stress response in cork oak (Sebastiana et al. 2018). Plants do not show enhanced photosynthesis, suggesting no effect in sustaining stomatal opening under water stress, and drought tolerance was independent of the water status and on ROS levels: *P. arenarius* did not increase plant water uptake or nitrogen concentration.

Aureobasidium pullulans, a naturally occurring epiphyte or endophyte (apple, cucumber, green beans, cabbage), is a yeast-like fungus resident on grapevine, required to prevent grapevine trunk disease, possibly due to a priming effect (Pinto et al. 2018): the colonization of grapevine by the strain Fito F278, persisting on plant roots for several months at epiphyte and endophyte level, allowed the plants to grow at various pH and under salt stress.

21.5 Microorganism Regulation of Plant Hormones

There is a variety of molecules with hormone-like activity affecting plant immunity and interaction with the microbiome. PGP microorganisms stimulate plants through the production of phytohormones such as auxin, JA, and ABA, biostimulants, and volatiles such as acetoin (Rudrappa et al. 2010) and 2,3-butanediol (plant growth-promoting compounds) or through modulation of phytohormone activity (Taghavi et al. 2010). Dimethyl disulfide and other volatiles (VOCs), such as 2-nonanone and 3-hydroxy-2-butanone from *Paenibacillus polymyxa* (Rybakova et al. 2017), in rhizobacteria volatiles, are able to control plant pathogens (Lugtenberg et al. 2017). VOCs from *Bacillus cereus*, *B. subtilis*, and *B. amyloliquefaciens* have been described in control of plant pathogens, triggering plant defense responses. This adds variety to the weapons synthesized by microorganisms, in the aerial warfare, as protein elicitors, as in the case of PeBA1 from *B. amyloliquefaciens* reported to trigger plant defense responses (Wang et al. 2016).

Roots are extensively affected by hormones: auxins, indole-3-acetic acid (3-IAA), controlling the elongation of the primary root, with an indirect effect on plant growth; jasmonates, affecting primary roots; and brassinosteroids, regulating lateral roots. *Enterobacter* sp. SA187, a desert plant endophyte that colonizes the surface and inner tissues of *Arabidopsis* roots and shoots, was shown to induce salt stress tolerance, through production of 2-keto-4-methylthiobutyric acid (KMBA), a compound able to modulate plant ET signaling pathway. *Enterobacter* sp. SA187 and KMBA were shown to enhance alfalfa crop yield under salt stress (Khare et al. 2018).

ABA biosynthesis and the ABA-dependent signaling pathways are regulated by beneficial microorganisms in plant endosphere, promoting plant growth under salt stress. The ABA-dependent signaling was shown regulated by halo-tolerant *Dietzia natronolimnaea*, responsible for salinity tolerance in wheat plants, with upregulation of ABA-responsive genes and *12-oxophytodienoate reductase 1* (OPR1), involved in the alternative pathway of α-linoleate and oxylipin catabolism (Khare et al. 2018), with scarce reducing activity on 9S,13S-OPDA, the precursor of jasmonic acid.

21.6 Simultaneous Stresses and Plant Hub Genes

Combined stresses are the most common state to which plants undergo in their exposure to environmental conditions (Santino et al. 2013; Rejeb et al. 2014). Researchers have shown how combined biotic and abiotic stresses result in alteration of plant behavior and exploited a systems biology approach to identify the crosstalk between molecular mechanisms involved in plant response to biotic and abiotic stresses, individuating the common components and specific agents of the signaling crosstalk (Nejat and Mantri 2017). The researchers showed how abiotic stresses components can induce plant susceptibility to pathogen infections, through changes in microRNA levels, in heat shock proteins, and in epigenetic marks (Dangi et al. 2018).

Under a collaborative project supported by EU Commission under the Framework Programme 7 (FP7) "Empowering root-targeted strategies to minimize abiotic stress impacts on horticultural crops" (http://www.rootopower.eu/), plant metabolic plasticity was studied by focusing on modifications of primary and secondary metabolism, with the aim to cope with biotic and abiotic stresses. Plants adapt to stress through changing the assimilate allocation between energy-producing (source organs) and energy-consuming (sink) tissues, affecting biomass partitioning between different organs, with consequence on crop yield. The relationships between source and sink organs influence the adaptation to abiotic and biotic stresses. Mechanisms affecting these relations have an influence on crop yield and stress tolerance. Roots, sensing salt or other osmotic stress, can influence shoot physiology sending signals (nutrients, hormones) that lead to a differential assimilate partitioning and changes in "source-to-sink" relationships, with influence on plant physiology (leaf growth, senescence, etc.).

Researches focused on root-to-shoot signaling, and to "source-to-sink" relationships, following adaptation to different biotic and abiotic stresses, identifying genes controlling different types (hydraulic, ionomic, and hormonal) of root-to-shoot signaling positively affecting shoot performance, and studying natural genetic variability and quantitative trait loci (QTL) that allow tomato roots to confer crop resistance to a range of abiotic stresses, alone or in association with arbuscular mycorrhizal fungi (AMF) and rhizobacteria. Attenuated accumulation of jasmonates was shown to modify stomatal responses to water deficit (De Ollas et al. 2018). A positive effect of microbial inoculants (AM fungi and PGPR) in improving tomato water stress tolerance was shown (Calvo-Polanco et al. 2016). Vascular brassinosteroid receptors were shown to confer drought resistance without negative side effects on growth (Fàbregas et al. 2018).

The FP7 EU project "Improving the resistance of legume crops to combined abiotic and biotic stress" ABSTRESS (https://secure.fera.defra.gov.uk/abstress/) studied the gene networks responsible either in drought stress response and following *Fusarium* challenge in legumes. *Medicago truncatula* (Mt) and pea were subjected to combined drought and *Fusarium oxysporum* infection. Associated high-throughput phenotyping data was captured from these plants as they were growing. Medicago and other legumes present several syntenic blocks, i.e., genomic regions with conserved sequences. Output of the project was to identify the orthologs of Medicago (Zahaf et al. 2012; Thatcher et al. 2016) and pea genes involved in simultaneous stress response (Rispail and Rubiales 2014; Castillejo et al. 2015; Bani et al. 2018) and their orthologs in major crop genera of the Brassicaceae (oil seed rape), Poaceae (wheat), Solanaceae, Compositae (sunflower), and Chenopodiaceae (sugar beet) as well as other legume crops. The transcription factor HSFA1b has been identified as a major hub controlling resistance to biotrophic pathogens, increased tolerance to drought stress preserving crop yield (Bechtold et al. 2013; Albihlal et al. 2018), and has applied a systems biology approach to find hub genes implicated in controlling networks responsive to environmental stresses (Formey et al. 2014).

Nodule gene expression studies were conducted by quantitative PCR. The nitrogen fixation genes nifA, nifH, and fixJ of the symbiotic bacteria *Sinorhizobium medicae* and *Rhizobium leguminosarum* were analyzed. Inhibition of N_2-fixation in both Medicago and pea root nodules as a result of drought stress and *Fusarium* attack was analyzed by monitoring nitrogenase gene expression (*nifH*). Water stress mainly affected shoot compartments, while carbon allocation to roots and its biomass were maintained. The situation observed was reversed with the addition of the biotic stress (*Fusarium*-infected plants), which might have resulted as a strategy of the pathogen to “preserve” its host. While water stress negatively impacted nodule number in Medicago, mean nodule biomass reduction was observed in pea: *Fusarium* effect was principally detected on pea plants where nodule number was decreased while mean nodule weight increased. Drought stress led to significant downregulation of genes involved in nitrogen fixation, an effect that was amplified by *Fusarium* attack. IAA production by rhizobacteria is one of the traits promoting plant growth under biotic stress conditions. The infection of three different legume plants with the specific IAA-overproducing rhizobia strains greatly improved the stress resistance of these plants.

Twenty-six homolog sequences in the pea genome corresponding to 26 Medicago HUB genes with high sequence similarity were identified, and sequences for 6 Medicago hub genes with no similarity in pea showed they are nodule specific. Genomic maps and localization of pea homologs of 22 Medicago HUB genes were determined, individuating genes in orthologous relationship between Medicago and pea by co-linear genomic location. For seven gene sequences mapped in non-syntenic genomic regions, the genes were considered paralogs.

The study of plants’ responses to multiple stresses is starting to give results. In particular, data have been provided for signaling crosstalk, describing how drought and heat can increase susceptibility of plants to pathogens (Nguyen et al. 2018). In studies on legumes, researchers analyzed the interaction of leaf damage (herbivore attack) and salinity on Medicago, to find differences in hormone biosynthesis and crosstalk, studying spatiotemporal events occurring between leafs and roots in the activation of proteins involved in JA synthesis, such as lipoxygenases (LOX), allene oxide synthases (AOS), and AO cyclases (AOC) (De Domenico et al. 2019). It was previously shown how specific isoforms of LOX contributed to the long distance travel of the signal and to the synthesis of JA in distal leaves in *Arabidopsis* (Chauvin et al. 2013). Other genes in the JA synthesis pathway have been associated with abiotic stress tolerance, 12-OXOPHYTODIENOATE REDUCTASE 3 (AtOPR3) in wheat chill tolerance (Pigolev et al. 2018) and AOC in wheat salinity tolerance (Zhao et al. 2014), in addition to effects of methyl jasmonate, avoiding the decrease of root hydraulic conductivity during water stress (Sánchez-Romera et al. 2014, 2016).

21.7 Management of Saline Soil Conditions Through a Combined Approach

Various physical-chemical techniques may be applied in the management of saline soil conditions to favor crop yields. In case of soil salinity depending on application of fertilizer or irrigation water with high electrical conductivity, methods that may improve the soil quality are drainage improvement; leaching practices, with or without gypsum applications, limestone, and sulfuric acid; deep tillage; subsoiling; and inversion of the soil profile. The application of organic matter (used on rows or seed beds) may dilute the concentration of salts in the soil. This latter technique is also useful when soils are inherently saline (Gupta Choudhury et al. 2014).

Other approaches to mitigate salt stress include rotation of crop, the supplementation of crop residues and application of biosolids and biochar to dilute the concentration of ions in the soil, and the sustainment of plant microbiome, favoring beneficial microorganisms in plants under salt stress, through production of growth hormones, osmolytes, and stress-ameliorating chemicals and through the capturing ability of microorganisms, temporarily sequestering the salt ions. Organic matter increases the CO_2 availability thus allowing photosynthesis even under conditions of partial stomata closure, thus allowing production of energy, antioxidants, and osmolytes: it has been proposed to exploit microbial inoculants to deliver plant nutrients, to attenuate plant abiotic and biotic stresses, and to minimize the negative impact of chemical input (Egamberdieva and Lugtenberg 2014; Alori and Babalola 2018).

21.8 Perspectives

Plant-microbial interactions are at a starting point of knowledge; advancements on the understanding of the interactions may help to develop a sustainable agriculture to preserve crop yield and improve food security. In the era of -omic techniques, monitoring of the transformations in rhizosphere microbial community and of effects on plant development can be achieved. Furthermore, additional microbial species interacting positively with plants will be found. These strains can have application as biofertilizers and biocontrol agents. Under a similar perspective, soil-borne microorganisms, fungi, and bacteria may be exploited and applied into the rhizosphere for suppression of root and foliar pathogens and through plant-induced systemic resistance. Microbial strains isolated from the root microbiome of resistant tomato plants transferred to varieties sensitive to *R. solanacearum* have been shown to induce a protective state and increased resistance to the pathogens (Kwak et al. 2018). Therefore, it is envisaged that a combined approach, exploiting physicochemical techniques for the management of soils, water adjustments by dripping methods, and an appropriate addition of plant microorganisms supporting plants in relieve from stress, may improve crop yields under ever-changing climate conditions.

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Chapter 22
Role of Plant Endophytes in Conferring Abiotic Stress Tolerance

Laccy Phurailatpam and Sushma Mishra

Contents

Abstract Plants, being stationary, are continuously challenged to extreme environmental conditions like drought, salinity, heat, cold, etc. that limit crop productivity by about 50–70%. The response of plants to these abiotic stresses is complex and involves a plethora of physiological, biochemical, and gene expression changes. A number of approaches like plant breeding, genetic engineering, mutagenesis, and *omics*-based approaches have been used to understand and impart tolerance against various types of stresses in economically important plant species. This chapter would, however, focus on the application of endophytes (a group of beneficial microorganisms present within the plant tissues) in conferring selective advantage to host plants with which they are associated in harsh environmental conditions. Numerous reports have proposed that endophytes impart stress tolerance to plants either by activation of host stress response system or by biosynthesis of anti-stress metabolites like plant growth regulators, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatiles, bioactive compounds, etc. Depending on the types of abiotic stress, different types of endophytes adopt different signaling mechanisms which may include synthesis and accumulation of compatible solutes, adjustment in ion transport, expression of stress-responsive genes, generation of ROS scavenger

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molecules, etc. In particular, the role of endophyte-mediated stress tolerance against drought, salinity, and heavy metal conditions has been discussed in detail.

Keywords Abiotic stress tolerance · Endophytes · Drought stress · Osmotic stress · Heavy metal stress · Salinity stress · Signaling mechanism

Abbreviations

ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
AMF	Arbuscular mycorrhizal fungi
APX	Ascorbate or thiol-dependent peroxidase
As	Arsenic
CAT	Catalase
Co	Cobalt
Cr	Chromium
Cu	Copper
DHAR	Dehydroascorbate reductase
EC	Endophyte colonized
GAs	Gibberellic acid
GR	Glutathione reductase
Hg	Mercury
IAA	Indole-3-acetic-acid
LEA	Late embryogenesis abundant protein
MDHAR	Mono-dehydroascorbate
Ni	Nickel
Pb	Lead
PGP	Plant growth promoter
PGPR	Plant growth-promoting regulator
POX	Peroxidase
ROS	Reactive oxygen species
S-Ado-Met	*s*-Adenosyl-methionine
SOD	Superoxide dismutase
Zn	Zinc

22.1 Introduction

Abiotic stress in plants is generally described as unfavorable conditions imposed by various nonliving agents on plant growth and metabolism, contributing to vast losses in agricultural yield (Dhumal et al. 2018). Some of the major abiotic stresses to which plants are generally exposed include osmotic stress, salinity, drought, heat, cold, heavy metals, etc. (Boyer 1982; Wang et al. 2003; Khare and Arora 2015). In order to cope with sudden, hostile changes in the environment, plants usually initiate a number of molecular, cellular, and physiological modifications which will help them adapt to the changing adverse conditions. Almost all abiotic stresses are interlinked and may instigate nearly identical damages to plant cells like production of reactive oxygen species (ROS), denaturation of proteins, alteration of enzyme activity, loss of membrane stability, etc. (Wang et al. 2003; Mishra et al. 2019). This book chapter would mainly deal with the role of beneficial microorganisms residing within plant tissues, called endophytes, in imparting stress tolerance to their host plants. We begin this chapter by giving an introduction about the major types of abiotic stresses faced by the plants, followed by a brief note on the general plant defense mechanisms, and finally the role of endophytes in providing selective advantage to plants exposed to abiotic stresses in comparison with the non-endophytic plant.

Among the major abiotic stresses mentioned above, *drought stress* is considered to be the most prevalent limiting condition for crop yield and production in various temperate and tropical regions of the Earth (Saranga et al. 2001). It has been calculated that about one-fourth of total land area is dry and about one-third of the Earth's arable land is exposed to water-deficit conditions (Kirigwi et al. 2004). The standard and annual yield of crop is also greatly affected by the changing climatic conditions resulting in frequent water shortage conditions making the scenario more critical for plant's survival (Hongbo et al. 2005). Since nutrients are absorbed from the soil by roots and transported to the shoot region with the help of water, stress restricts accessibility and mobilization of soil nutrients. Drought stress thus reduces nutrient translocation and bulk accumulation of water-soluble nutrients like calcium, magnesium, nitrate, silicon, etc. Drought stress also contributes in increasing the emergence of ROS, which are reactive chemical elements containing oxygen, such as peroxides, superoxides, hydroxyl radicals, and singlet oxygen, that damage cellular components like membrane lipids, proteins, and nucleic acids, thereby resulting to oxidative stress (Dat et al. 2000).

Salt stress is another important abiotic stress which extensively affects plant growth and maturation. It has been reported that out of the overall world's irrigated and cultivated cropland, about 33% and 20%, respectively, are destroyed by extreme salinity or sodicity. In addition, the degree of salinity and its affected land have been expanding regularly by about 10% per annum due to several climatic changes. Considering the recent dramatic changes and current prevailing situation, it is predicted that more than 50% percent of the total cultivable land will be affected by salinity stress till 2050 (Jamil et al. 2011). The quantity or amount of salt present in the soil can affect various soil processes in the soil solution (salinity) which determines the osmotic potential and the amount of sodium present on the exchange complex of the soil (sodicity) which influences soil structural stability. Salinity can also lead to sodicity condition over a period of time (Shi and Wang 2005). It is very common and natural for most of the irrigated soil to be in saline or sodicity condition; however, its intensity and corresponding harmful effects have been increasing in the recent decades due to agricultural undertaking like deforestation and irrigation. Salinity reduces the capacity of plant to utilize water, resulting in reduced growth rate as well as changes in plant metabolic processes. The cause of reduced growth of crop in saline soils may be due to imbalance in mineral nutrition, reduction in water uptake, and ion toxicity to plants. Salinity is also the main cause of ionic and osmotic stress in plants which retards plant development. Osmotic stress happens instantly in plant when there is formation of hypertonic solution outside the cell of plant, but ionic stress occurs when the plant is exposed to salt stress for long duration resulting in excess accumulation of Cl^- and Na^+ ion within the plant's cell.

Another abiotic stress is *high temperature*, which significantly affects plant's efficiency, especially in barren desert lands (Bray 1997). A 10–15 °C rise in temperature above the normal is called *heat stress*. Such extreme temperatures interfere with basic physiological functions like photosynthesis, membrane fluidity, protein stability, etc. Photosynthetic machinery is the most thermosensitive structure of plant. It causes denaturation of enzymes involved in photosynthesis and stomatal dynamics and decline in photosystem II-mediated electron transport (Schlau-Cohen et al. 2015).

Cold stress refers to suboptimally low temperatures (chilling and freezing) in the environment that are detrimental to plant growth and development. According to the degree of temperature, cold stress can be categorized into chilling and freezing stress (Salinas 2002). Plants' exposure to temperature ranging from 0 to 10 °C induces chilling stress, whereas temperature below 0 °C induces freezing stress. In general, cold stress induces loss of membrane integrity mainly by increasing the amount of saturated fatty acids in membrane lipid composition that ultimately leads to alteration in membrane fluidity. Another major effect of cold stress is the freezing-induced cellular dehydration that not only results in osmotic stress but also leads to damage to cellular components (Balestrasse et al. 2010; Wang et al. 2006; Mishra et al. 2019).

Heavy metals are primarily generated due to mining, smelting, agriculture, and other industrialization and urbanization activities. Apart from these anthropogenic sources, weathering of rocks also contributes to the natural sources of heavy metal pollution (Shahid et al. 2015). The major heavy metal pollutants present in the environment include mercury (Hg), arsenic (As), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn), nickel (Ni), and cadmium (Cd) (Wuana and Okieimen 2011). In particular, Pb pollution is a global problem due to its use as an additive fertilizer and gasoline since 1920. Although some heavy metals such as Cu, Mo, Zn, cobalt (Co), Ni, and Mg are essential for plant growth and development, these can cause unusual morphological changes and metabolic disorders when their concentration exceeds threshold levels (Xiong et al. 2014; Pierart et al. 2015; Salla et al. 2011). The detrimental effects of heavy metals are exhibited basically due to the accumulation of ROS that results in uncontrolled oxidation and radical chain reactions, ultimately damaging the cellular biomolecules like nucleic acids, lipids, and proteins (Gill and Tuteja 2010; Pourrut et al. 2011).

All the major abiotic stresses, described above, together limit the crop production by as much as 70% (Boyer 1982). In fact, it has been reported that the amount of reduction of crop yield due to abiotic stresses was significantly higher than the standard productivity of many of our economically important crops. The adaptation of plant to highly stressed ecosystem involves a combination of phenotypic flexibility and genetic modification (Rodriguez et al. 2004). Some of the stress tolerance mechanisms of plants include the production of osmolytes, phytohormones, and enzymes, altering water mobilization, scavenging (ROS), etc.

22.2 General Plant Defense Mechanisms Against Abiotic Stress

Plants, being sessile, need to develop and evolve mechanisms to acclimatize to their changing environmental conditions. The adaptations and management strategies allow plants to not only survive under abiotic stress conditions but also enable to colonize diverse habitats on Earth. Plants respond to abiotic stress by changing physiological, biochemical, and molecular mechanisms (Zhu 2016). Several abiotic stresses like salinity, drought, heavy metal, heat, and cold create a secondary stress condition in plants called osmotic stress. Osmotic stress refers to sudden change in solute concentration in cells accompanied by movement of water, which mainly results in loss of turgor pressure required for cell growth.

During abiotic stress conditions, plants show specific changes in metabolism, physiology, and gene expression as a response to changing environmental conditions. As, for example, the genes activated by abiotic stresses could be categorized into *functional genes*, which encode for products like osmoprotectants, molecular chaperones, detoxifying enzymes late embryogenesis abundant (LEA) protein.; *regulatory genes.* These genes generally act upstream to several functional genes and mainly encode for protein kinases and transcription factors. Apart from the changes in gene expression, abiotic stress responses in plant cells also include rapid and transient rise in cytoplasmic Ca^{2+} levels, which function as secondary messenger in abiotic stress signaling. The influx of Ca^{2+}, either from extracellular sources or from intracellular stores (endoplasmic reticulum or vacuole), leads to a series of phosphorylation and dephosphorylation events, thereby facilitating the transmission of stress within the nucleus, where changes in corresponding gene expression could take place (Chinnusamy et al. 2004; Knight 1999). Plants also respond to salinity stress at the phenotypic as well as at the cellular level. Under extreme saline conditions, heavy roots are quite beneficial to plant as they could absorb higher quantity of salt and may not allow them to translocate to the shoot portion of the plant. So, it is very important to maintain a proper root-to-shoot ratio for the survival of plant under high salt concentrations (Munns and Tester 2008). These morphological modifications in plants are controlled by different phytohormones, namely, auxin, gibberellin, ethylene, abscisic acid, etc. (Khan et al. 2011). At the cellular level, salt ions are being accumulated within the vacuole, leading to disturbance in the osmotic balance inside the cell. As a result, water overflows from the cytoplasm into the extracellular region resulting in cell dehydration. So, in order to establish such type of osmotic condition, plants absorb light-molecular-weight organic compound like proline, sucrose, mannitol, glycine betaine, etc. in the cytoplasm, which go well along with the metabolic pathway (Hussain et al. 2008; Bartels 2005).

22.3 Function of Endophytes in Conferring Abiotic Stress Tolerance in Plants

22.3.1 What Are Endophytes?

Endophytes are a class of plant-associated microorganisms (mainly bacteria and fungi) which live inside roots, stems, leaves, petioles, and other internal cells, without causing any disease symptoms or injury (Hallmann et al. 1997). Endophytes may colonize plant apoplast, intercellular spaces of plant's tissues, and xylem vessels; these were isolated from various plant parts like roots, stems, leaves, flowers, fruits, and seeds (Compant et al. 2011). It has been established that the intimate relationship between host plant and endophytes is mediated through interaction and mutualism between microorganisms and host cells (Reinhold-Hurek and Hurek 2011; Brader et al. 2014). Some major functions of endophytes in plants include plant growth enhancement (Hardoim et al. 2008); bioremediation (Lumactud et al. 2016); synthesis of bioactive metabolites, especially when isolated from medicinal plants (Aravind et al. 2009); and induced systemic resistance (Uppala et al. 2010a), improving biometric properties, crop production (Govindarajan et al. 2008; Uppala et al. 2010b), and pathogenic repulsion (Ziedan 2006; Uppala et al. 2010c). Endophytes enable increased availability of macro- and micronutrients that occur in complex forms in the environment. For example, plant root exudates are metabolized by ammonifying bacteria to provide nitrogen to the plant for amino acid synthesis. Endophytes including in genera *Enterobacter*, *Pseudomonas*, *Acinetobacter*, *Ralstonia*, and *Pantoea* also promote plant development by increasing phosphate solubilization, through enhanced productions of essential plant growth-promoting agents like indole acetic acid (IAA), cytokinins, and gibberellin (GAs) (Jha et al. 2011). Endophytes also enhanced production of siderophores and provide essential vitamins during nutrient-limiting conditions (Jha et al. 2011). Moreover, endophytes also possess the capability to degrade organic components including cellulose, hemicellulose, and lignin which mediate nutrient cycling (He et al.2012).

A huge diversity of *bacterial endophytes* such as *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Serratia* spp., *Acetobacter diazotrophicus*, *Enterobacter* spp., *Bacillus* spp., etc. are present in many crop species (Gyaneshwar et al. 2001; McInroy and Kloepper 1995). These microbes were reported for their function in enhancing plant growth through nutrient acquisition and their plant growth-promoting activities like production of IAA, GA, and cytokinin like compounds (Maheswari et al. 2013; Latif Khan et al. 2016). Many of the bacterial endophytes are also known to be engaged in nitrogen fixation, phosphate solubilization, and stress tolerance production of siderophores as in case of eggplant by *Pseudomonas fluorescens* (Döbereiner et al. 1995; Szilagyi-Zecchin et al. 2014; Ramesh et al. 2009; Kumar et al. 2016). They also impart abiotic stress tolerance in plant by making them resistant to the changing environmental conditions. It has also been reported that *Achromobacter xylosoxidans* and *Bacillus pumilus* induce the development of sunflower plant during exposure to water stress period (Forchetti et al. 2010).

The most common *fungal endophytes* include *Trichoderma* spp., *Epicoccum nigrum*, *Penicillium* spp., *Xylaria*, *Alternaria*, *Cladosporium*, *Fusarium* spp., etc. (Romão-Dumaresq et al. 2012; Uppala 2007; Paul et al. 2012; De Lima Fávaro et al. 2012). Several plant PGP traits were described for such endophytic fungus in several plants. For example, *Trichoderma virens* produces endochitinases which contradict the activity of *Ceratocystis paradoxa*, which are responsible for pineapple disease in sugarcane (Romão-Dumaresq et al. 2012). It was proved that the endophyte *Fusarium oxysporum* strain EF119 express anti-oomycete property toward the late blight pathogen of tomato (Kwon et al. 2007). Some of the fungi produce bioactive compounds which are beneficial in controlling several plant diseases. Some of them were studied in *Oryza sativa* and *Gossypium* species (Naik et al. 2009; Ek-Ramos et al. 2013). Antagonist activities induced by *Phytophthora capsici* against various microbial pathogens have also been reported on black pepper. Microscopic filamentous fungi, also known as arbuscular mycorrhizal fungi (AMF), are also a prominent class of fungal endophytes that inhabit the roots and rhizosphere of plants simultaneously (Nasim 2010). AM fungi are in symbiotic relationship with almost all land plants, and, therefore, their symbiotic association with the host plant has been most extensively studied (Barrow et al. 2008). Their filamentous hyphae occupies the interior as well as the exterior portion of the roots which allow the plants to absorb more amount of water with nutrients available in the soil. Numerous studies indicate that AM fungi induced resistivity to host plant toward various environmental stresses, micronutrient imbalances, sulfur dioxide fumigation, biocide treatment, slurry application, industrial effluents, and pathogens (Sylvia and Williams 1992; Charest et al. 1993; Siddiqui and Singh 2005; Siddiqui and Singh 2004).

22.3.2 Mechanism of Stress Tolerance Conferred by Endophytes

Although endophytes are adapted to live inside specific plant species, numerous reports indicate that the structure of endophytic community is affected by a number of abiotic factors like drought, temperature, light, and heavy metals and by biotic agents like microbe's symbiotic relationship with host plant and microbe–microbe associations (Ryan et al. 2008). Plants growing in limited environmental conditions allow to recruit some specific microbiome only according to their symbiotic relationship. Along with this the bacterial/fungal endophytes provide abiotic stress resistivity in both the host plant and other crop plants (Redman et al. 2011; Marasco et al. 2013). Endophyte isolated from some plants such as rice, panic grass, dune grass, tomato, etc. increase plant biomass in drought-stressed plants while comparing with non-endophytic plant. This drought resistance is provided by endophytes by increasing the levels of solutes in plants (Malinowski and Belesky 2000). Endophytes provide resistance in plants against osmotic stress by inhibition of shoot growth, thereby enhancing root growth which allows fast mineral solubilization, uptake and transport of metals, etc (Zhao et al 2016).

Endophytes impart stress resistivity to plants mainly through two important steps: (1) activating stress-regulating system within the host plants (Schulz et al. 1999; Redman et al. 1999) and (2) self-synthesis of anti-stress compounds (Miller et al. 2002; Schulz et al. 2002). Abiotic stress like drought, heat, and salinity affects plants and deteriorates plant at cellular and molecular level by decreasing yield with productivity (Wang et al. 2003; Bray 1997). In response to these abiotic stresses, endophytes upregulate some stress-responsive genes, generates scavenger molecules like ROS, synthesizes anti-stress metabolites, and increase accumulation of some compatible solutes in plants (Lata et al. 2018). Phytohormones produced by endophytes such as IAA, gibberellic acid, jasmonic acid, salicylic acid, and brassinosteroid provide abiotic stress resistance to plants (Waqas et al. 2012; Wani et al. 2016). Endophyte decreased the level of electrolytic leakage in plants under drought conditions. Proline accumulation is very common in endophyte-associated plant during water stress period. It has also been observed in tall fescue plant and wheat associated with the endophyte *Neotyphodium* and *Chaetomium globosum*, respectively (Bayat et al. 2009; Cong et al. 2015; Elmi and West 1995). In drought-sensitive *Theobroma cacao* seedlings inoculated with an isolate of *Trichoderma hamatum*, the quantity of glutamic acid and aspartic acid and of γ-aminobutyric acid and alanine was measured (Bae et al. 2009). The reduction in osmolyte secretion is one of the important strategies employed by the drought-resistant plant to escape from the harmful effects of stress period (Augé and Moore 2005; Ruiz-Sánchez et al. 2010). It was also reported that *Theobroma cacao* plant inoculated with *Trichoderma hamatum* show increased root water content under water-limiting condition (Bae et al. 2009). Fungal mycorrhiza are known to enhance plant growth and fitness by several mechanisms when subjected to water-limiting conditions. Mycorrhizal plants may mitigate the obstacle of drought stress to some extent through promoting

Table 22.1 The table given below is listed with several endophytes along with the plant in which they reside imparting different abiotic stress resistance to their corresponding host plant species

Host plant	Endophytic organisms	Abiotic stress	References
Oryza sativa	*Fusarium culmorum*	Water deficit	Redman et al. (2011)
Brassica campestris	*Piriformospora indica*	Water deficit	Sun et al. (2010)
Theobroma cacao	*Trichoderma hamatum*	Water deficit	Bae et al. (2009)
O. sativa	*Curvularia protuberate*	Water deficit	Rodriguez et al. (2008)
Lycopersicon esculentum	*C. protuberate*	High temperature	Rodriguez et al. (2008)
O. sativa, L. esculentum	*F. culmorum*	Salinity	Rodriguez et al. (2008)
Hordeum vulgare	*Piriformospora indica*	Salinity	Waller et al. (2005)
Festuca arizonica	*Neotyphodium*	Water-limiting condition	Morse et al. (2002)
Medicago sativa	*Sinorhizobium meliloti*	Drought	Naya et al. (2007)
T. cacao	*T. hamatum* DIS 219b	Water-limiting condition	Bae et al. (2009)
Brassica campestris	*P. indica*	Water deficit	Sun et al. (2010)
Saccharum officinarum	*Gluconacetobacter diazotrophicus*	Water deficit	Vargas et al. (2014)
Oryza sativa	*Trichoderma harzianum*	Water-limiting condition	Pandey et al. (2016)
Capsicum annum	*Arthrobacter* sp.	Osmotic stress	Sziderics et al. (2007)
	Bacillus sp.		
O. sativa	*Pseudomonas pseudoalcaligenes*	Salinity	Jha et al. (2011)
Zea mays	*Pantoea agglomerans*	Salinity	Gond et al. (2015)
Nicotina tabaccum	*Pseudomonas* sp.	Osmotic stress	Sarma (2011)
Burkholderia phytofirmans	*Burkholderia phytofirmans*	Cold stress	Su et al. (2015)
Arabidopsis thaliana			
Solanum lycopersicon	*Pseudomonas frederiksbergensis, Pseudomonas vancouverensis*	Cold stress	Subramanian et al. (2015)

water absorption effectively even at less moisture-containing soil. Such severe drought conditions are escaped in case of onion by increasing phosphorus nutrition, while in *Bromus* and rose, some other mechanism prevails (Nelsen and Safir 1982; Bildusas et al. 1986). The mutualistic interaction between mycorrhiza and host plant is an indispensable condition in enhancing plants to tolerate unfavorable environmental conditions (Augé and Moore 2005). Arbuscular mycorrhizal fungi commonly dwell in areas with high concentration of salt (Wang and Liu 2001). Earlier studies have already clarified that AM fungi have the priority role in attenuating the effects of salinity stress (Al-Karaki et al. 2001) and help the plants to deal properly with salt stress (Jahromi et al. 2008). A consortium of common plant endophytes involved in conferring abiotic stress tolerance has been listed in Table 22.1.

22.3.3 Drought Stress Tolerance

Endophyte enhanced drought tolerance to plants by conferring improved water use efficiency through reducing water consumption so that the plant can maintain greater water content under stress conditions. Hence this signifies the role of endophytes in delaying desiccation and damage of plant tissues during stress period (Rodriguez et al. 2008). Some of the signaling mechanisms triggered by the plant–endophyte association has been described in Fig. 22.1. Phytohormones, apart from being involved in overall plant growth and development, also confer stress tolerance to plants. Under water-limiting conditions, a number of plant varieties in association with IAA-producing bacterial endophytes reflected significant increase in root development, generation of adventitious roots, and root hairs, which contributes in increasing absorption of solvent and minerals or organic and inorganic components from the soil. So, IAA is regarded as the most physiologically active form of auxin in increasing shoot and root growth and cytokinesis of young plantlets in presence of cytokinin. A number of plant species like *Lactuca sativa* have become drought resistant when inoculated with IAA-producing bacteria, *Azospirillum* (Dimpka et al. 2009). Treatment of *Phaseolus vulgaris* L. with *Azospirillum brasilense* Cd increased the surface area of root and its potential of spreading over larger area more significantly than control plant when both are exposed to drought stress (German et al. 2000). Likewise, the young seeds of maize treated with *Azospirillum brasilense* show greater potential of relative and absolute water-holding capacity, while the control plant does not have such property when exposed to drought stress. When plants are inoculated with promising endophytic bacteria, they exhibit lower water potential but improved the length and girth of root and leaf's surface area and promoted proline uptake by leaves and roots (Casanovas et al. 2002). Application of rhizobacterium, *Pseudomonas putida* H-2-3, of which is a GA-producing bacteria on *Glycine max* seedlings enhanced plant development during water deficit period (Kang et al. 2014). Likewise, maize plants inoculated with *Azospirillum lipoferum* alleviated the effect of drought stress through the secretion of ABA and gibberellins (Cohen et al. 2009).

Accumulation of *volatiles* in plants occurs when they are suffering from several abnormal and uncomfortable conditions in their ecology or habitat (Loreto and Schnitzler 2010; Holopainen and Gershenzon 2010). Some of the important stress induced volatiles like 2*R*,3*R* butanediol function as signaling component in developing various systemic adaptations within the plant itself as well as with neighboring plants when they are subjected to wide ranges of abiotic stresses (Heil and Bueno 2007; Choudhary et al. 2008; Niinemets 2010). Root colonization of *Arabidopsis* with *Pseudomonas chlororaphis* O6 prevents dehydration when the

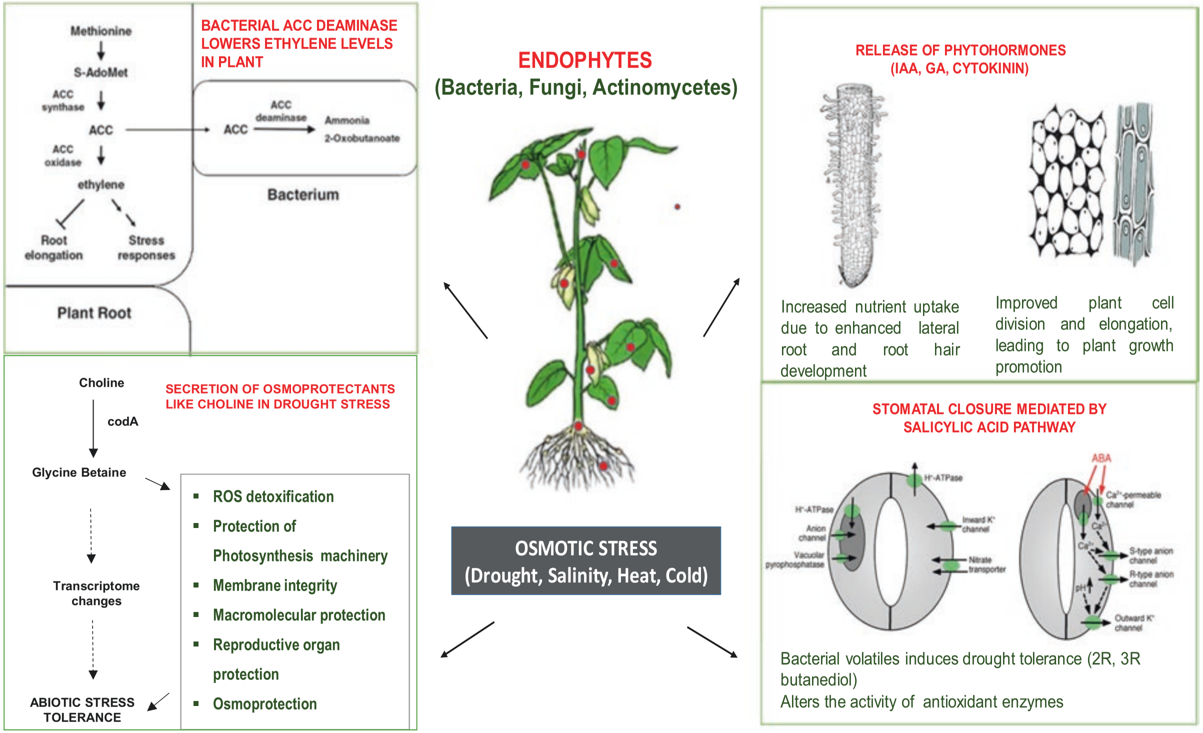

Fig. 22.1 Endophytes and abiotic stress tolerance. The plant endophytes (bacteria, fungi, and actinomycetes) trigger various signaling pathways to impart tolerance against osmotic stress resulting due to water deficit conditions, high salinity, and extreme temperatures

stomata are closed. This particular response might be induced by volatiles, 2*R*,3*R*-butanediol, secreted by the colonizing bacteria through SA-dependent pathway, whereas non-colonized *Arabidopsis* plant does not exhibit such response of drought tolerance indicating less production of the volatiles in the plant (Cho et al. 2008). It has also been shown that ethylene and jasmonic acid signaling pathways enhanced drought tolerance activities in mutant *Arabidopsis* plants. The induction of the closing of stomata and resistivity to drought stress were mediated by Aba-1 and OST-1 kinase (Cho et al. 2008).

Ethylene levels play a crucial role in various plant's activities, and their biosynthesis is modulated by various environmental and biotic stresses (Hardoim et al. 2008). During the synthesis of ethylene, *S*-adenosylmethionine (*S*-AdoMet) is transformed by 1-aminocyclopropane-1-carboxylate synthase (ACS) to 1-aminocyc lopropane-1-carboxylate (ACC), the immediate substrate of ethylene biosynthesis. During stress conditions, the phytohormones, ethylene, internally regulate plant equilibrium leading to reduction in root and stem development. During such conditions, plant ACC is accumulated and destroyed by ACC deaminase-secreting endophytic bacteria thereby contributing to minimize the harmful effect of ethylene. Moreover, the microbes also supply with nitrogen and energy, making the host plant drought resistant and promote its growth (Glick 2005). So, treatment of plants with bacteria expressing ACC deaminase activity increases the number and yield of seed and nitrogen accumulation. It can also even restore nodulation in legumes which was depressed under drought stress period (Dodd et al. 2004). ACC deaminase producing PGPR *Achromobacter piechaudii* ARV8 help in increasing the fresh and dry weights of tomato and pepper plants and minimized ethylene generation during dry conditions (Mayak et al. 2004). Colonization of *Pisum sativum* growing on dry lands with the ACC deaminase-producing *Variovorax paradoxus* 5C-2 was much beneficial (Dodd et al. 2004).

Plants adaptations to drought stress is engaged with *osmotic adjustment* resulting due to absorption of many compatible solutes or osmolytes, amino acids, and water stress proteins like dehydrins (Yancey et al., 1982; Close, 1996). During water stress conditions, PGPR (plant growth-promoting regulators) secrete osmolytes such as sugars, proline, quaternary ammonium compounds, polyamines, polyhydric alcohols, and betaines that respond in association with plant-generated osmolytes and enhanced plant development (Vurukonda et al. 2016). The inoculation of *Pseudomonas putida* GAP-P45 with maize during drought stress period shows much significant improvement in plant's biomass, relative water content, and leaf water potential signifying the role of proline accumulation by the host plant (Sandhya et al. 2009). Tomato (*Lycopersicon esculentum* Mill) cv. Anakha treated with phosphate-solubilizing bacteria, *Bacillus polymyxa*, secreted excess proline in order to resist the drought period (Shintu and Jayram 2015). During extreme water-deficient conditions, even the growth of drought-tolerant plant species is influenced by endophytes. As, for example, two cultivars of rice species, i.e., Sahbhagi (drought tolerant) and IR-64 (drought sensitive), when treated with *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81, and *Arthrobacter nitroguajacolicus* strain YB3, strain YB5 expresses improved production of proline and enhanced proper growth of plant (Gusain et al. 2015). Similarly, when maize plants are inoculated with *Pseudomonas putida* GAP-145 and *Azospirillum lipoferum*, improved growth rate can be observed due to the uptake of amino acids and soluble sugars (Bano et al. 2013; Sandhya et al. 2010).

Fungal endophytes also respond in different ways according to the conditions of the drought stress and types of plants in which they exist. In some plants they enhanced plant growth and increased biomass under water-deficit condition, as in case of drought-sensitive rice plant that when inoculated with *Fusarium culmorum* and *Curvularia protuberata* shows higher biomass (Redman et al. 2011). In some of the plant colonized with promising endophytes, the secretion of stress-associated genes like abscisic acid, 9-cis-epoxycarotenoid dioxygenase 3, ABA aldehyde oxidase 3, and zeaxanthin epoxidase suddenly dropped down during drought stress period. On the contrary, increased production of GA3 for promoting plant growth and yield can be observed through modification of host plant's physiological pathway (Khan et al. 2015). In few cases fungal endophytes produced higher amount of auxins which conferred increased plant development during water-limiting period (De Battista et al. 1990). This positive effect can be competed with plants treated with GA3 exogenously on the basis of enhancing plant developments and increasing crop productions during stressed situations by altering host's metabolic pathway However, it has also been reported that some specific endophytes do not confer plant growth and development in drought stress period, but they help the host plant to recover rapidly on rewatering (Ren and Clay 2009).

The activities of endophyte-associated plant under drought stress can be influenced by the action of several drought-related genes. During drought conditions, endophytic and non-endophytic plant enhance the activity of drought-related genes present within their tissues differently. Some of the activity of drought-related genes may include Ca_2P signaling, other regulatory pathways secreting enzymes which synthesize several metabolites which activate signaling mechanism, and other corresponding proteins (Singh et al. 2011). Various stress tolerance genes are known to be regulated by endophyte during drought period, and the delayed expression of some of them, namely, TcCESA3, TcPR5TcTPP, and TcSOT, in roots and leaves has been reported (Bae et al. 2009). CAS protein and the quantity of CAS mRNA present in the thylakoid membrane associated with Ca^{2+}-sensing regulator also contribute significantly to endophyte-mediated drought tolerance to plant (Sun et al. 2010). Chinese cabbage plants in association with *Piriformospora indica* exhibit increased expression of drought-associated genes such as RD29A, CBL1, DREB2A, and ANAC072 specially by their leaves. *Arabidopsis* plants, inoculated with *Piriformospora indica*, show the expression of various stress-mediated genes, including ANAC072, CBL1, DEHYDRATION-FINGER1, HAT, 29A, etc., which confer drought tolerance (Sherameti et al. 2008). Under extreme saline condition, it has been reported that the biosynthesis of ABA and its mediated signaling pathways have been increased significantly in presence of endophytic microorganisms resulting in plant growth enhancement. Moreover, regulation of an ABA signaling pathway by halotolerant *Dietzia natronolimnaea*, responsible for salt stress resistance in *Triticum* plants, was proved through increased expression of *TaABARE*

(ABA-responsive gene) and *TaOPR1* genes (Ilangumaran and Smith 2017). Inoculation of endophytic *Trichoderma harzianum* in rice plants confers tolerance against abiotic stress through the upregulation of genes encoding for aquaporin, dehydrin, and malondialdehyde (Pandey et al. 2016). The induction of stress-related gene array in grapevine associated with PsJN bacteria increased earlier at higher levels along with increase in nutrient at lower temperature conducting carbohydrate metabolism (Fernandez et al. 2012). During the proper growth and development of *Dichanthelium lanuginosum* growing in extreme temperatures of Yellowstone National Park, stress has been reported to be due to its associated fungus, *Curvularia protuberata* (Márquez et al. 2007). Likewise, *Burkholderia phytofirmans* strain PsJN, an endophyte isolated from potato plant, demonstrated regulated expression of genes encoding for a cell surface signaling element, which allowed the bacteria to adjust their metabolic pathway according to changing environment (Sheibani-Tezerji et al. 2015).

22.3.4 Salinity Stress Tolerance

Symbiotic rhizobia and roots of leguminous plants significantly enhanced plant growth during severe salinity stress conditions in stress-sensitive plants (Dardanelli et al. 2010). Fungal endophytes have the property to colonize legumes, imparting salt resistance to the host plant by producing various secondary metabolites. Various flavonoid groups are important classes of compounds which mediate root interaction with fungi and plant defense against environmental pressure (Pimentel et al. 2006; Khan et al. 2011). In plants GA (gibberellic acid) secretion by endophytic fungus may increase the significance of phenylalanine ammonia lyase enzyme enhancing self-secretion of isoflavones by plant (Barnes and Jones 1984). Soybean grows poorly in saline soil, but fungal symbionts were found to be engaged in regulation of some physiological pathway and stress tolerance activity. When fungal endophytes are associated with plants during high-salinity stress, the plant shows improvement in almost all aspects such as dry and wet biomass, shoot growth, chlorophyll content, photosynthetic activity, etc. in comparison with non-endophytic plants (Khan et al. 2011). It was also proved that *Aspergillus fumigatus* enhanced increased elongation of the aerial part of soybean growing in saline conditions and also made the plants resistant to salt stress of 70 and 140 mM compared with control species (Khan et al. 2011). Resistance of plants to salinity stress is conferred by endophytes through alleviated secretion of various antioxidants such as tocopherol, ascorbate, catalase, superoxide dismutase, glutathione reductase, etc. (Rouhier et al. 2008). Endophytic bacteria *Piriformospora indica* confers salt stress resistance in barley through promoting the production of various antioxidative elements (Baltruschat et al. 2008). It has also been reported that plants in symbiotic association with inoculated *Fusarium culmorum* have no significant effect of salt stress like cell shrinkage as long as they are not treated with 0.5 M sodium chloride solution for 2 weeks (Rodriguez et al. 2008).

Reactive oxygen species (ROS) are the by-products of metabolic reactions released during mitochondrial electron transport chain. Basically, they are very unstable and reactive free radicals procured from O_2. They may also be produced by oxidoreductase enzymes and metal-catalyzed oxidation. ROS are important signaling components present within plants engaged with several activities like development, stress resistance, cell division, and apoptosis by promoting the improvement of the activities of corresponding genes. During abiotic stress conditions, ROS are highly synthesized which alter amino acid sequence and also degrade fatty acids, nucleotides, etc. finally contributing to oxidative stress (Gechev et al. 2006; Gill and Tuteja 2010). Some of the ROS, whose excess secretion is responsible for the damage of plant cell and even death of plant during drought period, are singlet oxygen, hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), and superoxide (Smirnoff 1993). Plants have evolved complex mechanism to defend themselves from the negative effects of extra ROS with the help of several complex strategies such as ROS-accumulating activity or indirect signaling pathway induced by several related hormones. Several enzyme-catalyzed and enzyme-independent molecules having antioxidative properties are engaged as *ROS scavengers* (revised in Miller et al. 2010; Scheibe and Beck 2011). In many recent studies, it has been reported that association of endophytes with host plant enhanced a much prominent ROS scavenging property within plants which are exposed to stress conditions. Such association may help in protection of biological components from the deleterious effects of ROS. It has also been reported that tomato plants colonized with endophytes produced lower range of ROS in comparison with control plant under drought stress condition (Azad and Kaminskyj 2016). Increasing activities of superoxide dismutase, catalase, and peroxidase within the leaves of plants inoculated with *Piriformospora indica* under extreme drought condition were also shown (Sun et al. 2010).

Several free-living and symbiotic bacteria inhabit the plant rhizosphere and positively regulate their development with yield by production of several growth-promoting regulators and by contributing in nutrient acquisition from soil (Nadeem et al. 2014). The important beneficial effects contributed by endophytes during salt stress conditions may include osmotic regulation, protection from toxic elements, phytohormone activities, and nutrient accumulation by plants contributing in the reduction of the detrimental impacts of salinity stress. Bacterial endophytes have contributed significantly in conferring plant growth under various stress conditions. Under salinity stress conditions, they exhibit the property of *absorption of osmolytes* and antioxidant components. Such osmolytes are known to be engaged with osmotic regulation by stabilizing various structures and organelles present within the cell. It has also been shown that the endophytes, *Bacillus* and *Arthrobacter* species, confer the absorption of proline in *Capsicum annum* under osmotic stress (Sziderics et al. 2007). Bacterization also enhanced the activities of stress-related genes in pepper in comparison with the nonbacterial-inoculated plants, which signify the role of both bacterial strains in alleviation of osmotic stress conditions. Bacterization of *Pseudomonas pseudoalcali* genes with glycine betaine has also been shown to promote the uptake of a component which has the potential to impart resistance in *Oryza sativa* sp. against salt stress (Jha et al. 2011). In the examination of proteomic profile of *B. napus* (canola) treated with *Pseudomonas putida* UW4 under salinity condition, Cheng et al. (2007) reported at the proteomics level that the gene array expresses the rate of increase of carbohydrate and protein synthesis, antioxidative activity, mobilization of metabolites within membranes, and pathogenic activities of proteins. Treatment of endophytic bacterium *Burkholderia phytofirmans* PsJN strain with wheat plants contributes to promote its antioxidant role reducing the effect of drought stress (Naveed et al. 2014). In a previous investigation, De Zélicourt et al. (2018) showed that *Enterobacter* sp. SA187, living in association with plant growing in desert, invades the outer membrane and internal tissues of *Arabidopsis* aerial portion and roots. Such type of interaction enhanced salt stress tolerance through the generation of bacterial 2-keto-4methylthiobutyric acid, modulating the plant ethylene signaling mechanism. This specific strategy was shown to be very beneficial in increasing the production of crops during salt stress period.

Bacterial endophytes also have the ability of balancing the primary *phytohormone levels* in plants which confer plant growth during stress under nutrient-limiting conditions. Many endophytic bacteria were also shown to produce several plant growth promoters specially IAA and GA. IAA are generally known as rooting hormone, and they promote plant growth significantly (Ali et al. 2009). In previous research, 51 endophytic bacteria present in ginseng stem were reported to produce IAA, and the particular isolates were classified as *Actinobacteria*, γ-*Proteobacteria*, *Firmicutes*, and α-*Proteobacteria* (Vendan et al. 2010). Miliūtė and Buzaitė (2011) also reported several endophytic bacteria present in the floret of apple which express IAA-producing activity in the range of 1.2–2.4 μg/mL. A particular endophyte, *Burkholderia kururiensis*, has also been reported to confer IAA-induced activities at the genomic level due to increased expression of corresponding genes in the rhizosphere of genetically modified rice plants (Mattos et al. 2008). Gibberellins are also known for their property of stimulating shoot growth. Co-inoculation of GA-producing bacteria, *Sphingomonas* sp. LK11, present in leguminous plant with seeds of tomato plant leads to increase in plant growth and chlorophyll amount present in plant (Khan et al. 2015). In previous literature, it was found that ABA-producing endophyte, *Bacillus amyloliquefaciens* RWL-1, possesses the ability to induce salt stress resistivity in rice plants. Their symbiotic association highly promotes the activity of essential amino acids and helps in in planta secretion of salicylic acid in the plant exposed to salt stress conditions (Shahzad et al. 2017).

22.3.5 Heavy Metal Stress Tolerance

A number of fungi are known for their potential in imparting tolerance to heavy metal stress (Ezzouhri et al. 2009; Oladipo et al. 2018; Chen et al. 2015; Hassan et al. 2017). This property is conferred to them because of their unique cell wall which has the potential to readily bind metal with the help of negative charges present on various functional groups like carboxylic acid, amine, or sulfydril phosphate in different cell wall components (Tiwari and Lata 2018; Ong et al. 2017).

Association of *Aspergillus niger* var. *tubingensis* Ed8 with Cr(VI) mainly in reduction and absorption process has also been reported in a recent study (Coreño-Alonso et al. 2014). In another case it was also observed that symbiotic relationship between *Trichoderma* sp. and chickpea resulted in significant relaxation in arsenic-induced stress in the host plant (Tripathi et al. 2013). Arbuscular mycorrhizal fungi are soil microorganisms having crucial role in plant physiology with notable significance providing many beneficial activities to host's plant. The property of AM fungi in imparting resistance to plants against heavy metals is influenced by various physical and biological factors (Meharg 2003). These include the plant and fungal species and their corresponding ecotypes, edaphic conditions, types and quantity of metal obtainable by the plant, and growth conditions of the host plant like solidity of root tissues and potency of light (Pawlowska and Charvat 2004). AM fungi establish a direct physical association between soil and plant roots, thereby increasing the root surface area of the plant (Tiwari and Lata 2018). As in case of PGPR (plant growth-promoting regulators), several signaling pathway are also postulated for the management and allocation of hazardous heavy metal within the root's tissues of plants in the presence of various arbuscular mycorrhizal fungi which may include accumulation of metal within cell wall and their storage inside the vacuoles of the fungal hyphae (Leyval et al. 1997). Siderophores also have a prominent contribution in metal sequestration in soil or into root apoplast.

Several studies have been reported where bacterial endophytes like *Pseudomonas*, *Bacillus*, *Methylobacterium*, and *Streptomyces* conferred heavy metal stress tolerance (Dary et al. 2010; Tiwari et al. 2016, 2017). These microbes are capable in conferring plant growth through alleviating the deleterious effects of heavy metals through limitation of the availability of metal elements in the ecology of various plants in the form of conjugates like siderophores, substrate for plant's growth, and microbial transporting agents (Rajkumar et al. 2010; Sessitsch et al. 2013). They have also developed various pathways to get rid of heavy metal components present in biosphere like transportation of metals within the membrane of cell's cytoplasm, their bio-absorption and accumulation in the cell walls, and their entrapment in vesicles and capsules which are later extruded by endocytosis or exocytosis. Detoxification of heavy metal is also carried out by some bacteria through oxidation–reduction reaction, and some of them can also be precipitated to some extent (Zubair et al. 2016). A previous study has reported a promising result from the interaction of As- and Pb-resistant *Bacillus* species and cadmium-repellant *Ochrobactrum* species having various plant growth-promoting activities with rice cultivar. Their association helps in the dilution of the hazardous effect of heavy metal and improvement in the maturation of the host plant (Pandey et al. 2013). Different types of rhizobacteria are known for their activity in the accumulation of metals and their role in conferring heavy metal stress tolerance to host plant by establishing symbiotic association with them (Thijs et al. 2017). They are also very beneficial in inducing biocontrol and bioremediation in polluted soil as they can be used in place of chemical additives to reduce the quantity of heavy metal present in soil to some extent (Mishra et al. 2017). It has also been mentioned in earlier research that interaction of *Brassica juncea* and *Lupinus albus* with metal-tolerant microbes in presence of thiosulfate conferred transfer and absorption of As and Hg inducing bioavailability, phytoextraction, etc. (Franchi et al. 2017).

22.4 Conclusions and Future Perspectives

A number of reports have emphasized the potential applications of endophytic microbes in agriculture and industries, based on their ability to synthesize plant growth-promoting substances like phytohormones and nutrients, plant secondary metabolites, enzymes, etc. under in vitro and in vivo conditions. Compared to the challenges involved in identification of microbes in the past, recent advances in genomic technologies have helped in making this process faster and easier. Moreover, the next-generation sequencing-based metagenomics have enabled the unraveling of microbial diversity of even the uncultivable endophytes. Further, the transcriptomics- and proteomics-based approaches can contribute in enhancing our knowledge of the plant–endophyte interactions. This knowledge would then be used to exploit these beneficial microorganisms in tackling the abiotic stress-related yield losses. Stress tolerance imparted to host plants by microbes is an eco-friendly strategy for improving crop productivity in a sustainable manner. However, there are still certain issues that need to be addressed, some of which have been discussed here. The endophytic population of aquatic plants has been largely unexplored. Also, the mechanism(s) by which microorganisms switch over from pathogenic to the endophytic lifestyle or adopt to endophytic mode in the host plant is still unknown. Further research in these areas would help us to tap the enormous potential of microbes in a more organized and efficient manner.

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Chapter 23
Dark Septate Endophytic Fungi (DSE) Response to Global Change and Soil Contamination

Federico N. Spagnoletti and Romina Giacometti

Contents

Abstract As a consequence of global change, distribution of species and interaction between organisms are altered. Organisms share their environment with hundreds of species, some of them displaying pathogenic, neutral, or benefic behavior. Due to the adapting ability of organisms to live in diverse natural scenarios, they present a wide array of responses to climate change and soil contamination. Some biological interactions, like plant–insect subjected to diverse environmental conditions, have been moderately well explained. However, plant–fungus associations have received less attention, particularly plant–dark septate endophytic fungi (DSE) relationship. Since DSE may reduce plant infection by pathogens, increase nutrient uptake, and reduce the detrimental effects of stressful environments allowing plant to establish in adverse environments, changes in plant–DSE interactions could have important consequences for ecosystem function. In this chapter, we summarize current knowledge on how global change, including anthropic contamination, global

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warming, concentration of CO_2 in the atmosphere, and drought or heavy rainfall events, affects plant–DSE interactions. Understanding the specific responses of DSE will allow us to focus on possible lines of research that in a near future will help to develop tolerance to climate change.

Keywords Global change · Heavy metals · Dark septate endophytic fungi · Plant–microbe interaction

Abbreviations

CAT	Catalase
Cd	Cadmium
CO_2	Carbon dioxide
Cs	Cesium
DSE	Dark septate endophytic fungi
H_2O_2	Hydrogen peroxide
HMs	Heavy metals
HO•	Hydroxyl radical
$O_2^{\bullet-}$	Superoxide radical
P	Phosphorous
Pb	Lead
POD	Peroxidase
SOD	Superoxide dismutase
Zn	Zinc

23.1 Introduction

One of the most daring challenges facing our society is global environmental change. This detrimental event is being caused by human activities enhancing the greenhouse effect that will continue impacting negatively on the environment. Some of the adverse effects are simply increasing atmospheric carbon dioxide (CO_2) concentration and temperature, as well as severe variations in rainfall and drought (NASA 2016). It is unquestionable that human activity had a quantifiable effect on climate of the planet, due to higher CO_2 levels produced by fossil fuel burning and land use (Kerr 2001).Worldwide researchers have studied the effects of elevated CO_2 concentrations and high temperature on plants, mainly analyzing plant growth changes and carbon fixation rate.

On the other hand, pollution of soils by heavy metals (HMs), as a consequence of activities such as mining, fossil fuel production, and agriculture, has become one of the most severe environmental issues (Ruttens et al. 2006). High levels of HMs detected in soil display harmful effects on ecosystems and agroecosystems particularly, putting at risk human health as they enter the food chain through HMs enrichment of drinking water and contaminated food (Cerbasi and Yetis 2001). However, it was reported that adapted HMs-tolerant microorganisms can improve plant development and nutrition in such contaminated soils (Ma et al. 2011).

The effects of global change and the presence of heavy metals in soil systems have been less studied. It is known that soil organisms not only interact between themselves but also with plant community in different ways, maintaining ecosystem functions, since they can shape landscape patterns of plants richness and abundance (Smith and Read 2010). In this chapter, we will focus on global environmental change and soil contamination research done on dark septate endophytic fungi (DSE) and their interaction with plants. Jumpponen (2001) described DSE as conidial or sterile ascomycetes with dark pigmentation, melanized septate hyphae, and microsclerotia that colonize the root epidermis and cortex inter- and intracellularly in the host roots without causing apparent negative effects. Though DSE fungi can be found worldwide, they are more common in environments subjected to stress, such as semiarid and arid ecosystems (Mandyam and Jumpponen 2005). Several authors suggested that their prevalence in those harsh environments implies that their role might be fundamental. Here, we present a thorough analysis of the

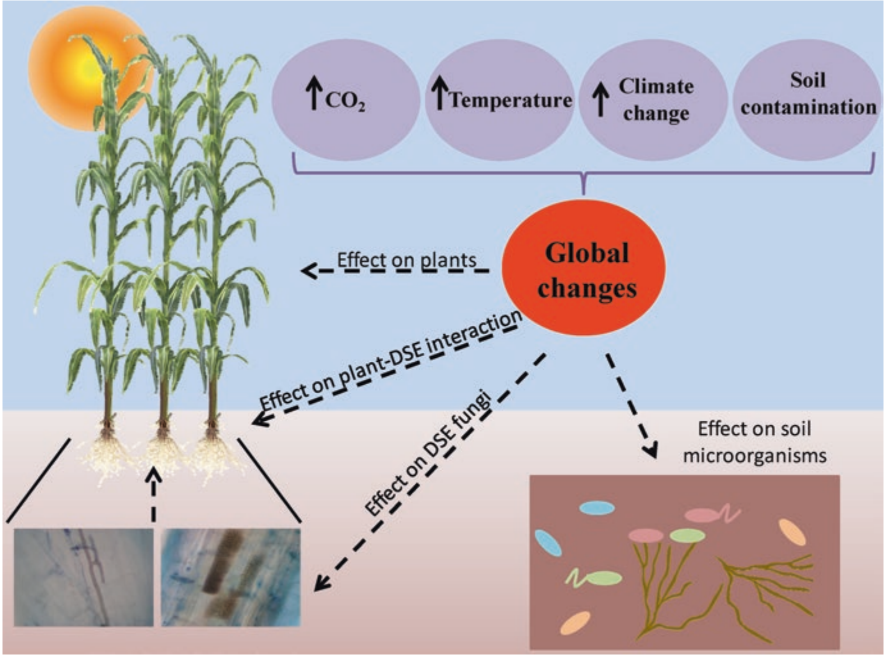

Fig. 23.1 Simplified scheme of the impacts of global change on DSE and plant–DSE interactions

knowledge to date on the effects of global change and heavy metal soil contamination on DSE and plant–DSE interactions (Fig. 23.1).

23.2 Dark Septate Endophytic Fungi (DSE)

All plants display symbiosis with fungal root endophytes (Rodriguez et al. 2009). Among this extremely diverse group of endophytic fungi, DSE fungi have received a lot of consideration in the current years. DSE are a group of cosmopolitan fungal endophytes that are sterile or conidial ascomycetes hosted by plant roots where they produce microsclerotia and dark septate hyphae (Berthelot et al. 2018). These types of fungi have been observed in more than 600 plant species spanning 100 plant families (Mandyam and Jumpponen 2005). DSEs are able to colonize both woody and herbaceous plants and more particularly roots of orchids, halophytes, macrophytes, or metal hyper-accumulators existing in several environments, for instance from artic and boreal to temperate, tropical, or arid ecosystems (Mandyam and Jumpponen 2005). Plant–DSE symbiosis has important distinction regarding arbuscular mycorrhiza since DSE fungi do not have specific intraradical structures for carbon and nutrients exchange, and more important responses of plants to DSEs are variable and seem to depend on the plant–DSE specific combination, so DSE may have a positive, neutral, or even negative impact on the host plant (Ruotsalainen 2018). However, numerous reports suggest that DSE in general may benefit their host plants by competing and reducing plant infection by pathogens (Barrow 2003; Terhonen et al. 2016), increasing the uptake of water or nutrients like nitrogen and phosphorous (Upson et al. 2009; Mandyam and Jumpponen 2015) and decreasing the detrimental effects of stressful environments (Sonjak et al. 2009; Jin et al. 2018). The relatively high presence of DSEs in stressed environments would suggest a significant play in host survival in these habitats (Likar and Regvar 2013). Although interest on the study of DSE has increased in the last years (Card et al. 2016; Spagnoletti et al. 2017), the knowledge on the molecular mechanisms taking place in the interaction process with plants is really scarce; so it is necessary to deepen these studies analyzing the function of DSE in different hosts and environments.

23.3 Impacts of Heavy Metals on DSE Fungi

The presence of HMs in soil leads to an important selection pressure that can drive the evolution of tolerant fungal ecotypes in metal-contaminated soils. Some authors have reported that fungal strains from uncontaminated sites show lower metal tolerance than strains isolated from metal-enriched environments (Carrillo-González and González-Chávez 2012). It is important to note that the tolerance to HMs corresponds only to the HM that pollutes the soil from which DSE were isolated. Results obtained by Xu et al. (2015) revealed that DSE isolated from HM-contaminated soil showed higher Cd tolerance in vitro than those from slightly polluted sites.

23.3.1 Mechanisms Involved in DSE Tolerance Against Heavy Metals

23.3.1.1 DSE Fungal Walls

One possible reason of DSE abundance in extreme environments may be due mainly to the natural components of DSE fungal walls, like chitin and melanin, which have the ability to increase wall rigidity and decrease permeability (Mandyam and Jumpponen 2005; Ban et al. 2012). Melanins are dark-brown or black pigments formed by oxidative polymerization of phenolic compounds. This pigment contains phenolic, carboxyl, amino, and hydroxyl groups rendering possible binding/biosorption sites for HMs (Fogarty and Tobin 1996). Melanin is frequently accumulated in hyphal walls under stressful conditions (Robinson 2001), and due to that, component fungal hyphae can defend itself from adverse temperatures, UV radiation, hydric stress, and high concentration of salts (Zhan et al. 2011). Years ago, some studies have shown that fungal melanin has a capacity to bind HM ions (Bruenger et al. 1967). More recently, it has been found that melanin content on DSE fungal wall increases in correlation with Cd or Pb concentration (Zhang et al. 2008; Zhan et al. 2011; Ban et al. 2012). In addition, DSE produce melanized cell walls inside the host's roots, creating complexes with oxygen radicals formed during stress, resulting positive to host growth and survival in stressful environments (Redman et al. 2002). Nevertheless, little is known about the role of fungal melanin in the metal tolerance of DSE fungi. For instance, Zhan et al. (2016) showed that melanin protects DSE against Cd; however, the melanin content did not have any effect on metal accumulation in DSE biomass.

23.3.1.2 Changes at Biochemical Level

Several reports have shown that HMs induce oxidative stress by generating elevated concentrations of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), and superoxide radical $O_2^{\bullet -}$, causing unbalanced cellular redox status and leading to lipid peroxidation and fungal membrane damage. To counteract oxidative stress, the fungal antioxidant defense mechanism triggers a nonenzymatic antioxidant system, in which ascorbate, carotenoids, and glutathione (GSH) play a role; also an enzymatic antioxidant system counts on the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) (Ban et al. 2012).

Glutathione is the main HM-responsive thiol, protecting cells subjected to oxidative stress (Pócsi et al. 2004). Although there are few studies that analyzed the levels of glutathione in the DSE fungi subjected to HMs, it has been found that the content of glutathione was increased in DSE fungi exposed to Pb (Ban et al. 2012) and Zn (Diao et al. 2013).

On the other hand, antioxidant enzymes such as SOD and CAT are vital for cellular detoxification, regulating the levels of O_2^- and H_2O_2. Studies carried out by several authors found that the tolerance of DSE (*Exophiala pisciphila* and *Gaeumannomyces cylindrosporum*) to HMs could account for a complex process where SOD and CAT activities play an important role, with positive correlations with Cd and Pb concentrations (Zhang et al. 2008; Ban et al. 2012). Moreover, Diao et al. (2013) found that the activity of SOD and POD enzymes in two DSE fungal strains (*Exophiala* spp.) increased quickly with the addition of higher Zn concentrations. Presumably, these antioxidant enzymes play efficient roles in protecting the DSE fungi from the oxidative stress induced by high HMs concentrations. Mechanisms of tolerance to HMs in DSE fungi are extraordinarily complex; thus further studies are needed.

23.3.2 Association with DSE Fungi in Heavy Metal Contaminated Soils

Recently, Newsham (2011) published a meta-analysis about the effects of DSE on their hosts and exposed that DSE colonization has positive impacts for the plant. This study supports the idea that DSE fungi are related with improving plant development. However, more recently, Mayerhofer et al. (2013) suggested negative to neutral effects of DSE colonization on hosts biomass and nitrogen content. All published articles in the field seem to present contradictory data on DSE effects on their hosts in HMs-contaminated soils, though some works showed that DSE inoculation may enhance the nutritional status of the hosts under heavy metal presence. For instance, Usuki and Narisawa (2007) revealed that DSEs can give nitrogen to the plant in exchange for photosynthates. Furthermore, a search article from our group showed that DSE are able to leave available phosphorous (P) for plant uptake, due to solubilization of insoluble P forms, such as calcium, aluminum, and iron phosphates (Spagnoletti et al. 2017), and produce hormones as auxin or organic compounds which may stimulate plant growth (Berthelot et al. 2016). All these benefits provided by DSE colonization could increase the tolerance of their hosts to adverse environmental conditions, such as HMs-contaminated soils.

Dark septate endophytes are particularly frequent in heavy metal-contaminated soils (Zhang et al. 2013; Ban et al. 2015). Despite challenges associated with high levels of HMs, DSE colonization was found in these stressful soils and presented HMs tolerance, suggesting that DSE have evolved to adapt to harsh environments. There are several DSE species that were identified in soils contaminated with heavy metals. For instance, *Cadophora* sp., *Leptodontidium* sp., *Exophialapisciphila*, and *Phialophora* sp. were isolated from Pb-, Cd-, and

Zn-contaminated soils (Zhang et al. 2008; Ban et al. 2012; Likar and Regvar 2013; Berthelot et al. 2018). Several studies have demonstrated that plant responses to DSE inoculation vary according to the DSE strain and their host. Ban et al. (2015) found that DSE colonization was negatively correlated with translocation of Pb, being accumulated in six different plant species growing on Pb–Zn mine tailings. It was also suggested that the colonization of DSE may cause the accumulation of Zn and Pb in roots (Ban et al. 2015). On the other hand, Diene et al. (2014) analyzed the potential use of DSE in phytoremediation for cesium (Cs) and found that the presence of DSE reduced metal accumulation in inoculated tomato plants but improved phytoremediation of Cs by Chinese cabbage. Furthermore, other authors showed that the inoculation of *Salix caprea* with DSE fungi decreased HM uptake and enhanced the physiology of their host plants by increasing chlorophyll concentrations and transpiration rates (Likar and Regvar 2013; Berthelot et al. 2017).

Several reports have shown that DSE could take over and sometimes substitute arbuscular mycorrhizal fungi in contaminated soils (Regvar et al. 2010; Deram et al. 2011). For example, Deram et al. (2011) found that arbuscular mycorrhizal association was reduced when Cd concentrations augmented in the soil, while DSE colonization was not affected in *Arrhenatherum elatius*. Nevertheless, the strategies underlying DSE tolerance to extreme conditions have not been studied in depth, mainly at the molecular level.

DSE inoculation/colonization induces biochemical changes in plant roots. An increasing number of reports suggest that reinforcing the antioxidant activity of host plants by interaction with beneficial microorganisms is an efficient strategy to gain tolerance to HMs (Ma et al. 2016). At biochemical level, Zhu et al. (2018) found that inoculation of tomato leaves with two different DSE strains (*Phialophora mustea*) triggered the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase (POD), which reduced the membrane lipid peroxidation damage of plants caused by the presence of HMs.

In view of the fact that DSE fungi are common root endophytes, their effects on host plants should be taken into account and considered advantageous. As we expressed above, DSE fungi can influence the HM uptake protecting the host plants and improve their tolerance to HM stress. Nevertheless, few researchers studied whether the mitigation of HM plant toxicity can be accredited to DSE fungi per se or to the consequence of the interactions between DSE strain and host plants. The mechanisms by which DSEs assist their hosts to improve HM tolerance could be more significant than the operating self-protection mechanism.

23.4 Dark Septate Endophytes in Drought Environments

Drought conditions are a severe environmental problem, affecting more than 45% of agriculture soils (Singh et al. 2012). Drought stress frequently takes place overlapping rapid growth and grain-filling stage, leading to a significant loss (10%) in total grain output worldwide (Celebi et al. 2010). It is known that this kind of stress was increased in the last years, as a result of human activities and climate change (Gong et al. 2013). While plants display enhanced drought resistance directly by means of changed physiology and morphology, indirect plant responses to drought through fungal endophytes interaction have obtained less attention. To date, pure culture and molecular analyses showed that a variety of plant species in areas prone to drought stress have a diversity of DSE fungi association (Lugo et al. 2009, 2015; Knapp et al. 2015). Studies using crops showed that DSE inoculation might augment the tolerance of their host plants to water deficiency. For example, Zhang et al. (2017) showed that drought had negative effects on DSE colonization in sorghum roots; drought stress reduced the hyphal spread, microsclerotia development, and dispersal of propagules for the DSE fungus *E. pisciphila*. Nevertheless, DSE inoculation enhanced plant height, collar diameter, and aerial biomass in sorghum subjected to drought conditions (Zhang et al. 2017). Another study carried out by Valli and Muthukumar (2018) also showed that DSE inoculation (*Nectria haematococca*) of tomato plants improved general plant growth parameters like height, stem girth, biomass, and leaf characteristics under drought stress.

Several possible reasons can explain the enhanced growth of host plants inoculated with DSE. The first could involve phytohormone production by DSE fungi or their indirect regulation affecting their synthesis. It is known that DSEs improve hormone signal transduction and hormone-regulated gene expression in their host plants (Andrade-Linares et al. 2011) and affect the regulation of plant hormones under scarce water availability (Zhang et al. 2017). Second, DSE may increase the nutrient uptake of host plants. Several authors have shown that the presence of DSE fungi in the rhizosphere creates a fungal network linked with a mucilaginous complex on the plant root surface that might improve nutrient transport (mainly nitrogen and/or phosphorus) in plants subjected to drought stress (Barrow 2003; Newsham 2011). Moreover, Li et al. (2018) found that DSE inoculation promoted the uptake of potassium in the roots of an evergreen broad-leaf shrub (*Ammopiptanthus mongolicus*). Potassium plays a critical function in the opening and closing of stomata in plant leaves, and its deficit can affect plant–water relation; hence, the higher tolerance to drought found in DSE-inoculated plants could be partially linked to a higher content of potassium in plant tissues. The third reason implies the maintenance of water balance between availability and demand during soil drought. Zhang et al. (2017) indicated that DSE inoculation in sorghum led to opening of stomata, enhancing transpiration fluxes, improving gas exchange and photosynthesis. Therefore, literature on DSE–plant interaction simply says that host plants tolerate adverse environmental conditions more than non-inoculated plants due to their capacity to maintain higher water condition and a better stomata regulation. Another possibility could be related to the promotion of plant secondary metabolism which is one of the most robust defense mechanisms. Some researchers found that DSE inoculation improved the content of flavonoids, anthocyanin, and phenols in *Saussurea involucrata* seedlings and in sorghum under drought conditions (Wu et al. 2010; Zhang et al. 2017).

Finally, another physiological response found in many plants under drought conditions is proline accumulation. Usually, plant proline content augments under adverse conditions to maintain the osmotic potential and is a consistent indicator of environmental stress. In this sense, it was shown that proline content in roots and shoots of tomato was increased in DSE-inoculated plants showing more proline in their tissues than non-inoculated plants under water-limiting conditions (Valli and Muthukumar 2018).

23.5 Dark Septate Endophytes and Heavy Rainfall Events

As mentioned previously, there is a consensus opinion on how global warming will increase extreme weather events, mainly as heavy rainfalls (Shepherd 2015). Nevertheless, available studies on the effects of heavy rainfalls on soil ecosystem are limited. It is known that water saturation of soils can reduce its oxygen levels, influencing the diversity of soil microorganisms and changing plant productivity leading also to plant death. Studies carried out with arbuscular mycorrhizal fungi or saprophytic fungi showed that they are not affected by waterlogging conditions or even thrive in this stressed environments (Yang et al. 2016). However, fungi display an array of responses to variations in soil water conditions. For example, some ectomycorrhizal fungi cannot survive in waterlogging soils (Coutts and Nicoll 1990; Tedersoo et al. 2009). Barnes et al. (2018) showed that extreme rainfall events in the UK caused a considerable reduction in the relative abundance and richness of the ectomycorrhizal fungal community and a simultaneous augment in the relative abundance and richness of saprophytes and pathogens. Nevertheless, those authors did not detect any changes in fungal endophytes community. Therefore, the study of the way in which heavy rainfalls affect DSE fungi remains unknown.

23.6 Dark Septate Endophytes in CO_2-Enriched Environments

Several authors have exposed that the rising level of atmospheric CO_2 will have substantial impacts on plant growth and the functioning of ecosystems. Sieber and Grünig (2006) suggested that elevated CO_2 as a consequence of anthropic activities could influence the effects of DSE fungi on their host plants but observed that there is no information about the direction and magnitude of potential effects. Most recently, Alberton et al. (2010) analyzed the effects of different DSE strains on growth and nutrient acquisition by *Pinus sylvestris* seedlings under conditions of elevated CO_2 and found that DSE inoculation had a positive (*S. vaccinii* and *M. vraolstadiae*) or neutral (*Phialocephala fortinii*, *Cadophora finlandica*, *Chloridium paucisporum*, and *Meliniomyces variabilis*) effect on plant biomass under elevated CO_2. Results regarding the relationship between DSE fungi and their hosts show a large variation, revealing that the ecological function of DSE fungi is still poorly understood. Alberton et al. (2010) exposed that DSE did not increase nutrient uptake under elevated CO_2 conditions. DSE inoculation decreased nitrogen content of the pine seedlings, and since elevated CO_2 levels allowed the DSE to generate more fungal biomass, these results suggest high competition for nitrogen between DSE and the host (Alberton et al. 2010). Therefore, a possible mechanism underlying plant biomass enhancement, even when plant nutrient uptake declines, is the production of phytohormones by DSE fungi (Schulz and Boyle 2005; Alberton et al. 2010). The meta-analysis carried out by Kivlin et al. (2013) showed that high atmospheric CO_2 levels increased plant biomass, despite the presence of fungal symbionts, and that their presence did not affect plant response to CO_2 concentrations. Further studies are desired in order to fully comprehend the functional basis of DSE under high CO_2 concentrations.

23.6.1 Dark Septate Endophytes in Warming Conditions

Several authors have exposed that increases in soil temperature can influence a large number of soil and plant parameters, as nutrient availability, water status, plant phenology, root growth and architecture, respiration rate, and nutrient uptake; however, the effects on soil fungi are diverse and in some cases unpredictable (Fujimura et al. 2008).

In general, warming has low effect on plant biomass, although plant biomass is improved in plants inoculated with DSE fungi. As expected, the increase in temperature accelerates the metabolism of both DSE and host, enhancing growth, potentiating the symbiosis, as well as increasing nutrient and photosynthates exchange. Possibly, under higher temperatures, fungal symbionts will supply their hosts with a larger net of benefits due to their ability to provide nutrients from soil to hosts (Hawkes et al. 2008).

23.7 Conclusion

DSE fungi occur to be present in a variety of biomes, exposed to all sorts of climates, and are relatively common in extreme and stressed terrestrial ecosystems. This chapter is the first to summarize our knowledge on the impact of global change and soil contamination on DSE fungi and their interaction with host plants, in terms of their function and ability to improve the tolerance of plants to stressful conditions. We show that DSE responses to human activity are extremely context dependent, leading to a complex interpretation of results. In this chapter we explain that plant responses cannot be directly related with global warming or soil contamination, since detected responses can be the result of interactions influenced by other microorganisms like DSE fungi. We show that DSE fungi have the ability to improve plant tolerance to heavy metals and some to global changes. Human impacts present direct and indirect effects affecting DSE fungi and plant–DSE interaction, respectively. It is important to mention that responses to those impacts are vital to comprehend as they can affect numerous ecological process. As we mentioned, global change and other anthropic activities affect all soil organisms and physical soil properties; therefore, plant–DSE fungi symbiosis cannot be analyzed by itself. Since DSE fungi play significant roles in the soil system, more in-depth studies on their potential effects on plants subjected under stressed environments are consequently required.

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Chapter 24
Can Mycorrhizal Symbiosis Mitigate the Adverse Effects of Climate Change on Crop Production?

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Contents

Abstract Climate change, growing world population, and finite natural resources represent a sizable challenge to global food security. Increased temperature associated with more extreme rainfall, especially in the tropics, alters the relationships between crops, pests, pathogens, and weeds resulting in increased use of pesticides. This may promote contamination of agricultural soils by xenobiotics and potentially toxic metals (PTM). At the same time, aridity levels in many regions of the planet increase progressively, which will potentiate problems with drought and soil salinity. This situation harms the future sustainability and resilience of agricultural crops. Soil microorganisms perform various functions in the soil, improving plant nutrition

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and health, as well as soil quality, even in disturbed environments. Many economically important agricultural crops establish mutual symbiosis with arbuscular mycorrhizal fungi (AMF). AMF improvement to plants is derived from nutritional and nonnutritional effects. Such effects can ensure better yields in environments altered by climate change. In this chapter, we wish to highlight the potential of AMF for the maintenance of agroecosystems in environments modified by climate change, whether under drought stress, salinity stress, or soil contaminated by PTM or xenobiotics. AMF are ubiquitous, adapt to a wide variety of adverse environments, and therefore provide important ecosystem services. Under stress conditions, these fungi can improve water and nutrient uptake, plant growth and grain yield, and photosynthetic rate and promote biochemical and molecular changes in plant metabolism so that plants become more tolerant to biotic (pests, pathogens, nematodes) and abiotic (drought, salinity, PTM) stresses. In addition, AMF stimulate other microbial communities, increasing C sequestration in soil and, consequently, contributing to improvements in soil structure. In this scenario of climate change, exploring the wide range of these ecosystem services and their interaction with agricultural crops against finite natural resource and need to increase food production at the lowest environmental costs is a relevant question to ask. The results make evident the feasibility of the AMF application in drought conditions, high salinity, and in PTM- or xenobiotics-contaminated soils. The application of microbial biotechnology in agriculture has promoted many achievements; however, many barriers still have to be transposed for future sustainable agricultural development. From this, it is expected to be possible to exploit the AMF improvements to reduce agricultural inputs and promote food production even in the face of the climate change challenges.

Keywords Drought stress · Salinity stress · Xenobiotics · Potentially toxic metals · Arbuscular mycorrhizal fungi

24.1 Introduction

Climate change concept, widely discussed in recent years, encompasses changes in the climate system characteristics, compared to previous long periods of time, regardless of cause. It is possible that such changes may affect carbon dioxide concentration in the atmosphere, temperature, precipitation patterns, crop productivity, soil quality, and plant diversity on the planet (Altieri et al. 2015; Delcour et al. 2015; Knox et al. 2016).

The imminent climate change associated with an increasing world population and finite natural resources poses a considerable challenge to global food security (Thirkell et al. 2017). Temperature increase associated with more extreme rainfall, especially in the tropics, alters the relationships between crops, pests, pathogens, and weeds, resulting in greater use of pesticides and can promote the contamination of agricultural soils by xenobiotics. These risks raise concerns about the future sustainability and resilience of agricultural crops in metal- and pesticide-contaminated soils, under excess salts or in environments with drought stress (Altieri et al. 2015; Myers et al. 2017).

Soil microorganisms perform various functions in soil, including in agricultural areas, improving plant nutrition and health, as well as soil quality, even in disturbed environments (Barea 2015). Thus, effective exploitation of beneficial microbial services should comprise environmentally friendly strategies to mitigate the negative effects of climate change. Many economically important agricultural crops establish mutual symbiosis with arbuscular mycorrhizal fungi (AMF), and this has motivated the development of new approaches of the agricultural practices to stimulate the AMF native populations' development (Thirkell et al. 2017). AMF improvements to plants are derived from nutritional and nonnutritional effects and are, therefore, categorized into bio-fertilizing, bio-regulating, and bio-protective effects. The bio-fertilizer action is the main and precursor of the others (Siqueira and Klauberg Filho 2000). Such effects can ensure better yields in agricultural environments altered by climate change.

In this chapter, based on the recently available research data, we highlight the AMF potential as an important component for the agroecosystems' maintenance in environments modified by climate change, whether they are drought stress, contaminated soils by potentially toxic metals and xenobiotics, or with higher salinity.

24.2 Mycorrhizal Symbiosis

The arbuscular mycorrhizal association is a mutualist symbiosis performed between higher plants and AMF, which colonize the radicular cortex, intracellularly (Claassens et al. 2018; Guerrero-Galán et al. 2018). AMF belong to the phylum Glomeromycota, and its species are included in the orders Glomerales, Diversisporales, Archaeosporales, and Paraglomerales (Redecker et al. 2013). These fungi establish mutualism with another 80% of the terrestrial vegetal ecosystem (Schmitz et al. 2019). Likewise, most of the plants are colonized simultaneously by several AMF species, which are interconnected by the network of extra-radicular mycelium in the soil (Zeng et al. 2018). Mutualism occurs as a result of the greater nutrient uptake from the soil by the fungus to the plant, and, in its turn, the plant directs to this microorganism sugars and lipids. AMF are obligatory biotrophic; therefore, they need this symbiosis to complete your life cycle (Aloui et al. 2018; Guerrero-Galán et al. 2018; Luginbuehl and Oldroyd 2017; Rich et al. 2017).

AMF have important functional structures that characterize the functionality of the symbiosis: arbuscules, hyphae, vesicles, and spores (Claassens et al. 2018); however, not all species produce vesicles (Guerrero-Galán et al. 2018). In the pre-symbiotic phase, one of the observed structures is the fungal appressoria, which penetrates the radicular cortex and, from the hyphae, develops the intra-radicular mycelium. In subsequent stages, this mycelium will give rise to the arbuscules and vesicles (Yurkov et al. 2015). When intracellular colonization is established, the extra-radicular mycelium formation begins (Guerrero-Galán et al. 2018), which uptakes nutrients and water from the soil solution (Aloui et al. 2018; Li et al. 2013).

In intracellular colonization, only the cell wall is penetrated by fungal hyphae, limited to the plasma membrane (Guerrero-Galán et al. 2018). This membrane, however, recognizes the AMF presence, preparing for its colonization by the biochemical signal transduction. Thus, around the arbuscules, the periarbuscular membrane is formed with transporting proteins responsible for affecting the nutrient and molecule exchange (Aloui et al. 2018; Dormann et al. 2014). Aloui et al. (2018) studied 82 membrane-bound proteins and symbioses, which are, largely, sugar carriers and, apparently, participants in lipid synthesis. The authors identified the sucrose transporter SUT2, which is one of the determinants of nutrient supply to the fungus. There are plant proteins with nutritional connotations in the symbiosis, as observed per Luginbuehl et al. (2017) that identified in the protein RAM2 a function of providing fatty acids to AMF.

After mycorrhizal symbiosis establishment, the extra-radicular mycelium formation expressively increases the zone of nutrient uptake by the plants. This AMF improvement is widely known as a bio-fertilizer function. In *Glycyrrhiza uralensis* under low level of soil nutrients, Chen et al. (2017b) verified that *Glomus mosseae* inoculation increased K content in shoots (+97%) and P in roots (+139%). Yurkov et al. (2015) observed greater P uptake already in the first stage of AMF colonization in *Medicago lupulina* plants. Zhu et al. (2016) showed that *Rhizophagus irregularis* inoculation improved N and C uptake in wheat plants (*Triticum aestivum*).

The bio-fertilizer function is characterized as the most reported ecosystem service in AMF researches around the world. Nonetheless, AMF improvements to plants may also result from nonnutritional effects known as bio-regulatory and bio-protective functions, which are secondary and, generally, result from the effects of bio-fertilizer function. In the bio-regulatory function, AMF can favor the production of growth substances and promote biochemical and physiological changes in plants to increase their tolerance through some stress. On the other hand, in the biocontrol action, these fungi act to improve soil aggregation, reducing biotic and abiotic stresses, such as contaminants and salts excess in the soil (Siqueira and Klauberg Filho 2000). Table 24.1 shows the range of ecosystem services achieved by arbuscular mycorrhizal fungi (AMF) on plant species and ecosystems.

24.3 Salinity Stress

Aridity levels in many regions of the planet increased progressively, which potentiated the problems of drought and soil salinity. In these regions, climate change could further intensify the salinity problems (Barea 2015), including those in agricultural areas. Salinity stress affects crop growth and productivity. Fortunately, research has shown that AMF reduces the negative effects of salinity by increasing growth rate and physiological processes and maintaining ionic cell balance (Borde

Table 24.1 Published research identifying the extent of ecosystem services performed by arbuscular mycorrhizal fungi (AMF) on plant species and ecosystems

Ecosystem services	Published research
Nutrient and water uptake improvement	Burducea et al. (2018), Chen et al. (2017b), Ebrahim and Saleem (2018), Gomes et al. (2014), Haque and Matsubara (2018), Jespersen et al. (2019), Liu et al. (2015), Sun et al. (2018), Yurkov et al. (2015), Zhang et al. (2017), Zhu et al. (2016)
Plant growth and grain yield improvement	Ahmad et al. (2018), Burducea et al. (2018), Chen et al. (2017b), Cobb et al. (2016), Fileccia et al. (2017), Hashem et al. (2019), Lehnert et al. (2018), Lin et al. (2018), Rozpadek et al. (2016), Sun et al. (2018), Zeng et al. (2018), Zhang et al. (2017)
Molecular and biochemical changes	Burducea et al. (2018), Chen et al. (2017b), Hashem et al. (2018), He et al. (2017), Jespersen et al. (2019), Kobae et al. (2018), Lehnert et al. (2018), Lin et al. (2018), Luginbuehl et al. (2017), Mohammadi-Dehcheshmeh et al. (2018), Rivero et al. (2018), Rouphael et al. (2015), Rozpadek et al. (2016), Volpe et al. (2018), Zeng et al. (2018)
Biotic stress tolerance	Anda et al. (2015), Ceustermans et al. (2018), He et al. (2017), Volpe et al. (2018)
Xenobiotic tolerance	Chhabra and Jalali (2013), Helander et al. (2018), Huang et al. (2007), Joner and Leyval (2003), Lenoir et al. (2016), Makarian et al. (2016), Nivelle et al. (2018), Pánková et al. (2018), Wu et al. (2008)
Drought tolerance	Duc et al. (2018), Ganjeali et al. (2018), Lehnert et al. (2018), Liu et al. (2018a), Mathur et al. (2019), Moradtalab et al. (2019), Sun et al. (2017), Symanczik et al. (2018), Wang et al. (2017b)
Metal tolerance	Aguirre et al. (2018), Chang et al. (2018a), Chen et al. (2019), Christie et al. (2004), Debeljak et al. (2018), Gai et al. (2018), Gomes et al. (2014), Hristozkova et al. (2017), Liu et al. (2018b), Shahabivand et al. (2016), Sharma et al. (2016, 2017), Spagnoletti et al. (2016), Sun et al. (2018), Vilela et al. (2018a), Zhang et al. (2018)
Salinity tolerance	Aguirre et al. (2018), Ahmad et al. (2018), Borde et al. (2017), Debeljak et al. (2018), Ebrahim and Saleem (2018), Gai et al. (2018), Haque and Matsubara (2018), Hashem et al. (2019), Hristozkova et al. (2017), Huang et al. (2018), Lin et al. (2018), Ren et al. (2019)
Soil C sequestration	Malekzadeh et al. (2016), Rillig and Mummey (2006), Vilela et al. (2014)
Soil aggregation	Muchane et al. (2019), Rillig and Mummey (2006), Vilela et al. (2014)

et al. 2017; Lin et al. 2018). Table 24.2 highlights some results of the last 5 years on the contributions of AMF to plants under salinity stress.

Although it varies according to plant species, plant growth parameters are strongly inhibited by excess salinity in the soil. However, AMF can mitigate these effects by promoting both root and shoot growth. The *G. mosseae* inoculation in *Citrus tangerine* increased the stem diameter (+14%), plant height (+6%), shoot biomass (+37), and root biomass (+31%) in 100 mM NaCl. In contrast, inoculation with *Paraglomus occultum* promoted increases of 15%, 9%, 37%, 33%, and 34%, respectively (Wu et al. 2009). Abdel Latef and Chaoxing (2011) subjected tomato plants (*Solanum lycopersicum*) to salinity levels (0, 50, and 100 mM NaCl) and

Table 24.2 Results of recent research on the contributions of arbuscular mycorrhizal fungi (AMF) to plants under saline stress

Plant specie	AMF species[a]	Salinity levels	Main results	References
Zea mays	*Glomus mosseae* and native species	2.0, 2.5, 3.5, 5.0, 8.0, 12.0 dS m^{-1} of Hoagland's Solution (NaCl, Na_2SO_4, $CaCl_2$ and $MgSO_4$—7:9:3:1 ratio, respectively).	Root and shoot dry weight of inoculated plants was higher than control plants, even with increasing salt concentration	Boyacıoglu and Uyanöz (2013)
			G. mosseae promoted greater benefit in plant growth than native AMF	
			Saline stress did not reduce colonization of *G. mosseae*; however, it reduced spore density at 12.0 dS m^{-1}. In native AMF, salinity did not affect these parameters	
			At the end of the study, the reduction of mycorrhizal colonization was proportional to the increase of salinity. Native AMF showed more spores than *G. mosseae*	
			G. mosseae had higher K content in shoot and roots at all salinity levels	
			P content was higher in plants inoculated at 2.5, 3.5, and 12.0 dS m^{-1}	
			G. mosseae inoculation and salt addition increased P content in the shoot, while native AMF increased P content in the roots	
Vigna unguiculata	*Funneliformis mosseae*, *Rhizophagus intraradices*, and *Claroideoglomus etunicatum*	0 and 200 mM NaCl	Inoculation mitigated the negative impact of salinity. At 200 mM NaCl, AMF increased shoot height, shoot dry weight, leaf area, *a* and *b* chlorophyll, carotenoid, and total pigment contents. In addition, inoculated plants had higher leaf water content, membrane stability index, and higher proline and malondialdehyde levels	Abeer et al. (2015)
			AMF prevented excess Na^+ uptake and simultaneously increased the antioxidant enzymes' activity, improving the reactive oxygen species scavenging. Moreover, AMF has also increased the mineral elements uptake	
Solanum lycopersicum	*Glomus fasciculatum*	0, 25, 50, 100, and 200 mM NaCl	AMF inoculation attenuated the adverse effects of salinity. It increased leaf contents of N, P, K, and Mg and reduced Na. There was also an increase in *a* and *b* chlorophyll concentration and reduction of the enzyme activity of the antioxidant defense system (superoxide dismutase, catalase, and peroxidase)	Ebrahim and Saleem (2018)
Triticum durum	Mixture of *Rhizophagus irregularis* and *Funneliformis mosseae*	0 and 10 g Na L^{-1}	AMF inoculation increased shoot biomass (+5%), root biomass (+14%), and N absorption (+18%). In addition, there was an increase in the SPAD index, even under saline stress	Fileccia et al. (2017)

Plant specie	AMF species[a]	Salinity levels	Main results	References
Cucumis sativus	*Glomus versiforme*	100 mmol NaCl L^{-1}	AMF inoculation improved the growth parameters in the two cultivars tested, with more pronounced results in the salinity-tolerant cultivar. Even so, the inoculation of the salinity-sensitive cultivar improved shoot length (+10%), root length (+0.1%), shoot fresh weight (+16%), root fresh weight (+57%), shoot dry weight (+35%), and root dry weight (+63%). For the tolerant cultivar, the increments were 12%, 17%, 16%, 82%, 31%, and 70%, respectively	Ahmad et al. (2018)
Fragaria × ananassa	*Gigaspora margarita*	0 and 200 mM NaCl	Salinity reduced mycorrhizal colonization. However, mycorrhizal plants had greater shoot and root dry weight	Haque and Matsubara (2018)
			Mycorrhizal plants had no reduction in chlorophyll content as occurred in control plants under saline stress	
			Na accumulation and Na^+/K^+ ratio were lower in mycorrhizal plants than in control plants submitted to salinity	
Cucumis sativus	*Claroideoglomus etunicatum*, *Rhizophagus intraradices*, and *Funneliformis mosseae*	0 and 200 mM NaCl	Salinity reduced the number of mycelium (−55%), vesicles (−61%), and arbuscules (−72%)	Hashem et al. (2018)
			Even under salinity stress, AMF inoculation increased content of *a* chlorophyll (+27%), *b* chlorophyll (+18%), total chlorophyll (+27%), carotenoids (+42%), proline (+24%), and total phenol (19%). Moreover, it increased the stomatal conductance and relative leaf water content. On the other hand, it reduced the content of hydrogen peroxide (−38%), lipid peroxidation (−21%), and electrolyte leakage (−34%) in cucumber cells	
Solanum lycopersicum	*Claroideoglomus etunicatum*, *Rhizophagus irregularis*, and *Funneliformis mosseae*	0, 75 and 150 mM NaCl	AMF inoculation attenuated the impact of saline stress and all fungal species-colonized tomato roots	Rivero et al. (2018)
			C. etunicatum obtained higher mycorrhizal growth response than *R. irregularis* and *F. mosseae* at both salinity levels	
			Roots colonized by AMF had lower leaf Na^+ concentrations than those not inoculated, even under severe stress. In this aspect, *C. etunicatum* was more efficient in protecting the plants against the Na^+ excess	
			Nontargeted metabolic analysis showed that plants inoculated with *R. irregularis* and *F. mosseae* had metabolic responses overlapping with salt stress, with profiles obviously separate from those of non-colonized plants. More evident alterations occurred in plants colonized with *C. etunicatum* that exhibited distinct metabolic rearrangement, grouping separately from any other experimental condition	

(continued)

Table 24.2 (continued)

Plant specie	AMF species[a]	Salinity levels	Main results	References
Zea mays	*Glomus tortuosum*	0, 150, and 300 mM NaCl	At 150 mM, AMF inoculation suppressed the negative effects of salinity on leaf area and dry mass, which did not occur at the 300 mM dose	Xu et al. (2018)
			In all salinity conditions, AMF inoculation attenuated the negative effects of stress favoring *a*, *b*, and total chlorophyll content	
			Salinity reduced drastically the net photosynthetic rate, transpiration rate, stomatal conductance, and rubisco activity. But in mycorrhizal plants, this reduction was less drastic	
Glycine max	*Funneliformis mosseae*, *Rhizophagus intraradices*, and *Claroideoglomus etunicatum*	0 and 200 mM NaCl	In both tolerant and salinity-sensitive cultivars, AMF inoculation attenuated the negative effects on nodule number, dry and fresh weight nodules, leghemoglobin content, nitrogenase activity, and chlorophyll and carotenoid content	Hashem et al. (2019)
			At 200 mM, the mycelia, vesicles, and arbuscules percentage in the salt-tolerant cultivar was greatly affected. In the salt-sensitive cultivar, these parameters were almost completely suppressed	

[a] AMF nomenclature used in the original paper

G. mosseae inoculation. This AMF increased shoot dry matter and leaf area in plants under salt stress with a more evident effect on shoot biomass. Furthermore, the fruit fresh weight and production under salt stress also increased.

AMF bio-fertilizer function mitigates salinity impacts on the soil. Through its hyphae, the fungus promotes greater nutrient and water uptake, making the plant more tolerant to stress (Djighaly et al. 2018). Increase in nutrient uptake was observed in cucumber (*Cucumis sativus*) inoculated with *Claroideoglomus etunicatum*, *R. intraradices*, and *Funneliformis mosseae* and spiked NaCl (gradually, 25 mM/day until 200 mM NaCl) (Hashem et al. 2018). Despite the salt stress, mycorrhized cucumber plants had higher K (+23%), Ca (+37%), Mg (+23%), Zn (+6%), Fe (+19%), Mn (+7%) and Cu (+25%) in the leaves than those not inoculated. Chang et al. (2018b) showed that *Elaeagnus angustifolia*, inoculated with *R. irregularis* under 300 mM NaCl, obtained a substantial increase in K, Ca, and Mg uptake and maintained a higher K/Na ratio, which is beneficial for the maintenance of the ionic balance of the cell (Borde et al. 2017). On the other hand, wheat plants (*T. aestivum*) inoculated with *R. irregularis* and submitted to 2 g NaCl kg^{-1} did not get a positive effect on K, Mg, and Ca leaf contents, which can be explained by the higher plant growth, causing nutrient dilution effect on shoot (Zhu et al. 2018). Also, the tomato (*S. lycopersicum*) reached a significant increase of Ca and Fe content under moderate salinity (50 mM NaCl) and a meaning reduction of K and Ca content under severe salinity (100 mM NaCl) (Balliu et al. 2015).

Soils with high salt concentration usually have lower water potential due to salt accumulation, resulting in the closure of the stomata. Therefore, osmotic adjustment is an important physiological mechanism responsible for the protection of plants against salt stress (Chen et al. 2017a). In this aspect, increase in water absorption promoted by AMF hyphae is highlighted (Mbodj et al. 2018). According to Chen et al. (2017a), *Robinia pseudoacacia* inoculated with *R. irregularis* obtained a 9% gain in the relative water content in relation to uninoculated plants in 200 mM NaCl. Djighaly et al. (2018) showed that inoculation with *R. intraradices*, *R. aggregatus*, and *R. fasciculatus* improved the water uptake of *Casuarina glauca* and *C. equisetifolia* submitted to 150 and 300 mM NaCl. Also, there was a higher water uptake in cucumber (AMF, *C. etunicatum*, *R. intraradices*, and *F. mosseae*—200 mM NaCl) (Hashem et al. 2018) and in pepper (AMF, *G. irradicans*—25 and 50 mM NaCl) (Hegazi et al. 2017).

According to Chen et al. (2017a), inoculation of *R. pseudoacacia* with *R. irregularis* improved the photosynthetic rate under 100 mM NaCl (+106%) and 200 mM NaCl (+81%). In addition, there was attenuation of stress in photosystem II, with an increase in quantum efficiency in electron transport, which resulted in higher photosynthetic capacity of plants under salt stress. Similarly, *C. glauca* and *C. equisetifolia* increased expressively the total chlorophyll content when inoculated with *R. fasciculatus*, improving its photosynthetic potential (Djighaly et al. 2018).

Salinity affects the photosynthetic activity because it reduces the pigment content in the leaves, impairs the electron flow in photosystems I and II, and reduces the light use efficiency (Chen et al. 2017a). Likewise, the increase of chlorophyllase activity promotes higher chlorophyll degradation, reducing drastically the photosynthetic rate. Increased nutrient uptake, such as Mg, by AMF hyphae may attenuate these effects and, thus, cause higher photosynthetic rates in mycorrhizal plants, even under high salt stress (Borde et al. 2017; Hashem et al. 2018). Chen et al. (2017a) showed that *R. irregularis* inoculation in *R. pseudoacacia* improved the photosynthetic rate under 100 mM NaCl (+106%) and 200 mM NaCl (+81%). Furthermore, there was attenuation of stress in photosystem II, with an increase in quantum efficiency in electron transport, which resulted in higher photosynthetic capacity of plants under salt stress. Similarly, *C. glauca* and *C. equisetifolia* increased expressively the total chlorophyll content when inoculated with *R. fasciculatus*, improving its photosynthetic potential (Djighaly et al. 2018).

Salinity stress also results in nutritional and ionic imbalance that result in oxidative stress, which occurs because excess salts trigger the reactive oxygen species (ROS) formation in the cell. ROS accumulation promotes oxidation of several molecules, such as lipids, proteins, and chlorophyll (Abeer et al. 2015; Li et al. 2017). Typically, ROS generation and accumulation are low in stress-free conditions. Thus, for its elimination, the plant has antioxidant defense mechanisms, which involve enzymatic and nonenzymatic components. The nonenzymatic antioxidant defense system includes molecules such as ascorbic acid, glutathione, phenolic compounds, alkaloids, tocopherol, carotenoids, etc. In the enzymatic system, on the other hand, among many enzymes, we highlight the superoxide dismutase, guaiacol peroxidase, glutathione peroxidase, catalase, glutathione reductase, ascorbate peroxidase, monodehydroascorbate reductase and dehydroascorbate reductase (Abdel Latef and Chaoxing 2011; Mbodj et al. 2018). The *F. mosseae*, *R. intraradices*, and *C. etunicatum* inoculation in *Ephedra aphylla* increased the antioxidant enzyme activities at all salt levels (75 and 150 mM NaCl). Compared to control, inoculated plants increased the activity of catalase (+52%), ascorbate peroxidase (+47%), peroxidase (+43%), glutathione reductase (+52%), and superoxide dismutase (+55%) (Alqarawi et al. 2014). Additionally, in *Vigna unguiculata* at 200 mM NaCl, inoculation with *F. mosseae*, *R. intraradices*, and *C. etunicatum* increased the activities of these same enzymes by 83%, 153%, 66%, 122%, and 52%, respectively (Abeer et al. 2015).

Proline accumulation is also a defense mechanism of plants under stress conditions. This organic solute is an osmolyte with important function in the establishment of the plant under salt stress because it acts in the cell osmotic adjustment. Researches show that AMF increases the proline concentration in the plants, through the greater activity of synthesizing enzymes of this osmolyte. This substance is mainly concentrated in the roots, for they are areas of primary contact with excess salts in the soil (Mbodj et al. 2018). Pollastri et al. (2018) found that the proline accumulation in *Arundo donax* was significantly higher in plants inoculated with *F. mosseae* and *R. irregularis* under saline stress (25–200 mM NaCl). In *V. unguiculata*, Abeer et al. (2015) showed that, in 200 mM NaCl, AMF inoculation increased proline synthesis by 26% compared to uninoculated plants. In corn, the results do not corroborate with the data previously mentioned. Sheng et al. (2011) found that maize under salinity (0, 0.5, and 1.0 g NaCl kg^{-1}) and *G. mosseae* inoculation showed lower proline concentration than non-colonized plants at all salinity levels. Similarly, basil (*Osmium basilicum*) under salinity stress (0, 5, and 10 dS m^{-1}) and *G. deserticola* inoculation showed significantly lower proline content, both shoot and root (Elhindi et al. 2017).

24.4 Potentially Toxic Metals

Hydrology fluctuations as a result of climate change will result in variations in land management, which will affect the release and leaching of potentially toxic metals (PTM) in the soil (van der Perk et al. 2018). Moreover, to ensure crop productivity in the face of climate change, it is likely to increase the amount of fertilizer applied in agricultural areas, which also represents a form of entry of these elements into the soil. The PTM may be essential to living beings, participating in metabolic processes such as cobalt (Co), copper (Cu), iron (Fe), molybdenum (Mo), manganese (Mn), and zinc (Zn). Nevertheless, elements like chromium (Cr), silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), lead (Pb), antimony (Sb), strontium (Sr), and mercury have no obvious function in biological processes. Regardless, all these elements, in high concentrations in the soil, are toxic for plants, animals, and microorganisms. In the soil, these elements can be as free ions, chemical or organic metal complexes, exchangeable in the colloidal complex, forming oxides, carbonates and hydroxides, generally insoluble, or even integrating the crystalline structure of primary and secondary minerals (Alloway 2013; Kabata-Pendias 2001).

Whereas AMF establish a symbiotic association with most terrestrial plants and their biocontrol function in ecosystems, it is clear that these microorganisms can protect host plants in PTM-contaminated soils (Vilela et al. 2018b). Similar to plants, AMF developed strategies to mitigate the adverse effects of PTM toxicity (Garg and Bhandari 2013), which results in abundant positive effects both on plant growth and on physiological processes in the most plant species and at many contamination levels, as explored in Table 24.3.

AMF-colonized plants confer higher tolerance to PTM through mechanisms such as metal immobilization and chelation in hyphae, increased root colonization, and compartmentalization of these metals into fungal cells (Garg and Bhandari 2013; Kaur and Garg 2017). Huang et al. (2018) found that the hydroxyl, amide, carboxyl, and phosphate groups played predominant roles in increasing Cd tolerance in response to *G. clarum* inoculation. This species had greater Cu, Cd, and Pb retention capacity in their mycelium compared to *Gigaspora gigantea*, which retained more Zn. On the other hand, consideration should be given to the metal type and concentration, since retention may be inhibited in severe cases of toxicity. In cucumber (*C. sativus*), inoculated with *G. etunicatum* and *G. claroideum*, extraradicular mycelium development reached the maximum length at the lowest Cd level (5 mg kg^{-1}), and inhibition occurred at 25 and 50 mg Cd kg^{-1}(Gai et al. 2018).

Metal retention in the glomalin (glycoprotein) produced by AMF represents an important strategy to mitigate the PTM toxic effects (Garg and Bhandari 2013). In a field study in a Cd-contaminated area, Gonzalez-Chavez et al. (2009) found retention of 0.028 mg Cd g^{-1} in glomalin extracted from the soil. For immobilizing PTM in biomass itself (retention/adsorption on the hyphae cell walls), AMF acts as a barrier against the translocation of these metals to the shoot (Andrade and Silveira 2008). In As-contaminated soil (539.33 mg kg^{-1}), Gomes et al. (2014) detected that the legume *Anadenanthera peregrina*, inoculated with *Acaulospora scrobiculata*,

Table 24.3 Results of recent research about the effects of arbuscular mycorrhizal fungi (AMF) on plants exposed to potentially toxic metals (PTM)

AMF species[a]	Plant species	Research conditions	Main results	References
Acaulospora scrobiculata	*Anadenanthera peregrina*	Contaminated soil with As 539.33 mg As kg^{-1}	AMF inoculation improved plant growth and increased P concentration in shoot	Gomes et al. (2014)
Glomus versiforme	*Solanum nigrum*	Cd levels (0, 25, 50, and 100 mg kg^{-1})	Mycorrhizal colonization rates were high (71–82%) at all Cd levels	Liu et al. (2015)
			AMF inoculation increased the acid phosphatase activity in the soil and, consequently, P acquisition and plant growth at all Cd levels	
Funneliformis mosseae and *Rhizophagus intraradices*	*Robinia pseudoacacia*	Pb levels (0, 500, 1000, and 2000 mg kg^{-1})	AMF inoculation reduced the Pb concentration in the leaves and promoted the biomass accumulation and photosynthetic pigments content	Yang et al. (2015)
			Mycorrhizal plants also showed greater capacity of gas exchange, photochemical efficiency, and greater activity of the antioxidant defense system enzymes (superoxide dismutase, ascorbate peroxidase, and glutathione peroxidase). On the other hand, they presented lower hydrogen peroxide and malondialdehyde production	
Rhizophagus irregularis	*Phragmites australis*	Cd levels (0, 0.01, 0.5, 1, 2, 5, 10, and 20 mg L^{-1})	Mycorrhizal symbiosis alleviated Cd toxicity by increasing root biomass and photosynthesis rate and reduced malondialdehyde and proline levels in plants	Wang et al. (2017a)
Acaulospora colombiana, *Acaulospora morrowiae*, *Acaulospora scrobiculata*, *Dentiscutata heterogama*, *Gigaspora margarita*, and *Rhizophagus clarus*	*Chrysopogon zizanioides*	Contaminated soil with Cd (17.3 mg kg^{-1}), Pb (125 mg kg^{-1}), Zn (422 mg kg^{-1}), As (8.60 mg kg^{-1}), Cr (11.3 mg kg^{-1}), and Cu (17.7 mg kg^{-1}).	*R. clarus* had the highest mycorrhizal colonization (28.6%), followed by *A. colombiana* (7.2%) and *G. margarita* (6.4%). *A. scrobiculata*, *A. morrowiae*, and *D. heterogama* reached only 0.8% of colonized roots	Meyer et al. (2017)
			Plants inoculated with *R. clarus* had the highest spore density	
			With the exception of *A. scrobiculata*, AMF species increased the P content in the leaves	
			A. morrowiae promoted the highest Cr accumulation and *R. clarus* the highest Pb accumulation in the leaves. Plants inoculated with *A. colombiana*, *A. morrowiae*, and *A. scrobiculata* presented the highest Cu and Zn levels in the roots	
			Plants inoculated with *G. margarita* showed 35% less Zn in the roots when compared to the control	

AMF species[a]	Plant species	Research conditions	Main results	References
Rhizophagus clarus and *Claroideoglomus claroideum*	*Physalis peruviana*	Contaminated soil with Cd (6.7 mg kg^{-1}), Pb (230 mg kg^{-1}), and Zn (199.5 mg kg^{-1})	AMF inoculation considerably reduced the Cd and Pb contents in the fruits	Hristozkova et al. (2017)
			C. claroideum inoculation increased the weight and number of fruits in the plants and acid phosphatase activity and the glomalin content in the soil	
			Both AMF species reduced the H_2O_2 content in the fruits	
			Only *C. claroideum* inoculation increased the activity of catalase, superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidase	
			R. clarum reduced the total phenols and flavonoid concentration	
			Water-soluble metabolites and lipid content with antioxidant activity in fruits were higher in the two AMF species	
Species of *Glomus*, *Entrophospora*, and *Scutellospora*	*Lactuca sativa*	Hg levels (0, 10 and 100 mg kg^{-1})	AMF inoculation improved root growth and seedling development, but did not hinder cell damage in the roots and the hypocotyl caused by oxidative stress	Aguirre et al. (2018)
Claroideoglomus etunicatum	*Zea mays*	Soils spiked with La (100 mg kg^{-1}) and/or Cd (5 mg kg^{-1})	The individual and combined applications of La and Cd significantly reduced root colonization by 22.0% and 35.0%, respectively	Chang et al. (2018a)
			In the La + Cd treatment, AMF inoculation increased maize biomass (+26.2%), N content (+20.1%), and P (+76.8%)	
Glomus sp.	*Zea mays*	Contaminated substrate with Hg (50 mg g^{-1})	AMF inoculation did not favor the plant growth in contaminated soil. In addition, mycorrhizal plants showed higher Hg uptake	Debeljak et al. (2018)
Claroideoglomus etunicatum and *G. claroideum*	*Cucumis sativus*	Cd levels (0, 5, 25 and 50 mg kg^{-1})	Both AMF isolates showed relevant root colonization	Gai et al. (2018)
			Total length and active length of the extra radicular mycelium were greater at 5 mg kg^{-1}, and from 25 mg kg^{-1} there was inhibition at this parameter	
			G. claroideum has been shown to be more tolerant to Cd toxicity	
			There was no effect of AMF inoculation on shoot growth, but root growth was stimulated by inoculation with *G. claroideum*	
			Total plant P uptake was increased by AMF inoculation, and inoculation with *G. etunicatum* had a much more pronounced effect than with *G. claroideum*	

(continued)

Table 24.3 (continued)

AMF species[a]	Plant species	Research conditions	Main results	References
Rhizophagus irregularis	*Phragmites australis*	Cd levels (0, 1 and 20 mg L^{-1})	At 1 mg L^{-1}, inoculation increased the Cd uptake in shoot. On the other hand, in 20 mg L^{-1}, mycorrhizal plants accumulated more Cd in the roots	Huang et al. (2018)
Funneliformis mosseae and *Rhizophagus intraradices*	*Oryza sativa*	Soil spiked with Cd (0 and 10 μM)	Mycorrhizal colonization was not changed by Cd in both AMF species	Chen et al. (2019)
			Cd addition decreased root biomass of plants inoculated with *F. mosseae* but not of *R. intraradices*	
			F. mosseae and *R. intraradices* decreased Cd concentration in root (−18% and −38%, respectively) and shoot (−20% and −23%, respectively)	
			R. intraradices reduced Cd extractable soil and Cd root content, but the Cd shoot accumulation did not differ from the other treatments	
			In contrast, *R. intraradices* enhanced Actinobacteria community by 15%, while *F. mosseae* and the control reached only 2 and 1%	
Glomus etunicatum	*Sesbania rostrata*	U levels (0, 80, 150, and 300 mg kg^{-1})	U levels did not affect mycorrhizal colonization	Ren et al. (2019)
			Mycorrhizal plants showed higher U levels in the shoot and roots and, consequently, a higher U extraction rate of the soil	

[a] AMF nomenclature used in the original paper

accumulated greater amount of As in the roots and presented low translocation index for the shoot.

In addition to direct actions to attenuate PTM toxicity, AMF also favor the reduction of phytotoxic effects through the promotion of plant growth, which allows the dilution of metal concentration in plant tissues (Andrade et al. 2008; Andrade and Silveira 2008; Christie et al. 2004). Meyer et al. (2017) assessed the AMF effects on the *Chrysopogon zizanioides* growth and the phytoextraction capacity of trace elements in degraded soil by coal mining in southern Brazil. The results showed that some AMF species (*Acaulospora morrowiae*, *Gigaspora margarita*, and *R. clarus*) promoted greater biomass production and P uptake (+300%), evidencing the potential of these species for the rehabilitation of degraded soils by coal mines.

Mycorrhizal symbiosis also changes in the plant the response of the antioxidant system to PTM toxicity. Researches indicate positive results of AMF performance in the enzymatic and nonenzymatic system of the antioxidant defense system. In wheat submitted to As levels (25, 50, and 100 mg kg^{-1}), inoculation with *R. intraradices* and *G. etunicatum* reduced reactive oxygen species (H_2O_2) generation and lipid peroxidation. In the enzymatic system, there was an increase in the activity of superoxide dismutase, catalase, and guaiacol peroxidase. In the nonenzymatic system, AMF inoculation increased the production of proline, carotenoids, α-tocopherol, and ascorbate (Sharma et al. 2017). The reduction of the production of reactive oxygen species promoted by AMF inoculation was also reported in corn (Merlos et al. 2016), wheat (Shahabivand et al. 2016), and sunflower (Abd_Allah et al. 2015). In order to evaluate the effects of AMF on the enzymatic and nonenzymatic systems, it has been reported in tomato (Hashem et al. 2016), sorghum (Huang et al. 2016), corn (Merlos et al. 2016), wheat (Shahabivand et al. 2016; Sharma et al. 2016), and soybean (Spagnoletti et al. 2016).

The results presented clearly demonstrate the benefits of mycorrhizal symbiosis in sites with PTM contamination, demonstrating the potential of the AMF to promote the plant growth agricultural ecosystems. However, the main obstacle is in large-scale AMF inoculation mainly due to its obligate biotrophism. Despite the challenge, managing the indigenous population in these areas can be a viable strategy. Moreover, for perennial crops, there is the possibility of inoculation of seedlings in nurseries, favoring the increase of AMF diversity in the soil of these areas.

24.5 Xenobiotics

In agriculture, pesticides are widely used to prevent or control pests, diseases, and weeds. The use of these inputs promotes a competitive advantage for agricultural crops and, therefore, ensures higher yields (Delcour et al. 2015). However, as a result of climate change, rising temperatures in some areas of the globe will increase the survival of some pests, pathogens, and weeds, even in winter, compelling the increase in application volume and frequency of pesticide use in these areas (Myers et al. 2017). Besides, most pesticides have synthetic chemical compounds known as xenobiotics that can accumulate in the soil (Moreira and Siqueira 2006). As a consequence, climate changes can increase the xenobiotics residual concentration in the soil, especially in agricultural areas (Delcour et al. 2015). Plant protection against xenobiotics toxicity is also reported as the AMF biocontrol role. Research has shown that AMF-colonized plants show higher tolerance in soils with high xenobiotics concentrations when compared to non-colonized soils (Makarian et al. 2016). Lenoir et al. (2016) points out that AMF benefit plant growth by increasing the water and nutrient uptake, making them more tolerant to abiotic stresses. Individual application of bavistin, thiram, captan, and aldrin drastically reduced the growth and nutrient uptake of wheat plants, but *G. fasciculatum* inoculation expressively attenuated these effects (Chhabra and Jalali 2013). In corn, roots colonized by *G. caledonium* accumulated more atrazine than those not colonized. In contrast, inoculation reduced herbicide concentrations in shoot. This reduction reached approximately 56% at the highest atrazine dose (5 mg kg^{-1}). In parallel, the soil close to the rhizosphere of mycorrhizal plants showed higher atrazine dissipation, irrespective of atrazine application (Huang et al. 2007). Similar results were found by Wu et al. (2008) in alfalfa plants inoculated with *G. etunicatum* and submitted to the application of organochloride insecticide dichlorodiphenyltrichloroethane (DDT, 1,1,1-trichloro-2,2,bis(*p*-chlorophenyl)-ethane).

Some researchers suggest that the AMF mechanisms involved in the remediation of xenobiotics-contaminated soil are mainly through biodegradation (Binet et al. 2000; Chibuike 2013; Gao et al. 2011; Joner and Leyval 2003). Even though AMF have very limited saprophytic capacity, there are effects on the oxidative enzyme activity in roots and rhizospheric soil (Joner and Leyval 2003). Furthermore, AMF may favor the activity of other soil microorganism populations (Chen et al. 2019; Chibuike 2013; Harrier and Watson 2004; Rillig and Mummey 2006), favoring the biodegradation processes. Directly, AMF affects bacterial communities by deposition of mycelial products that act as substrates for bacterial growth. Indirectly, the modification in the rhizodeposition, quantitative and qualitative, promoted by the mycorrhizal symbiosis results in the alteration of the bacterial community composition (Rillig and Mummey 2006). The AMF influence on the soil microbial community was verified by Chen et al. (2019). These researchers confirmed that *R. intraradices* inoculation in rice at spiked soil with 10 μM Cd enhanced by 15% actinobacteria community diversity. Furthermore, AMF can attenuate xenobiotics toxicity by two other mechanisms: phytostabilization and phytoextraction. However, phytostabilization is more efficient in soils with low concentration of these compounds (Chibuike 2013). Advantageously, AMF are ubiquitous organisms and adjust to many adverse conditions in a wide variety of environments. Thus, several AMF species could be used to protect plants in xenobiotics-contaminated soils. Unfortunately, experimental evidence on the AMF impact on the xenobiotic degradation, mainly in agricultural areas, is very scarce.

Although the risks of pesticide toxicity to nontarget organisms are poorly considered, increasing the volume and frequency of pesticide application in crops may damage the AMF development (Table 24.4). Druille et al. (2013a) found that the application of low doses of glyphosate (0.8 and 3 l ha^{-1}) and, mainly, their soil residence time reduced the spore viability and the mycorrhizal colonization rate of

Lolium multiflorum. Since AMF spores are important sources of propagules, viability spore reduction can alter the fungi diversity and the symbiosis functionality in ecosystems. Also, changes in carbohydrate flow from the plant to the fungus can affect the quality of mycorrhizal symbiosis (Karpouzas et al. 2014). Although the glyphosate application to soil adversely affected mycorrhizal colonization (Druille et al. 2013a), the same did not occur in glyphosate application in plant foliage (Druille et al. 2013b).

Table 24.4 Results of recent research of the effects of xenobiotics on the development of arbuscular mycorrhizal fungi (AMF)

Xenobiotic	AMF species[a]	Main results	References
Glyphosate	Native AMF	Glyphosate application (0.8 and 3 L ha^{-1}) reduced spore viability in 5.8- and 7.7-fold, respectively	Druille et al. (2013a)
		Mycorrhizal colonization and arbuscules percentage reduced after 30 days of glyphosate application at both concentrations	
		Glyphosate application did not affect the vesicles percentage	
Glyphosate	Native AMF	Only plant foliage application did not decrease AMF spore viability, but glyphosate soil application did	Druille et al. (2013b)
		Glyphosate application (0.8 and 3 L ha^{-1}) did not affected root colonization, vesicles, and arbuscules percentage of *Paspalum dilatatum* and *Lotus tenuis*	
Nicosulfuron	Native AMF	In culture cycle 1, nicosulfuron application (60,000 g a.i.^{2} ha^{-1}) induced a significant reduction in hyphae colonization. In subsequent cycles, high phytotoxicity allowed no further measurements	Karpouzas et al. (2014)
		At 6000 g a.i. ha^{-1}, nicosulfuron induced an increase in hyphae colonization in cycle 2. However, in subsequent cycles, the same dose reduced hyphae colonization	
		Increasing doses reduced the AMF diversity, especially in subsequent cycles	
Glyphosate	*Funneliformis mosseae*, *Claroideoglomus etunicatum*, *Septoglomus constrictum*, and *Funneliformis caledonium*	At 384 and 1440 g a.i. ha^{-1} reduced proportionally the spore viability of *F. caledonium* and *S. constrictum* and did not affect the other species	Druille et al. (2015)
Fenhexamid, folpel, and deltamethrin	*Funneliformis mosseae, Claroideoglomus etunicatum* and *C. claroideum*	Abundance of all species was reduced after application of the pesticide cocktail	Rivera-Becerril et al. (2017)
		F. mosseae has been shown to be more tolerant of exposure to pesticides	

(continued)

Table 24.4 (continued)

Xenobiotic	AMF species[a]	Main results	References
Glyphosate	Native AMF	Glyphosate application (0.8 and 3 L ha^{-1}) reduced the spore density by 100%. On the other hand, it did not affect the arbuscules and total mycorrhizal colonization percentage	Druille et al. (2016)
Metribuzin	*Glomus mosseae*, *Glomus intraradices*, and *Glomus fasciculatum*	AFM inoculation increased the dry weight, height, and chlorophyll content of corn and barley at low herbicide concentrations (175 g a.i. ha^{-1}) compared to uninoculated treatments	Makarian et al. (2016)
Glyphosate	Native AMF	Application of 450 L ha^{-1} decreased the total mycorrhizal colonization by 23% in *Festuca pratensis* and 25% in *Elymus repens* and reduced the arbuscules percentage (*F. pratensis* 44% and *E. repens* 27%) Glyphosate did not affect the vesicles in *E. repens*	Helander et al. (2018)
Glyphosate	Native AMF	720 g a.i ha^{-1} did not affect percentage of root length colonized by AMF	Nivelle et al. (2018)
Carbendazim	Native AMF	Continuous application of the fungicide for 7 years reduced the arbuscular mycorrhizal fungi infectivity	Pánková et al. (2018)

a.i: active ingredient
[a] AMF nomenclature used in the original paper

In addition to the functionality of mycorrhizal symbiosis, high xenobiotics concentrations may alter the AMF diversity. For instance, at high concentrations of nicosulfuron herbicide, Karpouzas et al. (2014) verified that the AMF community was dominated by *Glomus* genus, which has often been verified on agricultural soils with major disturbances (Freitas et al. 2018). In contrast, some research show that in super low levels, xenobiotics as herbicides can stimulate organisms. The herbicides hormesis effects on plants have been reported (Belz and Duke 2014). Unfortunately, little attention is given to the hormesis studies of these molecules in AMF. Hage-Ahmed et al. (2018) emphasize that detailed studies are essential to clarify AMF–plant–xenobiotic interrelationships.

24.6 Drought Stress

Climate change will in many ways affect the water resource availability. For crop production, the most disturbing modification will be the change in precipitation patterns. Predictions indicate precipitation reduction in the arid and semiarid regions; higher precipitation in the polar regions and higher frequency of flooding are also expected (Boyer et al. 2015; Myers et al. 2017).

To mitigate the negative effects in crop production, research is focused on developing strategies to make crop more resistant, optimizing ecosystem services from root-associated microbial communities, among others (Balestrini et al. 2018).

Likewise, stimulating mycorrhizal symbiosis may attenuate the effects of drought stress, since AMF increases the water uptake by plants and, consequently, its tolerance to water deficit (Fernández-Lizarazo and Moreno-Fonseca 2016), as shown in Table 24.5. Usually, native plant roots in arid and semiarid ecosystems have a high level of mycorrhizal colonization, which highlights the relevance of symbiosis for performance under drought stress (Tchabi et al. 2008).

Under drought stress conditions, the most reported result in AMF research is about its contribution to plant growth. This microbial service acquires great importance, especially for crop species, in the face of the climate change scenario. In lettuce, *R. irregularis* inoculation promoted the plants' growth in moderate (+39%) and severe (+31%) drought stress. In tomatoes under the same conditions, the increments reached 33% and 30%, respectively (Ruiz-Lozano et al. 2016). In another research with tomato, native AMF inoculation did not affect shoot growth; however, it increased fruit yield by 25% under water stress (Bowles et al. 2016). Increased data on growth and productivity are reported for several other agricultural crops: soybeans, wheat, citrus, beans, sorghum, and strawberry, among others (Duc et al. 2018; Ganjeali et al. 2018; Grümberg et al. 2015; Liu et al. 2018a; Mathur et al. 2019; Moradtalab et al. 2019; Ruiz-Lozano et al. 2016; Sun et al. 2017; Symanczik et al. 2018; Wang et al. 2017b; Zhou et al. 2015).

The positive results of AMF in plants under drought stress can derive from several mechanisms. The AMF's ability to maintain an adequate hydration status of inoculated plants stands out as one of these mechanisms (Ruiz-Sánchez et al. 2010). Effectively, AMF extra-radicular mycelium can easily access soil micropores and significantly expand the water uptake zone of the plant (Rillig and Mummey 2006; Wang et al. 2017b). Furthermore, it is likely that AMF promote higher hydraulic conductivity in colonized roots (Ganjeali et al. 2018). *F. mosseae* inoculation in *C. trifoliata* under drought stress improved leaf water relative content by 4.1% (Wang et al. 2017b). A similar result was observed in tomato. Plants inoculated with *Septoglomus deserticola* or *S. constrictum* showed higher water leaf potential and relative water content, especially those inoculated with *S. constrictum* (Duc et al. 2018).

Also, researches show that mycorrhizal plants submitted to drought stress present less damage to the photosynthetic apparatus, and, therefore, they can develop better than non-inoculated plants (Wang et al. 2017b). Under drought stress conditions, many data show that mycorrhizal plants show an increase in the content of pigments (*a* and *b* chlorophyll and carotenoids content), in the efficiency of photosystem II, in stomatal conductance and, consequently, a higher photosynthetic rate (Duc et al. 2018; Ganjeali et al. 2018; Mathur et al. 2019; Moradtalab et al. 2019; Ruiz-Lozano et al. 2016; Zhou et al. 2015).

Changes in the metabolites production are too reported in mycorrhizal plants submitted to drought stress. In *C. trifoliata*, *F. mosseae* inoculation increased the indole acetic acid content in the roots (+37%), which shows one of the mechanisms of AMF in promoting the growth of plants under these conditions. In a study with the same fungus and plant species, Wang et al. (2017b) found an increase in indole acetic acid content in the leaves (+28%). Also, in this study, *F. mosseae* increased abscisic acid (+6%) and zeatin riboside (+18%) leaves content. Remarkably, abscisic

Table 24.5 Results of recent research about the effects of arbuscular mycorrhizal fungi (AMF) on plants exposed to drought stress

Plant specie	AMF species[a]	Main results	References
Glycine max	*Septoglomus constrictum*, *Glomus* sp., and *Glomus aggregatum* (individual inoculation and their mixture)	*S. constrictum* and *Glomus* sp. had very high colonization rates under drought stress (95 and 91%, respectively), while *G. aggregatum* had only 60%. The mixture of the three species reached 80% of colonized roots. These colonization rates were similar to those found in under well-watered plants	Grümberg et al. (2015)
		However, under drought stress, all AMF inoculation conditions reduced root dry mass and shoot dry mass	
Solanum lycopersicum and *Lactuca sativa*	*Rhizophagus irregularis*	Under moderate and severe drought stress, mycorrhizal colonization rate increased significantly and steadily over time (4–8 weeks)	Ruiz-Lozano et al. (2016)
		Inoculated plants had higher growth than those not inoculated, even under water stress. In lettuce there was an increase of 39 and 31% under moderate drought or severed drought stress conditions, respectively. In turn, tomato plants had 33 and 30%	
		AMF inoculation did not affect stomatal conductance under drought stress	
		AMF inoculation of both species promoted the highest values of the photosystem II efficiency in treatments under water stress	
Triticum aestivum	Mixture of *Glomus intraradices*, *Glomus mosseae*, *Glomus geosporum*, *Glomus claroideum*	Drought stress did not affect AMF colonization in both wheat cultivars	Zhou et al. (2015)
		AMF inoculation, under drought conditions, increased plant height, shoot growth, and grain yield	
		In addition, inoculation increased rates of photosynthesis, transpiration, and stomatal conductance	
Citrus trifoliata	*Funneliformis mosseae*	Six-week drought stress treatment suppressed mycorrhizal colonization	Wang et al. (2017b)
		Inoculated plants had higher decrease in plant height, leaf number, and leaf, stem, and root fresh weight caused by drought stress	
		On the other hand, these plants had larger stem diameter, plant height, number of leaves per plant, and fresh weight of leaves and roots, regardless of the water regime	
		AMF inoculation significantly increased leaf relative water content by 6.0% and 4.1% under well-watered and drought conditions, respectively	
		AMF-inoculated seedlings showed leaf ABA level 3.3% and 5.6% higher than those not inoculated in well-watered and drought conditions, respectively. AMF inoculation increased leaf indolacetic acid level by 21.6% and 28.3% and zeatin riboside level by 18.8% and 17.9% in well-watered and drought conditions, respectively	

Plant specie	AMF species[a]	Main results	References
Sorghum bicolor	*Funneliformis mosseae*	Mycorrhizal plants showed larger shoot and root dry mass and specific leaf area. The root/shoot ratio of all plants were similar	Sun et al. (2017)
		Mycorrhizal colonization was strongly reduced by drought stress. After 10 days of severe water shortage, many collapsed arbuscules were observed in the roots	
Phaseolus vulgaris	*Glomus mosseae*	AMF inoculation increased leaf area, shoot dry matter, leaf P content, photosynthetic rate, carotenoid content, and transpiration rate. However, it did not affect root growth, *a*, *b*, and total chlorophyll concentrations	Ganjeali et al. (2018)
Solanum lycopersicum	*Septoglomus deserticola* and *S. constrictum*	*S. constrictum* inoculation in plants under drought stress increased root and shoot dry weight. In addition, stomatal conductance was almost twice as high as that observed in non-inoculated plants. In contrast, there was a substantial reduction in the growth of plants inoculated with *S. deserticola*	Duc et al. (2018)
		In general, inoculated plants had higher leaf water potential and relative water content, notably in plants inoculated with *S. constrictum*	
		Mycorrhizal symbiosis increased considerably the photochemical efficiency of photosystem II in tomato plants, particularly when inoculated with *S. constrictum* (+4%). Colonization by *S. deserticola* did not alter the photochemical efficiency of photosystem II	
Citrus trifoliata	*Funneliformis mosseae*	Drought stress reduced mycorrhizal colonization by 9.4%	Liu et al. (2018a)
		Under drought stress AMF inoculation did not favor the plant growth but incresed length (+15%), diameter (+25%), and density (+40%) of root hairs. In addition, indole acetic acid concentration also increased in the roots by 37%	
Sorghum bicolor	*Rhizophagus irregularis* or *Rhizophagus arabicus*	AMF colonization did not affect the shoot biomass, but *R. arabicus* improved transpiration efficiency and drought tolerance of plants more than *R. irregularis*	Symanczik et al. (2018)
		Moreover, *R. arabicus* was able to extract more N and P from the soil under both water regimes	
Triticum aestivum	*Funneliformis mosseae*	Drought stress reduced the mycorrhizal colonization and the AMF spore density. However, the inoculation increased leaf chlorophyll content and relative water content in leaf and soil. In addition, *F. mosseae* increased the quantum efficiency of PSI and PSII.	Mathur et al. (2019)
Fragaria × *ananassa*	*Rhizophagus clarus*	Joint application of Si and AMF increased plant biomass production, photosynthesis rate, water content and use efficiency, and enzyme content of the antioxidant defense system and improved the nutritional status of plants	Moradtalab et al. (2019)

[a] AMF nomenclature used in the original paper

acid acts on the stomatal opening regulation and, therefore, on the transpiration rate of the plant (Ruiz-Lozano et al. 2016). In turn, zeatin riboside can delay the cellular senescence process, ensuring the tonoplast membrane integrity and the photosynthesis process (Wang et al. 2017b).

Finally, previous research indicates that, despite numerous ecosystem services, drought stress can harm the AMF spore germination, the root exudates production, and the supply of roots carbohydrates to the fungus, resulting in the reduction of AMF colonization in the host plant (Wang et al. 2017b). There are reports that, even under severe drought, mycorrhizal colonization was not affected (Grümberg et al. 2015; Ruiz-Lozano et al. 2016; Zhou et al. 2015). On the other hand, representative reductions in mycorrhizal colonization of plants under drought stress were also reported (Liu et al. 2018a; Mathur et al. 2019; Sun et al. 2017; Wang et al. 2017b). Thus, it is clear that the root colonization level and the amplitude of the benefits or attenuation of the stress effects may differ according to the AMF species, plant species, soil conditions, and drought stress intensity.

24.7 Conclusion

Arbuscular mycorrhizal fungi are ubiquitous microorganisms, adjust to a wide diversity of adverse environments, and, hence, provide important ecosystem services. In the face of climate change, exploring the immense range of these ecosystem services and their interface with agricultural crops in a finite natural resource and requirement to increase food production at the lowest environmental costs is a pertinent question. The results demonstrate the feasibility of AMF applicability to increase plant tolerance in drought conditions, high salinity, and in PTM- and xenobiotics-contaminated soils. However, there is much to be uncovered as the results of the research do not allow direct conclusions as to which species of AMF are more tolerant or more suitable for certain types of stress conditions. Application of microbial biotechnology in agriculture promoted many achievements; even so many difficulties still have to be transposed for future sustainable agricultural development. Opportunely, molecular techniques and the omic sciences will allow the understanding of several mechanisms that, to date, are not yet clear to researchers. Based on the results achieved with mechanistic research, it is hoped that it will be possible to explore the ecosystem services provided by AMF to mitigate possible negative effects caused by climate change and, if possible, reduce the use of synthetic agricultural inputs to promote positive economic and environmental impacts.

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Chapter 25
Plant-Microbe Interactions in Wastewater-Irrigated Soils

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Abstract With increase in urbanization and industrialization, there has been an enormous increase in the amount of wastewater being discharged into the environment without any pretreatment, especially in developing countries. In such regions of the world, farmers are using this wastewater for irrigation purposes in agricultural fields due to unavailability and unsuitability of clean irrigation water. The wastewater discharged by the industries is loaded with huge amount of heavy metals, dyes, organic compounds, and pathogens. It has been found that soils irrigated with wastewater harbor a diverse community of microbes resistant to toxic metals, which not only contribute in the remediation of wastewater-polluted soils but also play their role in plant growth promotion. Most of them are found in close vicinity of plant roots and some of them enter in root cortical cells to act as endophytes. These microbes utilize various strategies to remediate contaminated soils such as biosorption, biotransformation, biovolatilization, bioleaching, biodegradation, etc. On the other hand, they also promote plant growth by utilizing numerous mechanisms such as phosphate solubilization, growth hormone synthesis, zinc mobilization, induction of stress tolerance, ACC deaminase activity, siderophore production, nitrogen fixation, biocontrol activity, etc. This chapter elaborates the potential of beneficial microorganisms to remediate the soils irrigated with wastewater and interaction of these microbes with plants in order to promote their growth by relieving them from toxic effects of pollutants.

Keywords Beneficial interaction · Microbes · Remediation · Soil · Wastewaters

Abbreviations

ACC-1	Aminocyclopropane-1-carboxylic acid
BNF	Biological nitrogen fixation
GA	Gibberellin
HCN	Hydrogen cyanide
IAA	Indoleacetic acid
NF	Nitrogen fixation
PGP	Plant growth promotion
PGPR	Plant growth-promoting rhizobacteria
TSS	Total suspended solid
UV	Ultraviolet

25.1 Introduction

During the past few decades, the rapid industrialization and urbanization has led to the release of increased amount of wastewater into the environment. This wastewater has been extensively utilized for irrigation purposes in the developing regions of the world due to the unavailability of clean water and unsuitability of underground water. The industrial wastewater predominantly contains large amount of toxic organic pollutants such as benzene, toluene, phenol, naphthalene, and chloroform as well as inorganic heavy metals that adversely affect the plant growth, human health, and environment (Mishra et al. 2017). The heavy metals such as arsenic (As), chromium (Cr), copper (Cu), cadmium (Cd), mercury (Hg), lead (Pb), etc., present in wastewater also alter the physicochemical properties of the soil and affect many ecological interactions, thus making the soil unfit for agricultural practices (Dixit et al. 2015). Within soils, the mass of heavy metals can be inferred using the following formula:

$$M = \left(M_{\mathrm{a}} + M_{\mathrm{f}} + M_{\mathrm{p}} + M_{\mathrm{ow}} + M_{\mathrm{ag}} + M_{\mathrm{ip}}\right) - \left(M_{\mathrm{l}} + M_{\mathrm{cr}}\right).$$

where M is the total mass of heavy metal; M_{a} is atmospheric deposition; M_{f} is fertilizer source; M_{p} is the parent material; M_{ow} is organic waste source; M_{ag} is agrochemical source; M_{ip} is inorganic pollutant; M_{l} is losses by leaching, volatilization, and other processes; and M_{cr} is crop removal (Dzionek et al. 2016). Heavy metals are the heterogeneous group of elements that not only affect the plant growth and yield but also have carcinogenic and mutagenic effects for humans. Thus, the treatment of wastewater-contaminated soil is necessary in the developing countries, where there are limited water and agricultural resources in order to feed the continuously increasing population (Sposito and Page 1984; Khan 2005; Kavamura and Esposito 2010).

Among various available treatment strategies, the bioremediation of the wastewater-contaminated soil is the cheapest and eco-friendly approach (Molinuevo-Salces et al. 2019**)**. The microbe-mediated detoxification and degradation of toxic chemical pollutants present in the soil to improve its health in an eco-friendly manner is termed as bioremediation. Due to the greater benefits of bioremediation, it is gaining global attention of researchers, industrialists, and policy makers (Mahmood et al. 2017; Shahid et al. 2018). Bioremediation utilizes the innate capabilities of beneficial microbial community to degrade, chelate, and transform toxic compounds into harmless or less toxic forms. The bioremediation process can be either performed in situ or ex situ (Coelho et al. 2015). In situ bioremediation technique is the removal of toxic compounds present in environment. This process includes the conversion of organic contaminants to less toxic form using natural metabolic processes of fungi and bacteria. In situ bioremediation includes various processes such as biosparging, bioventing, bio-augmentation, and biopiling (Das et al. 2016). Ex situ bioremediation strategy can also be used to degrade organic pollutants under aerobic conditions. Ex situ process requires the excavation of contaminated soil and pumping out of water from the location at which it was found (Francis and Nancharaiah 2015; Kuppusamy et al. 2016).

It has been found that the microorganisms resisting heavy metals and other toxic compounds are indigenous to contaminated soils (Mahmood et al. 2017). Among microbial community, bacteria and archaea are found most proficient that perform the functions of toxins and pollutants degradation in the presence or absence of oxygen especially in harsh environment. Different bacterial species (including *Escherichia coli*, *Paenibacillus* sp., and *Bacillus thuringiensis*) and fungal strain (*Trichoderma inhamatum*) resistant to Cd, Cu, and Zn have been reported to exist in contaminated environment (Abdu et al. 2017). The continuous increase in the concentrations of pollutants is the real hazard for plants due to the toxicological threats of these contaminants that could pose damaging effects to plant morphology, biochemistry, and physiology, consequently decreasing plant growth and yield (Chibuike and Obiora 2014).

There is an utmost need to understand the fundamental mechanisms that microorganisms utilize in order to degrade pollutants and toxic chemicals. The phyto-beneficial microbes interact with plants to improve their growth and survival in stressful environment. Microbes, specifically plant growth-promoting rhizobacteria (PGPR), develop mutualistic associations with the rhizospheric, endospheric, and phyllospheric regions of the plants. The associated microbes degrade the harmful chemicals through various mechanisms like heavy metal detoxification, biodegradation, biotransformation, and bioleaching and consequently promote plant growth under such contaminated environments. In turn, the rhizospheric microorganisms associated with the external surface of plant roots get nutrition from root exudates. Some of them enter into the internal plant tissues and develop symbiotic interaction to help in plant growth and development (Coelho et al. 2015). The association/interaction between microbes and plants in contaminated soils depends upon various factors such as concentration of specific pollutant, nature of soil, diversity of microbes, etc. The microbes not only decontaminate the polluted soils but also harbor numerous mechanisms of plant growth promotion. Different plant growth-promoting rhizospheric bacterial species including *Pseudomonas aeruginosa, Bacillus* spp., *Burkholderia* spp., and *Enterobacter* spp. have been reported to improve the growth of *Brassica napus* and *Solanum nigrum* plants through various phyto-beneficial mechanisms like nitrogen fixation, phytohormone production, and detoxification of heavy metals like Zn, Cu, Ag, and Cd in wastewater-contaminated soils (Ashraf et al. 2017). Wastewater-contaminated soils also contain numerous fungal species of *Basidiomycota* and *Ascomycota* (Mishra et al. 2017) having roles in soil reclamation. Fungi also play an important role in carbon cycle and regulating nutrients. Mycorrhizal fungi like *arbuscular* and *ericaceous* provide 80% of the total phosphorus to plants which is important for plant growth and survival. Almost all species of plants depend upon symbionts for nourishment. Mycorrhizal fungi influencing plant diversity and productivity develop underground mutualistic association with plants allowing movement of resources among plants (Van Der Heijden et al. 2015). The plant-beneficial microbes employed various mechanisms like biological fixing nitrogen, phosphate solubilization, phytohormone production, providing protection against pathogens, and degradation of numerous pollutants in order to promote plant growth under stressful environment (van der Heijden 2015).

25.2 Wastewater Contamination in Agricultural Soils

A large amount of wastewater is produced by municipalities, industries, and agricultural activities in urban areas, and due to the lack of any pretreatment plans especially in developing countries, this wastewater is discharged into the nearby agricultural fields through drains (Sahay et al. 2019). Textile industries are the largest consumer of water which after the dyeing process releases it as wastewater into the environment. The wastewater from textile industries contains chemicals and practically unfixed dyes which are unhealthy for plants and humans. It also contains heavy metals which have carcinogenic nature and can alter metabolic, physiological, and genetic responses both in plants and animals (Siddiquee et al. 2015). Thus, the treatment of wastewater is required prior to its use for irrigation of agricultural crops. Various physical, chemical, and biological strategies are available for the decontamination of industrial wastewater, which are described below.

25.2.1 Physical Methods

Physical wastewater treatment methods consist of filtration techniques (screening, filtration, green filtration, multimedia filtration, microfiltration, ultrafiltration, nano-filtration, and reverse osmosis), sedimentation, flotation, adsorption, and nanoparticles- and ultrasound-mediated treatment (Kesari et al. 2011). The major aim of physical treatment methods is to protect clogging of different processes which are used to remove large suspended and floating materials. Other methods cannot easily remove large materials present in wastewater. In physical process, 65% of greases and oil can be removed. Organic phosphorus, nitrogen, and heavy metals associated with solid particles are removed in this process without any chemical and biological change in water like other methods. Moreover, larger objects are removed by coarse screening and sedimentation (Sonune and Ghate 2004).

25.2.1.1 Membrane Filtration Technique

It utilizes semipermeable membrane, which is used to remove the total suspended solid (TSS) particles present in the wastewater. The larger particles cannot pass through the membrane and, thus, separated from the wastewater. The advantage of this technique is that no chemical change occurs in this process and it removes large suspended and floating materials (Crini and Lichtfouse 2019; Sonune and Ghate 2004), while disadvantages are membrane fouling, expensive cleaning, necessity of regeneration scheme, and equipment cost (Dickhout et al. 2017)

25.2.1.2 Multimedia Filtration Technique

It is specialized for the separation of suspended solid particles. During filtration process, as the wastewater passed through the filtration unit, the suspended particles are trapped into the filter, while smaller particles passed through the filter. A secondary filtration process is also required to clean up the non-eliminated pollutants (Crini and Lichtfouse 2019; Guyer 2017). Advantages of this technique are that it consists of multiple layers which enable to retain and stop large particles and can be operated for longer period of time as compared to other conventional methods (Jafarinejad 2017). This technique removes larger particles easily; however, smaller ones remain in treated water which is a major limitation of this technique hindering its commercialization (Dickhout et al. 2017).

25.2.1.3 Nanoparticles-Based Technology

Nanoparticles are the nanoscale entities that have at least one structural dimension of less than 100 nm. In wastewater treatment technology, use of nanoparticles is cost-efficient and eco-friendly approach (Anjum et al. 2016). They have unique potential for decontamination of industrial effluent, drinking water, and groundwater. Smaller size of nanoparticles increases adsorption capacity of metals on their surface. Heavy metals and organic pollutants are adsorbed by nanoparticles under equilibrium condition. Zinc oxide, titanium oxide, carbon nanotubes, manganese oxide, and activated carbon have been frequently reported due to their potential applications in wastewater treatment (Tyagi et al. 2017).

25.2.1.4 UV Light-Based Strategies

This technique degrades different organic and inorganic compounds present in soil and wastewaters. UV light degrades a range of pollutants and pathogenic microbes in aqueous solution by powerful oxidizing species (hydroxyl radicals), which are produced through photolysis of hydrogen peroxide (H_2O_2) (Ibrahim et al. 2019). No chemical changes occur during this process and it has no impact on dissolved oxygen content of water. This process is cost-effective for short-term application, but long-term operation requires capital investment (Matafonova and Batoev 2018).

25.2.2 Chemical Methods

These methods use chemicals to treat wastewater in order to make it reusable. Chlorination is one of the chemical methods commonly used for the disinfection of the wastewater (Decol et al. 2019). During chlorination process, chlorine destroys different types of bacteria and viruses present in wastewater. Chemical processes utilize numerous chemical reactions like chemical oxidation, chemical precipitation, chemical coagulation, ion exchange, and stabilization for the treatment of wastewaters (Ahmed et al. 2017). These methods have numerous advantages such as pollutants being transformed into nontoxic forms efficiently, and there is a change in the chemical characteristics (i.e., solubilities and volatilities) of the pollutants that causes it to be removed from the water and soil. Furthermore, these strategies are most effective and useful in case of various inorganic compounds produced from industries which are not easily removed by the biological and physical techniques (Eckenfelder and Cecil 2013; Crini and Lichtfouse 2019). The limitations associated with these methods which hinder their large-scale applications include high cost of chemicals used for treatment, production of toxic sludge which is difficult to be removed, and not being as eco-friendly as biological methods because of the production of hazardous by-products (Eckenfelder and Cecil 2013; Miklos et al. 2018).

25.2.3 Biological Methods

Biological methods include several techniques such as biological aerobic treatment (in the presence of oxygen), activated sludge treatment, trickling filters, aerated lagoons, oxidation pond, and biological anaerobic treatment (in the absence of oxygen) for removal of pollutants from wastewater (Grandclément et al. 2017). Different microbial populations such as bacteria, algae, fungi, protozoa, and nematodes have a substantial potential of wastewater remediation. Bioremediation is an important step in industrial wastewater treatment often called secondary treatment process which includes the treatment of primary contaminants. Moreover, biological method is more effective than physical and chemical methods because of its cost-effective, eco-friendly, and less laborious nature (Gunatilake 2015; Dhokpande and Kaware 2013).

Microorganisms, indigenous to wastewater-contaminated soils, harbor the natural potential of heavy metals decontamination into their nontoxic forms as well as biodegradation, biotransformation, and bioaccumulation of organic contaminants present in soils (Zhu et al. 2017). They produce several degradative enzymes to mineralize organic contaminants into carbon dioxide which is one of the primary substrates for cell growth in plants. Bioremediation process includes various mechanisms such as biosorption, biomineralization, bioaccumulation, biotransformation, and bioleaching. It can take place on the particular site by understanding metabolic capability, controlling mechanism and response of microbes to specific environmental changes (Dixit et al. 2015). *Basidiomycota* spp., *Saccharomyces boulardii*, and *Ascomycota* spp. are the fungal species that are commonly used for the decontamination of heavy metals from wastewater (Mishra et al. 2017), whereas bacterial species including *Escherichia coli*, *Enterococcus faecium*, *Bacillus* spp., and *Staphylococcus* sp. are widely employed for the treatment of wastewater contaminated with heavy metals (Kanamarlapudi et al. 2018; Shahid et al. 2018).

25.3 Microbe-Based Strategies for Remediation of Wastewater-Contaminated Soils

The following microbe-based strategies are used to treat contaminated soils (Fig. 25.1, Table 25.1).

25.3.1 Biosorption

Biosorption is referred to as the removal of contaminants from solution using biological material. This technique depends on physicochemical interactions (such as metal ion chelation and ion exchange) between the sorbents and functional groups on cell surface (Guiza 2017). Biosorption is different from bioaccumulation process and biomass used for biosorption may be living or dead. Dead biomass including algae, seaweeds, aquatic fern, and mycelial waste from different industries is extensively used as biosorbent for removal of heavy metals because it reduces the complexity and influences the metabolic processes on contaminated sites (Macek and Mackova 2011). It has been reported that *Ulva lactuca* has potential to biosorb different heavy metals including Cu^{2+}, Cd^{2+}, and Pb^{2+} from wastewater. Removal efficiency of heavy metals was 64.5 and 84.7 mg/g for Cu, 62.5 and 84.6 mg/g for Cd, 68.9 and 83.3 mg/g for Pb (Carolin et al. 2017). Biosorbent should have high adsorption ability and readily available and must be cost-effective (Lesmana et al. 2009; Dhankhar and Hooda 2011). Several advantages of this technique such as eco-friendly nature, low cost, easy removal of biosorbed metals, biomass reusability, and easy handling render this technique as the method of choice for soil remediation worldwide (Fomina and Gadd 2014; Ahluwalia and Goyal 2007).

Other advantages of this technique include selective removal of metals, minimal amount of chemical and biological sludge, rapid kinetic adsorption, low capital and operational cost, as well as high efficiency for dilute solution (Macek and Mackova 2011; Ahluwalia and Goyal 2007). This is economically best method for remediation of heavy metals-polluted soil and waterbodies, however, not successfully commercialized yet because of being complex in nature. Moreover, different attempts to make it more beneficial may result in the increase in the cost and raise environmental issues (Fomina and Gadd 2014).

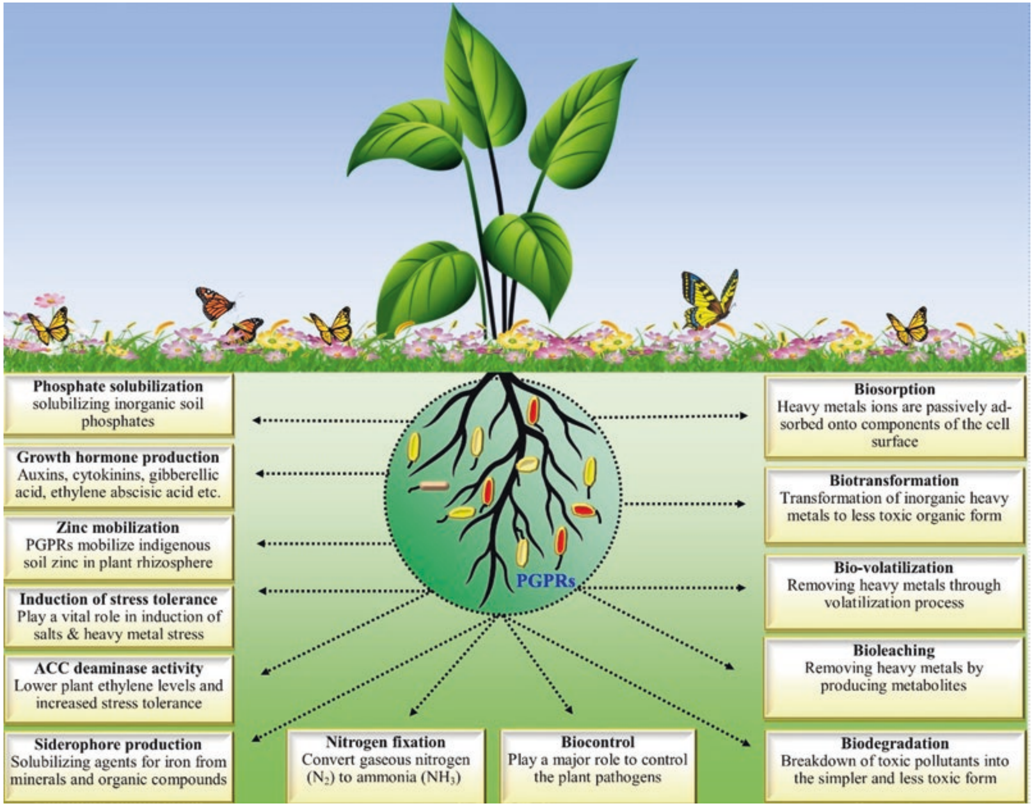

Fig. 25.1 Plant-microbe interaction in wastewater-contaminated soil

Table 25.1 Rational comparison of different biological strategies for remediation of wastewater-contaminated soils

Serial no.	Strategies	Advantages	Disadvantages	References
1	Bioaccumulation	• Low energy consumption • Eco-friendly and no harmful chemical produce	• Low performance • High capital costs • Skilled person and regular monitoring required • High production of sludge that must be thickened	Dhokpande and Kaware (2013); Gunatilake (2015); Wiszniowski et al. (2006)
2	Bioleaching	• Does not affect soil condition and fertilizing value of sludge • Give higher yield of metal extraction and 80% low chemical cost than natural chemical process • Remove Cu, Mn, Zn (91%, 93%, 96%) eco-friendly	• Require strict monitoring of parameters (temperature, aeration) • Plenty of oxygen required for aerobic bacteria • Applicable only for low solid concentration	Babel and del Mundo Dacera (2006); Bosecker (2001); Gu et al. (2018)
3	Biotransformation	• Produce large amount of biomass and various enzymes • Microbes produce stable enzymes towards heat and acid • Operate at neutral pH and atmospheric pressure • Environment friendly	• Specific organism required • Organism selection is a laborious process • Sometime not economical • Low yield	Chaturvedi et al. (2015); Hegazy et al. (2015)
4	Biosorption	• Cost-effective, high efficiency, regeneration of biosorbents, minimum chemical and biological sludge production	?????	Alluri et al. (2007); Macek and Mackova (2011)
5	Biovolatilization	• Stress tolerance to various factors • Remove arsenic from liquid media • Improved efficacy in the treatment	• Volatile compounds exhibit higher toxicity • Lead to an uncontrolled distribution of toxicants in the environment	Michalke and Hensel (2004); Tripathi et al. (2013)

(continued)

Table 25.1 (continued)

Serial no.	Strategies	Advantages	Disadvantages	References
6	Biodegradation	• Cost-effective • Low sludge production • Metal removal and recovery efficiently	• Treatment time longer • Controlling volatile compounds is difficult • Sensitive to toxicity level and environmental conditions	Dadrasnia et al. (2017); Whitlock (1989)

25.3.2 *Biotransformation*

Biotransformation is an excellent method for decontamination of heavy metals from sediments, soils, and wastewaters. The biotransformation utilizes various enzymatic processes of microbes like hydrolysis, oxidation, and reduction to decontaminate heavy metals into less toxic form (Madakka et al. 2019). The microbes with inherent potential of biotransformation are equipped with several types of extracellular enzymes that convert the toxic form of heavy metals into their harmless form (Yadav and Garg 2019). During biotransformation, the natural capabilities of bacteria and fungi can be exploited to decontaminate the heavy metals-polluted wastewater, which can ultimately be used for irrigation of cropland (Chaturvedi et al. 2015; Alexander 1994). Bacteria and fungi chelate heavy metals with cellular ligand and transform toxic form of heavy metals into nontoxic form. For example, archaea and eubacteria have been reported to decontaminate Mn (IV), SeO_3, AsO_2^{4-}, Fe (III), and Co (II) and convert these metals into their nontoxic forms by oxidation. Certain bacterial species of *Pseudomonas*, *Bacillus*, and *Alcaligenes* have been reported to reduce U (VI) to U (IV), Se (VI) to Se, and Hg (II) to Hg (0) (Chaturvedi et al. 2015; Jobby et al. 2018). Similarly, some algal species (*Ascophyllum nodosum, Cladophora glomerata, Oedogonium oedogonium*) have greater potential to remediate heavy metals like Cu, Cd, Ni, Co, and Mg than bacteria (Yadav et al. 2017). Fungi detoxify and resist the toxic effects of heavy metals by active transport of metal into the cell, valence transformation, and intracellular and extracellular precipitation. Different fungal species such as *Aspergillus niger*, A*spergillus fumigatus*, and *Aspergillus niveus* accumulate heavy metals (like Cu, Pb, Zn, Cd, and Hg) into their spores and mycelium (Rajkumar et al. 2012; Yadav et al. 2017). They degrade heavy metals into their nontoxic form by changing their valence state. Biotransformation utilizes the natural capabilities of bacteria and fungi to degrade heavy metals which makes it a cost-efficient and eco-friendly process for the remediation of heavy metal-contaminated wastewater (Chaturvedi et al. 2015).

25.3.3 Biovolatilization

Gases and methylated compounds are highly volatile in nature. Process of releasing methylated compounds and gases from cell to the surrounding environment is considered as biovolatilization. Several well-known compounds such as As, Se, Hg, bismuth, antimony, and tellurium are methylated by microorganisms, but the effect of valence properties of such elements on their biovolatile properties is not clear (Zhang et al. 2019). Biomethylation is a natural process occurring in living organisms in which methyl group is transformed enzymatically and linked to the metal and metalloid. The process of biomethylation involves series of oxidative methylation and metabolic reduction reactions of metals and metalloid. Fungal strain named *Scopulariopsis brevicaulis* is the methylator of antimony and arsenic (Boriová et al. 2014). Different elements such as tellurium, selenium, arsenic, and antimony cannot be completely accumulated by microbial cells. The process of biomethylation is the best alternative for the elements, which can neither be accumulated nor biosorbed and transform these elements into alkylated and methylated products which consequently are volatilized into the atmosphere (Bentley and Chasteen 2002).

25.3.4 Bioleaching

There are different methods like hydrometallurgy and pyrometallurgy for the extraction of metals from sulfides and metal oxides. These methods are not easy to handle due to several problems like environmental pollution, high operating cost, and low recovery yield (Pathak et al. 2009). Bioleaching is the use of microorganisms to solubilize sulfides and metal oxides from polluted areas. Biosolubilization is a simple process that requires low-cost investment as compared to the conventional processes of bioremediation (Bosecker 2001). At industrial level, different processes including heap, dump, and in situ bioleaching are employed to extract purified form of metals from its raw forms or mineral ores (Gu et al. 2018). Scientists are trying to employ bioleaching process for remediation of solid waste, but slow reaction kinetics of this technique hinders its large-scale application. To apply this process for bioremediation of solid waste, different operating parameters should be optimized such as pH, temperature, particle size, pulp density, nutrient requirement, reaction time, and mass transfer. Proper pretreatments are necessary to improve the efficiency of bioleaching.

At industrial scale, bioleaching had been applied for decades; however, the role of microbes in mineralization of metals was not well understood. A bacterium named *Acidithiobacillus ferrooxidans* was discovered in 1961 in leachates that can oxidize Fe^{2+} to Fe^{3+}. The Fe^{3+} is an electron acceptor that can solubilize metal sulfides. An archaebacterial species *Acidianusbrierleyi* discovered in 1965 has been reported to oxidize iron and sulfur (Gu et al. 2018). At industrial scale, bioleaching is effectively practiced since 1980 for better understanding of the metabolism of microorganisms (Mishra et al. 2005). Mesophilic bacterial species of *Leptospirillum* and *Acidithiobacillus* genera are widely used in industry for bioleaching. These gram-negative, non-spore-forming, and rod-shaped bacteria use sulfur compounds such as sulfide and thiosulfate as energy source and airborne CO_2 as carbon source. They do not need organic carbon supplement which is the main advantage of such bacteria in bioleaching. Low pH conditions are most appropriate for them in bioleaching of metal sulfides and oxides. Some important species of genus *Acidithiobacillus* in bioleaching are *A. albertis, A. caldus, A. ferrooxidans, A. thiooxidans, A. acidophilus, A. prosperus*, *and A. concretivorus*. These species work most appropriately under alkaline conditions (Hoque and Philip 2011).

Two species named *A. ferrooxidans and A. thiooxidans* grow under acidic conditions making them more suitable for the bioleaching of heavy metals. The *A. ferrooxidans* has substantial potential of bioleaching of heavy metals because it utilizes Fe^{2+} as an energy source and oxidizes reduced sulfur compounds for obtaining energy. Archaea are heat-resistant bacteria which grow at high temperature (i.e., above 60 °C), are important candidates for bioleaching at high temperature, and can provide better yield of metals from minerals (Kaksonen et al. 2017). Various bacteria use organic compounds as energy source during their metabolic pathway. They secrete several types of organic acids such as lactic acid, gluconic acid, oxalic acid, and citric acid and enzymes to form soluble metal complexes (Rastegar et al. 2016; Schmidt and Schaechter 2012). Due to advancements in microbiome, it is considered that bioleaching will be the groundbreaking technology of the future for the treatment of heavy metal-contaminated wastewater (Gu et al. 2018).

25.3.5 Biodegradation

The process of biodegradation is termed as the implementation of various microbial groups such as bacteria, algae, and fungi independently or in consortia to degrade organic compounds present in wastewater-contaminated soils. The microbes utilize various metabolic and/or enzymatic reactions in order to mineralize organic pollutants into carbon dioxide, water, and methane. Anaerobic bacteria use carbon dioxide as energy source and aerobic bacteria use oxygen for their survival and growth (Jacob et al. 2018). Wastewater-contaminated soils contain oxides of nitrogen and sulfur; microorganisms degrading these compounds are known as nitrate-reducing bacteria and sulfide-oxidizing bacteria, respectively (Arivalagan et al. 2014). Heterotrophic bacteria degrade organic matter by forming clusters or biofilms on the surface of organic pollutant. Microorganisms produce extracellular polymeric substances upon consumption and absorption of organic substances from wastewater environment, cell secretion, cell lysis, and degradation. The polymeric substances such as nucleic acids, proteins, and phospholipids secreted by microbial cells help to protect and sustain biomass against toxic substances by constructing the diffusion barrier (Dadrasnia et al. 2017). These substances have the potential of degradation and biosorption of toxic contaminants present in the wastewater-irrigated soils (Geyik et al. 2016).

25.4 Beneficial Microbes in Wastewater-Contaminated Soils

In addition to their service as bioremediation tool for wastewater-contaminated soils, microbes also act as biofertilizers when applied to root surface, seed, or soil. They have to compete with the indigenous microbes for rhizospheric colonization. After successful colonization, they promote plant growth directly by providing plants with nutrients and phytohormone production and indirectly by biocontrol activity. Rhizosphere is that area of soil where complex biochemical interactions take place. It is referred to as nutrient-rich area as various nutrients such as amino acid, carbohydrates, chitinases, and antibiotics are exuded by plant roots as root exudates (Kalam et al. 2017). The microbial community colonizing this area is termed as rhizospheric microbes. The most predominant population among rhizospheric microbes is the plant growth-promoting bacteria (PGPR). It has been revealed that some bacterial species of *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Bacteroidetes*, and *Firmicutes* are present in the rhizosphere of plants (Foesel et al. 2016). PGPR have been reported to improve plant growth by increasing nutrient and mineral uptake and nitrogen fixation and providing resistance against diseases. Rhizobial species interacting with legumes and forming nodules exert a positive effect on plant growth. For example, *Burkholderia cepacia* when applied to soil increases maize growth by production of siderophores. Similarly, *Penicillium bilaii* is a fungal strain and has the potential to improve plant growth in wastewater-contaminated soils (Kuiper et al. 2004; Verma et al. 2017).

25.5 Microbe-Based Strategies for Plant Growth in Wastewater-Contaminated Soils

Some of the strategies utilized by microbes to improve plant growth in wastewater-contaminated soils are discussed below (Table 25.2).

25.5.1 Phosphate Solubilization

Phosphorous (P) is an important macronutrient for plants as it is a vital part of ATP, DNA, and phospholipids. The P cycle is different from nitrogen cycle which circulates in the atmosphere in gaseous form. The P cycle mostly begins

Table 25.2 Microbe-based strategies for plant growth in contaminated soils

Serial no.	Strategies	Microbes involved	Advantages	References
1	Phosphate solubilization	*Pseudomonas, Agrobacterium, Bacillus, Rhizobium, and Micrococcus* Some fugal species are *Penicillium daleae, Aspergillus* sp., *Aspergillus versicolor*	• Eco-friendly, enhance agriculture and forest production	Alori et al. (2017); Hayat et al. (2010); Ahemad (2015)
2	Growth hormones production	*Acetobacter, Bacillus, Xanthomonas, Enterobacter, and Arthrobacter* Some fungal species are *Aspergillus, Eurotium,* and *Penicillium*	• Increase crop yield • Nutrient uptake and growth	(Arshad and Frankenberger Jr (1997); Saharan and Nehra (2011); Glick et al. (2007)
3	Zinc mobilization	*Lupinus albus and Azospirillum lipoferum* Some fungal species are Agaricomycete *Grifola frondosa* and Ascomycete *Helvella crispa*	• Enhance metal mobility • Biodegradable • More efficient in lowering the pH of soil	Pérez-Esteban et al. (2013); Tariq et al. (2007)
4	Induction of stress tolerance	*Burkholderia phytofirmans, Paenibacillus polymyxa, and Curvularia protuberata*	• Show resistance against pathogens, heat, drought, limiting nutrients, and herbivores	de Zelicourt et al. (2013); Yang et al. (2009)
5	Biocontrol	*Streptomyces*, *Bacillus* Some fungal strain are *Phenazines, Pyrrolnitrin, and Pyoluteorin*	• Environment friendly • Target specific, biodegradable • Antagonistic activity	Berg (2009); Bloemberg and Lugtenberg (2001)
6	ACC deaminase activity	*Pseudomonas putida* UW4	• Make plant disease free and enhance growth rate	Arshad et al. (2007); Glick (2014)
7	Siderophore production	*Enterobacter, Pseudomonas,* and *Rhodococcus*	• Increase the plant growth and enhance iron absorbing ability	Abbas et al. (2019); Grinter and Lithgow (2019)
8	Nitrogen fixation	*Enterobacter, Pseudomonas,* and *Rhodococcus*	• Enhance plant growth and disease management	Prasad et al. (2019); Pankievicz et al. (2015)

with rocks as it is present in soil in the form of rock phosphate as organic and inorganic phosphates. Phosphorus is present in very low concentration in soil (up to 1 ppm) which is the ultimate source of P for plants. If soil fails to provide the required amount of P to the plants, the growth of plants becomes stunted (Rodríguez et al. 2006). Phosphate solubilization is the conversion of unavailable inorganic form of phosphates into its soluble form which is readily available and easily accessible to plants. Plants can only take phosphates in the form of orthophosphates such as $H_2PO_4^-$ and HPO_4^{2-}. Microbial phosphate solubilization can be done via enzymatic processes in case of organic phosphate mobilization, while extracellular synthesis of organic acids is involved in inorganic phosphate solubilization (Shahid et al. 2012, 2015, 2018; Mahmood et al. 2017; Hanif et al. 2015). Certain microbial groups such as bacteria and fungi have innate potential of phosphate solubilization. Among these, bacteria have been widely explored for the solubilization of inorganic phosphates. The most widely studied bacterial genera in terms of phosphate solubilization are *Pseudomonas, Agrobacterium, Bacillus, Rhizobium, Micrococcus, Burkholderia, Achromobacter, Erwinia, Flavobacterium*, *and Aerobacter* (Kalam et al. 2017). Some fungal species involve in phosphate solubilization in wastewater-contaminated soil are *Penicillium daleae*, *Aspergillus* sp., and *Aspergillus versicolor*. Among these, the strains of *Pseudomonas*, *Rhizobium*, and *Bacillus* have been reported as the most proficient candidates for the solubilization of inorganic phosphates (Hayat et al. 2010; Shahid et al. 2018). The process of inorganic phosphate solubilization involves the acidification of the soil, which leads to the lowering of pH. The bacteria secrete variety of organic acids such as gluconic acid, glycolic acid, oxalic acid, malonic acid, succinic acid, citric acid, acetic acid, isobutyric acid, isovaleric acid, and lactic acid to lower the pH of the soil (Shahid et al. 2012). These acidic conditions de-chelate the P from metal ions and made P available to plants (Rodríguez and Fraga 1999). Microbial organic phosphate solubilization is carried out through the hydrolysis of organic substrates into inorganic phosphates in the presence of enzyme phosphatases and phytases, which cause hydrolysis of phosphodiester bonds. Plant and animal remains are the sole source of organic phosphates and their decomposition by saprophytes releases radical orthophosphates (Whitelaw 1999; Hanif et al. 2015). Recently, it has been shown by experimental evidences that phosphate solubilizing bacteria can also be inoculated in contaminated soils to nourish the plant with phosphates under harsh conditions (Shahid et al. 2018; Mahmood et al. 2017).

25.5.2 Growth Hormone Production

Plant hormones are a group of signal molecules which are synthesized by plants by various metabolic processes; that helps in the coordination of cellular activities and plant development. They interact with specific tissues and produce physiological responses. These organic substances regulate growth, germination, metabolism, and many other physiological activities. Plant hormones are generally referred to as phytohormones. There are five well-established classes of plant hormones including auxins, gibberellins, cytokinins, abscisic acid, and ethylene. Each one of them has a vital function in maintaining the growth, counteracting abiotic stress, and upholding the plant in harsh conditions (Davies 2010). Higher plants produce a considerable amount of plant hormones indigenously, while in some cases these hormones are provided exogenously by soil microbiota. Nowadays, microbial preparations are available commercially which increase crop yield through phytohormone production (Shi et al. 2017). In order to increase crop yield, specific microbial preparations are being inoculated which produces plant hormones. This practice is common in the USA, Australia, Belgium, New Zealand, Soviet Union, the Netherlands, and India (Akram et al. 2016). Most widely studied microbes for phytohormone production are the members of *Azotobacter* and *Azospirillum*. These are involved in in vitro production of auxin, gibberellins, and cytokinins as well as involved in nitrogen fixation. Rhizobia are diazotrophic bacteria which produce indoleacetic acid (IAA) and are also involved in dinitrogen fixation (Dinnage et al. 2019). Other microbes which are studied for plant hormones production include *Acetobacter, Bacillus, Xanthomonas, Enterobacter, Arthrobacter*, and *Pseudomonas* (Arshad and Frankenberger 1997; Akram et al. 2016).

Some fungal species reported to produce IAA include *Hebeloma* sp., *H. cylindrosporum*, and some actomycorrhizal fungi (Saharan and Nehra 2011). Phyto-beneficial bacterial species also have a great potential to exhibit phytohormone synthesis when inoculated under harsh conditions of contaminated soils (Javed et al. 2019; Shahid et al. 2018). Some fungal species like *Aspergillus, Eurotium,* and *Penicillium* are phyto-beneficial agents found in wastewater-contaminated soil and have the ability to produce growth hormones. It is reported that different species of *Aspergillus* are efficient for production of plant growth promoters like gibberellin (GA) indoleacetic acid (IAA) (Samah et al. 2019).

25.5.3 Zinc Mobilization

Zinc (Zn) is a micronutrient required in adequate concentration by plants for their enhanced growth and productivity. It is present on the surface of clays, organic matter, and anhydrous oxides. Plant roots absorb Zn by process of diffusion when present in accessible form in soil. The soil conditions and properties, root growth, and surface area have the tremendous effect on the solubilization and availability of Zn. With an increase in pH, availability of Zn decreases as its adsorption on soil particles increases (Straczek and Hinsinger 2004). Zinc is present in many forms like soluble Zn and insoluble Zn in form of crystalline lattice, chelated and absorbed onto metal oxides. PGPR serve as the best alternative to chemical fertilizers in terms of enhancing Zn mobility to plants. It has been studied that biofertilizer containing microbial consortium of *Azospirillum lipoferum*, *Pseudomonas* sp., and

Agrobacterium sp. enhanced number of plant root and shoot weight and root length and volume (Tariq et al. 2007; Pérez-Esteban et al. 2013). Higher concentrations of heavy metals are toxic for microorganisms. Soil becomes polluted with heavy metals when irrigated with metal-contaminated industrial wastewater (Barakat et al. 2019). It has been reported that different bacteria species like *Bacillus megaterium*, *Rhizobium rhizogenes*, *Rhizobium leguminosarum*, *Azotobacter vinelandii*, and *Nocardiopsis dassonvillei* have great ability to remediate Zn from wastewater-contaminated soil. It has been proved that bacterial species *Bacillus megaterium* have greater potential to remediate Zn from contaminated soil as compared to the remaining species of microbes (El-barbary and El-Badry 2019). Some fungal species also have been reported in accumulating and detoxifying Zn in wastewater-contaminated soil as Agaricomycete *Grifola frondosa* and Ascomycete *Helvella crispa* (Leonhardt et al. 2019).

25.5.4 Induction of Stress Tolerance

Plants have to face different biotic and abiotic stresses such as infections caused by phyto-pathogens, salinity, heat, drought, and herbivorous activity. Therefore, plants must be equipped with certain defending mechanisms to thrive under such conditions (Akilan et al. 2019). Plants have evolved various mechanisms which help them to understand and respond to changing environmental conditions when it comes to developing the habit of living under harsh conditions such as wastewater-contaminated soils (Gururani et al. 2015). In such conditions, the plant-associated microbes play a vital role in plant survival by triggering protective mechanisms in plants. Most commonly plant-associated microbes are mycorrhizal fungi and PGPR, which provide resistance to plants against harsh environment. They stimulate the host defense system by exopolysaccharide, phytohormone, ACC deaminase, and signal transduction (de Zelicourt et al. 2013).

Plant growth-promoting fungi provide resistance against pathogens, heat, drought, nutrient-limiting conditions, and herbivores in wastewater-contaminated soils. Most common bacterial strains helping plants under harsh conditions are *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Burkholderia* (Javed et al. 2019; Shahid et al. 2018). In certain areas, drought and water loss due to high temperature limit the growth and productivity of plants. The *Paenibacillus polymyxa* inoculation to *Arabidopsis thaliana* showed better resistance to water-deficit conditions than control plants (Yang et al. 2009). In wastewater-contaminated soil, heavy metals are reduced to immobilized form by microbial action (Cherian et al. 2012). Connection between plants and bacteria shows many benefits for host plant such as development and growth enhancement. It is reported that *Neorhizobium huautlense* T1–17 and *Bacillus megaterium* H3 reduced 79–96% concentration of Cd in wastewater and enhanced grain biomass (Javed et al. 2019).

25.5.5 ACC Deaminase Activity

Ethylene is produced in plants and improves plant growth in low concentrations, but under stress conditions (wastewater contamination), the cellular ethylene level is increased. Ethylene hinders plant growth at high concentration in all plant parts like root, shoot, leaves, flower, and fruit (Afridi et al. 2019). Ethylene production within cell is directly linked with the concentration of 1-aminocyclopropane-1-carboxylic acid (ACC), which acts as ethylene precursor. Bacteria that possess enzyme ACC deaminase help plants to regulate ethylene concentration under wastewater-contaminated environments by breaking ACC into α-ketobutyrate and ammonia, thus reducing ethylene level in plants under stressed environment (Mahmood et al. 2017; Glick 2014). It is reported that ACC deaminase producing *Pseudomonas putida* UW4 improved the growth of tobacco plants. The inoculated plants showed better ability to accumulate heavy metals when irrigated by metal-contaminated wastewater (Arshad et al. 2007).

25.5.6 Siderophore Production

Plants require iron (Fe) for their optimum growth and development. To overcome the deficiency of iron, microbe-oriented siderophores helped plants to uptake iron from natural resources. These are compounds having low molecular weight with strong iron-binding ability (Rizzi et al. 2019). Microbes provide iron by forming soluble Fe^{3+} complex. It is reported that both gram-negative and gram-positive bacteria have the ability to produce siderophores, e.g., *Enterobacter, Pseudomonas*, and *Rhodococcus* genera (Grinter and Lithgow 2019). Studies showed that siderophores produced by PGPR increase the plant growth in stress conditions including wastewater-contaminated soil and enhance iron absorbing ability at low concentrations (Abbas et al. 2019).

25.5.7 Nitrogen Fixation

Nitrogen is a main element for plant growth and also an important constituent of amino acid, membrane lipids, and nucleotides. It is present in gaseous N_2 form that is inaccessible for animals and plants. Biological nitrogen fixation (BNF) is an essential source which provides plants with nitrogen subsequently used by plants for their growth (Pankievicz et al. 2015). Plant-beneficial microbes have the ability to fix nitrogen and make it available for plant use by utilizing the nitrogenase enzyme activity. Nitrogen fixation (NF) is a mutualistic relationship between plants and microbes. Plant-beneficial microbes that fix atmospheric nitrogen are *Rhizobium, Bradyrhizobium, Sinorhizobium, and Mesorhizobium*. Under wastewater contamination, microbes may help nourish plants with nitrogen by fixing nitrogen in order to enhance plant growth (Prasad et al. 2019).

25.5.8 Biocontrol Activity

Biological control is the elimination and reduction of pathogens by using living organisms. Many environmental risks are directly involved in order to make plants resistant against diseases (Shukla 2019). Moreover, breeding new varieties is a time-taking process and it does not provide resistance against all diseases. On the other hand, agrochemicals are costly and also eliminate the non-targeted organisms which might be useful for plants (Hause and Schaarschmidt 2009). For this purpose, biocontrol technique becomes much important for control of plant pathogens. HCN (cyanogenesis) is a volatile biocontrol agent that is used to inhibit metalloenzyme. Hydrogen cyanide produced by gram-negative bacteria as a secondary metabolite is catalyzed by HCN synthase (Munir et al. 2019). It is reported that cyanide produced by *Pseudomonas* strains is used to inhibit canker of tomato (Lanteigne et al. 2012). Hydrolytic enzymes that degrade several types of compounds might play a vital role in order to control pathogens in wastewater-contaminated soils. Hydrolytic enzymes produced by rhizobacteria are ß-1-3-glucanases, chitinases, cellulases, lipases, and proteases (Guenoun et al. 2019). These enzymes efficiently degrade fungal cell wall to control the fungal diseases of plants (Kenneth et al. 2019). It has been studied that many bacterial strains have proved themselves as potential biocontrol agents. The bacterial genera, *Streptomyces* and *Bacillus*, produce antifungal metabolites such as Phenazines, Pyrrolnitrin, and Pyoluteorin for effective biocontrol activity (Bloemberg and Lugtenberg 2001).

25.6 Conclusion

Rapid increase in industrialization and feeding mouths not only increased the consumption of water but also increased the discharge of wastewater into the nearby agricultural fields, which also forced the farming community to use unfit water for irrigation purposes especially in developing countries, where availability and suitability of irrigation water is a question mark. Under the given circumstances, farmers have no options except remediation of soil by physical, chemical, and biological methods in order to achieve maximum yield per unit area. Biological methods are the most suitable due to being cost-efficient, eco-friendly, and less laborious. These methods use microorganisms such as bacteria, fungi, algae, etc., to provide a quick relief to plants from stressful conditions. These microorganisms interact with soil-plant system and utilize various strategies such as biosorption, bioaccumulation, biovolatilization, biotransformation, bioleaching, biodegradation, etc., in order to mitigate the ecotoxicological effects in wastewater-contaminated soils. In addition to the bioremediation strategies, microorganisms are also equipped with phytobeneficial potential to enhance plant growth in harsh condition employing various mechanisms such as phosphate solubilization, growth hormone production, ACC deaminase activity, exopolysaccharide production, siderophore production, and biocontrol activity. Due to the heterogeneous nature of pollutants present in wastewater-contaminated soils and complexity of soil-plant-microbe interactions, deep mechanistic investigations are needed by researchers to make microbe-based soil remediation strategies more efficient, applicable, and acceptable. Due to the importance of the subject, policy makers need to think on the growing land areas being irrigated with wastewaters and economically support the stakeholders involved in active research to biologically remediate these land areas for efficient and profitable agricultural outputs.

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Chapter 26

Phytoremediation of Heavy Metals: An Overview and New Insight on Green Approaches

Anindita Mitra, Soumya Chatterjee, and Dharmendra K. Gupta

Contents

Abstract Phytoremediation is a cost-effective, eco-friendly green technology exploiting plants with their natural uptake capabilities through root organization, along with the translocation, bioaggregation or detoxifying abilities to decontaminate the soil, water and groundwater from toxic pollutants. The plant approaches to cope with several xenobiotics include phytostabilization, phytoextraction, phytovolatilization, rhizofiltration, phytodegradation and phytostimulation. Some plants can survive under severe metal stress, and higher metal tolerance can be achieved either by reduced uptake or improved plant internal sequestration of metals, an outcome of interaction between specific plant genotype and its environment. Molecular

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genetics have led to better understanding of mechanisms of heavy metal tolerance or accumulation in plants, and genetic engineering has unlocked new gateways in phytoremediation strategies by producing elite plants with enhanced metal remediation capabilities. This article reviews the recent developments in phytoremediation of heavy metals addressing the genetic basis of metal hyperaccumulation and tolerance and a discussion on the likelihood of transgenic plants in phytoremediation of heavy metals.

Keywords Phytoremediation · Heavy metals · Hyperaccumulation · Genetic basis · Transgenic plants

26.1 Introduction: Phytoremediation—An Overview

Extensive anthropogenic activities from mining, agronomy, industry and military operations have discharged toxic compounds in large extent into the environment regularly, among which heavy metals or metalloids and organic pollutants are challenging the survival of the plants, animals and human (Chatterjee et al. 2017). Contamination of soil by heavy metals like lead (Pb), mercury (Hg), nickel (Ni), cadmium (Cd), chromium (Cr) and arsenic (As) has adverse effect on plant growth and metabolism, thus reducing agricultural production. Furthermore, wildlife inhabiting in these areas and human also get affected from the adverse effect of these metals (de Vries et al. 2013; Sharma et al. 2010, 2014). Therefore, an immediate attention is required for effective removal of these contaminants from the polluted sites. The conventional physical and chemical methods of remediation generally used, such as soil excavation, filling of land, washing of soil and physicochemical immobilization or extraction of metals, all have certain limits, i.e. expensiveness, requirement of extensive labour, disturbance of natural habitats of soil microflora and leaving unpleasant scars on the landscape (Cherian and Oliveira 2005). Among the varied concepts of the in situ ecological reformation of heavy metal-contaminated soils, especial consideration is intended to the alternative approach of phytoremediation (Mahar et al. 2016). Phytoremediation is an environment friendly and economically sound approach in correlation with physical and chemical remediation that uses plants and accompanying soil microbes to clean up environment (Kramer 2005). Phytoremediation techniques exploit natural or transgenic plants and competently clear away hazardous pollutants, i.e. HMs including radionuclides, pesticides, polychlorinated biphenyls (PCB) and polynuclear aromatic hydrocarbons (PAH) from the ambient converting them into safer compounds (Mahar et al. 2016). On the basis of economic execution, the target of phytoremediation can be viewed in three layers: (1) phytoextraction of metals with financial benefit, i.e. Ni and Ti; (2) abatement of hazardous metals, i.e. Cd and Hg (phytostabilization); and (3) renewal of soil properties by increasing soil fertility for sustainable crop growth (Van Aken 2009). Estimated value of removal of HMs through phytoremediation per ton of soil is about 25–100 US dollars (Movahed and Maeiyat 2009). The annual dividend earned from phytoremediation market is approximately 235–400 million US dollars (Yadav et al. 2010). The goal of phytoremediation, therefore, depends on the selection of suitable plant species that can efficiently absorb and translocate HMs to the above-ground parts (Kramer 2010; Milner and Kochian 2008). The association of plant and soil microbial community (such as mycorrhizal fungi and growth-promoting bacteria) also plays significant role in promoting HMs to the aerial parts as reported by several workers (Luo et al. 2014; Ma et al. 2014; Rajkumar et al. 2012). Plants utilize different strategies to decontaminate xenobiotics including phytostabilization, phytoextraction, phytovolatilization, rhizofiltration, phytodegradation and phytostimulation (Tsao 2003).

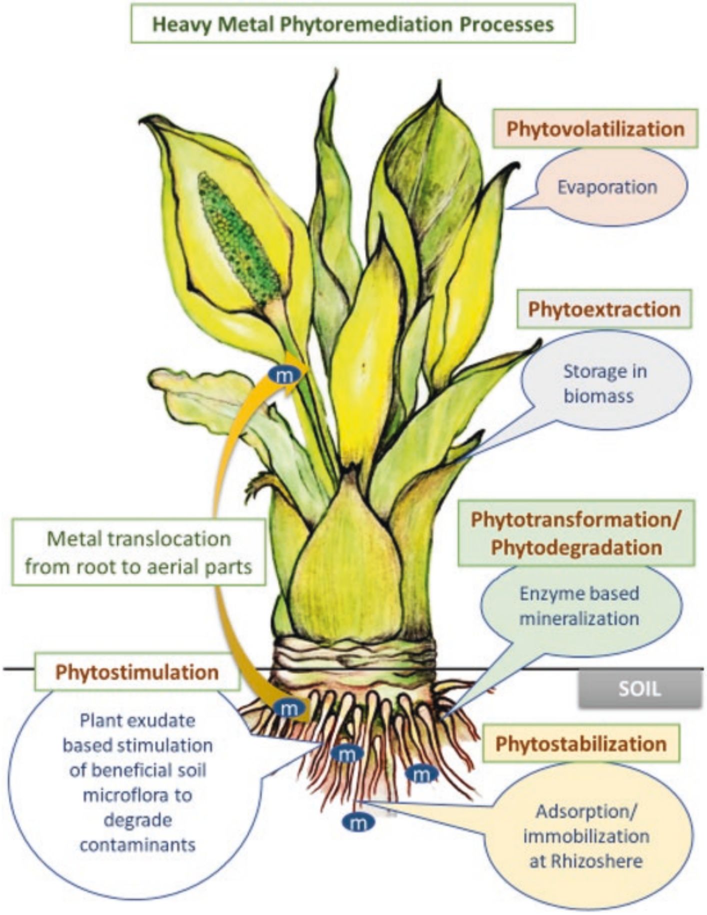

Fig. 26.1 Schematic diagram of different methods of phytoremediation

Phytoextraction (also termed as phytoaccumulation) uses the hyperaccumulating plant varieties that uptake and remove HMs from soil or water by their roots and translocate and accumulate in aerial part, i.e. shoots or leaves (Fig. 26.1) (Kramer 2005; Ghosh and Singh 2005; Kotrba et al. 2009; Chatterjee et al. 2013a) that can be plucked at the end of the growth of the plant. It is most effectual among other phytoremediation methods, although practical adversities persist for its application (Kramer 2005). Small, organic compounds secreted by plants rhizospheric region are called phytosiderophores that are high-affinity iron-chelating metal transporter in the cell membrane translocating metals from root to shoot (Hider and Kong 2010; Dotaniya et al. 2013). Selection of suitable plants that are capable of translocating HMs to their shoots is crucial for successful phytoextraction because harvesting of the root biomass is not a practicable approach (Yang et al. 2005). The term "hyperaccumulator" was first created for the plants accumulating Ni more than 1000 mg kg^{-1} dry weight (0.1%) (Brooks et al. 1977). The natural hyperaccumulators proficiently absorb 100–500 times more HMs than their counterpart which has led to the profound development of phytoextraction technology (Baker and Brooks 1989). The most popular natural hyperaccumulators plants belong to the families including Asteraceae, Brassicaceae, Caryophyllaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae and Euphorbiaceae (Bolan et al. 2014). Examples are alpine pennycress *Thlaspi caerulescens*, chiefly hyperaccumulating Zn^{2+} and sporadically Cd^{2+} and Ni^{2+} (Milner and Kochian 2008), serpentine endemic shrub *Alyssum* sp., Indian mustard *Brassica juncea* (Brassicaceae) and *Astragalus racemosus* (Leguminosae) and Asian stonecrop *Sedum alfredii* (capable of accumulation of Zn, Cd and Pb) (Lu et al. 2008; Deng et al. 2008). Highest absorption capacity for Cd in *Datura stramonium* as found was 0.1498 mg kg^{-1} Cd, whereas in *Amaranthus paniculatus* it was 68.04 g ha^{-1} with the maximal absorption potentiality per unit area (Li et al. 2015). Plants characterized by widely branched root system, high and fast-growing biomass, ability to grow everywhere, comparatively facile to cultivate and repugnant to herbivores (thereby, avoiding the outflow of accrued metals into the food chain) are intended for phytoextraction (Buscaroli 2017). An effective application of phytoextraction for remediation of Ni soiled area surrounding a plant in Rustenburg, South Africa, by using Ni hyperaccumulator *B. coddii* has been reported by Antony et al. (2015). An efficient removal of Pb, Cr and Ni from the polluted soil by the well-known sunflower plant has been reported by Dhiman et al. (2017).

On the other hand, in phytostabilization (Fig. 26.1) process, agility and bioavailability of the contaminant in the soil or water are reduced by immobilizing the pollutant by using plants through adsorption onto roots, accumulation by roots or precipitation within the root zone (Tangahu et al. 2011; Chatterjee et al. 2013a), thereby preventing the escape of metals by percolation, weathering or dissipation through soil, water or air to avoid contamination to aquifers and ambient habitat (Ernst 2005). Plants competent for phytostabilization should have an expanded root system for providing improved soil cover, higher endurance to the toxic metals, preferably arresting the HMs in the rhizospheric region (Kramer 2005) and endurable to aridity and heat. Phytostabilization technique exploits grass species varieties tolerant to HMs such as *Agrostis capillaries* and *Festuca rubra* (Kidd et al. 2009), although leguminous species *Lupinus albus* also has been recommended as a potential agent for clean-up of Cd- and As-polluted soil (Vazquez et al. 2006). Applying grass species is more advantageous for phytostabilization in comparison to dicot species as they bioaccumulate less metals in the aerial parts, thereby, minimizing the risk of wildlife to come in contact to these noxious metals (Pilon-Smits 2005). The more toxic soluble Cr^{6+} compounds are effectively reduced to insoluble Cr^{3+} by the deep-rooted plants, thereby reducing environmental risk (James 1996). By an unknown mechanism, bioavailable form of Pb in a hugely contaminated Pb/Zn mine wastes is converted into insoluble and nonbioavailable pyromorphite by *Agrostis capillaris* using soil lead and phosphate, thus minimizing the risk of escape of lead into the food chain (Cotter-Howells and Capom 1996). These reports prove that Cr and Pb can be successfully phytostabilized. Soil amendments are a prerequisite to reach the goal of phytostabilization on a long-term basis by (1) increasing the soil pH greater than 5 by liming with $CaCO_3$ and/or $Ca(OH)_2$ (Mench et al. 1994); (2) reducing mobility of the HMs by the application of soil additives such as compost, Fe, Al and Mn hydroxides (Vangronsveld et al. 1995); and (3) enriching soil property by applying fertilizer (Li and Chaney 1998). Plant's root exudates also have an important role in detoxification of HM-contaminated soil as they regulate the pH of rhizospheric soil (Yan et al. 1996) and promote chelation of minerals (Dakora and Phillips 2002), acting as nutrient source and chemoattractant for the microbes in soil (Girkina et al. 2018).

In the process of rhizofiltration, plants remove the HMs from contaminated water by the roots through the process of adsorption or precipitation and restricted the mobility of HMs within root (Jeevanantham et al. 2019). This method is appropriate for in situ and ex situ removal of the metals like Pb, Cd, Cu, Fe, Ni, Mn, Zn and Cr and radionuclides (90 Sr, 37 Cs, 238 U, 236 U) from groundwater, surface water and wastewater and can be applicable in even the contaminants present in a very low concentration in large volume of water (Dushenkov et al. 1995, 1997a, b; Dhir 2013). A perennial sedge *Carex pendula* that preferably grows in moist habitats can accumulate considerable amount of Pb in the roots at in situ conditions (Doty et al. 2007). This herb can grow to a higher concentration of Pb in sewage (10 mg L^{-1}) showing stunted growth and a potential candidate for decontamination of Pb-contaminated sewage (Ghosh 2010). Several factors such as pH, rate of water flow and amount of pollutant in waterbody and dumping of the contaminated plant biomass may be the limiting factors for effective application of this process.

In phytodegradation, also known as phytotransformation (Fig. 26.1) as the name implies, plants are able to metabolize and degrade the contaminants from soil, sediments, sludges or groundwater and surface water through enzymatic process (Spaczynski et al. 2012). Enzymes involved are dehalogenase, oxygenase, nitroreductase, peroxidase, laccase and nitrilase catalysing and accelerating the degradation process (Schnoor et al. 1995). Phytodegradation is limited to remove chiefly organic compounds such as trinitrotoluene (components of explosives), chlorinated solvents and pesticides (Burken and Schnoor 1997; Thompson et al. 1998; Campos et al. 2008). The plants characterized by extensive root system able to secrete substantial quantity of metabolites for catalysing the xenobiotics, tolerant to high concentration of xenobiotics, fast growth and a relatively high biomass are ideal for phytodegradation (Wang and Chen 2007). An earlier report showed phytodegradation of TNT by nitroreductase enzyme present in *Myriophyllum aquaticum* (Schnoor et al. 1995).

Phytovolatilization (Fig. 26.1) is a modified form of phytoextraction method where the pollutants are released into the atmosphere in volatile and less toxic form by leaves instead of accumulating in the above-ground parts (Kramer 2005). Following uptake by roots, contaminants are transported into less toxic water-soluble forms to the leaves where metals get accumulated into the mesophyll tissue between the epidermal layers. In hyperaccumulators, metals are not allowed to retain in mesophyll tissue for longer times and therefore volatilized. Most promising result is obtained in phytovolatilization of selenium (Se) and mercury (Hg) (Bhargava et al. 2012). Genetically engineered *A. thaliana* (Yang et al. 2003) and *Nicotiana tabacum* (Ruiz and Daniell 2009) overproducing bacterial *mer*A and/or *mer*B gene products prove to be highly tolerant to Hg^{2+} and R-Hg^{+} and volatilize ten times of Hg^{0} (elemental mercury) than to non-transgenic plants (Ojuederie and Babalola 2017). Selenocysteine methyltransferase (SMT) gene of selenium hyperaccumulators converts selenite into volatile dimethylselenide (DMSe) form, less toxic than selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) present in contaminated soil (Terry et al. 2000). Transgenic *Brassica juncea* harbouring SMT gene of Se-hyperaccumulator *Astragalus bisulcatus* releases a higher DMSe in comparison to the control plants (Le Duc et al. 2004). Members of Brassicaceae family, e.g. *Brassica juncea*, were known to successfully clean up about 40 g Se ha^{-1} through phytoevaporation from Se-contaminated soil (Padmavathiamma and Li 2007).

Phytostimulation (Fig. 26.1) is the process of bioremediation in which plant root exudates endorse the growth of microbial communities in the rhizosphere which ultimately degrade the contaminant, also known as rhizospheric phytodegradation (Anderson et al. 1994). In this process plants are not involved directly in the degradation process, but, instead, promote to intensify the profusion of soil microflora in the rhizosphere in higher extent in respect to the circumambient soil mass; microbial enzymatic and metabolic processes then convert the chemicals adjacent to rhizosphere in greater range than the adjacent slack soil (Walton et al. 1994; Salt et al. 1998). For example, microbes, metabolizing PCB to non-toxic form, are reported to house in mulberry plant's (*Morus rubra*) root system (Wenzel et al. 1999). Polynuclear aromatic hydrocarbons (PAH) were removed from contaminated site in volatile form or by producing humus by rhizospheric microorganisms (Cunningham et al. 1995; Dec and Bollag 1994). Phytostimulation can be successfully achieved by genetically engineered plants intended for encoding the enzymes or factors connected to detoxification process (phase I and phase II) (Spaczynski et al. 2012).

26.2 An Insight on the Mechanisms of Phytoremediation for Ameliorating Heavy Metal-Contaminated Soils

26.2.1 Sequestration and Accumulation of Heavy Metal in Plants

After uptake and transport, plants accumulate HMs in different organs such as roots, stems, leaves, seeds and fruits which have long-term effects on plant growth and development (Datta and Sarkar 2004; Gallego et al. 2012; Mitra et al. 2014). The phytotoxicity exhibited by HMs includes stubby growth, chlorosis, reduced photosynthesis, increased peroxidation of lipids, greater mobilization of proteins, imbalance between reactive oxygen species (ROS) and antioxidants and finally cell death (Luo et al. 2016; Gallego et al. 2012). Several approaches are utilized by plants to minimize the harmful effects induced by HMs, such as isolation and conversion to non-toxic product. Within plant organs, the cell wall seems to be the major site for accrual followed by cellular chambers, i.e. vacuoles and Golgi apparatus (Ovečka and Takáč 2014). The complex polymers (cellulose, hemicellulose, lignin, suberin and pectin) of cell wall can bind to HM ions, preferably the polyphenolic complex, i.e. suberin, and the heteropolysaccharide pectin (Baxter et al. 2009; Chen et al. 2013). For example, Zn is deposited as simple Zn salts or in complexation with protein or carbohydrate along the walls of vascular bundles in stem, whether in roots it is stored along the cell walls (Shao et al. 2010). Similarly, binding of Cd with phosphorus (P) formed precipitate in the cell wall of the root cortex in *A. thaliana* (Van Belleghem et al. 2007). In other hyperaccumulators, such as *A. halleri*, *N. caerulescens* and *Crassula helmsii*, precipitation of Zn, Cd and Cu in the cell walls has been reported (Huguet et al. 2012; Küpper et al. 2009; Vázquez et al. 1992). Within cytosol heavy metals are detoxified in the presence of strong-affinity binding agents like glutathione (GSH), phytochelatins (PC) and metallothioneins (MTs) (Saraswat and Rai 2011) and are lead to vacuolar transportation where they are reformed to low molecular weight and/or high molecular weight complexes (Song et al. 2014). Further, decoupling of these HM complexes was found in acidic medium within vacuoles and the free HM ions get further chelation with organic acids (e.g. citrate, oxalate and malate and amino acids) (Choppala et al. 2014). Besides vesicular deposition in plants, immediate contact of HMs with several enzymes and metabolic intermediates was further prevented through the mediation of the Golgi apparatus that helps in exocytosis of HMs (Peiter et al. 2007).

26.2.2 Genetic Basis of Metal Hyperaccumulation and Tolerance

A number of genes present in both hyperaccumulator and non-accumulators play prime role in heavy metal tolerance and hyperaccumulation abilities of the plants by differentially expressing in both plants (Hanikenne et al. 2008; Mishra et al. 2017; Singh et al. 2019). The members of gene family which are constitutively overexpressed in hyperaccumulators include ZIP (ZRT, IRT-like) proteins, metal tolerance protein (MTP) families, natural resistance-associated macrophage proteins (NRAMP), P1B-type ATPase (HMA), cation diffusion facilitators (CDF) and yellow stripe-like (YSL) proteins associated with increased take-up of metals, transportation to shoots and vacuolar or cell wall confinement (Indriolo et al. 2010;Mishra et al. 2017; Peng et al. 2017; Zhang et al. 2018; Ahmed et al. 2019).

ZIP protein, one of the primary metal transporters associated with metal uptake, is noticed in many plant species (Mäser et al. 2001). Members of ZIP family translocate divalent cations (Fe, Zn, Mn and Cd) across cell membranes and show broad-spectrum difference in substrate specificity (Mäser et al. 2001). The ZIP proteins consist of eight domains traversing the membrane with outward directed amino- and carboxyl-terminal ends (Guerinot 2000). ZIP transporters are responsible for higher tolerance of metals and enhanced accumulation of metals in plants as evidenced from the augmented upregulation of three zinc transporter genes, namely, ZTP1, ZNT1 and ZNT2, in root and leaves of *Thlaspi caerulescen*s compared to non-accumulator *T. arvense* (Assunção et al. 2001). In *Noccaea caerulescens* roots the ZIP transporter *NcZNT1* was found to involve in Zn/Cd uptake and highly active in the root and shoot suggesting its need in xylem loading of the metal (Milner et al. 2012). The differential cellular expression of another ZIP protein family, ZNT1 and ZNT5, has been reported by Küpper and Kochian (2010) where ZNT1 was found to involve in micronutrient uptake and ZNT5 was involved in hyperaccumulation. Accelerated cellular expression of ZNT1 and ZNT5 genes following Cd exposure supporting their role in plants tolerance to Cd toxicity.

The transporters participating in vacuolar sequestration of heavy metals are P1B-type ATPase also called HMAs, which play key role in metal tolerance in hyperaccumulators. In *A. thaliana*, HMA3 transporter in vacuolar membrane (Miyadate et al. 2011) participates in sequestration of multiple metals (Pb, Co, Cd and Zn) within the vacuoles (Morel et al. 2009). Following Cd exposure, HMA3 gene was found to overexpress in the roots and shoots of *T. caerulescens* (Ueno et al. 2011) and *A. halleri* (Becher et al. 2004) than in non-accumulator species and thus aid vacuolar sequestration of Cd. Similar observation has been reported in tonoplastic SpHMA3 expression in the shoots of *Sedum plumbizincicola* to reduce toxic effect of Cd and retain the natural growth of leaf buds in Cd-contaminated soils (Liu et al. 2017). Peng et al. (2017) observed that the HMA4 and HMA2 were expressed at higher level in hyperaccumulator *S. plumbizincicola* than its non-hyperaccumulating ecospecies *S. alfredii* (Peng et al. 2017).

Cation diffusion facilitator (CDF) proteins are a family of heavy metal transporters accompanying the transport of Zn, Co and Cd present in bacteria and eukaryotes (van der Zaal et al. 1999; Mitra et al. 2014). Members of this family show extreme variation in size and possess six transmembrane domains, a signature sequence at N-terminal end and a C-terminal cation binding domain (Mäser et al. 2001). Although four phylogenetic groups of CDF gene family are found in plants, however only groups I and III participate in heavy metal efflux and metal tolerance and homeostasis (Kramer et al. 2007). The MTP1, MTP8 and MTP11 genes of CDF family are reported to be expressed at enhanced level in *A. halleri and T. caerulescens* than the non-hyperaccumulator counterpart (van de Mortel et al. 2006). AhMTP1 gene in the leaves of *A. halleri* was constitutively overexpressed following exogenous exposure of Zn and resulted in higher accumulation. On the other hand, knockdown of AhMTP1 gene leads to increased susceptibility toward Zn and Cd as found in *A. thaliana* (Drager et al. 2004). Hypertolerance to higher concentrations of Zn has been achieved by the overexpression of MTP1 protein in plants hyperaccumulating Zn (Ricachenevsky et al. 2013). Greater accumulation of Ni^{21} ions within shoot vacuoles in *T. goesingense* than the non-accumulators plants *A. thaliana, T. arvense* and *B. juncea* was found to be associated with boosted TgMTP1 activity in *T. goesingense* (Persans et al. 2001). *NgMTP1* gene isolated from the phytoremediator plant *Nicotiana glauca* also supports the role of this gene in the sequestration of Zn and Co into the vacuoles, thereby reducing the phytotoxic effects of these metals (Shingu et al. 2005). NRAMP transporters are engaged in the transporting of multiple metal ions (Zn, Mn, Cu, Cd, Fe, Co and Ni) and homeostasis (Migeon et al. 2010; Ovečka and Takáč 2014). Plants can uptake and store a significant amount of Cd from contaminated soil through different cationic transporters including NRAMP (He et al. 2015). In hyperaccumulator *T. caerulescens*, a homologous TcNRAMP3 was found to involve in Fe and Cd transport (Wei et al. 2009). Like other transporter proteins, overexpression of TcNRAMP3 and TcNRAMP4 in *T. caerulescens* was found in comparison to non-accumulator *A. thaliana* (Oomen et al. 2009). Overexpression of NcNramp1 gene in Cd hyperaccumulator *N. caerulescens* (Ganges ecospecies) was due to difference in the number of copies of the gene present in plants (Milner et al. 2014). An intensified Ni^{21} sensitivity and Ni^{21} concentration was found in *T. japonicum* due to elevated expression of TjNramp4, approving the role of NRAMP transporters in the Ni^{21} homeostasis in Ni hyperaccumulators (Mizuno et al. 2005). The enhanced activity of LeNRAMP3 gene in tomato leaves at higher Cd concentration (150–200 µM) observed by Meena and co-workers (Meena et al. 2018) elucidates that Cd is sequestered within vacuoles of root cells till it reaches safer boundary to protect from metal stress and beyond that most of the Cd is translocated from root to aerial part like shoot and leaves.

The ABC transporters (ATP-binding cassette) are conserved integral membrane protein families driven by ATP and constitute a diverse family of membrane proteins that allocate a wide array of metabolites (including ions, sugars, lipids, peptides, pigments, xenobiotics, antibiotics and heavy metals) that are transported into the vacuole (Hall and Williams 2003; Liu et al. 2013; Mitra et al. 2014). ABC proteins ship the metals coupled with either GSH or PCs. Augmented expression of the ABC transporter AtMRP2 in the shoots of *A. halleri* has been revealed by microarray (Becher et al. 2004). An iron-regulated tonoplastic transporter PgIREG1 in *Psychotria gabriellae,* a Ni^{2+} hyperaccumulator, exhibited higher expression in respect to non-accumulator orthologue (Merlot et al. 2014). Similarly, an oligopeptide transporter TcOPT3 in *T. caerulescens* encodes an influx transporter for a range of metals (Zn/Cd/ Fe/Cu) that plays important role in metal hyperaccumulation (Hu et al. 2012). In situ hybridization reveals TcOPT3 expression in the vascular systems of leaves and stems, thereby witnessing its participation in long-distance transportation (Hu et al. 2012). Park et al. (2012) illustrated the role of AtABCC1 and AtABCC2 gene in phytochelatin-dependent vacuolar sequestration of Cd and Hg to confer detoxification of these metals. Oda et al. (2011) reported that OsABCG43/ PDR5 in the rice root is responsible for compartmentalization of Cd into organelles to detoxify Cd. A recent report on ABC transporters of *Brassica napus* (BnABC) revealed upregulation of BnABC under Cd stress which are involved in Cd uptake or transport (Zhang et al. 2018).

YSL (yellow strip-like) oligopeptide transporters of plants play important role in transporting metal-nicotianamine complexes over long route from root to shoot and are, therefore, involved in heavy metal stress response. As observed by Wang et al. (2013), tobacco plants transgenic for BjYSL7 gene showed extensive growth of root and augmented translocation ability to shoots found for Cd^{21} and Ni^{21} in comparison with wild-type plants. Another YSL member, SnYSL, was found to overexpress in Cd^{21} hyperaccumulator *Solanum nigrum* and proclaimed higher translocation ratios of Fe and Cd under Cd exposure compared to non-hyperaccumulator (Feng et al. 2017). Another observation from Peng et al. (2017) showed enhanced level of expression of YSLs in *S. plumbizincicola* than in *S. alfredii.*

26.3 Transgenic Approaches for Improved Efficiency of Phytoremediation

Plants are genetically engineered by manipulating the potential pathways for improving phytoremediation such as metal deposition within biomass, higher metal mobilization and root absorption from contaminated soil, complexation with metal ligands and chelators, prevention of contamination by sequestration within vacuoles and further translocation to shoots via symplastic or apoplastic pathways (Chatterjee et al. 2013a, b; Nakamura et al. 2014; Das and Jayalekshmy 2015). The possible pathways of transgenic approaches for effective phytoremediation are clearly represented in Fig. 26.2. The genes that are targeted extensively to introduce in transgenic plants include metal transporters (related to metal uptake, translocation, sequestration) and/or metal chelators (Shukla et al. 2013; Mani and Kumar 2014; Guo et al. 2012; Das et al. 2016).

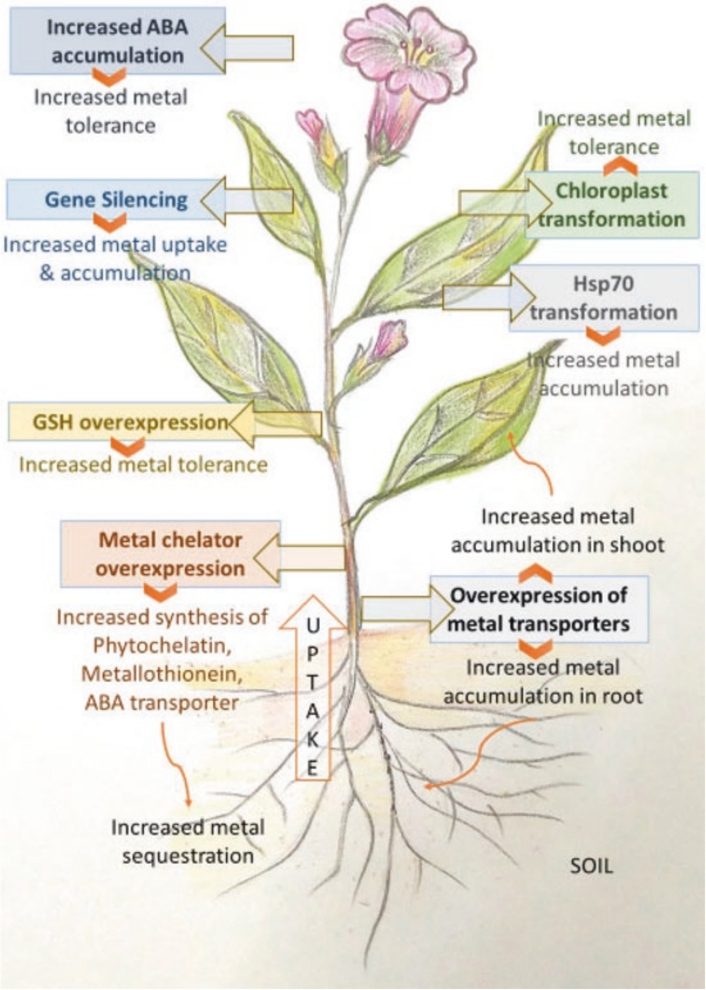

Fig. 26.2 Schematic representation of promising transgenic approaches for improved phytoremediation

A popular approach for enhanced phytoremediation involves the genes encoding heavy metal transporter. Manipulation may be through enhancing metal deposition within roots (phytostabilization), or in the shoots (phytoextraction), or by constraining heavy metal uptake and amassment. A recent report on transgenic tobacco plant overexpressing OsMTP1 gene of *Oryza sativa* (L. cv. IR64 variety) was shown to hyperaccumulate Cd as well as become tolerant to and accumulate exogenous As stress, suggesting wide-range substrate specificity of OsMTP1 gene (Das et al. 2016). Similarly, ZIP genes are also exploited to improve phytoremediation of soil profoundly contaminated with heavy metals. In an earlier report, overexpression of AtIRT1a gene in *A. thaliana* was found to trigger nearly 150% rise in accumulated amount of Cd and Zn (Conolly et al. 2002). Recent approaches are directed toward the manipulation of metal transporters from hyperaccumulator species for environmental clean-up. For instance, overexpression of NcZNT1 gene from *Noccaea caerulescens* responsible for a Zn transporter caused higher amassment of Zn and Cd in the transgenic *A. thaliana* (Lin et al. 2016).

The major group of metal chelators in plants are PCs (peptides synthesized from glutathione) and MT (ubiquitous cysteine-rich proteins directly synthesized from gene) (Gupta et al. 2013a). A strategy is to enhance heavy metal tolerance in plants by manipulating two crucial enzymes catalysing the generation of PCs which are phytochelatin synthase (PCS) and c-glutamylcysteine synthetase (cGCS) (Hirata et al. 2005). Transgenic representative such as tobacco plant (*Nicotiana glauca and Nicotiana tabacum*) is either proficiently accumulating higher amount of Cd and Pb or hypertolerant to Cd and Pb with little accumulation of HMs depending on the source of the transgene (Huang et al. 2012; Chen et al. 2015). For example, PCS gene from *Populus tomentosa* attenuated cadmium transport to shoots of transgenic tobacco (Chen et al. 2015), while PCS from *Ceratophyllum demersum* (an aquatic macrophyte) augmented the translocation process in tobacco plant (Shukla et al. 2013). The differential expression issues of the transgene may be overcome by co-transformation of multiple genes associated with metal homeostasis for production of efficient metal hyperaccumulator. An evidence of co-transformation was provided by the report of Guo et al. (2012) where A. thaliana was transformed with two genes, PCS and YCF1 (an ABC metal transporter), and the transgenic plant was found to be co-tolerant for both As and Cd as well as hyperaccumulator for these trace metals. As proved from this report, improved plant tolerance was achieved due to diminished hypersensitivity reaction that resulted in the accumulation of PC-metal chelates; overexpression of ABC transporter gene helps to sequester these complexes within vacuoles. Another report of co-transformation of PCS with glutamylcysteine synthetase (GCS) genes proved advantageous rather than sole transformation of PCS gene, as overexpression of GCS boosted the catalytic activity of PCS, resulting in the synthesis of phytochelatins in larger amount (Zhao et al. 2014). A recent experiment by Chen and co-workers (Chen et al. 2019) showed that YSL6 gene from blueberry (*Vaccinium* sp.) in transgenic tobacco plant takes part in the transport of Cd from the root to the aerial parts and increases the Cd content in the leaves of tobacco up to 15.57% at high-concentration Cd stress.

Other important high-affinity metal chelators in plants are MTs, generally engaged in maintaining steadiness of vital metals (Se, Zn, Ni and Cu) and detoxification of hazardous metals (Cd, Hg and Ag) (Tripathi et al. 2015). Recently, MTs transgenes are exploited to improve HM tolerance in plants and a large number of MTs and MTs-like gene sequences are accessible in databases (Leszczyszyn et al. 2013). Expression of MTs genes from *Prosopis juliflora* was found to promote higher Cd aggregation and metal sufferance in transgenic tobacco plants (Balasundaram et al. 2014). Current research is intended to transform MTs genes from halophytes that are of particular interest because they can withstand high salinity. Advantage of using these plants lies on the fact that the cellular processes facilitate plant's proliferation under salt stress and also mitigate plant responses to abiotic stresses inclusive of heavy metal stress (Mariem et al. 2014; Taamalli et al. 2014; Guo et al. 2017). For instance, IlMt2a gene (MTs gene) of a halophyte *Iris lactea var. chinensis* when incorporated into *Arabidopsis* genome led to higher tolerance of Cd and Cu in the transgenic plants (Gu et al. 2014, 2015). An improved heavy metal endurance was observed in tobacco plant transformed with SbMt2 gene from an extreme halophyte *Salicornia brachiate* which is due to higher efficiency of antioxidant defence system resulting lower level of ROS production (Chaturvedi et al. 2014).

Heavy metal-mediated formation of reactive oxygen species (ROS) is a well-known fact causing oxidative stress in plants (Hall 2002; Gupta et al. 2013b). In response to HMs exposure, ROS are derived from the activities of membrane localized NADPH oxidase, peroxidases and amine oxidases (Keunen et al. 2011) which have an adverse effect in plant physiology such as altered photosynthetic efficiency (Sebastian and Prasad 2015). A promising approach may be directed toward the production of transgenic plants with enhanced antioxidant activity assuring heavy metal resistance. Among different non-enzymatic antioxidants, GSH plays major role in detoxification of HMs including redox regulation, metal chelation and phytochelatin production (Hasanuzzaman et al. 2017). Therefore, overexpression of GSH may be reasonable approach to augment phytoremediation. The role of GSH-based transgenic approach was evidenced from the study of Liu et al. (2015), where enhanced accumulation and higher tolerance of Cd, Zn and Cu were found in genetically modified sugar beets overexpressing γ-glutamylcysteine synthetase-glutathione synthetase (StGCS-GS) gene from *Streptococcus thermophilus*. As suggested by Hasanuzzaman et al. (2017), strategies accepted for transgenesis targeting on metal tolerance by increasing GSH pool can also be accomplished via targeting the genes encoding glutathione synthetase (GSH2), ATP sulfurylase, cystathionine synthase, serine acetyltransferase (SAT) and glutathione reductase (GR). Higher Ni tolerance as well as enhanced GSH content was achieved in transformed *Arabidopsis* harbouring SAT gene from *Thlaspi goesingense* validating the role of GSH-based transgenic approach toward improved phytoremediation (Na and Salt 2011).

However, manipulation of antioxidative genes in transgenic plants often consequences to several phenotypical and physiological disorders such as delayed growth, bizarre root structure and declining photosynthetic efficacy (Iannone et al. 2015; Gao et al. 2016). Even some genes like catalase were reported to have no protective role in tobacco plants against Cd toxicity (Iannone et al. 2015). A different strategy can be applied to overcome this issue by overexpressing the genes related to DNA repair and transcription by which higher metal resistance in targeted plants can be achieved by upregulating other genes chiefly those encoding antioxidant enzymes (e.g. SOD and CAT), metallothioneins and metal transporters including the enzymes involved in DNA repair mechanism (Faè et al. 2014; Charfeddine et al. 2017). Following this strategy, overexpression of a transcription factor encoded from drought-responsive element binding (DREB) family and an improved resistance to Cu and Cd in transgenic potato have been reported by several authors (Ban et al. 2011; Charfeddine et al. 2017).

26.4 Emerging Trends of Improved Phytoremediation Using Transgenic Approaches

26.4.1 Gene Silencing by RNA Interference

An emanating transgenic concept for developing higher heavy metal tolerance may be achieved by gene silencing. In this process, small RNA molecules are used for suppressing the expression of genes by punctuating the translation of functional mRNA (Saurabh et al. 2014). Gene silencing via RNA interference aids in uptake and hyperaccumulation of heavy metals. In *Arabidopsis* following knockout of arsenate reductase, gene translocation efficiency of As to shoot was higher in respect to control plants (Dhankher et al. 2006). Similar finding was obtained by Takahashi et al. (2014) by silencing the Cd-transporter gene in the root (*OsNRAMP5*) and resulted in greater Cd translocation to the shoots. However, gene silencing may restrict the accumulation of HMs as proved from the study of Li et al. (2007), where silencing of PCS gene resulted in drastic reduction of grains Cd content.

26.4.2 Transformation of Chloroplast Genome

Chloroplast transformation is another promising approach for effective phytoremediation and especially ideal for generating transgenic plants when transformation of multiple genes is considered (Kozminska et al. 2018). Plant cells are modified by transgenic plastid genome termed as transplastomic plants. Plastid genome can be modified by homologous recombination enabling site-specific exchange of endogenous plastid genes; thereby, foreign gene can be precisely inserted within plastid DNA (Bock 2015). Homologous recombination facilitates deciphering the activity of chloroplast gene in vivo and can be equally competent in plant to that with nuclear transformation (Lu et al. 2013; Bock 2015; Daniell et al. 2016). Moreover, this method is advantageous in overcoming the problems of gene silencing and position effect of nuclear transformation (Daniell et al. 2016). Pioneer experiment with tobacco chloroplast gene, transformed by native bacterial merA and merB genes, instigated plant tolerance to highly concentrated (400 mM) phenylmercuric acetate (PMA) (Ruiz and Daniell 2009; Hussein et al. 2007) without exhibiting any phytotoxic effect.

26.4.3 Transformation of Heat Shock Protein (HSP)

HM stress consequences to disruption of cellular homeostasis by interrupting vital enzymes and by subduing protein function (Hossain et al. 2012). HM stress also induces heat shock proteins (HSP) to accumulate within cell (Hasan et al. 2017) and plays a key protective role, by restoring normal protein function and cellular homeostasis (Rhee et al. 2009). Among the major classes of HSPs, HSP70 family members have been found to accumulate in response to HM stress in a wide range of plant species (Gupta et al. 2010). This protein plays its role as a chaperone, thereby, hindering the toxic effects of HMs on proteomes as well as assisting in the metal exchange system for sequestration of metal ions. Recently, role of BAG3 proteins in coordinating the chaperone proteins to increase the potentiality of HSP70 to refold the denatured proteins has been established by Rauch et al. (2017). Therefore, potentiality of HSP70 as a target gene for transgenic approaches for upgradation of metal agglomeration capabilities s in plants cannot be ignored (Sebastian et al. 2019).

26.4.4 Targeting ABA Accumulation Within Plant Cell

Heavy metal stress leads to interference in water balance which triggers the accumulation of phytohormones like abscisic acid (ABA), auxin and ethylene in plants (Bucker-Neto et al. 2017). The higher concentration of ABA protects the guard cell by retaining its water potential and restricting water balance related to detention in plant growth. A recent report by Xu et al. (2018) showed that transgenic *Arabidopsis* overexpressing G subunit of *Juglans regia* (JrVHAG1) implicated in ABA synthesis become tolerant to higher Cd concentration. Therefore, promoting ABA production and accumulation may be advantageous for higher metal deposition in the plants used for phytoremediation.

26.5 Conclusion

Heavy metal contamination of soil and aquatic ecosystem is a global burden, and therefore promising remediation methods are required which can be successfully applied in the field. Phytoremediation is an advantageous eco-friendly technology with wide acceptance and less distressing than existing chemical and physical process. It is a new approach to remediate the contaminated soil, water and ambient air (Van Ginneken et al. 2007) and also applicable for improvement of infertile soil with higher concentration of aluminium and salt (Tangahu et al. 2011). An updated knowledge about movement of heavy metals from soil to plants will support successful phytoremediation. Subsisting floral diversity should be scrutinized for effective metal hyperaccumulators. Further studies should be directed toward better understanding about the role of major rhizospheric factors and low-cost eco-friendly chelating chemicals regarding metal uptake and also the fate of metal ions within plant biomass for improvement of metal hyperaccumulation and plant tolerance. Finally, extensive research works are required to curtail the limitation of these techniques for successful application of phytoremediation method.

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Chapter 27
Phytoremediation of Metal-Contaminated Sites

Govind Gupta, Jabbar Khan, and Naveen Kumar Singh

Contents

Abstract Contamination of metals in the soil and water has become a serious issue for the human health and environment safety. Toxic metals are released into the environment from anthropogenic and developmental activities, viz., mining, industrial manufacturing, vehicular exhaust, and domestic and agricultural uses of chemicals. Metals like lead, cadmium, chromium, mercury, and arsenic are very toxic in nature for living being and can cause teratogenic, mutagenic, and endocrine disruptors and neurological disorders in human beings. Untreated or partially treated industrial effluent containing high concentration of metals contaminates water bodies or sometimes soil through unregulated discharge. Metals due to recalcitrant in nature get accumulated in living organisms and biomagnify in the organisms at top

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trophic level. Traditional wastewater treatment processes are not so efficient to remove metal from contaminated water; therefore, to remove metals from contaminated water it needs some advance and viable methods. Using efficient plants and microbes to remove or reduce contamination and detoxifying metals from contaminated sites is called phytoremediation. Various plants have been identified as efficient metal accumulator and are used to remove metals from contaminated water either individually or in combination. Phytoremediation is an eco-friendly and economic technique as it is solar driven and requires energy for operation. Developing more viable techniques to remove metals from contaminated sites employing efficient plants would be helpful in conserving water and soil quality and minimizing the negative effects of human health. Further, advancement in understanding the mechanisms of bioabsorption, bioconversion, and tolerance in plants helps us to select and apply the best combination of plants for removing metals from contaminated sites more efficiently.

Keywords Metal · Toxic · Hyper-accumulator · Tolerance · Accumulation

27.1 Introduction

Metal contamination has now became a worldwide concern; however, the concentration and effects of metal contaminants vary with place and time. Various metals are grouped as toxic metals due to their recalcitrant nature and cause detrimental effects on human health. Arsenic, cadmium, chromium, lead, copper, nickel, zinc, and mercury have been considered as more common metal contaminants in wastewater. Discharge of metal-containing wastewater without any treatment into water bodies or using it for irrigation leads to enriching metal concentration in water and soil. High concentration of metals in soil and water resulted in food chain contamination and threat to human and other animals. Continuous exposure of metals to the human through contaminated food and drinking water affects growth rate, causes cancerous and neural illness, and sometimes leads to death. Lead and mercury exposure may cause suppression of immune system, arthritis, and circulatory and pulmonary diseases in human beings (Oelofse et al. 2007; Johnson and Hallberg 2005; Rajendran et al. 2003). Metals remain in water and soil for long time due to their persistance nature and translocate, accumulate, and biomagnify in the plants and animals at higher trophic level through the food chain including human beings which cause different illnesses. The exposure of zinc and cadmium results in respiratory, gastrointestinal, and pulmonary disease (Lone et al. 2008; Nomanbhay and Palanisamy 2005). Irrigation of land with wastewater containing metals or direct discharge on land damages plant tissues and leads to risk of human health and other animals (Athar and Ahmad 2002). Some metals like copper, iron, and zinc are essential for plant growth, development, and metabolism; however, at higher concentration these metals have toxic effects as reduced growth and chlorosis (Alva et al. 1995). Detrimental effects on plants and microbes have been reported under lead and nickel contamination (Jada and Fulekar 2009; Khan and Moheman 2006; Boonyapookana et al. 2005; Kuzovkina et al. 2004). With the foundation of a couple of water establishments and standards around the globe (South Africa Water Act, US Clean Water Act, Australian Water Quality Guidelines, etc.) joined with the prerequisite for ecological entities (Goal 7 of the Millennium Development Goals), a couple of stringent components of water quality for domestic and wastewater are required (Pivetz 2001). Since substantial metal contamination influences the nature of wastewater release and drinking water supply, incredible endeavors have been made over the most recent two decades to decrease contamination origins and cure dirtied water assets. In this chapter we emphasized phytoremediation techniques and their applications for the reclamation and remediation of metal-contaminated sites.

27.2 Metal Contamination and Mobilization in Soil and Water

Contamination and mobilization of metals in the soil and water takes place through geogenic and anthropogenic activities which include weathering of rock and soil, mining, electroplating industries, biological process activities and volcanic eruption, etc. Since the EPA 1990 came into power in England in 2000, it has been risks over how to define "contaminated land" ought to be interpreted (Defra 2008). Remediation of metal-contaminated land may be expensive and time consuming. Precipitation by corrosion can be a good method for centralizing metals from contamination (Mannings et al. 1996; Floor et al. 2011). Notwithstanding, corrosive downpour driven librarian of metals is not more common in many countries due to the size of corrosive storm guidelines to reduce SO_2 emission (Menz and Seip 2004; Shilland et al. 2012). Another possible mechanism for metal mobilization-contaminated areas is reductive crumbling of Mn and Fe oxides (Erbs et al. 2010; Neidhardt et al. 2014; Ni et al. 2009). Nanometer-sized Mn and Fe oxides in the soils provide more surface binding different metals (Kumpiene et al. 2008; Waychunas et al. 2005). For example, Phuengprasop et al. (2011) reported that 1 g of Fe oxide absorbed 14.7, 17.3, and 42.4 mg of Cd, Cu, and Pb separately. Contaminated terrains are having consistently scarce vegetation and prone to soil crumbling, which results in mobilization of metals and subsequently transported low-lying topographic soil through water during precipitation. Under diminished conditions, oxides of Mn and Fe in the soils disintegrate through various physical and microbial mechanisms (Roden and Wetzel 2002). Fe^{3+} may act as an electron acceptor at terminal end in microbial decomposition process and reduce to Fe^{2+} (Lovely and Phillips 1986). Mobilization and cycling of Iron (Fe^{3+}) and Manganese (Mn^{4+}) in the soil and has been reported through microbial mediated reduction processes (Lovely 1991). Different metals associated with oxides Mn and Fe and their disintegration depend on various factors; most factors are unknown (Ma and Dong 2004; Douglas et al. 2002). More often, by mechanical process metals from contaminated soils mobilize and reach into the water bodies and subsequently gets absorb, bio-magnify in aquatic flora and fauna.

27.3 Effects of Metal Contamination in Water and Soil Quality

Metal contamination of water and soil occurs due to unregulated discharge of trade effluents in the land and water bodies or geologic processes which pose harmful effects on plants and animals. Further, anthropogenic activities include mining and purifying of metals, consumption of nonrenewable energy sources, utilization of composts and use of batteries, pesticides in horticulture, and sewage discharge (Shen et al. 2002; Raskin et al. 1994). Toxic effects and reduced growth of plants have been reported under metal-containing soils (Oancea et al. 2005; Chatterjee and Chatterjee 2000; Oncel et al. 2000). Metal stress leads to slower plant development and poor yield of crops which results in the long term food insecurity. Therefore, remediation of heavy metal contaminated soils should be taken by using advance techniques. Overwhelming metals show metallic properties, i.e., pliability, flexibility, cation steadiness, ligand and conductivity, etc. Metals are portrayed by generally high thickness and relative nuclear load with a nuclear number more than 20 (Raskin et al. 1994). Some metals like Fe, Zn, Cu, and Mn are essential for living organisms up to a certain extent; however, these metals may be toxic at higher concentration. Metals like Cd, Hg, Cd, As, and Pb are nonessential for growth and development of living beings and are therefore termed as the “primary dangers” since they are harmful to the plants and animals. Soil properties influence metal accessibility in various ways. Harter 1983 revealed that dirt pH is the central point influencing metal accessibility in soil. Accessibility of Cd and Zn to the underlying foundations of *Thlaspi caerulescens* diminished with increments in soil pH (Wang et al. 2006). Natural issue and hydrous ferric oxide have appeared to diminish heavy metal accessibility through immobilization of these metals (Yi et al. 2007). Huge positive connections have additionally been recorded between substantial metals and some dirt physical properties, for example, dampness substance and water holding limit (Sharma and Raju 2013). Different variables that influence the metal accessibility in soil include the thickness and sort of charge in soil colloids, the level of complexation with ligands, and the dirt’s relative surface zone (Marques et al. 2009). The substantial interface and explicit surface zones given by soil colloids help in controlling the convergence of overwhelming metals in regular soils. Also, dissolvable convergences of metals in dirtied soils might be diminished by soil particles with high explicit surface zone; however this may be metal explicit (Marques et al. 2009). For instance, Mcbride and Martínez (2000) reported that expansion of revision comprising of hydroxides with high responsive surface territory diminished the dissolvability of As, Cd, Cu, Mo, and Pb while the solvency of Ni and Zn was not changed. Soil aeration, microbial action, and mineral organization have additionally been appeared to impact overwhelming metal accessibility in soils (Magnuson et al. 2001). There are disparities in studies looking at the impact of substantial metals on soil organic properties. While a few scientists have recorded negative impact of substantial metals on soil natural properties (Friedlová 2010; Nannipieri et al. 1997; Smejkalova et al. 2003), others have emphasized no connection between high overwhelming metal focuses and some dirt (micro)biological properties (Castaldi et al. 2004). A portion of the irregularities may emerge in light of the fact that a portion of these investigations were led under research center conditions utilizing misleadingly defiled soils while others were done utilizing soils from regions that are really contaminated in the field. Notwithstanding the root of the dirts utilized in these tests, the way that the impact of overwhelming metals on soil natural properties should be considered in more detail so as to completely comprehend the impact of these metals on the dirt biological system remains. Further, it is to better understand and utilize a wide range of methods (e.g., microbial biomass, C and N mineralization, breath, and enzymatic exercises) when considering impact of metals on soil natural properties as opposed to concentrating on a single method since results got from use of different methods would be progressively thorough and definitive. The nearness of one overwhelming metal may influence the accessibility of another in the soil and thus plant uptake. Abedin et al. 2002 reported that the nearness of arsenite firmly smothered the take-up of arsenate by rice plants growing on a contaminated soil.

27.4 Metal Hyper-accumulator Plants

More than 400 species dispersed in 45 common families have been considered as metal hyper-accumulators (Brooks 1998). By definition, hyper collectors are the plants which efficiently uptake metals from contaminated sites without any noticeable symptoms and translocate and accumulate into shoot and leaves about 0.1% of biomass for Cu, Co, Pb, Ni, and Cr while 1.0% for Mn and Zn (Baker and Brooks 1989; Baker et al. 2000). Metal hyper-accumulation has advanced in plants everywhere throughout the world and critical locales for gathering germplasm are, among others, Australia, New Caledonia, South and Central Europe, the Mediterranean Area, Cuba, Southeast Asia, Dominican Republic, Zimbabwe, California, Transvaal in South Africa, Hokkaido in Japan, Newfoundland in Canada, and Goiàs in Brazil (Baker and Brooks 1989; Ernst 1974). Metal hyper-accumulation potential of various plants has been extensively evaluated and updated. It turns out to be evident that diverse assorted instruments of metal gathering, prohibition, and settlement into compartments exist within the plant. For example, Zn accumulates in the vacuoles of epidermal cells in a solvent structure in the case of *T. caerulescens* (Frey et al. 2000). However, in the case of *A. halleri* Zn has been found accumulated in the mesophyll cells of leaves (Sarret et al. 2002; Kupper et al. 2000; Zhao et al. 2000).

Cosio et al. (2004) evaluated Cd and Zn uptake and accumulation in the mesophyll cells of *T. caerulescens* and *A. halleri* and reported the regulation processes in the plasma layer of leaf mesophyll cell. Puschenreiter et al. (2003) reported chemical mechanisms in the rhizosphere of hyper-accumulator *T. goesingenseand*, *T. caerulescens*, and *T. arvense* under contaminated and non-contaminated soils. Uneo et al. (2004a) compared Cd accumulation in the shoot of *T. caerulescens* and reported that there was no any affect in excess of Zn, while the Cd accumulation in the roots diminished with higher concentration of Zn in the growing medium. In other study, Uneo et al. (2004b) investigated the Zn and Cd accumulation potential by *T. caerulescens* under soil having insoluble and dissolved Zn and Cd and reported that there was no more variation in Cd accumulation in the shoot which indicated utilization of insoluble Zn and Cd blends in soils. Whiting et al. (2000) observed an augmented root growth of plants *T. caerulescens populace* and reported more accumulation of Cd grown in Cd-contaminated soil as compared to soil not having Cd. It has been reported that the plant *T. caerulescens* may alter rhizosphere soil conditions which influence the accessibility and uptake of metals in other plants growing along with them (Gove et al. 2002). Similarly, when *S. alfredii* is grown along with *Z. mays* (a grain crop) content of Cu and Zn decreased in the grains as compared to grains grown through monoculture which indicated that intercropping of metal hyper-accumulator and with edible crops may improve the advancement of both plant species (Liu et al. 2005). Metal hyper-accumulation in plants greatly influence through rhizosphere mechanisms, as increase Ni accumulation has been reported in *T. geosingense* (Wenzel et al. 2002) which indicated that root exudates of natural ligands promoted Ni uptake. *Sedum alfredii* Hance has been recognized as Zn and Cd hyper-accumulator in China, and its metal accumulation potential is well investigated and documented under metal-supplemented solution as well as contaminated and non-contaminated soils (Yang et al. 2004, 2006; Liu et al. 2005; Xiong et al. 2004; Li et al. 2005, 2005a, 2005b). The mined ecotypes of *S. alfredii* showed better tolerance, Cd uptake, and accumulation as compared to the non-mined (Xiong et al. 2004). Uptake and transport of Zn through tonoplast into the root and uptake in the leaves are the significant instruments related with the strong Zn hyper-accumulation in the *S. alfredii H.* (Yang et al. 2006). Root morphology and physiological response of the *S. alfredii H.* has been reported from the mined locale and rural domain for Zn and Pb accumulation in hydroponics which indicated flexibility in immobilization with root morphology, bioavailability, and physiology (Li et al. 2005, 2005a, 2005b). The *Alyssum* species has been identified as Ni hyper-accumulator (Kupper et al. 2001. Similarly, Kidd and Monterroso (2005) evaluated the potential of *Alyssum serpyllifolium* sp. *lusitanicum* (Brassicaceae) for its possible utilization in reclamation of metal-contaminated soils and suggested it as suitable hyper-accumulator. The plants of Pteris genus, i.e., *P. vittata*, and two cultivars of *P. cretica*, *P. longifolia*, and *P. umbrosa* have been reported for arsenic hyper-accumulator (Zhao et al. 2002; Caille et al. 2005). In another investigation the speciation and allocation of As of brake greenery was studied (Zhang et al. 2002).

27.5 Metal Tolerance and Toxicity in Plants

There has been a consistently expanding mindfulness in the course of the most recent years of the intensity of overwhelming metals as ecological contaminations. Their diligence in the earth and nearness in an assortment of inorganic and complexed concoction shapes result in them getting to be joined into natural cycles where they can apply long haul harmful impacts. Overwhelming metal contamination can achieve extreme phytotoxic activity; it can likewise go about as an incredible power on plant populaces prompting the directional determination of tolerant genotypes. Significant consideration has been centered around these transformative procedures (Baker 1987) and the physiological reactions of plants to the toxic metals included. In every such investigation, there is a need to evaluate the dangerous impacts of the metals concerned. A wide scope of plant reactions has been utilized for this reason and it is the point of this audit to unite such unique data. The tests depicted may discover significantly more extensive utilization in different investigations of phytotoxicity and bioavailability of dangerous components. The ecophysiological importance of trial estimations of substantial metal resilience requires mindful understanding especially in the light of bizarre reactions. All plants react to increments in substantial metal focuses in their quick condition. The nature, bearing, and extent of these reactions will rely upon the affectability of the individual, the power (focus and span) of presentation, the metal concerned, and the structure in which it is available. From exploratory examinations it is conceivable to develop yield-portion reaction bends, where yield can speak to a development parameter extending from biomass generation in the long haul to appraisals of root development restraint in the present moment. Metal uptake plants cause phytotoxicity, lesson, and physiological changes (Berry and Wallace 1989). The defense reactions in plants rely upon relative metal species sensitivities which, in tum, provide tolerance and in bioassay consideration for a specific metal toxicity. Differential metal accumulation in plants indicate their suitability for execution in phytoremediation of metal contaminated soil for both affectability and resilience.

27.6 Application of Plants for Metal Removal from Wastewater

Using plants to remediate and remove metals from contaminated soil and water is called phytoremediation. This process involves uptake and accumulation of metals in plant tissues more efficiently when grown on contaminated sites (Pivetz 2001). Phytoremediator plants may transform, degrade, extract, stabilize, or filter metals for detoxification and removal from contaminated sites (UNEP 2010; Lasat 2000).

27.6.1 Phytodegradation

In this process metal contaminants may degrade or immobilize in the rhizosphere and break down from interior and outer metabolic forms for plant uptake. It involves plants to uptake and accumulate metals inside the tissues. Amid procedure, plants really use what's more and pulverize pollutants inside their tissues. A few contaminants can be consumed by the plant and are at that point separated from plant proteins (Newman and Reynolds 2004). Plants can take up metal straightforwardly from soil and water which is supported by root exudates in the rhizosphere. For efficient application of phytodegradation, it is imperative that the metabolites formed in the plant should be nontoxic or possibly no more harmful than the parent compound (Schnoor 1997).

27.6.2 Phytoextraction

The process of phytoremediation includes, metals uptake driven through roots and get translocate, and accumulate in shoot and leaves and, therefore, metal extracted from contaminated sites termed as phytoextraction. Phytoextraction method is primarily used for metal-contaminated sites. Various plants are often used for metal uptake due to their high level of tolerance. Metals like Cu, Zn, and Ni have been successfully phytoextracted; however, more research are being carried out on Cr and Pb phytoextraction (UNEP 2010; Lasat 2000). Utmazian and Wenzel (2006) reported that process of phytoextraction applied as a continuous process employing hyper-accumulator plants or assisted with chelates to increase bioavailability and subsequent phytoextraction.

27.6.3 Phytostimulation

In this process of phytoremediation, plants release root exudates or other ingredients to stimulate the metal mobilization and uptake in rhizosphere by microorganisms. It is a harmonious connection among plants and associated microorganisms to metal uptake and tolerance in plants (Lasat 2000; Miller 1996). This process augments microbial interaction for metal uptake and accumulation.

27.6.4 Rhizofiltration

Rhizofiltration process involves bringing nurseries of aquatic metal accumulator plants with their underlying foundations in water. It is the degradation of contaminants in water by means of improved microbial movement in the rhizosphere. The process of rhizofiltration is more effective for metal removal from contaminated groundwater than the contaminated soils. Metals are either adsorbed on the root or absorbed inside the root of plants. Plants utilized specifically are not planted in situ but rather are first adapted to the contaminant (Lasat 2000; Miller 1996). An appropriate plant for applications of rhizofiltration may expel harmful metals from arrangement over an all-inclusive timeframe with its quick development root framework. An assortment of plant animal groups have been observed to be successful in evacuating lethal metals (Dushenkov and Kapulnik 2000). Demarco et al. (2019) reported the most reasonable rhizofiltration system of the plant *S. montevidensis* which showed attributes for metal uptake and subsequent accumulation.

27.6.5 Phytovolatilization

Phytovolatilization method is a capacity of plants to take up pollutants from the growing medium through roots and volatilize them into the atmosphere through its aerial parts. Metals taken up by plants volatilize from leaves into environment with moderately low fixation (Ghosh and Singh 2005). This process depends overwhelmingly on the physical state of the contaminant itself. In order to get absorbed by the root of the plant, the contaminant must be accessible in the rhizosphere. After getting absorbed inside the plant metals would then have the option to be volatize into the atmosphere through the stomata (Scheper and Tsao 2003).

27.6.6 Phytostabilization

Phytostabilization, additionally alluded to as setup inactivation, is essentially utilized for the remediation of metal-contaminated soil. This is the utilization for roots of plants to restrict bioavailability and pollutant mobility in the rhizosphere. Amid this procedure, pollutants are ingested and amassed from roots or accelerated in the rhizosphere. At the point when this occurs, there is the counteractive action of portability of pollutants, thus decreasing their accessibility in natural pecking order (Jada and Fulekar 2009; Lasat 2000). The process of phytoremediation relies upon the metal accumulation capacity of phytoremediator plant for metal contaminant. Notwithstanding whether the plant really remove all metals from contaminated soil, however, they are useful, if they immobilze metals in the contaminated sites (Scheper and Tsao 2003).

27.7 Metal Accumulation from Soil by Plants

Substantial metals make a noteworthy commitment to condition because of human exercises, for example, mining, purifying, electroplating, vitality and fuel creation, control transmission, serious agribusiness, muck dumping, and softening tasks

(Samarghandi et al. 2007; Welch 1995). Page et al. (1982) revealed each significant metal at high centers has strong toxic effects and is seen as natural defilements. According to Gurbisu and Alkorta (2003) phytoremediation, uses efficient plants to remove metals from contaminated soils through uptake, sequestration and accumulation in their tissues. Mostly, plant growing in contaminated soil, remove metals by methods of filtration, adsorption, and cation exchange and through plant-microbes interaction and changes in the rhizosphere (Wright and Otte 1999; Dunbabin and Bowmer 1992). There is evidence that plants can store significant metals in their tissues, for instance, *Sebera hone* and *Thlaspi caerulescens* (Cunningham and Ow 1996), *Arabidopsis thaliana* (Delhaize 1996), *Typha latifolia*, and *Phragmites australis* (Ye et al. 2001). Ye et al. (1997a, 1997b) reported the phytoremediation of Pb/Zn mine effectively utilized by *T. latifolia* and *P. australis*. Metal amassing from plants is influenced by numerous elements. Huibers et al. (2004) reported that in creating nations, this water may, in uncommon cases, show up as debilitated unrefined sewage, paying little respect to whether this preparation is seen as unlawful. In any case, the nature of the wastewater used and the possibility of its usage vary enormously, both between and inside countries. In various low-pay countries, in Africa, Asia, and Latin America, the wastewater will by and large be untreated, while in focus pay countries, for instance, Tunisia and Jordan, treated wastewater is used (Qadir et al. 2010; Al-Nakshabandi et al. 1997). Wastewater framework is similarly known to contribute through to the significant metal substance of soils (Mapanda et al. 2005). Plant classes have an assortment of abilities to expel and accumulate metals; accordingly, there are reports demonstrating that specific classes may aggregate explicit substantial metals, making a genuine hazard human well-being when plant-based foodstuffs are devoured (Fytianos et al. 2001). The wastewater including sewage consistently discharge and use for irrigation of crops including vegetables. Discharge of sewage containing metals into water and land leads to increasing metal concentration therein, for instance, Fe, Cu, Mn, Zn, Cr, Pb, Ni, Co, and Cd, are getting enrich into the soils (Rattan et al. 2005). Chary et al. (2008) reported there is an expanding risk of discharge of metal-containing sewage. Various studies indicate that increase of metal contamination in soil and water due to wastewater discharge (Hani et al. 2010; Tijani 2009; Srinivasan and Reddy 2009; Khan et al. 2008; Sharma et al. 2007). Hazard appraisals have been performed utilizing different hazard evaluation methods, for example, the danger remainder (Chary et al. 2008), the dismalness status (Srinivasan and Reddy 2009), health risk index (Khan et al. 2008), advancement factor, level of sullying, insights, geostatistics and geographic data frameworks (Hani et al. 2010), and the take-up/exchange factor (Tijani 2009). Kanwar and Sandha (2000) reported the contaminated water with lead, zinc, copper, cobalt, boron, arsenic, chromium, manganese, and molybdenum is harmful to plants and animals. Continuous use of untreated and treated water has brought high concentration of metals in the soil (Ullah et al. 2012; Khan et al. 2008). Similarly, leachate from dump sites contaminate groundwater through leaching process (Oyeku and Eludoyin 2010) and their subsequent accumulation in vegetables and oats leads to potential risk to the consumers (Kumar Sharma et al. 2007; McGrath et al. 1994). Metal focuses in plants developed in contaminated water-flooded soils were altogether higher than in plants developed in the reference soil. Singh et al. (2010) and Khan et al. (2008) have presumed the utilization of untreated and treated contaminated water for irrigation containing Cd, Ni, and Pb, may accumulate in the edible part of plants, causing risks for human health in the long term. Khan et al. (2012) and Sachan et al. (2007) have investigated high bioaccumulation of Cr and Pb in plants than the maximum permissible limit and provide less calories. Vegetable crops grown in metal-contaminated soils assemble higher concentration of metals than those grown in uncontaminated soils since they acclimatize these metals through their leaves (Al Jassir et al. 2005). For examples, cabbage, cauliflower and spinach, reported as hyper accumulators growing in contaminated water (Cobb et al. 2000). According to Kapourchal et al. (2009) radish can efficiently grow and accumulate metals from contaminated water. Vegetables grown under irrigation with contaminated water accumulated substantial metals, which cause genuine well-being perils to consumers (Avci 2013). Agarwal et al. (2003) reported that Okra gum made up of an anionic polysaccharide (seedpods of *Hibiscus esculentus*) utilized as a flocculant for the removal of solid particles from tannery efffluent. Using wastewater in farming for irrigation purpose leads to enrichment of P, N, and metals (Cu, Cd, Mn, Fe, Zn, and Pb), from sewage and industrial effluent (Devkota and Schmidt 2000) (Table 27.1).

Table 27.1 Sources of metal contamination of soil and water

Sources	Heavy metals
Cr	Sludge, tanneries, electroplating industry, solid waste (Knox et al. 1999)
Cd	Metal smelting and refining, application of phosphate fertilizers, fossil fuel burning, sewage sludge (Alloway 1995; Kabata-Pendias 2001), anthropogenic activities (Nriagu and Pacyna 1988), geogenic sources (Mench et al. 1997)
Cu	Electroplating industry, mining, biosolids, smelting, and refining (Liu et al. 2005)
As	Petroleum refining, semiconductors, wood preservatives, coal power plants, volcanoes, herbicides, animal feed additives, mining, and smelting (Walsh et al. 1979; Nriagu 1994)
Hg	Emissions from industries producing caustic soda, volcano eruptions, coal, peat and wood burning, forest fire (Lindqvist 1991)
Se	Combustion of fossil fuels, coal mining, glass manufacturing industry (e.g., varnish, pigment formulation), chemical synthesis
Ni	Land fill, bubble bursting and gas exchange in ocean, weathering of soils and geological materials, forest fire, volcanic eruptions (Knox et al. 1999)
Zn	Smelting and refining, biosolids, mining, electroplating industry (Liu et al. 2005)
Pb	Industrial wastes enriched in Pb, burning of leaded gasoline, municipal sewage, paints, mining and smelting of metalliferous ores (Gisbert et al. 2003; Seaward and Richardson 1990)

27.8 Applicability and Limitations of Phytoremediation

27.8.1 Applicability of Phytoremediation

Phytoremediation systems may likewise be all the more freely worthy, stylishly satisfying, and less problematic than the present methods of physical and compound procedure (Salido et al. 2003). Application of phytoremediation to remove metals from contaminated soil may be a better and sustainable option which includes biological treatment. The genuine great conditions of the considerable metal take-up advancement from biomass are its suitability in diminishing the assembly of overpowering metal particles to low measurements and the usage of modest biosorbent materials (Rakhshaee et al. 2009). Phytoremediation of water and soil is least expensive and eco-friendly technique to remediate contaminated sites (US EPA 2000). Phytoremediation envelops various distinctive techniques that can prompt contaminant corruption (Rodriguez et al. 2005). Phytoremediation is a minimal effort alternative and modest methodology for remediating natural media, especially fit to substantial destinations that have generally low dimensions of tainting (Van Ginneken et al. 2007). This innovation has been accepting consideration recently as a creative, financially savvy option in contrast to the more settled treatment techniques utilized at risky waste destinations (US EPA 2000). Phytoremediation conceivably offers one of a kind, minimal effort answers for some current issues of soil tainting (Liu et al. 2000; US Department of Energy 1994). It is cheap (60–80% or even less exorbitant) than traditional physicochemical techniques, since it doesn't require costly hardware or exceptionally concentrated faculty. It is savvy for extensive capacities of water taking low groupings of pollutants and for substantial regions taking low to reasonably polluted surface soils (Mwegoha 2008). Phytoremediation technique can be applied on varied scope to remove toxic radionuclides and metals (Liu et al. 2000) and furthermore helpful to treat a wide scope of natural pollutants, with natural and inorganic pollutants (Mwegoha 2008). Phytoremediation is viewed as an alternative method for treatment of contaminated water, soils and air (Van Ginneken et al. 2007). Phytoremediation study indicated improvement and treatment of aluminium contaminated soils (US Department of Energy 1994). It is pertinent to a scope of phytoremediation in removal of toxic metals and radionuclides from contaminated soil and water, in situ. Phytoremediation technique used in wide area of metal contaminated soil and water and have been associated with using hyper accumulator plants for removal of metals (Wang and Greger 2006). Phytoremediation is a solar driven, cost effective, sustainable technique for environmental cleanup through in situ treatment. In situ applications remove metals from contaminated soil by using advance treatment techniques. It will in general be performed with unimportant biological disrupting impact with topsoil left in a usable state and may be recouped for cultivating usage. Toxic metals may effects H_2O and CO_2 evacuation and cause toxicity in plants (Mwegoha 2008). Phytoremediation used to be an alternate option for wastewater treatment to the conventional methods which require energy, add chemical etc and finally generate sludge. Hinchman et al. (1998) reported that phytoremediation really remove metals, from soil leaving an upgraded, utilitarian mud biological community at expenses evaluated at roughly one-tenth of those presently embraced advancements. The most biological cleaning innovation for polluted soils is called a green innovation. Phytoremediation could be a practical choice to treat and clean metal-contaminated soils, especially than the biomass delivered amid in phytoremediation procedure can be financially value as bioenergy. Utilization of metal-collecting plants for bioenergy may be appropriate for energy generation. Furthermore, metals contaminated sites may be phytoremediated along with biodiesel, oil crops subsequent vegetation oil could be a suitable choice to produce bioenergy (Van Ginneken et al. 2007). In substantial scale applications, the potential vitality put away can be used to produce warm vitality (Mwegoha 2008). The achievement of the phytoextraction system relies on the distinguishing proof of appropriate plant species that can hyper-accumulate overwhelming metals and yield a lot of biomass utilizing setup harvest creation and management practices (Rodriguez et al. 2005).

27.8.2 Limitations of Phytoremediation

Phytoremediation being a biological technique depends on, the measure of biomass, the root profundity, soil science and the degree of tolerance the period of plant, the contaminant focus, the effects of planted vegetation, and climatic condition. Phytoremediation can be a tedious procedure, and it might take at any rate a few developing seasons to tidy up a site. The intermediates shaped from those natural and inorganic contaminants might be cytotoxic to plants (Mwegoha 2008). Phytoremediation is additionally constrained by the development pace of the plants. Additional time might be required to phytoremediate a site as contrasted and other increasingly customary cleanup advancements. Unearthing and transfer or cremation takes a long time to months to achieve, while phytoextraction or corruption may require quite a while. In this manner, for locales that posture intense dangers for humans and other biological receptors, phytoremediation may not be the remediation procedure of alternative methods (US EPA 2000; Mwegoha 2008). Phytoremediation may be most appropriate for remote territories where human contact is restricted or where soil defilement doesn't require a prompt reaction (Salido et al. 2003). Under the best climatic conditions, with water system and treatment, complete biomass productivities can approach 100 t $ha^{-1}year^{-1}$. One of the uncertain issues is the trade-off between harmful metal accumulation and profitability. This is frequently a worthy pace of contaminant expulsion, permitting site remediation over a couple of years to two or three decades, especially where the centralization of the contaminant can be brought adequately down to meet administrative criteria. These qualities for efficiency of biomass and overwhelming metal substance would restrict yearly harmful component expulsion limit somewhere in the range of 10 and 400 t $ha^{-1}year^{-1}$, contingent upon the contamination, plant species, climate, and different variables (US Department of Energy 1994). The accomplishment of phytoremediation might be constrained by variables, for example, developing time, atmosphere, root profundity, soil science, and level of defilement (Salido et al. 2003). Root contact is an essential constraint on phytoremediation appropriateness. Remediation with plants necessitates that contaminants be in contact with the root zone of the plants. Either the plants must have the option to stretch out roots to the contaminants or the sullied media must be moved to be inside scope of the plants (US EPA 2000). Confined to locales with shallow defilement inside establishing zone of remediative plants, ground surface at the site may be altered to forestall flooding or disintegration (Mwegoha 2008). Age enormously influences the physiological movement of a plant, particularly its underlying foundations. For the most part, foundations of a youthful plant show more prominent capacity to assimilate particles than do those of an old plant when they are comparative in size. It is imperative to utilize sound youthful plants for progressively effective plant evacuation. Be that as it may, this doesn't discount the utilization of bigger more established plants whose bigger size may make up for their lower physiological movement when contrasted with littler more youthful plants (Tu et al. 2004). High centralizations of contaminants may repress plant development and, in this way, may constrain application on certain destinations or a few pieces of locales. The phytotoxicity in phytoremediator plant could prompt a medicinal methodology wherein high-focus waste is taken care of with costly ex situ strategies that rapidly lessen intense hazard, while in situ phytoremediation is utilized over a more drawn out timeframe to clean the high volumes of lower contaminant fixations. A significant restriction in the phytoremediation of harmful components is the maximal level that can be gathered by plants. Plants with the most significant levels of toxic metal substance, known as "hyper-accumulators," for the most part show, on a dry weight premise, from around 2000 ppm (0.2%) for increasingly lethal components (Pb, Cd) to above 2% for the less dangerous ones (Ni, Z n, Cu). Confined to accumulation in tissues with low metal contaminant focuses, the treatment is commonly constrained to soils at 1 m from the surface and groundwater inside a couple of meters of the surface with soil treatment might be required (Mwegoha 2008). Some environmental presentation may happen at whatever point plants are utilized to associate with contaminants from the contaminated soil. The destiny of the metals in the biomass is a worry. Albeit a few types of phytoremediation include collection of metals and require treatment of plant material implanted with metals, most plants don't amass huge degrees of natural contaminants. Metal-removing plants should be disposed or discarded in ecofreindly manner with safe disposal guidelines, most phytoremediative plants don't require further treatment or transfer (US EPA 2000). Collected plant biomass from phytoextraction might be named a risky waste; subsequently, disposal ought to be appropriate. Utilization of phytoremediated biomass is a reason for concern; contaminants may at present enter the evolved way of life through creatures/creepy crawlies that eat plant material containing contaminants (Mwegoha 2008). Climatic and hydrological conditions may confine the pace and application of plants that can be used. Use of non-native species may influence biodiversity (Mwegoha 2008).

27.9 Conclusion

With increase of human population and corresponding anthropogenic activities, water and soil is getting contaminated through unregulated waste discharge. Developing and opting viable alternate methods for removing metals from contaminated water and soil could be helpful in restoring the natural environment in more economic and efficient way. Using plants through phytoremediation for tackling the problem of metal contamination could be a viable, eco-friendly, and low-cost technology. The most significant factor is a suitable plant species which can be used to take up, detoxify, and accumulate metals. The efficient plants may be utilized individually or in combination creating constructed wetland to remove and immobilize metals that will be contingent on the type of metal to be removed and site of application.

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Chapter 28
Current Trends of Phytoremediation in Wetlands: Mechanisms and Applications

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Abstract This study is being done to integrate global scientific research on phytoremediation and to assess current trends of pollutant removal by wetlands. It focuses on phytoremediation of excessive organic and inorganic pollutants from contaminated sites. The concecpt of natural and constructed wetlands based on phytoremediation is a cost-effective and green emerging technology for cleaning up organic (phosphorus, nitrogen, pathogens) and inorgagnic (metals and pesticides) pollutants. Wetlands can remove pollutants by absorbing and binding them and make them a part of sediment. Mechanisms of pollutant removal include phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration. Phytoremediation strategy used for removal depends on pollution level, plant species, and climatic conditions of that wetland. Some plants have natural ability to degrade or render harmless contaminants in soils, water, or air that pose serious threat to humans and animals. This review paper provides information on recent progress in research and practical applications of phytoremediation in wetland.

Keywords Phytoremediation · Wetland · Pollutant · Phytoextraction · Phytostabilization · Phytovolatilization · Rhizofiltration

28.1 Introduction

28.1.1 Wetlands

A part of land that is present between the water area and dry land is called wetland. It is mostly defined as too moistened to plow and extremely dry to swim. There are different kinds of wetlands like swamps, marshes, bogs, and fens and seasonal wetlands such as wet meadows, sedge meadows, and wet prairies.

Wetland is that area which is inappropriate for many plants but is appropriate only for those plants which can adjust in soil of that land and its anaerobic conditions. Water is the primary factor in area of wetlands which starts to fill the space between the soil particles and controls the environment. Wetlands are present on land where the water table is close to the surface of the land or where the water is completely spread on land. Special wetland soils are called hydric soils. Specific types of vegetation and specific plants are present in wetlands; those are known as wetland plants and vegetation. Wetlands are extremely productive ecosystems that give many life-supporting services which are very important for mankind.

The Ramsar Convention takes a wide access in finding the wetlands which come under its assistance. Ramsar Convention describes the wetlands as the areas of marsh, fen, peatland, or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish, or salt, including areas of marine water, the depth of which at low tide does not exceed 6 m. Wetlands are found everywhere, from the part of tundra to the tropics. The United Nations Environment

Program WCMC has suggested an estimate of about 570 million hectares, roughly 6% of the Earth's land surface of which 2% are lakes, 30% bogs, 26% fens, 20% swamps, and 15% floodplains.

Wetlands are involved in most fertile environments of the world. A large biological diversity is present in wetlands because wetlands provide water and efficient primary production. To survive, a large number of species of plants and animals are dependent on wetlands. Wetlands give protection to a large number of birds, mammals, reptiles, amphibians, fish, and invertebrate species. Wetlands have significant value because they are also important storehouses of plant genetic material. For example, rice is a common wetland plant and is the staple diet of more than half of humanity.

28.1.2 Types of Wetlands

Five notable types of wetlands accompany:

1. Marine wetlands include coastal wetlands. Tidal pools overlooking the sea, steep coasts, and coral reefs are busy in the wetlands facing the beach.
2. Estuaries, including deltas, tides, and mangroves.
3. Lake wetlands linked to lakes.
4. Ripe wetlands along watercourses and watercourses.
5. Palustrine incorporates marshes, swamps, and marshes.

With the typical wetlands, there are artificial wetlands or fake wetlands, such as fish and shrimp lakes, breeding lakes, irrigated land for horticulture, saltpans, shops, rock pits, farms, and drainage channels. The Ramsar tradition organized types of wetlands and adopted an order of wetlands that included 42 types, which are grouped into three classifications that are marine and coastal wetlands, inland wetlands, and man-made wetlands.

28.1.3 Benefits of Wetlands

Wetlands are essential to mankind, as they provide numerous products and businesses, yet are presented in a wide range of weights and dangers around the world. Wetlands provide us with many advantages, such as water supply, fishing, agriculture, maintaining groundwater levels, and additional maintenance on floodplains, creating wood and vitality activity, similar to peat and plant material, the heritage of an indomitable life, the travel industry, and the entertainment and transport offices. Wetlands have explicit characteristics as part of humanity's social heritage. They are connected with religious beliefs and cosmology, give an elegant tool of motivation, give shelter to wildlife, and constitute important establishment habits in the vicinity. These capabilities, benefits, and features should be monitored if the environmental exposures of wetlands can continue to function.

In recent decades, instead of earning extraordinary land, wetlands are constantly associated with the world engaged essentially biological systems due to continuous filtering, processing, pollution, and excessive use of its mining activities. Wetlands are noticeably identified with hydrological forms. This encourages the representation of wetlands as regular resources.

Wetlands play a fundamental role in maintaining the solidity of each of the true biological communities and, as a consequence, contributing to the prosperity of humanity. Being a standout among the most advantageous biological systems, wetlands make basic administrative elements of hydrological forms within river basins such as water quality management, water levels, flood routines, and integration levels and sedimentation.

Wetlands are known as the kidneys of the earth. Wetlands reach many capacities ranging from the reduction of soil disintegration to normal water purifiers. These urban wetlands have been used as specialists in common purges to reduce the additional levels of toxins presented in urban conditions. Poisons poison expanded point measurement sources, such as sewage systems, waste and strong mechanical waste vehicle wash, and even those available in rural areas and in open dumps or emergency clinics are from.

Some favorable circumstances acquired by the wetlands come together:

1. They are useful for controlling floods and storms of water. Wetlands help to sediment the soil and reduce disintegration by absorbing the overflow of water from the expanded surface.
2. Conserving water quality by separating waste, supplements, and poisons and then releasing water to reactivate groundwater.
3. Offer a habitat or common living space for certain types of natural life, including fish, insects, land and water creatures, reptiles, feathered creatures, and warm-blooded animals. This environment is used for reproduction, settlement, reinforcement, and propagation. Many endangered species depend on wetlands.
4. Provide numerous open recreational gates for angling, the sighting of winged animals, kayaking, climbing, and hunting. There are other favorable monetary circumstances, such as the cultivation of blueberries, wild rice, and wood.
5. Polluting treatment filling it like a compound and a bowl of organic oxidation.

28.1.4 Phytoremediation

The phytoremediation is a sort of bioremediation and is pertinent to all physical or synthetic procedures that incorporate plants for the putrefaction or immobilization of poisons in soil and groundwater.

The word comes from the Greek word phyto, which means plant, and the Latin term "remedy," which means to restore harmony. It is an innovation that uses live plants to sort the soil, air, and water contaminated with synthetic risk mixtures. Plants can be used to regulate, extract, degrade, or volatilize toxins.

Types of phytoremediation are the following:

28.1.4.1 Phytosequestration

(a) It is also called phytostabilization. Many different procedures fall into this classification that may include.
(b) Absorption by roots.
(c) Adsorption outside the roots.
(d) The creation of biochemical products by the plant that is discharged into the earth.
(e) Seizure, acceleration, or something else immobilizes the nearby pollutants.

28.1.4.2 Rhizodegradation

This happens in the ground or in the aquifers that immediately cover the roots of the plant. Plant exudates invigorate the microscopic organisms of the rhizosphere to construct the biodegradation of soil contaminants.

28.1.4.3 Phytohydraulics

Deep rooted plants are used to contain contaminants or contaminants from groundwater that come in contact with their underlying foundations.

28.1.4.4 Phytoextraction

It is also called phytoaccumulation. Plants absorb contaminants through their underlying foundations and store them in the tissues of the stem or leaves. Contaminations do not degrade but are removed from the ground when the plants are harvested. It is useful to expel metals from the ground. Metals can be recovered to be reused by burning plants in a procedure called phytomining.

28.1.4.5 Phytovolatilization

Plants absorb the unpredictable mixtures through their underlying foundations and pass similar mixtures, or their metabolites, through the leaves and then dump them into the air.

28.1.4.6 Phytodegradation

The contaminants are absorbed into the tissues of the plants where they are metabolized or biotransformed. The place of transformation depends on the type of the plant and can occur in the roots, in the stem, or in the leaves.

28.2 Mechanisms

28.2.1 Process of Plant Uptake of Contaminants

There are various wetland plants that are used for the uptake of different types of contaminants in the wetland. Wetlands provide as a living place for the various plant and animal species, especially for the water birds. For the elimination of the different emerging contaminants through phytoremediation, constructed wetlands are the eco-friendly and cost-effective tools. Usually wetland plants are hyperaccumulator; therefore they can uptake a maximum number of contaminants from the wetland and accumulate them into themselves through various phenomena. Marshes have been selected as area for the phytoremediation of metals. The providence of metals within plant tissue is an unfavorable issue for the successfulness of this procedure (Peddrick 2003).

Phytoremediation of metals is a cheap and efficient green application which depends on the use of specifically favorable metal-accumulated plants to separate hazardous metals from the soil. Different actions in the plants include phytoextraction, phytostabilization, phytodegradation, and phytovolatilization. These actions into plants are according to the plant used for the contamination uptake. There is a variety of wetland plants that is used for the emerging contamination uptake. Phytostabilization into the plant minimizes the movability and the bioavailability of the pollutants through accumulation into the roots and into the rhizosphere. This action takes place into the roots and saves the aboveground part of the plant from toxic contaminants. This type of process is only useful for the rooted wetland plants like *Typha domingensis* and *Phragmites australis* (Bertrand 2008).

Other processes of plants like phytodegradation and the phytotransformation are used to degrade and immobilize the pollutants into the roots and the shoots of the plants through the enzymes or the cofactors. Another important phenomenon in plants is the phytoextraction through which the contaminants are transported to above ground biomass or kept below in the ground. These mechanisms are done through various scientific processes in the plants like plant-soil-microbe interactions, cytoplasmic chelates, ion exchange, and symplastic loading (Rai et al. 2018).

28.2.2 Physicochemical Characteristics of Wetland

Most common and known attributes of wetlands are sustainable overflow near the surface and existence of physical, chemical, and biological characteristics. Three significant attributes of wetlands are water, physicochemical properties, and biota. Physicochemical properties of wetland include wetland with hydric soil, which have physical and chemical demonstration of prolonged saturation. These indications are developed from the stoppage of oxygen transport by the water into the substrate of wetland. This depletion of the oxygen into the saturated soil is done by the roots and the microbes. This activity of microbes and roots leads to the complete loss of oxygen. This hydric soil of wetland has low oxygen content or redox potential during the saturation time period which causes disturbance in the color of the soil. These properties of the soil are known as redoximorphic features (Lopez 1995).

Physicochemical properties of the wetland provide substrate to the flora and fauna of the wetland to grow and develop. Created wetlands are usually found as non-indicative of hydric soil. So they are lack of physicochemical characteristics (bulk density, moisture content, carbon and nitrogen content). As compared to the constructed wetlands, these characteristics are present in the natural wetland. But anthropogenic activities or any other change can disturb the substrate of a natural wetland. However, in constructed wetlands microorganisms have been found in the form of communities; therefore, constructed wetlands are involved in the biochemical functions. The diversity among the bacterial communities differs between the sites. However, a high variety of microbial communities found in the wetlands created many years ago (Peralta and Ahn 2009).

28.3 Applications

28.3.1 Pollutant Removal by the Wetland

Wetlands consist of plants that are hyperaccumulator so they can accumulate a high amount of heavy metals and many other pollutants into them. Most of the research shows that the wetlands can remove BOD, COD, suspended solids, and nutrients. Some of the researches also revealed that it can also remove emerging environmental contaminants like volatile organic compounds, pesticides, pathogenic microbes, and heavy metals by using global wetland plants (Farid et al. 2020a, b). Volatile organic compounds can be volatilized through the leaves of the plants by the process of phytovolatilization and nonvolatile compounds are degraded inside the plants through phytodegradation or phytoextraction (Jabeen et al. 2015; Rai 2008).

Macrophytes perform a significant part in the removal capacity in constructed wetlands. It is inconvenient to illustrate difference in achievement of macrophytes and other plant species that have similar life forms and sizes (Sallah-Ud-Din et al. 2017).

According to the study the distinct amounts of lead, zinc, copper, and cadmium were assembled by 12 plants of wetland that mainly include countless populations of *Leersia hexandra*, *Juncus effusus*, and *Equisetum ramosisti* observed in field state of China (Ahmad et al. 2019). According to the result obtained from the experiment, metal collection by different wetland plants varies between plant species, tissues, and populations (Deng and Wong 2004).

28.3.2 Inorganic Pollutant Removal

Wastewater treatment is necessary for clean drinking water and to protect water from eutrophication; therefore nutrient removal is crucial specifically for perspective use of the treated water (Farid et al. 2019; Ehsan et al. 2014). In a study constructed wetland has been arranged in series that mainly consist of a free water surface (FWS) and a subsurface flow (SSF) and operated for 8 months. The main purpose behind conducting this study was to check the performance of the system in the separation of inorganic nitrogen and phosphate from wastewater by providing different hydraulic rates. It was observed that nitrogen removal efficiency of that setup was very high about 86–98% for ammonium nitrogen and 95–98% for total inorganic nitrogen (TIN). Phosphate removal of 32–71% happened.

The multiple operations influence removal and impermeability of nitrogen in constructed wetlands. Mainly these operations include NH3 volatilization, nitrification, denitrification, nitrogen fixation, plant and microbial uptake, mineralization, nitrate reduction to ammonium, anaerobic ammonia oxidation, fragmentation, sorption, desorption, burial, and leaching. Still only some procedures are useful which completely eliminate total nitrogen from the wastewater while many procedures only change its kind by conversion of nitrogen to its different forms (Vymazal 2007).

28.4 Organic Contamination Removal

28.4.1 Removal Mechanisms of Trace Organic Contaminants

Sorption of trace organic pollutants bound two different procedures. Adsorption requires the exchange of a molecule with a material, generally through ion exchange or surface complexation, but absorption involves division into a particle-associated organic phase. Sediments and biofilms in constructed wetlands incorporate various materials that have potential for sorption of trace biological pollutants. If the pollutants show excessive attraction for a surface, they will finally be buried as decomposed plant debris assembles in the wetland. If the sorbent material does not break down, the pollutant will persist in the debris layer until it is washed away as a part of wetland conservation task. The weakly bound pollutants with the sorbent material results in the lower mobility of pollutants and provide much time for other transformations to happen in a wetland (Wang and Xing 2007).

28.4.2 Nitrogen Removal by Vegetation in Integrated Vertical-Flow Constructed Wetlands (IVCWs)

The eutrophication in water is principally originated by nitrogen that is unfavorable to the fish development and also aesthetic beauty of water. The artificial wetland in cooperation with wastewater treatment plants had been highly used for the withdrawal of nitrogen. The types of plants used for the uptake of nutrients and pollutants, wetland type and design of a wetland have affect on the removal efficiency (Godos et al. 2010).

The extension of heterotrophic denitrifying bacteria and microbial group richness could be hastened by the root exudates of aquatic plants and plant diversity had a reaction to root exudates which is cost-effective for the separation of nitrogen. The decomposition, microbial conversion, plant uptake, and volatilization, sedimentation, and adsorption-fixation reactions are the main N-transformation pathways in CWs. The combination of vertical downflow cell and vertical upflow cell increases the nitrification, denitrification, and removal efficiency of nutrients (Wu et al. 2017).

The biological nitrogen removal could increase the anoxic-anaerobic conditions. The nitrogen contaminants could efficiently be eliminated by wetland systems. The higher removal efficiency typically gains by planted vegitation according to IVCWs than unplanted CWs treatment. The microbial removal percentage in IVCW could efficiently be enhanced by vegetative plants. *Pseudomonas*, *Rhizobium*, *Bacillus*, and *Rhodopseudomonas* are liable for the high rate of biological separation rate of N (Du et al. 2017a, b).

28.4.3 Phosphorus Removal by Integrated Vertical-Flow Constructed Wetlands (IVCWs)

The water contamination issues connected with phosphorus released from domestic, agricultural, and industrial points to surface waters create eutrophication (Ayaz et al. 2012).

The constructed wetlands comprise of a downflow cell and an upflow cell, which are known as integrated vertical-flow constructed wetlands. The vegetation types, hydraulic retention time (HRT), and season that are design and running framework of CWs have a large impact on contaminant elimination percentage. HRT is a necessary variable of CWs that control response time that directly increases the removal percentage of CWs (Kotti et al. 2010).

IVCWs planted with various plants such as *Arundo donax* and *Typha orientalis* could decrease the concentration of phosphorus effectively but it is essential to select suitable HRT and vegetation type for constructed wetlands. The IVCW planted with *Canna generalis* was the top performer in P removal. Besides this, the removal efficiency in autumn is better in summer (Du et al. 2017a, b).

28.5 Biological Contamination Removal

28.5.1 Removal of Pharmaceuticals by Constructed Wetland

The constructed wetlands supply multiple advantages such as CO_2 depletion, formation of natural surroundings for plants and animals, leisure and academic advantages, and aesthetic value along with decontamination of pollutants (Macci et al. 2015).

The constructed wetlands are productive for eliminating organic (petroleum hydrocarbons and fertilizers) and inorganic (sulfur, metals, and metalloids) deterioration (Vymazal 2011).

PPCPs are pollutants which are category of compounds discovered in various aquatic environments and effluents in amount ranging from nanograms per liter to micrograms per liter (Matamoros et al. 2008).

The micro orgranic pollutant removal efficiency of full-scale constructed wetland supplied with secondary effluent resulted in the 40% removal of two PPCPs; such as carbamazepine (drug used in the treatment of epilepsy) and clofibric acid (a lipid-lowering agent used for controlling cholesterol and triacylglycerol levels in the blood) (Ganjo and Khwakaram 2010).

The wetland type, plant type and their combinations, climate, and management policies, such as harvesting regime, influence the removal efficiency. The very commonly used plant in CWs is *Phragmites australis* and *Scirpus* are also customarily used (Doni et al. 2013).

The ornamental plant types *C. indica*, *Carex hirta*, *Miscanthus sinensis*, and *Z. aethiopica* have possibilities of pollutant minimization and have beauty which is very attractive and increases aesthetic value. Furthermore, *P. australis* is chosen as reference species because of its universal utilization in wastewater treatment. Constructed wetlands with various high-value ornamental plants can be utilized efficiently for removal of nutrients and heavy metals (Gómez et al. 2007).

Due to the presence of highest amount of metal concentration in their tissues, plants like *C. hirta*, *C. indica*, and *Phragmites* are the most effective in minimizing metals. But plants like *Z. aethiopica* are less effective.

28.5.2 Mitigation of Pesticide Pollution in Artificial Wetland Ecosystems

The pesticide pollution affects the environmental quality, wildlife, and public health. The agricultural nonpoint-source pesticide contamination can be removed in artificial wetland ecosystems. To give a huge and thick rhizosphere root system, aquatic species cattail, bulrush, reed *Phalaris*, reed *Phragmites*, *Glyceria*, and rushs are beneficial for filtration and biological activities. The constructed wetlands need special plants with high tolerance level to increase water extent variation from 0 to 50 cm, because of its dependance on hydrological cycle. A variety of plants has displayed this quality but the common reed and the reedmace are specifically efficient. They have a high biomass both on the top and below the surface of the soil (Vymazal and Březinová 2015).

For nutrient and ions absorption through binding with soil particles, a large surface area is produced by the plant tissues when it grows horizontally and vertically in a wetland. The air passes from the top of plants like leaves to the downside like roots and to the nearby soil by hollow vessels in the plant tissue. Aerobic microorganisms proliferate in a thin zone (rhizosphere) around the roots and anaerobic microorganisms are available in the underlying soil. Natural filtration in the substrate also helps in the elimination of various contaminants and pathogenic microorganisms. By low flow rate and a high vegetation density, removal percentage of pesticide could increase. Pesticide elimination from subsurface flow artificial wetland systems incorporates biological processes like biological degradation, absorption by plants, and aquatic organisms. Chemical processes include sorption, photodecomposition, and degradation and physical types are volatilization and sorption. Those wetlands which consist of high vegetation have a powerful possibility to give to aquatic pesticide threat reduction (Maillard et al. 2011).

28.5.3 Pathogen Removal

The artificial wetlands have been constructed for a broad range of approach to enhance water quality, incorporating treatment of industrial and municipal wastewater as well as stormwater, agricultural runoff, and acid mine drainage (Ansa et al. 2012).

The leading cause of the large wetland systems is commonly a combination of nutrient removal and habitat formation. Progressively, the removal of trace organic pollution and pathogens is also associated as an advantage (Buth et al. 2009).

The design of wetland is the primary obstacle to perceive their ability to upgrade water quality and to increase aquatic habitat. In comparison with manual treatment procedures, the ecological, transport, and transformation processes happening in treatment wetlands are more complicated (Weber and Legge 2008).

Mechanistic research can provide the basis for designing artificial wetlands with each procedure modified to the removal of a special set of contaminants along with identifying the necessary limitation. For instance, mechanistic research incorporated with studies of full-scale systems has led to strong design approaches for unit process wetlands for denitrification. Such denitrification wetlands can be utilized to remove nitrified effluents or they can be staged after shallow aerobic nitrification wetlands. Similarly, hybrid wetlands consist of a vertical flow cell and a cell with calcite media has manifest to be efficient for removing both BOD and phosphorus. Moreover, deep detention ponds for particle removal and anaerobic digestion of solids before vegetated wetlands and slow sand filters are recommended to give efficient removal of pathogens (Malaviya and Singh 2012).

Wastewater effluents hold potentially contagious microorganisms, including viruses, bacteria, protozoan cysts, and helminth eggs. Inactivation of pathogens is important before treated effluents are reprocessed. Treatment wetlands that sustain wastewater which has previously been disinfected may give extra removal of pathogens that are resistant to disinfection. In this case, a wetland may be utilized to decrease the chemical disinfection requirements and to give an extra treatment barrier. On the other hand, treatment wetlands that collect polluted water which has not been treated can play a major role in pathogen removal (Cabral 2010).

28.6 Factors Effecting Phytoremediation

There are a few factors which can influence the phytoremediation component. By knowing about following factors, the take-up execution through plants could be extraordinarily progressed.

28.6.1 Species of Plant

Characteristics of plants and their different varieties are checked, and those with prevalent remediation properties are chosen (Farid et al. 2019; Vara Prasad and de Oliveira Freitas 2003). The take-up of a compound is influenced by plant species trademark (Burken and Schnoor 1996). The achievement of the phytoextraction method depends upon the distinguishing proof of reasonable plant species that hyperaccumulate substantial metals and deliver higher of biomass (Farid et al. 2018a, b; Rodriguez et al. 2005).

28.6.2 Characteristics of Medium

Agronomical techniques are created to upgrade amendatory techniques like pH alteration, expansion of chelators, and composts (Vara Prasad and de Oliveira Freitas 2003). For instance, the measure of lead consumed by plant species is influenced through the pH, natural issue, and the phosphorus substance of the soil. To decrease lead take-up by plants, the pH of the soil is balanced with the use of lime to a dimension of 6.5–7.0 (Rizwan et al. 2017a, b; Tangahu et al. 2013).

28.6.3 The Root Tract

The toot tract is of exceptional enthusiasm for phytoremediation. It can retain toxic agents and utilize it within the plant tissue and can store. Degradation of pollutants in the dirt by plant chemicals radiated by the roots is another type of phytoremediation technology (Farid et al. 2017a). A morphological adjustment to dry season pressure is an expansion in root distance across and diminished root prolongation as a reaction to minimal porousness of the soil which is not wet (Merkl et al. 2005).

28.6.4 Uptake by Vegetation

Vegetative uptake is influenced by the ecological surroundings (Burken and Schnoor 1996). Temperature influences development materials and therefore root growth and extent. Root composition and formation under field conditions contrasts with other nursery states (Merkl et al. 2005). The success of phytoremediation, all the explicitly phytoextraction, relies upon a pollutant-explicit hyperaccumulator (Tu et al. 2004). Mass equalization examinations and the metabolic destiny of poisons in plants are the basic factors to demonstrate the pertinence of phytoremediation (Shakoor et al. 2014; Mwegoha 2008).

Metal take-up through plants depends upon the bioavailability of the metal present in the water stage that relies upon the management time of the metal and additionally association with different components and material present in the water (Habiba et al. 2015). Moreover, when metals have been attached to the dirt, pH, redox potential, and natural issue substance will influence inclination of the metal to present in ionic and plant-accessible frame (Mwegoha 2008).

Plants will influence the soil due to their capacity to bring down pH and oxygenate residue that influences accessibility of metals (Fritioff and Greger 2003). Bioavailability of overwhelming metals can be expended through the expansion of biodegradable physicochemical components, for example, chelating operators and micronutrients (Van Ginneken et al. 2007).

28.6.5 Adding of Chelating Agent

Enhancing availability of substantial minerals by controlling physical and chemical factors, take-up of minerals through vitality products could affected, for example, heterocyclic compound transportor, essential nutrients and substantial mineral-take-up limit the microbes network surrounding roots. Faster mineral take-up gives more affordable remediation periods. Be that as it may, with the utilization of manufactured chelating specialists, the danger of expanded draining must be considered (Farid et al. 2015; Van Ginneken et al. 2007). Chelate utilization by substantial mineral-based land assist in decontamination of pollutants to dirt. Availability of overwhelming land mineral diminishes above acidic condition; chelate utilization justified, it may be needed for basic land. Presenting species to chelate more extended time can enhance mineral transfer to different parts and additionally general withdraw execution (Roy et al. 2005).

Underground plant's part oozes natural chemical, for example, oxalate and citrate that influence mineral's availability in land. Heterocyclic compounds helped in pollutant removal, for example, NTA and EDTA mixed with aim to improve extraction of pollutants from contaminated areas (Farid et al. 2015). Presence of ligand influences take-up of substantial minerals by arranging ligand buildings, altering possibility to drain minerals beneath the plant's root (Seuntjens et al. 2004).

28.7 Limitations in Wetland Phytoremediation

Not all metals are effortlessly expelled by wetlands. Profoundly chelated metals, for example, dissolvable nickel, generally present in an unequivocally chelated frame, can go through wetlands while different less firmly chelated metals, for example, lead or zinc, are immediately expelled in wastewater; there is bounteous chelating limit; thus the solvent metal is not encouraged in the wetland (Farid et al. 2017b). Furthermore, the convergences of all metals in mine squanders can be high, so even 90% evacuation of nickel still leaves a focus well over that attractive in common waters (Cunningham and Berti 2000).

Wetlands can't remove metals always, dissimilar to nitrate expulsion, which is hypothetically unbounded. A wetland will in the end come to harmony with any substance when official and discharge are equivalent. In this way, in a developed swamp, metal discharge may give a consistent benchmark that will impact the obvious expulsion rate. Drying out the wetland ought to make oxidizing conditions. Thusly, this should re-oxidize a few metals or metalloids into dissolvable structures that may turn out to be greatly perilous when the wetland is reflooded. Exhuming the sullied metal focus is monetarily plausible in littler built wetlands (Cunningham and Berti 2000).

Few events such as low inflow fixation can occur if clean stormwater weakens the metal, the wetland may really give off an impression of being a wellspring of the metal for a period. This is very normal and might be because of stacking of metals as particles from wet and dry climatic aftermath, particularly in urban wetlands (Cunningham and Berti 2000).

Analyzing capability of swampland is intresting (Angier et al. 2002) because of its irregular states of water cycle, land/dregs, plant-specie and variety, developing conditions and procedure of environmental progression (Williams et al. 1999).

Few moist lands having biological communities, prevailing types, total living matter, and efficiency stay fixed for quite a long time. However, marshes which are liable to different water streams, sudden changes in chractristics can happen (Williams et al. 1999).

28.8 Current Trend of Wetland Phytoremediation

Phytoremediation is a broad field and accepted throughout the world and very eco-friendly approach for different kinds of polluted fluids like residential, commercial and agricultural. This method has the potential for the reduction of trace metal, organic, solid, and nutrient concentration. By this potential this method has been used to clean industrial effluent and also improves the water quality. Various aquatic plants had been used in this treatment method and they exhibit a pollutant removal with high percentage in the wastewater such as palm oil mill effluent. So, this study will be the review of phytoremediation potential and treatment system and their important component of contracted wetland (Sa' at 2017).

Biological agent has been used to clean the environment and this is called bioremediation. Worldwide, metallic contamination is basic that requires instant attentiveness; therefore, environmental degradation will be rectified. The fundamental thing in phytoremediation is the plants which play a vital role in treating the metal pollution that effected our surrounding. For various emergent problems phytoremediation is the best solution (Wani et al. 2017).

Phytoremediation is considered a method of soil coordination. However, significant progress has been made to achieve high efficiency in achieving atmospheric as well as financial maintenance. The present intervention often provide anticipated results to determine whether the misconceptions about this approach resolved or not. The latest researches are being conducted continuously to investigate microbial communities of soil pollution. This opens a new perspective on the detection of fragile biomass and gives us new methods of intervention in the microbiological community to enhance biodiversity. This review shows the visibility and future of studies of specific micronutrients related to microorganisms and the failure of scientific research. Application of phytoremediation to land makes handling easy. Continous increase in the removal of pollutants from surrounding is needed. Phytoremediation shows maximum efficiency for natural pollution and bioenergy plant productivity (Meagher 2000).

Today, the cleaning plant is not considered to be effective in removing all organic compounds, including PPCPs. Wastewater with POPs has a negative impact on freshwater and milk, even on concentrated stations. Mixed wetlands included in forest biogas technology could be a great opportunity to eliminate the restriction in water protection. On the other hand, if they enter the water, it is important to understand the ability to restore the system itself. The effectiveness of CWs is the cleaning phase of the irrigated areas and the possibility of elimination of toxins when it comes to salt soil. In both cases there is the same principle. Wet defined as an artificial ecosystem is designed and constructed for monitoring biological processes in natural wetlands and natural environments. An example case demonstrates the potential for restoring the target of CPC in both environments, i.e., electronic wastewater equipment and clay extract (LECA). Similarly, biological surfaces are planted with a single plant, but a theory layer. The presence of bodily and/or synergistic subsidies may reduce the levels of toxins in irrigated areas or in environments that are similar to the environment. The absorption of plant-absorbing plants and the reception of protein bio-/rhizoremediation have a highly hypothetical interpretation of the reduction in toxicity in CW and salts. This chapter discusses the idea of generating energy from CWs as a way to promote CW's competitiveness, other treatment systems that effectively integrate with a technology that can reduce energy costs (Ferreira et al. 2017).

Phytoremediation is a new technology based on the use of greenhouses for removing and destroying the damaging effects of greenhouse gases. More recent events in Europe and America show that this approach has a slight difference on both sides of the Atlantic. Europe focused on phytoremediation search engine and based on application identified results. In contrast, this approach in the USA is more appealing than other old practices and experiences. Despite its successful progress, there is a further business demonstration project that phytoremediation is effective in measuring the economics and phenomena to expand its programs. We need more basic research to understand complex interactions of soil, plant roots, and microbial rhizosphere, better levels to increase the bioavailability of contamination, in order to fully exploit the digestive tract of plants and thus place this new green technology (Schwitzguébel et al. 2002).

The process of breaking down or removal of pollutants by various plants is seen as a robust environmental strategy for controlling the ecosystem. This review discusses the current state of several subgroups of phytoremediation, including: (a) phytoextraction, a process that uses bio-plants to accumulate and transport soil to the part of the plant to be harvested; (b) a phytofiltration process in which root crops are used for concentrating rainwater and toxic-contaminated water; (c) phytostabilization, wherein the plants are resistant to pollutants which should be thrown away; and (d) phytovolatilization, wherein the plant absorbs toxins and converts it into gas compounds through transport. The advantages presented by these technologies are also demonstrated to understand the processes that affect the presence of pollution, reactivity, absorption, and prevention of toxic substances (Nwoko 2010).

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Chapter 29

Mechanisms of Arsenic Hyperaccumulation by Plants

Manju Shri, Debasis Chakrabarty, and Giti Verma

Contents

Abstract Arsenic (As) which is a heavy metal is ubiquitously present in soil as well as in water. As has been ranked as a potent carcinogen and is found to be very harmful to all the living beings ranging from bacteria to plant to animals as well as humans. All the organisms possess various defense mechanisms to combat such types of stresses. However, if it remains detoxified in plants, it may lead to oxidative stress, misfolded proteins thus disrupting the functioning of the proteins, mutations in the genetic material which ultimately results in the inhibition of the growth, disruption of photosynthe-

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sis, and loss in crop yield. Plants are sessile creatures of nature so they are more vulnerable to any type of stress. However, they possess a very strong defense system that fights against these stresses. There are various mechanisms responsible for defense against As stress such as phytochelatin (PC)-dependent defense in which As forms complex with PCs and these complexes are sequestered inside the vacuole. The antioxidant defense system is a very basic and strong player in this defense system. One of the interesting parts of this system is the hyperaccumulation of As. However, hyperaccumulation is not common to all the plants. This is a trait of some specific plant species which had gained a very high capacity of accumulation of As in the aboveground part without suffering phytotoxic effects during evolution. Hyperaccumulator plants differ from normal or non-accumulator plants in various ways. Among them, very fast translocation of As from root to aboveground part, much higher detoxification ability, and higher sequestration capacity of As in aboveground part are the main mechanism which differentiates hyperaccumulator plants to non-accumulator plants. In particular, a determinant role in driving the uptake, translocation to leaves, and, finally, sequestration in vacuoles is played in hyperaccumulators by constitutive overexpression of genes encoding transmembrane transporters, such as members of arsenical compound resistance 3 (ACR3). In this chapter, we will discuss mainly the As toxicity in the plants along with the mechanisms that are involved in hyperaccumulator plants, detoxification of As in plants, as well as the tolerance of As in plants.

Keywords Arsenic · Hyperaccumulator · Heavy metal toxicity · Oxidative stress · NOS · Signaling molecules

29.1 Introduction

Soil and water are primarily contaminated by arsenic due to volcanic eruption, weathering of arsenic-rich rocks, and various anthropogenic activities, viz., mining; use of pesticides, herbicides, fertilizers, and preservatives; etc. Arsenic exists in both organic and inorganic forms. The inorganic forms of arsenic are found either in the oxidized form, i.e., arsenate (AsV), or in the reduced form, i.e., arsenite (AsIII); they are profusely found in water and soil in comparison to the organic forms, i.e., arsenobetaine, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenosugars (Panda et al. 2010). However, the solubility of As depends upon the pH and ionic milieu. AsV is found abundant in aerobic soils, while AsIII is more toxic and mobile, found profusely in anaerobic conditions like paddy soil at $pH < 8$ in general (Souri et al. 2017). The methylated forms of As were earlier used as pesticides and herbicides consequently accumulating more MMA and DMA in soil, which is now reported to be more toxic than inorganic forms. The DMA was found to be more toxic than AsV in wheat. Duncan et al. (2017) reported that the germination rates of wheat are 80% and 30% under AsV and DMA stress, respectively. In the same report, the grain yield also decreased by 20–50% when compared to AsV. Similarly, Naranmandura et al. (2011) reported MMA to be almost 18 times more toxic than arsenite especially in animal cells. The problem of As toxicity is alarming in South and Southeast Asian countries, as the groundwater as well as soil in these countries are highly contaminated. Apart from consuming As-contaminated water, rice has been identified as the second major source of arsenic to humans (Meharg et al. 2009). As arsenic-contaminated groundwater is also used for irrigation purposes, it further loads As into the soil and leads to its buildup in vegetables/agriculture products, eventually posing a significant health risk to humans as well as animals because fodder offers an alternate route for arsenic entry in the food chain. Populations of As-contaminated areas suffer from several deformities and diseases (Meharg et al. 2009).

Arsenic is non-essential and highly phytotoxic, with threshold concentrations usually varying from 5 to100 mg kg^{-r} DW (dry weight). The roots are the first tissue for As exposure, where it inhibits the root extension as well as its proliferation. Further, As translocation to shoot severely reduces or arrests growth, biomass reduction, fertility loss, loss in fruit production, and yield loss (Garg and Singla 2011). An interesting paradox of As toxicity is that the low concentrations of As resulted in growth enhancement under axenic conditions (Garg and Singla 2011). It has been reported by Chen et al. (2010) and further suggested that the growth enhancement observed in *Arabidopsis thaliana* is possibly due to the As participation with plant metabolism and with other plant nutrients. However the mechanism remains unclear; another explanation was given by Tu and Ma (2003) that the growth enhancement by arsenic is due to activation of Pi uptake as well.

Arsenic removal mainly relies on the chemical process of excavation and treatment of soil; however, this method of remediation is costly and cannot be used regularly (Wuana and Okieimen 2011). On the other hand, phytoremediation uses hyperaccumulator plants for remediating toxic metal compounds from soil and water in a very cost-effective and environmentally safe manner (Jiang et al. 2015). The accumulation of > 1000 mg As kg^{-1} DW in above ground parts, without any symptoms of phytotoxicity, is rare in terrestrial plants and considered as hyperaccumulation. Hyperaccumulation is rare in terrestrial plants, and they exhibit elevated growth rates, higher tolerance, and greater accumulation of heavy metals (Ghori et al. 2016). Same report mentions that plant hyperaccumulators entail both root-to-shoot and a root-to-soil heavy metal concentration ratio greater than one. Numerous plant species are known for their capability of detoxifying and hyperaccumulating heavy metals. However, few species of *Pteris* genus, viz., *Pteris vittata*, *Pteris criteca*, *Pteris longifolia*, *Pteris umbrosa*, *Pityrogramma calomelanos*, and *Isatis cappadocica*, are found to be As hyperaccumulators (Xie et al. 2009; Karimi et al. 2009).

Among these, *P. vittata* (Xie et al. 2009) and *I. cappadocica* (Karimi et al. 2009) are the most studied and will help elucidate mechanistic features in the arsenic hyperaccumulation and tolerance. Increase in thiol synthesis and the chelation of arsenic with glutathione (GSH) and phytochelatins (PCs) are the two important strategy of Isatis cappadocica which provide As tolerance to this hyperaccumulating plant (Karimi et al. 2009). However, the fern *Pteris vittata* utilizes the efficient system of

AsV/AsIII sequestration in vacuoles (Xie et al. 2009). In this chapter, we will discuss the mechanism utilized by As hyperaccumulator plants specially *Pteris vittata*, how it can accumulate such high concentration of As, and the various factors which are responsible for this trait. Also, the involvement of reactive oxygen species (ROS), nitric oxide (NO), and antioxidants in arsenic detoxification and tolerance will be discussed. Arsenic uptake, its transport, and translocation from root to the aboveground parts and detoxification mechanism in plants emphasizing on hyperaccumulator plant will also be discussed here.

29.2 Arsenic Toxicity in Plants

Hydroponic experiments have helped to identify the strength of exogenously applied arsenic on the overall growth of the plant; they eliminate the intricate and difficult factors occurring due to the variation in the mobility of several arsenic species. The arsenic uptake by plants is in the respective order, AsIII>AsV>MMAV>DMAV, while further translocation to the aboveground parts has the order DMAV>MMAV>AsV≥AsIII (Raab et al. 2007). The order of arsenic phytotoxicity is inconsistent; this is possibly due to differences in chemical reactivity of As species with the available nutrients.

29.2.1 By Substituting Phosphate

As and arsenate possess structural similarities with Pi; AsV could be substituted for Pi in biochemical reactions, possibly disturbing the basic cellular processes. Potentially, AsV-sensitive reactions would interfere with the pathways requiring Pi as key factors, like in glycolysis, phosphorylation/dephosphorylation, phospholipid metabolism, and DNA and RNA metabolism. Plants are generally equipped with both low- and high-affinity Pi transport systems. As soon as AsV enters the root and comes in contact with cell surface, the Pi transporter is probably the first site where AsV competes with Pi. Competition between AsV with Pi has been observed in monocots as well as dicots and also in both As hyperaccumulators and non-hyperaccumulators (Clark et al. 2000; Wang et al. 2002; Bleeker et al. 2003). It has also been reported by Guo et al. (2008) that AsV can pass the *Arabidopsis* AtPHT4 (phosphate transporter) which is localized in the plastid and Golgi complex.

29.2.2 *Reacting with Thiols*

AsIII, MMAIII, and DMAIII are highly reactive toward thiol groups. AsIII binds to the thiol groups of proteins with enzymatic properties of enzyme co-factors altering or inhibiting their activity or structural properties. AsIII can crosslink by binding up to three monothiol molecules, like GSH (Kitchin and Wallace 2006), or it can bind a single molecule like PCs with polythiol properties. Arsenite toxicity is aggravated due to its binding capacity to other thiol-containing proteins and co-factors (Gupta et al. 2011). The stability of arsenite-thiol complexes depends upon the number of bonds and the preferential binding of AsIII to the proteins possessing three or more Cys residues in their zinc-finger motifs (Zhou et al. 2011). Also, the binding of AsIII to proteins alters their folding pattern and thus their function (Cline et al. 2003). These proteins include proteolytic, metabolic, and redox-regulatory enzymes, structural proteins, transcription factors, and signal-transducing proteins. Styblo et al. (1997) reported that MMAIII acts as a potent inhibitor like glutathione reductase. A similar report was published for thioredoxin reductase by Lin et al. (1999). In comparison to inorganic arsenic (AsIII and AsV) the methylated form of arsenic can displace Zn^{+2} from Zn finger protein at a low micromlar concentration so there is a need of understanding the plants ability to chnage less toxic form of As to more toxic methylated form (Schwerdtle et al. 2003).

29.2.3 *Oxidative Stress*

Arsenic toxicity further imposes oxidative stress (Zhao et al. 2010). By definition, during the oxidative stress, the cells are exposed to excessive molecular oxygen or the reduced form, i.e., reactive oxygen species (ROS). The overproduction of ROS such as superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH\bullet$), peroxyl radicals ($ROO^{\bullet}$), and alkoxyl radicals ($RO^{\bullet}$) and also some non-radical compounds such as hydrogen peroxide (H_2O_2), the singlet oxygen ($^1O_2$), ozone (O_3), and hypochlorous acid ($HOCl^-$) (Halliwell and Gutteridge 2015). ROS production can also be triggered by heavy metals (Sandalio et al. 2012). The regulation and expression of these proteins change depending upon varied oxidation states of different heavy metals (Cuypers et al. 2011). ROS are the intermediates of basal metabolism including mitochondrial respiration, photosynthesis in chloroplast, and photorespiration in peroxisomes and by NADPH oxidase (NOX) associated with the plasma membrane (Torres and Dangl 2005).

In general, overproduction of ROS oxidizes proteins, lipids, and nucleic acids, modifying their activity or function. Their levels are tightly regulated byenzymatic and non-enzymatic ROS-scavenging mechanisms (Apel and Hirt 2004). The enzymatic ROS scavengers include superoxide dismutases (SOD), catalase (CAT), and components of the ascorbate-glutathione (ASC-GSH) cycle. ASC-GSH comprises of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase

(DHAR), and glutathione reductase (GR). The non-enzymatic antioxidants include glutathione (GSH) and ascorbic acid (AsA), involved in maintaining H_2O_2 levels and also helping in maintaining the cellular redox state (Apel and Hirt 2004).

ROS overproduction by arsenic leads to (a) generation of intermediate arsenic with different valencies, (b) perturbations in mitochondrial electron-transport chain, (c) overexpression of NOX, and (d) altering the levels of GSH and GSH/GSSG ratio and affecting the redox pool.

The overexpression of PCs has also been observed during As toxicity (Singh et al. 2006). Ha et al. (1999) reported that PC has significant role in regulating As toxicity, as the mutant of PC synthase gene (PCS; *cad1–3*) in exhibited enhanced sensitivity to arsenate as compared to the control *Arabidopsis* plant. Overexpression of PCS in *Arabidopsis*, *Brassica juncea*, and *Nicotiana tabacum* resulted in enhanced tolerance toward arsenic toxicity (Li et al. 2004; Gasic and Korban 2007; Wojas et al. 2010). Conversely, PC overexpression can also diminish cellular GSH levels, disturbing the cellular redox pool and the overall antioxidant capability (Hartley-Whitaker et al. 2001).

Box 29.1 Mechanism of As Hyperaccumulation in Plants
- Higher affinity of As with their transporter
- Increased uptake and translocation of As
- Efficient reduction of AsV to AsIII
- Higher accumulation in aboveground part with root to shoot translocation of >1
- Decreased efflux of As to external medium
- Efficient loading of As to xylem

29.3 Mechanisms Irresponsible for As Hyperaccumulation

As hyperaccumulator plants behave differently with other non-hyperaccumulator plants in various traits. They can withstand such a higher concentration of As which is very much toxic to other plants, and toxicity is so much high that the non-accumulator plants are even not able to survive. Higher uptake of As, efficient translocation, and accumulation are the key features of hyperaccumulator plants. Along with these, the mechanism which is responsible for withstanding such a higher concentration of As without showing any toxic effects is also important. *Pteris vittata* member of the Pteridaceae family is the first As hyperaccumulator fern identified by Ma et al. (2001). This plant is very efficient in accumulating the As in its fronds without showing any phytotoxic effects (Lombi et al. 2002; Tu and Ma 2002). If we compare this plant with the normal or non-accumulator plant, we can say that it possesses a very high rate of As translocation from root to aboveground parts (Caille et al. 2005). Box 29.1 is showing important mechanisms of As hyperaccumulation in hyperaccumulator plants, while the major difference in As hyperaccumulator and non-accumulator plants has been listed in Table 29.1.

29.3.1 Arsenic Uptake, Transport, and Translocation

There are various factors such as soil characteristics, soil pH, As speciation (AsV, AsIII, and methylated As), plant species, water content, etc. that are responsible for the rate of uptake of As from soil (Zhao et al. 2009). As previously discussed, arsenic exists in the environment in organic and inorganic forms. These different forms of As are differentially taken up by the plants via their respective transporters (Farooq et al. 2016). AsV which is a phosphate analog is transported with the help of high-affinity Pi transporters (Fig. 29.1) following Michaelis-Menten kinetics in *P. vittata* as well as other higher plants (Karimi and Souri 2015). There are various evidences available in the literature for the transport of AsV via phosphate transporter. The two independent types of research had shown that a competitive inhibition exists for the uptake of AsV by Pi with the help of radiotracer 73 AsV (Abedin et al. 2002; Karimi et al. 2009). *Arabidopsis thaliana* mutants who are defective for phosphate transporters were also defective for AsV transport (Catarecha et al. 2007). Pi is very much important for the plant so its uptake is highly regulated. However, this transporter is also responsible for AsV transport; the uptake of AsV must also be under higher regulation with the same mechanism (Sun et al. 2012).

AsIII at neutral pH remains as arsenous acid, and this is mainly uptaken with the help of aquaporins which transport water, urea, as well a neutral molecule, and the inhibition studies had confirmed that glycerol competitively inhibits AsIII uptake. This arsenous acid shows structural similarity with silicic acid, and thus it shares the transporters which are transporting and uptaking silicic acid. Nodulin 26-like intrinsic proteins (NIPs), an important subclass of aquaporins, have been shown to transport AsIII from soil to roots of plants (Zhao et al. 2009; Chen et al. 2016). In *Arabidopsis* also the uptake and transport of arsenous acid occur via various members of NIPs such as

Table 29.1 Difference between hyperaccumulator and non-accumulator plants

S. no.	Mechanism	Hyperaccumulator plants	Non-accumulator plants
1	Uptake of As	High	Low to medium
2	Efflux of As	Low	High
3	Reduction of AsV to AsIII	Very efficient	Moderate to low
4	Sequestration in root vacuole	Very less	Efficient
5	Accumulation in root	Low	High
6	Accumulation in above ground part	Very high	Low
7.	Translocation from soil to root to shoot	>1	<1
8	Volatilization of As	Less	Moderate to high

NIP1;1, NIP1;2, NIP3;1, NIP5;1, NIP6;1, and NIP7;1 (Bienert et al. 2008; Xu et al. 2015). Not only in *Arabidopsis* the same family of transporters is also involved in AsIII transport in other plants also such as HvNIP1;2 in *Hordeum vulgare* and OsNIP3;3 (Katsuhara et al. 2014) Lsi1, and Lsi2, in rice roots (Ma et al. 2008). However, in *P. vittata* hyperaccumulator plant, neither glycerol nor silicic acid affects AsIII uptake suggesting that AsIII uptake and transport differ a lot in this plant in comparison to other plant species (He et al. 2016). In *P. vittata*, tonoplast intrinsic protein (TIP), a member of aquaporins called PvTIP4, is involved in AsIII uptake (He et al. 2016). The second difference between hyperaccumulator and non-accumulator plants is the affinity of transporters with AsIII. Hyperaccumulator plants show higher affinity in comparison to non-accumulator. The affinity of AsIII transports in *P. vittata* is much higher for

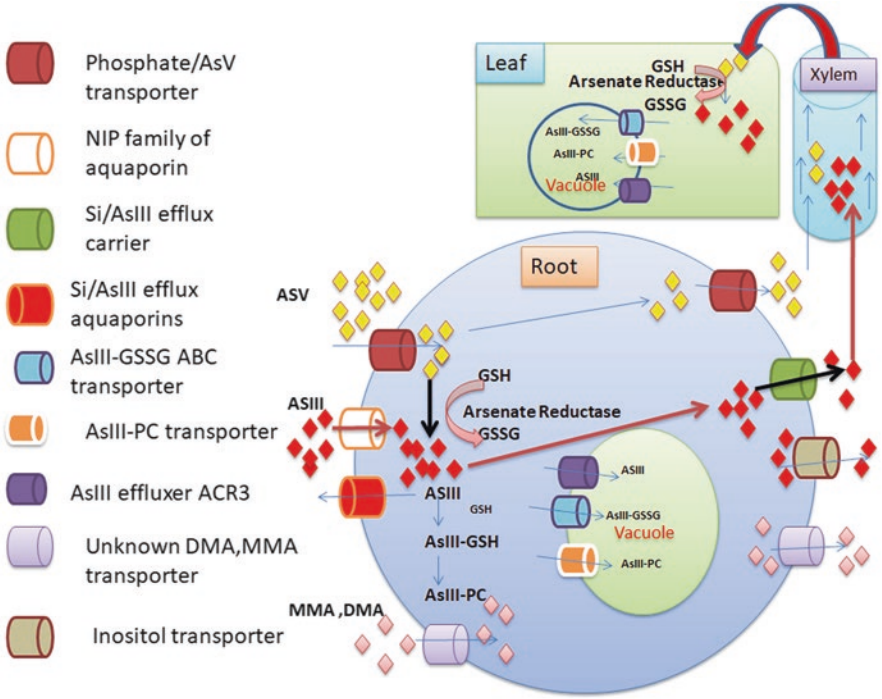

Fig. 29.1 Diagrammatic representation of As uptake, transport, translocation, and detoxification mechanism in plants. AsV being phosphate analogue enters the cell via phosphate transporters. As a detoxification mechanism, arsenate is reduced to arsenite via arsenate reductase using GSH as reducing agent. AsIII uptake is taken place via nodulin 26-like intrinsic (NIP) aquaglyceroporin channels. Transporters of methylated species (DMA/MMA) are either unknown or taken up by NIP. AsIII forms complexes either with phytochelatins (PCs) or GSH, and these complexes are sequestered in vacuole via ABC transporter. In *P. vittata*, AsIII is sequestered inside the vacuole via ACR3 effluxer. As a hyperaccumulation strategy, loading of AsIII to xylem must be efficient and is mediated by the Si/arsenite efflux transporters or inositol transporters (INT). Efficient translocation of As from root to shoot and sequestration capacity in vacuole of aboveground part makes hyperaccumulator plants to accumulate higher As in aboveground part. Dark lines represent efficient pathways of As transport and translocation in hyperaccumulator plants

AsIII in comparison to other plants explaining its extraordinary capacity for AsIII uptake (Chen et al. 2016). In *Arabidopsis* INT transporters (At INT) which are responsible for inositol uptake and transport are also responsible for AsIII distribution in phloem siliques and seeds (Duan et al. 2016). However, the presence of the same inositol transporter in hyperaccumulator plants is still doubtful. Another difference between the hyperaccumulator and non-accumulator plants is the site of accumulation of As. In hyperaccumulator plants, As is accumulated more in the aboveground part, while in non-accumulator plants, it is restricted in roots only, and translocation is also reduced from root to shoot and thus accumulation more As in roots. As hyperaccumulators, such as *I. cappadocica* and *P. vittata*, accumulate a higher amount of As (approximately 60–80%) in aboveground parts. In these plants, the As shoot-to-root ratio was found to be greater than 1 (Karimi et al. 2013; Chen et al. 2016). On one side, the As hyperaccumulator plants are accumulating approximately 60–80% of As in shoots. However, conversely, the non-accumulator plants, such as *Arabidopsis* (Isayenkov and Maathuis 2008) and *P. tremula* fern (Caille et al. 2005), accumulate comparatively very less proportion of As in shoots (5–10% of total As). Once As enters the plants, its efflux to root or volatilization can reduce As translocation and thus As accumulation to aboveground parts. Efflux of As to external media is also a very important strategy of non-accumulator plants for reducing As accumulation. In contrast to non-accumulator plant, translocation rate of As from root to shoots in hyperaccumulator plants is very efficient, and the efflux rate of As to external media is very less (Chen et al. 2016). As a detoxification mechanism as early as AsV enters the cell it is converted to AsIII (either enzymatically or GSH dependent) and this AsIII then forms complexes with either PCs or GSH and sequestered inside the vacuole and thus further reduces translocation of As to shoot and above-ground parts (Fig. 29.1). In hyperaccumulator plants, reduction of Asv to AsIII is very efficient; however their sequestration inside the vacuole is compromised for the efficient translocation of As from root to aboveground parts. Reports suggest AsIII is the main form of As transported from root to shoot through xylem sap, regardless of any inorganic form which is supplied to the plants (Su et al. 2008). For translocation and As accumulation in shoots, it is very important to have efficient loading of As to the xylem which is an important feature of As hyperaccumulator plants. Organic forms of As are also taken up by the plants. Some of the methylated forms of As are more cytotoxic in comparison to inorganic forms, yet their translocation in xylem and phloem is much higher in comparison to AsIII and AsV. However, in As hyperaccumulator plants, the key transporters for methylated species have not been identified in these tissues (Huang et al. 2008; Li et al. 2009). Uptake of methylated form of As in rice roots occurs via Lsi1 (Li et al. 2009).

29.3.2 Accumulation of Arsenic in **Pteris vittata**

Arsenic hyperaccumulator accumulates As in a different manner in comparison to non-accumulator plants. In *P. vittata*, most of the As is accumulated in aboveground biomass of the fern. There is a report stating that in these plants most of the As (almost 93%) was found to be accumulated in the fronds of the plant (Ma et al. 2001). Even in fronds, accumulation of As was found to be higher in the lamina of pinnae in comparison to midribs and spores. If we talk about the pinnae, it is the base of midrib which has a significantly higher amount and accumulation of As than those that are accumulated in the central or apical part of the pinnae. This can be explained based on the development of pinnae as the base of pinnae develops earlier than the central part while the apical parts develop recently. Accumulation of As was found to be least in spores. At the cellular level, accumulation of As in the pinnae of *P. vittata* also follows the same pattern as other hyperaccumulator plants; As was majorly accumulated in the upper and lower epidermal cell. The accumulation and distribution pattern of As differ a lot from other elements such as Mg, S, and Ca. The distribution pattern of As and K was found to be positively correlated in upper epidermis cells of *P. vittata*. A probable explanation of this fact could be the counterbalance of negative charge of As by positive charge of potassium ion. However, at acidic pH of vacuole, AsIII has to be undissociated from the complexes (either with PCs or with GSH) and requires locations (other than the undissociated protons) for balancing charge, unless it forms negatively charged complexes. It is observed that the restriction of As in the central part of the cell which is the vacuole was reported by X-ray microanalyses. This observation for the accumulation of As inside the vacuole is in agreement with the tentative distribution of heavy metals and metalloids in hyperaccumulating plants. It depicts the accumulation of both metals and metalloids inside the vacuoles which play a key role in providing tolerance against these heavy metals/metalloids. The XANES analyses showed AsIII is the predominant form of As accumulated inside the fronds (75%) while the rest was AsV.

29.3.3 Regulation of Arsenic Accumulation in Roots of Hyperaccumulator Plant **Pteris vittata** *at the Transcript Level*

Potdukhe et al. (2018) had done a root transcriptome analysis of *P. vittata* and identified 554,973 transcripts associated with As uptake transport translocation and accumulation. Various transcription factors, transporters of metals along with genes responsible for the biosynthesis of GSH and PCs, were found to be crucial in this study. Some of the genes which were found to be highly regulated in that study such as cysteine-rich receptor-like kinases and G family of ABC transporter further need validation.

In root transcriptome data, the transcripts having a role in As uptake and transporters have been identified. As previously in this chapter we had discussed the uptake and accumulation of As in hyperaccumulator plants, this study also confirms the previous facts that the AsV transport pathways share transport pathways of inorganic phosphate. Transcripts of the transporter that were associated with inorganic phosphate (PiT) and a large number of members of aquaporins which transports AsIII are upregulated. The higher modulation of the transcripts associated with AsIII transport and translocation might be accountable for the direct influx and accumulation of AsIIIe (Abedin et al. 2002). Arsenic further results in oxidative stress to the plant and activate various stress-responsive genes and molecules via kinase signaling (Rao et al. 2011). Oxidative phosphorylation also plays a very crucial role in activating stress-responsive genes.

Cysteine-rich RLK (receptor-like protein kinase) is induced by Cr stress (Trinh et al. 2014) which has also been upregulated by As stress in *P. vittata* indicating its role in heavy metal stress. This kinase has also been shown to be affected by oxidative stress (ROS) (Burdiak et al. 2015). This cascade further may induce various transcription factors such as asWRKY, bZIP, and MYB families which will further regulate the expression of various genes responsible for providing the tolerance against As stress (Thapa et al. 2012).

29.4 Arsenic Detoxification

29.4.1 Chelation of Arsenite

As detoxification is carried by chelating arsenate to arsenite, this chelation leads to a subsequent loss of toxicity. Generally, chelation is done by glutathione and PCs, and their biosynthesis is common in plants. However, As exposure leads to a substantial increase in the biosynthesis of both glutathione and PCs (Srivastava et al. 2007) indicating its importance as a key detoxifying mechanism. Similarly, upregulation of cysteine, GSH, and PC biosynthesis genes leads to tolerance toward arsenic toxicity (Shri et al. 2014; Tripathi et al. 2007; Wojas et al. 2008). However, the augmented PC biosynthesis has been observed constitutively in both hypertolerant and non-tolerant and in non-accumulator as well as in hyperaccumulator plants (Tripathi et al. 2007; Gupta et al. 2011). Hypertolerance and amassing of As in *I. cappadocica* due to >50% PC complexation indicate the presence of systematic tolerance machinery (Karimi et al. 2009). Above reports indicate that PCs are instrumental in arsenic detoxification. On the other hand, exceptionally low concentration of phytochelatins was perceived in hyperaccumulator *P. vittata* and *P. cretica*, indicating a minor or no role in direct As detoxification (Zhao et al. 2009). Interestingly several reports are indicating that PCs shave little or no role in As tolerance in certain hypertolerants (*H. lanatus* and *Silene paradoxa*; Raab et al. 2007). Phytochelatin-dependent As detoxification system has also been observed in certain non-accumulator plants like

Helianthus annuus, *Ceratophyllum demersum*, and *Hydrilla verticillata* (Raab et al. 2004; Srivastava et al. 2007, 2011; Mishra et al. 2008. In *Arabidopsis* root, Liu et al. (2010) reported that binding of AsIII with phytochelatins withheld the further translocation of As to shoot and also its efflux to the external environment.

29.4.2 Arsenate Reductase

Arsenate reductase (AR) reduces arsenate to arsenite by deploying glutathione as a reducing power (Finnegan and Chen 2012). Several AR genes from *Arabidopsis*, rice, and *H. lanatus* were found to regulate arsenic accumulation, viz., *AtHAC1/ATQ1*, OsHAC1;1, OsHAC1;2, and *HlAsr*, respectively) (from rice) (Chao et al. 2014; Shi et al. 2016; Bleeker et al. 2006). Hyperaccumulator *P. vittata* showed almost sevenfold greater AR activity when compared to As-susceptible *Oryza sativa* and *A. thaliana* (Danh et al. 2014). The expression analysis of AR and AsIII transporters in *P. vittata* indicated higher expression, thus possibly explaining the mechanism for hyperaccumulation by deploying vacuolar transporters in sequestering arsenic (Song et al. 2010).

29.4.3 Sequestration of Arsenic to Vacuoles

The sequestration of complexes As-GSH-PCs in the vacuoles is critical in arsenic detoxification (Gupta et al. 2011). Raab et al. (2004) reported that the vacuoles of hyperaccumulating and tolerant plants like *P. vittata* and *H. lanatus* contain a large amount of non-complexed arsenite, thus suggesting that hyperaccumulators don't save As in complexed forms and also indicating the role of vacuolar arsenite transporters (similar to bacterial extrusion pumps). A study demonstrated the role of arsenical compound resistance 3 (AsIII effluxer, ACR3), found on vacuolar membrane, in arsenic hyperaccumulation in *P. vittata* (Indriolo et al. 2010). Knockdown studies in yeast were performed to analyze the role of ACR in the gametophyte; it ensued arsenite-susceptible phenotype. PvACR3 imparted tolerance to ACR3-deficient yeast, signifying the importance of ACR3. Nevertheless, the absence of these transporters in flowering plants probably is the reason that As hyperaccumulators discovered till date are from Pteridaceae.

For the first time, Song et al. (2010) identified vacuolar PC transporters AtABCC1 and AtABCC2 in *A. thaliana*; they reported that they are vital for As detoxification. Further, double knockout of *atabcc1 atabcc2* of comparable characteristics and *cad1* mutant, lacking phytochelatin biosynthesis, implies the importance of vacuolar sequestration of phytochelatin-arsenite complexes and is as important as the synthesis of PCs for arsenic detoxification. Song et al. (2014) reported similar transporter OsABCC1 with an identical function in rice. Localization of OsABCC1was reported on tonoplast, and it enhanced As resistance in yeast cells expressing PC synthase, while the knockout of OsABCC1 resulted in significantly higher As hypersensitivity.

29.5 Arsenic Tolerance

29.5.1 ROS

Initially, ROS were considered toxic; during the last decade, ROS has been established to play an important role in the signal transduction pathways (Verma et al. 2019). In the last decade, research has been done on the heavy metal toxicity and its role in elevating ROS; however, lacunae regarding their effect on the regulation of cellular responses as well as signaling cascades remain to be done. Kidwai et al. (2019) reported that arsenic can elevate ROS production, viz., $O_2^{\bullet -}$, OH•, H_2O_2, and singlet oxygen (). ROS, primarily H_2O_2, has been established as a signaling molecule playing an important role in cellular response to arsenic stress (Sharma 2012). A proteomic analysis performed by Requejo and Tena (2005) in *Zea mays* roots also indicates that oxidative stress is associated with As toxicity as well; Gupta et al. (2017) reported that by generating ROS, NADPH oxidase C (NOXC) helps in regulating both the translocation and uptake of arsenic in *Arabidopsis thaliana*. H_2O_2 also activates mitogen-activated protein kinase (MAPK) signal transduction cascade under arsenic toxicity (Huang et al. 2012). During arsenate treatment in rice, out of the 11 reported MAPK kinases (MAPKKKs), one MAPK and ten phosphatase genes were upregulated. Recently, Kidwai et al. (2019) also documented the importance of class III peroxidase in arsenic tolerance and eventually low accumulation in rice. Another important report is by Dixit et al. (2015), who reported that high-sulfur treatment enhances the activities of antioxidant enzymes and mitigates the effect of oxidative stress due to As stress (Dixit et al. 2015).

29.5.2 Nitric Oxide

Nitric oxide (NO) is a hydrophobic, diffusible, and gaseous free radical molecule and plays an effective role as a signaling molecule in the plant system. NO can pass through the biological membranes, without requiring any specific membrane transporter, making it a suitable candidate for a signaling molecule. NO, a free radical, acts as a ROS scavenger, and being a redox-related signaling compound, it can alleviate oxidative stress by inducing antioxidant machinery, and it also exhibits immune responses against pathogen (Romero-Puertas and Sandalio 2016a, b; Bellin et al. 2013). NO derivatives are named reactive nitrogen species (RNS); nitrosonium ion (NO^+) and nitroxyl radical (NO^-) are the result of losing and gaining electron by NO, respectively. Reactive nitrogen species (RNS) are generated by the reaction between

NO and ROS, such as peroxynitrite and the NOx compounds (($ONOO^-$, NO_2, N_2O_3, and N_2O_4; Romero-Puertas and Sandalio 2016a). NO can interact with different molecules resulting in modulation of gene expression and protein function. This NO interaction brings the post-translational modification (PTM), which leads to *S*-nitrosylation, nitration, and nitrosylation of proteins. It has been reported that *NO*-dependent *S*-nitrosylation can change the levels of H_2O_2 either by modifying the ROS scavenging (or perturbing the ROS-generating enzymes (GOX and NOX; Romero-Puertas and Sandalio 2016a, b)). Similarly, APX and SODs are regulated by $ONOO^-$ (Romero-Puertas and Sandalio 2016a). The emerging data from the past few years suggest that NO is instrumental in heavy metal tolerance (Fancy et al. 2016). In a study, SNP treatment (a NO donor) suppressed O_2^- levels during metal toxicity (Kopyra and Gwóźdź 2003). Exogenously supplied NO provides protection against AsIII toxicity by attenuating oxidative stress in rice (Singh et al. 2009). Similarly, in *Vigna radiata*, arsenic toxicity is prevented by mitigating ROS generation and accumulation and preventing membrane damage by lowering malondialdehyde (MDA) content (Ismail 2012). Besides, NO can also regulate arsenic accumulation by modulating silicone (*OsLis1* and *OsLis2*; Singh et al. 2016) and ABC transporters (Hussain et al. 2016) in rice. NO can help in sulfate uptake (Farnese et al. 2013) and PC synthesis (Singh et al. 2016). Like H_2O_2, it can also activate MAPK signaling pathway which is also instrumental in combating metal and As stress (Ye et al. 2013). According to Dixit et al. (2015), AsV supplementation significantly hampers thiol metabolism, reduces GSH content and GSH/GSSG ratio, and also enhances the PC level. NO co-treatment with AsV helped in maintaining the GSH/GSSG ratio and reduce PCs.

29.6 Conclusions

In nature, few plants are equipped to survive extremely high levels of arsenic by either accumulating or detoxifying it. Research performed on arsenic metabolism in plants has helped in answering basic questions like how As is taken up and translocated in the plant, how it is chelated and sequestered in the fairly less toxic state, why it is transported through phosphate transporters, and how arsenite binds to sulfhydryl groups and aggravates oxidative stress. With the work done in a couple of decades, the mechanism of As hyperaccumulation without poisoning the plant has almost been decoded. As mentioned above, these hyperaccumulators have evolved well-synchronized approaches for As uptake, efficient transport, and translocation to aboveground parts. Another important mechanism is followed by *P. vittata*, where rapid arsenic uptake and chelation are done either with GSH and/or with PCs, and then this complex is further sequestered to vacuoles; a higher antioxidant capacity has also been reported in this hyperaccumulator plant. However, few important questions remain unanswered like how the cells can mask AsIII and prevent any interference with various metabolic targets while reaching the vacuole for its sequestration or how the AsIII concentrations are kept under check to prevent its harmful effect while translocation. A comprehensive study of other hyperaccumulator plants is also required so that the exact mechanism can be deciphered which will help us to utilize them for phytoremediation. However, in the future, the detailed physiological, biochemical, genomic, metabolomic, and proteomic approaches will help to achieve better insight into the contrasting features of As hyperaccumulators and non-accumulators and fill the lacunae in our understanding of the overall mechanism.

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Chapter 30
Biochar: A Sustainable Product for Remediation of Contaminated Soils

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Contents

Abstract Combustion of biological residue under less condition of oxygen resulting in less density carbon material is biochar. As thermal decomposition of biomass under limited oxygen condition is responsible for producing biochar, this method is getting attention in soil remediation and waste disposal in recent years. On the advantages of biochar, interest increases nowadays and it may discover multidisciplinary field in science and engineering. Biochar has large area of surface and has

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greater capacity to absorb heavy metals from contaminated soil. It can be used to reduce the availability of heavy metals and organic pollutants in soil through adsorption as well as other physicochemical reactions. Basically, biochar is an alkaline material which can increase the pH of soil and responsible for heavy metal stabilization. Phytoremediation and biochar are two sound environmental technologies which could be at the forefront to mitigate soil pollution. For remediation of polluted soil, biochar applications may provide new solution for contaminated soil problems. The provided biochar application may include sequestration of carbon, improvement fertility of soil, remediation, and recycling of agricultural waste. For controlling its properties, the key parameters include pyrolysis temperature, time of residence, heat transfer rate, and feedstock type. This article will provide an overview of the biochar impact on the environment and movement of heavy metals in polluted soil as well as methods for remediation of contaminated soil through biochar. Also, in this review, a succinct overview of current biochar use as a sorbent for contaminant management in soil will be summarized and discussed.

Keywords Biochar · Phytoremediation · Soil contamination · Organic matter · Soil remediation · Carbon sequestration

Abbreviations

Ca^{2+}	Calcium
CEC	Cation exchange capacity
Cu^{2+}	Copper
GHG	Greenhouse gases
IBI	International biochar initiative
Mg^{2+}	Magnesium
NH_4^+	Ammonium
Pb^{2+}	Lead
PLB	Poultry liter biochar

30.1 Introduction

30.1.1 Biochar Production, Properties

Biochar is a highly absorbent carbon-rich substance formed by organic biomass or agricultural residue after pyrolysis. Biochar production is a suitable alternative for the management of disease and waste. The presence of 50% of original carbon in biochar makes it highly intractable in the environment which supports the plant in fastening the carbon present in the plant biomass in carbon sequestration (Anon 2018).

Biochar can be produced from the variability of feedstock and collective procedures which give rise to a distinctive set of biochar having exclusive chemical properties and complexity (Joseph and Amonette 2012).

1. The cation exchange capacity, C concentration, and surface area of biochar produced are relatively less than the nitrogen content, potassium, calcium, phosphorus, and magnesium and biochar PH which is relatively high (Bird et al. 2011).
2. The sorption capability of soil is also amplified by the application of biochar; also it is noticed that the nutrient availability for plant uptake also increased (Nigussie et al. 2012).
3. Soils contaminated with heavy metals at the rate of 0, 2.5, and 5% by weight for 210 d were incubated by the biochar prepared from the dairy manure. An alternative activated carbon which is commercially prepared could also be used (Cao et al. 2011).

Biochar composition involves oxygen (O), nitrogen (N), carbon (C), hydrogen (H), sulfur (S), and ash in various quantities. It is vastly used to increase the nutrient content in soil along with carbon sequestration. Its property to mend water holding capacity of the soil and exceedingly porous structure makes it highly operative in biology, biotechnology, and sustainable growth choice for soil improvement and remediation (Anon 2018). There are certain physical properties of the biochar which subsidize to the management of the soil environment in several aspects. These features can affect differently the soil properties and system as each soil has diverse nature and physical properties along with the variable amounts of organic matter present. The addition of biochar to soil then affects differently according to the soil assembly, depth, and manipulating consistency, thus altering the soil compactness, soil porosity, and stuffing (Chan and Xu 2009). The movement of most organic and inorganic matter can be efficiently abridged by the practice of biochar (Beesley et al. 2011).

Biochar is also used as an energy source to power homes and automobiles as it produces bio oil and gases, i.e., hydrogen during their productions, which are used as a fuel. Its application in the soil not only increases the quality of soil and nutrient content but also helps in building up utmost potential for the long-lasting carbon sequestration (Anon 2018). Disease confrontation to crops can effectually be abridged through the application of biochar (Tang et al. 2013).

Biochar provides various benefits to the environment; i.e., it has the capacity to remediate the contaminated soil. The mechcanisims of biochar activity are still poorly unstated even though the practice of biochar reported back to thousands of years (Anon 2018).

Wood husk and grass is used to manufacture biochar beneath normalizes atmospheric circumstance in an experimental scale pyrolysis kiln according to Black carbon steering committee (Downie et al. 2009).

30.1.2 Applications of Biochar

Biochar has been used in a large number of applications which range from power production to soil remediation. The use of biochar has been increased in recent years for a number of environmental solicitations including removal of pollutants, carbon sequestration, and soil amelioration.

Biochar has been used since thousands of years as a soil amendment tool in terra prera a region of Amazon. It is not only used as a soil conditioner to improve soil quality but also used for a number of purposes due to its numerous beneficial qualities.

Some of the significant applications of biochar are as follows:

- Waste management tool
- Wastewater treatment
- Carbon sequestration
- Soil amendment
- Metallurgy
- Industries, i.e., food and cosmetics
- Soil conditioner
- Catalyst development (Anon 2018)

30.2 Soil Contamination: A Significant Issue

Today, we all are aware of the significant issues of the air and water pollution and related health impacts. However, the soil pollution impacts on health are still underlying because of the complex science involved. In certain soils heavy metals exist naturally although there are certain circumstances where the soils have plant species that takes up the heavy metals in large quantities in some localities where they established (Farid et al. 2017a, b). Researchers are now working on soil to develop our understanding about various issues related to soil such as soil erosion, sealing, and soil contamination and their impact on health as well (Sallah-ud-Din et al. 2017; Ahmad et al. 2019).

In Europe various researches have been done on population in different locations, being affected by contaminated soil, around the world and the impacts on them have been calculated broadly by epidemiologists and toxicologist. Dose-response relationships are often comparatively forthright to determine in these cases. As the relationship of soil and human health is a complex attempt, the impact of contaminated soil is harder to determine.

The primary focus of this report is the contamination of soil through various anthropogenic activities involving mining, industrial activities, household waste, and animal and human pharmaceuticals. It also gives an overview of soil contamination through heavy metals and remediation through biochar. Biological contamination through soil, which results in various human health impacts, involves pathogens such as tetanus and parasites, i.e., hookworms. However, it is not involved in this report.

30.3 Biochar and Phytoremediation

Heavy metal concentration has been increasing due to various anthropogenic activities (Farid et al. 2020). They are being accumulated in the environment as they cannot be degraded easily and have the capacity to contaminate the food chain (Ali et al. 2013). Various issues related to heavy metals are soil quality, plant survival, and human health which needs much attention to be remediated. But a high cost is impaired with it. In order to overcome this issue two sound environmental technologies, the use of biochar and phytoremediation, could be used to lessen soil pollution (Farid et al. 2019).

Biochar is the by-product of the pyrolysis process of organic matter. Feedstock, i.e., sludge and manures, could be used but the pre-used method involves the use of wood biochar (charcoal). Biochar has numerous benefits to soil properties; it also results in the increased biological activity of soil. Normally biochar is alkaline in nature and has high cation exchange capacity, fading greenhouse gases from soil via agricultural sources consequently increasing carbon sequestration. Soil quality will be altered when biochar will be added to the contaminated soil along with the enhanced capacity to upsurge in agricultural yield.

The liming influence of biochar is primarily accountable for the immobilization of heavy metals in soil. As the procedure goes on it results in the declined mobility of metals in soil due to aging reactions (Houben et al. 2013a). Heavy metals do not completely diminish by the addition of biochar although it results in reduced bioavailability and mobility of heavy metals and metolloids.

When the biochar is added to the contaminated soil the concentration of heavy metals, i.e., arsenic, cadmium, and lead, decreased in the maze shoot plants. A very interesting thing that happened to plants after biochar application is that the arsenic concentration suddenly increased with the addition of biochar but uptake by plants decreased which is an important factor. This leads to the fact that biochar can increase the heavy metal concentration in plant soil but also reduce the transfer of heavy metals in food chain. Leaching of arsenic could occur to nearby plants which is an important undertaking factor (UNEP 2018).

Phytoremediation is an operative and cost-effective approach to ingather heavy metals from soil (Farid et al. 2018a, b; Farid et al. 2015; Sarwar et al. 2017). Phytoremediation is the direct technique which involves the use of plants for the exclusion and degradation of heavy metals from contaminated soils, water, sludge, and sediments (Habiba et al. 2015). Plants have significant absorption and metabolic abilities to take up and transport nutrients or contaminants from soil (Jabeen et al. 2015). Plants are required to grow in a contaminated media for a specific time period to remove, immobilize, or degrade the heavy metals (Rizwan et al. 2017; UNEP 2018).

30.4 Biochar for Carbon Sequestration

The world's most pressing environmental downside is climate change and given world dependence on fossil fuels, one among its most balking issues. Renewable energy sources within the sort of star, wind, hydro, geothermal, and biomass energy should ultimately replace fossil fuel combustion. Energy obtained by combustion of biomass (plant matter made within the recent past) is constantly renewable and is ultimately carbon neutral—carbon oxide discharged in combustion is ultimately absorbed by new plant growth. Biochar production may be a variation of biomass energy, i,e., whenever plant material combusted for energy, charcoal residue act as biochar.

The carbon in this biochar is extremely immune to any decay, in order that once applied to agricultural soils, a little of the first biomass carbon is effectively sequestered for many years, centuries, or maybe millennia. Biochar is therefore considered as carbon-negative energy resource whereas at the same time it enhances carbon sequestration. Given the difficulty of the temperature change downside, biochar seems to represent a promising technology.

The IBI (International Biochar Initiative) states biochar as:

Solid substances attained from the thermochemical transformation of biomass in an environment with limited oxygen. Biochar can be recycled as a product itself or as an constituent within a mixed product, with a variety of applications as an agent for soil enhancement, amended resource use efficiency, remediation against precise environmental pollution, and as an possibility for greenhouse gas (GHG) mitigation (IBI 2015).

Biochar is made by transmutation, where biomass is heated within the absence of adequate oxygen for complete combustion, the residue can be used a biochar (Brown et al. 2015). Transmutation temperature and period have an effect on the proportion and qualities of the biochar made, similarly as proportions and qualities of the gasified and liquid coproducts. Coproducts embody energy-rich fuels, similarly as hydrocarbons with different probably helpful properties. There's a good kind of biochar and coproduced characteristics, and also the term "biochar" truly describes a family of connected merchandise rather than one uniform product.

30.5 Properties of Soil Affected by Biochar

30.5.1 Effect on Porosity of Soil

The permeability of soil is the quantitative relation of the pore volume to total soil volume. It is very significant in soil to feature plant growth. There are three variations of pores in soil categorized on the idea of size. These are important for aeration, movement, retention of nutrient, and water. It also offers shelter to microbes within the soil. The overall stability of soil amplified by application of biochar though this will increase in level of soil prosperity will depend on procedure of biochar used and on type of soil (Herath et al. 2013). Comparative contribution of three types of pores varies in total growth of soil uniformity relying upon biochar and kind of soil (Glaser et al. 2002). The rise in porosity level of soil was due to nature of biochar which is highly porous (Mukherjee and Zimmerman 2013). Some scientists embodied that soil consistency can drop by biochar application due to soil pore blockage by biochar mud.

30.5.2 Effect on Water Retention Property

Soil water retention capability is a measure of water that a soil will retain. If a soil will hold large amount of water, it drops the irrigation frequency of crop and this way plants grow well in such kind of soil. It was considered that biochar application boosts up to 97% with available content of water in the soil and saturated contents of water up to 56% (Uzoma et al. 2011). Some experimental analysis suggests that biochar application exaggerated the water retention capability of the soil due to chemisorptive nature of biochar (Herath et al. 2013).

30.5.3 Effects of Feedstock and Pyrolysis Temperature on Biochar Adsorption of Ammonium and Nitrate

Biochar is created by conversion and can be consumed to determine the contamination of nitrogen (N). The current analysis studied the impacts of temperature and feedstock on qualities of biochar and capacity to intake nitrate and ammonium. Diverse kinds of biochar were created from straw of wheat and corn and also the nutshell at different transformation temperatures which range from 400 to 700 °C. The biochars were developed for N adsorption tests. The outcomes demonstrated that substance of N and yield of biochar, oxygen (O), and Hydrogen (H) declined as temperature expanded from 400 to 700 °C, while substance of fiery debris, pH, and carbon expanded with more prominent pyrolysis temperature. All biochars could adsorb considerable measures of NH4 ± N, and the adsorption qualities were very much fitted to the Freundlich isotherm show. The capacity of biochars to adsorb NH4 ± N pursued C-BC>P-BC>W-BC, and the adsorption sum diminished with higher pyrolysis temperature. The capacity of corn straw biochar to absorb ammonium (NH4 ± N) was the most astounding on the grounds that it had the biggest cation exchange capacity (CEC) among all biochars.

30.5.4 Effect of Biochar on the Mineralization of Soil Organic Matter

Biochar is formed by natural fires, as a time starting from slightly burnt stuff to charcoal, sod, and carbon, and also it's typically represented as black carbon (Seiler and Crutzen 1980). Black carbon additionally seems to own a variety of positive effects on soil properties, like a high cation exchange capability, pH, and water holding capability (Glaser et al. 2002).

Black carbon or biochar in soil for long duration of time enhances soil excellence as well as carbon sequestration from the atmosphere (Lehmann et al. 2009). Incubation experiments have specified that exclusively a little fraction—is degraded at laboratory incubation intervals, relying each on the fabric from its been made and process conditions (Bruun et al. 2008; Kuzyakov et al. 2009; Nguyen et al. 2009; Zimmerman 2010). However, future stabilization is tough to gauge from incubation studies.

Nevertheless, associated experiment from Sweden has recently specified that biochar will proliferate the plant litter degradation (Karhu et al. 2011). If this is often the case, greenhouse emission discharged from the litter, as a result of the biochar addition, might balance the carbon sequestered within the biochar (Zhang et al. 2013).

30.6 Mechanism of Interaction Between Heavy Metals and Biochar

Functions of biochar depend on various factors like feedstock's particle size, its type, temperature, and pyrolysis. For the remediation of heavy metals, biochar may be more suitable for some specific substances as compared to others due to its characteristics. So for selection of biochar for remediation, not only soil characteristics and type are enough but also there should be awareness about properties of biochar. Also the essential properties of biochar like pH, carbon content, and surface area should be considered as posttreatment can affect them and to immobilize heavy metal ability of biochar enhanced (Lima et al. 2014).

It is crucial to note before knowing the way of interaction among heavy metals and biochar that on the bioavailable portion of soil heavy metals, biochar acts and also their reachability can be lessened by it.

Biochar characteristic of having large surface areas that indicates more capacity of complex metals on the surface. By using scanning electron microscopy, heavy metal's surface sorption has been revealed and it happen due to the complexity of heavy metals with diverse functional groups in biochar because physical sorption and the exchange of cations present in biochar with heavy metals like Mg^{+2}, Ca^{+2} etc. On biochar surface, heavy metals are also stabilized by oxygen functional groups especially for softer acids such as Cu^{+2} and Pb^{+2}. Moreover, raised oxygenated surface groups are related to Cu^{+2} sorption along with higher apparent charge density, pore diameter, and Mg^{+2} and Ca^{+2} exchange content. Cations present in soil and biochar and soil type are the factors on which sorption mechanisms are highly dependent. Heavy metals can also be stabilized by precipitation of some compounds like sulfates and carbonates present in the ash with the pollutants. Alkaline nature of biochar also helps in lowering bioavailable metal concentrations in biochar-amended land. After biochar addition, heavy metal precipitation in soil can be resulted from higher pH values (Cantrell et al. 2012).

Biochar pH and pyrolysis are directly linked, as with temperature biochar pH increases that is linked with more fraction of ash content (Paz-Ferreiro et al. 2014).

30.7 Combining Biochar with Phytoremediation

Literature review describes the beneficial use of lime and compost which is used to reduce the bioavailability of heavy metals by combining with phytoremediators (Komárek et al. 2013). Biochar can also stabilize heavy metals in soils by many processes; biochar proves beneficial for the growth of plants, their cation exchange capacity, water detainment, and nutrient confinement while increasing the production of phytoremediators because it is highly heterogeneous in nature in accordance with the type of plant (de Abreu et al. 2012). Majorly, phytoremediation is used to upgrade ecosystem functioning, as the chemical and biological properties of soil demonstrate the quality of soil and environmentally sensitive issues, including heavy metal contamination (Jeffery et al. 2011; Liu et al. 2013).

According to previous research, a sample of soil is tested which is contaminated with cadmium in a laboratory to check heavy metal concentration by the addition of biochar and phytoremediation using *Amaranthus tricolor* L (Houben et al. 2013b). Two types of biochar are used in the soil including eucalyptus pyrolysis and poultry litter (PLB).

As a result, the processes, biochar, and phytoremediation enhance and boost soil's biochemical characteristics while it depends on enzyme type and PH alterations of soil (Graber et al. 2010; Elad et al. 2011). Soil having greater acidic value with a fine medium texture enhance the yield of crops, as scientific research also proved that biochar boosts the plants to be stronger to fight with diseases as it changes the activity of microbes present in soil which promotes the growth of useful microorganisms. It results in the productions of antibiotics which are beneficial for the protection of plants antagonistic to pathogens. One of the contraptions of biochar as approached by researchers includes the following compounds in biochar: quinone, 2-phenylethanol, hydroxybutyric acids, and ethylene glycol for the quelling of pathogens occurred in microbiota (Graber et al. 2010).

Biochar which is obtained from the sludge deposited in sewage inhibits the quality of crop production because the heavy metals which occur in sewage sludge are in stationary form (Jeffery et al. 2011). Areas enriched with heavy metals are unsuitable for the productivity of food crops there for the plantation of biological crops (poplar, willow) upgrade organic matter and reduced pollutants from soil, which is the core motive of phytoremediation as it uptake heavy metals in a faster speed (Al Agely et al. 2005; Hartley et al. 2009).

Currently both the techniques (phytoremediation and biochar) are widely used to treat cadmium-contaminated soil by addition of *Brassica Napus* L with Miscanthus biochar. It is one of the feasible methods to collaborate phytoextracter and biochar for the treatment of multiple pollutants which are enriched in soil and to enhance the colonization of acidic soil. Biochar has the capacity to ameliorate the stability of greenhouse gases for some bioenergy crop productivity, for example, Miscanthus (Novak et al. 2018).

In contrast to remediate water, only few studies are accessible on the use of biochar for soil's remediation which is enriched with organic pollutants (Ahmad et al. 2013). Predominantly it is observed that the application of biochar has been mostly used for organic pollutants which are microbial degraded (Qin et al. 2013; Xin et al. 2014).

30.8 Conclusion

In phytoremediation and biochar, both techniques either combine or separately have the ability to treat the heavy metal-enrich soil. Biochar works as a filter and reduces the availability of heavy metals in the soil. Phytoextraction, on other hand, is a technique used to reduce the heavy metals present in the polluted soil. As both biochar and phytoremediation are new techniques, these techniques may be combined in the future to reduce the heavy metals present in the polluted soil. As biochars have heterogeneous properties, there is a need to understand this to maximize the efficiency of biochar. We should understand the mechanism of biochar and how we can treat the polluted soil with combination of both phytoremediation and biochar technique. Experiments are conducted to use these techniques separately but not in combined form. In phytoremediation case, heavy metal extraction can be obtained. But in the case of biochar, both experiments, i.e., in field and in laboratory, have been done which clear the fate of these heavy metals. It could be expected that, by increasing the time, the ability of heavy metal sequestration reduces. More research is needed to understand this process.

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Chapter 31
Phytoremediation Potential of Oilseed Crops for Lead- and Nickel-Contaminated Soil

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Contents

Abstract Production of crops has been facing many environmental stresses from the past few decades, hence reducing the productivity and nutrient enrichment of crops worldwide. Climatic changes and environmental stress have led to interpret the stress on plants and the purpose of its low productivity. Today the most impor-

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tant type of crops are the oilseed crops. Oilseed crops are mostly grown for the oil contained in the seed. The oil content in grains like wheat is only 1–2% as compared to the oilseed crops which contain primarily 20–40% of oil. Major types of oilseed crops include sunflower, brassica, olive plant, soybean, safflower and rapeseed. Due to the environmental stress like shortage of rainfall, providing wastewater for irrigation purpose, dumping of solid waste on crop lands and excessive use of fertilizers lead to the contamination of oilseed crops by mainly lead and nickel which are the heavy metals. In order to treat the crops contaminated by heavy metals, a modern, efficient, novel and environment-friendly technology is introduced named as phytoremediation. Phytoremediation is the technique in which different plants are used in order to extract the heavy metals from their roots and aerial parts. Usually the green plants have more ability to remove the pollutants from the plant and can achieve decontamination by various mechanisms. Plants usually extract the heavy metals though phytoextraction, phytofiltration, phytovolatilization, phytodegradation and phytostabilization processes. There are some chemicals which assist the phytoremediation process. These hormones are present within the plants and are mostly present in the root's rips and in the shoots. These organic chemicals not only assist the phytoremediation mechanism but also increase the growth and nutrient rate of oilseed crops. The heavy metals like nickel and lead affect the oilseed crops in any way such as decreasing the growth of plant and disturbing its natural processes like photosynthesis. Effective uptake of lead and nickel in oilseed plants is beneficial and acts as a good hyper-accumulator while excessive uptake harms the crops in various ways. Rotation of crops is another effective method in food crops in order to decrease the accumulation of heavy metals in crops and in food chain.

Keywords Oilseed crops · Climate change · Productivity · Contamination of pollutants · Phytoremediation · Heavy metals · Hyper-accumulator

31.1 Introduction

31.1.1 Oilseed Crops

31.1.1.1 Sunflower

The word *Helianthus* means "sunflower" and the species name "*annuus*" means "annual". It originates from the Greek word "Helios Anthos" (Kindscher 1987). In many parts of the world *Helianthus annuus* L. (sunflower) is considered most imperative in all oilseed plants (Farid et al. 2020a, b). After rehabilitating the genetic arrangements of plant species, the Indians improved the size of seeds (Yarnell 1978). Plant height of sunflower at flowering is usually between 1.5 and 2.5 m and flower head has 15–30 m diameter with yellow and disc or tube flowers. On the stem of maximum 25–35 leaves one apical inflorescence is present. Leaves of *Helianthus* are roughly heart shaped and dark green and they have large surface (Murphy 1994). In sunflower average protein content is 20–30% with 90% oleic and linoleic acids and oil content is 40–50% (Lide 1991). There are many factors which affect the yield and production due to underprivileged weather conditions (Farid et al. 2019; Mwale et al. 2003). At sowing time due to shortage of rainfall and moisture sometimes seeds are present in scattered form in seedbeds (Angadi and Entz 2002). California natives used the sunflower as a food and also used after mixing with other seeds in pinole (Strike 1994). In Mexico the seeds of *Helianthus* not only used as a food but also had believed medical worth in relaxing the pain in the chest (Heiser 1976).

31.1.1.2 *Brassica*

In the family *Brassicaceae* the genus *Brassica* is one of the 51 genera. *Brassica* is belonging to the cruciferous crops. In the family of *Brassiceae* it is the most vital genus economically. There are lots of other crop plant species which are involved in the *Brassica* genus and it included 37 different such species. This type of different species gives edible stems, buds, flowers, roots, leaves and seeds (Farid et al. 2015; Gomez-Campo 1980). After soybean and cotton seed the *Brassica* has the vital source of vegetable oil. The production of *Brassica* seeds is also different in different parts of world such as in Europe it has highest seed yield and in India has the lower than the China and Canada (Kimber and McGregor 1995). The combination of *Brassica* and *B. oleracea* produces the vegetables such as collards, savoy, cabbage and broccoli (Kristal and Lamp 2002). Sulphur-containing compounds are present in the *Brassica* due to its glucosinolate content and pungent smell (Higdon et al. 2007). Radish, watercress, daikon, Japanese mustard and wasabi are also included in the other species of *Brassica* (Lampe and Peterson 2002). Region/ area of growth, cooking situations, harvest era and variety are such factor on which the Brassica vegetables are depends (Gupta 2011).

31.1.1.3 Soybean

Scientific name of soybean is "*Glycine max* L". In Sothern Africa its production number has intensified speedily. There are some types of soybeans which are grown in small farms such as cowpea and groundnut, but they have no good storing worth of grains and have less ability to resist the disease and the pests. These types of soybeans also have less biomass of leaves which is necessary for enhancing the soil fertility (Mpepereki et al. 1996). Trade of soybeans is basically initiated from the United States. On land of 30 million hectares in the United States about 46% soybeans were grown, which were about 52% source of world oilseed production in 1999. In Brazil and Latin America this soybean production is also source of foreign currency (Ploper 1997). About 30–60% earning comes from the trade of soybean crop in many areas of Asia (Lancon 1997). In the West and East there are different types of soybeans which are prepared into numerous food items and used as food for the human; some are used to make the defatted meal and oil. Two types of soybeans such as food beans and oil beans are formed due to dissimilarity in soybean use (Liu et al. 1995; Orthoefer and Liu 1995).

31.1.1.4 Olive Plants

Scientific name of olive is "*Olea europaea* L". Throughout the world it is mostly present in temperate and tropical regions with 600 species and about 25 genera which belongs to Oleaceae family (a medium-sized family) (Besnard et al. 2009). Because of production of essential oil in their flowers, evergreen trees and bushes, some other genera of family such as lilac, privet and jasmine are economically very significant (Heywood 1985). Three vital tissues such as exocarp, endocarp and mesocarp help the olive in a specific form of growth and are responsible for its composition. Between the mesocarp and endocarp a loop of vascular bundles is present in the ovary (King 1938; Rallo and Rapoport 2010; Rosati et al. 2012).

Since the last ice age, in agroecosystem oleaster has vanished but in natural ecosystem it is present due to some factors such as it is not a threatened species and impartments in agricultural activities (Hannachi et al. 2010). During the Holocene it is evident that oleaster is present in West and became abundant (Carrión et al. 2010).

31.2 Phytoremediation

Phytoremediation is associated with microbes present in soil to decrease the attentions or toxic properties of chemicals in the environments (Farid et al. 2019; Greipsson 2011). It is the use of plant capabilities to remove and sequestrate the heavy metals from the wastewater as well as for organic pollutants (such as polynuclear aromatic hydrocarbons, polychlorinated biphenyls and pesticides). Phytoremediation of heavy metals may take one of the several forms such as phytoextraction, phytoaccumulation, rhizofiltration, etc.; plants that are used for remediation and uptake of these heavy metals are called hyper-accumulator (Farid et al. 2018a, b; Glick 2003). Heavy metals are non-biodegradable, which coagulate in the environment and add in the food chain and create the health risk for humans. These heavy metals are released into the water through industries; different physical and chemical process are used to remove these heavy metals. These methods are very costly and require labour and large amount of equipment but phytoremediation is a simple and environment-friendly biological process and public acceptance process (Ali et al. 2013). Phytoremediation is an emerging technology which used microorganisms and plants. It is cost-effective and has aesthetic benefits of long time (Farid et al. 2017a; Jadia and Fulekar 2009). In phytoremediation technique plants are used to remove toxic chemicals from different growing mediums like soil, water or sediments through physical, chemical and natural conditions (Wang et al. 2007).

Toxic metals are removed by the phytoremediation process which has different subgroups, which are applicable: rhizofiltration is used to accumulate the heavy metals from wastewater by plant roots; phytoextraction remediates the metals from the soil and phytostabilization helps to reduce the heavy metal biodiversity in the soil media (Farid et al. 2017b; Salt et al. 1995). It is a fast growing field plant, especially in developing countries like Pakistan and India, suitable due to its ability to grow in the toxic environment, these plant uptake the metals from the water with their roots under certain conditions.

31.2.1 Phytoremediation Assisted by Organic Chemicals

The major five types of plant hormones are given: auxins, cytokinins, gibberellins, ethylene and abscisic acid. Each hormone has a dissimilar action for oilseed, pulse and cereal crops. Auxins and cytokinins can importantly recover plant growth, promote growth of roots and shoots and decrease stress.

31.2.1.1 Auxins

Auxin is a hormone present in plants which stimulate many processes in plants such as expansion of cells, apical dominance, and particularly in root and shoots. When the auxins have low concentration, it endorses the growth of plant, and higher attentiveness shows damaging effect on growth. Auxins are more effective in growth of oilseed crops, swelling the production of oilseed crops. On other hand, auxins also improve the asset of the fibres and plants become better. So, auxins have possible effects on the growth of oilseed crops (Fu and Harberd 2003; Bennet and Bennet 2011).

31.2.1.2 Cytokinins

A hormone called cytokinins is present in all parts of plants, like roots, shoots and leaves, etc. Cytokinins are typically current in the young seeds, in theu root tips, and in the shoot's apical meristem. Cytokinins are able to persuade cell division in plants. Cytokinins move from one place to another according to the need of plants. Cytokinins are produced in lateral roots known as primordial cells with initial cell division on neighbouring pericyclic cells (Bennett and Kottasz 2011).

31.2.1.3 Gibberellins

Gibberellins have various topographies inside the plants, such as breaking the seed dormancy, beginning of flowers and also in regulating plant growth. Gibberellins induce the expansion of cells and increase the growth of plants as well as plant parts. Most of the oilseed crops like *Arabidopsis* depend upon gibberellins for their growth. Due to high retention of gibberellins few seeds per pod reduction in the weight and length of the pod (Silverstone et al. 2001). The gibberellins and cytokinins, both hormones, play a role for handling the wall growth of pod (Bennette et al. 2011). Gibberellins involve in managing the growth of stem, flowering and ventilation in different plant classes. High concentration of gibberellins upsurges the growth of plants and low attentiveness constrains the flowering and growth.

31.2.1.4 Ethylene

Ethylene is involved in various developmental processes especially in organ senescence timing and germination of seeds. Ripening of fruits and plant growth can be enhanced by ethylene. Ethylene assists response of plants toward other hormones, including auxins, cytokinins, gibberellins and abscisic acids that produce biotic and abiotic stress (Oeller et al. 1991; Joaquin et al. 2007).

31.2.1.5 Abscisic Acids

Abscisic acid is linked with abiotic and biotic stress of plants, and as a result it slows down the process of growth and in the parts where no stress is found, it promotes the cell growth. Grain rate can be affected due to imbalance abscisic acid and ethylene ratio. Abscisic acid is important for plants in environmental stresses including drought, cold tolerance, soil salinity, heat stress, freezing tolerance and heavy metal ion tolerance (Xiong and Zhu 2003).

31.3 Metal Toxicity and Phytoremediation in Different Oilseed Crops

31.3.1 Sunflower

31.3.1.1 Toxicity of Lead in Sunflower

One of the major causes of health problems and environmental issues in our society is the aggregation of heavy metals in the food chain. Fossil fuel burning, mining, melting of metallic ores, municipal waste, fertilizers, pesticides and sewage are the key origin of pollution (Peng et al. 2006). A toxicant which has the ability to assemble within soils and sediments is lead. Factors which influence lead concentration in plants include pH, cation exchange capacity, particle size, root transpiration factor and many other physiochemical factors (Antosiewicz 1992). High level of lead causes various effects on plant's growth and on the root system and chlorosis. Sunflower plants which are served by lead usually show retarded growth and decreased leaf extension (Nandakumar et al. 1995).

31.3.1.2 Toxicity of Nickel in Sunflower

Nickel is a pervasive element in tissues of plants. Usually the concentration of nickel in plants is 0.01–5 mg kg^{-1} (Mishra and Kar 1974). The most important element for plant growth and nutrition is nickel (Brown et al. 1987). Wheat, cotton, paprika, tomato and potatoes can have a favourable effect of low level of nickel (Mishra and Kar 1974). Despite this, great amount of nickel is being deposited to the environment because of increased industrialization, organic fertilizers and pesticides, mining processes and dumping of urban solid waste (Alloway 1990). On the other hand, biofertilizers also play a vital role in uptake of nickel in sunflower plants (Abou-Shanab et al. 2003). At increased concentration, nickel is poisonous for plants (Baycu et al. 2006). Due to increased nickel, chlorosis and necrosis happens and forms white strips in leaves (Seregin and Kozhevnikova 2008). During photosynthesis, electron transport chain is disturbed due to excessive nickel. Due to this, it intercepts electron establishment and stomatal transaction (Chen et al. 2009a, b).

31.3.1.3 Phytoremediation of Lead by Sunflower

Lead contamination emerges from smelting and mining activities. Paints, gasoline, explosives and the dumping of sewage sludge contain high amount of lead (Jackson and Watson 1977). The remediation of lead from contaminated soil represents an important expense to industries and governmental agencies. Soils contaminated with lead can be remediated with a range of engineering-based technologies (Sallah-Ud-Din et al. 2017; Cunningham et al. 1995; Salt et al. 1995). Recently, plants are being used for remediating the heavy metals from contaminated soil, called as phytoremediation, which has been gaining increased attention among a modern world (Chaney 1983, 1993; Cunningham and Berti 1993; Baker et al. 1994; Raskin et al. 1994). Phytoremediation of lead-contaminated soil has two main strategies: phytostabilization and phytoextraction (Cunningham et al. 1995; Salt et al. 1995). Phytostabilization lowers the inseparable toxicants which are produced due to the heavy metal contamination and lowers the effect without removing it from the actual site. On the other hand, phytoextraction uses the plant body to remove a harmful toxicant from soil. Lead is not a known essential element for plants; however there is a precise evident indicating that some plants have capability to absorb lead by roots and then transport the lead from roots to aerial parts of plants (Reeves and Brooks 1983; Qureshi et al. 1985; Baker and Walker 1989).

It is being reported that in order to reduce the bioavailability and toxicity of a metal in a plant, inorganic changes are considered more effective than the organic alterations. It is due to the fact that inorganic alterations usually give more binding sites (Grawboska 2011; Arunakumara et al. 2013).

In sunflowers, lead uptake increases due to the exertion of EDTA under lead stress, while it also enhances the shoot and root length. An author summarizes that due to the hindrances of Pb with EDTA, plant's biomass increases while it also reduces the injurious effects of lead in sunflower plants. However, increased concentration of Pb and EDTA is harmful for sunflowers (Azhar et al. 2009). Fassler et al. (2010) describe that EDDS crucially decreases lead and zinc uptake in sunflowers and also promotes plant's growth, root system, surface area and also the length. Additionally, EDDS plays a major role in nutrient solution for increase in the sunflower's dry weight of its shoots and roots under stress condition of Pb, Zn and Cu (Tandy et al. 2006).

31.3.1.4 Phytoremediation of Nickel by Sunflowers

Soil pollution is usually caused due to excessive addition of heavy metal such as nickel (Morel and Hering 1993; Lasat 2002). Soil pollution is considered as one of the greatest threats to the environment (Kimbrough et al. 1999) as it adversely effects ecology of soil, land fertility, quality of crop and water resources, hence causing serious health problems for humans and animals (Thawornchaisit and Polprasert 2009).

Metal toxicants are not biodegradable, unlike organic pollutants, and the purification of soil by these metals is a necessity (Lasat 2002). Soil polluted with heavy metals can be solved through physical, chemical and biological methods (McEldowney et al. 1993). Physical and chemical methods cause irreparable effects on soil properties, annihilate biodiversity and also are costly. In phytoremediation, hyper-accumulator plants are of great importance (Shen et al. 1997). The requirement for nickel in non-hyper-accumulator is so low that it has never been reported for grown crops, yet Ni is an essential element. Since nickel is a water-soluble metal, it is easily bioavailable and bioaccumulated.

Nickel content in soil usually varies between 5 and 150 mg kg^{-1} (Kabata-Pendias and Pendias 2001). The major cause of nickel emission is anthropogenic activities which include smelting, burning of fossil fuel, vehicle emissions, household waste disposal, municipal waste, metal mining fertilizers and organic manures (Salt et al. 2000). The total uptake of Ni by plants usually depends on Ni^{2+} concentration in soil solution, plant metabolism and presence of other metals and on organic matter composition (Chen et al. 2009a, b).

For cleaning the contaminated soil, current technologies which are used are physical and chemical remediation including processes like pyrometallurgical separation, chelate-enhanced leaching, etc.; these are efficient methods but are costly and labour intensive and can have probable environmental loss (Mulligan et al. 2001; Krämer 2005; Wu et al. 2010).

Through harvesting of biomass accumulated by Ni, a "bio-ore" can be produced; the process is usually called as "phyto-mining". Due to the clean viability and the market value of nickel, this method is sustainable and environmental sound approachable (Brooks 1998; Li et al. 2003; Sas-Nowosielska et al. 2004; Chaney et al. 2007). Plants can also uptake nickel directly in the form of ion pairs and in the form of ion complexes in soil solution (Kabata-Pendias and Pendias 1992). In order to access heavy metal concentration in soil various biosensors have also been designed (Michel et al. 2006).

31.3.2 Brassica

Globalization all over the world has resulted in the contamination of the arable land with heavy metals. The urge to meet the food demand leads to the use of contaminated soils that poses threats to food security (He et al. 2005). The genus *Brassica* comprises of more than 30 species including certain hybrids and species, several of which are eaten by a majority of people (Ebbs and Kochian 1997). Species of genus *Brassica* are considered as very vital agrarian crop in various regions of the world; along with this they are considered as heavy metal accumulators (Shakoor et al. 2014; Mourato et al. 2015). A number of studies published used *Brassica* species for phytoremediation that gave variable results. These species are taken into account of phytoremediation due to intrinsic properties of their stems to tolerate and accumulate heavy metals, easy fast growth that is easily harvestable and for above ground biomass production (Marchiol et al. 2004).

Expanding horizons of anthropogenic pressure have also raised attention towards the heavy metal contamination in which lead is highlighted as a potent heavy metal pollutant. Soils adulterated with lead demonstrate a drastic decline in crop yield. Generally, lead accumulation usually takes place in roots of a plant but a proportion of it is hauled to the areal plant parts. The availability of lead and its inhibitions relay on various plant and root factors such as soil pH, particle size of soil, cation exchange capacity of soil, soot surface, root depth, root exudates and the transpiration rate. The lethal concentrations of Pb, Pb flux, go in the vascular tissues. In aerial parts of plants, lead deposits are mainly present in cell walls, vacuoles and intercellular spaces (Eick et al. 1999). Lead results in the disproportion of minerals (K, Fe, Mg, Ca, Mn, Zn, Cu) within the plant tissues by substantially hindering the approach of the ions to the sorption sites of rhizomes (Szczygłowska et al. 2011). Detrimental effect of Pb on *Brassica* is that it lowers the plant's growth rate and nitrogen assimilation. Plants grown in lead-contaminated soils have elevated lead accumulation in the shoots (Xiong et al. 2006).

31.3.3 Soyabean

31.3.3.1 Toxicity of Lead in Soybean

Lead is one of the toxic heavy metals present in the environment and originates from diverse sources like mining and smelting of lead ores, burning of coal, effluents from storage battery industries, car exhausts, metallic plating and finishing operations, fertilizers, insecticides and from components in pigments and gasoline (Eick et al. 1999). The increasing concentration of Pb in soil inhibits germination of seeds and adversely affects the bloom and metabolism of flora (Godbold and Kettner 1991; Kastori et al. 1992). Many environmental stresses like soil salinity, drought, extremes of temperature and heavy metals are acknowledged to drive oxidative harm to vegetation both directly or indirectly by triggering an expanded level of manufacturing of reactive oxygen species (ROS) (Comba et al. 1998; Malecka et al. 2001).

The toxic Pb also causes formation of reactive oxygen species which leads to reduced plant growth. Similarly nickel causes inhibition of lateral root formation, harm photosynthetic process and plant above-ground biomass. Pb is considered as a common protoplasmic poison. Soil contaminated with Pb festally reduced the land capability to produce crops and also lowers the land fertility, which is causing a severe problem in the sector of agriculture (Kastori et al. 1992). Lead is available from soil and areal resources for the life of plants. Studies related to lead toxicant have proven that flowers have the capacity to carry huge quantities of lead but up to certain limit (Lane and Martin 1977).

The absorption of Pb inside the soil increases within 3.0–8.5, with the growing pH rate (Li 1998). The modern age studies have described the resources of Pb but these resources can change under physical, biological and unusual circumstances (Shalini and Dubey 2003).

31.3.3.2 Toxicity of Nickel in Soybean

Nickel holds a unique characteristic in heavy metals. Cd, Pb, Hg, Cr and many different metals that are not the additives of evaluation to plant enzymes are vital for a few species of plants. On the other hand, Ni is not necessary for plants as key metals such as Zn and Cu. However, as soon as other heavy metals accumulate within the plant body, it appears to be toxic. Nickel accumulates in soybean first through xylem and then transports to the shoot (Sagner et al. 1998; Yang et al. 1997). The heavy metals are available in minute quantities in soil. The excessive presence of heavy metals in the soil substantially affects the plant's biological and chemical properties and also causes disorders which leads to the increased soil pollution and reduced crop production.

31.3.3.3 Phytoremediation of Nickel by Means of Soybean

The first experiment was carried out using California *Streptanthus polygaloides* in California and it is found that 100 kg ha^{-1}sulphur-free nickel could be produced. We have used the same technique to experience the capacity of phytomining from South Africa and Italy.

Soil protection techniques encompass isolation and containment; however additionally the protection technique requires special devices and environmental catastrophe. Apart from this, these techniques aren't suitable when pollutants are in low concentration. Cadmium also can be imposed by means of lime and magnesium (Lin and Shroff 2005).

31.3.4 Olive Plant

31.3.4.1 Toxicity of Lead in Olive Plant

All metals have characteristics but most toxic metal lead not only accumulates in individuals and affects them but also moves in food chain and affects the health system of all plants, animals, phytoplankton and human beings which are linked to each other (Singh et al. 2010). The major source of heavy metals in the environment is mining. Due to mining procedures, manmade habitats are being created which are opposite to the natural system and by disrupting the natural order certain difficulties are experienced for planting and sustainable vegetation (Wong 2003). Lead comes into soil from different sources and may move from soil to higher parts of the plants through root system (Zimdahl 1976; Peterson 1978). There are various factors on which the quantity of lead in plants depends on such as chemical properties of soil, distance from emission source, wind direction and season. Each plant has a threshold capacity to accumulate heavy metals and when the limit is crossed, the plant no longer remains safe for cultivation and further production (Sharma and Gaur 1995; Salt et al. 1995; Rai et al. 1995; Ewais 1997; Burzynski and Buczek 1989). The variation in metal accumulation capacity is due to change in the environment, physiology and genetics and when this accumulation of heavy metals increases negative impacts start to show in plants (Manios et al. 2003). The observed high accumulation of heavy metals in olive plants reduced chlorophyll a and chlorophyll b (Manios et al. 2003). A number of researchers have observed high build-up of heavy metals in plants due to which plant photosynthesis ability is reduced and the fluorescence ability is also reduced due to decrease in plant chlorophyll *a* and *b* (Ehsan et al. 2014). Lead toxicity response is shown by the roots of the plants in both ecotypes of *E. argyi* (Islam et al. 2007). Some plants are mainly poor in growth due to higher concentration of lead which also reduces the uptake of minerals and activities of root system (Islam et al. 2007).

31.3.4.2 Toxicity of Nickel in Olive Plant

Nickel is ranked as 24th most abundant element of Earth's crust. Nickel is an essential nutrient found in mineral as a trace concentration (Bai et al. 2006). However, plants may show high level of toxicity in the presence of high nickel (Foy et al. 1978; Seregin and Kozhevnikova 2006). Naturally raised nickel level can be perceived in rocks made by serpentine (ultramafic) minerals (Kopittke et al. 2007). Major sources of nickel pollution in the environment are extraction, production and processing of nickel, reuse of nickel-containing products, recycling, disposal, sludge application on land and the high use of fertilizers in inorganic form and usage of pesticides (Gimeno-García et al. 1996). Photosynthesis in plants is inhibited due to excess level of nickel presence in the environment in which plants are being grown (Ahmed and Häder 2010). Bazzaz et al. (1974), Clijsters and Van Assche (1985) and Seregin and Kozhevnikova (2006) demonstrated that the reduced rate of photosynthesis in nickel-stressed olive plant is linked to decreased stomatal conductance.

31.3.4.3 Phytoremediation of Lead by Olive Plant

Pollution due to heavy metals is a worldwide problem, although harshness and levels of pollution fluctuate from place to place. Twenty metals are considered as toxic and half of them are released into the environment that contain excessive risks to human being's health (Akpor and Muchie 2010). Mostly known heavy metals such as Cd, Pb, Co, Zn and Cr, etc., which are phytotoxic at both high levels and very low level are detected in wastewater. These heavy metals may affect the health of plants, animals and human beings by entering in food chain from sediments of soil. If it is present in low then they are not harmful for living organisms because in small portion these elements are needed by plants, animals and humans for proper growth, but high concentration of these heavy metals may cause chronic or acute toxicity (Singh et al. 2010). Nowadays, there is a great threat to ecology of the world due to heavy metals (HM) which lead to develop a new remediation technology of economical plants. Previous methods of remediation such as physical, chemical and microbiological are very costly and difficult to operate (Danh et al. 2009). Lead is present in soil elemental and molecular form and found in air in aerosol form.

31.3.4.4 Phytoremediation of Nickel by Olive Plant

Small quantities of some elements are required by the plants for their growth such as Fe, Cu, Co, Ni, Mn and Mo. A research started early in 1990s at University of Botswana to identify that plants which accumulate high level of nickel in their body. By research, it is proved that nickel and copper more accumulate in *Blepharis diversispina* (Nees) C.B., *Helichrysum candolleanum* Buek (Asteraceae family) and clark (Acanthaceae family) than others (Takuwa et al. 1995). There is a natural system in plants; they have the ability to extract elements from soil and then distribute them into its different body parts like roots, shoot and fruits based on the procedures (Ximénez-Embún et al. 2002; Page et al. 2006; Fritioff and Greger 2006). Therefore, elements that include micro- and macronutrients, as well as heavy metallurgy, can be absorbed by soil plants and can easily enter food chain.

If we want to study how heavy metals transfer from roots to different plant body parts, the TF was calculated according to Cui et al. (2004) using a relation that is:

$$\mathrm{MTF}1/4\cdot\mathrm{MCp}=\mathrm{MCs}$$

where MTF is metal transfer factor, MCp metal concentration of plant (μg/g dry mater) and MCs metal concentration of soil (μgg^{-1} dry soil).

The tendency of the transfer of heavy metals from the soil to the plant according to the calculated TF was approximately the same for the olive fruits and for the leaves; that is, fruits: Cu>Zn>Mn>Fe>Ni>Pb>Cd and Fe sheets>Zn>Mn>Cu>Ni>Pb>Cd, suggesting a consistency of metal transfer from the soil to the plant. The absorption of heavy metals by olive plants (leaves and fruits) was not always related to the corresponding concentration of wastewater, suggesting a selective absorption (Batarseh et al. 2011).

31.4 Conclusion

Plants are not only as a source of food, fuel and fibre but also play an important role as an environmental neutralizer to industrial pollution. Phytoremediation is an appropriate technology and also a low-cost technique for diffusing pollution in large areas. The toxic heavy metals present in the soil can be extracted through different remediation technologies such as phytovolatilization, phytoextraction, phytofiltration, phytostabilization as well as phytodegradation. Some of the oilseed crops are also used for the remediation purpose. The most commonly used oilseed crops are rapeseed and mustard, sunflower, soybean, groundnut, sesame, linseed, castor seeds and safflower. These oilseed crops are also frequently cultivated in Pakistan. Oilseed crops are assisted by chemicals which influence the phytoremediation of these crops by enhancing the plant growth and reducing the toxicant stress. It is concluded that effective uptake of lead and nickel from contaminated soil acts as good hyperaccumulator. For metal contaminants, plants show the potential for extraction, uptake and recovery of contaminants into above-ground biomass (Anderson et al. 1998; Bañuelos et al. 1999; Huang and Cunningham 1996). The utility can likewise be investigated in crop rotation with the food crops to control biomagnification of harmful metals in the food chain.

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Chapter 32
Adaptation of Halophytes to the Gradient Conditions on the Northern Seas Coast

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Contents

Abstract Nowadays, it is relevant to study the mechanisms of rhythmic processes that are the basis of the vital activity of all organisms. From this point of view, it is necessary to study the functioning of organisms living in tidal conditions on the coastal zone of the northern seas. Twice a day, plants are flooded with water on the intertidal zone, and then they go out for drying—there is a change in the water and air environment, which is accompanied by changes in light, temperature, oxygen and CO_2 concentrations, pressure, and other parameters. These conditions have led to the formation of a unique halophyte complex of higher terrestrial plants, which have formed various adaptive mechanisms that provide the possibilities for normal existence. Three zones are distinguished on the coastal zone of the White Sea: supratidal, intertidal, and subtidal zones. For each zone, the following species was

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investigated: for supratidal—*Alopecurus arundinaceus, Atriplex glabriuscula, Carex subspathacea*; for intertidal—*Tripolium vulgare, Triglochin maritima, Salicornia europaea*, and three species of *Plantago* genus; for subtidal—*Zostera marina.* Species growing on intertidal and subtidal zones are resistant to flooding and have various structural and functional adaptations. The photosynthetic characteristics (CO_2 rate of gas exchange, transpiration, stomatal conductivity, and CO_2 concentration in the intercellular spaces of the leaves) were studied using the LCPro + portable gas analyzer at natural light intensity and air temperature. Analysis of CO_2 exchange curves by the model of Farquhar et al. showed a difference in the values of the maximum carboxylation rate (Vcmax), the electron transport rate at light saturation (Jmax), the utilization rate of triose phosphate, and a number of other parameters in plants of different zones. A comparative study of physiological parameters showed the highest functional activity in plants in the intertidal zone, which undergo daily tidal dynamics. The influence of environmental factors on the content of chlorophylls a and b and the amount of carotenoids was estimated. Evaluation of the implementation of photosynthetic activity in natural habitats in relation to the maximum values at saturating concentration of CO_2 showed that the species of higher vascular plants realize their potential in the range of 6–20% on the supratidal zone, from 10 to 70% on the intertidal zone, and from 30 to 60% on the subtidal zone. In the case of a global increase of CO_2 concentration, we can expect a significant increase in CO_2 absorption by the following halophytes: *T. vulgare, Plantago maritima, Z. marina, Al. arundinaceus,* and *At. glabriuscula,* as well as an increase in productivity of *P. maritima* and *Z. marina* among the studied halophytes of the coastal zones of the northern Holarctic seas.

Keywords Halophytes · Supratidal zone · Intertidal zone · Subtidal zone—photosynthesis · Pigments—ecological factors · Daily rhythm

Abbreviations

DM	Dry mass
ETR	Electron transport rate
Jmax	Electron transport rate at light saturation
LHC	Light-harvesting complex
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SP	Sample plot
TPU	Triose phosphate utilization
Vcmax	Maximum carboxylation rate

32.1 Introduction

The coastal zones of the northern Holarctic seas, which are influenced by the sea, are characterized by heterogeneity of habitat conditions for higher vascular plants. Combination of heterogeneity and gradients of environmental factors led to the formation of ecotopes that differ in the local flora and, accordingly, in the adaptation paths of the plants. These plants are from the ecotone zone, which consist of the following biotopes: supratidal, intertidal, and subtidal zones. All these species, according to Tolmachev and Yurtsev (1970), were originally terrestrial plants and have colonized this heterogeneous territory. The transect from the land to the sea makes it possible to observe the structural and functional changes of different species to the increasing effect of the water factor and the decreasing effect of the air environment in the dynamics of gradual changes, taking into account the tidal cycle. Salt marsh plants undergo constant changes in their habitats, controlled by geomorphology, physical, and biological processes (Bakker et al. 2005). Both biotic and abiotic factors play a fundamental role in determining the distribution of species along the gradient (Bockelmann and Neuhaus 1999) and the possibilities for their adaptation.

The literature describes in details the mechanisms of plant adaptation to habitat conditions during flooding and salinization in coastal areas. It is known (Minden et al. 2012) that various mechanisms are involved in the adaptation to salinity stress: the ability of plants to form aerenchym, water-saving tissue, exclusion of salt ions from metabolism, succulence, etc. Thus, some species form a special intracellular space (aerenchym), which connects root tissues with the aboveground part and ensures the delivery of oxygen to the roots during the flooding (Adam 1990). The aerenchym in salt marsh plants during their full flooding ensures their gas balance in coastal areas (Grigore and Toma 2017). One of the main models of salt tolerance is the accumulation of salts in vacuole and the simultaneous synthesis of compatible metabolites. Thus, the water potential in the metabolically active cell compartments (cytoplasm/organelles) is balanced and salt toxic effects are excluded. Compatible metabolites are able to maintain cell turgor and metabolism. The amount and type of compatible metabolites varies between species and habitats and depend on salt concentration in soil. Another common strategy to reduce the negative effects of salt is structural or functional changes that reduce or even eliminate the absorption of salts through the roots or release salt by special glands or hairs, as well as by dropping leaves, in case of a high salt concentration in them (Schirmer and Breckle 1982; Van Diggelen et al. 1986). Many species form large mesophyll cells, which is interpreted as a succulence (Kinzel 1982). The morphological adaptations of plants to osmotic stress include also twisting leaves or the presence of hairs on leaves to reduce absorption of solar radiation, which contribute to adaptation to water deficiency by reducing transpiration (Rozema et al. 1985). Minden et al. (2012) have verified three adaptation concepts (Scholten et al. 1987; Wright et al. 2004; Brouwer 1962) for salt marshes. This study is considering a wide range of conditions associated with the main factors on the gradient of salt marshes and mechanisms associated with adaptations to salinity and flooding, balance of the biomass ratios of individual organs, and the contribution to the formation of photosynthetic tissue mass. The authors reported a partial similarity in the response of terrestrial and salt marsh ecosystems.

Studies on the adaptation of coastal halophytes to a wide range of changing conditions consider the changes in the functional parameters of the photosynthetic apparatus of the plant along the salt marsh gradient, but only few species have been investigated. A systematic study of the adaptations of photosynthetic apparatus of salt marsh plants, depending on their biology, geography, and life form along the flooding gradient were not fully conducted. At the same time, the study of these issues will make it possible to consider the indicators of the photosynthetic apparatus activity and the degree of their realization in natural habitats, as well as to evaluate the potential possibilities under conditions of different CO_2 concentrations. The research interest in CO_2 effects on halophyte plants in the coastal zones is associated with its low availability for underwater plants and the discussion about their assimilation activity under flooding conditions, as well as the effect of increasing atmospheric CO_2 concentration on maritime plants. This study will focus on the reaction of certain dominant plant species of coastal zone in natural conditions and at elevated concentrations of CO_2 to a number of physiological and biochemical parameters.

The research aims included a comparative description of the photosynthetic apparatus parameters of halophyte plants on coastal zones of the White Sea at various levels of CO_2.

32.2 Materials and Methods

32.2.1 Research Area and Experimental Design

The present study was carried out on the west coast of the White Sea. According to the natural zoning (Ramenskaya and Shubin 1975), the research area belongs to the Loukh–Belomorsky and Kemsko–Belomorsky districts of Karelia. According to the forest growing schemes and geobotanic zoning (Atlas of the USSR 1985; Aleksandrova and Yurkovskaya 1989), the research area belongs to the taiga zone, the middle taiga subzone. According to climatic zoning (Atlas of the KASSR 1989), these territories are located in the temperate zone. The climate of the territory is temperate continental with marine features.

Due to the predominant action of the Atlantic air masses, this area is characterized by long warm winters, short cool summers, and unstable weather throughout the year. The average monthly air temperature in summer is 13–15 °C, while the average temperature in winter is 12–13 °C. The frost-free period lasts from the end of May till the end of August; the vegetation period is 109–119 days long. The investigated areas are characterized by excessive moisture; the average annual rainfall is 550–650 mm (Leontyev 1991; Romanov 1961).

The study is carried out at three points on the estuarine coast sections in the vicinity of Keret, Kolezhma, and Rastnavolok settlements (Fig. 32.1). In this study, settlement and rivers names are used as a convenient geographical reference. The study was carried out at a sufficient distance (more than 0.5 km) from the rivers confluence, where the sea influence (rather than river flow) prevails.

32.2.1.1 Keret Settlement: The Keret River Estuary (66°16′58″N 33°34′32″E)

The Keret Bay is characterized by complex hydrologic phenomena. Tidal dynamic is typical for the Bay. The average tide is about 2.0 m. The White Sea and the Keret River waters mix in the Bay and the estuary forms. Generally, the model territory is characterized by the presence of halocline, which determines a number of important

Fig. 32.1 Map of study site

ecological features for the coastal plants ecological niche formation. Within the estuary part of the Keret River, the transect is laid which has two types of substrates: sandy clay and rocky, with gentle slope and developed polydominant salt marsh communities. Water salinity at the research area was 7%.

32.2.1.2 Coast along Rastnavolok Settlement (64°32′16″N, 34°46′48″E)

Coast along Rastnavolok settlement is composed of monolithic rocks outcrops (gneissose granite) with extensive (up to 2 km wide) intertidal zone formed by muddy silty or sandy sediments that have well-developed drainage channels. These areas are occupied by halophyte communities. The research area is a transect 180 m wide on littoral, which includes a nameless stream. The study territory is silted and inhabited by small groups of seaside plants. At the water edge, there is a sandy-silty beach which is bordered with a stone block wall, 40–60 cm high. Water salinity at the research area did not exceed 10%; average tide value was 1 m.

32.2.1.3 Kolezhma Settlement: The Kolezhma River Estuary, Lopsky Island (64°22′81″N 35°93′14″E)

The study was conducted at the White Sea coast to the South–East of the river estuary and Lopsky Island. There is a well-developed boggy silted clay intertidal with numerous boulders, stretching almost 2 km from the shore in this part of the coast. Salt marsh with a mosaic of groups of seaside vegetation confined to micro-depressions and micro-elevations adjoins directly to the river estuary. Lopsky Island connected to the mainland through the Tombolo. The Tombolo is outside the daily and even syzygy tide but is subjected to direct marine influence during severe storms. There are extensive low-lying areas with clayey silty soil in the central part of the Tombolo. Here, vegetation is represented by unclosed halophyte communities, going into *Carex* and *Uncus* meadows at higher sections. The South shore of the Tombolo is clay with boulders, low, thin, with developed channels; the North one consists of less swampy sand sediments and pebbles areas. The Southern coast of the island is similar to the adjacent part of the Tombolo; the eastern and northern parts have rocky outcrops or very small sandy beaches with adjacent drainage. To the South of the river mouth and in the northern part of the Tombolo, the coast is represented by a series of small sandy beaches. The transect is laid on the Southwestern part of Lopsky Island; water salinity at the research area did not exceed 10%, and the average tide was 1.8 m.

32.2.1.4 Dolgaya Bay of Bolshoy Solovetsky Island (65°02′24″N 35°44′34″E)

The Dolgaya Bay is a partially isolated water area and extends deep into the Bolshoy Solovetsky Island. The Dolgaya Bay is connected to the open water area of the Onega Bay by two narrow straits (Northern and Southern Iron Gate). The main water area of the Dolgaya Bay is connected to the section adjacent to the straits (the Funnel), a narrow and shallow water channel (the Throat). The average salinity of surface water is 26.6% (Khaitov et al. 2013). The transect is laid on the silty-sand littoral near the fresh stream. Three sample plots (SPs) are selected on the transect: SP1 at littoral during low tide, SP2 at a depth of 30–40 cm, and SP3 at a depth of 60–70 cm. Studies are carried out at low tide. The magnitude of the low tide in the study period was 0.7–0.8 m relative to zero depth.

Model transects 10 m wide and the length from maximum storm-cast macrophytes (supratidal) coastline to the lower limit of the maximum low tide (intertidal) were laid in each geographical point of the study, capturing a small section of subtidal zone (no more than 2 m). In order to collect plant material, SPs were located at different distances from the coast and the corresponding supratidal zone of the middle part of intertidal and subtidal zones at each of the transects. SPs position relative to water edge significantly determines the strength and nature of all other factors impact, such as water and soil salinity, mechanical composition of the substrate, and aeration mode and heat factor. Typical and widespread plants of the White Sea western coast were selected on each of the SPs. They reached maximum vegetative development and they were in the flowering phase during the study.

32.2.1.5 Supratidal Zone

Supratidal zone is situated near the bedrock coast above the quadrature tide level. It is characterized by high humidity. This area can be poured during syzygy tides or strong storm winds only. Such episodic and short-term flooding has virtually no effect on vegetation. It forms a closed grass cover represented by halophyte and glycophyte species. Supratidal plants are limited by a line of storm-cast material divided into age groups on the White Sea coast. The oldest of them (over 3 years) are already well decomposed and formed the richest in mineral composition areas, inhabited by a large group of plants, including *Alopecurus arundinaceus* Poir., *Atriplex* species, *Elymus arenarius* L., and *Sonchus arvensis* L. On this SP, *Atriplex glabriuscula* Edmonds, *Carex subspathacea*, and *Alopecurus arundinaceus* were chosen for our study.

32.2.1.6 The Middle Part of the Intertidal Zone

Intertidal zone is a part of coast flooded with seawater at high tide and drained at low tide. It is located between the highest water level at high tide and the lowest one at low tide. This area is flooded with salty seawater twice a day. The species of halophytes adapted to fully submerged in water at high tide for several hours grow here. The dominant intertidal species of the western coast of the White Sea are as follows: *Plantago* species, *Triglochin maritima* L., and *Tripolium vulgare* Ness. It is possible to see here such species as *Ruppia maritima* L., *Salicornia europaea* L., and *Glaux maritima* L. *Triglochin maritima*, *Tripolium vulgare*, *Plantago maritima* L., *Plantago schrenkii* K. Koch, *Plantago subpolaris* Andrejev, and *S. europaea* L. were chosen for our study.

32.2.1.7 Subtidal Zone

The subtidal zone is located directly near the water line. It is a coastal zone, constantly covered with water. The algae grow here predominantly, but seagrass *Zostera marina* L. inhabits this zone as well. This plant is constantly submerged in water but retains the properties of the land plant. *Z. marina* was chosen as the object of the study in this area.

32.2.2 Objects of Study

The morphological description of the species is given according to Markovskaya et al. (2010), and an anatomical description is given according to Grigore et al. (2014) and own observations. The ranges of species and ecological groups for wetting are determined according to Sekretareva (2004), and ecological groups in relation to salinity are given according to Sergienko (2008).

32.2.2.1 *Atriplex glabriuscula* Edmonds (Chenopodiaceae)

At. glabriuscula Edmonds (Chenopodiaceae) is a hypoarctic, amphiatlantic species, obligate halophyte, mesophyte (Fig. 32.2). It is 20–60 cm high annual plant with creeping stem. Stems are branching freely, the branches nearly oppositely arranged. They are somewhat ridged and bluish green. The leaves are simple, lance-hastate, lanceolate, ovate-lanceolate or triangular-hastate, entire or slightly toothed, more or less succulent with salt bladders on the epidermis, the base cuneate to a short petiole, the leaf base from subcordate to obtuse. Leaves and stem are glabrous or sparsely powdery mainly on the underside and about the main veins on the upper side.

Fig. 32.2 *At. glabriuscula* Edmonds

The epidermis is monolayer. Occasionally, stomata can be found slightly sunken under the general epidermis level, located on both sides of the leaf. The epidermis has numerous salt-secreting vesicular hair that are made of two cells: a small, stalk cell and large bladder cell. It has been shown that, in the case of these vesicular cells, the concentration of sodium and chloride is higher than that from the cells of the mesophyll and from the external environment. Moreover, hairs got filled with water in the wet periods, and in dry periods they would consume their reserves. The walls of these cells, emptied and flattened, formed a coating that protected the plant against dehydration. The mesophyll is more or less homogeneous. Numerous vascular bundles are embedded in the mesophyll, all surrounded by assimilating sheath.

A. glabriuscula occurs in stony and sandy seashores (supratidal zone): in *Carex* and *Phragmites* communities, also on sandy beaches, seaweed piles, and storm-cast macrophytes.

32.2.2.2 *Alopecurus arundinaceus* Poir. (Poaceae)

Al. arundinaceus Poir. (Poaceae) is a plurizonal, Eurasian species, facultative halophyte, hygrophyte (Fig. 32.3). It is 70–110 cm high perennial plant with rhizome. The roots grow into the soil down to a depth of 1 m, but most of the roots are located in the upper horizon of the soil and form elastic turf. Stems are tufted arising from among tussocks of leaves. Stems are erect. Plants are warm grey. Leaf blades are glabrous above, scabrous below, plane, 6–40 cm long, and 8–12 mm wide, with upper sheaths somewhat inflated. Ligules are 1.3–5 mm long, truncate.

The upper epidermis has right-angled elongated cells with straight lateral walls, disposed in parallel stripes; in some of these stripes, among the epidermal cells, there are also halteriform stomata in the same row. The very long cells alternate with some very small ones on the nervures. The lower epidermis has very long cells and the stomata are more numerous on the surface unit. In cross section, the leaf has a waved band shape on the upper face. The lower epidermis has larger cells and in the upper epidermis, and groups of five to six very large bulliform cells may be observed deeply stuck into the mesophyll. The mesophyll is homogenous, of spongy type.

Species occurs in damp, loam and clay, sandy, and saline soils. The plant is spring frost resistant and flood resistant. It occurs in high marsh and also on sandy beaches in storm-cast macrophytes, outside daily flood area.

Fig. 32.3 *Al. arundinaceus* Poir.

32.2.2.3 *Carex subspathacea* Wormsk. ex Hornem. (Cyperaceae)

C. subspathacea Wormsk. ex Hornem. (Cyperaceae) is an arctic species with circum-area, obligate halophyte, hygrophyte (Fig. 32.4). It is 3–5 (20) cm high perennial plant with rhizome. Rhizome branch lengths is 1–5 cm. Culms are obtusely angled, 3–15 cm, glabrous, usually shorter than the vegetative shoots. Leaves are 3–5 (8) cm long, 2–3 mm broad at the base, ending in a pale brown, narrow tip, involute (V-shaped in cross section), densely papillose on both surfaces, margins scabrous, olive green to rusty red.

The upper epidermis has rectangular cells and stomata. In the lower epidermis, the cells are longer and stomata absent. The mesophyll is homogenous, of spongy type. In certain places, the mesophyll begins to disorganize itself, generating irregularly shaped small air-containing cavities between the vascular bundles.

Species occurs in coastal lower salt marsh areas and their sites at rocky outcrops outside the daily flood area or on the upper part of the intertidal zone (at the level of the slow rise of water) on damp, loam and clay, sandy, and saline soils. This species is located at the southern limit of distribution in the studied territory.

Fig. 32.4 *C. subspathacea* Wormsk. ex Hornem.

32.2.2.4 *Plantago maritima* L. (Plantaginaceae)

P. maritima L. (Plantaginaceae) is a highly variable species. The variability led to the segregation of few species on the White Sea coast (Sergienko 2008): *P. maritima* is boreal species, *P. schrenkii*—hypoarctic species, morphologically isolated from *P. maritima,* and *P. subpolaris* is stabilized hybrid species, which has a number of transient signs.

32.2.2.5 *Plantago maritima* L.

P. maritima L. is a boreal species with circum-area, obligate halophyte, mesophyte. It is 30 cm high perennial plant with rhizome-caudex (Fig. 32.5). Caudex grows vertically. Leaves form a dense rosette of stems. Each leaf is lanceolate, succulent, 10–25 cm long, and 2–6 cm wide, with acute apex and a smooth and distant oral tooth margin.

The epidermis has a single layer. The anizacitic, diacytic, and anomocytic stomata are present in both epidermises. The stomata are small, oval, and randomly located. Leaf is isobilateral. Mesophyll is slightly differentiated into palisade parenchyma cells and spongy parenchyma cells. The central mesophyll region contains a water-saving tissue consisting of numerous colorless cells.

Species occurs in middle salt marsh, sandy and silty coasts, and pebbles. It is dominant and codominant species of salt marsh communities in the intertidal zone.

Fig. 32.5 *P. maritima* L.

32.2.2.6 *Plantago schrenkii* C. Koch (*P. maritima subsp. borealis* (Lange) Blytt et Dahl)

Plantago schrenkii C. Koch. is a hypoarctic, European-American species, obligate halophyte, mesophyte. It is 12 cm high perennial plant with rhizome-caudex (Fig. 32.6). Caudex grows vertically, less than 8 cm long, branchy, covered with dead leaves. The leaves are succulent, linear, smooth, 3–14 cm long, and 1–5 cm wide, usually reaching half the length of the flower-bearing stem, collected in the rosette. The upper side of the leaf has violet- or reddish-brown spots.

The mesophyll is homogenous, of spongy type. The central mesophyll region contains a water-saving tissue consisting of numerous colorless cells. The epidermis has a single layer. The stomata are present in both epidermises. The stomata are small, oval, randomly located.

Species occurs in low and middle salt marsh, sandy and silty coasts, and pebbles and also at rocky outcrops above the level of daily flooding.

32.2.2.7 *Plantago subpolaris Andrejev* (Plantaginaceae)

P. subpolaris Andrejev (Plantaginaceae) is a hypoarctic European species, obligate halophyte, mesophyte. It is 25cm high perennial plant with rhizome-caudex (Fig. 32.7). The leaves are succulent, linear, smooth, 10–20 cm long, and 2–5 cm wide. A distinctive feature of the *P. subpolaris* from the *Plantago mari-*

Fig. 32.6 *P. schrenkii* C.

Fig. 32.7 *P. subpolaris* Andrejev

tima is the similar height of flower-bearing stem and leaves, when *P. maritima* has flower-bearing stem above the leaves. The anatomical structure of the leaf is similar to *P. maritima*.

Species occur in low and middle salt marsh, sandy and silty coasts, and pebbles above the level of daily flooding.

32.2.2.8 *Salicornia europaea* L. (Chenopodiaceae)

S. europaea L. (Chenopodiaceae) is a plurizonal, Eurasian-American species, obligate halophyte, mesophyte. It is 10 cm high annual plant. They grow prostrate to erect; their simple or branched stems are succulent, glabrous, and apparently jointed (Fig. 32.8). The opposite leaves are fleshy, glabrous, sessile, basally connate, and decurrent, enclosing the stem (thus forming the joints). The leaf blades are reduced to small collar-like scales with narrow scarious margin. Many species are green, but their foliage turns red in autumn. The root system is shallow, located in the surface layers of the soil (at a depth of 1–5 cm).

The epidermis is thin and naked, the cuticle is relatively thick, and the stomata are superficial, randomly located. The cortex is very thick, differentiated in chlorenchyma, parenchyma of a palisade type (with two or three layers) with tall cells, water-storage parenchyma of the meatic type with very big cells, and endodermis of the primary type. The differentiation of the cortex in external palisade parenchyma and internal water-storage parenchyma has led some authors to believe that this

Fig. 32.8 *S. europaea* L.

structure belongs to the leaf and only central cylinder belongs to the particular structure of the stem. The central cylinder is very thin and starts with a layer of extremely small pericyclic cells, with all the walls moderately thickened, but intensely lignified. Inside this central cylinder, one may notice an axial medulla (many of the cells are disorganized, generating an air-storing lacuna), six vascular bundles, and a very thick sclerenchyma ring at their periphery, where several very small phloem islands are embedded.

Species occurs in clay, silty, or sandy coasts, the lower limit of plant growth corresponding to the average height of the quadrature tide; only seagrass grows below this value. Also *S. europaea* occurs in "salt pits." Salt pits are formed in low relief on salt marshes and in seawater puddles that dry out and form a thick layer of salt. The salt content in such usually free from vegetation soils exceeds the salt content in the sea itself.

32.2.2.9 *Triglochin maritima* L. (Juncaginaceae)

Triglochin maritima L. (Juncaginaceae) is a plurizonal, circumarctic species, obligate halophyte, hygrophyte. It is 20–70 cm high perennial plant with a short-branched rhizome (Fig. 32.9). The species has stolons in the axils of rosette leaves, which form small turfs. *T. maritima* propagated by seeds and vegetatively, forming coenopopulation due to the circular growth of the rhizome (Kirillova 2013).

Fig. 32.9 *T. maritima* L.

Leaves are basal, fleshy, narrow linear, grooved, with parallel venation, covered with a layer of cuticle. The epidermis has a single layer. The epidermis has isodiametric or slightly radially elongated cells, with very thick external walls. The paracytic stomata arranged in parallel rows at a relatively equal distance from each other. The hypodermis is composed of small, isodiametric cells. The leaf has an isobilateral structure of a mesophyll. The palisade tissue has two layers with high and relatively flat cells under both epidermises. The central parenchyma of meatic type has various air-storing cavities (lacunae) with different diameters. Also, aerenchym is located in the root system and flower-bearing stems. Proline is synthesized as compatible metabolites (Gorham et al. 1980).

Species occurs in seaside clay or sandy salt marshes, at all levels of flooding (can penetrate deep into marches). Dominant and codominant in the area of daily flooding.

32.2.2.10 *Tripolium vulgare* Ness. (Asteraceae)

T. vulgare Ness. (Asteraceae) is a boreal, Eurasian species, obligate halophyte, mesoxerophyte (Fig. 32.10). It is 15–80 cm high biennial plant with short, swollen, suberect rhizome. Leaves are fleshy lanceolate with entire or toothed margins. Stems are erect, branched, hollow, and grooved. The leaves are covered with a layer of cuticle; the epidermis has a single layer. The anisocytic, tetracytic, and anomocytic stomata are present in both epidermises but are more numerous on the surface unit, in the lower epidermis. The mesophyll is slightly differentiated in a palisade tissue, with small cells on the upper side and spongy tissue on the lower side; in some places, the hypodermic cells from the lower side are also palisade-like, but they are clearly smaller. The central mesophyll region contains a water-saving tissue. The root system has aerenchym. Glycine betaine, proline, and free sugars are compatible metabolites (Gorham et al. 1980)

The species grows individually or in small groups and propagates vegetatively, less often by seeds. *T. vulgare* is dominant and subdominant in coastal communities on the intertidal zone and is rarely found on the supratidal zone. Species occurs in low and middle salt marshes.

Fig. 32.10 *T. vulgare* Ness

32.2.2.11 *Zostera marina* L. (Zosteraceae)

Z. marina L. (Zosteraceae) is a plurizonal proper oceanic species, obligate halophyte, hydatophyte (Fig. 32.11). It is perennial plant with a long rhizome. Leaves are linear and thin with parallel venation. The upper and lower sides of the leaf are identical in anatomy. The mesophyll is homogenous, of spongy type, and chloroplasts are in the epidermis. Stomata are absent. The cuticle is a thin porous layer. The central parenchyma has air-storing cavities (lacunae). The lacunae keep the leaves upright. The leaves are well-developed mechanical tissue. Sclerenchyma fiber clusters are located near the edges of the leaf (Larkum 2006; Davies et al. 2007; Smith 2008). Osmotic regulation is performed due to the accumulation of sodium ions in the large vacuoles of mesophilic cells, and in the cells of the epidermis with small vacuoles such as proline and sucrose and potassium ions (Jagels 1983; Dawes 1998; Touchette 2007).

Z. marina grows in sandy shores at subtidal zone, at salty sea shoals, and puddles on clay or sandy intertidal zone. It is dominant and codominant in the subtidal zone (Kravchenko 2007). *Z. marina* grows to a depth of 5–7 m on the White Sea (Vekhov 1992).

Fig. 32.11 *Z. marina* L.

32.2.3 Research Methods

32.2.3.1 Photosynthesis Intensity Studies

To determine the rate of photosynthesis and transpiration, 3–4 sheets of each plant were taken from each SP and immediately measured using the LCPro + portable gas analyzer from ADC BioScientific Ltd. (UK). The middle part of 2–3 leaves was placed in a sheet chamber with a clip. CO_2 rate of gas exchange, transpiration, stomatal conductivity, and CO_2 concentration in the intercellular spaces of the leaf were measured when stationary values were established. All measurements were carried out from 11 to 15 h when the plants were in the air, with a natural light intensity of 1000–1200 μmol photon m^{-2} s^{-1} and air temperature 22–25 °C. CO_2 dependencies of the photosynthesis rate were obtained by changing the CO_2 concentration in the air, supplied to the assimilation chamber of the gas analyzer, within 50–1600 μmol CO_2 $mole^{-1}$ at the saturating light intensity. Using a microprocessor gas analyzer, levels of CO_2 concentration were set and sequentially changed: 400, 200, 100, 50, 400, 800, 1200, and 1600 μmol $mole^{-1}$. The analysis of CO_2 gas exchange curves was performed according to the Farquhar model (Farquhar et al. 1980) with modification (Caemmerer and Farquhar 1982; Harley and Sharkey 1991) and using Photosyn Assistant Ver. 1.1.2 programs (Parsons and Ogston 1999). In accordance with the authors' equations, the model allows to determine maximum carboxylation rate (Vcmax), electron transport rate at light saturation (Jmax), the rate of triose phosphate utilization (TPU), and some other parameters. The study was performed in a 3–4-fold biological replication. Each value in the tables represents the arithmetic mean for 3–4 plants and standard errors.

32.2.3.2 Pigment Apparatus Study

The pigment content was determined on the leaves of five plants (from similar leaves as for photosynthesis measurements) from each of the SPs. The content of chlorophylls a and b and the sum of carotenoids were determined in samples of plant tissues extracts in accordance with standard methods (Sapozhnikov et al. 1978; Maslova et al. 1986; Lichtenthaler 1987). The sample consisted of five sections of the middle part of leaves weighing 0.2 g in 2–3-fold biological replication. Further, the samples were fixed with boiling 96% ethyl alcohol. Simultaneously, the samples were taken to determine the dry mass. Photosynthetic pigments content was calculated from their absorption spectra on SF-26 spectrophotometer (Russia) in the alcohol extract. Chlorophylls concentration (Chla, Chlb) in alcohol extract was determined spectrophotometrically (SF-26, Russia) with maximum absorption at 665 and 649 nm (Maslova et al. 1986). Carotenoids concentration was determined at 470 nm, with allowance for the correction of absorption spectra (Maslova et al. 1986). Pigment content was calculated per gram dry weight. Chlorophyll proportion in the light-harvesting complex (LHC) was calculated by assuming that almost all chlorophyll is in the LHC and the ratio of a/b chlorophylls in it is 1.2 (Maslova et al. 1986).

32.3 Results and Discussion

32.3.1 Habitat Features and Characteristics of the Photosynthetic Apparatus of the Leaves of Plants in the Supratidal Zone of the White Sea Coast

Supratidal zone is a land area where seawater falls during wind surges and storms in the form of splashes or seeping through the ground. Salinity of the White Sea ranges from 25–27% to 0% in estuary, and soil temperature can rise to 25 °C in summer. Line of storm-cast materials from various algae, seagrasses, and other deposits are formed at the border of the supratidal zone (Markovskaya et al. 2010). The width and thickness of storm-cast line depend on the coast nature, the degree of coast openness, and the storm level. Storm-cast macrophytes lines reach 10 m in width and up to 2 m in height. Their mass varies widely: from 1.5 to 5 kg/m^2 (Berger 1995; Berger 2007). On the line of storm-cast materials, there are decomposing seagrass, thallus of Fucus, Laminaria, and filamentous algae species thrown out by the waves. These conditions are associated with unique species of grass communities formed by nitrophilic species, including many annual plants (Yamlaev 2010). The following halophytes and glycophytes are found on these ecotopes remote from the water edge: *Leymus arenarius* (L.) Hochst., *Al. arundinaceus* Poir., *Festuca ovina* L., *Atriplex* species, *Carex subspataceae*, and other species. Coastal salt marshes are developed from line of storm-cast materials to the sea on the silt and sandy-silt substrates of the White Sea. They include 25–40 species of higher vascular plants and several species of green mosses. Peat accumulation processes are locally observed (the peat horizon can reach 5–30 cm) in such communities. Species occupying this ecotope are adapted to the high level of soil salinity. Specific coastal supratidal vegetation on sandy, sandy-silty, and clay substrates is represented with a wide belt of well-developed coastal marshes of various levels. Marine ecosystem originates from their low level. Obligate halophytes *At. glabriuscula* and *C. subspathacea* and facultative halophyte *Al. arundinaceus* were used in our study. Both *Al. arundinaceus* and *At. glabriuscula* grow close to line of storm-cast macrophytes on substrates richer in mineral nutrition. *C. subspathacea* occurs in low sea salt marshes on clayey, silty, or sometimes sandy substrates.

Studied halophyte species had different values of physiological parameters (Table 32.1). *C. subspathacea* had photosynthesis intensity almost two times lower (2.4 mmol) than other halophyte species and high values of the transpiration rate. It was combined with high stomata conductivity and high CO$_2$ concentration in the intercellular space (Table 32.1). *Al. arundinaceus* and *At. glabriuscula* are marked

Table 32.1 The rate of photosynthesis (Pn), transpiration (E), leaf conductance (g_s), efficiency of the use of water (Pn/E), and intercellular CO_2 concentration (C_i) by the plants: *At. glabriuscula*, *Al. arundinaceus*, *C. subspathacea* under natural habitat conditions

Species	Pn, μmol CO_2 m^{-2} s^{-1}	E, μmol H_2O m^{-2} s^{-1}	Pn/E	g_s, mol H_2O m^{-2} s^{-1}	C_i, μmol CO_2 mol^{-1}
At. glabriuscula	5.9 ± 0.2	1.4 ± 0.1	4.2 ± 0.1	60 ± 3	174 ± 8
Al. arundinaceus	6.4 ± 0.4	2.8 ± 0.1	2.3 ± 0.1	96 ± 8	246 ± 14
C. subspathacea	2.4 ± 0.2	4.0 ± 0.2	0.6 ± 0.1	130 ± 10	320 ± 12

Table 32.2 Pigment contents in leaves of *Atriplex nudicaulis*, *Al. arundinaceus*, *C. subspathacea*

	Species		
Characteristics, M ± SD	*At. glabriuscula*	*Al. arundinaceus*	*C. subspathacea*
Chl*a*, mg/gDW	2.0 ± 0.1	3.0 ± 0.2	2.2 ± 0.1
Chl*b*, mg/gDW	0.6 ± 0.1	1.5 ± 0.2	1.5 ± 0.1
Chla+*b*, mg/gDW	2.6 ± 0.2	4.5 ± 0.4	3.7 ± 0.1
Car, mg/gDW	0.6 ± 0.1	0.9 ± 0.1	0.5 ± 0.1
Chl*a*/Chl *b*	3.2 ± 0.3	2.1 ± 0.2	1.4 ± 0.1
Chl*a* + *b*/Car	4.3 ± 0.1	5.0 ± 0.5	8.3 ± 1.1
LHC, %	52.6 ± 3.3	70.7 ± 4.8	91.0 ± 3.7

with high values of photosynthesis intensity and lower values of transpiration intensity, stomatal conductivity, and CO_2 concentration.

Pigment content studies showed that *Al. arundinaceus* had the highest values of chlorophylls and carotenoids sum, whereas *At. glabriuscula* had the smallest ones (Table 32.2). Chlorophylls(a/b) ratios were very low in *C. subspathacea* (1.4). Thus, it is defined as a shadow species. High Chl/Car ratios are associated with a very low carotenoids content. It is known that chlorophyll a prevalence forms chloroplast with a predominance of stromal thylakoids and chlorophyll b forms chloroplasts with a prevalence of gran thylakoids (Lichtenthaler 1987). The low ratio of Chla/Chlb shows that most of the chlorophylls belong to the LHC of photosystems. According to the calculation (Table 32.2), *C. subspathacea* has a large LHC value per 1 reaction center, which is associated with shadow growing conditions (i.e., the need to maximize the use of light with relatively lower intensity in the plants thickets) and manifests itself in lower values of CO_2 assimilation compared with other species. All indicators of *At. glabriuscula* adaptive strategy are related mostly to a water regime: low transpiration rate, stomatal conductivity reduction, and as a result more economical water consumption comparatively with other species. Special attention should be paid to *Al. arundinaceus*—the largest plant in this group, which habitat is confined to the extreme zone or coastal emissions. High level of photosynthesis intensity, average values of transpiration rate, stomata opening, and high content of internal CO_2 are against the background of photosynthetic pigments high content. Though, judging by chlorophylls ratio and large LHC (70%), the species is experiencing some shading.

Species reaction sharply differs under conditions of increased CO_2 concentration (Table 32.3). The calculation of photosynthetic apparatus activity implementation of the studied plant species in natural growing conditions as a percentage of maximum values at saturating CO_2 concentration showed that *Al. arundinaceus* realizes the photosynthetic apparatus capabilities by 6%, *At. glabriuscula* by 6%, and *C. subspathacea* by 20%. The most positive reaction to an increase in CO_2 concentration is observed in *Al. arundinaceus* and *At. glabriuscula*. The rate of photosynthesis, respiration, and electron transport increases abruptly. In *Al. arundinaceus*, intensively high photosynthesis, high pigment content, and almost three times higher rate of TPU against the background of a high CO_2 compensation point provide this species with a successful existence in coastal areas.

At. glabriuscula is marked by an increase in carboxylation speed and efficiency but with a decrease in triose phosphates consumption against the background of a lower CO_2 compensation point value, which does not give this species a significant advantage over other plants. Under conditions of high CO_2 content, potentially high carboxylation activity of *C. subspathacea* is not fulfilled by plants due to the low efficiency of the carboxylation reaction against the background of a low CO_2 compensation point and possibly lack of macroergs at a low rate of photosynthesis light reactions. These species differ in halophilicity and geographic range. *C. subspathacea* is an arctic species, obligate halophyte, that can determine its lower values of pigment content and low photosynthesis rate. *C. subspathacea* has aerenchym allowing to store CO_2 from the air environment; and compared to their species, it has a fewer stomata located only on the leaves surface.

However, their high activity provides high values of stomatal conductivity. Other indicators are combined with this feature: low carboxylation efficiency and rate of

Table 32.3 Parameters of approximation of CO_2 response curves of the leaves of *At. nudicaulis*, *Al. arundinaceus*, and *C. subspathacea* calculated by the Farquhar et al. model

Parameters	*At. glabriuscula*	*Al. arundinaceus*	*C. subspathacea*
Maximal rate of CO_2 uptake, μmol CO_2 m^{-2} s^{-1}	95.8 ± 7.7	103 ± 13.9	12.5 ± 1.5
Rate of dark respiration in the light, μmol CO_2 m^{-2} s^{-1}	−2.5 ± 0.3	−2.8 ± 0.4	−1.4 ± 0.3
Maximal rate of carboxylation, μmol CO_2 m^{-2} s^{-1}	25.4 ± 1.6	19.1 ± 1.8	28.4 ± 1.5
Effectiveness of carboxylation, μmol CO_2 m^{-2} s^{-1} Pa^{-1}	0.30 ± 0.04	0.19 ± 0.03	0.14 ± 0.06
Rate of electron transport at light saturation, μmol m^{-2} s^{-1}	53.8 ± 4.0	46.0 ± 3.4	31.6 ± 2.1
Rates of TPU, μmolm^{-2} s^{-1}	2.4 ± 0.1	7.1 ± 0.3	2.0 ± 0.1
CO_2 compensation point, μmol CO_2 mol^{-1}	104 ± 6	136 ± 8	108 ± 4

TPU are the processes that are closely related to the energy reserve and plant productivity. Arctic origin of the species is aimed at the survival of the plant but not at achieving high productivity. *At. glabriuscula* is a hypoarctic plant, for which the conditions of middle taiga are suitable for relatively high assimilation activity. It is an obligate halophyte that is out of flooding and has a high rate of TPU against the background of low carboxylation values, which indicates optimal habitat conditions. The species has air bubbles on the leaf surface for salt disposal. *Al. arundinaceus* stands apart. It is a Eurasian species, a faculty halophyte, which does not include salt in the main metabolic processes in these habitat conditions. The higher intensity of photosynthesis, the higher pigment content; the rate of TPU almost being three times higher against the background of high CO_2 absorption ensures this species a successful existence in coastal areas.

32.3.2 Habitat Features and Characteristics of the Photosynthetic Apparatus of the Leaves of Plants in the Intertidal Zone of the White Sea Coast

Intertidal zone and its natural features are dependent on the tidal cycle. It is an ecological seabed zone that is flooded at high tide and drained at low tide. It is situated between the lowest ebb and the highest tide water levels and is covered with water and released from it twice a day. Depending on sea bottom slope and the intertidal zone amplitude, its width can be from a few meters to a few kilometers. The time between the highest and the lowest water on the White Sea is 6 h 12 min and the highest tide (Karelian and Pomeranian shores) reaches 2 m. Being a part of the seabed, it is directly adjacent to land and is influenced by desalinated and saturated organic matter continental water flow. These territories are inhabited by organisms that can withstand drainage and flooding, storm waves, salinization, and insufficient or excessive aeration. As attached organisms, plants are subjected and adapted to this negative impact. Three horizons are distinguished within intertidal zone. They are characterized by a sharp gradient of environmental factors daily fluctuations caused by regular flooding, different flooding duration, solar radiation (Armstrong et al. 1985), soil salinity, and depth of flooding (Adam 1990), which are the main limiting factors determining the distribution of species. More southern latitudes show that the competition is one of the leading factors in the upper parts of marches, and in lower ones (near the water edge) the role of stress conditions increases. Highly competitive species monopolized coastal marshes and shifted other species towards increasing stress factors (Bertness 1991). It is introduced in the “physiological and ecological amplitude” concept (Scholten et al. 1987), which justifies the species position on a gradient of conditions (Pennings and Callaway 1992; Bockelmann and Neuhaus 1999). In North high latitudes, the competitive factor is practically not discussed due to the limited species diversity in these areas and sparser vegetation even at high marshes. This concept can only be limited by stress conditions increasing towards the water edge. According to Austin (1990), species niches are generally limited by physiological resistance to extreme environmental gradients. Natural stresses on salt marshes can be associated with either the peculiarities of water factor determined by ebb and flow periodicity, immersion degree, water and soil salinity, temperature, light, spectral composition of light, and draining (Armstrong et al. 1985; Adam 1990) or with soil nutrition factor, where stress may be associated with its low level on sand and clay marshes. In the conditions of northern seaside ecotopes of this area, 15–30 species of flowering plants grow and form various combinations. The lower horizon of the intertidal zone includes areas that occupy the lowest level and are subjected to daily flooding. Characteristic species of this territory are the pioneer ones like *Eleocharis uniglumis* (Link) Schult., *S. europaea* L., and *Bolboschoenus maritimus* (L.) Palla. The most interesting of them is *S. europaea.* This species grows on edge of the distribition area and has green or red leaves. The plants taken into the study are: *T. vulgare* Ness., three *Plantago* species (subspecies), *T. maritima* L., *S. europaea* L. Studied species differed in all the parameters and united into the groups.

Plantago species are characterized with the highest values of photosynthesis intensity, transpiration intensity, high values of stomatal conductivity, and a very low concentration of intracellular CO_2. *P. maritima* showed the most activity in these parameters. Lower photosynthesis rates and higher values of intracellular CO_2 concentration were noted in *T. vulgare* and *T. maritima*. However, these species differed in other indicators: in *T. vulgare*, transpiration intensity was almost two times higher and stomatal conductance was 2.5 times higher (as in *Plantago*) comparing to *T. maritima* (Table 32.4). The lowest values of photosynthesis intensity and transpiration intensity were obtained in *S. europaea*, which is characterized by the lowest values of stomatal conductivity and, like in other species, high values of intracellular CO_2 concentration. Practically for the whole group of littoral species, an economical water exchange is characteristic, and according to the reduction degree of this indicator, they are arranged in the following order: *S. europaea, T. vulgare, P. subpolaris, T. maritima, P. schrenkii*, *P. maritima*. The mechanisms of its maintenance are primarily the presence of water-saving tissues (in *S. europaea*, it is located in the depth of the palisade parenchyma and at the base of the reduced

Table 32.4 The rate of photosynthesis (Pn), transpiration (E), leaf conductance (g_s), efficiency of the use of water (Pn/E), and intercellular CO_2 concentration (C_i) by the plants: *T. vulgare*, *T. maritima*, *S. europaea* and species of *Plantago* under natural habitat conditions

Species	Pn, μmolCO_2 m^{-2} s^{-1}	E, μmol H_2O m^{-2} s^{-1}	Pn/E	g_s, mol H_2O m^{-2} s^{-1}	C_i, μmol CO_2 mol^{-1}
S. europaea	4.0 ± 0.3	1.82 ± 0.1	2.2 ± 0.1	60 ± 5	273 ± 12
T. vulgare	11.5 ± 0.8	4.7 ± 0.2	2.4 ± 0.1	280 ± 10	298 ± 14
T. maritima	8.4 ± 1.2	2.8 ± 0.2	3.0 ± 0.1	80 ± 10	207 ± 15
P. maritima	36.9 ± 1.1	9.4 ± 0.4	4.0 ± 0.2	255 ± 6	64 ± 4
P. subpolaris	24.7 ± 1.9	9.3 ± 0.5	2.6 ± 0.1	270 ± 4	50 ± 3
P. schrenkii	25.2 ± 1.0	7.0 ± 0.3	3.6 ± 0.2	264 ± 4	30 ± 2

leaves; in *T. maritima*, there is a layer of hypodermal cells in the leaf; in *T. vulgare* and *Plantago* species, there is a tissue or a water-saving tissue in the leaf). Additional mechanisms for maintaining water balance are the magnitude of stomatal conductivity and transpiration intensity, which had low values in two species: *S. europaea* and *T. maritima*. It is known that salt ions also get into the water-saving tissue. Photosynthesis intensity is also associated with carboxylation substrate CO_2, which also gets into the body of plants through stomata. If we compare the values of photosynthesis intensity and stomatal conduction, it will turn out that these processes are generally consistent for *P. schrenkii, P. subpolaris*, and *T. maritima*. Stomatal conductivity is low for such a high value of photosynthesis intensity for *P. maritima,* and *T. vulgare* and *S. europaea* have relatively low values of photosynthesis intensity against the background of high conductivity. Participation of intracellular CO_2 reserves in these processes is not clear: in *S. europaea* and *T. vulgare*, they are very large, and photosynthesis is low.

The content of photosynthetic pigments in intertidal species is low in comparison with terrestrial species (Markovskaya and Shmakova 2017). The studied species (Table 32.5) differed but less significantly than in other indicators: the highest values of chlorophylls amount were noted in *P. maritima* (3.3 mg/g) and similar values in *T. vulgare*, *T. maritima*, *P. schrenkii*, and *P. subpolaris* (2.4–2.8 mg/g). In terms of chlorophylls ratio, the range turned out to be wider: for all *Plantago* species, the ratio about 1.6:2.2 indicates a shadow reaction; for other species, this ratio is closer to 3 or to light living conditions. The ratio of chlorophylls to carotenoids is high in almost all species. It indicates a low content of carotenoids in species of the intertidal zone and their limited participation in the photosynthetic apparatus. For all species of the intertidal, LHC values in the range from 42 to 57% are characteristic.

The study of photosynthetic apparatus activity of intertidal species in conditions of elevated CO_2 concentration showed (Table 32.6) that the species differ greatly. If we evaluate their realization degree of maximum photosynthesis intensity in natural conditions of CO_2 in the atmosphere, it turns out that *P. schrenkii* implements its photosynthetic activity by 70%, *P. subpolaris* by 54%, *P. maritima* by 44%, *T. vulgare*, *T. maritima* by 15%, and *S. europaea* only by 10%. It depends primarily on the

Table 32.5 Pigment contents in leaves of *T. vulgare*, *T. maritima*, *S. europaea*, *P. maritima, P. subpolaris*, and *P. schrenkii*

Characteristics, M ± SD	Species					
	S. europaea	*T. vulgare*	*T. maritima*	*P. maritima*	*P. subpolaris*	*P. schrenkii*
Chl*a*, mg/gDW	1.7 ± 0.1	2.0 ± 0.1	2.1 ± 0.2	2.1 ± 0.4	1.7 ± 0.3	1.6 ± 0.1
Chl*b*, mg/gDW	0.4 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	1.3 ± 0.2	0.8 ± 0.2	0.7 ± 0.0
Chla+*b*, mg/gDW	2.1 ± 0.2	2.6 ± 0.2	2.8 ± 0.1	3.3 ± 0.6	2.5 ± 0.4	2.4 ± 0.1
Car, mg/gDW	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
Chl*a*/Chl *b*	4.2 ± 0.1	3.0 ± 0.3	3.0 ± 0.6	1.6 ± 0.1	2.1 ± 0.7	2.2 ± 0.2
Chl*a* + *b*/Car	3.7 ± 0.1	4.2 ± 0.1	5.1 ± 0.9	5.9 ± 0.7	4.9 ± 0.6	4.2 ± 0.2
LHC, %	42.7 ± 0.9	55.3 ± 3.5	56.7 ± 9.3	52.2 ± 8.8	49.8 ± 8.8	41.5 ± 1.8

Table 32.6 Parameters of approximation of CO_2 response curves of the leaves of *T. vulgare, T. maritima, S. europaea, P. maritima, P. subpolaris,* and *P. schrenkii* calculated by the Farquhar et al. model

	Species					
Parameters	*S. europaea*	*T. vulgare*	*T. maritima*	*P. maritima*	*P. subpolaris*	*P. schrenkii*
Maximal rate of CO_2 uptake, μmol CO_2 m^{-2} s^{-1}	38.1 ± 4.4	76.9 ± 4.6	48.7 ± 3.4	85.0 ± 4.8	45.2 ± 7.5	36.9 ± 3.2
Rate of dark respiration in the light, μmol CO_2 m^{-2} s^{-1}	−4.1 ± 1.3	−5.8 ± 0.6	−17.2 ± 3.4	−9.8 ± 1.7	−7.4 ± 2.6	−4.2 ± 1.7
Maximal rate of carboxylation, μmol CO_2 m^{-2} s^{-1}	65.4 ± 4.9	62.6 ± 2.6	56.2 ± 9.8	123.0 ± 20.9	142.9 ± 13.8	69.5 ± 18.4
Effectiveness of carboxylation, μmol CO_2m^{-2} s^{-1} Pa^{-1}	0.63 ± 0.15	0.73 ± 0.05	1.38 ± 0.33	1.78 ± 0.24	0.53 ± 0.14	0.48 ± 0.03
Rate of electron transport at light saturation, $μmolm^{-2}s^{-}$	151.8 ± 7.7	220.2 ± 18.4	140.3 ± 14.4	428.0 ± 93.7	181.1 ± 3.4	253.8 ± 14.5
Rates of TPU, μmol m^{-2} s^{-1}	10.9 ± 1.2	14.3 ± 1.2	13.2 ± 1.8	29.3 ± 4.7	14.6 ± 1.5	15.3 ± 1.4
CO_2 compensation point, μmol CO_2 mol^{-1}	160 ± 6	118 ± 4	196 ± 9	115 ± 5	115 ± 6	98 ± 4

activity of all biochemical components and their response to an increase in the concentration of CO_2. It turned out that the most actively working in vivo *P. schrenkii* has a relatively high carboxylation rate, and in ETR it is second only after *P. maritima*; it has one of the highest rate of TPU indicating a high photosynthetic productivity, the average rate of dark respiration, and, most importantly, lower CO_2 compensation point value, which enables active uptake of CO_2 at low content compared to other species. High realization of photosynthetic activity in *P. maritima* is associated with its initially high activity exceeding other species. And for photosynthetic apparatus of *P. subpolaris*, the highest rate of RuBisCO enzyme but the low values of carboxylation efficiency are characteristic, which may be related to its oxygenase activity. Relatively high rate of phosphate utilization and low CO_2 point provide this species with higher productivity compared to other plants. Activation of RuBisCO oxygenase function may also be associated with the need to increase the stability of this species in the North conditions. The geographical range of *Plantago* species allows us to explain some of the patterns. *P. maritima* showing a high integrated activity in almost all indicators is a representative of the boreal group; the conditions in the studied areas of Karelia are optimal for such species, although several indicators show that a number of its needs are approaching the range edge conditions. The data on the high photosynthetic realization of *P. schrenkii* and *P. subpolaris* species were unexpected. They belong to the group of more northern hypoarctic species, vegetating in coastal stony ecotopes and only partially flooded for a shorter time (compared to *P. maritima*), that have been able to adapt and be highly competitive with other species.

The second group on the implementation of photosynthetic potential includes *T. vulgare* and *T. maritima*, which are completely different species in biochemical components. *T. vulgare* and *T. maritima* have both similar data on the maximum photosynthesis rate, carboxylation (slightly higher in *T. vulgare*), and rate of TPU but very significant differences in carboxylation efficiency and dark respiration rate 2–3 times higher in *T. maritima*. However, against the background of lower CO_2 absorption values, *T. vulgare* plants can work more efficiently. As these two species greatly differ biologically, it is difficult to compare them. First of all, *T. vulgare* is an annual or biennial plant, unlike the perennial *T. maritima*, which life strategy is not only related with current metabolic transformations and the reproductive organs generation, like *T. vulgare*, but with the need to create nutrients for overwintering. The distinctive feature of *T. maritima* is a high respiration intensity that indicates the need for large energy expenditures for spare metabolites. However, *T. vulgare* belongs to the boreal species of the Eurasian areas, which cannot be well adapted in the flood intertidal zone condition according to their requirements, including the temperature factor. *T. maritima* is a plurizonal species with a circumarctic areas, which is situated at the limit of its distribution. Supplying of all its organs with metabolites can be limited by CO_2 availability, especially since the species grows at the greatest depth of flooding. However, even in such conditions, it belongs to large intertidal plants. Perhaps, that is the reason for its such a complex shoot and underground organs systems, the supply of which with metabolites complicates donor–acceptor relations and only partial realization of the species under these conditions.

The study with increased CO_2 content showed a good reaction of *S. europaea* in all indicators of photochemical activity. Nevertheless, they were lower than in other species. This is a plurizonal, Eurasian-American species that is at the limit of its distribution. Plant sizes do not exceed 10 cm and its distribution along the transect is limited to coastal areas with a small but steady flood. These facts indicate limited possibilities for adaptation of the plant. The main limiting factor is the temperature that, apparently, to a greater extent determines the low pigment synthesis, which is a serious limitation of *Salicornia* photosynthetic apparatus. The plant strategy is to survive in these conditions.

32.3.3 Habitat Features and Characteristics of the Photosynthetic Apparatus of the Leaves of Plants in the Lower Intertidal and Subtidal Zones

The subtidal zone is considered to be the most complex zone of the White Sea divided into photic and aphotic zones. For higher vascular plants, only the upper part of the photic zone is of interest, the position of which is determined by the kelp belt. However, the seagrass *Z. marina* also grows in this zone. As an aquatic plant, *Z. marina* can grow permanently in the aquatic environment and reach a depth of 5–7 m in the White Sea, besides in the north it can grow in the littoral and low tide almost completely drained (Vekhov 1992). At the depth in the canopy of the leaves, the illumination can be only 1% of the spectral composition of the light and shifts to the long-wavelength part of the spectrum—blue and green light dominate, and this type of higher vascular plants adapts to such light conditions (Carty 2003).

Studied *Zostera* plants were growing at different depths in the lip of Dolgaya, Bolshoi Solovetsky Island, and three sampling plots were laid on the transect: one on the drying (intertidal) zone, SP1, and two on the subtidal zone, SP2 (depth 30–40 cm) and SP3 (depth 60–70 cm). A study of a number of photosynthesis parameters showed that *Z. marina* growing at different depths differed in the parameters of the photosynthesis light curve. So, the leaves taken from SP1 in the conditions of natural light (400–450 μmol photon m^{-2} s^{-1}) had the maximum rate of photosynthesis at PPFD saturation: 16.8–1.7 μmol CO_2 m^{-2} s^{-1}. The PPFD saturation point was observed at 109–8 μmol (photon) m^{-2} s^{-1}, and compensation point was 25–3 μmol (photon) m^{-2} s^{-1}. The rate of dark respiration, maximal rate of photosynthesis at natural CO_2 concentration in these plants was high (Table 32.7). It should be noted that, against the background of the high functional activity of the photosynthetic apparatus of plants, under these conditions, they accumulated a very low biomass (aboveground biomass was 16.3 ± 7.5 g DW m^{-2}).

In plants that were submerged at low tide (SP2), the intensity of photosynthesis decreased to 10.8 ± 1.4 μmol CO_2 m^{-2} s^{-1}, while the saturation and compensation

Table 32.7 Parameters of the curve relating Pn to PPFD in (*Z. marina* taken at different sea depths. Each value is the mean ± S.E. based of three or four determinations

	Depths for plants sampling		
Parameters	SP1 0 cm	SP2 30–40 cm	SP3 60–70 cm
Pn at PPFD saturation, μmol CO_2 m^{-2} s^{-1}	16.8 ± 1.7	10.8 ± 1.4	8.0 ± 0.8
Rate of dark respiration in the light, μmol CO_2 m^{-2} s^{-1}	−5.0 ± 0.1	−3.9 ± 0.8	−0.9 ± 0.4
Photosynthetic efficiency, μmol CO_2 $μmol^{-1}$ photon	0.25 ± 0.06	0.20 ± 0.03	0.07 ± 0.01
PPFD compensation point, μmol (photon) m^{-2} s^{-1}	25 ± 3	21 ± 2	12 ± 1
PPFD saturation point, μmol (photon) m^{-2} s^{-1}	109 ± 8	80 ± 6	120 ± 5

points shifted down to 80 ± 6 and 21 ± 2.2 μmolphotonm^{-2} s^{-1}, respectively. The intensity of respiration and photosynthesis quantum efficiency of plants of this experience did not change significantly as compared to SP1. The aboveground biomass of these plants increased 5 times, compared to SP1, and it was 102.1 ± 29.8 g DW/m^2.

A further increase in the depth of growth (SP3) slightly affected the intensity of photosynthesis (8.3 ± 0.8 μmol CO_2 m^{-2} s^{-1}). But an increase in saturation point was observed (up to 120 ± 5 μmol photon m^{-2} s^{-1}) and saturation point decreases to the lower values. These plants show a sharp decrease in respiration rate and photosynthesis quantum efficiency. The aboveground biomass of these plants significantly increases to 606.7 ± 152.3 g DW/m^2 and the proportion of generative shoots increases.

Thus, the assimilation activity of plants in natural conditions was the highest for plants that during the period of low tide were on drying (SP1), and almost all indicators of photosynthesis naturally decreased with depth of growth, as well as for respiration. However, the most significant drop in the intensity of photosynthesis was observed in plants at a depth of about 40–50 cm (SP2) and then it changed slightly. Quite unexpected result was obtained for PPFD saturation point, which increased in SP3 plants compared with SP2 plants. This analysis showed that if the greatest differences in photosynthesis parameters were obtained between the functional activity of plants on SP1 and SP2, then in terms of respiration and photosynthesis rate, more significant changes were obtained in SP3 plants growing at depth.

The measurement of pigment content showed that the highest values of pigment concentration were obtained for plants on lowest depth (SP1), decreased at SP2, and began to increase deeper at SP3 (Table 32.8). In plants at a depth, the ratio Chl/Car increases: on SP2, it depends on a relatively larger decrease of content of carotenoids and on SP3 on relatively large increase in the content of chlorophylls. LHS sizes with depth increase and the largest value is noted at 60–70 cm (SP3), which indicates the plant response to shading. A higher content of carotenoids in plants at the lowest depth (SP1) and relatively high by 30–40 cm (SP2) may indicate the contribution of yellow pigments to the protection of the photosynthetic apparatus in conditions of higher illumination.

Table 32.8 Pigment contents in leaves of *Z. marina*, growing in different depth

Depths for plants sampling	Chl*a*, mg/gDW	Chl*b*, mg/gDW	Chla+*b*, mg/gDW	Car, mg/gDW	Chl*a*/Chl*b*	Chl/Car	LHC,%
SP1 0 cm	3.35 ± 0.11	1.14 ± 0.04	4.49 ± 0.14	1.35 ± 0.05	2.94 ± 0.12	3.33 ± 0.21	56.6 ± 1.6
SP2 30–40 cm	2.20 ± 0.18	0.83 ± 0.10	3.03 ± 0.29	0.75 ± 0.07	2.66 ± 0.11	4.05 ± 0.16	60.2 ± 1.9
SP3 60–70 cm	2.64 ± 0.02	1.09 ± 0.02	3.73 ± 0.04	0.76 ± 0.01	2.41 ± 0.04	4.91 ± 0.02	64.5 ± 0.7

For a detailed elucidation of the activity of individual stages of the photosynthesis, an approach was used consisting of the analysis of CO_2 curves using the Farquhar et al. model (1980). An increase in the concentration of CO_2 led to an increase in the rate of photosynthesis in *Z. marina*, but the nature of its change remained with depth. Calculation of the implementation of the photosynthesis intensity showed that at a depth of 0 cm it is about 40%, at 30–40 cm about 60%, and at 60–70 cm about 30% of the maximum possible value. Compared to the results with the natural concentration of CO_2, the rate of photosynthesis on SP1 increased 3–4 times, in SP2 2–1.5 times, and in SP3 3–4 times. *Z. marina*, as well as all terrestrial C3 plants, shows an increase in the intensity of photosynthesis with increasing CO_2 concentration (Mokronosov 1999). With an increase in the depth of growth, the intensity of photosynthesis dropped sharply in plants at SP2 and then changed slightly in SP3 plants. The observed changes in the activity of the photosynthetic apparatus of plants with an increased concentration of CO_2 such as a decrease in the intensity of photosynthesis in samples under water (SP2 and SP3) were due to a change in the rate of the different stages of the carboxylation reaction. Thus, in plants at SP2 and SP3, similar and higher values of RuBisCO activity are noted. At the same time, in plants, the efficiency of carboxylation decreased linearly: from 5.4–0.3 at SP1 to the lowest values 0.7–0.3 at SP3. The rate of electron transport decreased in plants at SP2, but with an increase in the depth of growth at SP3 it did not change. The rate of utilization of triose phosphates at SP1 and SP2 was the same and then sharply decreased in plants at a depth at SP3.

Z. marina is an oceanic species, hydrophyte, obligate halophyte, which is in optimal conditions in the White Sea but closer to the edge of the area and, according to the literature, is capable of accumulating larger biomass (Maksimovich et al. 2004). The response to an increase in CO_2 concentration in the environment is moderate and the increase in the intensity of photosynthesis is not as high as in the other studied species, which is also associated with low compensation point compared to other investigated species. Very high values of indicators (Table 32.9) in the variant 0 cm (SP1) indicate a limitation of the development of this species to a greater extent by temperature.

However, this conclusion does not apply to the TPU (high values at all depths) and, hence, to the productivity of the species. Thus, plants growing on the border of the intertidal and subtidal zones and subjected to periodic changes in the level of illumination due to tidal dynamics form a labile photosynthetic apparatus differing in their functional characteristics.

32.4 Conclusion

Investigations have shown that, along the transect of the White Sea coast from the supratidal to the subtidal zones, there are species of higher vascular plants that have different habitat conditions associated with the gradual transition from air to water environment. The similarity of these species is the photosynthetic apparatus of the

Table 32.9 Parameters of approximation of CO_2 response curves of the leaves of *Z. marina*, calculated by the Farquhar et al. model

Parameters	Depths for plants sampling		
	SP1 0 cm	SP2 30–40 cm	SP3 60–70 cm
Maximal rate of CO_2 uptake, μmol CO_2 m^{-2} s^{-1}	43.0 ± 5.7	16.6 ± 4.2	23.9 ± 7.6
Rate of dark respiration in the light, μmol CO_2 m^{-2} s^{-1}	−18.4 ± 2.8	−5.1 ± 1.1	−2.2 ± 1.1
Maximal rate of carboxylation, μmol CO_2 m^{-2} s^{-1}	152 ± 24	399 ± 32	337 ± 24
Effectiveness of carboxylation, μmol $CO_2 m^{-2}$ s^{-1} Pa^{-1}	5.4 ± 0.3	3.3 ± 0.2	0.7 ± 0.3
Rate of electron transport at light saturation, μmol m^{-2} s^{-1}	134 ± 15.3	40.8 ± 4.6	60.4 ± 8.0
Rates of TPU, μmol m^{-2} s^{-1}	4.3 ± 0.5	5.1 ± 0.4	0.3 ± 0.1
CO_2 compensation point, μmol CO_2 mol^{-1}	62 ± 4	44 ± 3	50 ± 5

terrestrial plants that makes it possible to compare them. Studies of the rate of CO_2 gas exchange were carried out on the dominant species of three zones: supratidal (3 species), intertidal (6 species), and subtidal (1 species) zones under natural environmental conditions and at elevated CO_2 concentrations in the assimilation chamber. An analysis of ten species showed that nine of them belong to obligate halophytes and one species to facultative halophytes. Studied species have different latitudinal and longitudinal characteristics and include plurizonal, boreal, hypoarctic, and arctic representatives and have Euro-American, European, and circumarctic areas. The analysis of the main physiological parameters of plants in different zones showed that all species have a photosynthetic apparatus that is differently adapted to their habitat conditions. The main indicator for all species activity is the photosynthesis intensity, which varies greatly in plants of different zones, but the maximum values are noted for the *Plantago* species growing in the tidal cycles in the intertidal zone. This group includes three halophytes species (subspecies) with different latitudinal and longitudinal areas (Sergienko 2008). Analysis of species under conditions of elevated CO_2 concentration showed that maximum photosynthesis intensity values are achieved in different ways related to coordination of various biochemical mechanisms. The assessment of the photosynthesis intensity degree of realization in natural conditions from its maximum at elevated CO_2 concentration showed that in supratidal species this indicator ranges from 6 to 20%, in intertidal ones from 10 to 70%, and in subtidal ones from 30 to 60%. This issue is resolved for *Z. marina* in subtidal conditions and it is experimentally proved that the species has a carbon concentration mechanism (CCM) (Larkum et al. 2017). This mechanism was shown for *Z. marina*, which has a photosynthetic apparatus structure similar to terrestrial plants (Larkum 2006), is adapted to low light conditions (Starodubtseva et al. 2011), and is capable of more active CO_2 utilization with the participation of RuBisCO. There is no CO_2 deficiency for supratidal plants within its concentration in the atmosphere under natural conditions. If it is raised, the plants will be able to utilize it, but the quantitative indicators of its consumption will be different. A wide variation range in the photosynthetic apparatus activity obtained in the intertidal zone is associated primarily with the degree of CO_2 availability in plant species under flooded conditions. According to our and literature data obtained on flooding species, it has been shown that some species have an optional connection to the CCM, which can deliver CO_2 from a seawater medium to the main carboxylation center (RuBisCO). Based on indirect data in our previous studies on the *T. vulgare* photosynthesis, a hypothesis was expressed on the carbonic anhydrase activity under flooded conditions. The carbonic anhydrase provides RuBisCO with an additional substrate (CO_2) due to a facultative connection of CCM with PEP carboxylase under flooding (Markovskaya et al. 2015). The literature discusses CCM operation (Bowes et al. 2002) and the possibility of using dissolved HCO^{3-} (Maberly and Madsen 2002) or deposited CO_2 (Pedersen et al. 1995) as a source of CO_2 in most plants under flooded conditions. In modern works (Colmer and Pedersen 2008; Kurokawa et al. 2018), another mechanism that plants use when flooded is discussed: the presence of a gas film on leaves of a "hydrophobic" type. It is noted that the leaves of most salt marsh and aquatic plants are hydrophobic (Neinhuis and Barthlott 1997) and this property seems to be associated with the development of a gas film on their surface during flooding. Perhaps, *P. maritima* has leaf gas film in intertidal conditions. Currently, the LGF1 (leaf gas film) gene is discovered on rice leaves, which controls the synthesis of vegetable wax with nanostructures that can preserve a thin air film (Kurokawa et al. 2018). It can be assumed that the group with such mechanism may include *T. vulgare* and *Plantago* species.

In the case of a global increase of CO_2 concentration, a significant increase in CO_2 absorption can be expected by the following species: *T. vulgare, P. maritima, Z. marina, Al. arundinaceus,* and *At. glabriuscula,* and there is a possibility of an increase in productivity of *P. maritima* and *Z. marina* among the studied halophytes of the coastal areas of the northern Holarctic seas.

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Retraction Note to: Physiological Role of Gamma Aminobutyric Acid (GABA) in Salt Stress Tolerance

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