Rhizosphere Biology

Anita Sharma *Editor*

# Microbes and Signaling Biomolecules Against Plant Stress

## Strategies of Plant- Microbe Relationships for Better Survival

Springer

# Rhizosphere Biology

**Series Editor**

Anil Kumar Sharma
Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

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Anita Sharma
Editor

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# Preface

The ever-increasing human population is constantly putting pressure on agriculture production globally. Plants encounter various biotic and abiotic stresses during their life span which significantly affect the overall agricultural produce. Although various agrochemicals provide instant solutions to the problem and help plants to cope up with major biotic and abiotic challenges, their injudicious use also threatens the sustainability of the environment. The present state of environmental conditions demands eco-friendly approaches that help to restore environmental sustainability by using microorganisms as biofertilizers and biopesticides which can be a good alternative for the same. The plant is directly or indirectly affected by its rhizospheric microbiome, a major constituent of soil–plant interaction. A better understanding of plant microbiome, their interactions, mechanisms, and signaling pathways involved in microbe-driven stress alleviation in plants is necessary to develop such eco-friendly approaches. The content of the book revolves around the plant–microbe interaction and the mechanisms involved in stress management by both the partners. Besides, this book also focuses on communication and signaling between plant and associated microorganisms under stress. The content of the book may help in developing a better knowledge regarding cross-talk between plant and its associated microflora and may also help in developing new approaches and techniques that might help in combating the pressure of extreme environmental conditions on plants. Exploring plant–microbe communication and signaling pathways can pave a path for future research as well as rhizosphere engineering which may help in the maintenance of agricultural sustainability as well as harmony with the environment. The synergistic effect of plants and microbes in phytoremediation is also covered in the book. The book helps to uncover the knowledge of molecular (omics) and physiological aspects of the various processes in improving strategies employed by the plants under stress/extreme environmental conditions and ultimately optimizing plant tolerance to various environmental stresses that pose a major threat in agricultural productivity in an eco-friendly way by microbe-driven methods.

Pantnagar, Uttarakhand, India
Anita Sharma

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# Editor and Contributors

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**Anita Sharma** is presently working as a Professor at the Department of Microbiology, GBPUA&T, Pantnagar. She is an alumna of Pant Nagar University and obtained her M.Sc. degree in 1981 in Microbiology with a minor in Biochemistry. She has been a recipient of merit scholarships throughout her academic career. She obtained her PhD from BHU in 1990. She was awarded a two-year CSIR Fellowship for her project on Nitrogen Fixation by Cyanobacteria, after which she explored plant growth promotory rhizobacteria in a DBT-funded project at the Department of Microbiology, Pantnagar. She joined the Department of Microbiology, Gurukul Kangri University, in 1997, and took on her current position at the Department of Microbiology, Pantnagar, in 1999.

Her main research interests are in plant–microbe interaction and biodegradation; in this regard, she has worked as PI for three projects and Co-PI in more than 10 projects including one INDO SWISS project. Her most recent focus is on soil metagenomics under the influence of nanocompounds and pesticides. She has published more than 60 papers in national and international journals, book chapters, and research articles. She has also edited one book: *Microbial Biotechnology for Environmental Cleanup*. She has presented her work at various national and international conferences and is a life member of the AMI (Association of Microbiologist of India) and a member of several other societies. She has served as Coordinator of the Women Empowerment Cell, Faculty Secretary for the College of Basic Science and Humanities, and as a member of the University's Academic Council.

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# Abbreviations

| | |
|---|---|
| 2DE | Two-dimensional electrophoresis |
| AAS | Atomic absorption spectroscopy |
| ABA | Abscisic acid |
| ABC | Antigen-binding cassette |
| ACC | 1-amino-cyclopropane-1-carboxylase |
| ACCD | Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta |
| AFP | Antifreeze proteins |
| AHO | Acetonyl hydroxyoindole |
| AM | Arbuscular mycorrhizae |
| APX | Ascorbate peroxidase |
| ATAF | Arabidopsis transcription activation factor |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| AzA | Azelaic acid |
| BR | Brassinosteroid |
| BTH | Benzothiadiazole carbothioic acid S-methyl ester |
| CDF | Cumulative distribution function |
| CDPKs | Calcium-dependent protein kinases/calmodulin-like domain protein kinases |
| CMC | Critical micelle concentration |
| COR | Cold-regulated genes |
| CREs | Cis-regulatory elements |
| DA | Dehydroabietinal |
| DAPG | Diacetylphloroglucinol |
| DHAR | Dehydroascorbate reductase |
| DREB | Dehydration responsive element binding |
| EBR | Epibrassinosteroid |
| EDS | Enhanced disease susceptibility |
| EEA | European Economic Area |
| ELISA | Enzyme-linked immunosorbent assay |
| EPA | Environment protection agency |

| | |
|---|---|
| EPS | Exopolysaccharide |
| ERD | Early responsive to dehydration |
| ERM | Extraradical mycelium |
| ESI-LC-MS/MS | Electrospray ionization liquid chromatography-mass spectrometry |
| ESTs | Earlier expressed sequence tags |
| ETC | Electron transport chain |
| ETI | Effector-triggered immunity |
| FAO | Food and Agriculture Organization |
| FT-ICR-MS | Fourier-transform ion cyclotron resonance mass spectrometry |
| FTIR | Fourier-transform infrared spectroscopy |
| GC-MS | Gas chromatography-mass spectroscopy |
| GOGAT | Glutamine synthase |
| GP | Guaiacol peroxidase |
| GPx | Glutathione peroxidase |
| GR | Glutathione reductase |
| GS | Glutamine synthetase |
| GSH | Glutathione |
| GST | Glutathione-s-transferase |
| HA | Hyperaccumulator |
| HAPs | Hyperaccumulator plants |
| HCMV | Human cytomegalovirus |
| HKT | Histidine kinase transporter |
| HM | Heavy metals |
| HNE | Hydroxynonenal |
| HPLC | High-pressure liquid chromatography |
| HR | Hypersensitive response |
| HSP | Heat shock proteins |
| ICP-AES/OES | Inductively coupled plasma-atomic/optical emission spectrometry |
| ICP-MS | Inductively coupled plasma-mass spectrometry |
| ICS | Isochorismate synthase |
| INA | Isonicotinic acid |
| IPCC | Intergovernmental Panel on Climate Change |
| IPLs | Isopyruvate lyases |
| IP-MS | Immunoprecipitation-mass spectrometry |
| ISR | Induced systemic resistance |
| IST | Induced systemic tolerance |
| ITPS | International Technology Professional Solutions |
| iTRAQ | Isobaric tag for relative and absolute quantification |
| JA | Jasmonic acid |
| LC-MS | Liquid chromatography-mass spectroscopy |
| LEA Protein | Late embryogenesis abundant protein |
| LEA | Late embryogenesis abundant protein |

| | |
|---|---|
| LPO | Lipid peroxidation |
| MALDI-TOF | Matrix-assisted laser desorption/ionization time-of-flight |
| MAMPs | Microbe-associated molecular pattern |
| MAPK | Mitogen-activated protein kinase |
| MDA | Malondialdehyde |
| MDHAR | Monodehydroascorbate reductase |
| MG | Methylglyoxal |
| MT | Metallothionine |
| NAA | Neutron activation analysis |
| NAM | No apical meristem |
| NGS | Next-generation sequencing |
| NMR | Nuclear magnetic resonance |
| NPR | Nonexpressor of pathogenesis-related protein |
| NRAMP | Natural resistance-associated macrophage protein |
| PAH | Polycyclic aromatic hydrocarbon |
| PAL | Phenylalanine ammonia-lyase |
| PAMPs | Pathogen-associated molecular pattern |
| PC | Phytochelatins |
| PCBs | Polychlorinated biphenyls |
| PCD | Programmed cell death |
| PEG | Polyethylene glycol |
| PFOA | Perfluorooctanoic acid |
| PGPR | Plant growth-promoting bacteria |
| PIP | Plasma membrane intrinsic protein |
| POPs | Persistent organic pollutants |
| PPI | Protein–protein interaction |
| PR | Pathogenesis-related protein |
| PRX | Peroxiredoxin |
| PSB | Phosphate-solubilizing bacteria |
| PUFA | Polyunsaturated fatty acid |
| PYR | Pyrrolidonyl arylamidase |
| QTL | Quantitative trait locus |
| RBOH | Respiratory burst oxidase homologs |
| RISR | Rhizobacteria-induced systemic resistance |
| RLKs | Receptor-like kinases |
| RND | Reflex neurovascular dystrophy |
| ROPs | Recalcitrant organic pollutants |
| ROS | Reactive oxygen species |
| RUBISCO | Ribulose-1,5-bisphosphate carboxylase/oxygenase |
| RWC | Relative water content |
| RYMV | Rice yellow mosaic virus |
| SA | Salicylic acid |
| SAA | Systemic acquired acclimation |
| SAR | Systemic acquired resistance |

| | |
|---|---|
| SDS-PAGE | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| SNPs | Single-nucleotide polymorphisms |
| SOD | Superoxide dismutase |
| TCTP | Translationally controlled tumor protein |
| TEM | Transmission electron microscope |
| TEM-EDX | Transmission electron microscopy energy-dispersive X-ray spectroscopy |
| TF | Transcription factor |
| TMV | Tobacco mosaic virus |
| TNT | Trinitrotoluene |
| TRXh | Thioredoxin |
| VOCs | Volatile organic compound |
| XRF | X-ray fluorescence |
| ZFP | Zinc finger protein |

# Chapter 1
# Rhizospheric Engineering for Sustainable Agriculture

**Stanzin Idong and Anil Kumar Sharma**

**Abstract** The mammoth soil entails seemingly high number of microbes in order of tens of thousands of species. The intricate plant-microbiome community is of paramount significance for crop health. Understanding, predicting, and controlling the structure and function of the rhizosphere will allow us to tackle plant–microbe interactions precisely and which may act as a means to increase plant ecosystem productivity, improve the mechanism to a wide range of environmental distress, and mitigate effects of climate change by designing ecosystems for long-term soil carbon storage. Modifying the rhizosphere helps to increase beneficial microbes that increases nutrient availability and reduces biotic and abiotic stresses. It is highly connected and interactive therefore engineering needs to be cautious.

## 1.1 Introduction

For several years components of rhizosphere (plant, soil, and microbes) are studied separately. The rhizosphere is a narrow zone and gets influenced by root secretions that contain up to $10^{11}$ microbial cells per gram and more than 30,000 prokaryotes. Rhizospheric soil is mesotrophic in nature which means it is a home for many bacteria, archaea, viruses, and fungi. Due to the intimate association with microbes, plants could be considered as meta-organisms or holobionts (between the plants per se and its interacting microbiota) and the genome of the plant microbiome is sometimes referred to as the second genome of the plant. Rhizodeposition may change qualitatively and quantitatively and affect the microbial component of the plant (Hartmann et al. 2009). This can be described as the characteristic rhizosphere feedback loop that maintains the rhizosphere in a dynamic equilibrium.

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Rhizosphere has its importance in different activities such as crop production, nutrient uptake, and carbon storage. The rhizosphere ecology is badly affected due to current changes in climate and weather, which ultimately leads to decrease in crop yield (Adl 2016). Abiotic stresses such as drought, salinity, and high temperature have detrimental effect on plant growth and development (Lobell and Field 2007). Additional environmental stresses show negative impact on plant growth and development in a number of ways like disturbing hormone balance and increasing susceptibility to diseases (Ashraf 2003; Glick et al. 2007). The physical and chemical context of rhizosphere are the result of many competing and interacting processes that depend on soil type and water content, the composition of microbial communities, and the physiology of the plant itself.

All the components of the rhizosphere can be engineered or manipulated to improve plant health and productivity or to limit the effect of various stresses of biotic or abiotic origins, which is of paramount interest in the current situation of global climate change and for the need of sustainable agricultural practices. Plants can be engineered through modifying rhizosphere pH or by releasing compounds that improve nutrient availability, protect against biotic and abiotic stresses, or encourage the proliferation of beneficial microorganisms. Rhizobacteria that promote plant growth have been engineered to interfere with the synthesis of stress-induced hormones such as ethylene, which retards root growth, and by producing antibiotics against soil borne root pathogens (Ryan et al. 2009). Type of crop species and the cultivars supporting the antibiotic producing microbes are helpful in decreasing different pathogens.

In order to understand the complex interaction in the rhizosphere, new molecular tools are being employed which ensure whether the strategies to engineer the rhizosphere are safe, beneficial to productivity, and substantially improve the sustainability of agricultural systems. This approach may reduce the use of agrochemicals and improve the quality and quantity of crop plants. Some of the materials are still to be developed while others are being tested in the field. Role of phytohormones should also be considered while dealing with rhizosphere engineering.

## 1.2 How Rhizospheric Engineering Works?

### *1.2.1 Engineering the Soil*

Since ages soil engineering has been in use and helpful in changing the physical properties of the soil and leading to beneficial growth. Classical amendment practices e.g. biochar improves the water retention capacity of the soil and increases the pH of acidic soil. Another successful amendment practice is with calcium silicate that leads to a partial control of sugarcane borer pest, *Diatraea saccharalis* on rice (Sidhu et al. 2013) and plant residues for the partial control of *Rhizoctonia*. Application of the ammonium-based fertilizers tends to acidify the rhizosphere whereas nitrate-based fertilizers result in an alkaline rhizosphere. Shifts in pH can alter soil chemistry around the roots and influence the growth and composition of microbial communities of the soil.

### 1.2.2 Engineering the Plants

Plant Engineering has gained attention from over 30 years with the development of genetic engineering. Genetic engineering is an accepted technique for plant rhizospheric engineering. A large part of it deals with resistance to pathogens. Transgenic tobacco (*Nicotiana tabacum*) and potato (*Solanum tuberosum*) plants overexpressing a *Trichoderma harzianum* endochitinase gene were generated and they were tolerant to fungal pathogens such as *Alternaria alternata*, *A. solani*, *Botrytis cinerea*, or *Rhizoctonia solani* most likely because the transgenic plants were more efficient in degrading the fungal cell wall (Nion and Toyota 2015).

Several attempts have been made to change root architecture as it is the main part where absorption of minerals takes place. Root architecture is influenced by temperature, nutrient availability, and internal system of plants. These factors are subject for modification that helps in developing resistance against drought and salinity. The exudates released from the plants are known as rhizodeposition and used as another method. Many genes have been identified controlling the root exudates which can be manipulated via genetic engineering so rhizosphere can be changed. Iron uptake in dicots and non-graminaceous monocots also involves the release of organic compounds that chelate $Fe3^+$ and facilitates its reduction to $Fe2^+$ for uptake (Hinsinger et al. 2003). Manipulating efflux of $H^+$ and organic anions from roots in transgenic plants help in engineering rhizosphere.

### 1.2.3 Engineering Microbes

Many studies have been dealt with microbes that surround roots specially with PGPR. Plant growth promoting rhizobacteria (PGPR), the soil microbes that influence plant growth have been known for more than 100 years (Hartmann et al. 2009). They stimulate plant growth through various direct and indirect mechanisms. These mechanisms include nutrient solubilization, biological nitrogen fixation, induction of systemic resistance, production of plant growth regulators, organic acids, and volatile organic compounds (VOCs) as well as enzymes like 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, chitinase, and glucanase.

PGPR are used as bioinoculants for many years but now bioinoculants involve microbial community or mixture of bacterial strains. Knowing the genes/proteins/metabolites involved in PGPR–plant interactions, responsible for abiotic stress resistance may allow creating engineered plants harboring genes that prevent stress, and/or microbes that could be used to alleviate stress. *Bacillus amyloliquefaciens* strain KPS46 was investigated for its ability to activate extracellular protein elicitors for enhanced plant growth and induced systemic resistance in soybean plants.

## 1.3 Conclusion

The Scientists related to agriculture research fields have sensed rhizospheric engineering as a tool that can help to meet out demands of growing population without disturbing the ecosystem. It provides an exciting opportunity to unleash the diverse rhizosphere and fills the gap to find solutions to the problems. Unravelling the plant–microbe interaction will be important to understand and exploit the full potential of a cropping system, understanding the mechanisms behind rhizosphere priming and managing the carbon cycle in the soil under current and future climate. Molecular techniques have allowed manipulation of genes that influence rhizospheric function and development in biotechnology but raised the bars to ensure that it is safe, beneficial, and improves sustainability of the agriculture. Major obstacle that hampers development is the complexity of the rhizosphere including chemistry and biology.

Molecular techniques that allow the direct manipulation of genes influencing rhizosphere functions will be progressive in future. High throughput and "omics" techniques further make it possible to screen and analyze large and complex microbial communities in the soil. Genomics has given rise to metagenomics, an approach that gives remarkable development in mass sequencing procedures and which will enable us to explore the microbial diversity of the rhizosphere more rapidly and in greater detail. The complexity of rhizosphere chemistry and biology continues to present a great challenge for further research. The complex relationships between the structure of microbial communities and their function make attempts to predict and manipulate their ecology very difficult, and will certainly remain for several more years the touch stone of rhizosphere ecology.

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# Chapter 2

# Stress Alleviation in Plants Using SAR and ISR: Current Views on Stress Signaling Network

**Damini Maithani, Harshita Singh, and Anita Sharma**

**Abstract** Plants are armored with several defense mechanisms that are inducible in the presence of stress stimuli to protect them from pathogen attack, insect mediated herbivory or to increase systemic tolerance towards abiotic stimuli such as heat, light, cold, mechanical injuries, etc. These inducible defenses include multifaceted molecular, biochemical, and morphological changes. SAR and ISR are the two forms of induced resistance pathways in which the plant defense system is hiked up against a variety of pathogens as well as abiotic stresses. Plant defenses are usually preconditioned by previous encounter with the pathogens. Molecular and biochemical studies have revealed the elicitors mediating both types of responses. These elicitors are commercially available and commonly used in conventional agriculture practices for disease management. Research on SAR and ISR over the past few years has elaborated the understanding of the mechanism of entire process at molecular level. Use of PGPR as well as exploiting ISR and SAR machineries for disease management proves to be a promising alternative for environmentally malignant chemical pesticides. This chapter summarizes response pathways of plants under stress conditions, role of ROS (Reactive Oxygen Species), metabolic cues including hormones involved in signaling cascade, and mechanisms of systemic protection in plants.

## 2.1 Introduction

Plants follow sessile lifestyle and are usually exposed to plethora of biotic and abiotic stresses. Thus to cope up with the extreme conditions, plants have inbuilt intricate machineries that help them to minimize, avoid, or tolerate the stress conditions. To elude the effect of various biotic stresses like attack by pathogenic microorganisms, insects, or herbivores, plants are armored with complex constitutive and inducible machinery involving interplay of genes, proteins, hormones, and

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various other chemicals at molecular, biochemical, and physiological levels. Some defense pathways are continuously operating in plants for maintaining plant immunity but some are activated or induced only under certain conditions. Defense pathway in plants on attack by any pathogen, insect, or herbivore is not linear but rather a complex signaling circuit, and these complex synergistic, cooperative, and antagonistic interactions determine the output response towards the stimuli (Kunkel and Brooks 2002; Rojo et al. 2003). On exposure to biotic stress, plants use a mechanism known as Induced Systemic Resistance (ISR) to elude the consequences of stress (Schenk et al. 2012). Experimental studies conducted on diseases caused by Tobacco Mosaic virus (Ross 1961b) and *Peronospora tabacina* (Cruickshank and Mandryk 1960) on tobacco plants were landmark studies demonstrating that plants could be preconditioned against a variety of diseases. These studies articulated the concept of SAR more clearly. Later, other plants were also studied as model plants to understand SAR. Studies conducted on biological control of soil borne diseases led to the discovery of another form of resistance known as Induced Systemic Resistance (ISR) which is associated with plant growth promoting rhizobacteria (PGPR). On encountering an attack by pathogen, herbivore, or insect at local area, plants produce certain chemical compounds that minimize the effect and may also protect plant from further attack. Response not only occurs at the site of attack (local response) but also at distant parts in plants (systemic response). An enhanced defensive state is shown by plants when appropriately stimulated. Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) are such induced responses of plants when stimulated by pathogen attack that provide resistance to the host plant against subsequent challenges offered by pathogens. Plant growth promoting rhizobacteria (PGPR) are able to suppress growth of pathogen by either antagonistic activity or by inducing systemic resistance in plant against soil borne and foliar pathogens. Pathogen induced SAR response is similar to ISR response induced by rhizobacteria, as in both cases plant becomes resistant against future pathogenic challenges.

Rhizobacteria can trigger systemic resistance in plants via Salicylic Acid (SA) dependent and independent pathways. SA independent pathway, which depends upon jasmonic acid (JA) and ethylene signals, is known as ISR. Combined effect of ISR and SAR produces an extended protection to plant against a broad spectrum of pathogens as compared to ISR or SAR alone. SAR and ISR can be differentiated on the basis of nature of the elicitor as well as cascade of signaling pathway followed after induction (Choudhary et al. 2007). Induced resistance in plants is a complex signaling cascade and the mechanism has been elucidated in *Arabidopsis*. Three pathways have been proposed for induced resistance in *Arabidopsis*, out of which two pathways involve the role of PR proteins. In one pathway, pathogenesis related (PR) proteins are produced as a result of pathogen attack whereas in other, as a result of mechanical injury, wounding, or necrotic pathogens. Pathogen induced pathway involves SA whereas pathway induced by wounding relies on JA as signaling molecule. JA dependent pathway, known as ISR is used for pathway initiated by root associated bacteria. A plethora of proteins collectively known as PR proteins are involved in both salicylate and jasmonate induced pathways. These proteins are chitinases, glucanases, thaumatins, phytoalexins, and oxidative enzymes viz., peroxidases, polyphenol oxidases, etc. Third pathway evoked by non-pathogenic rhizobacteria, known as rhizobacteria induced systemic resistance (RISR) is different from abovementioned pathways as compounds like PR proteins and antimicrobials like phytoalexins do not accumulate in the absence of pathogen. Local infection elicits salicylic acid dependent signaling cascade that leads to disease resistance against various pathogens in distantly located plant parts. Plants respond to local attack by de novo synthesis of pathogenesis related proteins as chitinase, glucanase, and other defense related compounds. PGPR besides inducing ISR can also protect germinating seedlings against variety of pathogens by inducing plant vigor. They colonize emerging seedlings and provide protection to the plants against a broad spectrum of pathogens. Effectiveness of SAR and ISR against a variety of pathogens has been demonstrated through various lab and field experiments (Murphy et al. 2000; Nandakumar et al. 2001; Zehnder et al. 2001; Niranjan Raj et al. 2003). SAR is generally induced in plants when foliar and root tissues are exposed to biotic and abiotic elicitors. Signaling cascade is mediated via salicylic acid and accumulation of PR proteins whereas ISR is induced on the exposure of plant roots to PGPR and signaling is dependent on the production of jasmonic acid and ethylene (Vallad and Goodman 2004). SA-JA crosstalk works in concentration dependent manner. Low concentration of SA interacts synergistically with JA for activation of various defense related genes. At higher concentrations, an antagonistic interaction occurs between two pathways which may lead to oxidative burst and cell death (Mur et al. 2006). ROS acts as a mediator between the two pathways, in simple terms thiol signaling and redox homeostasis play crucial role in SA-JA crosstalk. Different stress pathways are interconnected and an overlap exists between their signaling cascades (Mullineaux and Baker 2010; Straus et al. 2010). High light intensity is reported to increase immunity of the plants against pathogen infection and tolerance towards oxidative stress, thus indicating cross-link between two stress pathways (Rossel et al. 2007; Mühlenbock et al. 2008). Figure 2.1a, b represents SAR and ISR mechanisms in brief.

## 2.2 Local Versus Systemic Signaling

Different types of systemic signaling exist in plants based on different stimulus triggering the response. Systemic acquired resistance (SAR) is activated in response to the pathogen attack and provide immunity to entire plant against bacterial, viral, and fungal pathogens. Systemic wound response is activated in plants due to mechanical injury caused by insect attack and provides immunity to entire plant against further insect attack. Systemic acquired acclimation (SAA), stimulated in response to a variety of abiotic stress stimuli makes entire plant tolerant to abiotic stimuli such as heat, salt, light, etc. Systemic metabolic responses are triggered due to fluctuations in the levels of metabolites (Baxter et al. 2013). Defense mechanisms can not only be activated in those tissues which are directly exposed to stress but also

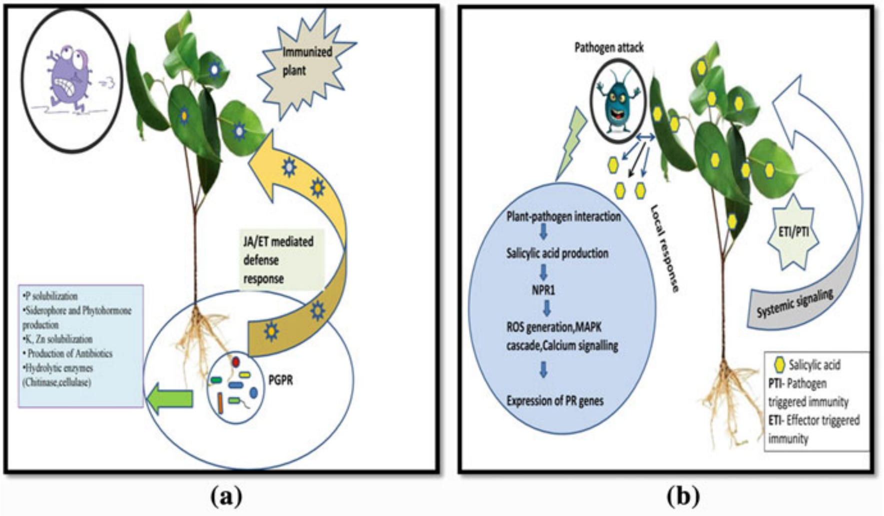

**Fig. 2.1** (**a**) Induced systemic resistance via JA/ET mediated signalling renders plant immune to herbivory, wounding, pathogen attack and increases tolerance towards abiotic stress stimui. (**b**) Plant pathogen interaction leads to production of SA that binds with NRP1 and leads to downstream signalling which results in expression of Pathogenesis Related proteins

in distant non-damaged plant parts and thus immunize entire plant systemically from further damage. Most of the ISR triggering rhizobacteria are beneficial and have plant growth promoting properties as compared to SAR, which is associated with accumulation of PRs and negatively affects plant growth (Kim et al. 2004). Long-distance signaling is a concept that shows local exposure of a plant to an environmental stimulus which induces physiological and molecular response systemically in organs that are not exposed to the stimulus (Heil and Ton 2008). It also provides a general mechanism for the coordination of responses in different plant parts and appears to be a more general sensing system for environment and/or nutrient status (Davies et al. 2005; Dempsey and Klessig 2012). Whereas, SA is required for the establishment of SAR. Grafting experiments have shown that SA is not a mobile signal (Vernooij et al. 1994). Putative phloem-mobile systemic SAR signals are reported to include methyl salicylate, glycerol-3-phosphate, lipid-transfer protein DIR1, and JA (Dempsey and Klessig 2012). ROS may also be involved in long-distance mediation of SAR responses (Alvarez et al. 1998). Recognition of virulent pathogens by a plant triggers a localized oxidative burst and cell death in hypersensitive response, which after a short delay is followed by a systemic oxidative burst and a micro-hypersensitive response. Changes in the levels of ROS in the local and systemic tissues are required for the expression of SAR. ROS appears to mediate SAR by regulating de novo synthesis of SA in systemic leaves. Accumulation of BR (Brassinosteroids), which functions as an immobile hormone induces a form of stress tolerance, independent of SA (Xia et al. 2011). BR-induced systemic resistance appears to be different from classic SAR response. Inhibition of ROS accumulation in BRs treated leaves abolished systemic responses.

## 2.3 ISR

Pathogenic attack to the plants causes great yield losses. Various disease management strategies like use of pesticides are being employed to minimize the losses. Agrochemicals are detrimental for the environment as well as for the consumers, thus use of bioresources such as plant growth promoting rhizobacteria (PGPR) is an appropriate alternative for disease management in economically important crops. PGPR compete with pathogens like fungi, bacteria, and nematodes for the substrate as well as for niche. Other mechanisms of suppression may involve secretion of allelochemicals which provide enhanced protection in plants against broad spectrum of pathogens by inducing ISR (Haas et al. 2002). Unlike SAR, ISR is stimulated by PGPR and does not involve accumulation of SA and Pathogenesis Related proteins (PR). Root exudates attract a variety of microorganisms in nutritious zone adjacent to root surface which is comprised of a complex dynamic network of synergistic and antagonistic interactions among the microorganisms. Induced systemic resistance provided by PGPR can be specific and nonspecific. ISR is a widespread mechanism of plant protection adapted by both monocotyledonous and dicotyledonous plants against a variety of pathogens. Enhanced defense capacity of the plants against pathogenic agents and development of resistance in plant tissues distantly located from site of infection are some of the hallmark features of ISR (Ryal et al. 1996). ISR is generally associated with a phenomenon known as priming, an enhanced defense response against phytopathogens. Salicylic acid or benzothiadiazole when provided to the plant cells generally induces priming and elicits a stronger response by activating defense related genes such as phenylalanine ammonia lyase. First encounter of the plant by pathogens causes accumulation of certain compounds in uninfected tissues; however, other biochemical changes are only expressed in response to further infections in plant parts where resistance is required, this phenomenon is known as priming (Conrath et al. 2001). Responses such as phytoalexin synthesis, cell wall lignification occur more strongly as compared to primary infection. Ethylene and jasmonic acid are reported to activate common responses against biotic stress in plants and their interaction pattern is determined by the type of pathogen. A group of beneficial microorganisms that colonize plant rhizosphere is able to immunize plant by providing systemic tolerance against a wide range of pathogens. Induced systemic resistance (ISR) pathway requires both ethylene and jasmonic acid (JA) (Rojo et al. 2003). Resistance induced by rhizobacteria is nonspecific and provides basal resistance to a variety of pathogens concomitantly. Jasmonic acid and ethylene during the defense response act in a sequential manner which was concluded by investigating ethylene and JA insensitive mutants (Pieterse et al. 1998). Ethylene and jasmonate converge at a point where activation of Ethylene Response Factor (ERF) gene occurs which acts downstream to regulate the response. This gene encodes a protein that is a transcriptional regulator of the genes and expressed on exposure of the plant to pathogen and thus prevents deleterious progression of disease. ERF1 gene can be activated by ethylene or jasmonate and also synergistically by both. Studies revealed that mutation in any pathway prevents the expression of ERF1 which proves that both ethylene and JA signaling pathways are required (Lorenzo et al. 2003).

When a pathogen attacks a plant it overcomes the host machinery and causes infection; this phenomenon is known as compatible response. In some cases pathogen attacks the plant but cannot establish disease successfully is known as incompatible response. During incompatible responses, pathogen elicits localized response at the site of infection. Pathogen is confined to the attacked site and prevents the infection to further escalate to other parts of the host (Heil and Bostock 2002). Generation of reactive oxygen species may sometimes lead to cell death and prevent pathogen invasion by inducing changes in the cell wall composition. Induction of de novo synthesis of antimicrobial compounds like phytoalexins and pathogenesis related proteins are some of the local responses (Kombrink and Schmelzer 2001). Following the local response, a response is also activated in distant unaffected plant parts through various signals, induced by activation and expression of genes. Phytoalexins are generally produced in local response but PR proteins are found in both local and systemic responses. PR proteins are used as ISR markers but no antimicrobial activity has been reported for them. Previously it was believed that PR proteins were absent in healthy plants and accumulate only after pathogen attack (Van Loon and Van Kammen 1970). PR proteins are reported in more than 40 plant species (Van Loon and Van Strien 1999) and are of two types. Acidic PR proteins are dominantly present in cytosol and basic PR proteins are localized in vacuoles. Some PR proteins have chitinase activity that contributes in plant protection from several fungal pathogens. Different studies suggest that plants overexpressing chitinase show decreased susceptibility to infection by fungal pathogens (Heil and Bostock 2002). However resistant phenotypes in the plants are not necessarily contributed by these proteins. Beneficial bacteria do not cause damage to host plants, thus elicitors produced by pathogens are absent in such kind of responses. Thus bacterial determinants act as elicitors in such kind of responses. Mechanism of elicitation shows similarity to that triggered by PAMPs. Both LPS and flagella of WCS 358 have been reported to elicit ISR in *Arabidopsis* plants when applied exogenously to the roots. Combination of siderophore, O-antigen, and flagella accounts for ISR response (Choudhary et al. 2007). Pathogen induced necrotic or chlorotic lesions were reduced in plants grown in soil treated with WCS 358 rhizobacterial strain (Meziane et al. 2005). Some rhizobacteria show similar response in all the plant species whereas others show narrow specificity that is an indicative of species specific recognition of the plants by bacteria. Both SAR and ISR show reduced disease severity by suppressing growth of the pathogens and reducing their colonization in induced tissues but they differ in signaling mechanism. Signaling in ISR is more complex than SAR. Defense signaling can be either SA-dependent or JA/ethylene dependent or both. SA is an important signaling molecule in local and systemic resistance in plants against pathogen but rhizobacteria mediated response is JA/ethylene dependent but phenotypically quite similar to SAR (Thomma et al. 2001). ISR was a more common response in case of necrotrophic pathogens while SAR was not much effective against *Botrytis cinerea* and *Alternaria brassicicola* (two common necrotrophic pathogens) (Thomma et al. 2000). This response is not always true and defense mechanism of different type can be effective against different pathogens attacking different plants. Arabidopsis plants when challenged with *Pseudomonas syringae* pv. tomato (Pst), SAR induced plants showed elevated expression of salicylic acid dependent PR-1. On the other hand, ISR induced plants showed accumulated mRNA of JA induced gene *vsp* to higher levels. This enhanced effect, also known as priming indicates that induced plants activate defense related gene expression to a greater extent than that of non-induced plants (Conrath et al. 2006). A study showed that *Pseudomonas chlororaphis* O6 upon colonizing cucumber root was able to trigger ISR against *Corynespora cassiicola* and the response was associated with faster and stronger accumulation of transcripts upon subsequent challenge inoculation (Kim et al. 2004).

## 2.4 SAR

Systemic acquired resistance (SAR) is a type of distinct long-distance signaling response in plants following the exposure to pathogens (Durrant and Dong 2004). It refers to a signaling pathway that is activated in plants as a response against attacking pathogen. Ross published the first systematic study on SAR in 1961 using TMV and demonstrated that resistance towards TMV infections was restricted to a prior infection. This resistance was effective against not only TMV but also against tobacco necrosis virus and certain bacterial pathogens. Ross coined the term "systemic acquired resistance" to refer to the inducible systemic resistance and "localized acquired resistance" to describe the resistance induced in inoculated leaves (Ross 1961a, b). Plants on exposure to a pathogen trigger formation of necrotic lesions which may lead to rapid cell death as a part of hypersensitive response (HR) or a symptom of disease. SAR is activated and results in the development of a broad spectrum systemic resistance (Neuenschwander et al. 1995). SAR is different from other defense responses in terms of type of genes induced as well as spectrum of protection it provides against the pathogens. This signaling cascade activates a set of genes known as SAR genes (Ryal et al. 1996). SAR appears to be distinct from pre-existing resistance mechanisms such as physical barriers or protein cross-linking and also from other inducible resistance mechanisms such as phytoalexin biosynthesis, the hypersensitive response, and ethylene-induced physiological changes. Furthermore, SAR is not related to responses induced by wounding or osmotic stress. SAR signal transduction pathway usually functions as a modulator of other disease resistance mechanisms. Tobacco has been well characterized for SAR. Other plants also show similar kind of responses. SAR is active in *Arabidopsis* against *Phytophthora parasitica*, *Pseudomonas syringa*e, and Turnip crinkle virus and the genes expressed are similar to those expressed in tobacco (Uknes et al. 1992).

Activation of SAR signaling cascade can transform a compatible plant–pathogen interaction to an incompatible one and the converse is also true (Mauch-Mani and Slusarenko 1996). A protein is characterized as SAR protein when its activity correlates with the maintenance of resistance in plants (Neuenschwander et al. 1995). Many proteins belonging to SAR consist of pathogenesis related proteins. Variety of pathogens like bacteria, virus, and fungi can attack the plants and trigger SAR response. This phenomenon is accompanied by expression of SAR genes in plants. A number of biochemical, physiological, and molecular changes are associated with plant pathogen infection such as oxidative burst, cell death, deposition of callose and lignin, synthesis of phytoalexins and other proteins (Dangl et al. 1996; Low and Merida 1996). Number of reports suggests a close correlation between SA and SAR response and exogenous application of SA can trigger SAR response. Salicylate acts as an important signaling molecule in initiating downstream signaling cascade of SAR response (Vernooij et al. 1995; Ryal et al. 1996). The most common evidence that implicates SA as a signal in SAR comes from the experiments on transgenic tobacco which expresses the enzyme salicylate hydroxylase, encoded by the *nah* G gene from *Pseudomonas putida*. This enzyme catalyzes the conversion of salicylate to catechol. Transgenic plants with this gene were found incapable of showing SAR response against a variety of pathogens (Friedrich et al. 1995). An increased SA concentration in the phloem sap was reported in plants infected with pathogens. Labelling studies also suggest accumulation of SA in uninfected tissues of host plants (Shulaev et al. 1995). Phenylalanine is converted to trans-cinnamic acid by enzyme phenylammonia lyase. Conversion of trans-cinnamic acid to salicylic acid involves an intermediary step in which benzoic acid is formed. $H_2O_2$ has been reported to act as a secondary messenger to induce SAR. The first step in the development of SAR is the recognition of pathogen infection by a plant. Once the plant reacts to the pathogen, signals are released that trigger resistance in adjacent and distant tissues. Only the compatible interactions can lead to SAR induction, thus, it is not necessary for the pathogen to induce a gene-for-gene resistance reaction. A useful approach in the study of SAR is to identify easy to measure markers that precisely correlate with the biological processes. To find appropriate markers for SAR, many c-DNA have been isolated that are expressed in uninfected tissues during SAR maintenance. Through tobacco/TMV system, steady state m-RNA levels from at least nine families of genes were induced in uninfected leaves of inoculated plants. These families are collectively referred as “SAR genes.” Abiotic agents that induce resistance, such as SA and 2,6-dichloroisonicotinic acid (Métraux et al. 1991) induce the same spectrum of SAR gene expression to levels comparable to those in SAR. Thus, expression of SAR genes tightly correlates with the onset of the resistant state. Along with reliable markers, some genes apparently have active role in developing resistance. Once the c-DNAs were isolated and encoded proteins purified, many genes were shown to have either direct antimicrobial or enzymatic activities of antimicrobial proteins. Several classes of SAR genes encode β-1,3-glucanases and chitinases (Linthorst et al. 1991). Thaumatin-like proteins were found to be active against fungi in vitro (Woloshuk et al. 1991) and their activity resides in the ability to disrupt membrane integrity, which is the basis for calling this class of proteins "permatins." A group of SAR genes, known to inhibit fungal growth is related to PR protein, known as PR-1. PR-1-related proteins from tobacco and tomato have in vitro activity against *Phytophthora infestans* (Cohen et al. 1992). Transgenic tobacco and *Brassica* seedlings that express a chitinase from bean were protected against damping-off caused by *Rhizoctonia* spp. (Broglie et al. 1991). High level expression of PR-1 in transgenic plants results in reduced infection by two Oomycete pathogens, *Peronospora tabaci* (which causes the downy mildew disease known as blue mold) and *Phytophthora parasitica* (black shank disease) (Alexander et al. 1993). Thus SAR genes encoding antimicrobial activities and expression of certain genes in transgenic plants that impart tolerance to the plants, strengthen the case that these genes play direct role in maintaining SAR. Each taxonomic group of plants has evolved its own set of SAR genes in response to evolutionary pressure from a specific spectrum of pathogens. One powerful method for the dissection of complex signal transduction process is the application of mutant analysis. SAR mutants have been isolated by screening ethylmethanesulfonate-mutagenized Arabidopsis plants for SAR gene expression (Lawton et al. 1993). Mutants with high constitutive PR-1 gene expression were called *cim* (constitutive immunity). Several *cim* mutants developed necrotic areas on the leaves even if they were grown under sterile conditions, a phenotype referred to as *lsd* (lesions stimulating disease). Defense response against pathogen attack involves various metabolic cues such as hormones, amino acids, and proteins. In local and systemic tissues, accumulation of SA, its glucoside derivative, and elevated expression of PR genes has been observed. Signals initiated in challenged tissues are translocated to distant parts via vascular bundles especially by phloem (Dempsey and Klessig 2012). Biologically active phloem-mobile chemical signals include Methyl salicylate (MeSA), glycerol-3-phosphate (G3P), azelaic acid (AzA), Jasmonic acid (JA), and pipecolic acid (Pip). These chemicals can induce systemic response when applied locally. Primary infection leads to accumulation of G3P and Aza which are not directly involved in SA biosynthesis and accumulation but precondition the plant for SA accumulation in response to secondary infection by the pathogen (Dempsey and Klessig 2012; Kachroo and Robin 2013). Dehydroabietinal (DA) and Pip are other two mobile SAR signals. Pip, a potential SAR signal is reported to induce its own biosynthesis and activate SAR by regulating SA accumulation in plants infected with *Pseudomonas syringae* pv. *Maculicola*. Similarly DA is reported to elevate level of SA, PR-1, SA biosynthesis enzymes and induce resistance to a variety of pathogens in tomato, *Arabidopsis*, and tobacco (Dempsey and Klessig 2012).

It is proposed that $H_2O_2$ acts as a second messenger in SAR signaling. A SA binding protein, identified as catalase inhibited catalase activity of this protein leading to elevated levels of $H_2O_2$ which further caused induction of PR-1 gene expression and was postulated to induce SAR (Chen et al. 1993, 1995). For $H_2O_2$ to function as a signaling agent of SA, $H_2O_2$ levels should be high in uninfected leaves of tobacco plants during SAR activation. This was tested by inoculating tobacco leaves with TMV and monitoring the accumulation of $H_2O_2$, PR-1 m-RNA, and the establishment of SAR. In the uninfected leaves of inoculated plants, SAR gene expression and establishment of SAR did not correlate with an increase in $H_2O_2$

levels. Apparently, induction of PR-1 expression by $H_2O_2$ was directly tested by infiltration of tobacco with $H_2O_2$. Substantial PR-1 m-RNA accumulation resulted after infiltration of 1M $H_2O_2$, a concentration that also caused severe tissue damage. However, high concentrations of $H_2O_2$ were found to induce SA synthesis in tobacco (Leon et al. 1995; Neuenschwander et al. 1995) and *Arabidopsis* (Summermatter et al. 1995), suggesting that $H_2O_2$ is not a second messenger of SA in the signal cascade leading to establishment of SAR.

## 2.5 Hormones and Plant Defense

Plant hormones play important role in regulating developmental processes and signaling network involved in plant responses to a wide range of biotic and abiotic stresses. Significant progress has been made in identifying the key components and understanding the role of salicylic acid (SA), jasmonates (JA), and ethylene (ET) in plant responses to biotic stresses. Recent studies indicate that hormones such as abscisic acid (ABA), auxin, gibberellic acid (GA), cytokinin (CK), brassino steroids (BR), and peptide hormones are also implicated in plant defense signaling pathways but their role in plant defense is not well studied. Salicylic acid (SA) is involved in the defense response to biotrophic and hemibiotrophic pathogens, while jasmonate (JA) and ethylene (ET) are the primary hormones involved in the response to necrotrophic pathogens (Neu et al. 2019). Confrontation of the plants to disease causing pathogens triggers plants to respond via activating complex cascades of signaling pathways that provide the plant with local and in some cases systemic immunity. Plant hormones are mighty chemical compounds that are an inevitable part of growth and development processes in plants. These molecules can show dramatic effect at low concentrations (Kucera et al. 2005). A set of plant factors including plant hormones (auxin, abscisic acid (ABA), ethylene, gibberellins, cytokinins, salicylic acid (SA), strigolactones, brassinosteroids (BRs), and nitrous/nitric oxide) are essential to make the plant tolerate the stress (Miransari 2016). In a study conducted by Hamayun et al. (2010), levels of ABA, jasmonate, and salicylate were high under drought stress. ABA, majorly involved in salinity and drought stress is known to play an important role in plant growth and development. It maintains dormancy, prevents precocious seed germination, regulates stomatal activity, leaf senescence, leaf hydraulic activity by regulating aquaporin activity (Tuteja 2007; Li et al. 2014). Three plant-specific hormones (salicylic acid (SA), jasmonic acid (JA), and ethylene (E)) are major endogenous signals involved in communicating the presence of an infection and triggering the defense responses in plants. Abscisic acid (ABA) and auxins may play a role in defense against pathogens. ABA negatively regulates SA-dependent resistance (Audenaert et al. 2002). However, involvement of ABA and auxins in pathogen defense is much less documented. Range of pathogen infections signaled through SA and JA or E are partially exclusive. SA has been found linked with the response towards the infection by viruses like tobacco mosaic virus (TMV) and turnip crinkle virus, as well as by biotrophic bacteria and fungi such as *Pseudomonas*, *Peronospora*, *Erysiphe*. Biotrophic pathogens require nutrients from live plant tissues. Hypersensitive responses in plants as a result of pathogen–plant interaction may lead to cell death and deprive pathogen from live host tissues (Dewdney et al. 2000; Kachroo et al. 2000). Contrary to this, HR-induced cell death proves to be beneficial for infection by necrotrophic pathogens (Govrin and Levine 2000). Other defense mechanisms against necrotrophic pathogens, evolved in plants are activated in many cases by JA and E signaling pathways. It is shown that JA and Ethylene signaling is required to develop resistance against the pathogens (mainly necrotrophic or saprophytic) such as *Alternaria*, *Botrytis*, *Septoria*, *Pythium*, *Erwinia*, *Plectosphaerella* (Berrocal-Lobo et al. 2002; Dıaz et al. 2002). However, generalizing the concept that defense against biotrophic pathogens is always attributed to SA signaling and against necrotrophic pathogens to JA and E signaling would be an oversimplification. JA and E signaling pathways have been reported in defense mechanisms against the biotrophic pathogens like *Erysiphe cichoracearum*, *Erysiphe orontii*, *Oidium lycopersicum*, *and Pseudomonas syringae* (Ellis and Turner 2001; Ellis et al. 2002). Similarly, reports exist in which SA is involved in resistance against the necrotrophic fungi *Botrytis cinerea* and *Plectosphaerella cucumerina* (Berrocal-Lobo et al. 2002; Audenaert et al. 2002; Dıaz et al. 2002). Moreover, not only these hormones have been shown to participate in activating parallel defense mechanisms against the same pathogen, but also many events of cross-talk among the SA, E, and JA signaling pathways have been reported and were shown to be significant in determining the resistance to pathogens. Recent studies demonstrated the involvement of brassinosteroids (BRs) in SAR and SAA in cucumber (Xia et al. 2011; Li et al. 2013). Although BRs are not directly involved in long-distance signaling but they affect other signals such as auxins and polyamines (Li et al. 2013). Local application of 24-epibrassinosteroid (EBR) can induce SAR and SAA to oxidative stress accompanied by local and systemic expression of known defense/acclimatory genes such as APX and catalase (Li et al. 2013).

## 2.6 Salicylic Acid

Salicylic acid (SA), a phenolic plant growth regulator is found to play an important role in flowering, leaf senescence, seed germination, and other physiological and developmental processes besides regulating activation of defense responses in plants under a variety of stress conditions to which plant is exposed to pathogens in particular. On exposure to pathogen, SA is synthesized in the chloroplasts via isochorismate pathway where ICS1 (isochorismate synthase 1) is the prime enzyme required for its biosynthesis. Salicylic acid turns on the transcriptional reprogramming by activating TGA transcription factors via NPR1 (non-expressor of pathogenesis-related genes 1) co-activators. Two models have been proposed for perception of SA. In one model, NPR3 and NPR4 perceive SA and then regulate NPR1 accumulation. In the second model NPR1 has itself been proposed to perceive SA and undergoes a conformational change which leads to increased transcription.

Besides the direct SA binding, NPR1 is also regulated by SA-mediated redox changes and phosphorylation. Emerging evidences show that pathogen virulence effectors target SA signaling and strengthen the importance of SA-mediated immunity (Seyfferth and Tsuda 2014). Salicylic acid at low concentration leads to generation of reactive oxygen species at non-toxic concentrations where these molecules act as secondary messengers during plant defense whereas high doses of salicylic acid lead to accumulation of reactive oxygen species at concentrations lethal for the survival of plants (Miura and Tada 2014). Hormone is able to enhance plant's survival under salinity as well as heavy metal stress by regulating redox homeostasis (Jibran et al. 2013). In plants SA is synthesized mainly via two pathways: Isochorismate (IC) and phenylalanine ammonia-lyase (PAL). Chorismate, an end product of shikimate pathway is used as a precursor of SA biosynthesis using critical enzymes, IC synthase (ICS) and PAL (Dempsey and Klessig 2012). Homologs of *ICS* and *PAL* genes are present in a variety of plants including *Arabidopsis*, tobacco, tomato, populus, sunflower, and pepper. Complete loss of SA accumulation was observed in *Arabidopsis* mutants showing dysfunctioning of ICS1 induced by pathogen (Cochrane et al. 2004; Uppalapati et al. 2007; Catinot et al. 2008; Yuan et al. 2009; Sadeghi et al. 2013; Kim and Hwang 2014). Similarly, *Arabidopsis* mutants with reduced PAL activity show reduced SA accumulation in comparison to wild type after pathogen attack (Chao et al. 2010). However, in higher plants, SA is majorly synthesized via IC pathway in chloroplast to provide immunity. ICS enzyme converts chorismate to isochorismate that is ultimately converted to SA (Strawn et al. 2007; Garcion and Métraux 2008; Dempsey and Klessig 2012). Isopyruvate lyases (IPLs) convert isochorismate to SA. Plants do not contain any gene encoding such enzyme or homologue. However the mechanism in plants for SA biosynthesis is more complex than that of bacteria (Dempsey and Klessig 2012). Export of SA from chloroplasts is mediated by EDS5 (Enhanced Disease Susceptibility 5) which has been confirmed by mutant analysis (Ishihara et al. 2008; Serrano et al. 2013). SA is synthesized from trans-cinnamic acid, via the intermediates ortho-coumaric acid or benzoic acid. Such pathway provides a link between pathogen induction of phenyl propanoid biosynthesis and SAR signal production. SA is a key player in immunizing plant against a variety of pathogens systemically and establishing SAR (Wang et al. 2006; Fu and Dong 2013). SA biosynthesis is tightly regulated as its elevated concentrations on constitutive expression can be harmful for the plant. It is often associated with stunted plant growth, resulting in reduction of plant fitness (Ishihara et al. 2008; Chandra et al. 2014). The biosynthetic pathway of SA appears to begin with the conversion of phenylalanine to trans-cinnamic acid catalyzed by PAL. The conversion of trans-cinnamic acid into SA proceeds via chain shortening to produce benzoic acid (BA) followed by hydroxylation at the C2 position to derive SA (Yalpani et al. 1993). The final step in SA synthesis is the conversion of BA to SA which is catalyzed by cytochrome P450 mono-oxygenase. Benzoic acid 2-hydroxylase is induced by either pathogen infection or by application of exogenous BA. Activity of benzoic acid 2-hydroxylase is induced by tenfold after pathogen infection and blocked by an inhibitor of protein synthesis (Leon et al. 1993). Once synthesized, the fate of SA in the cell is not clear. Considering the important role of SA as a signaling component, its biosynthesis and catabolism need to be further investigated. BA and SA can be conjugated to glucose which regulates SA level. In healthy tobacco plants, a large pool of conjugated BA is available which decreases transiently after pathogen infection. Decrease in conjugated BA level is correlated with an increase in free BA and SA (Yalpani et al. 1993).

Plant immunity can be characterized as pattern triggered immunity (PTI). Immunity triggered by recognition of patterns in pathogens specially microbes is known as microbe associated molecular pattern (MAMPs) and effector-triggered immunity (ETI). SA plays a key role in signaling associated with pathogen attack. Elicitors triggering the pathway may involve perception of PAMPs (Pathogen Associated Molecular patterns) or effector molecules produced by pathogens. Process of induction of PAMP or effector-triggered immunity involves certain molecular and biochemical events including expression of pathogen resistance, SA biosynthesis genes, production of toxic species like reactive oxygen species and NO, elevated levels of cytosolic calcium, and activation of MAPK cascades. PAMP triggered immunity induces a basal level of response whereas effector molecules may lead to hypersensitivity. Accumulation of ROS and SA influences each other in a positive manner. A loop shows that SA increases ROS accumulation and ROS in turn increases SA accumulation (Xia et al. 2015). Recognition of MAMPs is followed by a cascade of events such as calcium ion signaling, ROS generation, and activation of mitogen-activated protein kinases (MAPKs) which ultimately lead to SA production (Tsuda and Katagiri 2010; Macho and Zipfel 2014). Some pathogens can bypass plants PTI by secreting effector molecules via secretion systems such as pathogen *Pseudomonas syringae* pv. Tomato DC3000 can suppress PTI in *Arabidopsis* and tomato (Xin and He 2013). However, a second check is posed by plant to regain resistance. Plants possess intracellular receptors that recognize the effector molecules and generate a second layer of defense termed as ETI. Activation of ETI also induces SA accumulation and MAPK activation, which are also important for resistance against pathogens during ETI (Bonardi and Dangl 2012; Jacobs et al. 2013; Tsuda et al. 2013).

Salicylic acid pathway is also interlinked to other hormones like jasmonic acid (JA), ethylene, and abscisic acid. For example, JA and ethylene signaling negatively regulates SA biosynthesis at the transcriptional level (Zheng et al. 2012; Derksen et al. 2013). Salicylic acid perceiving receptors belong to a family of proteins named NPR (non-expressor of pathogenesis related genes). In *Arabidopsis*, three proteins (NPR1, NPR3, and NPR4) are bonafide SA receptors. NPR1 is a necessary protein for SA action in plants. It is an ankyrin repeat family protein comprised of BTB/POZ domain, nucleus localization signal, and an ankyrin repeat domain. It is a transcriptional co-activator. Redox change causes reduction of NPR1 oligomer in cytoplasm to monomers in the presence of SA. Monomers are then translocated to nucleus where they interact with transcriptional factors to enhance gene expression of PR proteins. Mutations in NPR1 result in complete loss of SA-mediated signaling pathway and increased susceptibility of plants towards hemibiotrophic pathogens (Dong 2004; Fu et al. 2012; Wu et al. 2012). SA-induced molecular and biochemical changes ultimately lead to activation of a downstream protein known as NPR1 which undergoes a change from oligomeric to monomeric state. NPR1 proteins then migrate towards nucleus where they interact with various transcription factors that are responsible for regulating PR gene expression. Further oligomerization of NPR1 is done by S-nitrosylation of protein which is then sequestered in cytoplasm (Mou et al. 2003; Tada et al. 2008). NPR4 has higher affinity for SA and NPR3 has low affinity in comparison to NPR1 which functions at intermediate levels of SA. ROS generation is involved in defense responses as well as cell death pathways in plants. It is not only induced by SA but also by NADPH oxidase. NADPH oxidase is involved in extracellular ROS generation whereas SA-mediated accumulation of ROS occurs in intracellular organelle such as mitochondria, chloroplast, and peroxisomes (Torres and Dangl 2005; Khokon et al. 2011). When SA signal is unavailable, NPR1 exists as an oligomer. SA perception triggers reduction of intermolecular bridges through TRXh3 and TRXh5 (Thioredoxins) causing monomerization of NPR1 followed by its transport into nucleus where it gets accumulated and regulate gene expression (Tada et al. 2008). Phosphorylation of NPR1 is also triggered in the presence of SA that leads to recruitment of ubiquitin ligase and proteasome mediates degradation of NPR1 then interact with transcription factors belonging to leucine zipper family of transcription factors, TGA (Gatz 2013). TGA2, TGA5, TGA6 repress PR1 transcription in the absence of SA and vice versa (Zhang et al. 2003).

## 2.7 Jasmonic Acid

Jasmonic acid (3-oxo-2-20-cis-pentenyl-cyclopentane-1-acetic acid) is an endogenous growth-regulating compound in higher plants. JA methyl ester and isoleucine conjugate (JA-Ile) are derivatives of a class of fatty acids and are collectively known as jasmonates (JAs). These compounds are vital for the plants and involved in the regulation of many physiological processes in plant growth and development. They can induce stomatal opening, transport of organic compounds like glucose, inhibit Rubisco biosynthesis, and enhance the uptake of nitrogen and phosphorus. As a signaling molecule, JAs can mediate responses against environmental stresses by inducing a series of gene expression. JA and its derivatives and the plant signaling molecules are closely related to plant defense and provide resistance against microbial pathogens, herbivorous insects, wounding, drought, salt stress, and low temperature. JAs and salicylic acid (SA)-mediated signaling pathways are mainly related to plant responses to biotic and abiotic stresses posed by environment. Response of plant towards mechanical injury and wounding is mediated via jasmonic acid pathway. Perception of signal during mechanical stress is mediated by stretch activated calcium ion channels and receptors that may lead to activation of downstream cascade of the events including Rho-GTPase, MAPK cascade, and activation of JA biosynthesis (Wolf et al. 2012). Studies on JA biosynthesis have been conducted extensively in tomato and *Arabidopsis*. Three pathways for JA biosynthesis exist in *Arabidopsis* and all of them require three reaction sites: the chloroplast, peroxisome, and cytoplasm. In the cytoplasm, JA is metabolized into different structures by various chemical reactions. These are Methyl jasmonate, cis-jasmone, and 12-hydroxyjasmonic acid. Initially, JA was identified as a stress-related hormone. Insect and pathogen attack generates oligouronides and polypeptides that initiate a signal transduction pathway involving synthesis of Jasmonic acid from linolenic acid (the octadecanoid pathway) which mediates the induction of defensive genes in plants. Hexadecane pathway starts from hexadecatrienoic acid. Cumulative evidences support a model for the activation of defensive genes in plants in response to insect and pathogen attacks in which various elicitors generated at the attack sites activate octadecanoid pathway via different recognition events to induce the expression of defensive genes in local and distal tissues of the plants (Doares et al. 1995).

Mechanical damage in plants can start accumulation of JA and its derivatives at the site of damage which activates signaling cascade and expression of defense response genes as a part of local response. Transmission of JAs to other parts of plant occurs via vascular bundles or airborne diffusion. After induction of JA biosynthesis, JAs are systemically transported via vascular bundles to distant parts of the plant (Thorpe et al. 2007). Some reports suggest that JAs are simultaneously resynthesized during transport (Heil and Ton 2008). Accumulation of JA within 15 min of mechanical injury has been reported in tomato plants by Malone (1996). JA cannot easily penetrate cell membrane but its derivative, MeJA is a volatile compound and can penetrate membrane easily. This compound can diffuse through airborne transmission to distant leaves in the same plant as well as in adjacent plants (Farmer and Ryan 1990). Jasmonic acid signaling involves interplay of various proteins and transcription factors (Li et al. 2017). Receptor for JA in JA signaling cascade that mediates its subcellular distribution is a high affinity ABC transporter (AtJAT1/AtABCG16) which is located on nuclear and on plasma membrane. It mediates JA transport across plasma membrane and bioactive isoleucine derivative (JA-Ile) across nuclear membrane. Higher JA concentration causes reduction in its intracellular concentration that in turn desensitizes JA signal. JA signal is then activated in other cells via transport across apoplast. When plant is under stress, nuclear AtJAT1/AtABCG16 transporter causes quick transport of JA-Ile into nucleus to regulate defense response. JA-Ile after entering nucleus through nuclear JAT1 transporter interacts with various MYC transcription factors by binding to JAZ, a repressor protein that blocks transcription of JA responsive genes (Chini et al. 2007). JAZ is then targeted for degradation via 26S proteasome complex. JAZ protein has two conserved domains namely Jas and ZIM. JAZ interacts with MYC2 with the help of ZIM domain and COI1 via Jas domain (Melotto et al. 2008; Chini et al. 2009). COI1 protein encodes an F-box protein, associated with SKP1 and Cullin to form a complex $\mathrm{SCF}^{\mathrm{COI1}}$ which tags the repressor with ubiquitin for degradation (Zhai et al. 2015). JAZ repressor blocks JA signaling cascade and thus JAZ degradation is the crucial step to activate JA pathway. COI1 and JAZ are proposed to be coreceptors of JA (Sheard et al. 2010). JAZ sequestered transcription factors are released after JAZ degradation and lead to activation of gene expression. Studies suggest that MYB transcription factors can also be released via JA-Ile induced JAZ degradation. Transcription factors such as NAC, ERF, and WRKY are the part of JA signaling. JA signaling also activates MAPK cascade, calcium channel, and many other physiological and biochemical processes that interact with plant growth regulators like ethylene, SA, ABA (Santner and Estelle 2009; Li et al. 2017). Enhancing UVB exposure has been reported to induce JA biosynthesis in *Nicotiana* and *Brassica* genus (Mewis et al. 2012; Svyatyna and Riemann 2012). JA pathway is also induced during low temperature adaptation in plants by activating cold responsive genes (Zheng et al. 2012). JA signaling under drought has also been reported in *A. thaliana* and *Citrus*. MeJA significantly regulates stomatal closure to reduce water loss under drought stress (Evans 2003; De Ollas et al. 2013). Under salt stress, tomato, potato, and Arabidopsis plants showed significantly high JA concentration. JA concentration in salt sensitive plant varieties was significantly high than the tolerant varieties. Exogenous JA application has been reported to enhance salt tolerance in plants (Ruan et al. 2019). Higher $CO_2$ concentration leads to increased JA release into environment in lima bean (Ballhorn et al. 2011). Ozone also enhances level of endogenous JAs in plants (Ruan et al. 2019). Proteomics study on *A. thaliana* treated with Me-JA expressed 186 proteins which are collectively involved in physiological processes such as photosynthesis, metabolism of carbohydrates and hormones, and defense under stress, etc. (Chen et al. 2011). In tomato, a polypeptide derived from hydrolysis of precursor protein named systemin having 18 amino acids was identified that accumulated in response to mechanical damage caused by insect herbivory, which activated JA signaling cascade (Pearce et al. 1991). A similar polypeptide, (AtPEP1) from *Arabidopsis thaliana*, also activates JA signaling pathway in response to mechanical damage as well as pathogen attack (Yamaguchi et al. 2010). Wounding response pathway also involves ATP that generates ROS via activation of NADPH oxidase along with induction of JA biosynthesis (Song et al. 2006). An interplay of calcium ions and ROS help in activation of MAPK pathway in early response. However silencing of MAPK pathway and reduced ROS generation inhibit expression of late wound related genes but JA synthesis is not affected (Xia et al. 2004).

## 2.8 ROS and Plant Systemic Responses

Reactive oxygen species (ROS) are unavoidable products of aerobic metabolism. ROS signaling network is highly conserved among aerobic organisms and controls a broad range of biological processes such as growth, development, and responses to biotic and/or abiotic stimuli (Mittler et al. 2011; Finka et al. 2012). Increased ROS concentration can cause oxidative damage to membranes as well as other cellular macromolecules and leads to oxidative stress in the cell. ROS-scavenging enzymatic and non-enzymatic pathways maintain redox homeostasis in the cell. Proteins like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PRX) and antioxidants such as ascorbic acid, glutathione (GSH) are some of the scavengers of ROS inside the cell (Mittler 2002). A balance between the process of ROS generation and its sequestration exists under normal conditions; however, this balance is disturbed under stress conditions. Tissues when exposed to stress stimuli, $Ca^{2+}$ influx inside the cell is marked as an early event in the signaling cascade followed by production of ROS along with other secondary messengers which may lead to activation of signaling cascades like MAPK pathways and protein phosphorylation that further amplify the response and decode the downstream response pathways that ultimately lead to transcriptional activation of genes involved in plant development as well as stress tolerance. ROS are well known toxic species and immense data is available regarding their toxicity and scavenging mechanisms in the cells. ROS also act as signaling molecules at non-toxic level where a balance is maintained between ROS generation and its sequestration. Activation of ROS signaling cascade occurs within few seconds or minutes on exposure to stress stimuli. ROS production in tobacco in heat stress has been reported to be triggered within 3 min of exposure. Activation of acclamatory responses of the plants to high light takes place within 5–20 min not only in local tissues but also in distant plant parts (Königshofer et al. 2008). Wounding responses in *Arabidopsis* are shown by elevated JA levels in local and in distant tissues within 30 s to 5 min after damage (Koo et al. 2009). In response to wounding, RBOHDs initiate a systemic signal that propagates at the rate of 8.4 cm/min (Miller et al. 2009). ROS accumulation plays a key role in hormone mediated signaling in plants when exposed to external stimuli (including stress) or during normal growth and development. Accumulation of these reactive species regulates synthesis, localization, translocation of hormone. Although early research involving ROS metabolism has focused on the potential toxicity of ROS and different ROS-scavenging mechanisms, but recent studies have focused on the role of ROS as signaling molecules. To utilize ROS as signaling molecules, non-toxic levels must be maintained in a delicate balancing manner between ROS production, involving ROS-producing enzymes and the unavoidable production of ROS during basic cellular processes, and the metabolic counter-process involving ROS-scavenging pathways (Mittler et al. 2011). In plants, NADPH oxidase, respiratory burst oxidase homologues (RBOHs), oxidases and peroxidases, processes occurring at the chloroplast, mitochondria, peroxisome via different pathways are involved in ROS generation (Vaahtera et al. 2014; Gilroy et al. 2016; Mignolet-Spruyt et al. 2016). RBOHs assorted to different tissues and their differential co-expression indicates that there is some sort of specialization. This spatial coordination of signals requires cell to cell communication. ROS involvement in such systemic plant immunity, response against mechanical damage, wound, high light acclimation, etc. has been reported by Alvarez et al. (1998) and Karpinski et al. (1999). Cell to cell signal transfer is dependent on extracellular accumulation of $H_2O_2$ that leads to the initiation of ROS wave that is a priming signal in systemic response when plant is exposed to abiotic stress stimulus. RBOHs are transmembrane proteins that span the membrane six times and possess a C-terminal domain having a characteristic NADPH and FAD binding domains. Plant RBOHs differ from mammalian RBOHs in the presence of cytosolic N-terminal domain with two calcium binding motifs and site for phosphorylation (Lin et al. 2009; Kimura et al. 2012). RBOH proteins generate superoxide, and superoxide dismutase enzyme converts it to membrane permeable $H_2O_2$ which then acts as signaling molecule (Xia et al.

2009; Wi et al. 2012). Cytosolic concentration of calcium positively regulates the activity of RBOH proteins. Besides RBOHs and NADPH oxidase, other mechanisms of ROS generation in plants involve photosynthesis, respiration, glycolate and oxalate oxidase, fatty acid oxidation, etc. (Mittler 2002). Peroxidases like PRX33 and PRX34 are involved in ROS generation during fungal and bacterial attack in *Arabidopsis*. Callose deposition in plants and expression of defense genes also involve ROS. Evidences for such facts have been derived from mutant studies where peroxidases remain dysfunctional in plants and lead to decreased callose deposition and disturbance in defense gene expression (Daudi et al. 2012; Wrzaczek et al. 2013). Fluctuating environmental conditions cause plants to regulate various metabolic, physiological, and developmental processes in order to survive and all these come under long term responses. In case of pathogen attack, ROS accumulation in cells has been observed for hours or days following the infection which confers protection to plants (Torres and Dangl 2005; Kobayashi et al. 2007; Wi et al. 2012). Long term responses in plants towards high light intensity are regulated by PQ pool and its redox state. High light also alters thylakoid composition via STN7 kinase (Pesaresi et al. 2009; Mittler et al. 2011). ROS production consists of a primary and a secondary phase; both the phases vary in their time duration where primary phase lasts for minutes and secondary phase for hours or days (Nishimura and Dangl 2010). Wound response involves initial generation of superoxide radical and its subsequent conversion to $H_2O_2$ after 6 h (Soares et al. 2009). In a study where transcriptomic levels in light challenged leaves were compared to distant leaves, more than 70% upregulated genes were similar in both the cases. Level of amino acids such as glycine, glycerate, and serine is altered in both the tissues similarly. Responses of tissues towards heat and cold stress involved similar signaling and stress induced changes (Mühlenbock et al. 2008; Suzuki et al. 2013). There is an overlap in signals within local and systemic tissues, but studies demonstrate significant difference in transcripts and metabolic profile in both types of tissues. Metabolic profiling in pathogen infected and uninfected tissues showed variation in distribution of metabolites (Simon et al. 2010). Burst of ROS generated in response to stress stimuli acts as a signal propagating from local to systemic tissues, as a result of cell to cell communication that carries signal to distant parts (Miller et al. 2009). Ethylene biosynthesis is positively regulated by RBOH proteins (Jakubowicz et al. 2010). Under stress, PQ pool redox state and a signal causing production of ACC (1-amino-cyclopropane-1-carboxylate) are disturbed. ROS accumulation causes bleaching and programmed cell death in plants (Karpiński et al. 2013). ABA and SA treatments have been shown to increase $H_2O_2$ production that helps plant to tolerate salt, heat, and other abiotic stresses (Xia et al. 2009). Acclimation of plants systemically when exposed to heat stress was characterized by ROS as well as ABA accumulation in non-challenged tissues. Mutants lacking ABA signaling showed attenuated systemic response towards heat stress. Interaction between ROS and ABA is involved in systemic response (Suzuki et al. 2013). Signaling induced by insect attack and wounding are also interrelated, as they both rely on JA biosynthesis at the site of attack, at the site of wounding, and its translocation to systemic tissues (Suzuki and Mittler 2012). Mutants that show dysfunction of ROS regulation, its production and elimination were found to be more sensitive towards a range of abiotic stresses and were also found to be impaired in systemic signaling (Davletova et al. 2005; Suzuki et al. 2013).

## 2.9 SAR and ISR in Agriculture

Ever increasing threats to agriculture system are posing a variety of abiotic and biotic stress that directly affect the productivity in economically important crops all over the world. Abiotic stress to which plants are exposed include prolonged droughts, flooding, heat stress, and frost injuries. Insects and pests also cause significant yield loss and crop damage. Increased food production demand is the need of the hour, which can be compensated by using environmentally unfriendly pesticides and agrochemicals. SAR can be triggered either by exposing plants to pathogenic or non-pathogenic microorganisms or by using synthetic chemical elicitors such as salicylic acid, 2,6-dichloro-isonicotinic acid (INA) or benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), SAR inducers, *N*-cyanomethyl-2-chloroisonicotinamide. These chemicals, at higher concentrations may show phytotoxicity (Nakashita et al. 2002; Vallad and Goodman 2004) and activate the expression of the same set of defense genes and induce resistance against the same spectrum of pathogens as in SA-induced SAR (Kessmann et al. 1994; Friedrich et al. 1996). A compound able to induce SAR was isolated from *Strobilanthes cusia* and chemically identified as 3-acetonyl-3-hydroxyoindole (AHO), which is a derivative of isatin. Resistance induced by this chemical depends on SA-mediated signaling when observed in inducing resistance against TMV and *Erysiphe cichoracearum* in tobacco. During resistance induction, AHO triggers PAL activity, elevates the SA level, and induces *PR-1* gene expression in tobacco plants (Li et al. 2008). Several reports have suggested that PAL is a key regulatory enzyme in the biosynthesis of SA and the establishment of SAR (Mauch-Mani and Slusarenko 1996). Chemical elicitors in general do not show direct antimicrobial activity against pathogenic microorganisms. However at higher concentrations these are reported to show antimicrobial activity (Rohilla et al. 2002). BTH (acibenzolar-S-methyl) was originally marketed for the protection of wheat and barley against powdery mildew (Görlach et al. 1996). Experimental studies revealed the effectiveness of BTH against a variety of diseases such as septoria leaf blotch and leaf rust in wheat, downy mildew of maize (Morris et al. 1998). Effectiveness of BTH against various bacterial, fungal, and viral diseases of solanaceous crops has been reported in experimental studies conducted in fields (Abbas et al. 2002; Matheron and Porchas 2002; Perez et al. 2003). In experimental studies, BTH was effective in reducing disease severity of bacterial spot and bacterial speck in tomato (Louws et al. 2001). Similarly, application of BTH was effective against bacterial spot in pepper and wildfire, blue mold, frogeye leaf spot, brown spot, and *Rhizoctonia* leaf spot diseases in tobacco (Buonaurio et al. 2002; Perez et al. 2003). Repeated application of INA reduced symptoms of white mold in *Glycine max* to some extent on susceptible cultivars but not against resistant varieties. BTH was also effective against the resistant varieties only when applied at tenfolds (Dann et al. 1998). Efficacy of BTH has also been checked for fruit crops such as *Malus domestica* and *Pyrus pyrifolia* (Ishii et al. 1999; Maxson-Stein et al. 2002). Application of BTH was able to control scab and rust in field grown Japanese pear trees. Generally, polycarbamate is used as a fungicide to control scab and rust diseases, but BTH was more effective as compared to polycarbamate in controlling scab disease but less effective in reducing severity of rust diseases as compared to polycarbamate (Ishii et al. 1999). Weekly spray of BTH on foliar tissues reduced fire blight caused by *Erwinia amylovora* more effectively as compared to streptomycin. Expression of PR proteins was induced after BTH application in apple seedlings under greenhouse conditions (Maxson-Stein et al. 2002). BTH application was studied in citrus fruits against scab, melanose, and *Alternaria* brown spot under greenhouse conditions by Agostini et al. (2003). Successful control of *Alternaria* leaf spot, bacterial blight, *Verticillium* wilt of cotton has also been reported with the use of BTH and INA (Colson-Hanks et al. 2000). In field trials on spinach, BTH was able to control white rust disease (Leskovar and Kolenda 2002). A number of PGPR are reported to elicit ISR response and systemically control various diseases when applied to plant roots using soil drench, transplant mix, root dip, or seed treatment. PGPR have been reported to control several diseases such as anthracnose caused by *Colletotrichum lagenarium*, angular leaf spot, and bacterial wilt in cucumber. Efficacy of most of the PGPR varies under field conditions; however, some strains like *Bacillus pumilus* INR-7 effectively protected cucumber plants for three years against anthracnose and angular leaf spot (Raupach and Kloepper 2000). This strain was also able to control downy mildew in pearl millet. PGPR strains when used against viral diseases, a limited success was obtained when applied under field conditions. However, greenhouse trials have provided some evidences of viral disease control. Application methods and efficacy of PGPR are interrelated. Repeated foliar application of *Bacillus pumilus* INR-7 and *Pseudomonas oryzihabitans* as well as seed treatment were equally effective against mixed infection of anthracnose and angular leaf spot on cucumber (Raupach and Kloepper 1998). Similarly, talc base formulations and fresh liquid suspension of PGPR provided similar results for the control of downy mildew in pearl millet (Niranjan Raj et al. 2003). Talc based formulations however allow easier storage and application in comparison to suspension. Most of the experiments conducted using BTH and INA led to the reduction of crop yield (Louws et al. 2001; Romero et al. 2001). In the field experiments on tomato across 22 fields, plots treated with standardized bacterial formulations and untreated control plots showed that BTH treated seeds were smaller than untreated plants (Louws et al. 2001). Similarly, reduction in yield, growth, and delay in plant maturity was observed in pepper plants treated with BTH (Romero et al. 2001). Resistance induced in monocots and dicots differs in the time duration for which resistance is maintained. Experimental studies showed that single application of BTH was sufficient to induce resistance in monocots for its lifespan whereas dicots required repeated application of BTH to provide longevity to the resistance (Louws et al. 2001; Romero et al. 2001). Control of disease using BTH was also influenced by stage of plant growth. Results of field experiments revealed that BTH application during late tillering stage controlled powdery mildew more efficiently as compared to that applied at mid tillering phase (Stadnik and Buchenauer 1999). Foliar application of jasmonic acid on radish under field conditions showed reduced tissue damage from subsequent insect herbivory. Jasmonate treated plants were more fit reproductively than damaged or untreated and undamaged plants (Agrawal 1998). Plant's growth stage and nutritional status do matter in physiological cost of induced resistance in plants (Heil 2001). Establishment of SAR requires a set of time that varies with type of elicitor as well as plant species. SAR is also associated with accumulation of salicylic acid and PR proteins throughout the plant. Plants deficient in ability to accumulate salicylic acid were found to lack PR proteins and showed attenuated SAR response (Uknes et al. 1992; Gaffney et al. 1993). Exposing plants to chemical elicitors like BTH and INA helped them to express PR proteins even in plants deficient in accumulating salicylic acid (Friedrich et al. 1996; Lawton et al. 1993). ISR is potentiated by beneficial PGPR and the response pathway does not involve accumulation of PR proteins and salicylic acid (Pieterse et al. 1996) but depends on ethylene and jasmonate signaling (Knoester et al. 1999; Yan et al. 2002). Some examples of ISR are also linked to production of siderophore and salicylic acid by PGPR strains (Vallad and Goodman 2004). SAR is effective across a broad range of plant species but ISR is specific for different PGPR strains of plant species and genotypes (Yan et al. 2002).

## 2.10 Conclusion

Till date, a number of studies have been conducted and have enlightened our understanding of plant responses towards biotic and abiotic stress stimuli. Transcriptomics, proteomics, mutant studies, and metabolic profiling has helped the researchers to understand the molecular mechanism of the process as well as genes, proteins, and other components involved to decode the events involved in downstream signaling cascade. Secondary messengers like ROS play an intermediate role in regulating biotic and abiotic stress individually as well as cross-linking the response pathways. Thus, the identification of new chemicals capable of inducing disease resistance would be useful, not only for elucidating the pathways, but also for developing new agents for plant protection. ISR and SAR have been recognized as an attractive tool in modern agriculture. Commercially available synthetic chemical inducers of SAR are commonly used to protect plant from pathogens. Similarly, ISR induced by plant growth promoting rhizobacteria are helpful in cultivating crops with optimal yield as well as reduced disease incidences. Thus, exploiting SAR and ISR and developing a combinatorial approach wherein elicitors of both the responses are used optimally to develop resistance and tolerance in plants towards different stress stimuli, can help to reduce the dependency on agrochemicals and prove to be a small step towards sustainable agriculture.

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# Chapter 3
# Plant–Microbe Interaction: A Sustainable Strategy to Elevate Salinity Tolerance in Plants

**Ajay Veer Singh, Amir Khan, and Manisha Joshi**

**Abstract** A large portion (approx 20%) of world's agricultural land is facing salinity stress and it is continuously increasing due to the natural and anthropogenic activities Salinity is one of the most dangerous abiotic stresses and characterized as enhanced concentration of sodium, calcium, magnesium, etc., in the soil. Saline soil loses its inherent capacity to support plant growth due to imbalanced nutrient content, high pH, and reduced water potential. Therefore, most of the crops except halophytes are highly susceptible towards saline conditions. High saline stress results in physiological and biochemical alteration of cellular metabolism in plants that ends up with reduced crop yield. To meet the future demands of food for burgeoning human population and bioremediation of saline soil, researchers are trying to develop cost effective, efficient, and environmental sustainable techniques. Halotolerant microorganisms have also gained much attention in the last few decades due to their ability to confer salinity tolerance in plants via mechanisms like production of compatible solutes, antioxidants and ACC deaminase enzymes, plant hormones, VOCs, etc., along with plant growth promotion through nutrient mobilization and plant protection. Therefore, application of such potent halotolerant plant growth promoting microorganisms is a valuable, proficient, and eco-friendly approach under saline stress. Current manuscript presents a comprehensive understanding about the soil salinization and the mechanisms adopted by the plant associated microorganisms in order to improve salt tolerance and productivity of plants in a sustainable manner.

## 3.1 Introduction

In the present era, agriculture system is experiencing various challenges such as global warming, drought, salinity, and pathogen attack, etc., and these are major causes responsible for reduced plant growth and yield. Among all, soil salinity is of

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major concern due to its drastic impact on food production. It refers to the prevalence of high magnitude of soluble salts in soil or water by means of natural and anthropogenic processes. It enhances osmotic pressure around the root and causes hindrance in water uptake and creates nutrient imbalance in soil and lowers down water and nutrient availability in plants. Deficiency of water and nutrients interrupts plant metabolic machinery which is ultimately reflected as reduced plant growth and productivity. Around the world, about 832 Mha land is suffering from salt stress. Out of this about 398Mha land is experiencing saline stress, whereas 434 Mha is affected by sodicity (Dagar 2014). According to FAO and ITPS (2015), about 50% soil of Pakistan, China, India, and United States are salt affected. Moreover, every year about 1–2% of agricultural land is assaulted by soil salinization in arid and semiarid regions (FAO 2002). Plants, except halophytes are unable to grow or poorly grow in such environment because it adversely affects the biochemical pathways of plants, seed germination rate, plant vigour, and yield in terms of quantity and quality. Generally saline soil is characterized with an electrical conductivity (EC) from 4–$<16$ dS/m to $<15$ ESR, but majority of crops are susceptible for low salinity, even EC $>$ 1 dS/m can decline the growth and yield of rice plants. Food from these crops can cause undernourishment and malnutrition in people. Hence, in order to enhance global food production and achieving sustainability in agriculture, maintenance of nutrient stress is extremely vital. Developing strategies effective in mitigating negative effects of salt stress is underway. Currently researchers are trying to develop such effective methods through plant breeding practices and by applying chemical fertilizers. Hybridized salt tolerant crops are reported to have very little success rate and they also come along with many problems such as huge labour demand and intense money expenditure. On the other hand, application of chemical fertilizers on a large scale proves to be dangerous to plants in long run since excessive fertilizers become inaccessible to plants. Recently, an eco-friendly and sustainable method, i.e. the application of beneficial microorganisms in crops, has gained researchers interest to improve the plant growth and productivity. Plant growth promoting microorganisms mineralize inaccessible nutrients and make them accessible to plants. Besides, they also protect plants from pathogens; improve soil structure and composition, and biotic activities of microbes make the soil ecosystem dynamic. These microorganisms are also involved in the improvement of salinity tolerance in plants by means of various direct and indirect mechanisms. Present manuscript illustrates a comprehensive role of salt tolerant plant growth promoting microorganisms in the improvement of salinity tolerance in plants.

## 3.2 Causes of Soil Salinization

The term “salinity” is marked as the surplus concentration of salts such as sulphates of sodium, magnesium, calcium, etc., in soil or water. Saline or sodic soils are called salt affected. Soil salinity is caused by natural or anthropogenic processes and the outcome of this is a massing of dissolved salts in total available water present in the soil which ends up in hindering plant growth and development (Isayenkov and Maathuis 2019). Soil salinization can be of three types in accordance to its cause. These are primary salinity (termed as natural salinity), secondary salinity (referred as dry land or anthropogenic salinity), and tertiary salinity (termed as irrigation salinity). Natural salinity results from the accumulation of salts for prolonged time period through natural processes in soil or ground water. The natural processes include weathering of rocks and accumulation of oceanic salt carried by wind or rain water. Weathering of parental rocks causes emancipation of various soluble salts like sodium, chlorides, calcium, magnesium, carbonates, sulphates, etc. Wind contributes to soil salinity as it carries “the cyclic salts” which are deposited in agricultural land by rainfall. The concentration of salt deposition depends upon distance from the coast and average annual rainfall in a particular area. An increment in average annual rainfall can result in rapid charging of water table and brings ground water in close vicinity to soil surface and causes accumulation of salts near the upper surface of the soil (Shrivastava and Kumar 2015).

Secondary salinity, also called as anthropogenic or dry land salinity, is the consequence of human actions that affect hydrologic equilibrium of soil. Dry land salinity is a massing of salts in soil and ground water. It drastically affects soil health, water quality, vegetation, crops, and biodiversity. Industrialization and rigorous farming practices have caused eradication of perennial vegetation such as deep-rooted native trees and shrubs which results in more leakage of water in water table. Rise in water table brings salts close to root zone and soil surface. As the ground water comes in close vicinity to the soil surface, salt enters plant root zone and results in water logging. Moreover, surplus concentration of soil amendments such as chemical fertilizers also leads to soil salinization. Third is irrigation salinity, which happens due to the leakage of water from rainfall and irrigation. This causes ground water recharge that brings soil salts close to the soil surface. Recharge rates in irrigation salinity are much higher as compared to dry land salinity and cause soil salinization at much higher rates. Inefficient irrigation and drainage systems are the major cause of excess leakage which may increase the risk of salinity and water logging in agricultural lands. Moreover, irrigation of agriculture lands with saline water or sea water is also a major cause of soil salinization. Factors influencing irrigation salinity include soil type, climate, abundance of deep-rooted perennial vegetation, etc. Annual average rainfall and temperature of particular area also determines the salinity rates. Usage of shallow rooted vegetation in an area causes more infiltration of water into water table and leads to high soil salinization.

## 3.3 Effect of Salinity on Plant Growth

Salinity is a major abiotic stress which adversely affects plant physiology and causes reduced germination rate, reproductive development, yield, and quality. Generally, many cereal crops such as rice, maize, potato, tomato, etc., are highly susceptible for high salt concentrations. On the other hand, some plants like mangrove, marshes, ice

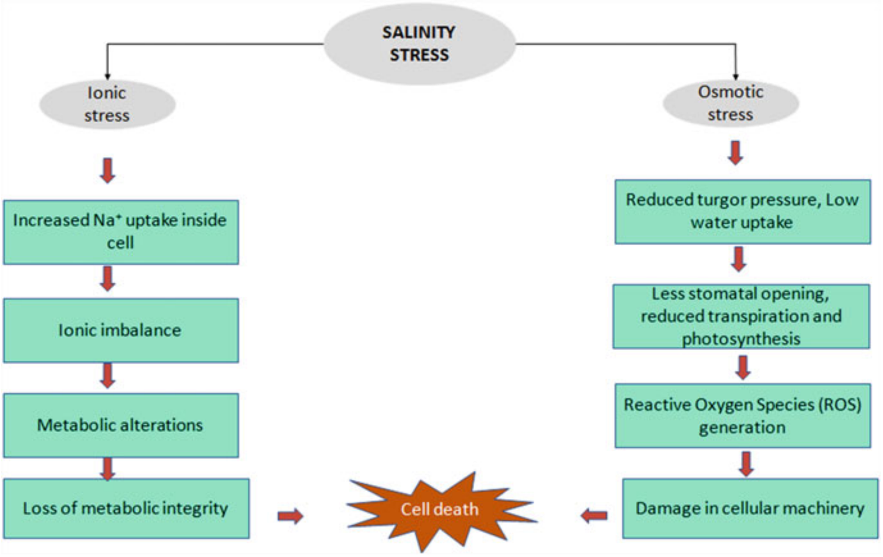

**Fig. 3.1** Effect of salt stress on plant physiology and metabolism

plant, Sarcocornia, etc., able to grow under saline environment are termed as halophytes or salt tolerant plants. During saline stress, the osmotic pressure of soil solution is high in comparison to plant cells and causes problems in water uptake through root zone and hence influences plants at various growth stages (Warrence et al. 2002). Moreover, plant cells experiencing decreased turgor pressure, reduced stomata size discourage stomata opening in order to conserve water. Transpiration rate and photosynthesis rate of the plants get reduced and induce the production of reactive oxygen species (ROS) such as singlet oxygen, superoxide, etc. Reactive oxygen species (ROS) exert their effects by causing damage in nucleic acid, lipids, proteins, etc., and result in disruption of cellular machinery and ultimately cause cell death (Xie et al. 2019). An increment in soil salinity disturbs the osmotic balance of plant cells and creates difficulties for plants to imbibe nutrients from the soil. Presence of salts readily affects the hormonal balance and causes premature senescence in plants (Fig. 3.1). High salt toxicity can negatively affect plant growth by causing necrotic patches, defoliation, leaf burn, and leaf bronzing. Overabundance of some salts can disturb the ideal ionic ratio in the cell and may compete for essential salts required for plant growth and development (Numan et al. 2018). For example, the uptake of additional amount of sodium and calcium inside the cell can hinder the uptake of potassium and iron, which may lead to the disease occurrence, poor quality seed, and low yield.

## 3.4 Effect of Salinity on Soil Health

Soil health is referred as soil status to sustain the nutrients, biodiversity, and plant growth in an environment. Presence of higher concentration of sodium, calcium, magnesium ions in the soil may cause salinity and it is reflected in the form of white patches on the top layer of the soil. Moreover, electrical conductivity of the saturated extract of a soil determines the degree of salinity stress (Table 3.1). An elevation in soil pH (up to 82) is often observed as a result of salinity, which is linked with the inaccessibility of some nutrients such as P, Fe, Zn, etc., that are essentially required for the growth and development of plants. Therefore, saline soil loses its inherent capacity to support plant growth up to some extent and leads to the eradication of native vegetation. Salinity may result in the loss of ground cover and makes soil more prone to erosion. Increased concentration of sodium ion causes separation of soil aggregates or clay dispersion by accumulating in between them, which results in reduced permeability, porosity, hydraulic conductivity, infiltration rate, and stability of the soil (Liu et al. 2014). Soil salinization also demolishes microbial community of that soil and decreases soil fertility. Because of variations in the ability to tolerate salinity, a shift of microbial community as compared to non-saline soil is observed. Fungi tend to be more sensitive to salt stress as compared to bacteria, thus an increase in bacteria/fungi ratio occurs in saline soil. Microorganisms residing in the soil play a major role in re-cycling of soil elements which are crucial for plant growth and metabolism. Microbes are involved in the processes like phosphate and zinc mineralization, metal chelation, sulphur oxidation, and nitrogen fixation (Khan et al. 2019a). These processes result in decomposition of litter which in turn restore organic matter and essential inorganic elements in the soil. Therefore, shifting of microbial community due to the salinity stress can cause alteration in total organic matter, carbonic content, and concentration of available minerals in the soil.

## 3.5 Plant Adaptations to Overcome Salinity Stress

Salinity is a major biotic stress and limits the plant growth and productivity significantly. Some plants such as halophytes have adapted different mechanisms at morphological, physiological, biochemical, and molecular level to survive under saline conditions. Salinity stress has severe effect on electron transport chain (ETC) and shows disruption of normal functioning of ETC in mitochondria and chloroplast.

**Table 3.1** Electrical conductivity of saline soil

| Salinity class | Electrical conductivity (dS/m) |
|---|---|
| Normal soil | 0–2 |
| Poorly saline soil | 2–4 |
| Moderately saline soil | 4–8 |
| Extremely saline soil | 8–16 |
| Very extremely saline soil | >16 |

Disturbed ETC is the main cause of accumulation of reactive oxygen species (ROS), i.e. hydroxyl radical, hydrogen peroxide, singlet oxygen, and superoxide and cellular damages. Under saline conditions, plants stimulate the production of different antioxidant enzymes (peroxidases (POD) (e.g. glutathione peroxidases and ascorbate peroxidase), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) which scavenges over produced reactive oxygen species (ROS). Moreover, brassinosteroids (BRs) possess hormonal properties and stimulate the accumulation of antioxidant enzymes thereby aiding in plant survival under saline stress. Halophytic plants are also unable to bear high salts concentration inside the cell but they survive under such conditions through compartmentalization processes. In these processes, overloaded salt is either captured in older tissues or shifted into the vacuoles through $Na^+/H^+$ antiporters through vacuolar V-ATPases. Wang et al. (2001), reported upregulated expression of V-ATPase in a halophyte (*Suaeda salsa*) Moreover, in another halophytic plant (*Mesembryanthemum crystallinum*), a HKT (histidine kinase transporter) family transporter was identified which regulates the efflux of $Na^+$ and influx of $K^+$ to maintain the ion homeostasis in plants (Yen et al. 2000). Elevated level of nitric oxide is also a marker for salinity stress tolerance in plants because under saline stress it enhances the synthesis of $H^+$ ATPase which triggers efflux of excess sodium ions through $Na^+/H^+$ exchange. Plants also synthesize various osmolytes such as proline, glycine betaine, sugars, polyols (referred as compatible solutes) against the osmotic stress caused by salinity. Compatible solutes are soluble, uncharged, and polar in nature. The major function of osmolytes is the maintenance of turgor pressure of the cell through steady water influx. Proline is a widely accumulating osmolyte, therefore considered as one of the parameters for measurement of salinity tolerance in plants. Besides the osmotic adjustment, proline also serves as a nitrogen source and antioxidant enzyme. Up regulation of several stress responsive transcription factors such as WRKY, AP2, NAC, bZIP, and DREB is also reported under saline stress, which enhances transcription rate of the proteins involved in tolerance of salinity stress (Abbas et al. 2019).

## 3.6 Strategies to Elevate Salinity Stress Tolerance

Salinity drastically affects agriculture and reduces crop productivity. To conquer the effect of saline stress, researchers are practicing various techniques in order to improve salinity tolerance in plants. Some of the techniques are summarized below:

### *3.6.1 Breeding Techniques*

In the present era, scientists are practicing several breeding and genetic engineering techniques to develop salt tolerant crop varieties to avoid drastic consequences of saline stress. Conventional breeding programme is the strategy to develop salt tolerant crops by cross-pollination of desired species. It involves two basic steps. The first step is to generate a breeding population that is highly impervious for salt tolerance and the second involves selection among the segregating progeny for the individuals that combine with most of the useful traits of the parent, i.e. high degree of salt tolerance. There are two important parameters (threshold and slope) to measure the salinity tolerance in plants. Threshold indicates the highest permissible salt level that does not affect crop production. Slope indicates percent reduction in yield per unit rise in salt level above the threshold (Hoang et al. 2016). Among the molecular breeding techniques, quantitative trait locus (QTL) is the major contributing factor to salt tolerance in crops. A major QTL designed "Saltol" was mapped on chromosome 1 of rice by using a population, generated from a cross between a sensitive variety IR29 and a salt tolerant rice, i.e. Pokkali (Waziri et al. 2016). Salt tolerance is a complex trait and the QTLs related with salt tolerance have a significant role in understanding the stress-response and to generate stress-tolerant plants. With a better understanding of the mechanisms and genetics of salt tolerance, more precise breeding approaches have been used for developing higher salt tolerance plant varieties. In order to develop salt resistance GMOs, in depth knowledge of stress responsive genes is required which may also lead to the transformation of responsible operons from salt tolerant varieties to salt sensitive plant varieties. Number of salt tolerant varieties has been released but their progress is slow. These varieties are not much successful as only a few responsible traits are considered in breeding and genetic engineering.

### *3.6.2 Microbial Mediated Amelioration of Salinity Tolerance*

Surplus saline condition of a soil reduces its fertility and plant growth by altering the availability of the nutrients and osmotic balance. Reduced productivity and quality of the crops limit the food quantity and cause inadequacy of food. They are major reasons and found responsible for decline in the market and economic values of crops. To conquer the problem, it is mandatory to recover the immobilized nutrients in saline soil which foster the salinity tolerance in plants. Role of plant growth promoting microorganisms such as bacteria, fungi, and actinomycetes has been well established in improving the growth, development, protection, and yield of plants by improving nutrient utilization ability, phytohormone production, reducing disease incidences, decomposing organic matter, membrane transporter alteration, and also by conferring abiotic stress tolerance (Fig. 3.2). Plant associated microorganisms are very diverse and dominated by plant growth promoting rhizobacteria (PGPR) and endophytes. Their composition varies according to the nature of plant exudates, native soil, and environmental conditions. It is well known that along with plants many microorganisms are also not able to grow in high salt concentration. Elevated level of salt ions in soil not only decreases soil microbial activity but also alters microbial community composition in native soil (Van Horn et al. 2014). The microbes able to survive under saline conditions are called halotolerant, whereas

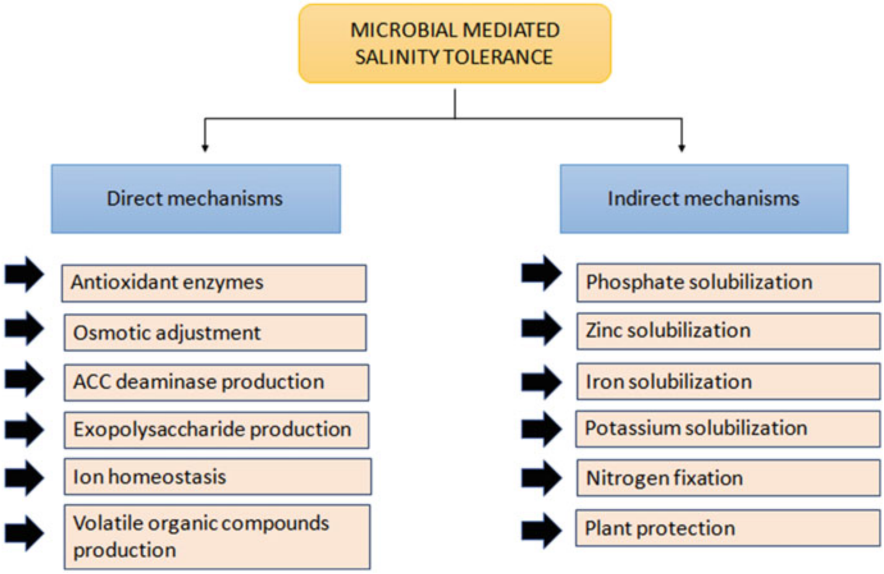

**Fig. 3.2** Mechanisms of halotolerant PGPRs to confer salinity tolerance in plants

other microorganisms that require high salt concentration for their growth are called halophiles. According to preceding information, various symbiotic and free living microorganisms associated with diverse genera such as *Achromobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Glomus, Klebsiella, Microbacterium, Rhizobium, Pseudomonas, Pantoea, Paenibacillus, and Variovorax,* etc., confer the tolerance to host plants during abiotic stresses (Abbas et al. 2019). These halophilic and halotolerant microorganisms reside around and inside the root and can foster salinity tolerance in plants to improve growth and development by lowering the negative upshot of saline stress via several direct and indirect mechanisms. Indirect mechanisms include phosphate solubilization, siderophore production, nitrogen fixation, zinc solubilization, etc., whereas direct mechanisms include production of exopolysaccharides, volatile organic compounds, antioxidants, ACC deaminase activity, ion homeostasis, osmotic balance, and induced systemic resistance (Fig. 3.2).

### 3.6.3 Direct Mechanisms of Salinity Stress Alleviation

Salinity tolerance prevails when plants maintain their metabolism even under high salt concentration, i.e. balance of $Na^+$ and $K^+$ ion, less reactive oxygen species (ROS) production, and maintenance of cell turgidity, etc. Many saline stress adaptive microorganisms are found around the root and release various substances that serve as signals to maintain plant metabolism. They maintain ion balance, rate of

ROS production, and ultimately improve root, shoot growth, and productivity under saline conditions.

### 3.6.4 Production of Antioxidant Enzymes

A plant continuously produces reactive oxygen species (ROS) during normal cellular metabolic processes. Main reactive oxygen species are hydroxyl radicals, hydrogen peroxide, and superoxide anionic radicals. Production rate of ROS becomes very high under stress of salinity, drought, and heat and which may lead to DNA damage, alteration in fatty acids structure, denaturation of membrane bound proteins, modulation in redox status, oxidative damage of pigments, etc. However, other biomolecules reduce membrane fluidity, change protein confirmation, damage the enzymatic activity, cell homeostasis and cause cell death (Sharma et al. 2016). Hydrogen peroxide ($H_2O_2$) stimulates ethylene synthesis in plants which leads to early senescence in plants. Many researchers have reported that seed bacterization with plant growth promoting microbes can efficiently enhance production of antioxidant enzymes (enzymatic antioxidants such as mono dehydroascorbate reductase, glutathione reductase (GR), superoxide dismutase (SOD), and non-enzymatic antioxidants like ascorbate tocopherols and cysteine) which can minimize the magnitude of ROS and reduce oxidative injury up to some extent and thus foster salinity tolerance in plants (Table 3.2) Recently, Feng et al. (2019) reported improvement

**Table 3.2** Role of halotolerant microorganisms in salinity tolerance

| Microorganisms | Plant | Mode of action | Reference |
|---|---|---|---|
| *Bacillus aryabhattai* H19-1<br>*B. mesonae* H20-5 | Tomato | High level of proline, ABA, antioxidant enzyme and upregulation of NCED1 and AREB1 proteins | Yoo et al. (2019) |
| *Streptomyces venezuelae* | Jasmine rice | Production of ACC deaminase, antioxidant enzymes, proline, MDA | Yoolong et al. (2019) |
| *Arthrobacter woluwensis* AK1 | Soybean | Production of phytohormones, siderophore, organic acids, induced expression of potassium, chloride, and $Na^+/H^+$ channel | Khan et al. (2019b) |
| *Glutamicibacter halophytocola* | *Limonium sinense* | Antioxidant production, compatible solute accumulation | Qin et al. (2018) |
| *Bacillus cereus, Bacillus aerius* | Safflower | Production of ACC deaminase, IAA | Hemida and Reyad (2018) |
| *P. Yonginensis* DCY84$^T$ | *Panax ginseng* | Production of Siderophore, IAA, organic acids, antioxidant enzymes, proline accumulation | Sukweenadhi et al. (2018) |
| *Pseudomonas frederiksbergensis* OS261 | Pepper | Reduced ethylene production, production of antioxidants | Chatterjee et al. (2017) |
| *Bacillus aquimaris* DY-3 | Maize | Accumulation of compatible solutes, antioxidant enzymes, IAA | Li and Jiang (2017) |

in saline tolerance in maize (bacterized with *Bacillus mojavensis* and *Rhodopseudomonas palustris*) with the elevation in antioxidant enzymes (Ascorbate peroxidase (APX), catalase (CAT)) under saline stress conditions. Sukweenadhi et al. (2015) observed induced expression of salinity responsive gene such as *AtRSA1* which is involved in degradation of ROS upon priming with *Paenibacillus yonginensis* in Arabidopsis seedlings. Several microorganisms belonging to genera like *Lactobacillus, Serratia, Pseudomonas, Enterococcus, Bacillus, Micrococcus, Streptomyces*, etc., having their own antioxidant enzymatic defence system are involved in eradication of ROS (Abbas et al. 2019).

### 3.6.5 Osmotic Adjustment

Some bacteria produce compatible solutes for maintenance of their cellular turgidity to govern regular cell functions smoothly. This is chief cellular machinery that lessens the osmotic stress in plants produced because of salinity. In saline stress, rhizobacteria accumulate compatible osmolytes such as glycine betaine and proline to assist plant growth. Proline is one of the prime osmolytes that accumulates in the plants after protein hydrolysis to lower down the osmotic stress (Krasensky and Jonak 2012). It plays multifunctional responsibilities in terms of decreasing lipid peroxidation, regulating cytosolic acidity, proteins maintenance, and scavenging of reactive oxygen species (Ilangumaran and Smith 2017). Trehalose, a compatible solute produced by some plant functions as an osmoprotectant under abiotic stress conditions, viz. osmotic stress, drought, salinity, and high temperature (Pan et al. 2019). Presence of glycine and betaine as compatible solutes in plants and microbes not only makes plants defensive against several abiotic stresses, even act as shield by stabilizing highly ordered structure of membrane and quaternary structure of proteins. Several salt tolerant microorganisms have extensive genes to endure under saline stress including $Na^+/H^+$ antiporter genes, etc. These genes are necessary for $Na^+$ detoxification and cell maintenance of cells, formation of $Na^+$ electrochemical gradient, adjustment of cell volume, cellular pH, and homeostasis (Abbas et al. 2019).

### 3.6.6 Production of ACC Deaminase

Ethylene, a gaseous plant hormone, also called a senescence hormone contributes significantly in diverse developmental processes of the plants. Under various biotic and abiotic stress conditions, production of ethylene in plants is very high which governs various activities in the plants to maintain ion homeostasis inside the cell and reduces root and shoot length ratio in plant. Elevated quantity of ethylene pessimistically affects plant physiology, reduces shoot growth, damages cell, induces early senescence, and ultimately condenses production rate. Some plants associated with microorganisms produce a unique enzyme called ACC deaminase, which catalyses the transformation of ACC (precursor for ethylene biosynthesis) into ammonia and α-ketobutyrate and suppresses the production of endogenous ethylene (Dodd and Pérez-Alfocea 2012). ACC deaminase production by microorganisms can reduce the adverse effect of ethylene and also confers the tolerance for salinity and drought stress (Bharti and Barnawal 2019). Till now, a number of ACC deaminase producing PGPR has been documented by various researchers which reduce salinity and induce shoot growth. Gupta and Pandey (2019) observed augmented root and shoot length, leaf ACC deaminase enzyme unit upon exposure of two ACC deaminase producing strains (*Aneurinibacillus* and *Paenibacillus*) in French bean under saline conditions. They correlated improved tolerance and productivity of French bean with ACC deaminase production and various metal solubilization. Similarly, enhanced salinity tolerance was also observed in tomato plants upon inoculation with ACC deaminase and IAA producing *Leclercia adecarboxylata* (Kang et al. 2019).

### *3.6.7 Exopolysaccharide Production*

Various expolysaccharides (extrapolymeric substances) are secreted by different microorganisms around the cell. On the basis of their biological functions they can be categorized into three types, viz. capsular polysaccharide (involved in pathogenesis), extracellular polysaccharide (involved in biofilm formation and pathogenesis), and storage polysaccharide. They govern cell to cell communication, assist in adherence on a solid support, and protect cells from desiccation and phagocytosis (Etesami and Beattie 2018). EPS producing microorganisms have potential to reduce the negative effects of salinity via formation of rhizosheath, in which EPS traps $Na^+$ ions present around the root via hydroxyl, sulfhydryl, phosphoryl, and carboxyl groups and prevents ionic movement inside the plants and therefore reduces $Na^+$ ion toxicity. Paul and Lade (2014) have documented the role of plant growth promoting rhizobacteria such as *Bacillus* sp. and *Pseudomonas* sp. in salinity stress alleviation via production of EPS. Different environmental conditions (salinity, drought, C/N ratio, oxygen rate, and pH) can alter the composition of EPS and can induce the production rate of EPS. Under high salinity level bacteria generally produce uronic acid and sulphates at a higher rate. It is well known fact that under saline conditions water availability declines and leads to desertification. Production of EPS by microbes imparts gluing property in the soil and improves soil aggregation, nutrient stabilization and retains moisture in soil as it can bind with water multiple times than its weight. According to Robertson and Firestone (1992), bacterial EPS can improve moisture retaining capacity of a sandy soil in comparison to sandy soil without EPS. Presence of high water content in soil was illustrated in various researches when inoculated with *Pseudomonas* sp., which occurred due the production of EPS (Chu et al. 2019). Moreover, Jalili et al. (2009) also confirmed the efficacy of EPS producing PGPR to confer salinity tolerance in plants under different salinity stress.

### 3.6.8 *Ion Homeostasis*

Saline condition leads to distortion of ion milieu in plants due to increased uptake of $Na^+$ ions and slighter uptake of $K^+$ ions which causes various changes in physiological and biochemical pathways of plants. $Na^+$ toxicity can be reduced by posing hindrance in $Na^+$ ions uptake and also by emitting excess $Na^+$ ions. Similar strategy has been adapted by salt resistant halophytes, which expel $Na^+$ ions from the leaves and avoid toxic effect of salinity in the plants (Horie et al. 2012). Therefore, any contribution of microbes in the hindrance of $Na^+$ uptake and its induced expel from the plant parts can improve salinity tolerance. Application of potential PGPRs can alter $Na^+$ ions uptake and improves nutrient mobilization via governing ion transporter expression regulation. It hinders uptake of $Na^+$ ions and improves the accumulation of selective $K^+$ ion in the plant cell, therefore, maintains $K^+/Na^+$ ratio, which is beneficial for accelerating plant growth and salinity tolerance (Qin et al. 2016). Abbas et al. (2019) documented that *Glomus* (arbuscular mycorrhizal fungi) induces selective upliftment of $K^+$, $Ca^{2+}$, and $Mg^{2+}$ and evades $Na^+$ uptake to enhance $K^+/Na^+$ ratio. Similarly, Zhang et al. (2008) also reported reduced $Na^+$ influx upon priming with *Bacillus subtilis* with *Arabidopsis thaliana* under salt stress conditions by down regulating the expression of HKT1/$K^+$ transporter. Therefore, it can be concluded that inoculation of PGPRs can improve ion milieu of plant cell and tolerance capacity and productivity of crop.

### 3.6.9 *Volatile Compounds*

Volatile compounds are low molecular weight organic compounds and have high vapour pressure at room temperature. Their composition and quantity may alter according to the environmental conditions. Concentration of volatile compounds is low under normal conditions and becomes induced under stress conditions. Induced expression of volatiles seems to be a protective mechanism against stress. Plant inhabiting microorganisms generally produce lipophilic VOCs which are slightly water soluble. These volatiles assist in cell to cell communication in order to promote growth. Besides, they induce choline, glycine betaine biosynthesis which takes part in osmotic adjustment under saline conditions. Accumulation of such osmoprotectants leads to rise in osmotic pressure of the cell thereby prevents water loss. Moreover, these VOCs also hinder $Na^+$ influx inside the plants and improve nutrient mobilization. A number of bacteria such as *Bacillus amyloliquefaciens, Pseudomonas chlororaphis, Pseudomonas simian*, and *Bacillus subtilis* have been documented to produce VOCs that involve in the salinity tolerance improvement and plant growth promotion (Timmusk et al. 2014; Liu and Zhang 2015). Bacterial VOCs do not directly interact with plant hormone or other growth stimuli but regulate auxin homeostasis and nutrient influx through roots (Zhang et al. 2008). Plant VOCs also assist in the plant protection from pathogens; improve biomass and other stress tolerance capacity of the plants. Cho et al. (2013) reported 2, 3-butanediol (VOC) mediated stress tolerance in terms of reduced water loss and high stomatal conductance in plants inoculated with *P chlororaphis* O6 under abiotic stress.

### *3.6.10 Indirect Mechanisms of Salinity Stress Alleviation*

Under high salinity, soil pH becomes basic (pH ~82) wherein, most of the freely available macro and micro nutrients form complexes with other compounds. These compounds are not available to the plants and thereby negatively affect plant growth and yield in terms of the nutrition value and quality of grains. Different types of microorganisms carrying different functions are found in the rhizosphere which mobilizes nutrients through various processes. These processes including phosphorous solubilization, nitrogen fixation, iron chelation, and potassium mobilization take place via producing organic acids and secretion of cation chelators.

### *3.6.11 Phosphorous Solubilization*

Phosphorous (P) is an important element for plant because it is involved in all the major pathways of a plant cell. It is abundantly present in the soil and found in both inorganic and organic forms About 005% of soil is comprised of P, but only 01% part of this fraction is available for plants as P is poorly soluble at neutral pH and forms insoluble complexes (Etesami and Beattie 2018). Besides, saline soil possesses high pH which enhances the complex formation capacity of P and thereby making P inaccessible to the plants. Plants influx only monobasic ($H_2PO_4^-$) and diabasic ($HPO_4^{2-}$) forms of phosphate (Parveen et al. 2018). This leads to a reduction in plant growth and phosphate deficiency induces disease incidences in plants. To overcome such problems, farmers generally apply NPK fertilizers but a very small amount of fertilizer is absorbed by plants and remaining fertilizer is converted into insoluble form. It is also one of the reasons for soil salinity. In this regard, application of halotolerant phosphate solubilizing PGPRs can be an effective approach to solubilize P complexes. These PGPRs solubilize P by acidification, by producing low molecular weight organic acids and by chelation and ion exchange. Whereas, to mineralize the organic phosphorous complexes bacteria secret various phytases, phosphatases (Singh and Shah 2013; Singh et al. 2018). Numerous bacteria isolated from saline environment and belonging to the genera *Arthrobacter, Azospirillum, Bacillus, Phyllobacterium*, and *Vibrio* have been documented to solubilize various phosphorous salts such as $Ca_3\ (PO_4)_2$, $AlPO_4$, and $FePO_4$ even under saline stress conditions (Abbas et al. 2019). Mayak et al. (2004) demonstrated that inoculation of *Achromobacter piechaudii* with *Solanum lycopersicum* under saline stress showed improvement in plant phosphorous content. Similarly, improved P content was also observed in a field trial of various crops when plants were inoculated with PGPR (Singh et al. 2013: Upadhyay and Singh 2015). Irfan et al. (2019) also communicated that instead of single bacterium inoculation, consortia (multiple bacteria) of phosphate solubilizing microorganisms (PSB) are more effective to solubilize the insoluble complexes of phosphorous under saline stress.

### *3.6.12 Siderophore Production*

Iron is another one of the important nutrients which played a significant role in plant respiration and $N_2$ fixation by acting as an essential component of different enzyme complex. Iron is generally found in $Fe^{2+}$ and $Fe^{3+}$ forms but plants can utilize only $Fe^{2+}$ form. Availability of iron in calcareous and saline soil is very low because most of the $Fe^{2+}$ gets converted into $Fe^{3+}$ form due to high pH of soil where it forms complexes such as hydroxides and oxyhydroxides and becomes inaccessible to plants (Upadhayay et al. 2018). This iron deficiency under saline stress drastically reduces plant growth and causes diseases in plants. Diverse microbial community inhabiting in rhizosphere and bulk soil possesses the ability to capture $Fe^{3+}$ form via secretion of various type of siderophores. Bacteria sometimes convert iron-siderophore complexes into free $Fe^{2+}$ form which is then sequestered subsequently into plants roots and assist in plant metabolic functioning. Rhizospheric region of the plants is highly capable to take up ferric iron complex due to these siderophores, and thereby improves iron availability in plants and enhances plant growth and yield even under saline stress conditions. Moreover, iron sequestration ability also assists in plant protection via competition of iron with pathogen. Yasin et al. (2018) documented that siderophore producing halotolerant *Bacillus* sp., inoculated with *Capsicum annuum* showed improved tolerance towards the saline stress. Similarly, improved salinity stress tolerance was also documented in wheat seedlings, inoculated with siderophore producing halotolerant PGPR strains (*Hallobacillus* sp. SL3 and *Bacillus halodenitrificans* PU62) (Ramadoss et al. 2013). Therefore, it can be concluded that siderophore secretion can improve iron availability in plants and improves plant growth and yield.

### *3.6.13 Nitrogen Fixation*

Under saline condition, soil shows depletion of various nutrients; therefore, partial availability, competitive influx, and transporters may cause physiological imbalance in metabolic pathways of plants. Nitrogen is a crucial element for the health of plants and can limit plant productivity. It accounts for more than 80% of minerals absorbed by plants. Around 78% $N_2$ is present in the atmosphere but it is inaccessible for direct use of plants. It can be converted into usable forms, i.e. nitrite and nitrate by physical, chemical, and biological mechanisms. One third of nitrogen is fixed by chemical and physical processes, whereas remaining is converted by biological means called as biological nitrogen fixation. Nitrogenase is found in nitrogen fixing microbes which converts atmospheric nitrogen into nitrate that can be directly utilized by plants. Microbes fix nitrogen in free living form as well as in association with plants. Free living nitrogen fixing microorganisms belong to genera *Azotobacter, Bacillus, Pseudomonas*, whereas associative nitrogen fixing such as *Rhizobium* and *Bradyrhizobium* form symbiotic association with legume plants and form nodules to fix nitrogen. Salinity generally reduces the symbiosis and also hinders the nodule formation in legumes. But salt tolerant PGPRs associated with halophytes and nonhalophytes are very crucial source for availability of nitrogen in saline soil (Sharma et al. 2016). A research by Navarro-Noya et al. (2016) concluded that even under extreme salinity and high pH, PGPR strains *Paenibacillus fujiensis* and *P. durus* act as excellent nitrogen fixers. Metwali et al. (2015) documented improvement in plant growth and yield of faba beans under saline stress upon inoculation with plant growth promoting *Pseudomonas putida, P. fluorescens and Bacillus subtilis*.

## 3.7 Conclusion

Sustainable agriculture system refers to the maintenance and improvement of agriculture to meet up the future demands and environment protection in an efficient and cost effective manner. In the present global situation, salinity is causing drastic effects on the quality of agricultural soil, biodiversity, environment, plant growth, and productivity. Moreover, continuous conversion of agricultural land into saline area is also an issue of huge concern. Plants possess several defence mechanisms against mild salinity stress but they are not able to cope up under high saline conditions. Therefore, world is demanding an ecologically compatible, cost effective, and efficient technique to overcome the said problems. Inoculation of potential halotolerant plant growth promoting microorganisms is a sustainable and effective alternative technique as compared to other traditional agricultural methods owing to the surviving ability of PGPRs under saline environment. PGPRs associated with plants recover saline soil, improve plant growth and vigour along with alleviation of negative effects caused by saline stress by means of various mechanisms such as osmotolerance, antioxidant metabolism, ion transporter regulation, production of VOCs and EPS, and nutrient mobilization. Therefore, present manuscript concludes that the application of potential halotolerant and halophilic PGPRs and their consortium may be a promising technique to conquer the future food demands, bioremediation of saline soil, and maintenance of environmental sustainability under saline stress.

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## Chapter 4

# Concepts and Application of Plant–Microbe Interaction in Remediation of Heavy Metals

**Geeta Bhandari and Pankaj Bhatt**

**Abstract** Level of anthropogenic chemicals is increasing day by day in the environment due to various human activities. Elimination of such chemicals is essentially required to save life on the earth through cost effective and eco-friendly approaches as these chemicals are hazardous and toxic for the living beings. Phytoremediation is considered as one of the promising methods for the removal of such chemicals from the soil. Plants and their rhizospheric bacteria mainly participate in the removal of such toxic chemicals from the soil and nearby environment. Root exudates from almost all kind of plants play important role in degradation of toxic chemical compounds as these chemicals also act as attractant and source of carbon and energy for the development and establishment of rhizospheric bacteria in and around the plant roots. In this chapter we have emphasized plant–microbe interactions and their importance in bioremediation of anthropogenic chemicals.

## 4.1 Introduction

Increasing population, urbanization, industrialization, and intensive agricultural practices over the last century have made consequential impact on the environment which demands a crucial solution worldwide. Heavy metal pollution has become a significant danger to the environment and food security due to developmental activities. Important sources of heavy metals include: mining and smelting activities, batteries, coal and leaded petrol combustion, fertilizers, pesticides, and hospital waste (Memon et al. 2001; Thangavel and Subbhuraam 2004; Khan et al. 2007; Wuana and Okieimen 2011; Rodrigues et al. 2012). Heavy metal leached from these

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sources then pollutes soils, air, ground and surface water and maybe transferred through the food chain by process of biomagnification. Metals having toxic effects include: mercury (Hg), zinc (Zn), copper (Cu), lead (Pb), manganese (Mn), chromium (Cr), cadmium (Cd), and aluminum (Al) (Omura et al. 1996; Adriano 2001; Duruibe et al. 2007) and some metalloids, such as antimony (Sb) and arsenic (As). United States Environmental Protection Agency (US EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) list of most hazardous materials comprise of some heavy metals and metalloids, e.g., As, Pb, Hg, and Cd (Smith et al. 1997; Park 2010; Wuana and Okieimen 2011). Being non-biodegradable, heavy metals persist in the soil and water for centuries which poses a considerable threat to human health (Sarwar et al. 2010). The vital health risks related to heavy metals comprise of cardiac diseases, chronic anemia, memory loss (Iqbal 2012), cancer, kidney damage (Wuana and Okieimen 2011), and impairment of nervous system, brain (Jarup 2003), skin, teeth, bones (Luo et al. 2012).

Several physicochemical approaches have been employed for remediation of toxic heavy metal, which consist of leaching, thermal treatment, excavation, electro-reclamation, and landfill. These technologies are fast but insufficient, expensive, and sometimes result in secondary pollution and thus have detrimental effects on the environment (Doble and Kumar 2005; Hooda 2007; Glick 2010; Ali et al. 2013). Due to adverse effects of the conventional physicochemical treatment methods, bioremediation has come into the existence. Bioremediation is regarded as a highly effectual technique for the remediation of toxic substances, because it is a natural, eco-friendly, and economical process (Doble and Kumar 2005). Bioremediation techniques comprise phytoremediation, bioventing, bioleaching, land farming, bioreactors, composting, bioaugmentation, and biostimulation. Phytoremediation uses plants for remediation of toxic substances from the environment. This method has been highly touted in the last decades (Glick 2010; Ali et al. 2013; Ma et al. 2016a; Sobariu et al. 2016). Phytoremediation works by several ways such as: (1) Phytoextraction is the translocation of metals from contaminated soils to the ground surface via the root system of the plants. (2) Phytostabilization employs specific plants to minimize the translocation and bioavailability of soil/water pollutants. (3) Phytodegradation is the transformation or breakdown of heavy metals by plant metabolic processes after uptake from the soils. (4) Phytovolatilization uses plants for metal extraction and conversion into volatile forms, which are then liberated to the atmosphere. (5) Rhizofiltration is associated with absorption, concentration, and precipitation of heavy metals from the water by aquatic or land plants. Several plant species have been identified as hyper accumulators due to their ability to absorb metal contaminants and accumulate them to unusually high levels. The hyper accumulator plants belong to *Pteris vittata, Sedum plumbizincicola, Thlaspi caerulescens, Alyssum serpyllifolium, Arabidopsis halleri, Phytolacca americana,* and *Solanum nigrum* (Ma et al. 2011a, b, 2015; Chen et al. 2014; Wei et al. 2014). However these hyper accumulators absorb high amounts of toxic metals but still are not competent for the remediation due to very low biomass production, slow growth rates, and selectivity for specific metals only (Abhilash et al. 2012;

Braud et al. 2006). Thus, it is of utmost importance to institute suitable phytoremediation techniques for remediation of multi-metal polluted field soils.

For upgrading the phytoremediation techniques, many researchers have focused on the interactions among plants–microbes and heavy metals in the rhizospheric soils (Glick 2010; Dharni et al. 2014; Ma et al. 2015). Inoculation of plants with screened bioaugmented microbes has accomplished eminence for phytoremediation of metal polluted soils (Lebeau et al. 2008; Glick 2010; Ma et al. 2011a, b). Microbial assisted phytoremediation comprises removing pollutants from contaminated soils by mutual interaction of plant roots and suitable microbiota, and is an effective eco-friendly, esthetic, and inexpensive method for treating a wide variety of contaminants.

## 4.2 Microbial Assisted Phytoremediation

The soil present in the vicinity of plant roots along with the roots is considered the rhizosphere, zone where enormous microbial activity has been described. Rhizosphere is a significant habitat and ecosystem for microbes (bacteria, fungi, algae, and protozoa). Rhizosphere is the hotspot of plant benefitting microbiome showing mutualistic plant–microbe interactions (Hinsinger et al. 2009). The rhizospheric microbes interact with the plants in several ways (Azevedo et al. 2000; Hao et al. 2012). Throughout all the developmental phases of plants, there is a mutualistic association among soil, plant, and microbes. This alliance develops when the rhizospheric microbes are triggered by the release of plant rhizo deposits. Root exudates ultimately stimulate specific microbial enzymes of the rhizospheric microbiome resulting in enhanced rhizodegradation. These microbial communities may work by influencing the metal availability and its uptake into the plant rhizosphere (Sarwar et al. 2017). Microbial revitalization using plant growth promoters had been achieved through various direct and indirect approaches including enhancing root growth, bio-fertilization and rhizoremediation, etc. (Gouda et al. 2018). According to Etesami (2018), PGPR increase plant growth and plant tolerance to biotic and abiotic stresses using different mechanisms including: phytostimulation (Glick et al. 2007; Spaepen et al. 2007), microbes assisted uptake of macro and micronutrients (Spaepen et al. 2007), and antagonistic effect of beneficial microbes against plant pathogens (Dawar et al. 2010). PGPR enhance plant growth by employing several mechanisms like phosphate solubilization, production of phytohormones, or nitrogen fixation that ultimately results in enhanced root growth; enzymatic activity; suppression of plant pathogens by production of antibiotics (Tabassum et al. 2017).

## 4.3 Phytoremediation of Heavy Metals Facilitated by Soil Bacteria (Table 4.1)

Plant growth promoting rhizobacteria aid the host plants to acclimatize to adverse condition of soil and elevate the ability of remediation by enhancing plant growth, reducing metal stress and metal phytotoxicity, improving metal bioavailability in soil and metal translocation in plants (Ma et al. 2011a, b). PGPR-aided metal remediation occurs by direct or indirect mechanisms. The direct mechanisms include: improved bioavailability, solubility, and accumulation of toxic metals. The indirect mechanisms involve promoting plant growth and protecting plant from pathogenic organisms. Some PGPR also help the host plants to adapt to suboptimal soil conditions and improve the capability phytoremediation by plant growth promotion, reducing metal toxicity, improving metal bioavailability in soil, and enhancing metal translocation within the plant.

## 4.4 Direct Mechanisms

Soil microbes are an integral part of various biogeochemical cycles of the essential nutrients (Khan et al. 2010). The direct mechanisms comprise biological nitrogen fixation, phosphate solubilization and phytohormone production and siderophores production. Some PGPR may also reduce toxic effects of heavy metals and thus promote plant growth and development through any of the abovementioned direct methods (Rajkumar et al. 2009; Pereira and Castro 2014).

### *4.4.1 Nitrogen Fixation*

The atmosphere consists of 78% of $N_2$; however, this atmospheric $N_2$ cannot be utilized by the plants in this form. Several diazotrophic microbes are capable of fixing the atmospheric $N_2$ and can do so even in heavily metal contaminated soils. The usage of such nitrogen fixing bacteria as in case of legume-Rhizobium symbiosis in metal contaminated soils not only promotes plant growth and development but also enhances soil fertility (Brígido et al. 2013). Nonnoi et al. (2012) have reported *Rhizobium bv. trifolii,* which is capable of fixing atmospheric nitrogen in heavy metal contaminated soils. It has also been reported that metal stress enhances nitrogen fixation rate and nitrogen accumulation rate in plants growing in nitrogen-poor ecosystems (Gupta and Panwar 2013). A number of heavy metal resistant microorganisms belonging to rhizobial strains, including *Bradyrhizobium, Rhizobium, Mesorhizobium, Azorhizobium,* and *Sinorhizobium*, have been reported till date from various metal contaminated soils (Zheng et al. 2004; Fan et al. 2010; Wani and Khan 2013).

**Table 4.1** Phytoremediation Of heavy metals assisted by soil microorganisms

| Bacteria | Source | Metal | PGPR | Reference |
|---|---|---|---|---|
| *Pseudomonas* sp. TLC | *Zea mays and Helianthus annuus* | Zn, Pb, Cu, As | IAA, phosphate solubilization, enhanced plant growth and Cu uptake | Li and Ramakrishna (2011) |
| *Serratia* sp. LRE07 | *Solanum nigrum* L. | Cd, Zn | IAA, ACC deaminase, phosphate solubilization, Siderophores, bioaccumulation of Cd and Zn | Luo et al. (2011) |
| Bacillus sp. MN3–4 | *Alnus firma and Brassica napus* | Zn, Ni and Cu | IAA, Siderophores, ACC deaminase, phosphate solubilization, bioremoval and bioaccumulation of Pb | Shin et al. (2012) |
| *Bacillus* sp. SLS18 | *Sorghum bicolor* L. | Cd, Mn | IAA, ACC deaminase, phosphate solubilization, Siderophores, enhanced plant growth and metal uptake | Luo et al. (2012) |
| *Bacillus thuringiensis* GDB-1 | *Alnus firma* | Zn, Pb, Cu, As, Ni, Cd | IAA, Siderophores, phosphate solubilization, enhanced plant growth and accumulation of As, Cu, Pb, Ni, and Zn | Babu et al. (2013) |
| *Rahnella* sp. JN6 | *Brassica napus* | Cu, Pb, Cd, and Zn | IAA, Siderophores, ACC deaminase, phosphate solubilization, increased uptake of Cu, Pb, Cd, and Zn in plant tissue | He et al. (2013) |
| *Bradyrhizobium* sp. YL-6 | *Lolium multiflorum* lam *and Glycine max* | Cd | IAA, Siderophores, phosphate solubilization, enhanced plant growth and Cd uptake | Guo and Chi (2014) |
| *Arthrobacter nicotinovorans* SA40 | *Alyssum pintodasilvae* | Ni | IAA, Siderophores, phosphate solubilization, growth promotion, and phytoextraction of Ni | Cabello-Conejo et al. (2014) |
| *Acinetobacter* sp. nbri05 | *Cicer arietinum* | As (V & III) | IAA, Siderophores, ACC deaminase, phosphate solubilization | Srivastava and Singh (2014) |
| *Acinetobacter* sp. RG30 and *Pseudomonas putida* GN04 | *Zea mays* | Cu | IAA, Siderophores phosphate solubilization | Rojas-Tapias et al. (2014) |
| *Sinorhizobium meliloti* CCNWSX0020 | *Medicago lupulina* | Cu | IAA, Siderophores, ACC deaminase, enhanced plant growth and Cu uptake | Kong et al. (2015) |
| *Bacillus pumilus* E2S2 | *Sedum plumbizincicola* | Zn, Cd | IAA, Siderophores, ACC deaminase, phosphate solubilization, enhanced plant growth and Cd uptake | Ma et al. (2015) |

(continued)

**Table 4.1** (continued)

| Bacteria | Source | Metal | PGPR | Reference |
|---|---|---|---|---|
| *Bacillus* sp. E1S2 | *Sedum plumbizincicola* | Zn, Cd, Pb | IAA, Siderophores, ACC deaminase, phosphate solubilization, enhanced plant growth and Zn uptake | Ma et al. (2015) |
| *Rhodococcus erythropolis* NSX2 | *Sedum plumbizincicola* | Zn, Cd, Cu | IAA, phosphate solubilization, enhanced plant growth and Cd uptake | Liu et al. (2015) |
| *Pseudomonas koreensis* AGB-1 | *Miscanthus sinensis* | Zn, Pb, Cu, As, Cd | Enhanced plant growth and metal uptake | Babu et al. (2015) |

### 4.4.2 Phosphate Solubilization

Phosphorous (P) is one of vital micronutrients for plants due to its involvement in several metabolic reactions of glucose transport, root growth and development, growth promotion of microbes and plants as well as in various physiological processes (Ahemad 2015). Soils are rich source of phosphorous; however, it exists in insoluble form and thus not accessible to the plants. Several microbes have been reported till date to produce organic acids capable of solubilizing the insoluble form of phosphate. *Pseudomonas, Bacillus,* and *Rhizobium* are some of the predominating phosphate-solubilizing microbes (Chen et al. 2006; Rodríguez et al. 2006). In heavy metal-contaminated soils, PGPR capable of phosphate solubilization play a vital role since the organic acids secreted by them for phosphate solubilization can also solubilize insoluble and stable metals. Therefore these phosphate-solubilizing microbes can enhance the heavy metal bioavailability for uptake by phytoextracting or phytoaccumulating plants (Becerra-Castro et al. 2011; Ahemad and Kibret 2014). Li and Ramakrishna (2011) have reported an enhanced copper accumulation and plant biomass in maize and sunflower plants after inoculating a copper-resistant strain *Pseudomonas* sp., TLC 6–6.5-4. In another study, on inoculation of canola plants growing in heavy metal contaminated soils with PGPR *Rahnella* sp. JN6, a significant increase in plant biomass and increase in concentrations and uptake of Cd, Pb, and Zn in aerial and root tissues of canola plants were observed (He et al. 2013).

### 4.4.3 Siderophore Production

Iron is one of the most abundantly found elements on earth; however, it is not accessible to plants and microbes for direct assimilation, since in natural conditions it is present mostly in $Fe^{3+}$ form. $Fe^{3+}$ exists in insoluble forms of hydroxides and oxyhydroxides (Rajkumar et al. 2010). Since iron is a vital micronutrient for growth and development of living organisms, several PGPR obtain iron by producing low-molecular weight iron-chelating molecules called siderophores. The siderophores show high binding affinity ($Ka > 1030$) for Fe and thus solubilize iron from minerals or organic insoluble compounds by functioning as iron solubilizing agents (Hider and Kong 2010). Besides iron, siderophores are also capable of forming stable complexes with several other metal ions, such as Al, Cd, Cu, Ga, In, Pb, and Zn as well as with radionuclides including U and Np (Kiss and Farkas 1998; Neubauer et al. 2000). Diverse group of bacteria have been reported to produce siderophores. Siderophores production by PGPR has also been reported from multiple environmental conditions such as heavy metal contaminated soils or nutrient deficient soils (Rajkumar et al. 2010; Ullah et al. 2015). Siderophores reported from *Streptomyces tendae* F4 have remarkably enhanced plant growth and metal uptake (Fe and Cd) by sunflower (*Helianthus annuus*) (Dimpka et al. 2009). A siderophore-producing bacterium *Kluyvera ascorbata* SUD165 has been reported to reduce heavy metal (Ni, Pb, and Zn) toxicity in canola, Indian mustard, and tomato. Beside this, a siderophore overproducing mutant, SUD165/26, of this bacterium showed greater metal toxicity protection, increased plant biomass, and chlorophyll content in plants grown in Ni-contaminated soil (Burd et al. 1998).

### 4.4.4 Production of Organic Acids

Low-molecular weight organic acids (Citric, oxalic, and gluconic acid) production has been reported by numerous PGPR (Ullah et al. 2015). These organic acids show the capability to enhance solubility and mobility of toxic heavy metals. In addition, these organic acids also increase heavy metal uptake by plants by regulating the complexion reactions (Rajkumar et al. 2010; Ullah et al. 2015). In this context, 5-ketoglutonic acid, a derivative of gluconic acid, produced by *Gluconacetobacter diazotrophicus* was found to increase the solubilization of Zn compounds.

### 4.4.5 Production of Biosurfactants

PGPR are also reported to synthesize biosurfactants, which are capable of enhancing mobility and subsequent phytoremediation of toxic heavy metals. The biosurfactants form complexes with several toxic heavy metals at surface of soil resulting in desorption of metals from soil matrix. This results in increase in the heavy metal availability to plants (Gadd 2010; Rajkumar et al. 2012). Biosurfactants produced by *Pseudomonas aeruginosa* BS2 have been reported to enhance the mobility and solubility of Cd and Pb (Ullah et al. 2015).

### *4.4.6 Bioleaching*

Oxidation-reduction reactions possess important capability to mobilize metalloids, metals, and influence compounds of organometallic nature (Bolan et al. 2014). A remarkable enhancement in the solubility of Mn(IV) and Fe(III) was reported by Ullah et al. (2015) when these metals are reduced to Mn(II) and Fe (II). Several PGPR are able to catalyze the enzymatic reduction of $Hg^{2+}$ to metallic mercury($Hg^0$) which is highly volatile in nature, thus enhancing the tolerance and detoxification process in soil (Gadd 2010). Similarly, PGPR can also solubilize Cu in Cu rich soils thus enhancing the bioavailability of Cu in the plants (Shi et al. 2012). Furthermore, bioleaching is also regarded as vital method of mobilization of minerals from their ores. *Pseudomonas fluorescence* and *Chromobacterium violaceum* capable of producing hydrogen cyanide are also able to carry out the mobilization of Cu and Ni by formation of different cyanide compounds and complexes by using various solid materials like electronic scarp and Cu-containing ores (Gadd 2010; Ullah et al. 2015).

Microorganisms can also fix $CO_2$ by carrying out reduction of sulfur containing compounds or oxidization of ferrous iron which results in acidification of the environment. In consequence the produced $H_2SO_4$ and Fe (II) improve metal solubilization (Gadd 2004). *Leptospirillum ferrooxidans* has been reported to oxidize iron, similarly *Thiobacillus ferrooxidans* has been reported to oxidize sulfur and iron and *Thiobacillus thiooxidans* to carry out oxidation of sulfur (Ullah et al. 2015). The metal oxides formed as a result of oxidation of sulfur and iron is solubilized in their acidified environment which in turn causes solubilization of various metal compounds. Metals can also be mobilized through biomethylation by microorganisms resulting in volatilization. A number of microbes possess the ability to mediate methylation of Sn, Tn, As, Se, Hg, and Pb by carrying out the transfer of methyl group to metals. The methylated metal compounds formed however differ greatly in toxicity, solubility, and volatility (Bolan et al. 2014; Ullah et al. 2015).

### *4.4.7 Phytochelatins*

Plants and fungi are capable of carrying out biosynthesis of phytochelatins (PCs) from glutathione under heavy metal toxicity (Gadd 2010). Phytochelatins rich in cysteine are important metal binding peptides. However some recombinant bacteria are also capable of producing phytochelatin synthase that gets activated in presence of various heavy metals such as Hg, Pb, Cd, and Cu. Phytochelatin synthase is responsible for increasing mobility and bioavailability of toxic metals by synthesizing thiol complexes able to bind metals (Kang et al. 2007). A recombinant *E. coli* strain was produced by Kang et al. (2007) by adding SpPCS of *Schizosaccharomyces pombe* that caused increased PCs synthesis. This recombinant strain was found to accumulate Cd 7.5-folds greater than wild type strain (Kang et al.

2007). Cysteine rich polypeptides (Metallothioneins) possess greater binding affinity for heavy metals such as Ag, Cu, Hg, Zn, and Cd (Ullah et al. 2015). Genes encoding metallothioneins are found in some prokaryotes, plants, and animals (Sriprang et al. 2002; Ullah et al. 2015). Bacteria having greater metal binding capacity through metallothioneins possess the potential to accumulate or sequester heavy metals. Metallothionein encoding genes have been expressed in diverse organisms including bacteria for increasing the accumulation of toxic heavy metals.

## 4.5 Indirect Methods

### *4.5.1 Induced Systemic Resistance*

PGP bacteria exhibit substantial ability to prevent plants from infection of pathogens, increase biomass and metal tolerance that enable plants to eliminate pollutants from the environment (Hassan et al. 2016). Several PGPR are known to diminish the stress effects in plants by suppressing damage caused by phytopathogens either via biological control of pathogens or by developing induced systemic resistance (ISR) of plants against pathogens (Harish et al. 2008). PGPR may effectively limit the phytopathogen infestation by producing antibiotics, siderophores, variety of hydrolytic enzymes (such as chitinases, proteases, and glucanases), and antimicrobial volatile organic compounds (Sheoran et al. 2015). In many biocontrol systems, one or more antibiotics have been shown to play important role in disease suppression. *Bacillus megaterium* BP 17 and *Curtobacterium luteum* TC 10 effectively suppressed burrowing nematode disease (*Radopholus similis* Thorne) by the synthesis of antibiotics (Aravind et al. 2010). PGPR can also act as effective competitors of pathogens for colonization in niches, availability of nutrients and by minimizing the adverse environmental impacts with nutrient loss and thereby indirectly promote plant productivity (Alvin et al. 2014). ISR refers to the state of systemically enhanced resistance in plants to a broad spectrum of pathogens (Kloepper and Beauchamp 1992). The ISR to various diseases caused by certain chemicals and plant growth promoting bacteria is a highly beneficial approach in protection of agroecosystem. ISR induced by PGPR has been demonstrated against various fungal, bacterial, and viral pathogens in many plant taxa (Alvin et al. 2014). Plants primed with bacterial inoculation induce a plant defense system (Sturz and Matheson 1996). Once the defense genes are expressed, ISR activates multiple potential defense mechanisms that include increased activity of chitinases, β-1,3-glucanases, superoxide dismutase, guaiacol, catalase (CAT), and peroxidases (POS) (Gechev et al. 2006). The activities of these enzymes are responsible to reduce reactive oxygen species production and protection of cell organelles against oxidative stress as a result of the activities of endophytic bacteria (Wan et al. 2012).

### *4.5.2 Phytohormones Production*

PGPR are also reported to regulate a number of physiochemical processes of plants including mineral uptake, osmotic modifications, stomatal regulation, and alterations in root morphology (Amaral et al. 2016; Ma et al. 2016a). Plants often face various environmental constraints including heavy metal stress that disturb physiochemical processes of the plants. A variety of PGP bacteria from rhizosphere are reported to produce phytohormones (gibberellins, cytokinins, IAA) even under abiotic stresses. Therefore, phytohormone producing bacteria promote significant alterations in endogenous plant hormones that shape the response of plants for different environmental constraints (Verbon and Liberman 2016). Ethylene is known to play essential role in regulating different physiological responses of plants but greater production of ethylene is lethal for plant growth. However, some PGP bacteria contain ACC deaminase enzyme that breaks down ACC into a ketobutyrate and ammonia. ACC is the precursor for ethylene biosynthesis (Hassan et al. 2016). Therefore, bacteria containing ACC deaminase generates ammonia that adds to nitrogen source of plants. Moreover, PGP bacteria also act as biocontrol agents because these bacteria prevent plants from phytopathogens (Glick 2012). Antifungal metabolites including tensin, 2,4-diacetylphloroglucinol, vicosinamide, pyoluterin, HCN, pyrrolnitrin, phenazines are produced by PGP bacteria (Ahemad and Kibret 2014). In nutshell, PGP bacteria enhance plant growth in soils contaminated with metals and metalloids that further enhance phytoremediation of heavy metals.

## 4.6 Mechanisms Responsible for Altering Plant Metal Uptake

### *4.6.1 Amelioration of Metal Stress*

The metal phytotoxicity is a critical factor affecting the success of phytoremediation (Shin et al. 2012). To overcome the metal stress, a number of bacterial mediated processes are involved in bacteria–host coevolution process either by alleviating metal toxicity or by conferring plant metal tolerance (Rajkumar et al. 2009; Ma et al. 2015). Recent studies suggest that some PGPR can reduce metal phytotoxicity via several mechanisms that include extracellular precipitation (Babu et al. 2015), intracellular accumulation and sequestration (Shin et al. 2012), biotransformation of toxic metal ions to less- or nontoxic forms (Zhu et al. 2014), and adsorption/desorption of metal ions (Guo et al. 2010; Luo et al. 2011). Abiotic or biotic stress can be alleviated or eliminated by the genes encoding metal or antibiotic resistance proteins. Shin et al. (2012) recently found that endophytic bacterial strain *Bacillus* sp. MN3–4 had evolved a better defined metal resistant mechanism, e.g. active export via a P-type ATPase efflux pump, which can transport metal ions across biological membranes against the concentration gradient using energy released by

ATP hydrolysis. Wan et al. (2012) and Zhang et al. (2010) found that bacteria could modulate the activity of plant antioxidant enzymes (such as POS, CAT, SOD, glutathione peroxidase, ascorbate peroxidase) as well as lipid peroxidation (malondialdehyde formation), which confronted plant defense mechanisms, especially resisted the heavy metal-induced oxidative stress in plants. In addition, certain PGPR can also use methylation as a metal resistance or detoxification mechanism. Some mercury resistant bacteria express MerB gene encoding organomercurial lyase which cleaves organomercurials into mercuric ion ($Hg^{2+}$) (Brown et al. 2003), and MerA gene encoding mercuric reductase which converts highly toxic ionic $Hg^{2+}$ into less toxic volatile $Hg^0$ (Cursino et al. 2000), thus alleviate metal toxicity and improve the efficiency of phytovolatilization. The genes specifying various functions needed for degradation of organomercurials and reduction of $Hg^{2+}$ are organized in the mercury resistance (Mer) operon, which is mostly found in gram-negative bacteria (Brown et al. 2003).Currently, several studies have focused on the role of bacteria on heavy metal tolerance and accumulation in hyperaccumulator and non-accumulator plants. However, it is not yet analyzed whether plants growing in metal polluted soils alter colonization/survival potential of specific metal resistant and/or beneficial microbes. Thus, it is critical to explore the diversity, distribution, and activity of endophytic microbial communities associated with various hyperaccumulator plants in phytoremediation studies.

### *4.6.2 Metal Biosorption and Bioaccumulation*

Various reports have exhibited that PGPR play a significant role in reduction of metal phytotoxicity by biosorbing and bioaccumulating heavy metals (Guo et al. 2010; Luo et al. 2011). A lead resistant bacteria *Bacillus* sp. MN3–4 was obtained from roots of *Alnus firma* (a metal hyperaccumulator plant) by Shin et al. (2012). This strain was found to improve phytoremediation capacity by decreasing metal phytotoxicity by extracellular sequestration and intracellular accumulation. In a similar study conducted by Luo et al. (2011), a cadmium resistant *Serratia* sp. LRE07 was reported to absorb about 65% of Cd and 35% of Zn in cells from single metal solution by the process of biosorption and bioaccumulation. This resulted in significantly reducing phytotoxic effects of the toxic heavy metals. In this aspect, the bacterial cell wall has been considered as a significant component for metal ion absorption (Bai et al. 2008). The process of metal biosorption by microorganism occurs in the following two steps: (1) Passive biosorption of metals by both live and dead/inactive cells that occurs by the cell wall owing to various metabolism-independent processes (Vijayaraghavan and Yun 2008). In the passive process, metal ions are first adsorbed rapidly on the microbial cell surface due to interactions between metals and functional groups (hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, amide, and phosphonate) present on the cell surface (Ma et al. 2011b). Several mechanisms such as ion exchange, microprecipitation, sorption, complexation, electrostatic interaction, coordination, and chelation are proposed to be responsible either synergistically or independently for the complexation processes (Volesky and Schiewer 1999); (2) Active biosorption (bioaccumulation) involves metal uptake (transport into cells, accumulate intracellularly, across cell membrane through cell metabolic cycle) by live cells by a slow active metabolism-dependent transport of metal into the microbial cells (Malik 2004). As soon as the metal is inside the living microbial cells, it may get bound, precipitated, accumulated, sequestered within specific intracellular organelles or may get translocated to specific structures, depending upon the type of organism and element concerned (Ma et al. 2011b). Different bacteria carry distinct and unique metal bioaccumulation abilities that can be utilized in plant–bacteria symbiotic system for facilitating heavy metal detoxification and enhanced phytoremediation efficiency (Guo et al. 2010; Shin et al. 2012).

### *4.6.3 Metal Bioavailability*

The translocation of heavy metals from contaminated soil to plant tissues depends on heavy metal bioavailability in the soils (Glick 2010). Lebeau et al. (2008) reported that bioavailability of heavy metals in soils is determined by different elements of soil such as particle size, nutrients, pH, redox potential, organic matter content, and presence of other metal ions. A number of studies proposed that PGPR having metal resistance/sequestration pathway (e.g. ncc-nre) may help in reduction in phytotoxicity of pollutants (Luo et al. 2011) and may enhance the phytoavailability of heavy metals by liberating metal chelating agents (e.g. siderophores, biosurfactants, and organic acid), acidification of soils, redox activity, and phosphate solubilization (Ma et al. 2011a). PGPR are able to increase metal and mineral solubilization from soils by producing low-molecular weight organic acids and metal-specific ligands like siderophore, which change the soil pH and enhance chelation efficiency (Rajkumar et al. 2009). In recent time, researchers have examined the function of microbes in heavy metal translocation by plants. Reports have indicated that organic acids production by microbes generally enhances under heavy metal stress conditions (Chen et al. 2014), thus suggesting the important role of these organic acids in heavy metal mobilization by plants. Sheng et al. (2008) examined the lead solubilizing potential of *Pseudomonas fluorescens* G10 and *Microbacterium sp.* G16 under in vitro conditions with Pb-amended and Pb-free media. They examined the application of Pb resistant bacteria on Pb uptake by *Brassica napus* and reported that both strains increased Pb availability by enhancing Pb accumulation in plant shoots from 76% to 131% (*P. fluorescens*) and from 59% to 80% (*Microbacterium* sp.), compared to the control treatments. Sheng et al. (2008) proposed that Pb accumulation in plants is due to synthesis of siderophore or organic acid by these bacterial strains.

Several endophytic bacteria have been reported to also produce biosurfactants. These biosurfactants have been reported to enhance the bioavailability of poorly soluble metals and to accelerate phytoremediation rate (Bacon and Hinton 2011).

Biosurfactants are low-molecular weight amphiphilic molecules comprised of a hydrophilic and a hydrophobic regions and contain different function groups such as mycolic acid, glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids, etc. (Banat et al. 2010). Biosurfactants are capable of reducing the surface tension, critical micelle concentration (CMC), and interfacial tension, thus influencing the dispersal of the metals among the different phases (Banat 1995). PGPR synthesize biosurfactants and excrete them to the host niches as root exudates. The secreted biosurfactants at first form metal complexes with the insoluble metals on the interface of rhizosphere soil particles, and then desorb metals from soil matrix, resulting in altered metal translocation and bioavailability in the soil (Rajkumar et al. 2009). The interactions of PGPR, toxic heavy metals, and biosurfactants can be considered as a vital element in facilitating metal uptake from contaminated soils (Bacon and Hinton 2011). It has also been proposed that it is feasible to enhance metal phytoextraction ability of hyper accumulating plant species by treating the plant seeds or rhizosphere soil with beneficial metal resistant bacteria.

*Methylobacterium oryzae* CBMB20 and *Burkholderia* sp. CBMB40 obtained from rice tissues were reported to reduce metal toxicity and accumulation and translocation of Ni and Cd in shoots of tomato plants on inoculation with these microbial strains. The reason of reduced accumulation of the toxic heavy metal may be due to immobilization of metals in rhizospheric soil by the bacterial strains. Production of various extracellular polymeric substances (EPS) mainly polysaccharides, proteins, nucleic acids, and lipids by microbes seems to play an important role in metal complexation and thus reduces metal bioaccessibility and bioavailability (Rajkumar et al. 2013). Joshi and Juwarkar (2009) have examined the capability of EPS, secreted by *Azotobacter* sp. in forming metal complexes by binding up to 15.2 mg $g^{-1}$ of Cd and 21.9 mg $g^{-1}$ of Cr, resulting in reduced metal accumulation by *Triticum aestivum*.

### *4.6.4 Metal Uptake and Translocation*

PGPR can alter heavy metal bioavailability and its translocation in plants, hence modify metal toxicity and plant metal uptake by secreting a variety of metabolites including siderophores, organic acids (e.g., citric, oxalic and acetic acids), etc. (Kuffner et al. 2010; Luo et al. 2012; Visioli et al. 2014). PGPR may also alter metal accumulation capacity and its translocation in plants by using their multiple plant growth promoting traits including metal resistance, detoxification, accumulation, transformation and sequestration, thus diminish metal phytotoxicity and alter phytoavailability of heavy metals in contaminated soils (Ma et al. 2016a, b). Sun et al. (2010) reported that inoculation with the copper-resistant endophytic bacteria having multiple plant growth promoting characteristics, such as ACC deaminase and arginine decarboxylase activity, enhanced plant growth and Cu transfer from root to above-ground tissues in *B. napus*, thus improving overall phytoextraction potential. Ma et al. (2011a) showed that Ni resistant endophytic *Pseudomonas* sp. A3R3 increased plant biomass (non-host *Brassica juncea*) and Ni accumulation in plants (host *A. serpyllifolium*), grown in artificially Ni-contaminated soil. This effect can be attributed to its ability to produce plant growth promoting substances (ACC deaminase, siderophores, IAA and P solubilization) and plant polymer hydrolyzing enzymes (cellulase and pectinase). Therefore, the plant associated PGPR are now being considered as a bio-tool to improve the efficiency of phytoremediation of metal polluted soils. The metal phytotoxicity is a critical factor inhibiting the success of phytoremediation (Shin et al. 2012). A lead resistant bacterium, *Bacillus* sp. MN3–4 isolated from the roots of the metal hyperaccumulator *Alnus firma* enhanced phytoremediation potential by reducing the phytotoxic effects of metals via extracellular sequestration and intracellular accumulation (Shin et al. 2012). PGPB also have great potential to enhance metal and mineral mobilization by the production of organic acids and metal-specific ligands (e.g. siderophores), and subsequently increase nutrient levels and heavy metal uptake, which may result in improved metal phytoextraction potential of host plants (Chen et al. 2014). Moreover, PGPB may also alter metal bioavailability and its translocation in plants, hence modifying metal phytotoxic effects and increasing plant metal uptake. Copper-resistant PGPB having multiple plant growth promoting traits enhanced plant growth and Cu translocation from roots to aerial parts of *B. napus*, thus improved overall phytoextraction potential of the plant (Sun et al. 2010). On the other hand, some PGPB are effective in reducing metal availability in soils and plant metal accumulation. A recent study reported that two metal resistant bacteria (*B. megaterium* H3 and *Neorhizobium huautlense* T1–17) decreased Cd bioavailability in rhizosphere soils, resulting in reduced Cd accumulation in polished rice (Li et al. 2017). Reduction in Cd uptake was dependent on the bacterial strain used and the level of metal contamination.

## 4.7 Reports of PGP Bacteria-Assisted Phytoremediation

Several reports on PGPR capable of enhancing phytoremediation are available in the literature (Glick 2010). A metal resistant PGPR was obtained from *Polygonum pubescens* plants growing in metal contaminated soil by Jing et al. (2014). These PGPR were then identified as *Enterobacter* sp. and *Klebsiella* sp. *Brassica napus* was observed to hyper accumulate toxic heavy metals (Zn, Pb, and Cd) on inoculation with these metal resistant PGPR strains. In another study, inoculating *Brassica napus* with *Pseudomonas tolaasii* ACC23, *Pseudomonas fluorescence* ACC9, and *Mycobacterium* sp. ACC14, an improved metal (Cd) uptake in aerial parts of plants was reported by Dell'Amico et al. (2008). Rye grass displayed an enhanced metal (Cr, Cd, Zn, Cu, and Pb) tolerance ability on inoculation with *Microbacterium arborescens*, isolated from the rhizosphere of *Prosopis juliflora* (Khan et al. 2015a, b). A notable amount of toxic metals were found in the root and shoot of the plants inoculated with *Microbacterium arborescens* strains. Enhanced plant growth and development was also observed on the inoculation with *Microbacterium* *arborescens* due to plant growth promoting properties (siderophore and IAA production, P solubilization, and ACC deaminase activity) of this strain. Inoculation of *Frankia* sp. decreased accumulation of Ni and increased nodulation in *Alnus glutinosa* (Wheeler et al. 2001). A *Streptomyces mirabilis* P16B-1 strain was isolated from soil of uranium mining area; it showed tolerance against multiple metals including Zn, Co, Cd, Ni, and Cu. Reduced metal bioavailability and enhanced plant growth were observed in *Sorghum bicolor* plants inoculated with *Streptomyces mirabilis* P16B-1 strain as compared to untreated plants (Schutze et al. 2013). Similarly, treating maize plants with *Streptomyces* sp. HM1 showed higher tolerance for Cd and exhibited significantly higher chlorophyll contents (El Sayed et al. 2015). In another study, *Rhodococcus erythropolis* was obtained from metal polluted soil; it was shown to decrease metal uptake and enhance plant growth in *Pisum sativum*. Improved plant growth was related to P solubilization and IAA production ability of *Rhodococcus erythropolis* (Trivedi et al. 2007). Likewise on treating green chili with *Cellulosimicrobium cellulans,* it showed reduced Cr uptake. *Cellulosimicrobium cellulans* possessing P solubilization and IAA production ability makes it an ideal candidate for plant growth promoting bacteria (Chatterjee et al. 2009). It has been proposed that heavy metals removal by phytobacterial systems is given more preference over traditional phytoremediation system alone. Accumulation of heavy metals by plants is determined by different elements such as soil pH, oxidation state of metal and bacterial species. PGPR that are also involved in phytoremediation are capable of synthesizing siderophores, IAA (Indole-3-acetic acid), ACC deaminase, and solubilize phosphorous which then results in improved plant growth in addition to increased solubility and bioavailability of heavy metals. Consequently, plants are able to hyperaccumulate heavy metals due to enhanced solubility and bioavailability of toxic heavy metals.

## 4.8 Phytoremediation Through Genetically Engineered Bacteria

A number of organic pollutants have been bioremediated in situ through a symbiotic relationship between plants and genetically transformed bacteria (Ullah et al. 2015). The application of such genetically engineered PGPR for phytoremediation of toxic heavy metals has been proposed as a novel phytobacterial approach for removal of toxic heavy metals. Genetically engineered PGPR carry one or more genes that are inserted so as to enhance heavy metal remediation. Genes responsible for metal chelation, transportation, metal homeostasis, degradative enzyme, and metal uptake regulators are significant for consideration for production of recombinant strains (Singh et al. 2011). Gene for phytochelatin synthase (PCS) from *Schizosaccharomyces pombe* was inserted in *Pseudomonas putida* KT2440. Phytochelatins are molecules having greater binding affinity toward several toxic heavy metals due to presence of cysteine-rich peptides. The recombinant strain

KT2440-spPCS was reported to increase tolerance to Ag, Hg, and Cd, accumulated Cd in greater quantities which subsequently enhanced the phytoremediation efficiency. In addition the recombinant strain KT2440-spPCS remarkably increased germination and plant growth in wheat. This indicates the symbiosis behavior of *Pseudomonas putida* KT2440 in improving phytoremediation of lethal metals (Yong et al. 2014). A recombinant strain *Pseudomonas putida* containing metal binding peptide (EC 20) was found to have higher Cd binding and mitigated Cd induced cellular toxicity. On inoculating sunflower roots with this recombinant strain *Pseudomonas putida* 06909 a significant decrease in phytotoxicity of Cd and 40% rise in Cd accumulation in plant roots was observed. Enhanced plant growth and phytoextraction ability for heavy metals through symbiotic association of recombinant PGP bacteria was reported by Wu et al. (2006). Sriprang et al. (2002) have constructed a recombinant strain of *Mesorhizobium huakuii* sb. Sp. *rengei* B3 by inserting human tetrameric metallothionein (MLT4) and reported improved heavy metal remediation ability through this symbiotic phytobacterial system. In another study, Sriprang et al. (2003) have constructed a recombinant of *Mesorhizobium huakuii* sb. Sp. *rengei* B3 by introducing the *Arabidopsis thaliana* gene AtPCS for enhanced production of PCS that showed significant increase in Cd uptake. Similarly, symbiotic association of recombinant bacteria with *Astragalus sinicus* was found to enhance Cd accumulation in root nodules (Ullah et al. 2015). Likewise, a recombinant strain of *Mesorhizobium huakuii* sb. Sp. *rengei* B3 with MLT4 and AtPCS resulted in enhanced accumulation of Cd in cells. Symbiotic association of these strains with *Astragalus sinicus* caused threefold increase in Cd accumulation in plant roots. Furthermore, abovementioned strains AtPCS or MLT4 strain B3 were modified by introducing iron-regulated transporter 1 gene of *Arabidopsis thaliana*. A significant rise in the buildup of As and Cu level in nodules than Zn and Cd was observed when AtPCS or MLT4 strain B3 (recombinant strains) established a symbiotic association with *Astragalus sinicus* (Ike et al. 2008).

## 4.9 Conclusion

Heavy metals induce serious effects on plant biosphere and increase the pollution load in the environment. Thus, there is dire need to find out some solutions to eliminate these pollutants from the environment. Different biological and physiochemical approaches are being employed to eliminate heavy metals from polluted soils. Of these approaches, microbial assisted phytoremediation is a promising sustainable, viable, inexpensive, and safe method. Phytobacterial extraction of heavy metals safely eliminates heavy metals without rendering any toxic effect on the environment. PGPR with multifunctional activities such as production of siderophore, IAA, ACC deaminase, organic acids, biomethylation, induce redox reactions, enhanced synthesis of biosurfactants, and anti-pathogen metabolites prove to be a better choice in assisting the phytoremediation process in metal contaminated soils. PGP bacteria transform heavy metals into sparingly soluble metal complexes and bioavailable forms thus accelerating phytoremediation. Moreover, recombinant bacterial strains and overexpression of genes responsible for metal degradation can also be explored for remediation of heavy metals. In this context, a combination of different bacterial strains could be more effective in enhancing reclamation of polluted soils. It is imperative to establish some novel strategies to eliminate pollutants from our environment so that we may provide safe environment to our future generations.

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# Chapter 5
# Abiotic Stress: Its Outcome and Tolerance in Plants

**P. Rawat, D. Shankhdhar, and S. C. Shankhdhar**

**Abstract** The onset of nineteenth century along with anthropogenic pressure paved the way for global climatic variation which is a major factor for global undernourishment, malnutrition and endangered food security. The major upshot of climate change is abiotic stress like salinity, flood and drought that declines the agricultural productivity. Abiotic stress hampers the survival of the plants and restricts their growth and development. Each abiotic stress confers negative impact on plants by altering its physiology, morphology and metabolism. Production of reactive oxygen species during stress condition alters the structure and metabolic function in plants and restricts its growth. Drought is one of the serious threats to crop productivity among the abiotic stress that imposes multidimensional effects on plants. Drought alters physiology and anatomy of the plants and is the main reason for economic loss in terms of livestock and grain yield in both developed and developing countries. Plants adapt several resistance mechanisms to cope up with the drastic impact of stress. Main tolerance mechanisms are alteration in membrane structure, escaping the unfavourable conditions, activation of antioxidant defense system, production of compatible solutes for maintaining osmotic balance of the cell. Present manuscript focuses on the outcome of major abiotic stress in plants and their tolerance strategies against the variable environmental conditions.

## 5.1 Introduction

Contemporary civilization and industrialization have led to global climate change whose consequences like flood, drought, high temperature and global warming are quite perceptible and hazardous for soil heath, microbial diversity and sustainable crop production. Climate fluctuation also threatens the vulnerable and endangered organisms in the ecosystem that are incompetent to endure such environmental changes in their habitat. Climate variability has immense impact on food security.

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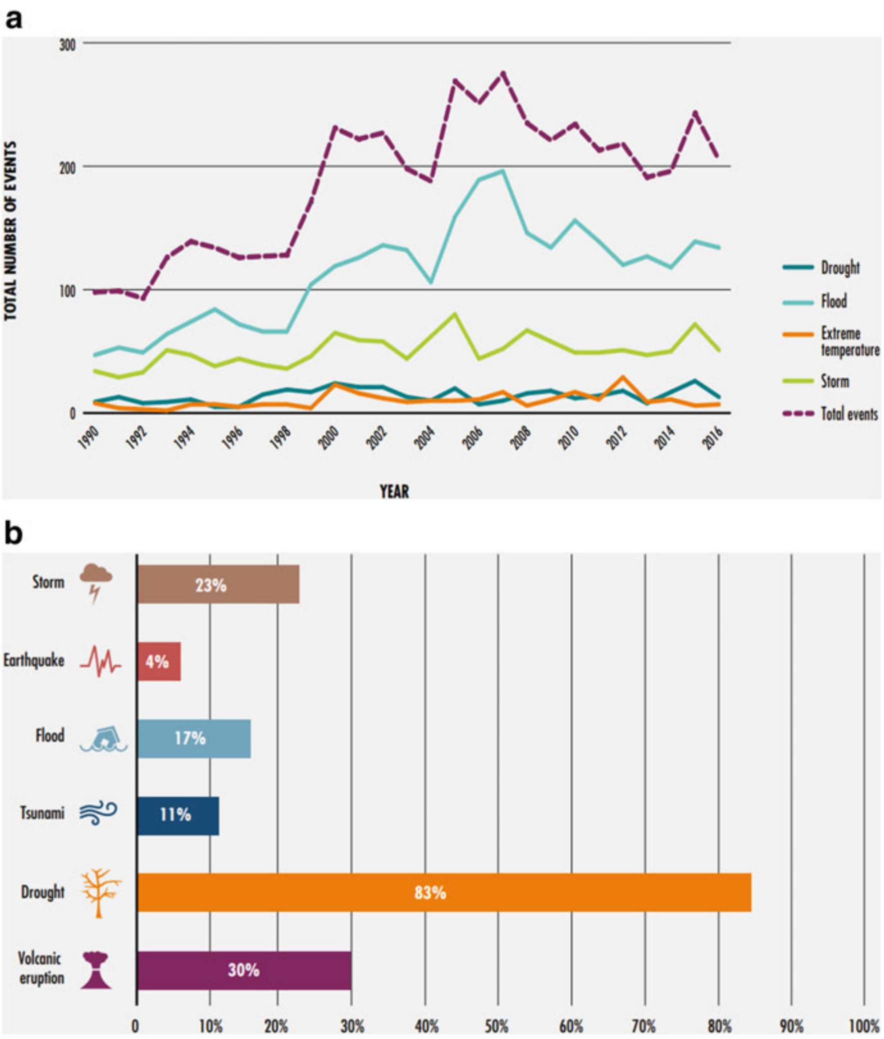

**Fig. 5.1** (**a**) Rise in the calamitous events (1990–2016). Adopted from FAO (2018). (**b**) Damage and loss in agriculture as share of total damage and loss across all sectors by type of hazards. Adopted from FAO (2018)

It hits the underdeveloped regions of the world causing yield and economic losses. It has been estimated that 26% of total loss was in agriculture sector due to calamitous events from 2006 to 2016 (FAO 2018). Figure 5.1a, b illustrates the severity of disastrous events in the past years along with its impact on agricultural sector. There has been a dire inflation in the number of undernourished people across the world from 806 million in 2016 to 821 million in 2017 as an outcome of climate shock (FAO 2018). In view of the above facts, the foremost challenge is to provide food security to the projected global population of about 9.7 billion in 2050 under climate fluctuation and dwindling crop productivity.

Abiotic stress is an aftermath of climatologically variation that ceases plant growth, development and productivity below optimum levels. A surfeit or scant in energetic, chemical or physical condition in the nature paved the origin of abiotic stress in plants. The utmost abiotic stress comprises of drought, salinity, flooding and oxidative stress that abolishes metabolic equilibrium of plants thereby threatening food security (Fedoroff et al. 2010). Economic forfeiture in the developing countries in terms of crop and livestock production was discerned up to USD 96 billion from 2005 to 2015 with a 2.6% decrement in national agricultural value added growth due to occurrence of each climatic hazards (FAO 2018). An alarming upshot of climate shock is forecasted to decline agriculture productivity and influence five billion people by 2050 (UNESCO 2018; Watts 2018).

## 5.2 Abiotic Stress in Plants

### *5.2.1 Salt Stress*

One of the most detrimental climate shocks is salinity stress which is prevalent in arid and semiarid regions in the world as depicted in Fig. 5.2. Approximately 33% of the world's irrigated land and 20% of overall cultivated land are devastated by salinity stress (Shrivastava and Kumar 2015). It has been forecasted that by 2050 around 50% of arable land would be influenced by salinity (Jamil et al. 2011). In India seven million hectares of land is sheathed by saline soil (Patel and Dave 2011). An electrical conductivity of precisely 4 $dSm^{-1}$ of saturation extract from root zone at 25 °C marks the saline soil (Jamil et al. 2011). Salinity damages the agricultural productivity as the major food crops are susceptible to it. A remarkable yield loss of

**Fig. 5.2** Salt affected regions of the world. Adopted from FAO (2016)

about 65% in wheat was evident as an outcome of salinity stress (Ahmad et al. 2013). Yield reduction of about 55% in corn and cotton was observed in case of salinity in the range of about 8–10 dSm$^{-1}$ and 18 dSm$^{-1}$, respectively (Satir and Berberoglu 2016; Zörb et al. 2019).

### 5.2.2 *Impact of Salinity Stress in Plants*

Salinity stress devastates the plants by two ways: (a) Osmotic effect that diminishes plant potential for water uptake due to excess salt in soil and (b) ion excessive effect that occurs due to accumulation of excess salt in the leaf tissues (Greenway and Munns 1980). Salinity amends the major physiological processes like photosynthesis, transpiration, nutrient regulation and osmotic balance as an outcome and affects plant growth and yield. Influence of salinity stress in plants has been illustrated in Table 5.1.

**Table 5.1** Outcome of salinity stress in plants

| Parameters | Alterations | References |
|---|---|---|
| Germination | Hinders water imbibition by seeds | Khan and Gul (2006) |
| | Alters the enzyme activity requisite for metabolic functions | Gomes-Filho et al. (2008) |
| | Deteriorates seed coat, diminishes the seed vigour index and surges seed dormancy | Panuccio et al. (2014) |
| Water relation | Osmotic imbalance leading to soaring salt concentration near roots | Munns (2005) |
| | Decrement in relative water content and turgor pressure | Ghoulam et al. (2002) |
| Plant growth | Minimizes water retaining capacity of plant | Munns (2002b) |
| | Enhances ion toxicity that results in early leaves senescence | Munns (2002a, 2005) |
| Photosynthesis | Accumulation of $Na^+$ and $Cl^-$ ions in chloroplasts | Sudhir and Murthy (2004) |
| | Alteration in electron transport chain by impeding photon phosphorylation and carbon metabolism | Farahbakhsh et al. (2017). |
| | Decrement in chlorophyll content | Saha et al. (2010) |
| | Modification in cytoplasmic structure, retardation in carboxylation reaction and generation of ROS | Maxwell and Johnson (2000) |
| Nutrient imbalance | High osmotic potential lessens nitrogen content in plants | Lea-Cox and Syvertsen (1993); Rozeff (1995); Bar et al. (1997) |
| | Low phosphorous content | Qadir and Schubert (2002) |
| | Reduced influx of $Ca^{2+}$ and $K^+$ ions | Suhayda et al. (1990); Hu and Schmidhalter (1997); Asch et al. (2000) |

## *5.2.3 Plant Tolerance Strategies against Salinity Stress*

Resistance strategies of plants to salinity rely on environmental conditions and plant species. Plants mainly endure salt stress by three ways:

(a) Endurance to osmotic stress
(b) Efflux of sodium from leaf blades
(c) Tissue tolerance

### 5.2.3.1 Osmotic Stress Endurance

Osmotic stress is marked by generation of osmolytes like glycine betaine, proline, polyols, etc. to maintain the osmotic balance. Osmotic tolerance also aids in balancing stomatal conductance and leaf expansion during salinity (Rajendran et al. 2009). Osmolytes stabilize the membrane integrity, proteins and pigments in the cell and also alleviate reactive oxygen species (Krasensky and Jonak 2012; Gupta and Huang 2014). It was evident that proline accumulation improved the salinity tolerance in maize (Tang et al. 2019). Likewise, trehalose accumulation also assisted in boosting salinity endurance in wheat (Sadak 2019).

### 5.2.3.2 Sodium Ion Efflux from Leaf Blades

Outcome of salinity stress in plants comprises ion toxicity due to sodium ion ($Na^+$) accumulation inside the cell. This can be overcome by down regulating the expression of ionic channels and $Na^+$ transporters (Munns and Tester 2008; Rajendran et al. 2009). An antiporter (AtNHX1) confined in tonoplast of *Arabidopsis* maintains $Na^+$ balance in the cell under salinity condition by directing the influx of $Na^+$ ions from cytosol to vacuole (Apse et al. 1999). Likewise NAX1gene in durum wheat impedes $Na^+$ translocation in the shoots and leaf sheath from roots, whereas NAX2 gene confers high potassium ion ($K^+$)translocation in leaf (Flagella et al. 2006). Sodium exclusion theory for salt resistance has been validated in rice (Ren et al. 2005) and durum wheat (James et al. 2006). It was studied that potassium transporter *(*PpHKT1) gene from rootstock of almond also enhanced salt tolerance in transgenic *Arabidopsis* by balancing ion homeostasis (Kaundal et al. 2019).

### 5.2.3.3 Tissue Tolerance

For hindering the senescence of leaves and enhancing its survival rate, tissue tolerance is a salient attribute during stress. Generation of compatible solutes that balances the osmotic homeostasis in the cell and antioxidant defense system that scavenges the reactive oxygen species under stress condition is an attribute of tolerance of plants to stress condition (Hasegawa et al. 2000; Zhu 2001; Sakamoto and Murata 2002). Tissue resistance also incites the ion compartmentalization in the cell to diminish toxicity of ion (Munns and Tester 2008). A notable rise in osmolyte production like proline up to 14.87 μmol $g^{-1}$ DW and an increment in antioxidants like ascorbate peroxidase up to 77%, catalase up to 15% and glutathione reductase up to 138% were recorded in chickpea as a tolerance response to salinity stress (Arefian et al. 2018).

## 5.3 Flooding Stress

Prolonged submergence and soil waterlogging result in hypoxic condition that constitutes flood stress in plants. One of the key limitations to crop productivity in high rainfall areas of the world is flood stress. Climate change promotes submergence to be more recurring and drastic (Pachauri et al. 2014). Approximately 10% of irrigated land in India, China, Russia and 16% of cultivable land in the USA are damaged by waterlogging (FAO 2015). Figure 5.3 explains the ubiquity of flood hazard in the world. According to statistical approximates of food and agriculture organization of United Nations, flood stress upshots 17% of total loss and damage in agriculture (FAO 2018).

It was discerned that annually 20–50% production loss in wheat occurred under waterlogging condition (Hossain and Uddin 2011). Overall 15% of maize production in South Asia is damaged by floods. India accounts for 25–30% maize production loss every year on account of flood stress (Bailey-Serres et al. 2012).

**Fig. 5.3** Global flood hazard prevalence. Adopted from SEDAC (n.d.)

## 5.3.1 *Outcome of Flood Stress in Plants*

The prime consequence of flood stress in plant comprises anoxia or oxygen limitation. Necrosis, senescence, low nitrogen fixation and ultimately plant death are the consequences of flood stress (Hasanuzzaman et al. 2012). Overall effects of flood stress in plants are outlined in Table 5.2.

## 5.3.2 *Adaptation of Plants to Flood Stress*

### 5.3.2.1 Morphological Alterations

For escaping anoxic conditions under flood stress, plants showcase morphological adaptations. Submerged leaves have low stomatal counts and are often dissected and filamentous (Sculthorpe 1967). Vigorous adventitious root growth is one of the prominent adaptation as evident in *Sesbania javanica* (Jackson 2006) and *Chrysanthemum* (Yin et al. 2009). Decrement in root hairs and diameter, rapid shoot elongation are some of the evident variations (Jackson 2008). Under submergence, formation of leaf gas film improves carbohydrate supply, generation of adventitious roots and aeration of roots. In rice, resistance to radial oxygen loss is enhanced by

**Table 5.2** Consequences of flood stress in plants

| Parameters | Alterations | References |
|---|---|---|
| Anatomical variation | Over growth of aerenchyma, lenticels and adventitious roots | Ashraf (2009) |
| | Decrement in stele and seminal root diameter, reduced cortex thickness | Grzesiak et al. (1999) |
| | Increment in width of phloem tissue and number of xylem vessels | de Souza et al. (2013) |
| | Formation of suberized exodermis on root cortex periphery | Kulichikhin et al. (2014) |
| Physiology and metabolism | Impaired stomatal conductance, $CO_2$ assimilation | Ashraf (2009) |
| | Decrement in net photosynthetic rate, transpiration rate, PSII efficiency and rubisco activity | Zheng et al. (2009) |
| | Low relative water content and membrane stability | Kumar et al. (2013) |
| | Accumulation of hexose sugars in roots and organic acids in leaves | Vandoorne et al. (2014) |
| Nutrient availability | Enhanced nitrogen content and reduced phosphorous and potassium content in plant tissues | Reddy and mittra (1985) |
| | Reduced uptake of copper and zinc | Tarekegne et al. (2000) |
| Growth and yield | Reduction in leaf number, diameter, height and leaf area of plant, leaf epinasty | Parent et al. (2008);<br>Gonzalez et al. (2009) |
| | Decline in dry matter accumulation, chlorosis, and flower abortion | Mensah et al. (2006) |

development of effective barriers. This aids in shielding the plants from toxins generated from anoxic environment and boosts root tip aeration under flood stress (Kirk et al. 2014; Yamauchi et al. 2018).

#### 5.3.2.2 Anatomical Alterations

To hinder the loss of radial oxygen from submerged roots, plants show formation of lateral diffusion barrier (Sauter 2013). Lenticels development is also evident in flood affected plants as this aids in oxygen diffusion in downward direction (Parelle et al. 2006). Aerenchyma formation is one of the unique characters of plants under flood or submerged condition as these gas spaces allow oxygen transport from roots to shoots (Shiono et al. 2008). Exodermis thickness was improved by 23.70% compared to control in *Garcinia brasiliensis* under flood state in addition to increment in root xylem vessels and phloem width (de Souza et al. 2013). In flood tolerant barley cultivar (TX9425 and Yerong), more well developed intercellular spaces in leaves and mesophyll cells were observed that enhanced photosynthesis due to quick gaseous exchange Zhang et al. 2016). In rice seedlings, rapid coleoptile elongation during germination is a marker for submergence tolerance that facilitates aeration of developing embryo (Guru and Dwivedi 2018).

#### 5.3.2.3 Biochemical Alterations

Under flood stress, switch in the metabolism of plants from aerobic to anaerobic condition with high activity of alcohol dehydrogenase, a key enzyme in anaerobic fermentation is observed (Sairam et al. 2008). Proline content in *Casuarina* was high in tolerant cultivar as it acts as a signal molecule, an osmolyte and showcases antioxidant defense response under stress condition (Bajpai and Chandra 2015). Enhanced activity of alpha aminobutyric acid has been evident in soybean nodules in flooded conditions as this metabolite balances intercellular pH, acts as nitrogen reservoir under flood condition and also serves as antioxidant (Souza et al. 2016). Elevated activity of enzymes like phosphoenolpyruvate carboxylase, glutamate dehydrogenase and alcohol dehydrogenase was observed in flooded nodules of *Sesbania herbacea* (Krishnan et al. 2019).

## 5.4 Heavy Metal Stress

One of the major aftermaths of industrial revolution in the twenty-first century is heavy metal pollution in the environment. Heavy metals are potentially noxious elements and present in trace amount in soil. They are comprised of metalloids, lanthanoides and transition metals with atomic density ⍰6 g $cm^{-3}$except selenium, boron and arsenic (Park et al. 2011). The root cause of heavy metals in the environment encompasses usage of pesticides and fertilizers, combustion of fuels, mining, sewage sludge, oil spills, etc. Heavy metals are diverse in their functioning. Metals like iron, zinc, copper, nickel, manganese, chromium impart proper functioning of plant's metabolic processes contrarily lead, arsenic, mercury have negative effect on plant growth and productivity (Pierart et al. 2015). These noxious elements enter the food chain and attack human, animal and soil health (Augustsson et al. 2015).

Uptake of heavy metal by plants interrupts the chief metabolic processes like photosynthesis, nitrogen fixation and nutrients uptake and results in biomass and yield reduction and also death of the plants (Buendía-González et al. 2010). In China, about 20,000,000 acres of farmland is polluted by heavy metal with an annual crop loss of 1,000,000 million tons per year (Wu et al. 2010). About 12% of world's agricultural land is estimated to be affected by heavy metal pollution (Dziubanek et al. 2015). One of the most detrimental heavy metal for human health is lead. Lead exposure resulted in loss in economic productivity of about $977 billion annually in low to middle-income count (Attina and Trasande 2013). Statistical estimates of Institute for Health Metrics and Evaluation disclosed that lead exposure caused nearly 0.5 million deaths and 9.3 million life years lost among adults of 15 years and older (Landrigan et al. 2018).

## *5.4.1 Outcome of heavy metal stress in plants*

Heavy metal stress deteriorates soil and plant health. Metal toxicity alters the morphology and physiology of plants at different growth stage as a consequence there is decline in crop productivity. Table 5.3 outlines the impact of heavy metal stress in plants.

## *5.4.2 Plant tolerance to heavy metal stress*

### 5.4.2.1 Antioxidant enzymes

To escape heavy metal toxicity plants evolve many alterations. Exclusion of metal ions from the cell or binding with ligand prevents entry of metal ions to the roots is the first line of defense mechanism (Zeng et al. 2011). Metal ions also make complexes with carboxyl group of pectin in the cell wall thereby restricting its entry in the cell (Jiang and Liu 2010). Secondary response for metal detoxification is generation of antioxidant enzymes like superoxide dismutase, catalase and peroxidase. Compounds like glutathione, proline, cysteine, ascorbic acid also serve as scavengers of reactive oxygen species (Shahid et al. 2012; Pourrut et al. 2013).

Glutathione is a tripeptide antioxidant with thiol group and forms mercaptide bond with metals due to its nucleophilic thiol group and its reduced form also

**Table 5.3** Out-turn of heavy metal stress in plants

| Parameters | Modifications | References |
|---|---|---|
| Germination | Inhibition of water uptake by copper, cadmium | Kranner and Colville (2011) |
| | Reduction in germination percentage | Chigbo and Batty (2013) |
| | Formation of abnormal embryos and decrement in seed viability | Stvolinskaya (2000) |
| | Slow activity of alpha amylases, phosphatases. Decrement in remobilization of storage reserves | Kalai et al. (2014) |
| | Lipid peroxidation and proline accumulation in the radical | Kalai et al. (2014) |
| Plant growth | Chlorosis, decline in growth and yield, reduced nutrient uptake and nitrogen fixing potential | Guala et al. (2010) |
| | Generation of reactive oxygen species | Romero-Puertas et al. (2004) |
| Photosynthesis | Disruption in energy transfer in light harvesting complex, decline in chlorophyll and carotenoid content | Kuzminov et al. (2013) |
| | Electron transport inhibition between photosystem I and II | Rama Devi and Prasad (1999) |
| | Deformation of chloroplast ultrastructure | Kalaji and Loboda (2007) |
| Nutrient uptake | Low concentration of zinc, iron, calcium and manganese | Chatterjee et al. (2004) |
| | Decline in shoot nitrate content and nitrate reductase activity | Xiong et al. (2006) |

scavenges peroxide radicals. Glutathione conjugates with compounds of heavy metals that are translocated to vacuole to protect cell from its harmful effects (Klein et al. 2006; Yazaki 2006). It was also studied that in response to cadmium toxicity there is a modulation in the activity of antioxidants like guaiacol peroxidase, ascorbate peroxidase and catalase in tolerant plants (Alves et al. 2017; Borges et al. 2018). In *Brassica juncea* L. such kind of high efficiency antioxidative defense system was evident during caesium toxicity (Lai and Luo 2019).

#### 5.4.2.2 Metallothioneins and Phytochelatins

Vacuolar sequestration of heavy metals is an important response of plants to metal stress. Complex formation of metal ion with ligand reduces the toxicity of heavy metals. Metallothioneins and phytochelatins rich in cysteine are natural metal chelators in plants that reduce metal toxicity by forming mercaptide bonds with metals and sequester them to vacuoles (Gupta et al. 2010; Jiang and Liu 2010)**.** In *Brassica napus,* cadmium toxicity results in strong expression of MT4 metallothionein in germinating seeds as a defense mechanism (Mierek-Adamska et al. 2018). Likewise in *Oryza sativa,* phytochelatin synthase genes (OsPCS5 and OsPCS15) were profoundly induced under cadmium stress as tolerance mechanism.

These genes encode phytochelatins that bind heavy metals in cytosol and detoxify them in the vacuoles (Park et al. 2019).

## 5.5 Low Temperature Stress

Chilling or low temperature stress amends the plant morphology, physiology and metabolism. Occurrence of chilling stress at temperature 0 °C to 15 °C whereas freezing stress at ambient temperature for ice crystal formation causes cellular injury and osmotic dehydration in the cell (Bhatla 2018).

### *5.5.1 Impact of low temperature stress in plants*

Chilling in plants is advantageous for breaking seed dormancy, for vernalization and cold acclimation. In contrast, chilling in susceptible crops has many aftermaths as listed in Table 5.4.

### *5.5.2 Adaptations to low temperature stress*

#### 5.5.2.1 Cold acclimation

Increment in phospholipid proportion and decrement in ceramide and sugar containing lipids in plasma membrane results in reduction in expansion induced lysis. Expression of sugar producing enzymes like acid invertase, galactinol synthase and sucrose synthase to improve membrane stability is induced in response to cold acclimation (Turhan and Ergin 2012). Accumulation of compatible solutes like

**Table 5.4** Consequences of chilling stress in susceptible crops

| Parameters | Modifications | References |
|---|---|---|
| Lipid membrane | Membrane becomes leaky and dysfunctional | Bhatla (2018) |
| | Membrane transport and receptors disabled | |
| | Cellular structure distorted | |
| | Osmotic dehydration of cell | |
| Photosynthetic apparatus | Impaired electron transport chain | Bhatla (2018);<br>Wise et al. (1987) |
| | Generation of reactive oxygen species | |
| | Cell plasmolysis | |
| | Chloroplast swelling and dilation of thylakoids, disintegration of chloroplast | |
| | Depletion of starch and phosphorylated metabolites in cell | |

hydroxyl proline and arabinose in the cell maintains osmotic balance during cold acclimation (Bhatla 2018). Expression of hydrophilic and LEA (late embryogenesis abundance protein) proteins declines the denaturation of proteins under cold and drought stress. In *Brassica napus,* dehydrin proteins, a class of LEA proteins was highly expressed under cold stress in tolerant cultivar. It is used as biomarker for selection of cold tolerant lines (Maryan et al. 2019).

### 5.5.2.2 Modification in gene expression

Expression of cold regulated genes (COR) confers cold tolerance in plants by stabilizing the plasma membrane during low temperature stress. Hydrophilic proteins encoded by COR genes expressed during low temperature stress are involved in the production of anthocyanin and play indirect role in cold acclimatization. Extracellular production of antifreeze proteins (AFP) prevents ice crystal formation during low temperature stress. Molecular chaperons like HSP90 restrict denaturation of proteins during chilling stress. Other proteins like MAP (mitogen activated protein) kinase and calmodulin related proteins are encoded by COR genes and impart role in cold temperature signalling in plants (Bhatla 2018). A chromatin remodelling factor PICKLE (PKL) was found responsible for cold stress tolerance in *Arabidopsis*. It also modulates expression of other cold responsive genes like RD29A and COR15A (Zhao et al. 2019). In *Brassica rapa* L., genes such as temperature-induced lipocalin-1, zinc finger protein ZAT12, dehydrin ERD10 and adenosylhomocysteinase 2 were analysed and found to be involved in cold stress tolerance (Ma et al. 2019).

## 5.6 Drought Stress

One of the most disastrous outcomes of climate change is drought that restricts plant growth and development in both developed and developing countries. The utmost driver of undernourishment and hunger is geographical and global drought that declines agricultural productivity (FAO 2017).US, a technologically advanced country encounters an annual loss of $ 6 billion in agricultural productivity due to drought and such losses are more severe in underdeveloped and developing countries (CIA 2017). Around 20% of world's cultivable land is hit by drought and more than 80% damage in livestock and agricultural production is because of drought stress (FAO 2018). Major drought exposed regions in the world have been delineated in Fig. 5.4.

Drought is a climatic variation with perpetuate periods of rainfall scarcity that results in hunger, malnutrition, deficit food productivity and ultimately food insecurity. Plants encounter severe drought when water loss via transpiration is high enough or when roots are deficient in water supply (Anjum et al. 2011). A severe drought can threaten the national food availability and results in pervasiveness of undernourishment**.** Approximate global yield loss of 21% in wheat and 40% in

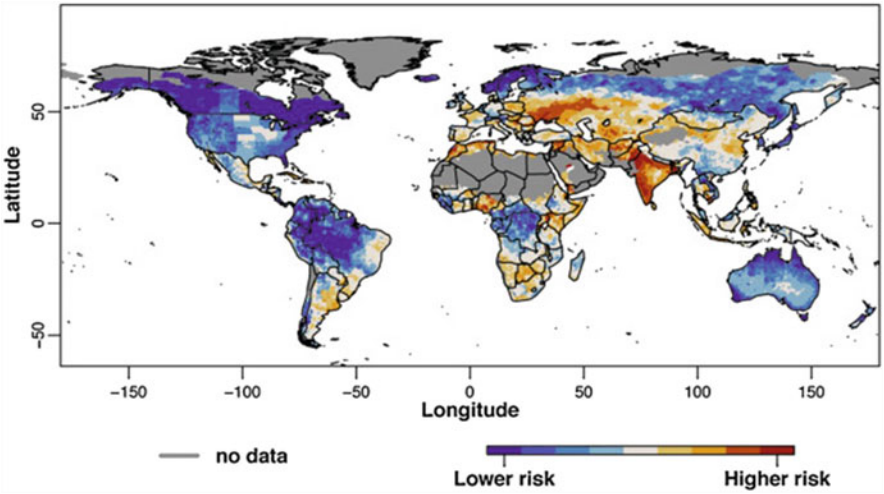

**Fig. 5.4** Global map of drought affected regions. Adapted from Carrao et al. (2016)

maize was observed from 1980 to 2015 (Daryanto et al. 2016). Table 5.5 illustrates susceptible and resistant crops under major abiotic stress.

Drought alters photosynthesis, water balance, nutrient acquisition, oxidative metabolism and assimilates partitioning in plants. This modification in plants depends on species, growth stage of the plants and environmental conditions. Apparent symptoms of drought in plants are depicted in Fig. 5.5. Reduction in grain filling rate, harvest index and solar use efficiency are some key drivers responsible for diminished yield under drought stress. Table 5.6 describes deleterious impact of drought stress in plants ranging from morphological, biochemical to molecular level and is discernible at every phenological stage of crop growth at any period of time (Farooq et al. 2009; Deepak et al. 2019).

### *5.6.1 Outcome of Drought Stress on Morphological Attributes*

The foremost impact of drought stress on morphology of plant is restricted seed germination and seedling growth (Farooq et al. 2009). Drought negatively alters seed vigour, seedling growth and also results in poor seed quality as reported in *Medicago sativa* (Zhang et al. 2019a, b), *Oryza sativa* (Bhavyasree et al. 2019), *Glycine max* (Reddy et al. 2019), *Zea mays* and *Sorghum* (Queiroz et al. 2019).

Drought impedes mitosis and cell enlargement because of restricted water flow from xylem to neighbouring cells (Hussain et al. 2008). Reduction in plant size and biomass is one of the major morphological alterations in plants during drought stress. Fifty percent reduction in biomass in wheat was observed as compared to control

**Table 5.5** Comparative representation of crops for resistance and tolerance to different abiotic stress

| Stress | Sensitive crop | Reference | Resistant crop | Reference |
|---|---|---|---|---|
| Salinity | *Brassica napus* L. | Dolatabadi et al. (2019) | *Hordeum vulgare* | Han et al. (2018) |
| | *Phaseolus vulgaris* | Lahaye and Epstein (1971) | *Echinochloa frumentaceae* | Williams et al. (2019) |
| | *Oryza sativa* L. | Khatun et al. (1995) | *Beta vulgaris* L. | Wu et al. (2018) |
| | *Triticum aestivum* | Ahmad et al. (2013) | *Vigna unguiculata* | Pan et al. (2019) |
| | *Lupinus albus* | Jeschke (1984) | *Solanum lycopersicum* | Amjad et al. (2019) |
| Flood | *Zea mays* | Panozzo et al. (2019) | *Saccharum officinarum* | Singh et al. (2019) |
| | *Solanum lycopersicum* | Ezin et al. (2010) | *Oryza sativa* L. | Dwivedi et al. (2018) |
| | *Glycine max* | Tewari et al. (2016) | *Solanum dulcamara* L. | Dawood et al. (2014) |
| | *Triticum aestivum* | Zhou (2010) | *Zea nicaraguensis* | Yamauchi et al. (2018) |
| | *Cicer arietinum* | Solaiman et al. (2007) | *Populus euphratica* | Yu et al. (2015) |
| Heavy metal | *Triticum aestivum* (Lead) | Alamri et al. (2018) | *Lupinus* (Cadmium, nickel) | Rathaur et al. (2012) |
| | *Oryza sativa* L. (Cadmium, arsenic) | Makino et al. (2019) | *Brassica napus* (Nickel, cadmium, copper) | Marchiol et al. (2004) |
| | *Lolium perenne* L. (Aluminium) | Parra-Almuna et al. (2018) | *Raphanus sativus* (Nickel, cadmium, copper) | Marchiol et al. (2004) |
| | *Brassica juncea* (Copper) | Yadav et al. (2018) | *Setaria italica L.* (Cadmium) | Chiang et al. (2011) |
| | *Solanum lycopersicum* (Cadmium) | Carvalho et al. (2018) | *Miscanthus sinensis* (Aluminium) | Ezaki et al. (2008) |
| Chilling | *Solanum lycopersicum* | Ronga et al. (2018) | *Arabidopsis thaliana* | Schulz et al. (2016) |
| | *Zea mays* | Bilska-Kos et al. (2017) | *Brassica juncea* L. | Sinha et al. (2015) |
| | *Cicer arietinum* | Selpair (2018) | *Mentha arvensis* | Dhawan et al. (2018) |
| | *Brassica oleracea* | Zhang et al. (2019a, b) | *Pisum sativa* L. | Zhang et al. (2016) |
| | *Vigna radiata* | Batra et al. (2018) | *Capsicum annuum* | Shawon et al. (2017) |
| Drought | *Oryza sativa* L. | Lawas et al. (2018) | *Setaria italica* | Han et al. (2019) |

(continued)

**Table 5.5** (continued)

| Stress | Sensitive crop | Reference | Resistant crop | Reference |
|---|---|---|---|---|
| | *Triticum aestivum* | Bakhshandeh et al. (2019) | *Sorghum* | Ohnishi et al. (2019) |
| | *Zea mays* | Daryanto et al. (2016) | *Arachis hypogaea* L. | Banavath et al. (2018) |
| | *Cicer arietinum* | Kaloki et al. (2019) | *Macrotyloma uniflorum* | Sharma et al. (2018) |
| | *Glycine max* | Popović et al. (2015) | *Brassica napus* L. | Mahmood et al. (2019) |
| | *Helianthus annuus* | Pekcan et al. (2015) | *Hordeum vulgare* | Carter et al. (2019) |

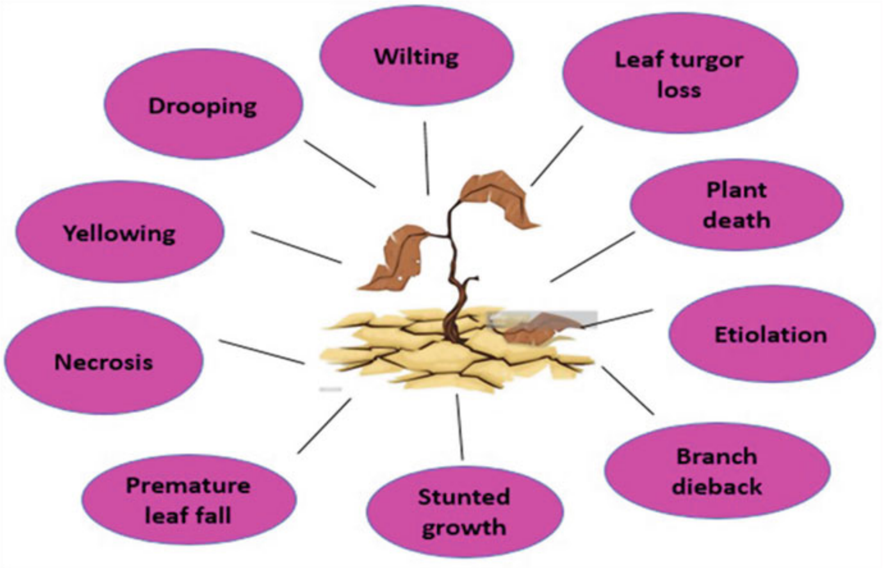

**Fig. 5.5** Drought stress prodrome in plants

under drought stress (Paul et al. 2019). Dry weight and fresh weight of root and shoot as well as root shoot ratio tend to cut down in drought induced *Brassica napus* L. (Khan et al. 2019).

Decrement in stomatal aperture, leaf rolling, cutinization of leaf surfaces are some observable drought stress induced morphological parameters (Hussain et al. 2008). Drought hampers the leaf size, grain yield in crops, for instance, yield parameters like panicle length, seed setting rate and grain weight declined in *Oryza sativa* L. under drought (Wei et al. 2017). Likewise in wheat, 62% of grain loss was observed in drought condition compared to well-watered conditions (Paul et al. 2019).

**Table 5.6** Salient modifications in drought stricken plants

| Parameters | Modification | Reference |
|---|---|---|
| Morphology | Inhibition of seed germination | Farooq et al. (2009) |
| | Reduce shoot and root dry weight | Manickavelu et al. (2006) |
| | Diminished leaf area and number of stomata | Jaleel et al. (2009) |
| | Impaired mitosis and cell elongation | Hussain et al. (2008) |
| | Loss of turgor pressure and cell growth | Taiz and Zeiger (2006) |
| Water and nutrient relation | Low water use efficiency and transpiration rate | Turner et al. (2001) |
| | Low uptake of phosphorous, calcium and magnesium | Barber (1995) |
| | Enhanced nitrogen uptake | Barber (1995) |
| Photosynthesis | Reduction in RUBP generation and photosystem II activity | Wise et al. (2004); Camejo et al. (2006) |
| | Low chlorophyll content | Din et al. (2011) |
| Assimilate partitioning | Decline in translocation of assimilates from source to sink | Kim et al. (2000) |
| | Disturbance in phloem loading and unloading | Zinselmeier et al. (1999) |
| | Low potential of sink to utilize the assimilates | Zinselmeier et al. (1999) |

### 5.6.2 *Physiological and Biochemical Alterations Under Drought*

Physiological attributes like transpiration rate, stomatal conduction, leaf water potential and relative water content are adversely affected by drought. Low water potential, transpiration rate, relative water content and enhanced leaf temperature were observed in *Oryza sativa* under drought (Fahad et al. 2017). Drought stress induces the formation of reactive oxygen species (ROS) along with enhanced generation of osmolytes like proline, glycine betaine. Production of ROS leads to oxidative stress that ultimately results in cell death in plants (Silva et al. 2019).

Another important trait influenced by drought is photosynthesis. Drought stress induces reduction in leaf expansion, damages the photosynthetic apparatus and activity of photosynthetic enzymes. Reduction in phosphorylation and disruption in ATP synthesis are key drivers for impaired photosynthesis under drought (Fahad et al. 2017). Reduction in thylakoid membrane proteins and PSII activity under PEG induced drought stress have been evident in many studies (Wang et al. 2019).

Activity of rubisco, a motor enzyme of photosynthesis is declined drastically under drought. Reduction in amount of substrate, i.e. ribulose 1, 5 bisphosphate for carboxylation, modification in ultrastructure of rubisco and chloroplast and acidification of chloroplast stroma are the driving factors for decrement in rubisco activity. Other enzymes like fructose-1, 6-bisphosphatase, NADP-glyceraldehyde phosphate dehydrogenase, phosphoribulokinase, phosphoenol pyruvate carboxylase and pyruvate orthophosphate dikinase also decline under drought stress (Farooq et al. 2009).

Drought restricts nutrient movement from soil to plants. Under drought, root growth and proliferation are hindered that limit nutrient translocation in plants. Limited flow of oxygen, carbon and nitrogen assimilation in root nodules impedes nitrogen fixing ability of legumes under drought (Ladrera et al. 2007). Nitrate reduction in leaves was limited by declined photosynthesis, which supplies reducing power, generated during photosynthetic via electron transport for the process of nitrate reduction (Chen et al. 2018).

## *5.6.3 Adaptation of Plants to Drought Stress*

### 5.6.3.1 Escape

Drought escape is characterized by short life cycle that enables plants to flower early before onset of drought. Growing season is dependent on environmental variation and plant genotype. Drought escape is enhanced when development of plant synergizes with duration when soil moisture is available. Although drought escape helps in plants survival during stress yet there is a decrement in the yield (Farooq et al. 2009). High grain yield in *Lens culinaris* was recorded under early drought as a result of drought escape. Maximum leaf area, stomatal conductance, high stomatal density, early flowering with short life cycle were also observed as an outcome of drought escape strategy in lentil (Sanchez-Gomez et al. 2019).

### 5.6.3.2 Avoidance

Avoidance is marked by maintaining the water potential of plants during drought. It is also characterized by reduced water deprivation through transpiration and balancing the water uptake from soil. For efficient water uptake, a deep and extensive root system is required along with thick cuticle whose formation requires high input of energy that ultimately results in low yield. Therefore, plants with avoidance strategies are generally of compact size (Lisar et al. 2012). In *Sorghum,* aquaporin genes PIP2;3 were strongly expressed in roots under drought compared to roots under watered conditions. Aquaporins are the membrane proteins that allow water and solute passage through the membrane into cell and their expression under drought is an adaptive strategy (Schulze et al. 2019).

### 5.6.3.3 Tolerance

Tolerance to drought stress is perceptible by limited area and number of leaves during water deficit condition. Plants show traits like formation of trichomes and hairy leaves which reduce leaf temperature during drought. Root architecture like root density, root proliferation, root growth rate are some key alterations in plants during drought. Reduction in stomata number, increment in root shoot ratio, accumulation of osmolytes and induction of antioxidant defense system are other drought tolerance traits in plants (Sapeta et al. 2013). In sorghum, leaf rolling, leaf waxiness, stay green trait, root architecture, abscisic acid metabolism, ion transporter, transpiration efficiency and osmotic adjustments are drought tolerant traits that enable it to survive under drought stress (Badigannavar et al. 2018).

## 5.7 Conclusion

Major constraint for sustainable agricultural productivity and global food security is abiotic stress which is an outcome of global climate change. Extreme weather conditions attack morphological, physiological and biochemical attributes of the plants and threat their survival in the environment. Multiple abiotic stresses like salinity, flood, heavy metal and drought impact the crop yield. Drought is the most devastating abiotic stress that declines the global crop productivity. Drought hampers seed germination, reduces the stomatal frequency and diminishes leaf area and water potential. At physiological level, drought restricts photosynthesis, thylakoid structure and enzyme production. All these modifications alter the metabolism and limit growth and development of plants. Despite such abiotic constraints, plants have inherent tolerance mechanisms that enable them to cope up and survive under extreme conditions. Activation of antioxidant defense system, changes in the membrane lipid composition and enzyme production as well as morphological alterations in plants are some of the tolerance traits in plants. In spite of such adaptive strategies, improvements in the tolerance of plants against the extreme calamities are required. Strategies like alteration in expression of stress responsive genes and transcription factors are potential candidates to develop stress tolerant crops. More emphases should be there on molecular research for exploiting the stress tolerance traits and minimizing the aftermath of stress in plants.

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# Chapter 6
# Physiological and Molecular Aspects of Retrieving Environmental Stress in Plants by Microbial Interactions

**Swati Rastogi, Sheel Ratna, Olfa Ben Said, and Rajesh Kumar**

**Abstract** Environmental stress is the foremost limiting factor for agricultural productivity. It is essential to alleviate the distress caused by environmental and edaphic conditions in plants, failing to which would affect their growth, development, and productivity. Microorganisms have enormous metabolic capabilities to lessen the environmental stress and their interactions with plants provide a local and systemic defense under various biotic and abiotic stresses. Due to an increase in adverse external conditions, it is imperative to study plant–microbe relationships at the physiological and molecular level to provide deeper insights into the stress-mitigating mechanisms.

## 6.1 Introduction

Decline in crop yield and ultimately agricultural productivity has resulted from extreme climatic conditions which burden the land with environmental stress. The geographical land area that is unaffected by any biotic or abiotic constraint is diminishing with the advent of industrialization, pollution, and moderation in the environment. Drought, salinity, acidity, alkalinity, low/high temperature, and nutrient deprivation are some of the paramount abiotic stresses along with biotic stress which includes pest and diseases and show a huge impact on world's agriculture (Latef et al. 2016; Meena et al. 2017). Plants being sessile are generally exposed to these environmental stresses that cause morphological, physiological, biochemical, and molecular alterations in them and may lead to diminution in average yields by 50%. To fight back such constraints, plants have excogitated and developed several intricate specific mechanisms that provide them resistance and minimize damage

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caused by stresses, thereby protecting invaluable resources for growth and development (Atkinson and Urwin 2012; Lenoir et al. 2016).

Though plants have their own internal mechanistic systems which activate specific and unique stress responses when subjected to multiple stress conditions but sometimes this mechanism cannot defy all the harmful stress factors. Complex surroundings where they flourish are comprised of numerous biotic and abiotic entities which tend to interact with them. Microorganisms are known to be ubiquitous in nature and plants are also found in many regions of the earth as they adapt to those environments that are otherwise not dwelled by humans and other larger animals (Coleman-Derr and Tringe 2014). Based on their presence and diversity, plants and microbes communicate with each other and have been observed to possess both negative and positive interactions. In order to cope up with antagonistic environment, plants and microbes have developed synergy to extract maximum benefit from one another. When exposed to environmental stress, such plant–microbe association shows mutual symbiotic relationship where plants may provide shelter, support, and nutrition to the microbes and microbes in return secrete exogenous metabolites and supplements such as primary nutrient sources, signaling molecules, chemo-attractants, etc. that would, otherwise, not be available to the plants (Babar et al. 2016).

The previous knowledge on plant–microbiome interactions at physiological, biochemical as well as at molecular level has provided significant support to establish and understand the complex integrated cellular paradox during such antagonistic variations and laid emphasis on more technological advancement towards plant–microbe interactions in relation to defense against environmental stresses (Nadeem et al. 2014; Ansari 2018). The present chapter summarizes the impact of environmental stress on plants and defense responses induced in plants due to plant–microbe interactions at the physiological and molecular level.

## 6.2 How Does Environmental Stress Affect Plants?

Plants need an optimum quantity of abiotic entity for their proper growth but they are said to be in a state of stress if certain determinants affect their growth and development, below or beyond optimum dosage or intensity. Plants are exposed to both abiotic and biotic stresses, and among them, abiotic stress is the main cause of abstaining agricultural productivity. These environmental stresses diminish the physiological processes of plants and bring about biochemical and molecular changes along with the generation of reactive oxygen species (ROS) (Ansari 2018).

The foremost effect of environmental stress in plants is the availability of low nutrient content which usually leads to poor growth and development. Drought and salt stress affect plant physiology by reducing soil water potential, leaf water potential (Doubková et al. 2013), stomatal conductance, photosynthetic rate, and chlorophyll pigment (Chakraborty et al. 2013), increased photorespiration, reduced seed germination index, leaf size, seed number, and viability (Aroca et al. 2013), root and shoot growth, decreased production of phytohormones (Palaniyandi et al. 2014; Shahzad et al. 2017), generation of free radicals as hydroxyl, superoxide, peroxide, etc. which disintegrate cell organelles membrane and interfere with metabolic signal pathways (Vurukonda et al. 2016; Lata et al. 2018). High salt concentration in arable soil causes a varying level of acidity or alkalinity, thereby increasing ion toxicity and burdening osmotic stress on plants, decreases crop yield, early senescence, production of oxidizing radicals (Brotman et al. 2013; Kim et al. 2014), affects the expression of metabolic genes (Nautiyal et al. 2013; Bharti et al. 2016), disturbs ion balance (Shukla et al. 2012). Heavy metal stress results in the production of ROS, affects cell's redox homeostasis and causing oxidative stress (Rastogi et al. 2019; De Souza et al. 2015; Fuentes et al. 2016). High or low temperature affects crop productivity by interfering with the biological activities of the soil, causes seasonal freeze-thaw effect leading to severe conditions, denaturation of membrane proteins (Subramanian et al. 2015; Lata et al. 2018). On the other hand, biotic stress is generally linked to the onset of diseases in plant and leads to severe morphological and internal damage, affects defense hormone levels, signaling pathways, and oxidative stress (Singh et al. 2013a, b; Waqas et al. 2015).

## 6.3 Plant–Microbe Interactions in Mitigating Environmental Stress: Beneficial Microorganisms

### *6.3.1 Arbuscular Mycorrhizal Symbiosis*

The most communal kind of symbiosis is arbuscular mycorrhizal (AM) symbiosis where arbuscular mycorrhizal fungi (AMF) provide water and nutrients to the plants and in return take up carbon (organic matter) and their interaction involves the production of significant signal molecules which activate symbiotic genes and develop the symbiotic association with plants. These mycorrhizal fungi belong to phylum Glomeromycota and can show both ecto- and endo-mycorrhizal symbiosis with their host plants. The term "arbuscular" denotes association of fungal hyphae into the internal cortex of roots in the form of branched structures "arbuscules" where the exchange of nutrients takes place (Kim et al. 2012; Pozo et al. 2013).

#### 6.3.1.1 Physiological Aspects of AMF

AMF form endosymbiotic relationships with plant's roots and help in amelioration of drought stress by direct or indirect mechanisms. Changes include underground and above the ground modifications in plants which may encounter changes in plant–water relations. Some examples of modifications are: changes in $CO_2$ assimilation, relative water content, growth and photosynthetic pigment parameters, stomatal conductance, leaf water potential, and improved efficiency of photosystem

II with enhanced photosynthetic rate and nutrient uptake (Rapparini and Penuelas 2014; Balestrini et al. 2017; Sun et al. 2017; Quiroga et al. 2017). It is proposed that physiological modifications in plants above the ground under drought stress by AM symbiosis largely depend on the type of host plant and the fungal species involved along with the involvement of hormone “abscisic acid” (ABA). An increased level of ABA is found responsible for bringing out such modifications. Chitarra et al. (2016) supported this fact and showed increased levels of ABA in non-mycorrhizal plants, which means that AM plants experience less drought stress. This theory was also supported by a study conducted on tomato plants. One of the indirect mechanisms by which AMF works in positive manner for plants is by improving soil structure in terms of soil porosity and organic matter. Drought stress is strongly linked with nutrient stress in plants and thus leads to poor soil structure under water deficit conditions and low nutrient availability (Kong et al. 2014; Bardi and Malusà 2012). Comparative studies on maize plants grown in two different types of coal mines under drought stress showed decreased plant dry weights, leaf moisture percentage, water utilization efficiency, nutrient content, and disturbed carbon:nitrogen:phosphorus (C:N:P) stoichiometry (Zhao et al. 2015). An underground association of AM with plant roots provides resistance by enhancing the absorption of water and nutrients from soil pores having low water potential through their hyphae (Hameed et al. 2014; Maiti and Ahirwal 2019). Fungi can form soil aggregates with the help of their hyphae and stabilize soil particles and enhance soil water retention potential. Besides this, extra mycelial growth along with AMF spores can contribute towards soil organic carbon and improve soil organic matter or structure. AMF colonization rate can range from 60 to 90% and can significantly increase the water use efficiency and leaf moisture percentage of fresh weight and improves C:N:P ratio (Zhao et al. 2015). A similar study where the presence of AMF “*Glomus constrictum* Trappe” in the rhizospheric region of marigold (*Tagetes erecta*) alleviated drought stress by improving phosphorus content and photosynthetic pigments (Asrar and Elhindi 2011). The growth of plants in arid or barren soil areas under drought stress can be improved by growing plants concomitantly with AMF which will solve the problem of water scarcity and will lead to restoration of ecosystem. Many studies have supported this fact and showed improved plant growth if inoculated with AMF. Plants generally have their own pathway for nutrient uptake but are depleted under drought stress and thus mycorrhizal uptake pathways come into action. High affinity uptake transporters of mycorrhiza absorb nutrients from soil and transfer them to plants and thus the nutrient level in plants for their growth is improved (Bücking and Kafle 2015). The role of AMF in phosphorus uptake is well established but recently many studies have also supported their role in nitrogen uptake, for example, in sunflower in the form of ammonium ions (Heidari and Karami 2014). Water deficit condition and its physiological impact and alleviation of water stress by AMF have been studied in many plants, *Robinia pseudoacacia* L. (Yang et al. 2015; He et al. 2016), *Zea mays* (Quiroga et al. 2017; Pavithra and Yapa 2018), *Vigna unguiculata* (Oyewole et al. 2017), *Rosa damascena* Mill. (Abdel-Salam et al. 2018).

Salinity is another environmental stress which affects the growth and development of plants. Though salt stress can negatively affect AMF but the constructive role of AMF in plants under salt stress is also well documented. Accumulation of excess salts in alkaline and saline soils generally found in arid and semi-arid geographical regions and also regions like estuaries and coastal fringes may lead to salinity. The physiological aspect of plant–mycorrhizal interaction results in high $K^+/Na^+$ content, improved photosynthetic and water status of the host plants, improved production of photosynthetic pigments, less ABA accumulation in AMF plants than non-mycorrhizal plants, other phytohormone production, nitrogen fixation, accumulation of compatible solutes and osmolytes, better host plant nutrition, improved antioxidant activity of enzymes to prevent generation of ROS (Porcel et al. 2012; Latef and Miransari 2014). Salinity affects nutrient uptake capacity of the plants (Miransari 2014) and AM fungi help in mitigating nutrient deficiency by assimilation of those nutrients in host plants. For example, elements like nitrogen are provided to plants by first assimilating it in the form of nitrate in their mycelia by using the activity of nitrate reductase enzyme that forms arginine which leads to the formation of other nitrogen containing substances along with reduction in toxic effects of sodium ions, thereby conserving the chlorophyll pigment of plant (Evelin et al. 2009; Latef and Miransari 2014). Latef and Miransari (2014) have also advocated that the other mineral elements like phosphorus, potassium, calcium, and magnesium are also provided to the plant by compartmentalization of sodium and chloride ions within vacuoles that would otherwise interfere with metabolic pathways in the plants and thus improved potassium-to-sodium ion ratio. Many studies have been conducted with crop plants like *Ocimum basilicum* L. (Elhindi et al. 2017), *Cucumis sativus* L. (Hashem et al. 2018), *Cicer arietinum* L. (Garg and Bhandari 2016), *Puccinellia tenuiflora* (Liu et al. 2018) and grass species like *Leymus chinensis* (Lin et al. 2016, 2017) and established the role of AMF in amelioration of salt stress.

#### 6.3.1.2 Molecular Aspects of AM Symbiosis in Alleviating Environmental Stress

Plants respond to drought stress through signal transduction pathway which involves ABA production. Transcriptomic studies showed the involvement of responsive to dehydration genes (rd) and early response to dehydration genes (erd) in response to stress. In fact, ABA signaling pathway is often found to be linked with nitric oxide (NO) pathway as removal of NO is important; otherwise, it would inhibit stomatal closure. Other physiological responses involved during stress in plants are: accumulation of compatible solutes and expression of osmolyte genes as they imitate for water and provide resistance against drought stress (Kim et al. 2012; Lata et al. 2018). A study based on earlier microarray assay followed by reverse transcriptase polymerase chain reaction (RT-PCR) confirmed the expression of four responsive dehydration genes in *Poncirus trifoliata* inoculated with *Glomus mosseae* (AMF) that led to relevant physiological and biochemical changes (increased plant height, chlorophyll content, etc.) in the plant during drought stress. Gene probe 401 was involved in activation of antioxidant system and genes 15,555 and 3652 had similar activities as MIOX1 (myo-inositol oxygenase) and GLX1 (glyoxalase I), respectively, which are involved in ascorbic acid (an antioxidant) biosynthesis and glutathione (GSH) based detoxification of methylglyoxal, respectively. Gene 6927 coded for chalcone isomerase enzyme was involved in flavonoid synthesis which modulates ROS levels under water deficit conditions. Their mRNA transcripts were higher in AM inoculated *P. trifoliata* plants (Fan and Liu 2011). Besides this, studies on significant genes which regulate important membrane proteins involved in water transport and uptake could provide insight into drought tolerance mechanism by AMF. These membrane proteins are called aquaporins which channelize water and other molecules in roots and leaves of the plants (Wang et al. 2018). Studies have revealed the presence of an increased concentration of such membrane proteins in plants and fungi which help in providing drought resistance. They can control hydraulic conductivity of the roots, moisture potential of the leaves, and transpiration rate in mycorrhized plants (Lenoir et al. 2016; Srivastava et al. 2016). A study conducted by He et al. (2016) on *Robinia pseudoacacia* L. when inoculated with mycorrhizal fungus (*Rhizophagus irregularis*) under water stress revealed that AM regulated the expression of aquaporin genes in host plant and its own genes and thus improved plant physiological parameters. Another study on two cultivars of maize focused on water conservation status by AM plants showed that up- and down-regulation of some aquaporin genes took place to minimize water loss and maintenance of water homeostasis (Quiroga et al. 2017).

#### 6.3.1.3 The Rhizo-Chemistry Between Plant and Plant Growth-Promoting Rhizobacteria (PGPR)

Beneficial plant–microbe interactions in the rhizosphere result in increased crop productivity. Plant growth-promoting rhizobacteria (PGPR) fall under such beneficial interactions. They can provide resistance to plants against biotic and abiotic stresses with the help of their characteristic properties like phosphate solubilization, production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC), biosurfactants, phytohormones, and exopolysaccharide, biological nitrogen fixation, siderophore production, production of many volatile compounds, and regulation of various stress-related genes (Vurukonda et al. 2016; Souza et al. 2015; Viscardi et al. 2016).

#### 6.3.1.4 Potential of Exopolysaccharides (EPS) as Bioadsorbent

Rhizobacterial exopolysaccharides are generally high molecular weight complex organic macromolecules that can bind soil particles to form aggregates which can easily occupy specific locations on the plant roots or hyphae of fungi and thus stabilize the aggregates and thereby minimize water stress. They reduce saline stress by binding to sodium ions and also reduce heavy metal toxicity (Selvakumar et al. 2012; Salwan et al. 2019). Exopolysaccharide mediated working mechanism of

*Bacillus amyloliquefaciens* FZB42, a PGPR in imparting resistance to disease and salt stress is not much explored in *Arabidopsis thaliana* (Lu et al. 2018). So, they explored the role of exopolysaccharide gene (epsC) in FZB42 when inoculated in *A. thaliana* during drought stress and found that inoculation of epsC containing PGPR enhanced plant growth with increased root and shoot length and their dry weights along with increased expression of drought protection marker genes such as RD29A, LEA14, etc. than epsC FZB42 mutants (Lu et al. 2018; Lal et al. 2018). Another study supported the inoculation of exopolysaccharide (eps) producing PGPR *Burkholderia cepacia* in conferring resistance to *Capsicum annuum* during drought and salt stress through the production of about 4.893 ± 0.06 mg/mg eps protein (Maxton et al. 2018). The alternative role of EPS in heavy metal remediation could be suggested in the form of bioadsorbents. The focus needs to be shifted in utilizing negatively charged EPS such as hyaluronan (*Pseudomonas aeruginosa*), gellan (*Sphingomonas paucimobilis*), etc. in biosorption and biomineralization of positively charged heavy metal ions as their surfaces have abundant anionic groups that can also be chemically modified by acetylation, carboxymethylation, etc. thus enhanced applicability. EPS production is associated with processes such as biofilm formation that beneficiates these biosorption process (Ojuederie and Babalola 2017). EPS of *Rhizobium radiobacter* was used in biosorption of lead ($Pb^{2+}$) and zinc ($Zn^{2+}$) ions (Wang et al. 2013). In another study, the role of EPS as bioadsorbent from *Klebsiella* sp. J1, *Pseudomonas aeruginosa* CPSB1 and *Azotobacter chroococcum* CAZ3 was demonstrated in removal and reduction of chromium, Cr (VI) and lead ($Pb^{2+}$) ions (Wei et al. 2018; Rizvi and Khan 2019). Therefore, the inoculation of rhizobacterial EPS as bioadsorbent could be helpful in phytoremediation and suppression of heavy metal stress in plants.

#### 6.3.1.5 Nitrogen Fixation, Phosphate Solubilization, and Production of Siderophore and Indole Acetic Acid

PGPR can work directly or indirectly for plant growth promotion and confer resistance against stress by fixing atmospheric nitrogen as done by a genus of *Rhizobium* or solubilize phosphates and chelate iron by secreting siderophores and provide nutrition to the host plant under stress environment (Dimkpa et al. 2009; Kumar et al. 2019). Rojas-Tapias et al. (2012) explored the effect of inoculation of *Azotobacter* strains in maize plants under salt stress. Under different salt concentrations they inoculated the plants with PGPR strains of *Azotobacter* and evaluated the changes in physiological parameters. Results indicated nitrogen fixation and phosphate solubilization irrespective of salt concentrations by two strains of *Azotobacter* C5 and C9 and auxin was also synthesized by C5 along with improved chlorophyll content and potassium-to-sodium ion ratio. A similar study was performed by Cardinale on barley (*Hordeum vulgare* L.) where PGPR improved physiological parameters of barley under salt stress. Their study also revealed that pure cultures might not alone show all PGP activities but hold plant growth promotion up to high levels and thus selection of strain should be done cautiously otherwise that would preclude new PGPR and novel plant growth mechanisms (Cardinale et al. 2015). The role of PGPR in providing protection against environmental stress has been studied by many researchers in many food and non-food crops to get insights of sustainable agriculture such as *Lavandula* and *Salvia* plants (Armada et al. 2014), *Bacopa monnieri* (Bharti et al. 2013), *Triticum aestivum* (Chakraborty et al. 2013; Kasim et al. 2013; Timmusk et al. 2014), *Capsicum annuum* (Del Amor and Cuadra-Crespo 2012), *Solanum tuberosum* (Gururani et al. 2013), *Helianthus* sp. (Shilev et al. 2012), *Glycine max* (Kang et al. 2014). Many studies revealed the inoculation of PGP bacteria having ACC deaminase was efficient in conferring resistance to plants against water stress (Choudhary et al. 2016). Salinity stress also results in declined production of phytohormones and use of IAA produced by PGP bacteria. PGPR can compensate for the decreased IAA levels from plants through their ACC deaminase activity, thereby improving root growth and absorption of nutrients in pea plants (Belimov et al. 2009; Wang et al. 2016). Inoculation of one of the common PGPR *Bacillus* sp. with characteristic features like siderophore, phosphate solubilization, and ACC deaminase activity can bring about modulation in gene profile and enzymes related to ROS scavenging (as found through mRNA profiling) and ethylene biosynthesis pathway thus improved physiological parameters during drought, salt, and heavy metal stress in potato plants (Gururani et al. 2013).

#### 6.3.1.6 Biosurfactants

Another important metabolite produced by PGP bacteria is biosurfactant that can be isolated from hydrocarbon-contaminated soil. Biosurfactants show tremendous results in terms of remediation of heavy metal stress in plants. Their industrial usage has increased in recent years as they are biodegradable, amphiphilic in nature, highly effective, and less toxic (Rastogi and Kumar 2020; Etesami 2018). Identification of heavy metal resistant PGPR strains with biosurfactant production ability could provide insights into novel methods through which plants can escape antagonistic environments (Abou-Aly et al. 2019). A study conducted by Yaseen et al. (2019) showed the role of biosurfactant producing *Pseudochrobactrum lubricantis*, *Lysobacter novalis* and a fungus *Aspergillus niger* in mitigating lead ions stress in plants. Plants employ several internal mechanisms to alleviate heavy metal stress which can be enhanced with the inoculation of biosurfactant producing rhizobacteria (Gupta and Kumar 2017). Bioaugmentation assisted phytoremediation of heavy metal and petroleum contaminated soil with rhamnolipid (biosurfactant) producing *Pseudomonas aeruginosa* has been reported in a study. The study reported 56–105% increase in shoot and root growth, respectively, and other physiological parameters in alfalfa (Agnello et al. 2016).

#### 6.3.1.7 Phytohormone Production

Bacteria use different mechanisms to produce phytohormones and contribute towards plant growth at root and shoot level (Egamberdieva 2013). Plant–microbe interaction does not involve any specific mechanism in coping up with environmental stress rather they work concomitantly involving multiple stress regulating mechanisms (Fahad et al. 2015). Khan et al. (2017) explored the role of *Sphingomonas* sp. LK11 and exogenous application of a phytohormone (jasmonic acid) in mitigating salt stress in *Solanum pimpinellifolium* and its non-isogenic mutant (Got-3). The improved physiological factors showed expression of glutathione-related genes in LK11 but stunted growth in Got-3 due to lack of glutamate oxaloacetate transaminase as its role lies in the assimilation of ammonia, aspartate, and in glutamate synthesis. Another study involving the co-inoculation of AMF (*Glomus etunicatum*) and PGP bacteria (*Methylobacterium oryzae* CBMB20) in maize plant has resulted in improved nutrient assimilation, phytohormone production, and decreased proline and sodium ion uptake during salinity stress (Lee et al. 2015). Symbiotic association of multiple microbes in the rhizospheric region of a plant provides a strong interactive environment to combat environmental stress. The interaction of AMF and *Bacillus subtilis* with *Acacia gerrardii* during salt stress resulted in greater root and shoot dry weight, improved nodulation for nitrogen fixation and nutrient acquisition (Hashem et al. 2016). In recent years, many studies were conducted to explain the interaction of valuable microorganisms with plants at physiological and molecular level to combat environmental stress as presented in Table 6.1.

#### 6.3.1.8 Volatile Organic Compounds (VOCs)

Some PGPR strains release VOCs in the rhizosphere. During salt stress, bacterial VOCs activate high affinity potassium transporter 1 (HKT1) that prevents sodium ion accumulation in shoot region of the plants and also induces expression of salt overly sensitive 3 (SOS3) gene which controls post-transcriptional activity of proton/sodium ion ($H^+/Na^+$), antiporter SOS1 that regulates $Na^+$ homeostasis in VOC-treated plants and also leads to acidification of rhizosphere and thus generating a $H^+$ gradient which allows SOS1 mediated $Na^+$ exclusion from roots (Liu and Zhang 2015). VOCs by PGPR *Pseudomonas simiae* AU triggered induced systemic tolerance (IST) and plant growth in soybean (*Glycine max* L. Merrill) during salt stress (Vaishnav et al. 2015). Detailed proteomic analysis and western blotting revealed the suppression of salt stress by the accumulation of vegetative storage proteins (VSP), ϒ-glutamyl hydrolase (GGH), and ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) enzyme that also helped in sustaining plant growth (Vaishnav et al. 2015). During water stress, expression of transcripts for enzyme phosphoethanolamine N-methyltransferase (PEAMT) that facilitates tolerance to dehydration and expression of vacuolar $H^+$ pumps is known to rise in VOC-treated plants (Farag et al. 2013; Liu and Zhang 2015; Vigani et al. 2019).

**Table 6.1** Plant–microbe interactions to alleviate environmental stress at physiological and molecular level

| S. No. | Stress | Detrimental effects | Affected plant | Microorganism | Physiological and molecular mechanism involved | References |
|---|---|---|---|---|---|---|
| 1 | Drought | Reduced leaf and water potential, nutrient deprivation | *Fragaria* × *ananassa* | AMF (*Rhizophagus clarus*) | Increased antioxidant enzyme defense system, photosynthetic machinery, and water status | Moradtalab et al. (2019) |
| 2 | Drought | Oxidative stress | *Triticum durum* Desf; cv. Mongibello, *Triticum aestivum*; cv. Chinese spring | AMF (*Funneliformis mosseae*) | Metabolite regulation, stimulated brassinosteroids biosynthetic pathway, cross-talk between phytohormones | Bernardo et al. (2019) |
| 3 | Drought | Oxidative stress | *Cicer arietinum* | PGPR (*Bacillus subtilis*, *B. thuringiensis*, *B. megaterium*) | Enhanced accumulation of amino acids and other metabolites such as proline, L-arginine, etc. | Khan et al. (2019) |
| 4 | Drought | Affected plant growth parameters | *Triticum aestivum* | AMF (*Glomus mosseae*) | Improved physiological aspects in terms of plant biomass and yield | Rani et al. (2018) |
| 5 | Salinity | Ion imbalance, accumulation of $Na^+$ in leaves | *Sulla coronaria* | AMF (*Rhizophagus intraradices*), PGPR (*Pseudomonas* sp., *Bacillus subtilis*) | Enhanced physiological processes in terms of improved nutrient uptake, reduced electrolyte absorption, malondialdehyde levels | Hidri et al. (2019) |
| 6 | Salinity | Ion toxicity, hormonal imbalance, nutrient deficiency | *Cicer arietinum* | *Rhizobium* sp., *Stenotrophomonas maltophilia, mycorrhizal* fungi | Improve nodulation, nitrogen fixation, and nutritional status | Abd-alla et al. (2019) |
| 7 | Salinity | Altered osmotic pressure, toxic $Na^+$ ion accumulation | *Alfalfa*, *Arabidopsis thaliana* | *Enterobacter* sp. SA187 | Induced systemic tolerance (IST) via ethylene signaling pathway by converting bacterial 2-keto-4-methylthiobutyric acid (KMBA) into ethylene | De Zélicourt et al. (2018) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | Salinity | Disturbed osmotic, $K^+$/$Na^+$ ion ratio | *Arabidopsis thaliana* | *Serendipita indica* (formerly *Piriformospora indica*) | $Na^+$ detoxification via ENA ATPases | Lanza et al. (2019); Gill et al. (2016) |
| 9 | Drought and salinity | Disturbed water potential, electrolyte imbalance, reduced photosynthetic rate | *Medicago truncatula* | AMF (*Funneliformis mosseae, Rhizophagus irregularis*) | Induced resistance by 14-3-3-like protein expression via genes $Fm_{201}$, $Ri_{14\text{-}3\text{-}3}$ and $RiBMH_2$ | Sun et al. (2018) |
| 10 | Nickel (Ni) and salinity | Electrolyte leakage, diminished chlorophyll, proline contents, etc. | *Helianthus annuus* | *Pseudomonas libanensis* TR1, AMF (*Claroideoglomus claroideum* BEG210) | Phytoremediation, improved physiological aspects | Ma et al. (2019) |
| 11 | Lead (Pb) | Decreased root and shoot dry weights, chlorophyll pigment, sugar content, etc. | *Lathyrus sativus* | PGPR consortium (*Rhizobium leguminosarum*, *Pseudomonas fluorescens*, *Luteibacter* sp., *Variovorax* sp. *Sinorhizobium meliloti*) | Mineral homeostasis, enhanced antioxidant enzyme activity, phenolic compounds biosynthesis, reduced oxidative damage | Abdelkrim et al. (2018) |
| 12 | Nutrient | Affected plant growth parameters | *Glycine max* | *Bradyrhizobium japonicum, AMF* | Nitrogen fixation and phosphate solubilization | Meena et al. (2018) |
| 13 | Allelopathic compounds | Effect on root and seedling growth | *Oryza sativa* | *Pseudomonas fluorescens* BRM-32111, *Burkholderia pyrrocinia* BRM-32113 | Increased plant growth parameters such as carbon assimilation, chlorophyll a and b levels, etc. | Rego et al. (2018) |
| 14 | *Aspergillus niger* | Suppression of plant growth and ultimately senescence | *Zea mays* | *Bacillus megaterium, Pseudomonas aeruginosa* | IST via defense related enzyme production such as phenylalanine ammonia lyase (PAL) and $\beta$-1, 1, 3-glucanase | Jha (2018) |
| 15 | *Tomato spotted wilt virus* and *Potato virus Y* | Plant growth suppression | *Solanum lycopersicum* | *Bacillus amyloliquefaciens* strain MBI600 | Induction of discrete gene expression patterns and genes related to salicylic acid (SA) or jasmonic acid (JA) signaling | Beris et al. (2018) |

PGPR induced drought tolerance was recently reported in pepper plants (*Capsicum annuum* L.). PGPR inoculation not only reduced water stress but also increased plant's vacuolar pyrophosphatase (V-PPase) pump enhancing photosynthetic activity and larger root system (Vigani et al. 2019).

At the molecular level, bacteria can provide stress resistance by modulating the gene expression of stress-causing genes. Growth and physiological responses of the plant can be accelerated by using recombinant technologies along with inoculation of multiple microbes as reported by Calvo-Polanco et al. (2016). They used four recombinant inbred lines of tomato and inoculated them with AMF and PGPR to see the amelioration effect of drought. These recombinant lines showed better stress-mitigating properties than wild ones (Calvo-Polanco et al. 2016). Another study on soybean (*Glycine max* L. Merrill) reported modulation and up-regulation of transcription factors such as dehydration responsive element binding (DREB) and ethylene responsive element binding (EREB), water uptake transporters like plasma membrane intrinsic proteins (PIP), and tonoplast intrinsic proteins (TIP) and many others due to inoculation with PGPR *Pseudomonas simiae* strain AU (Vaishnav and Choudhary 2019). In order to have a sustainable agriculture in future, the focus is on developing technologies and insight into plant–microbiome interaction at the molecular level without affecting the yield or productivity (Barea 2015). Plant and bacteria grow concomitantly in the soil where secretions of bacteria can trigger a series of internal reactions and contribute towards plant growth. Many genes have been reported in bacteria to promote plant growth such as auxin-induced genes (At4g36110, At2g33830) and techniques like DNA microarray, SAGE (serial analysis of gene expression), cDNA-AFLP (cDNA-amplified fragment length polymorphism), etc. are available to monitor plant and pathogen interaction during biotic stress (Verma et al. 2018).

## 6.4 Conclusion

Environmental stress causes a significant diminution in the growth of plants by hampering them at morphological, biochemical, physiological, and molecular level. The molecular aspects of plant–microbe interaction are the current focus in assessing stress-mitigating mechanism as it lays the foundation for breeding technology to pool out requisite genes, provides insights into pathways involved, in the production of miscellaneous novel compounds and in the development of new methods to ameliorate environmental stress. The alternate approaches for use of novel microbial compounds that are produced during stress and omics-based research including genomics, meta-genomics, metabolomics, etc. are highly recommended.

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# Chapter 7
# Plant–Microbe Interactions: An Insight into the Underlying Mechanisms to Mitigate Diverse Environmental Stresses

**Asifa Mushtaq and Seema Rawat**

**Abstract** Plants, being sessile, are often exposed to a range of biotic and abiotic stresses, which can negatively impact their growth and development causing humongous losses to the agriculture across the globe. Plants have evolved to sense and mitigate the adverse environmental challenges with an array of protective mechanisms, among which the plant–microbe interactions play a remarkable role in conferring tolerance to multiple stresses. The response of plant to a particular stress is governed by highly complex signaling pathways influenced by the associated microbes. As the abrupt climatic changes are posing a significant threat to the crop productivity, it becomes imperative to elucidate the plant–microbe interactions with respect to protection against environmental hostilities. Essentially, an exhaustive knowledge of the underlying mechanisms of microbe-mediated stress mitigation in plants can be translated into the development of resistant crop varieties, eventually paving the way for sustainable agriculture.

## 7.1 Introduction

Plants are often exposed to the diverse environmental stresses which affect their vital activities causing a substantial loss in crop quality as well as productivity. Even though a precise estimation of agricultural loss due to various stresses cannot be made, it has been very well documented that such stresses wallop large areas of land and consequently affect crop quality and quantity (Cramer et al. 2011). The varied stresses that a plant can experience have been broadly categorized into two groups, viz, biotic and abiotic. The biotic stresses are a manifestation of the interaction of a plant with pathogenic bacteria, fungi, or viruses as well as insects, and nematodes while the abiotic stresses are posed by drought, temperature extremes (high/low),

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salinity, light intensity, nutrient starvation, presence of heavy metals, and anaerobiosis. Although both biotic and abiotic stresses can impact the plant growth on various levels, some of the usual repercussions of these stresses on plants encompass hormonal and nutritional disparity, physiological disorders such as abscission, distorted growth, epinasty, senescence, and increased susceptibility to diseases (Nadeem et al. 2014). Plants initially perceive the effect of any hostile condition at the cellular level and the physiological symptoms become apparent with the passage of time. In a state of multiple biotic and abiotic adversities the plant metabolism can be put out of homeostasis which can stimulate the plant to make necessary advancements in genetic and metabolic mechanisms (Apel and Hirt 2004; Foyer and Noctor 2005; Gill and Tuteja 2010; Meena et al. 2017). Over the course of time plants have evolved to sense and mitigate the adverse environmental challenges with an array of protective mechanisms. The ability of plants to endure the challenging environmental conditions depends on a combination of processes comprising a wide spectrum of molecular and regulatory mechanisms (Duque et al. 2013; Mosa et al. 2017).

Plants interact with a myriad of soil microorganisms that bestow several benefits on the host plant, besides playing a quintessential role in quelling the burden of environmental stresses (Marulanda et al. 2006; Turner et al. 2013; Ngumbi and Kloepper 2014). Under the influence of several biotic and abiotic challenges, the consequence of plant–microbe interplay is regulated by the prioritization of physiological pathways. This cross-talk between plants and microbes is governed by a series of complex network of signaling pathways (Fujita et al. 2006; Pieterse et al. 2012; Mushtaq and Rawat 2017) which induce heterogeneous local and systemic responses that revamp metabolic capabilities of the plants to combat various forms of biotic and abiotic stresses (Kavamura et al. 2013; Nguyen et al. 2016). A variety of mechanisms have been proposed for underlying microbe-induced stress tolerance in plants (Fig. 7.1). The bacteria belonging to genera *Achromobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Methylobacterium*, *Microbacterium*, and *Serratia* have been reported to assist the host plants to adapt in different biotic and abiotic adversities through a wide spectrum of fine level molecular machinery (Joseph et al. 2007; Grover et al. 2011; Saharan and Nehra 2011). Goswami et al. (2016) reported that the plant-associated microbes employ either direct or indirect strategies to foster adaptive capacity in plants. Direct mechanisms comprise those in which plant–microbes enable an even distribution of plant growth regulators either by releasing the growth regulators themselves which are incorporated into the plant or by acting as a reservoir of hormones released by the plant itself and also by refining plant acclimatization via stimulating their metabolic activities (Govindasamy et al. 2011; Glick 2014). Indirect mechanisms involve the assistance of plant defensive metabolic processes primarily in the form of induced systemic resistance to plant pathogens (Choudhary et al. 2016). The main focus of this chapter is to get an insight into the underlying mechanisms which are employed by plant-associated microbes to impart resistance in plants against some of the major environmental constraints.

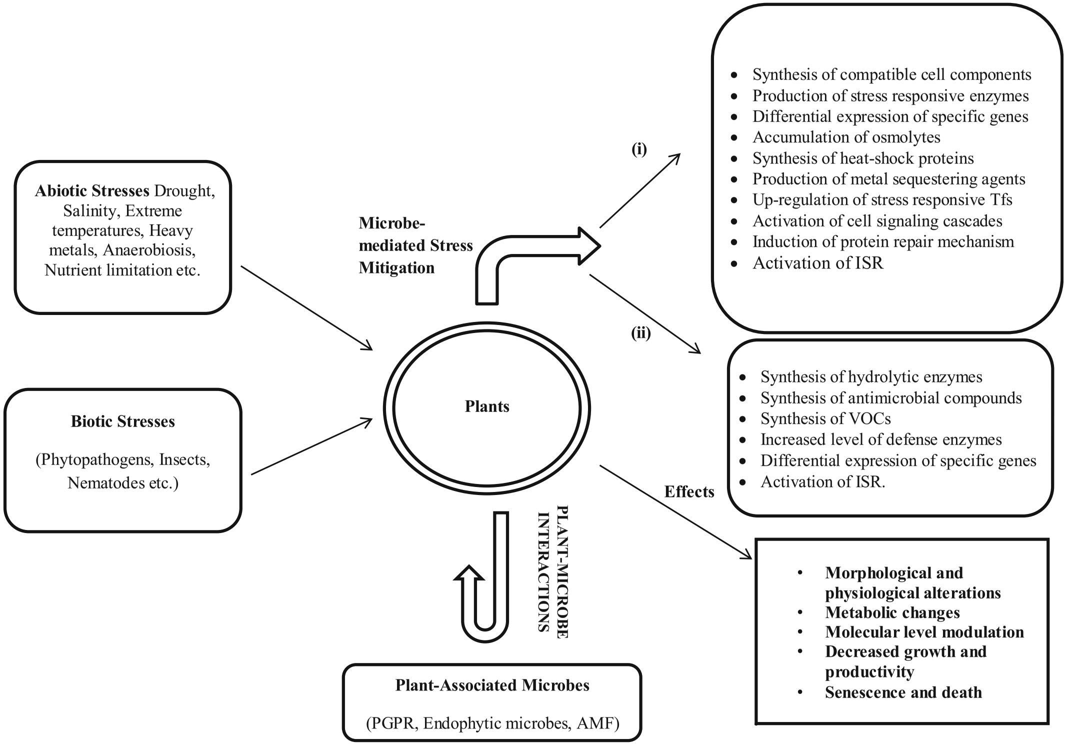

**Fig. 7.1** Schematic illustration of biotic and abiotic stresses encountered by plants, their effect, and the microbe-mediated alleviation of stress induced symptoms. (i) Abiotic stress responses. (ii) Biotic stress responses

## 7.2 Mechanisms Underlying Microbe-Mediated Stress Tolerance in Plants

### *7.2.1 Abiotic Stresses*

Hostile environmental conditions are becoming an abiding threat to agricultural crops across the globe. Abiotic stresses manifest a sequence of biochemical, physiological, morphological, and molecular alterations hampering plant growth and productivity (Wang et al. 2001). Over the years, role of connate metabolic and genetic capabilities of microorganisms to alleviate abiotic stresses in plants has become the intriguing area of research (Nadeem et al. 2014; Gopalakrishnan et al. 2015; Souza et al. 2015) (Table 7.1). This section will comprehend the mechanisms underlying microbe-mediated stress management in plants against the environmental hostilities that cause humongous losses to agriculture annually.

#### 7.2.1.1 Drought Stress Management

Drought being one of the primary abiotic stresses directly affects all the aspects of plant growth and development. The arid conditions induce a metabolic and osmotic variance which causes decline in cell turgor and stomata closure (Chaves et al. 2009) imposing a restriction on the uptake of $CO_2$ and thus resulting in suppressed cell growth and reduced photosynthesis (Shinozaki and Yamaguchi-Shinozaki 2000). The extended exposure of plants to these conditions can cause irreversible damage leading to plant death.

In order to survive the deleterious effects of drought, plants must respond in an appropriate manner. Plant-associated microbes are known to have a striking contribution in alleviating the drought stress symptoms. The role of plant growth promoting bacteria in eliciting the plant tolerance under arid and semiarid conditions has been widely recognized. It has been reported that plant growth promoting (PGP) bacteria confer Induced Systemic Tolerance (IST) in plants to cope with water deficit through diverse mechanisms. Plants are known to produce uncurbed levels of ethylene, when exposed to drought stress which modulates plant homeostasis internally leading to decreased root and shoot growth (Mahajan and Tuteja 2005). One of the defined properties of PGP bacteria is their ability to produce 1-aminocyclopropane-1-carboxylase deaminase, an enzyme that catalyzes the conversion of 1-aminocyclopropane-1-carboxylate (precursor of ethylene biosynthesis) to ammonia and α-ketobutyrate (Qin et al. 2016). Degradation of ethylene precursor by ACC deaminase releases plant stress and restores standard plant growth (Mayak et al. 2004; Naveed et al. 2014a, b). Plants, in order to overcome the drought-induced effects, employ a strategy of lowering the ethylene levels by associating with ACC deaminase producing microorganisms. The role of ACC deaminase producing PGPR strains to endow numerous plants with IST under drought stress has been thoroughly studied (Guo et al. 2015). Kohler et al. (2008) demonstrated that ACC

**Table 7.1** Microbe-mediated abiotic stress tolerance in plants

| Stress | Plant | Microorganism | Underlying mechanism | References |
|---|---|---|---|---|
| Drought | *Triticum aestivum* | *Pantoea alhagi* | Modulated accumulation of soluble sugars, proline, and malondialdehyde | Chen et al. (2017) |
| | *Triticum aestivum* | *Burkholderia phytofirmans* PsJN | Increased activity of glutathione reductase and CAT Increased lipid peroxidase and malondialdehyde. Enhanced accumulation of osmolytes | Naveed et al. (2014a, b) |
| | *Cucumis sativus* | *Bacillus cereus*, *B. subtilis*, *Serratia* | Efficient photosynthetic systems, antioxidases | Wang et al. (2012) |
| | *Phaseolus vulgaris* | *Rhizobiumetli* | Overexpression of trehalose-6-phosphatase synthase gene | Grover et al. (2011) |
| | *Phaseolus vulgaris* | *Rhizobium tropici* and *Paenibacillus polymyxa* | Upregulation of specific genes involved in stress tolerance | Figueiredo et al. (2008) |
| | *Solanum lycopersicum* | *Achromobacter piechaudii* ARV8 | ACC deaminase production | Kohler et al. (2008) |
| Heavy metals | *Triticum aestivum* | *Stenotrophomonas maltophilia* | Decreased proline and malondialdehyde, elevated activity of SOD, CAT, and POX | Singh and Jha (2017) |
| | *Brassica juncea* | *Pseudomonas brassicacearum*, *rhizobium leguminosarum* | Production of metal chelating molecules | Adediran et al. (2016) |
| | *Phragmites australis* | *Photobacterium* sp. | Increased activity of mercury reductase, IAA production | Mathew et al. (2015) |
| | *Vicia faba* | *Enterobacter cloacae* and *Pseudomonas* sp. | Modulated activity of SOD and CAT | Fatnassi et al. (2015) |
| | *Zea mays* | *Glomus* spp. | Increased SOD activity, high lead concentration in mycorrhizal roots | Zhang et al. (2010) |

(continued)

**Table 7.1** (continued)

| Stress | Plant | Microorganism | Underlying mechanism | References |
|---|---|---|---|---|
| Salinity | *Solanum lycopersicum* | *Enterobacter* sp. EJ01 | Rapid upregulation of conserved salt stress responsive genes | Kim et al. (2014) |
| | *Oryza sativa* | *Bacillus amyloliquefaciens* | Differential expression of specific set of genes | Nautiyal et al. (2013) |
| | *Glycine max* | *Metarhizium anisopliae* | Enhanced levels of endogenous JA, reduced malondialdehyde, elevated SOD activity | Khan et al. (2012) |
| | *Zea mays* | *Glomus mosseae* | Thicker root system and efficient root activity | Sheng et al. (2009) |
| | *Solanum lycopersicum* | *Glomus mosseae* | Elevated levels of SOD, POX, CAT, and ascorbate peroxidase | He et al. (2007) |
| Temperature | *Triticum aestivum* | *Enterobacter cloacae ZNP-3* | Synthesis of ACC deaminase, IAA, elevated levels of compatible solutes | Singh et al. (2017) |
| | *Triticum aestivum* | *Bacillus amyloliquefaciens*,<br>*Azospirillum brasilense* | Limited regeneration of ROS, preactivation of heat shock Tfs, modified metabolome | El-Daim et al. (2014) |
| | *Lycopersicum esculentum* L. (seedlings) | *Trichoderma harzianum* | Reduced accumulation of ROS | Mastouri et al. (2010) |
| | *Vitis vinifera* | *Burkholderia phytofirmans* | ISR activation | Barka et al. (2006) |

deaminase producing *Achromobacter piechaudii* ARV8 curtails the ethylene production in tomato seedlings exposed to conditions of drought and also shows a remarkable increase in the fresh and dry weight of tomato and pepper seedlings in water-deficit environment. Likewise, a consortium of PGPR species, namely, *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp.XY21 has been shown to impart induced systemic tolerance in cucumber plants under dry spell via retaining efficiency of photosynthetic systems, maintaining root vigor as well as increased activity of antioxidases, evading the involvement of ACC deaminase to reduce the levels of ethylene (Wang et al. 2012). Naveed et al. (2014a, b) have demonstrated that the bacterial inoculation of maize seedlings with endophytic strains *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17 minimizes the drought-imposed inhibitory effects besides strikingly increasing root biomass, shoot biomass, leaf area, chlorophyll content, and photochemical efficiency of Photosystem II.

Plants, under water-deficit conditions, elevate the osmotic potential by increasing the levels of osmolytes within the cells (Farooq et al. 2009). Dimkpa et al. (2009) reported that rhizobacteria produce osmolytes such as glycine betaine which might act in cooperation with glycine betaine produced by the plants in response to drought stress. Yuwono et al. (2005) demonstrated that rice plants inoculated with osmolyte-producing rhizobacteria on exposure to water-deficit conditions, exhibited more shoot and root dry weight along with number of tillers as compared to uninoculated plants. The roots in the inoculated rice plants proliferated vigorously (evidently to absorb more water) which could possibly be the effect of indole acetic acid (IAA) synthesized by osmotolerant bacteria, a hormone that stimulates the plant growth.

Plant root-associated bacteria have been acknowledged to influence various phenomena taking place at cell membrane interfaces. These processes are essential in shaping the physiological status of a plant including root cell membrane elasticity which plays a crucial role during the inadequate water availability. There are several studies which suggest rhizobacteria colonization directs plant root cell membranes to make necessary modifications in phospholipid composition modulating the cell membrane elasticity under water deficit. It has also been concluded that rhizobacteria-mediated modifications in the elasticity of root cell membranes could be one of the strategies implemented by plants in developing resistance against drought (Dimkpa et al. 2009). Sueldo et al. (1996) reported that wheat plants inoculated with *Azospirillum* restrain the rise in phosphatidylcholine and decrease in phosphatidylethanolamine content. Similar observations were reported by Pereyra et al. (2006).

Besides making significant metabolic and physiologic changes, plants also undergo various modifications at transcriptional level under water-deficit conditions. Timmusk and Wagner (1999) reported that the drought resistance in *Arabidopsis thaliana* was influenced by the PGPR strain *Paenibacillus polymyxa* which triggered transcription of drought-response gene ERD15 (Early Responsive to Dehydration) and Abscisic acid (ABA)-responsive gene RAB18 in the host plant. Likewise, another study carried by Rocha et al. (2007) on drought treated sugarcane highlights the differential expression of as many as 93 genes, encompassing conventional drought-responsive genes encoding MRB and WRKY transcription factors. Similarly, inoculation of *Rhizobium etli* (overexpressing trehalose-6-phosphatase synthase gene) was found to stimulate nodule formation with elevated nitrogenase activity, more biomass and drought resistance in *Phaseolus vulgaris* in contrast to the plants inoculated with wild type *Rhizobium etli*. Microarray analysis of 7200 expressed sequence tags of nodules from test plants divulged upregulation of genes entailed in stress resilience, suggesting the involvement of trehalose signal transduction (Grover et al. 2011).

Apart from plant-associated bacteria, arbuscular mycorrhizal fungi have also been reported to play a significant role in conferring drought resistance in the host plants through alteration of plant physiology and differential expression of specific genes (Ruiz-Lozano and Azcon 2000). Porcel et al. (2006) identified a 14-3-3 protein encoding gene from *Glomus intraradices* subjected to in vitro water deficit. The proteins regulating both signaling pathways as well as effector proteins were proposed to act in conferring drought resistance in the host plants. Aroca et al. (2007) reported the regulation of root hydraulic properties, in substantial correlation with plasma membrane intrinsic proteins (PIP) gene expression and protein phosphorylation by AM fungus *Glomus intraradices* in symbiotic association with *Phaseolus vulgaris* under arid conditions. Under inadequate water availability, amount of glutathione and ascorbate accumulation plays a critical role in maintaining the plant metabolism. Colonization of autochthonous drought resistant *Glomus intraradices* and *Glomus* sp. in lavender plants resulted in reduced accumulation of glutathione and ascorbate and increased tolerance against drought (Marulanda et al. 2007). A recent study conducted by Chen et al. (2017) highlighted the impact of colonization of a novel endophyte *Pantoea alhagi* in the drought exposed wheat plants. It was observed that the endophyte had a pronounced effect on the accumulation of soluble sugars, proline, and malondialdehyde (MDA) as well as on the degradation of the chlorophyll, thereby leading to the enhanced drought tolerance in wheat plants.

#### 7.2.1.2 Temperature Stress Management

Since temperature plays a critical role in the plant growth and development, any fluctuation in the optimal growth temperature (both below and above the optimum) might lead to severe repercussions in terms of plant growth and productivity (Koini et al. 2009; Pareek et al. 2010). Higher temperatures affect plant water relations, nutrient uptake, seed germination, membrane permeability, respiration and photosynthetic rate, and vigor of the organs involved in procreation and pollination (limiting fertilization), while extremely high temperatures can result in heat shock. Phytohormone levels may be elevated as a result of temperature fluctuations, revamping primary and secondary metabolites causing over expression of heat shock proteins, and generating reactive oxygen species (ROS) (Iba 2002). Prolonged exposure to such increased temperatures leads to excessive transpiration from the stomata leading to wilting and even death of the plant. At molecular level, heat shock can stimulate expression of otherwise unexpressed proteins altering DNA transposition recurrence and protein denaturation (Peterson and Higley 2000). On the other hand, excessive drop in optimal temperatures can cause intense mechanical and physical damages to the plants. Drop in temperatures below 0 °C leads to the formation of ice within the intercellular spaces which in turn increases the intracellular fluid accumulation resulting in increased cell membrane pressure and hence cell disruption (Sazzad 2007). Low temperatures also stimulate enhanced production of ROS within the cells causing oxidative damage to proteins, lipids, and DNA (Choudhury et al. 2013).

In order to cope with the challenging temperature fluctuations, plants undergo diverse physiological changes such as increased starch deposition, proline biosynthesis, and phenol accumulation which are the classic indicators of Induced Systemic Resistance (ISR) activation. Additionally, plants activate potential mechanisms that assist in upholding the cell membrane stability as well as induce MAPK (Mitogen-activated Protein Kinase) and CDPK (Calcium-dependent Protein Kinase) signaling pathways (Wang and Li 2006; Janska et al. 2010). Furthermore, increase in the level of antioxidants and appropriate solutes, synthesis of molecular chaperones, and activation of transcription factors are some of the prevalent phenomena occurring within the plants to cope up with harmful effects of temperature (Wang and Li 2006). Since chilling temperatures lead to reduced plant biomass and cell membrane injury resulting in electrolyte leakage, plants essentially accumulate cellular metabolites like sugars, proline (protects membranes and proteins), and anthocyanin in considerable amount to withstand the hostilities of chilling temperatures. Simultaneously, a variety of signaling pathways involved in the production of cold responsive proteins are also triggered, which include antifreeze proteins, detoxification enzymes, enzymes catalyzing biosynthesis of osmoprotectants, late embryogenesis abundant (LEA) proteins, lipid-transfer proteins, molecular chaperones, mRNA binding proteins, proteinase inhibitors, and transporter proteins (Shinozaki and Yamaguchi-Shinozaki 2000; Xiong and Zhu 2001; Wang et al. 2003; Grennan 2006; Nakashima and Yamaguchi-Shinozaki 2006; Yamaguchi-Shinozaki and Shinozaki 2006).

Plenty of literature accentuating the ability of plant-associated microbes to ameliorate the temperature induced stress tolerance in plants is available. Various studies advocating a positive correlation between fluctuating temperatures and microbe-mediated ISR have been put forth (Choudhary et al. 2016).

Colonization of bacteria with ice-nucleating activity can be an efficacious means of managing the cold temperature stress in plants. Lindow and Leveau (2002) reported that the epiphytic bacteria such as *Erwinia herbicola* and *Pseudomonas syringae* impeded the supercooling of plants at freezing temperatures by raising the ice-nucleation temperature, thereby, protecting the plants from frost damage. A plant growth promoting bacteria *Burkholderia phytofirmans* strain PsJN was reported to induce tolerance in *Vitis vinifera* exposed to heat stress (Compant et al. 2005) and cold temperature (Barka et al. 2006). The inoculation of *Burkholderia phytofirmans* PsJN strain into *Vitis vinifera* subjected to cold treatment lowered the biomass reduction rate as well as electrolyte leakage and also assisted the host plant in post-chilling recovery. The plants also showed increased accumulation of carbohydrates as compared to control plants. The marked increase observed in the rate of photosynthesis, starch deposition as well as higher levels of proline and phenols was suggestive of ISR activation.

Ali et al. (2009) reported the potential of a thermotolerant *Pseudomonas* sp. to mitigate the heat stress in sorghum seedlings by synthesizing high molecular weight proteins, improving biomass and increasing amino acid, sugar, proline, and chlorophyll content of the host plant. Ali et al. (2011) investigated the effect of inoculation of thermotolerant PGP *Pseudomonas putida* strain AKMP7 on the growth of wheat plants subjected to heat stress. Inoculated plants showed a marked difference in terms of root and shoot length, quantity of tillers, dry biomass, spike length, and grain formation as compared to uninoculated plants. Inoculated plants also had improved levels of cellular metabolites such as sugars, starch, amino acids, proline, chlorophyll, and essential proteins. These plants were also found to have minimum membrane injury with increased activity of antioxidases such as SOD, APX, and CAT to scavenge ROS. Selvakumar et al. (2012) demonstrated the ability of novel cold resistant PGPR, namely, *Exiguobacterium acetylicum*, *Pantoea dispersa*, *Pseudomonas fragi*, *Pseudomonas lurida*, and *Serratia marcescens* promoting growth of plants under extremely low temperatures (Tewari and Arora 2013). Similarly, inoculation of plant growth promoting *Azospirillum brasilense* and *Bacillus amyloliquefaciens* to *Triticum aestivum* plant seedlings was found to impart heat stress tolerance to the plants by causing a reduced regeneration of ROS, changes in the metabolome, and preactivation of heat shock transcription factors (El-Daim et al. 2014).

#### 7.2.1.3 Salinity Stress Management

Increasing soil salinity is a challenging environmental issue that affects the agricultural crops worldwide (Coleman-Derr and Tringe 2014). There occurs a three-fold effect of the salt stress reducing the water potential, affecting cellular metabolism, and causing ion imbalance and toxicity (Singh 2014). Some vital physiological processes including seed germination, germination time, $Na^+/K^+$ ratio in root and shoot, and root/shoot dry weight which are pivotal in the plant growth and development are also affected (Parida and Das 2005). As a result of the untimely exposure to salinity, ion toxicity occurs within the plant cells causing imbalance in the osmoregulation especially during the prolonged stress conditions (Munns and Tester 2008). Such deleterious conditions induce the generation of ROS which are detrimental for cell viability, thereby, limiting plant growth and development.

Ideally, both ionic and osmotic homeostasis need to be upheld within the plant cells to sustain the salinity stress. Plants usually combat the salinity stress by keeping susceptible tissues less exposed to the saline environment either by exuding ions from the roots or compartmentalizing ions distant from the cytoplasm of physiologically vital cells (Silva et al. 2010). At the molecular level, plants respond to salt stress via conserved signal transduction pathways triggering several stress forbearance and endurance processes (Xiong et al. 2002) encompassing the role of a stress phytohormone Abscisic acid (ABA). ABA is pivotal in such processes as it regulates the expression of genes whose promoter regions have ABA-responsive elements.

Several plant-associated bacteria have been reported to enhance salt resistance of their host. Such resistance is primarily explained by employing multiple mechanisms such as maintaining water and hormone homeostasis, accumulation of appropriate osmolytes, synthesis of antioxidants, production of volatile compounds and ACC deaminase as well as altering $Na^+$ uptake/transport (Yang et al. 2009; Dodd and Perez-Alfocea 2012). Interference in the production of ABA has been chiefly ascribed to boost the tolerance under saline conditions (Aroca et al. 2006; Jahromi et al. 2008). Similarly, the mechanisms employed by arbuscular mycorrhizal fungi to alleviate the adverse effects caused by salinity stress include alteration in physiological and enzymatic activities of the host plant, modifying root architecture to assist in water absorption, revamping the plant nutrition, and revising $Na^+$ and $K^+$ uptake (Evelin et al. 2009; Gamalero et al. 2010; Zhang et al. 2011; Zolfaghari et al. 2013; Krishnamoorthy et al. 2016).

Bano and Fatima (2009) reported that *Zea mays* plants inoculated with *Pseudomonas* sp. and *Rhizobium* sp. developed tolerance to salinity stress by increasing proline accumulation, reducing the electrolyte leakage, stimulating selective uptake of $K^+$ ions, thereby, retaining the water content of the plant leaves. ABA producing PGPRs isolated from halophytic weeds were shown to alleviate in vitro salt stress in *Glycine max* (Naz et al. 2009). *Pseudomonas* sp. with its desirable root colonizing ability along with its potential to produce exopolysaccharides has been demonstrated to confer increased tolerance to rice plants exposed to saline conditions (Sen and Chandrasekhar 2014). Similarly, *Enterobacter* sp. EJ01 was found to facilitate the growth in *Arabidopsis* and tomato subjected to salinity stress via activation of osmolytes accumulation, ROS scavenging, LEA (Late embryogenesis abundant protein) genes governing the restoration and conservation of proteins (Kim et al. 2014). The bacteria exhibited the property of in vitro ACC deaminase and IAA production. It was also observed that the short-term treatment of *Arabidopsis* with *Enterobacter* sp. EJ01 enhanced the expression of salt stress responsive genes such as DREB2b, RD29A, RD29B, and RAB18. The expression of genes involved in proline biosynthesis (P5CS1 and P5CS2) and priming processes (MPK3 and MPK6) was also up-regulated in response to stress stimulus. *Bacillus amyloliquefaciens* RWL-1 has been reported to confer tolerance in *Oryza sativa* against salinity stress via production of ABA and auxin phytohormones (Shahzad et al. 2017). Likewise, phytohormones producing endophytic bacteria along with exogenous application of jasmonic acid have been reported to enhance the growth of *Solanum pimpinellifolium* under saline environment (Khan et al. 2017). A promising soil bacterium *Pseudomonas frederiksbergensis* strain OS261 has been reported to mitigate salt stress in red pepper plants via reduction of ethylene production and regulating the activity of antioxidant enzymes (ascorbate peroxidase, superoxide dismutase, and catalase) (Chatterjee et al. 2017).

#### 7.2.1.4 Heavy Metal Stress Management

Heavy metals are metallic elements with relatively higher density (>4 g/cm$^3$) and high relative atomic weight (>20). They are non-degradable and even toxic at low concentrations (Duruibe et al. 2007; Chibuike and Obiora 2014; Ma et al. 2016a, b). Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are some of the heavy metals which are essential for the organisms in trace amounts (Wintz et al. 2002) but uncurbed quantities of these elements can have a negative impact on the organisms. Pb, Cd, Hg, and As, on the other hand, are the main cause of concern as they are highly toxic to the plants and animals, even in smaller amounts. Although heavy metals are naturally present in the soil, but continued industrialization, intensive agricultural practices, and anthropogenic activities have led to an increased concentration of heavy metals to the level where they become pernicious to the plants and animals. Although plants uptake certain heavy metals from the surrounding soil to facilitate their metabolic activities, accumulation of metal concentrations beyond the optimal levels can have adverse effects on plant growth and development such as inhibition of cytoplasmic enzymes, oxidative damage to the cellular structures (Jadia and Fulekar 2009), metabolism alteration, reduced biomass, chlorosis, decreased rate of photosynthesis, and senescence (Nagajyoti et al. 2010).

Plant growth promoting bacteria have been reported to exhibit bioremediation properties through various mechanisms such as detoxification of the metal pollutants via oxidation-reduction, biosorption and bioaccumulation at the cell wall, precipitation of heavy metals, transport of metals across the cytoplasm, and metal entrapment in the bacterial capsule (Zubair et al. 2016; Tiwari and Lata 2018). Many rhizobacteria have the ability to release metal chelators such as siderophores which form stable complexes with heavy metals that negatively impact the plant health such as Al, Cd, Cu, Ga, In, Pb, Ni, and Zn (Neubauer et al. 2000; Egamberdiyeva 2007; Dimkpa et al. 2008; Ahemad and Kibret 2013). Siderophore binding thus increases the concentration of soluble metal (Rajkumar et al. 2010) influencing bioavailability of toxic heavy metals, thereby, aiding the plant to overcome the constraints of excessive levels of heavy metals in the soil. Pishchik et al. (2002) observed the significant effects of PGPR *Klebsiella mobilis* CIAM inoculation on barley plants grown on Cd-contaminated soil. The inoculated plants showed higher grain yield and two-fold decrease in Cd content in the grain. It was observed that the ability of bacteria to bind free Cd ions seldom makes these ions available for the plants. Furthermore, the test bacterial strains also possessed the properties of nitrogen fixation, IAA, or ethylene production possibly playing a vital role in conferring stress tolerance to the plants. Interestingly, cell wall components of some of the heavy metal resistant PGPR (such as *Pseudomonas*) possess metal binding properties which might aid in intracellular accumulation of metals such as copper and cadmium. Association of such bacteria can limit the metal uptake by plants (Ganesan 2008; Sinha and Mukherjee 2008; Pabst et al. 2010), thereby helping the plant to acclimatize in hostile conditions. Fatnassi et al. (2015) demonstrated that the combined inoculation of copper accumulating *Rhizobium* and PGPR (*Enterobacter* *cloacae* and *Pseudomonas* sp.) was able to reduce the inhibitory effects of copper stress on *Vicia faba* plants. The copper tolerance in the plant was linked to the modulated activity of antioxidases (both superoxide dismutase and catalase) influenced by the co-inoculation. Kamran et al. (2016) demonstrated the potential of PGPR *Pseudomonas putida* to enhance the growth and tolerance of *Eruca sativa* under nickel stress.

AMF have also been reported to play a critical role in phytoremediation, especially in phytostabilization (Leyval et al. 2002; Orlowska et al. 2002; Regvar et al. 2003; Barea et al. 2005). AMF possibly sequesters heavy metals either by releasing chelators or by directly absorbing the heavy metals. Recently the role of AMF to hyper-accumulate heavy metals has generated a lot of interest among the researchers (Turnau et al. 2005). A recent study conducted by Hashem et al. (2016) has shown the role of AMF in mitigating the adverse effects of cadmium stress in *Solanum lycopersicum* by reducing malondialdehyde and hydrogen peroxide production, thereby palliating the oxidative stress as well as by increasing the production of proline and phenol content of the cells. Another study by Franchi et al. (2017) has revealed that the addition of thiosulfate with metal resistant PGPR improves the mobilization and uptake of arsenic and mercury in *Brassica juncea* and *Lupinus albus* fostering bioavailability and phytoextraction. Similarly, very recently, a metal resistant *Azotobacter chroococcum* strain CAZ3 has been shown to ameliorate the heavy metal (Cu and Pb) induced oxidative damage in *Zea mays*. The strain produced significant amounts of IAA, ACC deaminase, ammonia, and siderophores under metal pressure. The isolate also showed the metal chelating properties. Furthermore, the inoculation of the strain led to the decreased levels of proline, malondialdehyde, and antioxidases in plant leaves (Rizvi and Khan 2018).

### *7.2.2 Biotic Stress Management*

In addition to varying abiotic stresses, plants are also inflicted by the attack of pathogens (bacteria, fungi, viruses, protozoa, and nematodes) as well as by other plant pests (Atkinson and Urwin 2012). Abiotic stresses may also contribute to weakening the defense mechanisms of plants and thus making them susceptible to phytopathogens (Suzuki et al. 2014), for example elevated temperatures are known to facilitate pathogen transmission (Madgwick et al. 2011; Nicol et al. 2011). Likewise, chilling temperatures have been reported to compromise gene silencing, an efficient defense mechanism against plant viruses (Szittya et al. 2003; Suzuki et al. 2014). Biotic stresses are known to cause massive destruction of crop productivity annually (Sazzad 2007; Ramegowda and Senthil-Kumar 2015). Phytopathogens have been recognized as a main threat challenging the food productivity and ecosystem stability across the globe. Some of the common manifestations of these biotic factors are nutrient imbalance, imbalanced hormonal regulation, and various physiological disorders.

**Table 7.2** Microbe-mediated biotic stress tolerance in plants

| Plants | Pathogen | Biocontrol agent | Resistance mechanism | References |
|---|---|---|---|---|
| *Cucumis sativus* and *Triticum aestivum* | *Fusarium oxysporum*, *Botryosphaeria ribis*, *Alternaria* (Nees), *Rhizoctonia solani* | *Paenibacillus jamilae*HS-26 | Synthesis of hydrolytic enzymes, antifungal metabolites and VOC | Wang et al. (2019) |
| *Persea americana* | *Phytophthora cinnamomi*, *Colletotrichum gloeosporioides* | *Serratia* sp. | Synthesis of antifungal secondary metabolites | Granada et al. (2018) |
| *Triticum aestivum* | *Fusarium graminearum* | *Stenotrophomonas maltophilia* | Increased level of defense enzymes such as PAL, PO, PPO, β-1,3 glucanase | Singh and Jha (2017) |
| *Piper nigrum* | *Stemphylium lycopersici* | *Brevibacterium iodinum* | Increased expression of pathogen-related protein genes | Son et al. (2014) |
| *Cucumis sativus* | Cucumber mosaic cucumovirus | *Azotobacter* chroococcum, *Bacillus subtilis*, *Pseudomonas fluorescens* | Increased activities of POX and β-1,3 glucanase<br>Secretion of pathogen-related proteins | El-Borollosy and Oraby (2012) |
| *Oryza sativa* | *Xanthomonas oryzae* | *Bacillus* sp. | Enhanced accumulation of POX, phenylalanine lyase, and polyphenol oxidase | Chithrashree et al. (2011) |
| *Arabidopsis thaliana* | *Pseudomonas syringae* | *Bacillus subtilis* | Activation of induced systemic resistance | Rudrappa et al. (2008) |

Large body of literature eliciting the potential of plant-associated microbes in ameliorating the stress symptoms imposed by the attack of phytopathogens is available (Dobbelaere et al. 2003; Domenech et al. 2006; Saleem et al. 2007) (Table 7.2). The widely recognized mechanisms through which these microbes impart tolerance to the plants include activation of cellular component including cellular burst, accumulation of secondary metabolites, production of inhibitory allelochemicals, cell wall reinforcement, expression of genes encoding pathogenesis-related proteins, and induced systemic resistance (ISR) (Nurnberger et al. 2004; Compant et al. 2005; Lugtenberg and Kamilova 2009). The hormones that play a vital role in plant defense include ethylene, jasmonic acid (JA), and salicylic acid (SA) (Bari and Jones 2009; Verhage et al. 2010). *Bacillus* and *Pseudomonas* species have been widely recognized to potentiate ISR against varied phytopathogens via diverse mechanisms (Choudhary and Johri 2009; Bhattacharyya and Jha 2012). Majority of PGPR have been reported to elicit ISR potentially in the host plant via multiple signal transductions like SA-independent JA-ethylene-dependent (Pettersson and Bååth 2004) and NPR 1-dependent signaling pathways (Niu et al. 2011) as well as stimulating ISR-related gene expression.

PGPR strains have been reported to elicit ISR in a range of plant hosts via the activation of various defense related enzymes such as β-1,3-glucanase, chitinases, peroxidases, phenylalanine ammonia lyase, and polyphenol oxidase (Bharathi 2004). Many studies have highlighted the ability of rhizobacteria to contribute in the antibiosis of the host plant through the production of peptide antibiotics, siderophores, volatiles, and other organic metabolites (Maksimov et al. 2011). The ability to release a wide spectrum of lytic enzymes such as cellulases, chitinases, glucanases, and proteases to curb the growth of filamentous fungal pathogens by *Bacillus*, *Pseudomonas*, and other antagonistic bacteria makes them the appropriate biocontrol agents (Chernin and Chet 2002; Maksimov et al. 2011). Various reports are suggestive of ethylene synthesis inhibitors having an antagonistic effect against plant pathogens. It has also been reported that co-inoculation of PGPR with AMF curbs the repercussions of phytopathogens through stimulating growth attributes and increasing the resistance against plant diseases (Dohroo and Sharma 2012). PGPB have also been reported to evoke a series of other defensive strategies such as regulating quorum sensing phenomenon, activation of antioxidant enzymes, activation of phenylpropanoid pathway triggering the phenolic production, lignification of cell walls, and transgenerational defense response to overcome the hostilities of the parasite (Mishra et al. 2015).

Compant et al. (2005) reported that PGPM activate defense mechanisms in *Dianthus caryophyllus* against wilt causing *Cucumis sativus* and *Fusarium* sp. against foliar disease causing *Colletotrichum orbicular* pathogens. Rudrappa et al. (2008) reported that *Bacillus subtilis* FB17 protects the host plant against foliar pathogen *Pseudomonas syringae* pv. tomato Pst DC3000 through ISR. Many *Bacillus* strains have been shown to elicit ISR in rice against *Xanthomonas oryzae* pv. oryzae, the causative agent of bacterial leaf blight (Chithrashree et al. 2011). Similarly, Lee et al. (2015) reported the activation of ISR in *Panax ginseng* against *Phytophthora cactorum* by *Bacillus amyloliquefaciens* strain HK34. *Paenibacillus* P16 has been reported to be an effective biocontrol agent in cabbage against black rot caused by *Xanthomonas campestris*, simultaneously inducing systemic resistance (Ghazalibigla et al. 2016). Recently, *Serratia* sp. isolated from avocado plants has been demonstrated to produce secondary metabolites like serratamolide, haterumalide NC, prodigiosin with a significant inhibitory activity against *Phytophthora cinnamomi* and *Colletotrichum gloeosporioides* (Granada et al. 2018). A very recent study by Wang et al. (2019) has shown the biocontrol ability of *Paenibacillus jamilae* HS-26 isolated from cucumber rhizosphere against several fungal pathogens. The bacteria has been shown to produce hydrolytic enzymes and antifungal metabolites as well as volatile organic compounds essentially, N, N-diethyl-1, 4-phenylenediamine.

## 7.3 Conclusion

Plants share an intriguing relationship with the associated microbes. This interaction plays a quintessential role in maintaining the plant health both under biotic and abiotic stresses. Plant-associated microbes assist in reprogramming of plant physiology and metabolism via multiple and intricate signaling cascades for perceiving and responding to a diversity of biotic and abiotic adversities. Microbe-mediated stress mitigation provides an excellent model for apprehending the mechanisms underlying stress management. A comprehensive understanding of these interactions can be underpinning in developing biotechnological tools for a sustainable agriculture. Development of stress-resistant microbial bioformulations can be an efficient way to improve crop quality in challenging environmental conditions. As our understanding on the mechanisms governing plant responses to diverse environmental adversities expands, we will be able to get closer in comprehending the functioning of natural ecosystems where plants interact with biotic and abiotic factors. The use of OMICs in understanding the precise mechanisms of microbe-mediated stress mitigation in plants will strengthen the scope of developing crops capable of enduring extreme environmental conditions which have been anticipated to worsen in future.

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# Chapter 8
# Omics Technology: Role and Future in Providing Biotic and Abiotic Stress Tolerance to Plants

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**Abstract** Development and productivity of the plants are constantly threatened by abiotic and biotic stresses. Plants have in turn developed various strategies to combat these stressed conditions. Technological advancement has led to the integration of biology and computer science which has helped mankind significantly with various analytical advancements to understand plant system biology more precisely. Genomics, transcriptomics, proteomics and metabolomics are collectively known as 'omics'. These molecular tools help to integrate biological information encoded within the molecular system of the plants. These strategies act as powerful tools in deciphering the intricate, well-coordinated signalling pathways and understanding of plant phenotypic responses at cellular and molecular level towards various environmental stimuli. This chapter briefly addresses the current omics strategies used for deciphering the mechanisms involved in plant stress tolerance.

## 8.1 Introduction

Plants being confined to a fixed area are exposed to several biotic and abiotic stresses throughout their life cycles. This attack is just not over with the completion of their life cycles; even the produce obtained after harvesting is threatened by several storage pests. There is a need to understand the molecular mechanisms of biotic and abiotic stresses in plants in order to define a strategy to cope against them. Many conventional methods like agronomic managements, development of tolerant varieties through plant breeding practices have helped the agricultural scientists

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enormously in the past. But there is a continuous evolution in the agents causing biotic stress or increase in exposure to the degree of abiotic stresses on the plants. So, there is a need to adopt new technologies against these. Omics technologies have provided a way forward to biological science in this regard.

Omics are the emerging technologies to understand the behaviour and response of cells, tissues, organs and the whole organism at the cellular and molecular level using different modern-day technologies like genomics, proteomics, system biology, bioinformatics, as well as the computational tools needed to analyse and make sense of the data. *Omics* refers to the collective technologies used to explore the roles, relationships and actions of the various biomolecules that make up the cells of an organism. These technologies have potential to develop and integrate the available in vivo (phenotypic and other data such as inheritance pattern, etc.) and in vitro data (developed by several computational methods applied on living system like transcriptome or proteome data by using bioinformatics and molecular biology interventions).

These *omics* technologies measure the changes in the entire genome, gene, RNA, protein, metabolites or even at ionic level occurring at cellular level. Several fields of *omics* have evolved accordingly like the field of *omics* dealing with study at genome level is called as genomics and that deals with transcriptome is called as transcriptomics and so on. The field of *omics* includes: Genomics: study of structure, function of whole genome as well as comparison of genomes; Transcriptomics: study of change in gene expression profiling in different tissues; Proteomics: study of entire compendium of proteins; and Metabolomics: study of molecules involved in cellular metabolites

### *8.1.1 Genomics*

'Genome' term was first given by Hans Winkler and it stands for haploid DNA content in a cell including the representation from organelle genome. In simpler term 'Genomics' is the field of omics which deals with the study of genome (Keller 2011). It involves molecular characterization and evaluation of whole genome in order to study its structure, function and comparison with related organism. Structural genomics, functional genomics and comparative genomics are different subsidiaries of genomics (Rubin et al. 2000). (1) Structural genomics is the field of genomics which deals with the study of genome and protein structure. Genome structure analysis mainly includes gene mapping, gene density, genome complexity, repeat analysis, cot curve analysis and other such studies, while in protein structure analysis it mainly includes high throughput methods for determination of protein structure by using a combination of experimental and modelling approaches (Baker and Sali 2001). (2) Functional genomics is the field of genomics which deals with experimental strategies adapted in order to reveal the function of different genes which genomes possess. It is further classified into two groups: one is forward genetics approaches, adapted to identify the causal gene when the only phenotype is known. It is usually done by creating random mutation in the genome either through chemical mutagens (EMS is mainly used for chemical mutagenesis), physical mutagens (γ radiation are main physical mutagens) or biological mutagens (transposable elements and T-DNA insertions are mainly used, they create insertion mutagenesis) followed by mapping of mutation. Classical plant breeding such as QTL mapping is also a forward genetics approach. Reverse genetics approach is adapted to identify the function and phenotype produced by a gene when its sequence is known. In order to learn the influence a sequence has on the phenotype, or to discover its biological function, researchers can engineer a change or disrupt the DNA sequences specifically or alter the expression of the concern sequences followed by tracking the changes occurring in the phenotype. There are several approaches available for reverse genetics, e.g. site directed mutagenesis, target induced local lesions in genome, RNAi, virus induced gene silencing, etc. (Robinson et al. 2011). (3) Comparative genomics approaches are used to compare and contrast the genome or genes to study their evolution pattern. Synteny and collinearity analysis, consensus maps and comparative maps, etc. are some of the approaches used in comparative genomics (Hardison 2003).

### *8.1.2 Transcriptomics*

Gene expression inside a cell is highly spatio-temporal dependent. It is highly regulated by several environmental stimuli, for example, same tissue when uninfected and infected with a particular pathogen will have different gene expression profile which can be exploited to identify the causal genes (Geiger et al. 1996). Entire compendium of mRNA and small RNA expressing inside a cell constitutes transcriptome and a strategy adopted to study transcriptome is called as transcriptomics approach (Wang et al. 2009). Microarray analysis, serial analysis of gene expression, massive parallel signature sequencing, RNA-seq approaches are utilized for transcriptome analysis.

### *8.1.3 Proteomics*

Entire compendium of protein expression inside the cell including its post-translational modification, interactome profile and splicing products constitutes proteome (Blackstock and Weir 1999). Microarray, 2-dimensional gel electrophoresis, mass spectrometry, etc. are some of the strategies adopted to study proteomics. With the advancement in genomics several discoveries are being made day by day. These fields have been extensively exploited in order to develop resistance in plants against several biotic and abiotic stresses which they encounter in their life cycles. Changes at genome level, alteration in gene expression profiling (in terms of transcriptome and proteome) are the keys to find the genetic reasons for the harmful metabolic drift and to develop strategies to cope up with them (Mittler and Blumwald 2010). In this chapter we are presenting a comprehensive compilation of several discoveries made in regard to develop biotic and abiotic resistance, identifying their causes using different genomics approaches.

## 8.2 Application of Different Omics Technique in Biotic Stress

When plants are affected by environmental factors like extreme temperatures, light, salinity, etc., it is called abiotic stress (Mittler 2006) and when stress is caused by living organisms it is called biotic stress (Suzuki et al. 2014). The components of biotic stress are fungi, bacteria, virus, parasites, insects, weeds, etc. Upon recognizing the pathogens, plants also modulate their defence pathway accordingly in an effort to cope up those stresses. Hence, gene expression pattern, proteome dynamics and metabolite pool get altered from those at natural condition. To address how plants use their intercellular and intracellular mechanisms to combat these challenges, multi-omics techniques are the best way to take up (Seneviratne et al. 2017).

The omic approaches are extensively engaged in elucidating biological pathways which are actively involved in triggering responses of host and disease development. When plant senses pathogen attack and recognizes pathogen associated molecules it rapidly alters its behaviour and results into different signalling event. The components of basal defence line up get triggered (Mehta et al. 2008). On the other hand, pathogen also continues its effort to make the infection successful by interfering plant defence line. In the course of this confrontation, plant resets the level, form and activity of its macromolecules in order to better withstand the pathogen intervention. The system level understanding to pursue all those interrelated changes in plant cells can be achieved through the application of different omic techniques. In this portion we will focus on transcriptomics, proteomics and metabolomics techniques deployed to explore molecules involved in resistance development in different crop under biotic stress.

### *8.2.1 Transcriptome Analysis*

Transcriptomics study can provide a major understanding about why the same plant varies in resistance capability against the same pathogen at different ages or why within a genus some species can protect themselves whereas some are vulnerable to infection to a particular strain of pathogen (Dodds and Rathjen 2010). This also provides a fair idea about how genes are regulated at a particular time in a particular cell or tissue of an organism. Nowadays, transcriptome study is on high demand to screen out actively operating genes, pathways and interactions under any stress

**Table 8.1** Some examples of RNA-seq deployed to identify the underlying gene and pathway

| Crop | Pathogen | References |
|---|---|---|
| Poplar | *Marssonina brunnea* | Zhang et al. (2018) |
| Tomato | *Phytophthora parasitica* | Naveed and Ali (2018) |
| *Nicotiana benthamiana* | *Pseudomonas* spp. | Pombo et al. (2019) |
| Apple | Fungal pathogen<br>Apple stem grooving virus<br>*Erwinia amylovora* (bacteria) | Balan et al. (2018) |
| Rice (*Oryza sativa*) | *M. oryzae* Guy11<br>*M. oryzae* ZB13 | Cohen et al. (2019) |
| Sunflower | *Plasmopara halstedii* | Ramu et al. (2016) |

situation as it captures snapshot data on gene expression dynamics at a given time which becomes gradually instrumental to identify the underlying major gene(s). There are two main techniques in the field: microarray, which gives a relative quantitative value of the amount of transcript and identification based on a set of predefined sequences, and RNA-seq, which is proceeded through sequencing of transcript and mapping them in the reference, be it de novo or already existing Table 8.1**.** As the latter one provides very precise and absolute data about transcript read count and also works for exploring new transcript, it is adapted as a more popular technology in different molecular biology experiments (Tseng et al. 2012).

## 8.2.2 Proteomics Techniques

As and when pathogen entry gets initiated, many biological processes are triggered as well as many become suppressed. Functional genomics has been a boon to identify protein and corresponding gene by detecting those changes in transcript and protein level. Moreover, all the changes in transcriptome do not correlate with the proteome due to various factors like post-transcriptional modification, degradation, instability of the transcript, etc. Therefore, proteomics study has gained more focus to reveal the pathways and network of functional molecules in host plant.

Many proteomics tools and techniques have been exploited to date to detect pathogens and to shed light on host mechanisms under different biotic and abiotic stresses. These techniques have the potential to detect the proteins as well as define the underlying pathways and protein–protein interaction network. With the advent of enzyme linked immunosorbent assay (ELISA), detection of phytopathogen was remarkably easy (Lopez et al. 2003). 2D gel electrophoresis followed by mass spectrometry (MS) has long been exploited as a popular technique to identify the protein from stressed (biotic/abiotic/chemical) samples. Four defence-related protein (namely 14-3-3: master regulator active in biotic stress, Rab11: GTPase, RPN7: 26S proteasome subunit and an mRNA binding protein) and a ubiquitin pathway related protein were successfully detected and identified from *Capsicum annuum*

**Table 8.2** Proteomics utilized against different pathogens

| Plant–pathogen interaction | Strategy | References |
|---|---|---|
| *Brassica napus–Piriformospora indica* | LC-MS/MS | Shrivastava et al. (2018) |
| *Brassica napus–Alternaria brassicae* | 2DE and MS | Sharma et al. (2007) |
| Wheat–*Puccinia triticina* | 2DE and MS | Rampitsch et al. (2006) |
| Wheat–Fusarium graminearum | 2DE and LC-MS/MS | Zhou et al. (2005) |
| Rice–*Xanthomonas oryzae* | 2DE and MS | Mahmood et al. (2006) |
| Rice–*X. oryzae* pv. *Oryzae* | | Chen et al. (2007) |
| *Nicotiana benthamiana*–pepper mild mottle virus | 2DE and N-terminal sequencing | Pérez-Bueno et al. (2004) |

cv. Bugang inoculated with Tobacco mosaic virus (TMV) (Lee et al. 2006). In rice, infected with yellow mosaic virus (RYMV), Brizard and co-workers found the significant presence of 223 proteins which were later well categorized among 3 classes: defence category, metabolism category, protein synthesis category (Brizard et al. 2006).

IP-MS is a technique which can address protein–protein interaction (PPI) between host and pathogen. When pathogens come in contact with their hosts, they try to turn down the host defence mechanism. In this course of action direct or indirect PPIs take place at different levels. Identification and characterization of PPIs can give scientists a lead to discover drugs against that pathogen. Not only that, IP-MS can also be exploited to pursue how disease progression has taken place. Detection and analysis of different mechanisms sensitized upon attack of pathogen HCMV was also performed by exploring IP-MS technique (Beltran et al. 2017).

Proteomics can be deployed to decipher the type of plant–pathogen interaction. A study looking for the differential protein profile in resistant and susceptible *Pinus lambertiana* infected with fungus *Cronartium ribicola* (biotrophic fungi) unravelled the significant downregulation of two acidic proteins similar to ß-1,3-glucanase in susceptible plants whereas chitinase level got increased in resistant plant (Ekramoddoullah and Hunt 1993). In another study, SDS-PAGE, peptide mass fingerprinting and sequence determination by MS were utilized to get an insight on the change in the protein profile of the xylem sap of tomato (*Lycopersicon esculentum*) in response to *Fusarium oxysporum* (necrotroph) infection (Rep et al. 2002). To identify the differentially expressed proteins in rice upon the infection of *Magnaporthe grisea* (hemibiotroph), 2DE and MALDI-TOF analysis were sought in another study (Kim et al. 2004) (Table 8.2).

Proteomics approach has the ability to study post-translational modification (PTM) also. When pathogen interacts with host, many PTMs take place in both host and pathogen proteome as a signature of its dynamic nature under selection pressure. Studying those PTMs may lead to discover biomarker with respect to that specific host–pathogen interaction. In one study on animal system this concept has been proved (Zhang and Ge 2011).

### *8.2.3 Metabolomics Technique*

In spite of tremendous advancements in proteome strategies, many questions remain unanswered about the holistic changes in plant system that took place over pathogen attack. These questions can be addressed through the study of metabolome. Metabolome being more complex than proteome and metabolomic study to capture the changes in metabolite pool is still considered a tedious task due to its extremely dynamic nature albeit the evolution of more sensitive tools (Fessenden 2016). Other difficulties that come in the way of flawless insight into metabolome are: complex, sensitive, high level of fine-regulation and the presence of chemical contaminants (Lindon et al. 2011). Though current metabolomics techniques have their own advantages and disadvantages but scientists prefer to have deep insight on change in plant cell metabolome pool which is not possible with these techniques.

A study proved by metabolome analysis using LG-hybrid MS that in *Fusarium graminearum* infected yellow barley plant, resistance is directly correlated with the decrease in pathogen virulence factor and increase in detoxified product of virulence factor which is not the case for black barley (Kumaraswamya et al. 2011). In another study, in order to unravel the underlying biochemical activities in *Rhizoctonia solani* infected soybean orbitrap mass spectrometry coupled with GC/MS was carried out. For this experiment at first metabolite library of soybean was generated for quick reference of identified molecules. They detected movement of carbohydrates, disturbance of amino acid pools and elevation in the synthesis of various secondary metabolites like phenylpropanoid, a-linolenate and isoflavonoid, which in turn help plant to deter pathogen to colonize (Aliferis et al. 2014).

Pang et al. (2018) implemented another step to effectively differentiate plant metabolites from those of microbes before going for metabolic analysis to avoid the risk of contamination from pathogen metabolites. To study the effect of *Pseudomonas syringae* pv. tomato on *Arabidopsis thaliana*, the metabolome of microbes was labelled with $C^{13}$ and targeted analysis of plant metabolome was carried out. Their study unveiled that the alteration in primary metabolic pathways and plant signalling finally led to stomatal movement restriction prior to pathogen entry into the plant (Pang et al. 2018). Biotic stresses also have an impact on plant lipidome. Upon sensitization of pathogen attack plants start producing reactive oxygen species (ROS) which leads to lipid peroxidation (Zoeller et al. 2012). The accurate changes what this lipid peroxidation brings to limit pathogen invasion may be taken up by the scientists as a topic to explore in the near future.

The phytonematodes are responsible for the huge crop yield losses worldwide (Dhaliwal et al. 2015). These threatening crop insect pests cause vast economic losses which are projected around 10.8% globally, in many cases these losses are unpredictable and uncountable. These tiny creatures possess a challenge to the crop protectionists as most often they remain undiagnosed and their management seems to be a cumbersome task with available resources. To overcome these issues and to devise the new management strategies, one should have to reveal the hidden genetic information of these organisms. The omics studies of nematodes provide a deeper insight into the crop protectionist to understand their exact regulatory mode of life (Mosa et al. 2017). Once the secrets of genomes are unveiled, then the particular regulatory genes can be targeted to disturb nematode's life cycle; thus, the crop can be prevented from the potential harm. The sedentary nematodes which include the genus *Meloidogyne*, *Heterodera*, *Nacobbus*, *Tylenchulus and Rotylenchulus* are considered the most dangerous to the crops as they alter the host physiology (Nicol et al. 2011), while few other ectoparasites like *Xiphinema*, *Trichodorus*, *Paratrichodorus*, *Longidorus* and *Paralongidorus* are responsible for spreading crop-damaging viruses (Taylor and Brown 1997). In the world of nematodes, the genomic databases are available for the free-living model nematode *Caenorhabditis elegans* which help and outstretch its applicability towards the understanding of the basic biology and lifestyle of other nematodes. Unlike free-living nematodes, research on biological aspects of phytonematodes is a tough job because many of them form feeding structures inside their hosts and due to their obligatory nature, they cannot be reared on artificial media in the laboratory. However, the genomic research progress will enhance the databases and expand the scope of target-specific management strategies (Abad and McCarter 2011).

## 8.3 Nematomics Application in the Field of Nematology

The whole genomes sequencing of an organism helps in identifying the total gene pool of that species and in later stages, the work functions of those genes can also be predicted by bioinformatics analysis. Recently, the next generation sequencing technologies are making the whole genome sequencing economical and feasible. Sequencing platforms like Illumina, 454 sequencings, SOLiD, and Nanopore, all have certain advantages and limitations over one another and are used today invariably by the researchers (Berglund et al. 2011). In the late 1990s, the whole genome sequencing of free-living model nematode *Caenorhabditis elegans* boosted the research for future nematode genomics (Hodgkin et al. 1995). The smallest and largest genomic sequence is reported for the *Pratylenchus* spp. (19 Mb) (Burke et al. 2015) and animal parasitic nematode *Ascaris suum* (0.5 Gb) (Jex et al. 2011), respectively. The huge variation in the length of genomic sequences among the species under phylum Nematoda puts a challenging question to the molecular biologists and nematologists to understand its nature, molecular, biological and ecological aspects. Within the genus of root-knot nematode, two species like *Meloidogyne incognita* and *Meloidogyne hapla* exhibit huge genomic variation, which is attributed to its size and reproduction physiology? The first one is expert reproducing via parthenogenesis mechanism and exhibits polyploidy while the later one reproduces by amphimixis. Today the sequences of economically important sedentary endoparasitic nematode *Meloidogyne incognita* (Abad et al. 2008), *Meloidogyne hapla* (Opperman et al. 2008) and migratory endoparasite pine wilt nematode and *Bursaphelenchus xylophilus* are available. Many sequencing projects of other important plant-parasitic nematodes are under progress in various parts of the world. These available whole genome sequences help in documentation of novel proteins involved in parasitism, reproduction, feeding behaviour and other aspects of biology. Upon identification of these functional genes and protein, some of them can be chosen as effective targets for drawing management perspectives. Study of transcriptome (RNA profile) helps indirectly in identifying the expressed genes as all the genes of an organism are not functional (Berglund et al. 2011). The transcriptome data of some important nematodes are available on the internet like WormBase (Harris et al. 2009) or NEMBASE (Parkinson et al. 2004), which can be utilized to understand the transcriptomes of closely related other important nematodes.

The overall interaction mechanism between nematode and its host depends upon the protein–protein interaction at the target site and the secretion of specific proteins is affected by the environmental factors (Chen et al. 2011). Numerous effecter proteins for the sedentary and migratory endoparasitic nematodes have been documented and the proteome data is generated. The mass spectrometry analysis of proteins also helps in rapid detection of the unknown species, based on data comparison with available data resources Ahmad and Babalola 2014).

In an attempt to feed on plant or for the establishment of a permanent feeding site inside their hosts, the phytonematodes exhibit an interesting pattern of movement and behavioural adaptations (Lewis et al. 2002). These nematodes move randomly in soil and upon sensing the host's cues, they exhibit a directed movement towards the gradient of particular stimulant. Then they invade a preselected site on the roots and feed by extracorporeal digestions and initiate reproduction. Once the host becomes devoid of sufficient food, then the nematode leaves the host and moves in the soil in search of a host and the same pattern of the life cycle is repeated. Based on the life cycle, whole pattern is categorized into various stages like host search, invasion, secretion, digestion, multiplication and host evasion. For successful completion of each stage, a gene or specific sets of the gene are responsible. The identification of these essential genes is involved in plant–host interaction (Gheysen and Fenoll 2011) and their functions by omics and knocking them down could disrupt the normal physiology of the nematodes and indirectly inhibit their advancements. So, omics knowledge is essential here for devising the target-specific management approaches to prevent the menace of phytonematodes on commercially important agricultural crops.

The only way to manage nematodes in the field is to prevent them to get established above certain threshold limits. The correct and timely identification of problem causing nematodes is the first step to apply any management practices. Omics provides a way for correct and fast identification of the exact nematodes based on the availability of sequence databases. Further omics helps in identifying the resistant genes in the host against nematodes, effector targets of nematode or genes utmost needed for the survival of nematodes. Thus these genes can be targeted by gene silencing methods which may help in devising alternative management tactics. Knocking down the genes involved in pathogenesis by nematodes helps in preventing losses to plant (Kaletta and Hengartner 2006). The necessity of omics in the field of nematology cannot be underestimated and there is an immediate need to develop nematode's genomics databases.

## 8.4 Applications of Different Omics Techniques in Abiotic Stress

Any change in optimal conditions of non-living factors in the environment adversely affects plant growth and development and is called as abiotic stress (Jenks and Hasegawa 2005). Major abiotic factors causing stress to plants are drought, salinity, temperature (cold and heat), flooding, heavy metals, etc. (Jenks and Hasegawa 2005). Stress signalling to plant response includes a lot of players from receptors, secondary messengers, transcriptional actors, regulatory enzymes, stress responsive proteins, ROS, different organic and inorganic molecules, etc. (Knight and Knight 2001). Conventional breeding approaches are not always useful exercises owing to the complex nature of the stress response by plants. The difference in the genome favours different plants to respond differentially to abiotic stress even of the same species. This is basically attributed to changes in genome sequence of different genotypes. Abiotic stress is a complex trait. Omics approaches are considered better strategies to take over. Genomics, transcriptomics, proteomics, metabolomics and ionomics provide several tools to understand and utilize the variations for crop improvement and breeding (Tripathi et al. 2012; Deshmukh et al. 2014).

### *8.4.1 Genomics*

The complex nature of stress response by plants is not much efficient in improving the traits via conventional breeding practices (Varshney et al. 2011). The complex interactions occurring during abiotic response need to be understood in a holistic way to utilize that for crop improvement. Study of genome structure and function is necessary to gain a basic understanding of the molecular mechanisms involved in stress response of plants (Tripathi et al. 2012). Sequencing enables us to understand the whole genome of the organism and it paves way for more techniques to understand functional aspects of the genome and its evolution. Whole genome sequencing and resequencing enable identification of genome wide SNPs and SSRs and easy development of high density molecular maps (Gupta et al. 2008). Whole genome resequencing is a potential tool to find variations to develop molecular markers like SSR, SNPs and can be later used for genotyping by sequencing (Varshney et al. 2009). These techniques can be used for developing climate resilient crops (Henry 2014). QTL mapping for abiotic stress is a strategy to find chromosome regions contributing for a quantitative trait. Easy QTL mapping can be done using the genome wide high density markers developed previously using NGS technologies (Davey et al. 2011). Meta QTL analysis is done by combining many QTLs identified in a particular trait by using statistical tools in a single genetic map to predict the presence of QTLs (Goffinet and Gerber 2000). Genome wide association studies making use of genome wide SNP markers give more allelic diversity and increased resolution to QTL maps compared to biparental mapping methods (Myles et al. 2009). Genomic selection is another approach of crop improvement in which breeding values of different lines are predicted by using molecular markers (Desta and Ortiz 2014). This enables us to bring in all favourable QTLs even of minor effects together to develop plants tolerant to abiotic stress. Microarrays based SNP genotyping is another genomic tool which is less costly than NGS based short gun sequencing (Gunderson et al. 2005). SNP array is a hybridization-based technique and facilitates genome wide analysis and screening of a large number of genotypes easily. Sequencing and low-cost genome wide genotyping methods will fasten discovery of genetic variation, identification of genes and underlying molecular mechanisms and speed up crop breeding for stress tolerance.

### *8.4.2 Transcriptomics*

Analysing the genes expressed in response to stress in tolerant and susceptible plants will give a way to study the tolerance mechanisms involved in the process (Clement et al. 2008). Earlier expressed sequence tags (ESTs) developed by sequencing of cDNAs were used for preparing spotted arrays for the study of a large number of transcripts. But later, microarray was developed which is a high throughput technology where labelled RNA samples hybridized with thousands of synthetic probes on a chip (which is previously developed) represent different transcripts which is useful to profile expression patterns of RNA under various spatial temporal and developmental stages (Table 8.3). This is used as an effective way for genome wide

**Table 8.3** Some arrays developed in crop plants

| Crop | Size (technology) | References |
|---|---|---|
| Apple | 480 K (Affymetrix axiom) | Bianco et al. (2016) |
| Maize | 600 K (Affymetrix axiom) | Unterseer et al. (2014) |
| Rice | 1 M (Affymetrix) | McCouch et al. (2010) |
| Rice | 50 K (Affymetrix axiom) | Singh et al. (2015) |
| Soybean | 180 K (Affymetrix axiom) | Lee et al. (2014) |
| Wheat | 820 K (Affymetrix axiom) | Winfield et al. (2015) |
| De novo (applicable to multiple crops) | 50–300 K (GBS genotype-by-sequencing) | Elshire et al. (2011) |

**Table 8.4** RNA-seq in studying abiotic stress

| Crop: stress | Findings | References |
|---|---|---|
| Chickpea: drought | 261 and 169 drought stress responsive genes identified | Mashaki et al. (2018) |
| Sea buckthorn: drought | 1644 unigenes were differentially expressed, 519 unigenes were upregulated and 1125 were downregulated | Ye et al. (2018) |
| Mustard: high temperature and drought | Differentially regulated 19,110 transcripts | Bhardwaj et al. (2015) |
| Bread wheat: salinity | 5128 genes were differentially expressed due to salt stress | Amirbakhtiar et al. (2019) |

expression profiling under stress or any other specific conditions. Affymetrix chip of 61 k probes in soybean is one example of microarray (Le et al. 2012).

RNA-Seq: RNA sequencing is an advanced technology for transcriptome profiling. The entire transcript from a sample is deeply sequenced in NGS tools like ILLUMINA. It gives accurate information on the genes, expressed in a plant sample and its quantity which gives a detailed transcript profile by comparing RNA-seq data between tolerant and susceptible plants under stress conditions. It can be used to understand the genes involved in abiotic stress responses (Table 8.4). RNA-seq data also helps to understand molecular regulation, small RNA expression, alternative splicing, etc. (Deshmukh et al. 2014).

### 8.4.3 *Proteomics Techniques*

Stress reception, signal transduction, changes in gene expression, etc. involve many proteins including enzymes and regulation of these proteins is done by many mechanisms such as post-translation modification, protein–protein interactions, targeting, degradation, etc. (Marks and Klingmüller 2008). Profiling the proteins which are being synthesized, modified, targeted and made functional and non-functional according to the stress perceived by the plant will provide a better account on stress tolerance level of plants (Table 8.5).

### 8.4.4 *Metabolomics Technique*

Identification and quantification of metabolome (entire metabolites) at a particular stage in a tissue enable us to understand the biochemical pathways involved and the underlying molecular mechanisms (Hall 2006). Techniques like gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS), capillary electrophoresis mass spectrometry (CE-MS), Fourier transform ion

**Table 8.5** Application of proteomic techniques in abiotic stress

| Crop: stress | Techniques | Remarks | References |
|---|---|---|---|
| Tomato: thermotolerance | LC-MS fractionation protocol | Identified proteins involved in systemic and fundamental components of pollen thermotolerance | Jegadeesan et al. (2018) |
| Spinach: heat stress | iTRAQ Labelling, LC-20A HPLC system | Proteomic and phosphoproteomics of heat tolerant line were studied | Zhao et al. (2018) |
| Wheat: heat stress | 2DE | Characterization of heat tolerant and susceptible cultivars via flag leaf proteomics | Nandha et al. (2018) |
| Sugarcane: salt stress | ESI-LC-MS/MS | Analyse the proteomic effects of salt stress in micro-propagated shoots of two sugarcane cultivars | Passamani et al. (2017) |
| Spring wheat: drought | 2D-PAGE, LC-ESI-MS/MS | Effect of drought in proteome of susceptible vs. tolerant cultivar | Michaletti et al. (2018) |

**Table 8.6** Application of metabolomics tools in abiotic stress research

| Crop: stress | Techniques | Remarks | References |
|---|---|---|---|
| Spring wheat: drought | LC-MS | Effect of drought in metabolome of susceptible vs. tolerant cultivar | Michaletti et al. (2018) |
| Wheat: drought | Gas chromatography–time-of-flight/mass spectrometry | Mechanisms involved in wheat seedling drought tolerance | Guo et al. (2018) |
| Rice: salinity | GC-MS | Metabolomic responses of seedlings of tolerant varieties and susceptible varieties | Gupta and De (2017) |

cyclotron resonance mass spectrometry (FT-ICR-MS) and nuclear magnetic resonance (NMR) are generally used in plant sciences (Putri et al. 2013) (Table 8.6).

### 8.4.5 Ionomics

Ionomics deals with the identification and quantification of elemental composition of a tissue or an organism (Singh et al. 2013). Plants growing under different soil types have developed mechanisms to take up different elements. Ionomics studies will help to improve the understanding of functions of elements in biochemistry, physiology and molecular mechanisms of plants (Salt 2004). Availability of elements, uptake, transport, evapotranspiration, etc. makes the ionome profile very sensitive to the prevailing conditions. Exposure to various abiotic stresses may drastically change the elemental conditions in various plant tissues, so the study of ionomics in responses to abiotic stress conditions can give us a better understanding of underlying mechanisms. Some techniques used in ionomics studies are neutron activation analysis (NAA), X-ray fluorescence (XRF), inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-atomic/optical emission spectrometry (ICP-AES/OES) (Wu et al. 2013).

## 8.5 Conclusion

Integration of computer science and biology has resulted in the development of high throughput methods with fast data generation which help to identify, measure and quantify cellular molecules like DNA, RNA and proteins. These approaches are dependent on computational tools and have made an immense impact on our understanding of plant stress tolerance responses and signalling involved with the process. Omics measures change at gene, RNA, protein and even at ionic level within the cell. Further advancement and novel tools and computational models need to be developed in order to find the ultimate missing parts to decipher the complete mechanism of stress tolerance in plants and improve our understanding of plant system biology.

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# Chapter 9
# Role of Arbuscular Mycorrhizal Fungi in Amelioration of Drought Stress in Crop Plants

**Pallavi and Anil Kumar Sharma**

**Abstract** Present chapter addresses the importance of arbuscular mycorrhizal (AM) symbiosis in the mitigation of one of the most limiting environmental constraints in terms of global crop productivity loss, i.e. drought/water stress. Superior root morphology leading to enhanced water and nutrient uptake, better antioxidant machinery to fight the elevated reactive oxygen species (ROS), along with improved osmotic adjustment by accumulating high levels of compatible solutes like proline, glycine betaine, sugars, etc., are among the multifaceted factors which make AM association far more significant than any other symbiotic association in plants. So far little progress has been made on molecular level to understand the mechanisms of this miraculous organism which include the involvement of aquaporins, some binding protein genes (BiPs), MAPK pathway genes, and stress responsive genes like proline dehydrogenase, invertase, proline synthetase, etc. However, with the advancement of new age molecular techniques it seems possible that the unravelling of this mystery is not far away.

## Abbreviations

| | |
|---|---|
| AM | Arbuscular Mycorrhiza |
| ATP | Adenosine triphosphate |
| BiP | Binding proteins |
| CAT | Catalase |
| EEA | European Environment Agency |
| ERM | extraradical mycelium |
| GOGAT | Glutamine synthase |
| GS | Glutamine synthetase |

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|---|---|
| HNE | 4-hydroxy-2-nonenal |
| IAA | Indole-3-acetic acid |
| IPCC | Intergovernmental Panel on Climate Change |
| LPO | Lipid peroxidation |
| MAPK | Mitogen-activated protein kinase |
| MDA | Malondialdehyde |
| NR | Nitrate reductase |
| ROS | Reactive oxygen species |
| RUBISCO | Ribulose-1,5-biphosphate carboxylase/oxygenase |
| RWC | Relative water content |
| SOD | Super oxide dismutase |

## 9.1 Introduction

Plant–arbuscular mycorrhizal (AM) fungal interaction (symbiotic) is the most studied association, so far. Around 80% of land plants, especially agricultural and horticultural crop species establish symbiotic association with AM fungi belonging to the phylum Glomeromycota (Barea et al. 2002; Smith and Read 2008). Through this mutualistic beneficial association, AM fungi fulfil their carbon requirements in the form of photo-assimilates from the host plants and in return, fungi enhance plant's acquisition of water and mineral nutrients. Improved photosynthetic efficiency and antioxidant activity is detected in mycorrhized plants exposed to elongated water deficit conditions in comparison to non-mycorrhized plants. AM plants show changes in root architecture which is often accredited to the higher accumulation of indole-3-acetic acid (IAA), nitric oxide, methyl jasmonate, and calmodulin in roots (Zou et al. 2017), thus supporting the idea that AM fungal infection causes these morphological and biochemical changes in plants providing them a upper hand in facing drought conditions.

Amongst the yield deterring reasons, drought is the front runner among all the abiotic stresses. It is especially prominent in developing countries of tropical region where agricultural lands are primarily rain-fed and results in high production losses to the farmers. It can be defined as a stage of water deficiency resulting in stunted growth of plants. Drought or water stress happens when there is no significant rainfall or alternate irrigation facilities available. This leads to the deficiency of usable water to facilitate proper functioning of the plants in the soil which is further aggravated by atmospheric conditions causing continuous loss of water by transpiration or evaporation. According to the reports of IPCC (2007) and EEA (2015), climate change and growing water scarcity will result in further aggravation of drought conditions in coming years.

## 9.2 Plant Physiological Responses Towards Drought Stress

Basically, drought stress interferes with the normal metabolic activities of the plants by disrupting plant-water potential and turgor pressure (Rahdari and Hoseini 2012). Along the years, researchers have studied the impact of drought stress on all the major crops such as wheat (Farooq et al. 2014), rice (Korres et al. 2017), barley (Samarah 2005), and maize (Daryanto et al. 2016). Generally, intensity and time period of drought decide the effect it will have on the quantity and quality of plant growth by influencing the availability and uptake of soil nutrients to the shoot and carbon assimilating channels of the roots through water (Selvakumar et al. 2012). Drought stress changes chlorophyll contents and damages the photosynthetic machinery of plants (Ortiz et al. 2015**).** Root growth is severely hindered by water deficit. Similar to the other environmental stresses such as salinity, heavy metal toxicity, nutrient deficiency/excess, and biological stress like pathogen attack, drought also results in plant growth impairment by causing hormonal imbalance, susceptibility to diseases, nutrient deficiency, and metal toxicity (Nadeem et al. 2014).

All the developmental stages of plant are vulnerable to drought stress and this can be visualized on both morphological and molecular levels. Plants usually have some inherent drought tolerance but its extent varies in both interspecific and intraspecific cases. Drought causes loss in turgor pressure inducing stomatal closure which limits carbon fixation and reduces cell growth (Taiz and Zeiger 2006). In extended water deficient conditions, alteration in carbon and nitrogen metabolism results in reduced photosynthesis by inactivation of RUBISCO and retarded plant growth (Bota et al. 2004). Water deficit condition in the rhizosphere leads to an increased rate of root respiration, an imbalance is created inside the cell in the utilization of carbon resources, reduced ATP production and enhanced generation of reactive oxygen species (ROS) like hydroxyl radicals (OH-), superoxide anion radicals ($O^{-2}$), singlet oxygen ($O^2$), hydrogen peroxide ($H_2O_2$), and alkoxy radicals (RO) initiate the oxidative stress (Munné-Bosch and Peñuelas 2004; Nair et al. 2008). ROS may react with proteins, DNA, and lipids causing oxidative damage and destroy the normal functioning of the cells (Foyer and Fletcher 2001). Other parameters that are negatively influenced by drought stress are chlorophyll content (Anjum et al. 2011; Rahdari and Hoseini 2012) and tissue nutrient content (Garg 2003). Drought also increases ethylene biosynthesis, which inhibits normal functioning of the plants (Ali et al. 2014). Therefore, in order to achieve the designated goal of producing more food, amelioration of drought stress is extremely important.

## 9.3 Drought-Induced Oxidative Stress

Plant metabolic activities result in production of reactive oxygen species (ROS) and plant get rid of these by certain ROS scavenging activities, thus the balance is maintained. However, when plant is exposed to various abiotic environmental stresses (including drought), rate of production of these ROS becomes higher and the plant suffers from oxidative stress (Sies 1997). ROS are forms of oxygen that are more reactive than molecular oxygen ($O_2$). Plants use combination of signalling and survival tactics to deal with a variety of stressful conditions. Since, Earth's atmosphere consists of 21% oxygen, production of toxic reactive oxygen species as result of metabolism is inevitable (Finkel and Holbrook 2000). ROS are frequently produced as by-products of various metabolic pathways, for example, during photosynthesis oxygen is generated in the chloroplasts which after accepting electrons passing through the photosystems forms superoxide radical (O2•−) which has two unpaired electrons with same spin making it unstable, reactive, and harmful for the cells (Navrot et al. 2007). However, ROS are not harmful under normal circumstances, in fact, they act as key signalling molecules for cellular responses to developmental and environmental stimuli under non-stressed conditions (Pei et al. 2000; Carol and Dolan 2006), thus are also important in general metabolism of plants. Various stress conditions result in disruption of the balance between the production and the removal of ROS leading to loss in crop productivity worldwide (Shi et al. 2001).

## 9.4 Consequences of Oxidative Stress in Plants

Plants have evolved several mechanisms that permit them to adapt and survive the period of abiotic stress, particularly drought. As discussed in the previous section, one of the impending consequences of drought stress is enhanced ROS production in plant cell. ROS reactive nature makes them extremely dangerous, especially singlet oxygen and the hydroxyl radicals, unlike atmospheric oxygen, they are capable of oxidizing important building block of cellular machinery such as proteins, fatty acids/lipids, and nucleic acids. Uninhibited oxidation of these biomolecules can ultimately result in cell demise. Lipid peroxidation (LPO) is one of the first indications that the cell is under oxidative duress, wherein, a reactive radical initiates a chain reaction, by removal of a hydrogen atom from a polyunsaturated lipid, creating and rearranging the double bonds and is terminated by antioxidants. It is shown that LPO results in the release of small hydrocarbon fragments such as small aldehydes like malondialdehyde (MDA), and hydroxyalkenal like 4-hydroxy-2-nonenal (HNE), etc., as end products from the polyunsaturated precursors. (Garg and Manchanda 2009). Excessive LPO makes membrane porous thus increases its permeability and causes modification of membrane bound proteins, these changes disrupt the bilipid membrane arrangement (Møller et al. 2007). Another side effect of oxidative stress is protein oxidation or ROS mediated irreversible covalent modification of protein either by addition of nitrosyl moiety (nitrosylation), carbon monoxide molecule (carbonylation), disulphide bond formation, addition of glutathione (glutathionylation) or by reacting with breakdown products of poly unsaturated fatty acid peroxidation (Ghezzi and Bonetto 2003; Yamauchi et al. 2008). It advertently leads to cell death, which is characterized by disruption of the cell membrane and cellular organelles.

## 9.5 Physiological and Biological Responses of Plants towards Drought Stress Under AM Symbiosis

AMF facilitate alleviation of detrimental effect caused by drought stress in plants. Rapparini and Peñuelas (2014) in their review have compiled all the mechanisms that are speculated to play role in AMF mediated drought stress tolerance in plants. Among these mechanisms, the most important mechanism is, increase in water absorbing surface area of the roots by producing intensive intraradical and extraradical mycelium (ERM) for better utilization and effective transportation of nutrition and water to the plants. ERM has great ability to mobilize essential nutrients like phosphorus (P), zinc (Zn), nitrogen (N), or copper (Cu) which can be readily absorbed by host plants with the help of arbuscules and vesicles (Smith and Read 2010). One more benefit of fungal hyphal network is that they can enhance soil architecture by forming aggregates through the production of the glycoprotein glomalin (Miransari et al. 2007; Singh et al. 2013). These soil aggregates increase the water holding capacity of the soil (Augé 2001; Ruiz-Lozano 2003). Another parameter of drought tolerance is root hydraulic conductivity (L), it is found to be reduced under drought stress, but mycorrhized plants were partially able to avoid this diminution (El-Mesbahi et al. 2012; Bárzana et al. 2012).

Osmotic adjustment is the most vital biochemical adaptation by plants to resist drought stress. It is achieved by the accretion of osmolytes/compatible solutes like proline, glycine betaine, aspartic acid, protein, and soluble sugars which have the ability to decrease osmotic potential, while, maintaining a high relative water content (RWC), thereby optimal cellular environment required for plant survival (Farooq et al. 2009). Wu and Zou (2017) found that AMF were able to strongly alter plant tissue's sucrose and proline metabolism by regulating key enzymes important for osmotic adjustment of the host plant. Proline being an excellent osmoprotectant increases the plant tolerance by maintaining the osmotic balance in plant cells. Extensive studies reported that AMF-colonization-mediated proline accumulation on exposure to water deficit leads to subsequent drought tolerance in comparison to non-mycorrhizal counterparts (Yooyongwech et al. 2013; Doubková et al. 2013; Rapparini and Peñuelas 2014). In a study, drought-exposed *Poncirus trifoliata* when inoculated with *Funneliformis mosseae* resulted in decreased tissue proline accumulation and improved its growth performance along with biomass production (Zou et al. 2013). Researchers suggested that the inhibition of proline synthesis along with enhanced proline degradation might have resulted in lower proline concentration in the AM plants (Zou et al. 2013).

Oxidative stress occurs when plant's antioxidant defence system is overpowered and no longer able to maintain cellular redox balance (Rapparini and Peñuelas 2014). However, mycorrhiza-inoculated plants were reported to induce varied levels of major antioxidants/ROS scavengers and which helps in regaining the optimal cellular redox status (Rapparini and Peñuelas 2014). The amelioration of drought stress by AM symbiosis is often ascribed to the augmentation of antioxidant activities in plants (Ruiz-Sánchez et al. 2010; Baslam and Goicoechea 2012; Wu and Zou

2017). Drought-exposed mycorrhized plants were reported to have higher glutathione level which was correlated with a lowered cellular $H_2O_2$, decreased membrane lipid peroxidation, and enhanced photosynthetic performance. Also**,** although glutathione levels were increased, ascorbate levels decreased in mycorrhizal plants in comparison to control plants. It suggests differential up-regulation of various antioxidant systems, with preferential activation of the systems that are more effective in protecting plants against drought (Ruiz-Sánchez et al. 2010). This suggests that mycorrhizal infection offers protection against drought-induced oxidative stress (Ruiz-Lozano 2003). However, it has been contradicted by some studies. These antioxidant compounds can also be viewed as markers of drought stress. Less accumulation of glutathione and ascorbate in mycorrhizal plants of lavender under drought conditions was correlated with a high level of resistance in lavender plants against drought (Marulanda et al. 2007). Flavonoids are also found to play a defensive role against drought in mycorrhiza-inoculated plants (Abbaspour et al. 2012). Other compounds such as isoprenoids, specific isoprenoid-derived apocarotenoids, and strigolactones also contribute as a shield against abiotic stresses such as drought (Rapparini et al. 2008; Rapparini and Peñuelas 2014; Walter and Strack 2011; López Ráez et al. 2008).

It has been studied by various researchers that AM colonization alleviates ROS mediated oxidative burst by triggering the antioxidant machinery inside their host plant cell (Abbaspour et al. 2012; Baslam and Goicoechea 2012). Ascorbic acid is a powerful natural scavenger of OH that lowers the accumulations of malondialdehyde (MDA) in cell membrane, thus stabilizes the membrane integrity (Foyer and Noctor 2005). Increase in ascorbate content during salt stress in mycorrhized citrus plant was reported by Wu et al. (2010). Zou et al. (2015) observed significantly higher super oxide dismutase (SOD) or catalase (CAT) activity in leaves and roots in AM than in non-AM trifoliate orange seedlings, irrespective of water status, except for CAT activity that did not show any significant difference in AM and non-AM seedlings under non-stressed condition. These results suggest that the presence of AMF could induce higher antioxidant enzyme activities in the seedlings as a protective mechanism to prevent over accumulation of ROS under drought stress. Similar observations were reported by Bompadre et al. (2014) in pomegranate plants where *Rhizophagus intraradices* resulted in considerably higher SOD and CAT activity in shoots under irrigated and drought conditions.

Reports have also indicated that AM symbiosis under drought conditions enhances photochemical efficiency of photosystem II, which was assessed by chlorophyll fluorescence in rice, tomato, and lettuce (Ruiz-Sánchez et al. 2010; Ruiz Lozano et al. 2016), and in woody tree species (Yooyongwech et al. 2013). Such results indicate an improved performance of photosynthetic apparatus along with minimal drought-imposed damage in mycorrhized plants. These findings are consistent with another study investigating the effect of root inoculation of different tree species with a combination of both AM and ecto-mycorrhizal fungi (Fini et al. 2011).

Multiple studies have reported enhanced nutrient acquisition by plants after AMF inoculation which mitigates drought-induced deficiency of important nutrients especially with low mobility like P, Zn, and Fe. In addition, concentration of other nutrients like Ca, K, Mg in inoculated plants is also found to be improved (Bagheri et al. 2012; Gholamhoseini et al. 2013). However, the most significant role of AMF is observed in improving P concentration in plants which subsequently enhanced drought tolerance in plants (Gholamhoseini et al. 2013). Inoculation of *F. Mosseae* in sunflower improved P availability and minimized the drought-impact on seed oil percentage and oil yield (Gholamhoseini et al. 2013). AM symbiosis is considered as the most common strategy in enhancing P availability in the soil or capacity of P uptake (Smith et al. 2011). Recent findings have provided new evidences for the contribution of two well-recognized pathways (roots and fungal hyphae) by which P can be absorbed in mycorrhized plants. These results suggest a pivotal role of 'hidden P uptake' into plants via the AM fungal pathway (Smith et al. 2011), including when mycorrhizal plants experience conditions of drought stress (Smith et al. 2010). Wu et al. (2013) found diminished AMF colonization in roots of several citrus plants under drought. However, enhancement of plant growth by AM was still observed. In addition to P, in trifoliate orange (*Poncirus trifoliata* L. Raf.) seedlings, *G. versiforme* colonization significantly improved the concentration of Ca, K, and Fe in plants under well-watered and water stressed conditions (Wu and Zou 2011). Similarly, Pistachio plants, inoculated with *F. mosseae* and *R. intraradices* mediated significant augmentation in P, K, Zn, and Mn concentrations, irrespective of soil moisture conditions (Bagheri et al. 2012).

Mariotte et al. (2017) emphasized that drought induces changes in the nitrogen (N) and phosphorus (P) cycle making their availability to the plants sparse. Most plant species are unable to cope with limited nutrient supply causing an imbalance in optimal N:P ratio. They suggested that the plants which are able to sustain this imbalance under drought condition will be more successful in enduring drought. In a study, they observed that *Cynodon dactylon* with stronger symbiotic association was able to maintain the constant N:P, in comparison to *Paspalum dilatatum* with weaker association under drought condition.

AMF can also alter host water regulation through hormonal signalling modulation. Abscisic acid (ABA) reportedly play role in controlling stomatal conductance and AM symbiosis is found to enhance ABA production which result in stomatal closure and thus ensuring limited water loss in drought-exposed plants (Ludwig-Müller 2010; Doubková et al. 2013). Another method is by stimulating osmolytes or compatible solutes such as photosynthetic by-products and soluble sugars, enhanced levels of free polyamines and soluble nitrogenous compounds as compared to non-AM plants (Ruiz-Sánchez et al. 2010; Fan and Liu 2011; Rapparini and Peñuelas 2014).

According to Augé (2001), AMF inoculation improves the P status of the plant cells, thus the stomatal closure caused on exposure to the water deficit might be because of the elevated P concentration in the leaves possibly by either affecting the energetics involved in osmotic adjustments of the guard cell or by increased cell wall rigidity controlling the stomatal movements. This is further supported by the enhancement of soil acid phosphatase activity resulted drought stress alleviation in AMF inoculated plants (Wu et al. 2011). Additionally, plant tissues of mycorrhized maize cultivars exhibited higher nitrate reductase (NR), glutamine synthetase (GS), and glutamine synthase (GOGAT) activities than non-AM tissues under water stress conditions. Bernardo et al. (2019) observed metabolic responses of two AMF inoculated wheat cultivars exposed to water deficit conditions and reported some variations in the compounds like sugar and lipids in inoculated and un-inoculated wheat, metabolites involved in oxidative stress management along with modulation of phytohormones, specifically brassinosteroids biosynthetic pathway.

## 9.6 Molecular Mechanisms Involved in Stress Alleviation by Arbuscular Mycorrhiza

So far, many studies have confirmed the significance of aquaporins in modulation of root hydraulic properties under drought stress (Ruiz-Lozano and Aroca 2010; Barzana et al. 2014). Several studies confirmed that AM-inoculated plants can conserve more water under prolonged water deficit conditions. It was observed that hydraulic properties of the roots under AM symbiosis were regulated by aquaporin levels and state of phosphorylation. Authors argued that down-regulation of the activity of these proteins may provide a better explanation for the changes during water deficit. Additionally, the drought-induced reduction in the transpiration rate (observed in *Phaseolus vulgaris* mycorrhizal plants) occurred in conjunction with an increased free exuded sap flow and a higher water uptake in AM plants (Aroca et al. 2007; Rapparini and Peñuelas 2014). In another study, transformed carrot roots were cultured with *Glomus intraradices* and on exposure to drought, showed elevated expression of two aquaporin genes (GintAQPF1 and GintAQPF2) (Li et al. 2013). Studies on 'aquaporins and AMF' signify that simultaneous regulation of both expression and activity of aquaporins in host plants and fungi may represent an AM-dependent mechanism for enhancing plant tolerance to drought stress (Rapparini and Peñuelas 2014). Bárzana et al. (2012) reported activation of increased apoplastic water transport pathway in the mycorrhizal roots along with 'cell to cell' pathway during drought stress. Potential of AM plants to coordinate two transport pathways has been hypothesized as a mechanism that plays an important role in adding flexibility in drought responses of AM-plants in comparison to non-AM-plants (Rapparini and Peñuelas 2014). Aquaporins are also involved in nutrient transport during plant–mycorrhiza association (Maurel and Plassard 2011). Activation of genes encoding a crucial element of ER-luminal binding protein (BiP) is among the speculated mechanisms involved in drought stress alleviation by mycorrhized plants (Kapoor et al. 2013). From *G. intraradices*, a BiP gene has been discovered from the fungus growing in-vitro under drought stress (Porcel et al. 2007). Under in-vitro and in-vivo conditions, BiP expression was up-regulated during drought stress under natural symbiosis with plants. Liu et al., (2015) also reported a coordinated response in mitogen-activated protein kinase (MAPK) transcripts in mycorrhized soybean plant roots and fungi, suggesting a potential molecular mechanism responsible for mycorrhiza induced drought stress response. Table 9.1. summarizes the recent significant advances in the knowledge of molecular mechanism lying behind the AM-induced plant drought tolerance.

**Table 9.1** Molecular and physiological basis of AM induced drought tolerance

| S. No. | Crop | AMF | Effect/Mechanism | Study |
|---|---|---|---|---|
| 1 | Citrus (*Poncirus trifoliata*) | *F. Mosseae* | Mycorrhized roots were found to be having higher root unsaturation index which might have been achieved by the modulation of genes such as *FA desaturase 2 (PtFAD2), PtFAD6*, and *Δ9 FA desaturase (Pt Δ9)* under irrigated condition, while under water stressed conditions *PtFAD2, PtFAD6,* and *Δ15 FA desaturase (PtΔ15)* were activated | Wu et al. (2019) |
| 2 | Soyabean (*Glycine max*) | *Glomus intraradices* | Upregulation of MAPK transcripts in mycorrhizal soybean roots, suggesting the possibility of its potential role in communication between the two symbiotic partners | Liu et al. (2015) |
| 3 | Citrus (*P. trifoliata*) | *F. Mosseae* | Alternation in the gene expression profile of various tonoplast intrinsic proteins (TIPs) in mycorrhized plants indicating involvement of AMF in improving water absorption under drought | Jia-Dong et al. (2019) |
| 4 | Wheat (*Triticum aestivum*) | *F. Mosseae* | Effect on expression of proteins constituting the plant defence system against drought like in sugar metabolism, cytoskeletal organization, and Sulphur-containing proteins, as well as cell wall rearrangement | Bernardo et al. (2017) |
| 5 | Maize (*Zea mays*) | *Rhizophagus intraradices* | Expression of D-*myo*-inositol-3phosphate synthase (IPS) gene in association with 14–3GF gene was found to be responsible for the AM mediated drought tolerance in maize plants | Li et al. (2016) |
| 6 | Carrot (*Daucus carota*) | *R. Intraradices* | GintAQPF1 and GintAQPF2 aquaporin genes were found to be upregulated, thus, in turn, improving plant water relations under drought | Li et al. (2013) |
| 7 | Maize (*Z. mays*) | *R. Intraradices* | A wide number of aquaporin subfamily genes were regulated by presence of AMF and different water level | Barzana et al. (2014) |
| 8 | Trifoliate orange (*P. trifoliata*) | *F. Mosseae* | AM-inoculation elevated the synthesis of calmodulin in orange leaves and upregulated antioxidant enzyme activity | Huang et al. (2014) |
| 9 | Tomato (*Solanum lycopersicum*) | *F. Mosseae and R. intraradices* | Expression of LeEPFL9-STOMAGEN, and genes encoding intercellular signalling factors EPF1 and EPF2, which play role in stomatal development were found to be promoted by AM inoculation | Chitarra et al. (2016) |
| 10 | Citrus (*P. trifoliata*) | *F. Mosseae* | RT-PCR analysis revealed increase in mRNA abundance of genes involved in antioxidant activities like flavonoid biosynthase, lactoyl glutathione lyase, and oxido-reductase under water stress | Fan and Liu (2011) |

## 9.7 Conclusions

Importance of AMF in combating drought stress is undeniable and it has also been demonstrated in multiple studies by researchers across the world. Still, there is lacuna of information about the mechanism(s) behind AM mediated drought stress amelioration. Next generation molecular techniques like Single Molecule Real Time (SMRT) and nanopore sequencing approaches may generate new insight in stress alleviation by mycorrhizal symbiosis. They can help in identification of target genes for promoting growth under stress. This information can be used to transfer target genes into plants through biotechnology and developing stress tolerant plants.

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# Chapter 10
# Drought Stress Tolerance: An Insight to Resistance Mechanism and Adaptation in Plants

**Priyanka Khati, Pankaj Kumar Mishra, Manoj Parihar, Ashish Kumar Singh, Jaideep Kumar Bisht, and Arunava Pattanayak**

**Abstract** Water is essentially required by all the living systems including plants to maintain normal function of growth and metabolism. Drought stress in plants may affect several activities like cell elongation, morphology, nutrient availability due to the production of ROS and photo oxidation. Present study discusses various aspects of drought stress in plants and possible solutions to improve the tolerance. Application of plant growth promoting bacteria to ameliorate drought stress is undeniable in addition to several other ways to fight drought stress. Endophytic fungi are also reported to improve the stress management in plants as they colonize the plant roots and impart several benefits by providing essential metabolites related to resistance and development. Genetic engineering has always been a solution to stress management through the insertion or deletion of required genes. However, nanotechnology also offers ways to tolerate drought in plants as it improves the water use efficiency for plants and associated microbes. Future basic research at molecular level is still needed to explore the exact mechanisms lying beneath the stress tolerance strategies in plants. This may help in developing proper understanding between drought and plant metabolites and its application in future research.

## 10.1 Introduction

Global climatic changes accelerate the occurrence of a variety of abiotic (drought, salinity, heavy metals, and extreme temperatures) and biotic stresses (phytopathogens), which considerably affect agricultural productivity and bioremediation efficiency of plants even in forest ecosystems. Water is the basic requirement of plants which helps in maintaining normal function and turgor pressure to perform growth and metabolism. Drought, among all the other environmental stresses, is one of the major limiting factor for plant health and productivity worldwide. Drought stress

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arises in plants due to inadequate water supply during agricultural practices (Jaleel et al. 2007) and it is expected to cause serious problems in crop productivity in more than 50% of the arable lands by 2050 (Ashraf 1994; Kasim et al. 2013).

**Negative Impact of Drought Stress on Plants**

(a) Water stress may affect the plant growth as the turgor pressure is responsible for cell elongation.
(b) Drought may also affect the nutrient availability to plants which depends upon the availability of water.
(c) Increase in reactive oxygen species during drought stress may damage several cell functions and structures.
(d) The photo oxidation caused by drought may decrease chlorophyll content (Rahdari et al. 2012).
(e) Reduced growth due to obstructed cell elongation and impaired cell mitosis.
(f) Affects photosynthesis due to reduced leaf area and decrease in chlorophyll content.

Crop production in the rainfed region (~60%) of North West Himalaya is still a challenge due to insufficient utilization of water for irrigation (Sharma et al. 2017). Despite of adequate rainfall, this area shows water shortage for 3–4 confined months because of runoff water due to steep slopes. According to Gupta (2006), water availability in India is likely to be reduced to 1500 $m^3$/annum/person by 2025. Root development is affected by the availability of water (soil water deficit), however, root growth is less affected in comparison to aerial parts, so increase in overall root to shoot ratio is mostly observed. Root texture is mainly affected during drought as reduction of root weight in wheat, without an appreciable decline in their number was observed. Roots were fine and fibrous under dry conditions in comparison to favourable moisture conditions (Ashraf 1994). Seedling growth is affected by water scarcity as the turgor pressure which helps in cell elongation is absent. However, the root size and ratio of root/shoot improved under dry conditions. Coleoptiles growth is inhibited due to the reduction of water supply from the root to coleoptile under drought stress (Monayeri et al. 1984). Eziz et al. (2017) stated that drought significantly enhanced the root mass while creating negative impact on the leaf, stem, and reproductive mass of the plant.

## *10.1.1 Signalling Pathways During Drought Stress*

(a) *Abscisic acid (ABA) dependent pathway*: In plants, drought response is observed in the operation of ABA pathway which synthesizes ABA with the help of enzyme 9-*cis*-epoxycarotenoid dioxygenase (NCED). Once ABA is synthesized it activates ABA-responsive *cis*-element binding protein/ABA-responsive *cis*-element binding factor (AREB/ABF) which in turns activates ABA-responsive *cis*-element (ABER). ABER gets accumulated in the promoter region of drought responsive genes and upregulates their transcription.

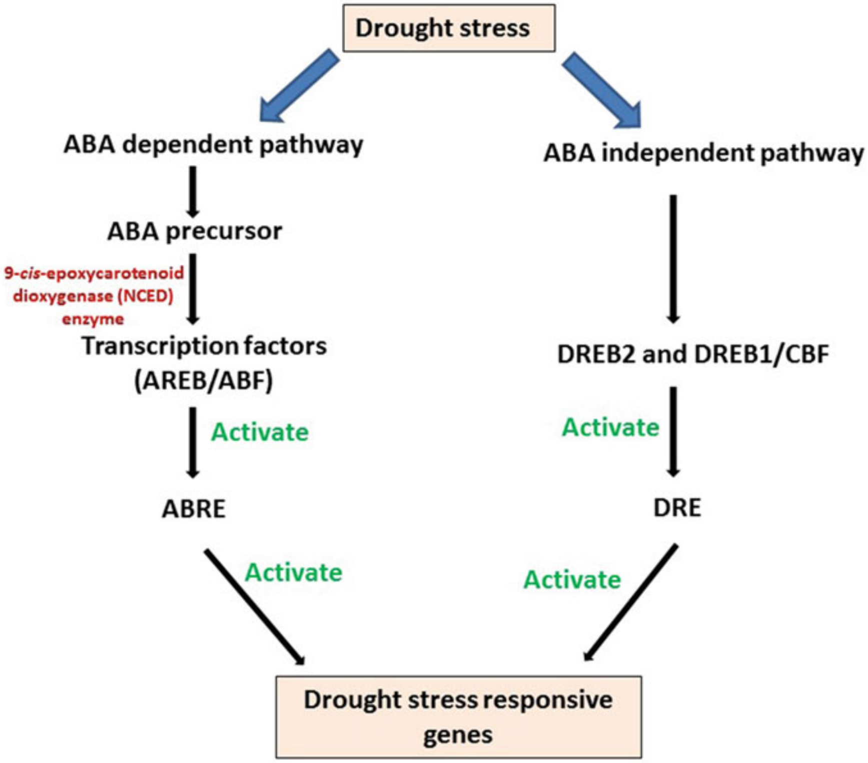

**Fig. 10.1** Signalling pathway involved in drought stress tolerance

(b) *ABA independent*: In the absence of ABA, drought stress is controlled by cold-binding factor/dehydration responsive (DREB2 and DEB1/CBF) transcription factors. These transcription factors through dehydration responsive element (DRE) regulate drought stress-responsive genes (Todaka et al. 2015). The signalling mechanism is shown in Fig. 10.1.

Plant tolerance to water stress may occur either by drought avoidance or drought tolerance mechanisms (Blum 2005, 2009). Drought avoidance is the maintenance of high water status or water conservation under water deficit conditions which promotes water use efficiency in plants (Karaba et al. 2007). Drought tolerance is the ability of one crop genotype to yield better than the other in a drought state (Blum 2005). According to Blum (2005) it depends on the following factors:

(a) Enhanced capture of soil moisture.
(b) Reduced water use.
(c) Osmotic adjustment and conservation of cellular water content.

### 10.1.2 Drought Stress Tolerance Mechanisms in Plants

Plants have several adaptation mechanisms to tolerate drought stress by secreting different metabolites, which may help in management of drought stress at different stages. Number of molecular mechanisms act for physical action of plants during drought stress. These mechanisms help in maintaining normal growth and metabolism of plants. Some of these are discussed below.

### 10.1.3 Physiological Traits

(a) Several metabolites like polyols, xylitol, and sorbitol, tyrosine, 2-hydroxypalmitate, flavonoid, vannilate, ϒ-glutamylglutamate, ϒ-glutamylphenylalanine, and ϒ-glutamylvaline from glutathione metabolism, erythritol, arabitol, glycerol, inositol 1-phosphate, and myo-inositol are affected during drought stress (Yobi et al. 2013; Pampurov and Dijck 2014).

(b) Signalling pathway induced by jasmonate (JA) is also found responsible for stress tolerance in plants (Ahmad et al. 2016). They showed role of JA (a signalling molecule) in plants under stress and non-stress condition. JA mediated stress response is caused by several cross talk molecules like nitric oxide, calcium, ABA, ethylene, salicylic acid, and reactive oxygen species.

(c) Curling of leaves fronds and folding of plasmalema to prevent water loss (Pampurov and Dijck 2014).

(d) Arrangement of organelle packaging (Pampurov and Dijck 2014).

(e) Accumulation of osmoprotectants like proline is very crucial step for water stress tolerance. These protectants help plants to maintain normal function of life even in extreme stress. Increase in proline content decreases the water potential of plant and reduces the water loss (Wang et al. 2019).

(f) Controlling stomatal conductance for water loss through transpiration (Taiz and Zeiger 1991; Pietragalla and Alistair 2012).

(g) Accumulation of antioxidants {superoxide dismutase (SOD), peroxidase (POD) e.g., guaiacol peroxidase and ascorbate peroxidase and catalase (CAT)} (Yuan et al. 2016) and reactive oxygen species {superoxide anion ($O_2.-$), singlet oxygen ($^{-}O_2$)}, and hydroxyl anion ($OH^-$), while non-radical forms include hydrogen peroxide ($H_2O_2$) (Sharma et al. 2012).

(h) Genes for antioxidant enzymes are induced by nitrous oxide (Siddiqui et al. 2011). Zhao et al. (2001) observed an increase in NOS-like activities in wheat seedlings under drought stress, and ABA accumulation was inhibited by NOS inhibitor. ROS and NO may be signal molecules by which the plants sense the drought stress, and may participate in the leaf water maintenance by stimulating ABA synthesis.

(i) Accumulation of potassium plays a central role as it acts as an osmolyte, aiding osmotic adjustment in plants (Kant and Kafkafi 2002; Marschner 2011).

(j) 2R, 3R-butanediol, a bacterial volatile induces systemic tolerance to drought stress by reducing the stomata aperture (Cho et al. 2008).

### 10.1.4 Molecular Mechanisms

The response shown by the plants during stress management is mainly regulated and controlled at molecular level. Stress factors cause the alteration of different stress specific genes and enzyme activities to cope up with stress conditions. A wide array of genes and gene products associated with stress response have been identified using experimental approaches.

(a) N-terminus Glycine Rich Ribosomal binding protein (Nt-GR-RBP 1) affects m RNA binding protein function (Jabeen et al. 2017). Ribosome inactivating proteins which remove adenine from rRNA control the drought stress at translation level (Jiang et al. 2008).
(b) Translationally controlled tumour protein (TCTP) and proliferating cells nuclear antigen (PCNA) induced systemic resistance in plants interact with DNA polymerase and enhance its fidelity and efficiency (Ghabooli et al. 2013).
(c) Late embryogenesis abundant proteins (LEA proteins) are one of the major groups of proteins upregulated during stress tolerance to help in survival of plants during extreme water loss (Battaglia et al. 2008). LEA proteins are responsible for protection of cellular and molecular structures including dehydration, buffering, ion sequestration, ROS scavenging, and metal ion-binding properties (Goyal et al. 2005; Wu et al. 2014).
(d) Wheat boiling stable stress-responsive protein (wBsSRP) was isolated from drought tolerant cultivar of wheat (PBW 175) under drought conditions (Rakhra et al. 2017).
(e) AtMYC2/JIN1, encoding the basic helix-loop-helix, leucine zipper transcription factor, is not only involved in ABA-mediated drought stress signalling pathway, but also in other wound response and pathogen defence responses. Over expression of AtMYC2 encodes ABA-mediated drought stress signalling (Wu et al. 2009).

## 10.2 Exogenous Solutions to Drought Stress

### 10.2.1 Plant Growth Promoting Rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are well known bacterial species which help in plant growth promotion in addition to elicit drought stress response in plants through alteration in biochemical parameters (Grover et al. 2011). These PGPR colonize the roots of plants and modulate the secretion of various hormones which help them tolerate different stress. Jaleel et al. (2007) investigated the role of plant growth promoting bacteria (PGPB) in combating drought stress of plants and suggested the role of *Pseudomonas fluorescens* which induced the production of ajmalicine in *Catharanthus roseus* under drought stress. They observed enhanced plant biomass and alkaloid content in *Pseudomonas* treated seedlings. Similarly, Garcíaa et al. (2017) also studied the effect of *Azospirillum* on the growth of maize under drought stress and observed that inoculation of seeds with the PGP strain resulted in better response to water deficit conditions. Polyamines are known to impart abiotic stress tolerance to plants. Increase in the level of 3 major polyamines, i.e. putrescine, spermidine, and spermine in *Arabidopsis thaliana* was observed after the influence of plant growth promoting *Pseudomonas putida* strain by Sen et al. (2018). These PGPB, known to modify phytohormonal activities in plants (Egamberdieva 2013), reduce the production of volatiles (Timmusk et al. 2014) and thus improve photosynthesis and produce antioxidants, osmolytes and exo-polysaccharides which help plants to combat stress like drought. PGPR have been reported to improve drought tolerance in *Arabidopsis* (Timmusk and Wagner 1999), pepper (Mayak et al. 2004), wheat (Yandigeri et al. 2012), tomato (Mayak et al. 2004), and pea (Belimov et al. 2009). PGPRs under stress trigger several mechanisms in plants like phytohormone synthesis, inhibition of plant pathogens and induction of systemic resistance (Sathya et al. 2017). Yang et al. (2009) proposed the term "induced systemic tolerance" (IST) to refer defence-related physical and chemical changes in plants induced by microbes under abiotic stresses. Certain phytohormones, such as indole-3-acetic acid (IAA), cytokinin (CTK), and gibberellin can be synthesized by PGPRs as well as by plants. Filamentous bacteria (Actinobacteria) might help improve the resilience of plants under water (Kohler et al. 2008; Yandigeri et al. 2012). *Streptomyces* species reportedly can produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, IAA, and siderophores, which can improve plant growth under stress conditions (Palaniyandi et al. 2014). The mechanisms underlying the drought tolerance-promoting effects of *Streptomyces* sp. in plants have been documented which include increasing the osmotic pressure in plant cells, callose accumulation, and lignification of the cell walls (Hasegawa et al. 2007, 2008).

Tolerance to drought was correlated with reduced water loss in *P. chlororaphis* O6-colonized plants and with stomatal closure, indicated by size of stomatal aperture and percentage of closed stomata. Stomatal closure and drought resistance were mediated by production of 2R, 3R-butanediol, and production of salicylic acid (SA)-, ethylene-, and jasmonic acid-signalling pathways. Increase in SA during drought stress also suggests the primary role of SA in management of drought stress (Cho et al. 2008). The mechanism of drought stress tolerance by PGPRs can be categorized by the following mechanisms:

(a) The PGPR secrete plant growth promotory substances (IAA and siderophores) that help plant to continue normal metabolism during stress (Egamberdieva 2013).
(b) Secretion of polyamines like putrescine, spermidine, and spermine is another mechanism to control drought stress (Sen et al. 2018).

(c) Induced systemic resistance (ISR), a combat mechanism of plant is induced by the microbial colonizers of plants.

(d) The phytohormone, ACC is secreted by PGPR that inhibits the conversion of ACC to ethylene, which is responsible to shut down the normal mechanism of plants during stress.

(e) The PGP are also known to secrete osmolytes and exopolysaccharides which protect the plant from environmental stress (Zhang et al. 2018a, b).

(f) 2R, 3R butanediol are the chemical compounds, which in addition to plants are also secreted by PGPRs and found responsible for stomata closure and hence reduced water loss.

(g) The microbes are also responsible for increasing the osmotic pressure in plant cells through callose accumulation and lignifications of the cell walls (Hasegawa et al. 2008). This mechanism thickens the cell wall and thus reduces the water loss.

(h) Hairpin proteins produced by several gram-negative bacteria induce defence response against pathogens and drought (Liu et al. 2016).

Extensive studies are still needed to explore more potential microbes which can address the drought stress management in plants.

### 10.2.2 Fungal Inoculants in the Form of Endosymbiont or Arbuscular Mycorrhiza (AM)

Ghabooli et al. (2013) studied the role of endophytic fungus *Piriformospora indica,* which imparts several plant health benefits and maintains productivity during drought conditions of Sebacinales. This endophytic fungus promotes plant growth and resistance to abiotic stress, including drought. Zhang et al. (2018a, b) studied the effect of *Piriformospora indica* inoculation on maize growth under drought stress. The maize after colonization showed improved leaf size, root length, and number of tap roots. Gene ontology reveals the role of carbon and sulphur metabolic pathways as major target. Higher transcripts for microtubule associated processes improved oxidative potential of roots and stimulated genes for hormone functions, including those which respond to abscisic acid, auxin, salicylic acid, and cytokinins.

Fungal symbionts have been found to be associated with every plant studied in the natural ecosystem, where they colonize and reside entirely or partially in the internal tissues of their host plants. Fungal endophytes can form a range of different relationships with different hosts including mutualistic, symbiotic, commensalistic, and parasitic in response to host genotype and environmental factors (Singh et al. 2011). In mutualistic association fungal endophyte can enhance growth, increase reproductive success, and confer biotic and abiotic stress tolerance to its host plant. Since abiotic stress such as, drought, high soil salinity, cold, heat, oxidative stress, and heavy metal toxicity are some common adverse environmental conditions that affect and limit crop productivity worldwide. It may be a promising alternative strategy to exploit fungal endophytes to overcome the limitations to crop production brought by abiotic stress. There is an increasing interest in developing the potential biotechnological applications of fungal endophytes for improving plant stress tolerance and sustainable production of food crops (Singh et al. 2011).

### *10.2.3 Nanotechnology*

According to Prasad et al. (2017), nanotechnology may revolutionize agriculture by innovative new techniques. Research in the area of application of nanotechnology in agriculture to reduce the water requirement for irrigation can be carried out to address drought stress in rainfed area of hills (Fig. 10.2).

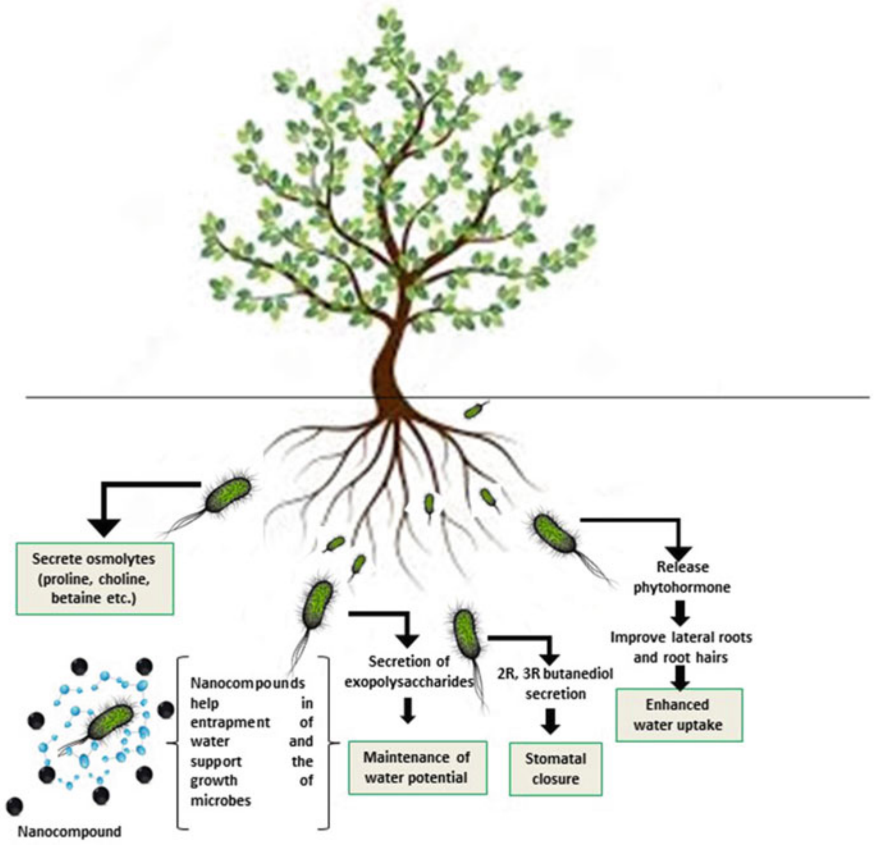

**Fig. 10.2** Impact of nanocompounds and microbial inoculants on drought stress tolerance mechanisms of plants

Dimkpa et al. (2017) assessed the micronutrient nanoparticles (ZnO, $B_2O_3$, and CuO) for their role in improving drought stress and observed positive results. They suggested the application of nanoparticles boosted the crop performance in drought stress. Bioformulations are already known for their potential to improve plant productivity, but due to the shelf life issue they still lack behind the chemical fertilizers. Meagre information is available regarding the shelf life of microbial population (especially vegetative cells of *Pseudomonas, Azospirillum,* etc.) after 6 months in nano-bioformulation, which brings the necessity for the research in this area. The soil based nanocompounds (nanoclay, nanozeolite) improve the soil structure and microbiome due to their good water and nutrient holding capacity (Khati et al. 2017).

The nanocompounds can support the life of microbial inoculants in formulation due to controlled release of nutrients to them. The poor survival of microbial inoculants in field condition is due to several factors, the major one is insufficiency of nutrients and moisture. A chelating system that can entrap the resources and allow their slow release to sustain the microbial population may solve the problem of nutrient and moisture scarcity in any formulation. The nanocompounds (nanozeolite and nanochitosan) due to their physical and chemical nature are reported to enhance the survival efficiency of bacteria in broth (Khati et al. 2019). Further research is needed to be carried to bring a better insight in this field and utilize it for the improvement of agriculture production. The role of nanocompounds and microbial inoculants to mitigate drought stress is elucidated in Fig. 10.2.

## 10.3 Genetic Engineering of Plants and Bacteria

The present technology has allowed us to engineer the genome of plants for the selected traits and impart any character of interest. The stress tolerance can also be improved by altering the stress-responsive traits to solve the management issue. Molecular chaperons like Glycine Rich RNA binding protein (GR-RBPs) were also reported to have important role in stress response management. The role of mRNA of GR-RBPs in drought stress response was reported by Khan et al. (2013). A Ribosomal inactivation protein (RIP), N-glycosidase inhibits protein synthesis by depurinating the rRNA. Jiang et al. (2008) studied the RIPs in *Oryza sativa* and reported their expression under biotic (pathogen) and abiotic stress (drought, salinity, and temperature).

Hairpin proteins are elicitors and produced by several gram-negative plant pathogenic bacteria, trigger multiple beneficial responses in plants, such as growth promotion, induction of defence against diverse pathogens and insects, and drought tolerance. The hairpin-encoding gene (popW) derived from *Ralstonia solanacearum* ZJ3721 was observed to impart tolerance to tobacco plant towards drought stress. These transgenic lines of tobacco displayed a significant increase in different enzymes like superoxide dismutase, peroxidase, catalase, and ascorbic acid content as compared to control plants under drought stress. Overexpression of popW in

**Table 10.1** Genes potentially responsible for drought stress tolerance

| Sl. No | Gene | Role | Reference |
|---|---|---|---|
| 1 | *PopW* | Hairpin-encoding gene that enhanced the relative transcript levels of oxidative stress-responsive genes (NtAPX, NtCAT1, NtGST, and NtCu/Zn-SOD) under drought stress | Liu et al. (2016) |
| 2 | *2b* | Encoded by cucumber mosaic virus and cause suppression of RNA silencing and improve drought stress tolerance | Westwood et al. (2013) |
| 3 | *AGO1* | Controls a microRNA-mediated drought tolerance mechanism | Westwood et al. (2013) |
| 4 | *LEA gene* | Improvement of growth characteristics under water deficient conditions, more biomass and more efficient water use | Wu et al. (2014) |
| 5 | *Sac B gene* | Encodes for levansucrase, which takes part in fructan synthesis. Fructan promotes the process of root branching, thus increasing root surface and water uptake | Pilon-Smits et al. (1995) |
| 6 | *P5CS gene* | Responsible for proline accumulation which is associated with abiotic stress | Delauney and Verma (1993) |
| 7 | *ADC* | In plants, polyamine, accumulate under several abiotic stress stimuli, including drought and salt | Kumar and Minocha (1998) |
| 8 | *DRE* | The dehydration responsive element (DRE) was identified as a cis-acting element regulating *gene expression* in response to dehydration | Van Rensburg and Kruger (1994) |
| 9 | *Fe-SOD* | Within a cell, the SODs constitute the first line of the defence against ROS. Transgenic tobacco plants containing oxidative stress-related genes showed elevated levels of glutathione reductase, superoxide dismutase and ascorbate peroxidase, resulting in enhance drought tolerance | Van Rensburg and Kruger (1994) |
| 10 | *WXPI* | The gene designated WXP1 is able to activate wax production and confer drought tolerance in alfalfa (*Medicago sativa*) | Zhang et al. (2005) |
| 11 | *MAPK* | Expression of mitogen-activated protein kinase gene (MAPK) genes activates an oxidative signal cascade and lead to the tolerance of freezing, drought, heat and salinity stressing | Shou et al. (2004) |
| 12 | *ERA1* | The gene identified in *Arabidopsis* encodes β-subunit of a farnesyltransferase and involves in ABA signalling | Wang et al. (2005) |
| 13 | *bZIP* | Act as ABA dependent transcription factor for drought responsive genes | Xiang et al. (2008) |
| 14 | *OsSIK1* | Enhanced tolerance to drought and salinity stresses | Ouyang et al. (2010) |
| 15 | *OsCPK4* | A calcium-dependent protein kinase showed enhanced tolerance to drought and salinity stresses | Campo et al. (2014) |
| 16 | *EDT1/ HDG11* | Encodes a homeodomain—leucine zipper transcription factor, which is likely involved in reproductive development during stress | Yu et al. (2008) |
| 17 | *SNAC1* | Responsible for reduced water loss due to increased stomatal closure and enhanced expression of a large number of stress-related genes | Hu et al. (2006) |
| 18 | *AP2/ ERF* | Enhanced photosynthesis and reduced transpiration under drought stress | Karaba et al. (2007) |

tobacco also significantly enhanced the relative transcript levels of oxidative stress-responsive genes under drought stress. (Liu et al. 2016).

Suárez et al. (2008) showed a new strategy to increase drought tolerance and yield in legumes by overexpressing trehalose-6-phosphate synthase activity, if inoculated with *R. etli* over expressing trehalose-6-phosphate synthase gene. *Phaseolus vulgaris* plants had more nodules with increased nitrogenase activity and higher biomass compared with plants inoculated with wild-type *R. etli*. They suggested the role of trehalose metabolism in rhizobia for signalling plant growth, yield, and adaptation to abiotic stress. Manipulation in trehalose metabolism using symbiotic rhizobia may show major agronomical impact on leguminous plants under drought stress.

Westwood et al. (2013) proved the role of RNA silencing in drought stress tolerance. Cucumber mosaic virus (CMV) encodes 2b protein, which plays a role in local and systemic virus movement, symptom induction, and suppression of RNA silencing. It also disrupts signalling regulated by salicylic acid and jasmonic acid. CMV induced tolerance to drought in *Arabidopsis thaliana*. The silencing effector ARGONAUTE1 (AGO1) controls a microRNA-mediated drought tolerance mechanism. Similarly, Wu et al. (2014) cloned an LEA gene (SmLEA), from *Salvia miltiorrhiza* Bunge. SmLEA-transformed *S. miltiorrhiza* plants showed better root elongation and a lower level of malondialdehyde. SmLEA-overexpressing transgenic plants showed a less rapid rate of water loss and had greater superoxide dismutase activity and a higher glutathione concentration (Table 10.1).

## 10.4 Conclusion

Under the influence of global warming all over the world, changes in the climatic condition are creating unusual weather phenomena often in the form of water stress. Moisture limitation significantly affects most of the metabolic processes in plants, including membrane conformation, chloroplast organization, and enzyme activity at cellular level, which affects whole plant growth and yield. Drought stress is a severe environmental constraint to agricultural productivity. Drought stress induces stomatal closure, decreases transpiration and photosynthetic rates, and leads to earlier crop maturity and poor productivity. To minimize the negative effects of water stress, plants have various signalling pathways. Plants respond immediately for any stress by changing their growth pattern, upregulation of antioxidants, accumulation of compatible solutes and by producing stress proteins and chaperones. Microbial bioinoculants (PGPR and Fungal endosymbiont) play important role in conferring resistance/tolerance and adaptation of plants to drought stresses and have the potential role in solving issues for future food security. The interaction between plants and microbes under drought conditions affects not only the plant but also changes the soil properties. Application of microbial inoculants is a potential and natural way to combat drought stress in plants. This technique still faces challenges due to its slow impact in actual condition. Development of drought tolerant crop varieties through genetic engineering and plant breeding is another important way to fight drought stress. The study also focuses on application of nanocompounds to mitigate drought stress, which may help in improvement of water use efficiency by plants and microbes. More investigations into the mechanisms by which microbes elicit tolerance to specific stress factors are needed. Studies are needed to elucidate the signal transduction pathways that result from treatment of plants with PGPR under stress conditions.

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# Chapter 11

# Phytoremediation: A Synergistic Interaction Between Plants and Microbes for Removal of Unwanted Chemicals/Contaminants

**Sheel Ratna, Swati Rastogi, and Rajesh Kumar**

**Abstract** Environmental pollution with obnoxious contaminants is detrimental to plant growth and poses health hazards to humans and other life forms. Thus, remediation of such antagonistic environment has become a key issue for environmentalists all around the world. Phytoremediation, a cooperative association between plants and microbes, is an emerging in situ cost-effective technology and provides a viable option in the treatment of such contaminated environments. Present chapter emphasizes on plant–microbes interactions during phytoremediation and how such beneficial interactions lead to improved plant growth and contamination free environment.

## 11.1 Introduction

Environmental contamination is one of the most intractable concerns worldwide to ensure the safest and healthiest environment. The source of pollution is natural and anthropogenic activities. Key anthropogenic sources of pollution are related to the burning of fossil fuels, mining and untreated or partially treated disposal of municipal solid wastes and wastewater discharges or use for irrigation and excessive utilization of fertilizers and pesticides (Pinto et al. 2016, 2018). Continuous and consistent increase in a variety of organic and inorganic pollutants has been reported to cause environmental pollution which results in severe health hazards in living beings. Inorganic contaminants are salts of nitrate, ammonia, sulfate, phosphate, cyanide, heavy metals (HMs), while alkanes, antibiotics, dioxins, phenols, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), Persistent Organic Pollutants (POPs), pesticides, synthetic azo dyes, polyaromatic, chlorinated, and nitro-aromatic compounds constitute organic contaminants.

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Among all the inorganic and organic contaminants, HMs and POPs are most problematic in the atmosphere due to their toxicity, bioaccumulation factor, resistance to degradation in an environment through natural processes. They are highly obnoxious and dangerous to animals, including humans (Ahmadpour et al. 2012; Saxena and Bharagava 2017; Arslan et al. 2017; Shayler et al. 2017). Most of the HMs are naturally occurring elements and have density higher than 5 g $cm^{-3}$. Heavy metals are not easily degradable as can lose their electron(s) to form cations and in addition to these, other organic toxic contaminants such as POPs exist persistently in the environment for several years before getting totally mineralized (Adrees et al. 2015; UNEP 2006). Carson (1962) reported the harmful effect of DDT first time on bird's eggs, which were unable to hatch due to the impact of DDT. Due to this phenomenal incidence, DDT was banned in 1973 (Carson 1962; Staniforth 2013). Recently, Stockholm Convention (COP9) on POPs held at Zeneva has issued a list of hazardous chemicals like dicofol, perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds in Annex A(elimination category) (the Stockholm Convention (COP9), https://www.meti.go.jp/english/press/2019/0514_001.html). Jamieson et al. (2017) have reported the presence of some POPs in fauna from the deepest ocean trenches (>10 km). Recently a research group has reported that consumption of leafy vegetables contaminated with PCBs and DDTs is not suitable for human health and causes death (Olatunji 2019). Soil acts as the hindmost acceptor of contaminants and these contaminants limit the development of plants through bioaccumulation and biomagnification along the whole food chain (He et al. 2014).

Phytoremediation concept was foremost postulated by Chaney. This term was used for the first time by Raskin and Salt to describe the use of plants for removal of toxic HMs (Raskin et al. 1994; Salt et al. 1995). Researchers used this promising approach for the remediation of soil and water, contaminated with organic and inorganic pollutants (Chaney 1983; Khan et al. 2014). Plants used in the process of phytoremediation of contaminants from the soil or water may be terrestrial and macrophytes or microphytes like free-floating, emerged or submerged (da Conceicao Gomes et al. 2017; Farid et al. 2017; Gu et al. 2017; Wani et al. 2017). Contamination of HMs and POPs in soil and water affects the phytoremediation efficiency of the plants (Ibanez et al. 2012; Khan et al. 2014; Gerhardt et al. 2017). Mutual relationships of the plants and microbes have been projected to enhance the efficiency of phytoremediation of HMs and POPs from contaminated soil and water (Becerra-Castro et al. 2013; Haslmayr et al. 2014; Kong and Glick 2017; Balazs et al. 2018; Boudh and Singh 2019; Gupta et al. 2019). In this chapter, we will discuss the toxic effects of HMs and POPs and their phytoremediation through synergistic interaction between plants and microbes in current scenario.

### *11.1.1 Toxicity of Heavy Metal and POPs*

HMs and POPs show their obnoxious effect on biotic and abiotic components of the ecosystem due to their less solubility and high carcinogenicity and mutagenicity (Yadav et al. 2017; Lal et al. 2018). Some metals such as As, Pb, Cd, and Hg may be toxic even at low concentrations (Ma et al. 2016a; Rastogi et al. 2019). They are absorbed by biotic/abiotic components of the environment and then transferred to the top consumers via food web from where they continue to accumulate in their vital organs like brain, liver, bones, and kidneys for years and ultimately lead to health hazards in human beings (Lal et al. 2018). POPs show toxic effects on both targeted and non-target populations and may lead to several physiological, biochemical, developmental, and behavioral stress responses at different trophic levels of the food web (Inostroza et al. 2016). Researchers have measured the extent of adversity of HMs, POPs, etc. in an ecosystem on the basis of their toxicity, concentration, and exposure period. Different studies have been conducted to elucidate the acute and chronic effects of a variety of POPs on living beings (Ashraf et al. 2017; Rajkumar et al. 2013). Recently a study reported that insulin resistance, obesity, and endocrine disruption are caused by acute exposure of POPs in humans (Gregoraszczuk and Ptak 2013). Effects of PCBs and PBDEs on life-history traits like somatic growth, spawning, and larval survival of Zebrafish have been analyzed by Horri et al. (2018). Numerous studies have demonstrated the adverse effect of different metal (loid)s on the microbial diversity of polluted soils (Kerfahi et al. 2019; Srut et al. 2019; Zhao et al. 2019). Excessive concentration of metal(loid)s in soils can affect the growth, morphology, and metabolism of microorganisms mainly through destruction of cellular membranes and organelles, enzyme denaturation, and functional or conformational disturbance (Rajkumar et al. 2013; Ashraf et al. 2017).

## 11.2 Phytoremediation

A variety of physical, chemical, and biological approaches for the remediation of persistent (organic) and non-degradable inorganic pollutants (heavy metals and metalloids) from water and soil have been in practice but because of some demerits, phytoremediation has gained a momentum and used as a good alternative for decontamination (Mahar et al. 2016; Coninx et al. 2017; Saxena et al. 2019). Phytoremediation is an efficient, inexpensive, and eco-friendly rehabilitation strategy that uses plants to absorb, accumulate, immobilize or biodegrade organic and inorganic pollutants from different environmental matrices (air, soil, and water) through physical, chemical, and biological processes (Vangronsveld et al. 2009; Cristaldi et al. 2017; Wang et al. 2017; Kumar 2019). Terrestrial and aquatic plants such as submerged/emerged and free-floating play an important role in phytoremediation of contaminants from the aquatic and terrestrial environment (da Conceicao Gomes et al. 2017; Farid et al. 2017; Wani et al. 2017).

Phytoremediation is therefore a suitable option to remediate soil and water bodies contaminated with HMs and POPs (Khan et al. 2018; Mahar et al. 2016; Mishra et al. 2020). Phytoremediation encompasses distinct mechanisms namely phytostabilization, phytodegradation, phytovolatilization, and rhizodegradation (Kong and Glick 2017) Fig. 11.1.

### *11.2.1 Phytovolatilization*

Phytovolatilization or phytoevaporation is the uptake of pollutants from the soil and water by plants and their conversion into volatile form and succeeding release into the atmosphere. It can be applied for organic pollutants and some volatile heavy metals, i.e. Selenium (Se) and Mercury (Hg) (Ali et al. 2013; Coninx et al. 2017). Complete removal of the pollutant from the site in gaseous form, without further need for plant harvesting and disposal holds a great promise as an attractive technology to control pollution (Pilon-Smits 2005; Lim et al. 2016). Phytoevaporation is the most controversial technology of the phytoremediation because this technique does not fix the contaminants completely but transfers pollutants from soil to atmosphere from where it can be redeposited (Padmavathiamma and Li 2007).

### *11.2.2 Phytostabilization*

Phytostabilization includes immobilization of the contaminants in soil either simply by preventing erosion, leaching, or dispersion or by transforming them through precipitation in the rhizosphere to less bioavailable forms (Pulford and Watson 2003). Phytostabilization is the best option for the remediation of large contaminated areas but excessive soil pollution may inhibit the survival of plants used for phytostabilization (Vangronsveld et al. 2009). Some hyperaccumulator (HA) plants accumulate HMs in the root and confine the HMs transport to aerial parts of the plants. Therefore, these HAPs are used for phytostabilization as they can help in reducing soil pollution thus survival of the plants (Vangronsveld et al. 2009; Coninx et al. 2017; Sheoran et al. 2010). Phytoremediation efficiency of *Sarcocornia fruticosa* is increased after bioaugmentation using autochthonous metal resistant *Saccharomyces cerevisiae* isolated from HMs (Cd, Pb, Ni, Cr, and Cu) contaminated /sedimented site (Said et al. 2018). Microbial assisted phytostabilization should be a promising technique which could be used for the removal of organic and inorganic contaminants. Inoculation of *Bradyrhizobium* and *Azospirillum* with *Glycine max* reduced the translocation and improved phytostabilization of As (Armendariz et al. 2019). *Achromobacter spanium* and *Serratia plymuthica* isolated from *Medicago sativa* nodules are some suggested

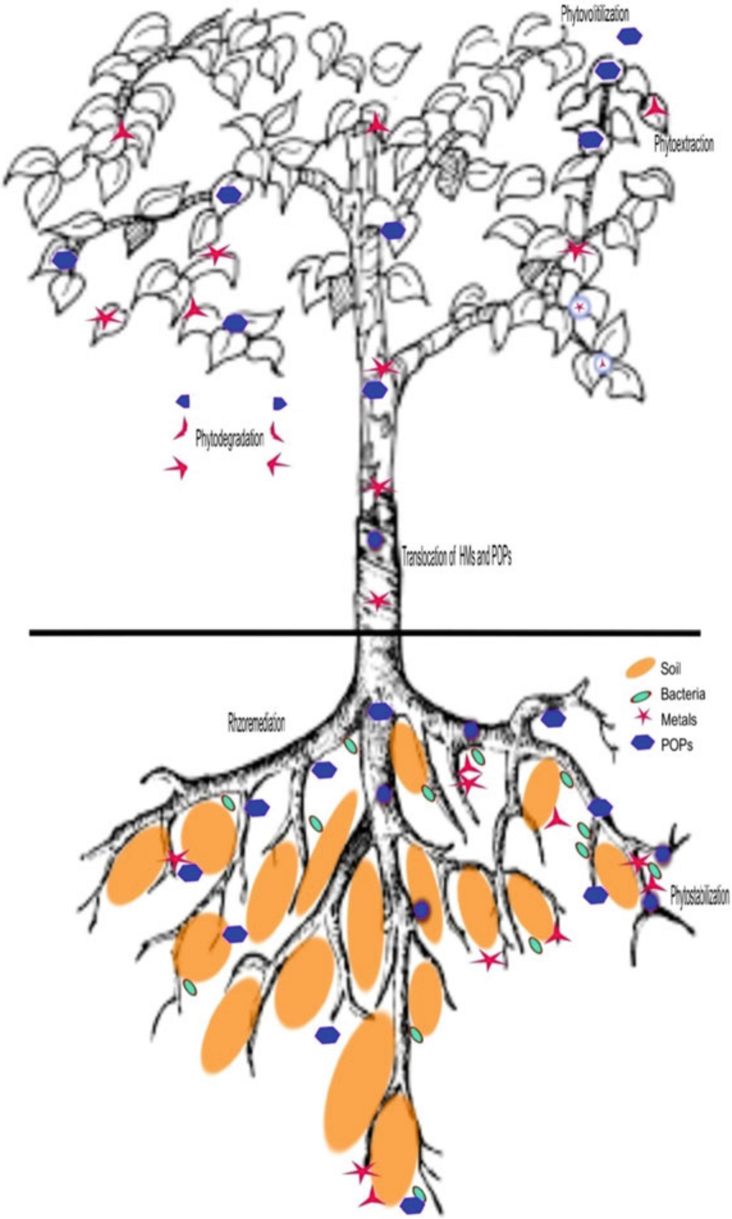

Fig. 11.1 A proposed model of plant and microbe interaction during phytoremediation

microbes which can be used as potential phytostabilizer for the pesticide-contaminated sites (Aroua et al. 2019).

### 11.2.3 Phytoextraction

Phytoextraction is popularly known as phytoaccumulation, phytoabsorption or phytosequestration. It is a type of phytoremediation through which uptake of the contaminants from soil, water, or sediments takes place by plant roots which are further translocated and accumulated in aerial parts, i.e., stem or other harvestable plant parts (Glick 2010; Pinto et al. 2015; Mahar et al. 2016). Phytoextraction is the most important and most useful phytoremediation technique for the removal of heavy metals and metalloids from polluted soils (Pinto et al. 2016) but it limits its use for non-food crop plants due to their accumulation in the aerial parts hence the plant biomass produced (Sharma and Pandey 2014; Burges et al. 2017). Some plants belonging to a particular family have the competence to grow and accumulate extremely high concentration of HMs and POPs without suffering from phytotoxic effect via enhancing uptake, translocation, detoxification, and sequestration in their airy parts and are known as Hyper Accumulator Plants (HAPs) (Samardjieva et al. 2015; Pinto et al. 2016). In environment, contaminants are present in complex mixed forms but HAPs can accumulate one metal from complex metal-polluted soils which often contains a mixture of metals and other organic pollutants (Pinto et al. 2018; Cristaldi et al. 2017). Some HAP (e.g. *Thlaspica erulescens*, *Alyssum bertolonii*, *Arabidopsis halleri*) species are able to accumulate contaminants but produce little biomass, and therefore it is possible to use those species that accumulate as well as produces more biomass like *Brassica* sp., *Arundo donax,* and *Typha* sp. (Pinto et al. 2016; Shiri et al. 2015) but the storage, treatment, placement, and disposal of the contaminated plant biomass are of great concern (Mahar et al. 2016; Ghosh and Singh 2005). In recent years, different biotechnological approaches have also been used to increase biomass and growth rate of hyperaccumulator plants through genetic engineering tools, i.e., synthesis of various metal-binding peptides (Mahar et al. 2016). The success of phytoextraction depends on high plant biomass production and ability to accumulate metals (Nayak et al. 2018).

### 11.2.4 Phyto/Rhizodegradation

In an integrated approach, phyto and rhizodegradation can be addressed as a mutually beneficial form of phytoremediation, where both plants and microorganisms mediate the breakdown of the contaminants via the use of their enzymatic machinery. Sampaio et al. (2019) reported the interaction between *Rhizophora mangle* and bacterial strains (*Pseudomonas aeruginosa* and *Bacillus*) as a potential association for the degradation of eight PAHs from diesel oil-contaminated site.

## 11.3 Phytoremediation Using Microbes (Fungi, Bacteria, Algae, and Mycorrhizae)

Plants and microbes reside together and interact with each other. A plant provides nutrients and space to the microbes and in return, microbes improve the bioavailability, mineralization, and detoxification of various organic and inorganic pollutants (Balseiro-Romero et al. 2017; Liao et al. 2016; Chirakkara et al. 2016; Wang et al. 2017). The interface between the microbes and plant roots in the rhizosphere is believed to vastly influence the growth and survival of the plants. Rhizospheric microbes have the potential to biodegrade organic pollutants through rhizodegradation, biotransformation, and volatilization (Ashraf et al. 2017). Optimization of the phytoremediation process is analyzed by the dynamics between the plants, microbes, and pollutants (Rajkumar et al. 2010, 2013; Ashraf et al. 2017). Recently, inoculation of plants with selected and acclimatized microbes (bioaugmentation) has gained attention for phytoremediation of metal(loid)s polluted soils and technologies equipped with phytoremediation techniques are gaining momentum to decontaminate polluted soils on large scales (Sarwar et al. 2017; Kong and Glick 2017; Rehman et al. 2018). Agnello et al. (2016) reported highest degree of bioaugmentation-assisted phytoremediation of heavy metals and petroleum hydrocarbons in alfalfa plants when inoculated with *Pseudomonas aeuroginosa.* PGP yeast *Candida* VITJzN04 inoculated with *Saccharum officinarum* showed the degradation of lindane via biostimulation thus helped in phytoremediation (Salam et al. 2017).

## 11.4 Mechanisms Between the Plant and Microbes During Phytoremediation

Plant-associated microbes (endophytic, phyllospheric, and rhizospheric) have a variety of interactions with host plants which are generally mutual beneficial associations for both the systems (Newman and Reynolds 2004; Weyens et al. 2010). Some species of bacteria that can resist the high concentration of metals interact with hyperaccumulators (HA) to promote their growth are of prime importance in phytoremediation (Wang et al. 2017). These bacterial species either directly or indirectly assist the HA plants to remediate the soil while growing well in a polluted environment (Kumari et al. 2016).

### *11.4.1 Plant Growth-Promoting Bacteria (PGPB)*

Plant growth-promoting bacteria (PGPB) are so named due to their potential to enhance plant growth. These bacteria may develop symbiotic/mutualistic associations with plants and may be found as free-living rhizospheric or endophytic bacteria. Genera including *Gluconacetobacter*, *Flavobacterium*, *Beijerinckia*, *Klebsiella*, *Erwinia*, *Enterobacter*, *Burkholderia*, *Pseudomonas*, *Serratia*, and *Bacillus* are among the beneficial PGP bacteria that control/promote plant growth (Dardanelli et al. 2010; Ashraf et al. 2017). Some species of microorganisms including bacteria (pollutant degrading and plant growth-promoting, etc.) and filamentous fungi alter the rhizospheric environment through bioavailability or stabilization and thus diminish the toxicity of HMs and POPs and ultimately appear to be beneficial in phytoremediation by increasing plant biomass (Wang et al. 2017; Cao and Liu 2015; Compant et al. 2010). PGPB help plant in the process of phytoremediation directly and indirectly (Glick 1995). Indirectly like acting as a biocontrol agent where they prevent or decrease the deleterious effect of pathogens on plants via several mechanisms such as competition for the binding sites on roots, production of cell wall-lysing enzymes against pathogenic fungi, iron depletion in the rhizosphere, antibiotic production, and providing systemic resistance in plants (Khasheii et al. 2016). The direct approach is through the production of substances that promotes plant growth and also increases mineral absorption. These functions are accomplished by the production of various metabolites such as siderophores, biosurfactants, indole acetic acid, ACC (1-aminocyclopropane-1-carboxylate) deaminases, and organic acids (Kong and Glick 2017). Secretion of different organic acids helps in solubilization and mobilization of HMs (Ma et al. 2016b). The role of siderophores in phytoremediation is to increase the availability of iron to the plant and simultaneously to decrease the iron for pathogens, besides this, they also act as scavenger of Reactive Oxygen Species (ROSs) generated by HMs (Chen et al. 2017; Ma et al. 2016b; Khasheii et al. 2016). On the other hand, ACC deaminase reduces the inhibitory effect of ethylene produced as a result of HM stress. It also increases the length and width of roots so as to increase the effectiveness of phytoremediation (Kumari et al. 2016). Indole acetic acid (IAA) induces the physiological changes and promotes root growth and metal absorption and finally upgrades the phytoremediation process. Nutrient deficiency is also an important factor that can limit phytoremediation. Many microorganisms play an important role in solubilizing minerals such as P, K, releasing nutrients and supplying them to plants and help in upgradation of phytoremediation (Schmalenberger et al. 2013; Grobelak et al. 2018).

Recently a research team reported that metal tolerant PGPB (*Ralstonia eutropha* 1C2 and *Chryseobacterium humi* ECP37) increases the Zn bioavailability in the soil along with enhancement in the bioaccumulation and biomass of Maize plants (Moreira et al. 2019). In addition to this, enzyme ACC deaminase synthesized by microorganisms are essential for plant growth since it facilitates plant growth by lowering plant's ethylene levels (Glick et al. 2007; Shahid et al. 2019). Usually microorganisms facilitate plant growth by using one or more PGP properties (Valentin-Vargas et al. 2014; Nayak et al. 2019). Fungi show a distinct advantage in phytoremediation due to their high tolerance for extreme pH, climate, nutrients, and heavy metals. Concomitant use of arbuscular mycorrhizae (AM) and *Aspergillus niger* is reported to increase the bioavailability and reducing the toxicity of HMs in plants (Gaur and Adholeya 2004; Shafiq and Jamil 2012). Ma and his research team inoculated bacteria (*Pseudomonas libanensis* TR1) and arbuscular mycorrhizal fungus (AMF) *Claroideoglomus claroideum* BEG210 with *Helianthus annuus* and found that this combination improves Ni exclusion, detoxification, phytostabilization and finally remediates nickel from contaminated saline soils (Ma et al. 2019). During plant–microbe interactions, POPs could be degraded by rhizobacteria (Glick 2010; Weyens et al. 2009). The interactions between plants and bacteria having catabolic genes have led to the evolution of a diverse variety of catabolic enzymes that can metabolize and detoxify the xenobiotics (Hong et al. 2015). The combined use of plants and bacteria has been exploited to enhance the phytoremediation of soil and water contaminated with different organic pollutants (Afzal et al. 2014a, b). Similarly, several studies were performed to explore the potential of plant-bacteria partnership for the remediation of POPs contaminated soil and water (Aken et al. 2009; Becerra-Castro et al. 2013; Jha and Jha 2015). Rhizobacteria capable of degrading different POPs have been isolated from rhizospheric soil of different plants and studied for POP degradation pathways and genes involved in POP degradation (Brazil et al. 1995; Fatima et al. 2015; Nicoara et al. 2014). A rhizobacterial strain *Bacillus* sp. PRB101 enhanced 92% endosulfan degradation after 120 days of inoculation (Rani et al. 2019). During POPs degradation, co-metabolism, i.e., similar metabolite secretions from the host plant supports bacterial population in the remediation processes (Bedard et al. 1986). Endophytic bacteria resides in plant tissues beneath the epidermal cell layers, from where they can colonize the internal tissues and form a range of different interaction thus help in the phytoremediation (Rajkumar et al. 2009). Endophytes also have PGPR properties like other rhizospheric microbes and they help in the phytoremediation process (Rajkumar et al. 2009; Ma et al. 2016b). The synergistic associations of different plant and microorganisms in the phytoremediation of POPs and HMs have been summarized in Table 11.1.

### *11.4.2 Biosurfactant*

Biosurfactants are low-molecular-weight amphiphilic molecules consisting of a hydrophilic and a hydrophobic moiety, comprising a wide range of chemical structures, such as mycolic acid, glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids, etc. (Ma et al. 2016b; Banat et al. 2010). Biosurfactants produced by endophytic and rhizobacteria seemed to increase the bioavailability of poorly soluble HMs and POPs and hence upgrade phytoremediation (Aslund and Zeeb 2010; Federici et al. 2012; Ma et al. 2016b; Rastogi and Kumar 2020). Mehetre et al. (2019) studied the role of individual and mixed cultures of thermophilic and thermo-tolerant biosurfactant-producing bacteria in biodegradation of polycyclic aromatic hydrocarbons (PAHs). They reported relatively higher degradation of four PAHs namely ANT (92–96%), FLU (83–86%), PHE (16–54%), and PYR (51–71%) at 50 °C by *Aeribacillus* sp. (UCPS2) and mixed cultures. Biosurfactants bind with POPs as soluble

**Table 11.1** Phytoremediation of POPs and HMs via synergistic interaction between plant and microbes

| S. No. | Plants | Microbe(s) | Mechanisms | Results | Targeted HMs and POPs | Source of sample | Research area | Study |
|---|---|---|---|---|---|---|---|---|
| 1 | *Silene vulgaris* | *Proteus vulgaris* H7, *Pseudomonas* sp. H15, *Pseudomonas helmanticensis* H16 | Phytoextraction with PGPB | *P. helmanticensis* H16 enhanced highest Zn and Cd accumulation in shoot by 43.8% and 112%, respectively | Cd, Zn | Soil | Katowice, Poland | Płociniczak et al. (2019) |
| 2 | *Glycine max* | *Mucor circinelloides* | Combined plant, microbe and through amendment material assisted phytoremediation | Bioavailability decreased by 4.7–20.50%, 4.3–11.9%, 21.9–183.9%, 13.6–27.3%, and 1–16.4% for Cu, Zn, Pb, Cd, and Mn, respectively | Cu, Zn, Pb, Cd, Mn | Mine tailings | Liaoning province, eastern China | Li et al. (2019a) |
| 3 | *Saxifraga stellaris* var. *alpigena* | *Mortierella* sp., *Stenotrophomonas maltophilia* | Endophytic fungus and bacterium influenced Cu tolerance of *S. stellaris* | Growth and survival rate enhanced by 70% and 130%, respectively, after microbial inoculation | Cu | Copper mine wastes | Vienna, Austria | Ryszka et al. (2019) |
| 4 | *Lactuca sativa* | *Rhodotorula mucilaginosa* CAM4 | Multiple PGP properties assisted phytoremediation | Removal efficiency up to 80–88% | Al | Culture medium | La Araucania Region, Southern Chile | Silambarasan et al. (2019) |
| 5 | *Sedum plumbizincicola* | *Rhodococcus* sp. NSX2 | Bacterial assisted phytoremediation | NSX2 promoted Cd accumulation in plant | Cd | Metal-contaminated soil | Guangdong Province, South China | Hou et al. (2019) |
| 6 | *Linum usitatissium* | *Serratia* sp. CP-13 | PGPB assisted phytoremediation | CP-13 inhibited the Cd uptake and sequestered it in rhizosphere zone and reduced lipid peroxidation and maintained the optimum level of nutrients in plant | Cd | Wastewater | Faisalabad, Pakistan | Shahid et al. (2019) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | *Brassica mapus* | *Cupriavidus necator* GX_ 5, *Sphingomonas* sp. GX _ 15 and *Curtobacterium* sp. GX_ 31 | PGPR associated phytoremediation | Increased dry weight of above ground tissues and root biomass by 40.90–80.55% and 1.099–103.13%, respectively, Cd uptake in aerial parts and roots by 7.38–11.98% and 48.09–79.73%, respectively | Cd | Pot experiment | Guangxi, China | Li et al. (2019b) |
| 8 | *Solanum torvum* and *Cymbopogon citratus* | *Bacillus cereus* T1B3 | PGPB assisted phytoremediation | Increased biomass, photosynthetic pigment content and act as Phytostabilizer of HMs in roots | Cr (VI), Fe, Mn, Cu, Cd, Ni, and Zn | Mine tailings contaminated soil | Bhubaneshwar, India | Nayak et al. (2019) |
| 9 | *Acacia mangium* and *Eucalyptus camaldulensis* | *Pseudomonas psychrophila* | Endophytic bacterial promoted phytoremediation | Increased Pb concentration in *A. mangium* root approximately 9.5% but not in *E. camalduensis* | Pb | Hydroponic | Bangkok, Thailand | Yongpisanphop et al. (2019) |
| 10 | *Lens culinaris* | *Bacillus* sp. KUJM2 | PGP assisted bioremediation | Bacterial inoculation lead to higher shoot length (7.1–27.61%), shoot dry weight (18.22–36.3%), seed production (19.23–29.17%), and uptake was reduced in shoot by 67.02–79.85% and 65.94–78.08% and in seed up to 72.82–86.62 and 68.68–85.94%, respectively, under single and combined PTEs | As, Cd, Cu, Ni | PTEs (potentially toxic elements) contaminated state | West Bengal, India | Mondal et al. (2019) |
| 11 | *Glycine max* | *Kocuria rhizophila* | PGPB and citric acid (CA) assisted phytoextraction | Co-application of *K. rhizophila and* CA enhanced plant biomass about 38.73% and accumulation of Cd, Cr, Cu, and Ni about 40.63%, 56.39%,59.1%, and 39.76%, respectively | Cd, Cr, Cu, and Ni | Multi-metal-contaminated soil | Islamabad, Pakistan | Hussain et al. (2019) |

(continued)

**Table 11.1** (continued)

| S. No. | Plants | Microbe(s) | Mechanisms | Results | Targeted HMs and POPs | Source of sample | Research area | Study |
|---|---|---|---|---|---|---|---|---|
| 12 | *Helianthus annus* | *Pseudomonas libanensis* TR1 and *Claroideoglomus claroideum* BEG210 | Salt resistant plant beneficial Bacteria (PBB) and Arbuscular mycorrhizal fungus (AMF) assisted phytoremediation | Enhanced plant growth changed physiological status as well as Ni and $Na^+$ accumulation potential of *H. annus* | Ni | Metal-polluted saline soils | Coimbra, Portugal | Ma et al. (2019) |
| 13 | *Medicago sativa, Festuca arundinacea, Lolium perenne* | *Pseudomonas putida* UW4 | PGPB enhanced phytoremediation (PEP) | UW4 significantly enhanced dissipation of phenanthrene and fluoranthene, and plant biomass dissipation depended on PGPB inoculum biomass, plant species, plant microbe specificity, and type of contaminants | PAH, Pb | PAH and HMs spiked soils | Birmingham, UK | Afegbua and Batty (2019) |
| 14 | *Helianthus annus* | *Paenibacillus* sp. IITOSM08, *Bacillus* sp. PRB77 and *Bacillus* sp. PRB101 | PGPB boosted phytoremediation | Plant biomass was increased and maximum degradation (92%) was found after inoculation with PRB10 | Endosulfan | Contaminated soil | Dhanbad, India | Rani et al. (2019) |
| 15 | *Arabidopsis thaliana* | *Mucor circinelloides* (MC) and *Trichoderma asperellum* (TA) | Endogenous fungi assisted phytoremediation | MC and TA strengthen *A. thaliana* tolerance to Cd and Pb with higher root length (40.19–117.50%) and shoot fresh weight (58.31–154.14%) of plant compared to non-inoculation, MC enhanced microbial diversity, and soil enzyme activity than TA | Cd and Pb | Contaminated soil | Jinan, China | Zhang et al. (2018) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 16 | *Rosmarinus officinalis* | *Glomus mosseae* and *Glomus interadices* | Bioaugmentation-assisted phytoremediation | Accumulation in root and shoot had a sequence Cu > Zn > Mn > Cd > Pb > Fe | Cd, Pb, Fe, Zn, and Cu | Ore mine soil contaminated with HMs | Kerman, Iran | Abbaslou et al. (2018) |
| 17 | *Pennisetum purpureum* and *Lumbricus terrestris* | VITMVCJ1*Klebsiella variicola* | Bioaugmentation-assisted bioremediation | Bioaugmentation process enhanced root, shoot length, chlorophyll content of plant and biofilm forming ability of microbes, reduction in the toxic effects of Pb on earth worm and plant, Pb accumulated mainly in the root of the plant and body moss of the earthworm | Pb | Industrial contaminated site | Vellore, India | Das and Osborne (2018) |
| 18 | *Solanum nigrum* | *Enterobacter* sp. LC1, *Kocuria* sp. LC2, *Kocuria* sp. LC3, *Enterobacter* sp. LC4, *Kocuria* sp. LC5, *Enterobacter* sp. LC6, and *Kosakonia* sp. LC7 | Microbe assisted phytoremediation | Microbial consortium most effectively increase plant growth and strong upregulation of an MRP (multidrug resistance-associated protein) transporter in the root by As + endophytes which mediated As (V) to As (III) for detoxification/transportation | As | As contaminated habitats | Kolkata, India | Mukherjee et al. (2018) |
| 19 | *Leptochloa fusca* and *Brachiaria mutica* | *Enterobacter* sp. HU38, *Microbacterium arborescens* HU33, and *Pantoea stewartii* ASI11 | Endophyte-assisted phytoremediation | Augmented endophytes increased root length (24–45%), shoot height (39–64%), chlorophyll content (20–55%), and overall biomass (32–61%) of plant | Cr | Contaminated soil | Faisalabad, Pakistan | Ahsan et al. (2018) |
| 20 | *Sarcocornia fruticosa* | *Saccharomyces cerevisiae* | Bioaugmentation with autochthonous yeast assisted phytoremediation | Yeast increased phytoremediation capacity of *S. fruticosa* | Cd, Pb, Ni, Cr, and Cu | Metal-contaminated sediments | Ria Formosa lagoon, Portugal | Said et al. (2018) |
| 20 | *Vetiveria zizanioides* | *Bacillus cereus* (T1B1) | PGPB assisted phytoremediation | Removal efficiency of *V. zizanioides* withT1B3 for $Cr^{6+}$, | | HM amended medium | Bhubaneshwar, India | Nayak et al. (2018) |

(continued)

**Table 11.1** (continued)

| S. No. | Plants | Microbe(s) | Mechanisms | Results | Targeted HMs and POPs | Source of sample | Research area | Study |
|---|---|---|---|---|---|---|---|---|
| | | | | Fe, Mn, Zn, Cd, Cu and Ni is 82%, 92%, 67%, 36%, 31%, 25%, and 43%, respectively | $Cr^{6+}$, Fe, Mn, Zn, Cd, Cu, Ni | | | |
| 21 | *Catharanthus roseus* | *Burkholderia cepacian* CS8 | PGPB assisted phytoremediation | CS8 treated plant show higher root-shoot length, fresh and dry weight, chlorophyll content, reduced levels of malondialdehyde (MDA) and $H_2O_2$ under Cd stress | Cd | Cd contaminated soil | Lahore, Pakistan | Khan et al. (2018) |
| 22 | *Saccharum officinarum* | *Candida* VitJzN04 | Bioaugmentation assisted phytoremediation | Removal rates in terms of half-life period were recorded as 7.1 days, 9.8 days, 43.3 days, and 13.3 days for immobilized yeast-plant, free yeast-plant, *Saccharum* and yeast, respectively, due to biostimulation of soil by PGP activity of yeast | Lindane | Doped garden soil | Cochin, India | Salam et al. (2017) |
| 23 | *Festuca arundinacea* and *Lolium perenne* | *Pseudomonas* sp. SB | Biosurfactant-producing bacterial-assisted phytoremediation | *Pseudomonas* sp. SB + fertilizer + tall fescue, fertilizer + perennial ryegrass, fertilizer + perennial ryegrass + Pseudomonas treatment effectively helped to increase the bioavailability of DDT and increased the removal rate about 65.6% than fertilizer control rate by 40.3% | DDT | DDT- contaminated soil | Nanjing, China | Wang et al. (2017) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 24 | *Populus* sp. | *Enterobacter* sp. PDN$_3$ | Endophyte-assisted phytoremediation | Inoculated popular tree exhibited increased growth and reduced TCE toxicity effects with increase in trunk diameter, excreted 50% more chloride ion into the rhizosphere and inactivation of increased TCE metabolism than non-inoculated tree | TCE | Trichloroethylene (TCE) contaminated ground water | Washington, United States | Doty et al. (2017) |
| 25 | *Brassica napus* | *Fusarium* sp. CBRF$_{44}$, *Penicillium* sp. CBRF$_{65}$ and *Alternaria* sp. CBSF$_{68}$ | Endophytic fungi assisted phytoremediation | CBRF$_{44}$ and CBRF$_{65}$ significantly increased the rape biomass and promoted the extraction efficacy of Pb and Cd, while CBSF$_{68}$ did not show similar results as above | Cd and Pb | Multi-metal-contaminated soils | Guangdong, Peoples of China | Shi et al. (2017) |
| 26 | *Catharanthus roseus* | *Pseudomonas fluorescens* RB4 and *Bacillus subtilis* 189 | Microbe assisted phytoremediation | Co-cultivation of *P. fluorescens* RB4 and *B. subtilis* 189 exhibited 121%, 102%, and 117% higher fresh weight in Cu, Pb and both elements together also showed higher accumulation of Cu and Pb in *C. roseus* shoot | Cu and Pb | Cu and Pb contaminated soils | Lahore, Pakistan | Khan et al. (2017) |

aggregates which ultimately release the pollutant from soil particles. However, the release of surfactants in the root exudates seems more promising as it may provide easy solubilization of POPs in plant rhizosphere (Passatore et al. 2014). These studies reveal that the combined use of plants and biosurfactant-producing bacteria can improve the bioavailability of organic pollutants through biosurfactant exudation and/or production and consequently the remediation of POPs contaminated environment. Rhizo-engineering has also gained attention for the removal of POPs from the environment (Thijs et al. 2016). A research team reported that biosurfactant amended phytoremediation may be a useful biotechnological approach for the remediation of petroleum hydrocarbon polluted soil (Liao et al. 2016). Biosurfactants work by decreasing the bonding between the metal and soil particles thus increasing the metal and POPs availability (Wang et al. 2017; Lal et al. 2018).

### *11.4.3 Exopolysaccharides (EPS)*

Extracellular polymeric substance (EPS) consists mainly of polysaccharides, proteins, lipids, and nucleic acids (Rajkumar et al. 2013; Joshi and Juwarkar 2009). EPS has the ability to reduce the accessibility and bioavailability of HMs in the soil by forming metal complexes. They also provide essential micronutrient based environment to the plant thus help in indirect phytoremediation of HMs (Ma et al. 2016b; Lal et al. 2018). Wani et al. (2019) explored the role of *Bacillus subtilis* $PAW_3$ in the reduction of Cr (VI) and cowpea growth through its various PGP substances such as EPS along with ACC deaminase, siderophore, etc. that reduced Cr (VI) to Cr (III) and also showed the presence of chromium reductase gene (ChR) in $PAW_3$.

## 11.5 Conclusion

A consequence of anthropogenic activities is observed as an increase of contaminated areas, which should be remediated to prevent or mitigate the transfer of contaminants into terrestrial, atmospheric, or aquatic environments. Point and diffuse contamination by organic and inorganic pollutants cause wide concerns, and intentional or accidental introduction of these substances in the environment may represent serious impacts on public health. Therefore, urgent action is needed to address the world about elimination, remediation, cleanup, and safe disposal of such chemicals/ contaminants. The use of transgenic plants and biotechnological potential of microorganisms to resist and/or remove metals directly from polluted media and their beneficial effects on plant growth may lead to environmental-friendly and cost-effective strategies toward the reclamation of polluted soils.

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# Chapter 12

# Involvement of Synergistic Interactions Between Plant and Rhizospheric Microbes for the Removal of Toxic/Hazardous Contaminants

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**Abstract** The abundance of toxic heavy metals, organic and inorganic pollutants, xenobiotic chemicals and contaminants pose a major threat to the environment which is linked to the health of the living systems including human beings and needs an eco-friendly remediation technology. This technology may not only reduce the pollution level by degradation or detoxification of such hazardous pollutants but also does not generate any secondary or intermediate pollutants. Phytoremediation is an advanced and efficient plant-based treatment technology that takes advantage of the remarkable ability of the plants to tolerate and grow at higher concentration of pollutants/contaminants. The mechanisms used by the plants for the removal of toxic components from the surroundings include accumulation, absorption or transformation of the toxic compounds in their tissues, leaf, stem and roots. In addition to this, plant-associated microbes also play an important role to enhance the efficiency of

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phytoremediation process. In recent years, understanding the knowledge of the synergistic relationship between plants and microbes for the removal of unwanted chemicals becomes an interesting area of research. Understanding the physiological and molecular mechanisms of plant–microbe interaction helps the environmentalists to get an insight of this technology and to improve and expand new horizons of phytoremediation.

## 12.1 Introduction

Widespread pollution of aquatic and terrestrial ecosystems with organic and inorganic pollutants becomes a major problem for the entire earth. These pollutants not only affect living beings but also disturb the ecological balance, which results in ozone depletion and problems of global warming. The number of toxic and restricted pollutants, chemicals and metals are used regularly in industrial and agricultural practices. For instance, long-term applications of pesticides, herbicides, chlorinated compounds, urea, phosphates, nitrates and chemical fertilizers for crop production and pest control are the principal sources of soil contamination. Wastewaters from textiles, tanneries, distilleries, electroplating and printing industries, pharmaceutical, food, paper and metallurgical industries contain tons of hazardous toxic pollutants, endocrine disrupting chemicals, phthalates, recalcitrant organic pollutants (ROPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated bis-phenyls (PCBs) hydrocarbons, absorbable organic halides (AOX), radioactive elements, organic solvents and corrosive infectious metals like cadmium, lead, chromium, arsenic and mercury (Dixit et al. 2015; Bharagava and Mishra 2018; Mishra et al. 2019).

These pollutants are highly dangerous, persistent, carcinogenic and mostly non-biodegradable in nature due to their chemical composition and toxicity. They get accumulated in aquatic and terrestrial ecosystems and cause severe soil and water pollution by deteriorating their natural and physicochemical qualities (Mishra and Bharagava 2016). Gradual increase in soil and water pollution leads to severe health hazards, poor soil fertility, unsafe food and water quality. Therefore, cleaning of the contaminated environment is a subject of utmost concern to human beings and needs an effective and advanced remedial technology that can treat all kinds of pollutants and chemicals in an effective and eco-friendly manner (Lee 2013). However, microbial degradation is an effective approach, many of these pollutants can be metabolized by bacterial, fugal and algal species but this process is very slow and inefficient because of the relatively low number of degradative microorganisms and after some time, the degradative ability decreases due to their stationary phase and tolerance capacity (Glick 2003). Besides, microbial remediation, “phytoremediation” could be the most promising and potential approach for the speedy degradation and mitigation of contaminated sites.

Phytoremediation is a relatively new concept for the removal of unwanted contaminants from the environment. It may be considered as advanced, solar-driven, environment friendly, cost-effective and economically feasible technology for the betterment of our environment (Glick 2012; Lee 2013). Phytoremediation, a plant-based technology directly utilizes green plants in situ or ex situ for the removal or transformation of toxic environmental pollutants into less toxic state (Ma et al. 2016). Generally, phytoremediation is carried out by using hyperaccumulators plants (Glick 2010; Lotfy and Mostafa 2014). Hyperaccumulators plants are capable to absorb high concentration of pollutants through roots. The root region of these plants is highly rich in microbial diversity including endophytes, mycorrhiza and plant growth promoting rhizospheric bacteria (PGPR). These root microorganisms are very helpful in phytoremediation process as they enhance plant growth and tolerance level for toxic chemicals and uptake of nutrients and supplements (Bhaduri et al. 2015).

Thus, the interaction between the plants and their root microorganism is a very interesting and extensive topic for the current research trend. Hence, this chapter describes about phytoremediation technology, which deals with not only plants but also root microorganism, plant–microbe interaction and their role in decontamination or degradation of environmental pollutants.

## 12.2 Classification of Environmental Pollutants

Based on the chemical nature, biodegradability and toxicity, environmental pollutants can be classified into two major classes: biodegradable and non-biodegradable (Table 12.1).

### *12.2.1 Biodegradable Pollutants*

Those pollutants, which are converted, degraded or transformed from their highly toxic state to non-toxic or less toxic states by microbial, plants or natural processing, are called biodegradable pollutants. These pollutants mostly originated from plants, animals or other organic sources and can be commonly found in municipal solid wastes like food waste, paper waste, manure, slaughterhouse waste, etc. Biodegradable pollutants can be of two types based on their degradability.

a. *Degradable non-persistent pollutants*: These pollutants are rapidly decomposed by natural processes.
b. *Degradable persistent pollutants:* These pollutants are degraded by natural processes, but their rate of decomposition is very slow and they persist in the environment for long time. Examples of this class are: pesticides, hydrocarbons, biodegradable plastics, etc.

**Table 12.1** Comparison between biodegradable and non-biodegradable waste

| S. No. | Properties | Biodegradable pollutants | Non-biodegradable pollutants |
|---|---|---|---|
| 1. | Origin | These pollutants are mostly of plant, animal or organic origin | These pollutants are mostly of inorganic origin |
| 2. | Nature | These are degraded and decomposed through microbial or natural processing | Non-degradable pollutants cannot be degraded by microbial actions |
| 3. | Persistence | They can be or cannot be persisted for a long time into the environment | They are persisted for a long time into the environment |
| 4. | Accumulation | Cannot be accumulated and if accumulated can be used up in short time as being a part of bio-geochemical cycle and give back rapid turnover | Rapidly accumulated into the environment, highly toxic and cannot enter into the biogeo-chemical cycle |
| 5. | Degradation rate | The degradation process is rapid | The degradation process is very slow and takes a long time to process |
| 6. | Toxicity | The toxicity is reduced by the degradation process | These are highly toxic and hazardous |
| 7. | Uses | After degradation process their degraded products are used to produce biogas, manure and organic compost | These can be recycled but the process is highly expansive and inefficient |
|  | Examples | Municipal waste, pesticides, hydrocarbons, sewage, etc. | Plastics and radio-nuclear waste |

### *12.2.2 Non-biodegradable*

The pollutants that cannot be degraded/converted or transformed by plants or microbial actions are called non-biodegradable wastes. These pollutants are highly toxic and hazardous to human health and the environment. Non-biodegradable pollutants persist in the environment for a long time and these are originated from inorganic substances.

## 12.3 Sources, Courses and Toxicity of Environmental Pollutants

### *12.3.1 Toxic Heavy Metals*

Heavy metals are highly toxic and belong to a heterogeneous group of elements having higher density ($<5$ g/cm$^3$) and atomic weight. Examples of heavy metals are arsenic (As) cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), mercury (Hg) and zinc (Zn). These are non-degradable in nature because of their complex chemical alignment and thus can persist in the air, soil and water for years (Nagajyoti et al. 2010). Industrial activities are mainly responsible for the entrance of these metals into the environment beyond their acceptable limits. Industrial processes like smelting, tanning and mining, pulping, dyeing, food processing, electroplating, chemical manufacturing release tons of metals in wastewater or in the solid waste form (Gadd 2010). Prolonged exposure of such toxic metals may result in their easy accumulation inside the living organisms which causes carcinogenic, genotoxic, mutagenic, teratogenic and neurogenic disabilities (Wuana and Okieimen 2011).

The excess concentration of Cd affects the metabolic rate of $Ca^{+2}$ ions resulting in low muscle power, bone fractures, cartilage disorders and kidney damage (ATSDR 2008). It also shows enzyme inhibiting reactions and causes nutritional deficiency in plants (Irfan et al. 2013). Lead causes infertility and memory dysfunction. It prominently pays for the severe Pb-poisoning in infants and young children, which may even lead to death by altering cell signalling, protein folding and inhibitory effects on di- and monovalent cations (Flora et al. 2007). Arsenic causes neurotoxicity, gastrointestinal toxicity and nephrotoxicity. Chromium toxicity may lead to severe health implications like nasal irritation, lung carcinoma and hearing impairment. It causes mutations and genotoxic abnormalities (IARC 1990; Tchounwou et al. 2003).

### *12.3.2 Polycyclic Aromatic Hydrocarbons (PAHs)*

The level of polycyclic aromatic hydrocarbons (PAHs), a group of predominantly recalcitrant organic compounds having two rings is found apparently increased in the environment because of several anthropogenic activities. PAHs are widely distributed in the atmosphere, volcanic eruptions and forest fire (Lee and Vu 2010). PAHs are well-known "*persistent organic pollutants*" that are continuously concentrated in the environment by incomplete combustion of fossil fuels, vehicular traffic, waste incineration and industrial processing (Zhang and Tao 2009). Ingestion and inhalation of smoked grilled vegetables and meats, tobacco smoking, polluted air and water are some common sources of PAHs exposure to humans and the environment (Lundstedt et al. 2007; Bansal and Kim 2015; Ramos and Moorthy 2005).

PAHs compounds are regarded as cancer and mutation causing agents and long-term exposure to these compounds results in potential health risks. These are acutely toxic even at very low concentrations to cause necrotic toxicity. Environmental factors such as solar radiation can considerably increase the toxicity level of some PAHS as these compounds absorb ultraviolet and visible light and generate reactive oxygen species (ROS), which can significantly harm living systems (Landrum et al. 1987; Marzooghi and DI Toro 2017).

Long-term exposure of hydrocarbons can severely affect central nervous systems in humans and animals and may cause skin, lung and kidney cancer, respiratory dysfunction, inhibits enzymatic activities and disrupts endocrine system (Singh et al. 2004; Gkorezis et al. 2016). Anthracene, benzopyrene and naphthalene are well-known skin irritant and sensitizer and cause allergic and inflammatory skin problems

(Unwin et al. 2006). Increased pollution of air, soils and water with excessive concentration of PAHs is a serious worldwide environmental problem, which drastically affects the normal functioning of human, animals and plants.

### *12.3.3 Pesticides and their Residues*

Extensive and widespread use of chemical pesticides in agricultural practices and households to kill pests, insects and weeds to protect plant crops is also contributing towards environmental pollution and contamination of soil and water ecosystems. Pesticides are highly toxic, recalcitrant and persistent organic pollutants, with low-biodegradability due to their high solubility, mobility, long-term stability and chemical complexity (Olette et al. 2008; Bolognesi and Merlo 2011). Owing to their extreme toxicity, chemical composition and mode of action, pesticides can be classified into four main classes like organochlorine, organophosphorus, carbamates and pyrethroids compounds (Barlow 1985). All these pesticides pose a dangerous health risk to target and non-target organisms and can cause endocrine disruption by interfering with the production, release and metabolism of hormones and enzymes.

Organophosphate and carbamate pesticides are reported for their neurotoxicity as they act as an inhibitor of acetylcholinesterase, which hydrolyses the neurotransmitter acetylcholine at the neuromuscular junction (Galloway and Handy 2003; Lushchaka et al. 2018), whereas organochlorines are endocrine disrupting chemicals and interfere with the functioning of molecular circuitry and cause dysfunction of the endocrine system (Sohail et al. 2004). Higher concentrations of pesticides get accumulated in human and animal bodies directly through intake of food items, dairy products, meats and vegetables and cause severe health hazards such as neuritis, psychiatric manifestations, immunological disorders resulting in genotoxic and carcinogenic diseases (Ascarrunz et al. 2006; Rusiecki et al. 2008). The excess use of pesticides in agricultural fields for high crop productivity has also led to increase the problem of soil, air and water pollution. It drastically affects the biological and physical properties of these environmental compartments, which adversely affect the life span of aquatic and terrestrial flora and fauna (Jayaraj et al. 2016).

## 12.4 Phytoremediation: A Synergistic Interaction Between Plant and Microbes

The use of plants for the treatment of contaminated soil and water has given a new way to the thought of clean, green, sustainable and safe environment. Phytoremediation has gained increasing attention since the last decade, as an emerging cheaper, environmentally friendly, aesthetically pleasing and cost-effective

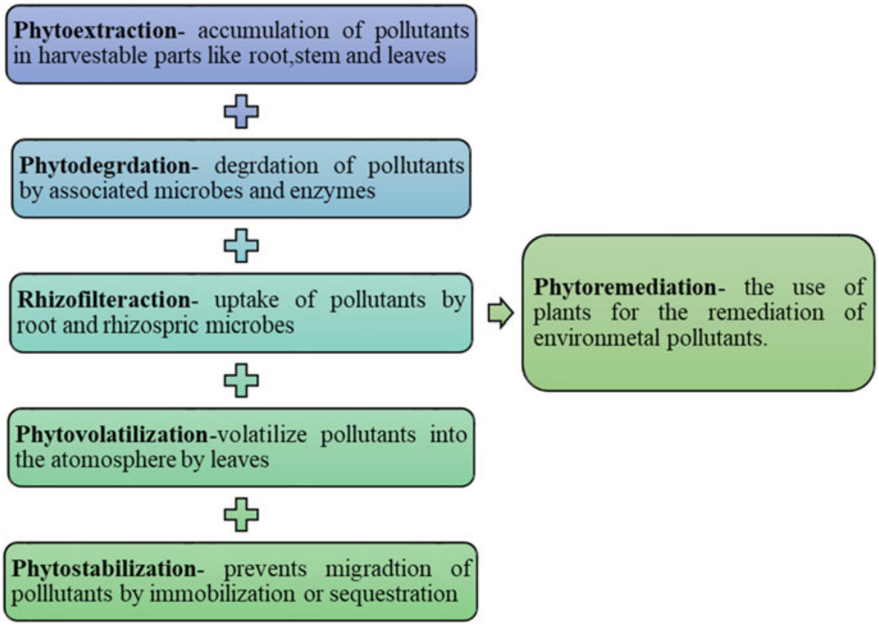

**Fig. 12.1** Different forms of phytoremediation for the remediation of environmental pollutants

technology (Shehzadi et al. 2015). It is a good alternative option and can be applied to clean organic and inorganic pollutants in air, soil and water systems. Phytoremediation is a very broad application, which may be sub-divided into five branches depending upon the nature of pollutants and treatment methodologies. Major branches of phytoremediation are phytoextraction, phytodegradation, rhizofiltration phytostabilization and phytovolatilization (Salt et al. 1998; Glick 2003, 2010) (Fig. 12.1).

a. *Phytoextraction*: The use of pollutant-accumulating plants to remove metals or organics from the soil by concentrating them in some harvestable parts. This technique represents one of the largest economical opportunities for the remediation of metal contaminated and radionuclides polluted sites (Lee 2013).
b. *Phytodegradation*: The use of plants and associated microorganisms to degrade organic pollutants like PCBs, PAHs, pesticides, etc. Plants and their associated microbes produce various enzymes like catalase, peroxidase, laccase, reductase which help in the uptake and degradation of the pollutants. This technique is highly suitable for the degradation of moderately hydrophobic organic chemicals (Morikawa and Erkin 2003).
c. *Rhizofiltration*: In this techniques plant roots are involved in the absorption and adsorption of toxic pollutants, mainly metals from water and soil ecosystems. In this technique rhizospheric microorganism like PGPRs, endophytes and mycorrhizal fungi play a vital role in the removal of pollutants.

d. *Phytostabilization*: The use of plants to reduce the bioavailability of pollutants, by preventing their leaching and spread into water, soil and air. This process cannot degrade pollutants, but it can only reduce the mobility of pollutants by immobilization and sequestration.
e. *Phytovolatilization*: This technique uses plants to volatilize pollutants and mostly used to remove pollutants from air. Toxic heavy metals like As and Hg can be bio-methylated to volatile compounds, which can then be lost to the atmosphere by leaf surfaces of plant (Raskin et al. 1997).

Plants have the ability to accumulate substantial concentrations of toxic pollutants through their above (leaf and stem) and below (roots) plant parts. After accumulation, pollutants are degraded, metabolized, sequestrated or excreted into non-toxic substrate. In this process of pollutant degradation, plant-associated microorganisms play an important and viable role by promoting plant growth and detoxification mechanisms (Weyens et al. 2015). The root region of any plant remains in direct contact with soil microbes and the rhizospheric region. This includes an area around the root region which is abundant with microbial community and activity. Relationship between a healthy plant and microbes depends on various important factors including temperature, pH, microbial growth, tolerance ability of plant, repression or induction of catabolic genes and enzymes, nature of pollutant, potentiality of the strain, etc. (Alexander 2000; Diaz Martinez et al. 2018).

The rhizospheric region of the plant contains a variety of microorganisms including plant growth promoting rhizobacteria, endophytes, mycorrhizae fungus. These microbes get their nutrition from root exudates and in reverse, they help plants through their metabolic detoxification mechanisms which convert toxic compounds that are detrimental to plant growth and life into non-toxic substrate (Adieze et al. 2012). The synergistic relationship between plants roots and their associated microorganisms has been well studied and found effective for improved and enhanced remediation of contaminated environments and pollutants (Muller and Shann 2006; Gkorezis et al. 2016; Lal et al. 2018). Mostly PGPRs endorse plant growth directly by enabling resource procurement or by diminishing the inhibitory effects of several pathogens on plant growth in the form of biocontrol agent (Glick 2012). *Pseudomonas*, *Bacillus*, *Brevibacillus* are well-known bacterial genera which promote growth and yield in different hyperaccumulator plants indirectly by acting as biocontrol agents. Biocontrol agents not only inhibit pathogens but also increase plant growth through improved recycling of nutrients and minerals such as nitrogen, phosphates and other nutrients (Ryan et al. 2008).

"*Mycorrhiza*" is the symbiotic relationship between the fungal species and roots of vascular plants. These fungi help in improving plant growth by increasing the surface area of the plant's roots which enhance nutrient and water uptake in roots from the soil. Mycorrhizal fungi can protect plants from abiotic (e.g., increased heavy metal concentrations) and biotic (e.g., soil-borne pathogens) stress through the enhanced nutrient supply (Kohler et al. 2007; Chibuike 2013).

## 12.5 Mechanisms Involved in Plant and Microbe Interaction

Soil-borne microorganisms like bacteria, fungi, algae, yeast and cyanobacteria show different functional activities and interactions with the rhizospheric region of the plants. Understanding the mechanisms between plants and microbes is a crucial topic of scientific research in reference to agriculture and environmental sustainability. The plants and microbes interact with each other for their existence into the stressful environment via competition or coexistence to reduce toxic pressure and adaptations through active secretions of substrates that are known to vary between plant species. Plant root exudates are a useful source of energy and nutrients for microbes (Ma et al. 2016). Microorganisms are benefitted from plant root exudates and can thus alleviate phytotoxicity of plants and help to stimulate their growth against higher concentration of toxic pollutants by direct or indirect mechanisms through the induction of defence enzymes such as catalase, SOD, dehydrogenase, urease, etc. (Bhaduri et al. 2015).

Microbes can also stimulate plant growth by solubilization of essential nutrients in soil like nitrogen, phosphate, potassium by secreting siderophores, plant growth regulating hormones and fixation of nitrogen. Microbes can also evolve various resistance mechanisms for the degradation of pollutants such as detoxification, redox reaction, acidification, chelation, precipitation. Siderophore production by bacterial cells enhances plant growth by increasing protein content and chlorophyll content and decreases the heavy metal accumulation (Burd et al. 2000). Rhizospheric bacteria enhance the ability to take up iron by plant roots from the complex ligand siderophore compounds, produced by them to increase the growth and development of plants and to remediate metal contaminated environment (Rashmi et al. 2013). Plant–microbe interaction plays an important role in the exploration and enhancement of phytoremediation (Mishra et al. 2019).

Plant–microbe partnership needs more attention and advanced molecular studies to treat contaminated sites, as microbes provide resistance ability, metabolic degradation pathways for decreasing phytotoxicity and improving phytoremediation effectiveness. Microbes play a vital role in the biogeochemical cycle of minerals/ nutrients and energy flow. The potentiality of biological nitrogen fixation as a low cost source of nitrogen and phosphorus solubilization by phosphate solubilizing bacteria and mycorrhizal fungi is very well studied and reported earlier (Bhaduri et al. 2015; Ozyigit and Dogan 2015).

## 12.6 Applications of Phytoremediation

### *12.6.1 In Metal Removal*

Increasing contamination of toxic heavy metal pollution is becoming a global challenge for the entire environment and living beings. Heavy metals are highly toxic, hazardous and persistent in nature due to their chemical reactivity and bioavailability (Lotfy and Mostafa 2014). Therefore, these can easily accumulate in soil and water ecosystems and can persist in these matrices for many years and affect the physicochemical and biological properties of soil and water. These metals can also accumulate inside the living bodies and cause severe respiratory, developmental, mutagenic and carcinogenic related problems. Excessive deposition of toxic metals has an inhibitory action on enzymatic and metabolic activities, which sometimes leads to death also.

According to previous studies various hyperaccumulator plant species like *Allium schoenoprasum* (Goland 2006), *Brassica juncea* (Singh and Fulekar 2012), *Brassica napus* (Sheng and Xia 2006), *Cucumis sativus* (Takeda et al. 2006), *Cicer arietinum* (Garg et al. 2014), *Zea maize* (Abhilash et al. 2016), etc. are reported for the uptake of higher concentration of Ni, Co, Ca, Cd, Zn, Pd, As and Cr toxic metals from soil (Lee 2013). These plants can accumulate >100 mg/kg Cd, 1000 mg/kg Ni, Cu and Pb and ~10,000 mg/kg Zn and Mn in their leaves and stems under metal contaminated soils (Baker and Brooks 1989). Aquatic plants species like *Eichhornia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce) are efficient accumulator of toxic metals and capable to transform into their less toxic form (Saleh 2012; Das et al. 2014).

### *12.6.2 In Hydrocarbon Degradation*

Hydrocarbons are organic, xenobiotic chemical compounds, whose excess concentration in the environment poses a serious matter of concern due to their ominously detrimental and deleterious impact on human, plants and on animals as well. Phytoremediation of hydrocarbons offers a safe and eco-friendly remediation process to degrade organic pollutants. The mutualistic interaction between plant roots and their associated microbes is more potent to endorse effective and efficient degradation of persistent organic pollutants (Asemoloye et al. 2017). PAHs, PCBs, 2, 4, 6-TNT and other organic pollutants can be degraded into stable ($CO_2$ and $H_2O$) and non-toxic intermediates by phytoremediation. Plants and their associated microbes produce various degradative enzymes that are capable in the complete mineralization of such pollutants.

Various plant species like *Helianthus annuus*, *Brassica juncea*, *Festuca arundinacea and Lolium perenne* are reported for the remediation of organic contaminants. These can degrade organic substances by means of releasing root exudates that promote microbial activity, certain degradative enzymatic activity in the vicinity for the potential biotransformation of organic compounds (Siciliano et al. 1998). Significant phytoremediation study of phenanthrene and pyrene was reported by Cheema et al. (2009) in acidic and spiked soil by single and combined plant cultivation system using *Zea maize*, *Medico sativa*, *Brassica napus.* These plants significantly enhance the adsorption of PAHs from polluted soils. *Cannabis sativa* (Campbell et al. 2002), *Oryza sativa* (Du et al. 2011) are also reported to have the potentiality for the reduction and degradation of benzopyrene and chrysene.

Meanwhile, application of grasses for the purpose of phytoremediation of organic pollutants has also been a promising approach to enhance this process. *Schizachyrium scoparium* (little bluestem grass) is capable to reduce the efficient amount of PAHs in combination with *P. virgatum*. *Lolium multiflorum* and *Cynodon dactylon* have been reported for the proficient degradation of naphthalenes (White et al. 2006).

### *12.6.3 In Pesticide Degradation*

Phytoremediation is the most efficient technique to prevent the migration of pesticide and their hazardous residues into the soil and water ecosystems via leaching and surface runoff. Aquatic plants have extraordinary potential for the degradation of pesticides due to their great functionality as biosinks and biofilters of the contaminants (Gao et al. 2000). Tondon et al. (2016) reported the degradation of N-methyl carbamate pesticide (carbofuran) degradation by three aquatic plants like *Chrysopogon zizanioides*, *Acorus gramineus* and *Scirpus cyperinus* at different concentrations and found that *Acorus gramineus* was the most efficient and potent plant for the phytoremediation. *Cyperus rotundus* and *Scirpus schoenoplectus* were found as a good candidate for the treatment of water, contaminated with highly toxic pesticide monocrotophos by Tondon et al. (2017).

Aquatic plants having a very rich root system and microbial diversity help in the absorption or uptake of pollutant and their residues. *Lemna minor*, *Elodea canadensis*, *Cabomba aquatic* and *Eichhornia crassipes* are free-floating, widespread aquatic plants that are useful in the phytoremediation of pesticides (Olette et al. 2008).

## 12.7 Economic Importance of Phytoremediation

Phytoremediation is not only an environmentally friendly technology but also a very efficient, feasible cost-effective technology from economical point of view because of its easy availability and less expansive methodology as plants can be easily grown without much effort and can be monitored easily. The use of plants and their associated microbes for the mitigation and reclamation of contaminated sites is a viable approach in cleaning and restoring our natural environment in a more healthy and green way (Sumiahadi and Acar 2018). Phytoremediation is not only beneficial for the environment but also for our society, farmers and agriculture. Phytoremediation contributes to biodiversity improvement, soil restoration, carbon sequestration, protection of environment stability and in reducing hazardous chemical and pollutants (Adams et al. 2013). Hyperaccumulator plants are highly functional and valuable in phytoremediation technique because of their potentiality to tolerate and grow at higher concentration of environmental pollutants. They are capable of accumulation and degradation of toxic pollutants into non-toxic or less toxic substance.

## 12.8 Future Perspectives

Phytoremediation is an undoubtedly tremendous approach for the remediation of contaminated environments, but the success of this technology depends upon its proper implementation at different environmental parameters with suitable and potent plant species that survive and tolerate such harsh stress conditions to a variety of contaminants. Selection of most efficient plant species to degrade a specific pollutant is one of the basic and most important concepts of this technology (Dhir 2013). It is a good treatment strategy for the degradation of a variety of toxic environmental pollutants but more advanced and molecular mechanistic information are required to understand the relationships between plants and microbes for accurate expected outcomes of this technology. Use of omics techniques like metagenomics, meta-transcriptomic, proteomic and metabolomics can provide deeper knowledge and understanding of the synergistic relationship between plant–microbe, microbe–metal and microbe–microbe (Bell et al. 2014).

Applications of GMOs along with greenhouse experiments in agronomic soil conditions ensure a better knowledge of plant biology and their capability to enhance phytoremediation at large scale projects. Furthermore, monitoring vegetation of native plants of contaminated sites provides a good summary about the environmental conditions, survival and management strategies under stress environment for better implementation of phytoremediating plants (Dushenkov 2003; Asemoloye et al. 2019).

## 12.9 Conclusion

The exploitation of phytoremediation technology for the mitigation and removal of toxic and hazardous pollutants from the environment is a very pragmatic and eco-friendly approach. It is a highly valuable and much easier technology for that does not require any expensive or huge equipment. Common peoples and farmers are easily connected to this technology, as it is more convenient than conventional physicochemical treatment approaches. Although plants are capable of removal or degradation of toxic pollutants but in this degradation process, soil-borne microorganism also plays a very important role in their growth promotion under stressful environment. Microbial associations can promote plant growth by siderophore formation, chelation, phosphate solubilization, acidification and redox reactions, which affects the bioavailability and solubility of pollutants. Therefore, the plant–microbe interaction is a very interesting and imperative relationship, which needs more attention and research in respect to understanding the deeper knowledge of their genetic and molecular level mechanism for such mutualistic relation in the remediation of contaminated environments and betterment of phytoremediation in the near future in a more sustainable way.

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# Chapter 13
# Understanding the Role of Microbes and Plants in the Management of Heavy Metal Stress: A Current Perspective

**Saima Saif, Almas Zaidi, and Mohd. Saghir Khan**

**Abstract** Significant amount of heavy metals is regularly added to the soils globally due to various natural and anthropogenic activities. The heavy metal uptake and accumulation in crops lead to yield losses. A common consequence of heavy metal toxicity to plants is an excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG). Both molecules may cause peroxidation of lipids, oxidation of protein, DNA damage, inactivation of enzymes, and/or affect other vital plant constituents. Higher plants have evolved a balanced antioxidant defence system and a glyoxalase system to scavenge ROS and MG. Besides plants, microbes can also be used to remove heavy metals from polluted soils. This chapter highlights the suitability of various strategies adopted by useful soil microbiota and plants to eradicate heavy metal toxicity and consequently to enhance crop production in metal stressed soils.

## 13.1 Introduction

### *13.1.1 Heavy Metal Pollution and Its Agro-Ecological Impact: An Overview*

Heavy metal pollution has emerged as a global challenge in developed and developing countries, which limits the economic growth and causes human health problems via food chain (Tchounwou et al. 2012; Khalid et al. 2017). Classically, heavy metals which are biologically and industrially important refer to a group of toxic elements having densities greater than 5 g $cm^{-3}$. Recently the term has also been used for metals and semimetals with potential human or environmental toxicity (Saunders et al. 2013; Mohmand et al. 2015). In soils, metals may be added through transport of continental dusts, emissions from volcanoes, and by weathering of metal-enriched rocks (Algreen et al. 2012). Natural inputs are unlikely to add

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considerably higher amount of HM to soils but due to rapid industrialization, there has been massive addition of heavy metals emanating from anthropogenic activities which has resulted in a greater public concern (Shaheen et al. 2017). Arsenic, lead, cadmium, and mercury are some of the notable soil heavy metals. Despite metal pollution arising from sewage application, sewage has primarily served as an important supplementary source to combat agricultural irrigation crisis in areas deficient in good quality waters (Li et al. 2017). Irrigation by wastewater in agriculture practices is common in many countries including China, India, Pakistan, Mexico, and Iran. The use of wastewater for irrigation has been found economical and to a certain extent has also solved the problem of effluent disposal (Navarro et al. 2015). Recent findings suggest that 35.9 Mha of irrigated crop lands are located in the vicinity of wastewater treatment plants and out of 82% of the agricultural fields, 75% are irrigated by wastewater (Thebo et al. 2017). Despite these benefits, use of wastewater for irrigation can pose substantial risks to the plants (Marrugo-Negrete et al. 2017). Although soil has some ability to clear and degrade pollutants via microbial metabolism and transformation but it is not enough to control heavy metal pollutants that accumulate in groundwater or soil solution due to continuous release of untreated pollutants or changes in soil pH. Hence, build-up of heavy metals in soils can restrict soil functioning (fertility) and result in toxicity to plants and physiological activities of microbes which in turn affect the quality and safety of foods severely and cause eventually human health problems (Meng et al. 2016).

### *13.1.2 Metal Toxicity to Human Health*

The non-biodegradability and lethal effect of heavy metals is a serious problem for human health worldwide (Azimi et al. 2017). Though metals like Zn, Cu, Ni, Co and Cr at small quantities play a pivotal role in metabolic and physiological processes of plants (Singh et al. 2016), humans (Yamada 2013), and microorganisms (Boer et al. 2014). For instance, they affect redox processes, regulate osmotic pressure, and stabilize molecules through electrostatic interactions and act as cofactors for numerous enzymes and electron transport systems (Emamverdian et al. 2015). In contrast, there are non-essential heavy metals like Ag, As, Cd, Pb, and Hg which do not have any biological importance to living organisms and are very toxic even at very low concentrations. Heavy metals usually enter the human body via different food chains, inhalation, and ingestion. Once inside the human body, they stimulate the immune system and may cause nausea, anorexia, vomiting, gastrointestinal abnormalities, and dermatitis (Megido et al. 2017). Metals like Cr, As, Zn, Ur, Se, Au, and Ni may also adversely affect the quality of soil, crop production as well as public health (Venkanna and Karthikeyan 2017). These pollutants in general are major cause of life-threatening degenerative diseases such as Alzheimer's disease, atherosclerosis, cancer, Parkinson's disease, etc. Due to the detrimental effects of heavy metals, there is urgent need to find strategies to effectively eradicate HM from the environment and stabilize the ecosystem.

## 13.1.3 *Metal–Microbe–Plant Interactions: A General Perspective*

### 13.1.3.1 Heavy Metals and Rhizospheric Microflora

Soil microorganisms play a key role in maintaining soil fertility through various activities such as organic matter disintegration, adsorption, and pH balance and hence optimize crop production (Shahbaz et al. 2017). However, soil microbiota are greatly influenced when exposed to stress factors like heavy metals (Gutierrez-Ginés et al. 2014), high pH (Wu et al. 2017), salinity (He et al. 2017), extreme temperature (Akkermans et al. 2017), and chemical pollution (Gianfreda and Rao 2017). Among microbial communities, the bacterial communities in general have been reported to be the most severely affected by high HM concentration as compared to fungal population (Rajapaksha et al. 2004). The beneficial or detrimental effect of HMs onto microbial cells however depends on concentration, speciation, and duration of exposure of metals.

### 13.1.3.2 Impact of HM on Soil Microbial Composition and Function

Soil microbial community structure serves as an important marker for polluted soil since long-term exposure to pollutants changes the microbial compositions and functions. On the contrary, the long-term exposure to metals may also help microorganisms to adapt to the stressed environment (Kaci et al. 2016). However, various in-depth analysis have shown that metal pollution had significant impact on bacterial community structure causing changes in the relative abundance of specific bacterial taxa, but not bacterial taxon richness and community composition revealing their resilient nature in the ecosystem. Therefore, various metagenomic studies have focused on identifying the microbial communities and their relationship to the changing soil properties (Azarbad et al. 2015). In a study, Zhang et al. (2017) using 16S rRNA gene sequencing observed a general reorganization of soil microbial communities persistently exposed to metals specifically Cd and Pb in rhizosphere. On the other hand, Azarbad et al. (2015) assessed functional and potential microbial diversity (using Geo Chip 4.2) along two different gradients of metal polluted sites in Southern Poland. It was found that metal pollution caused negative impact on the relative abundance of specific bacterial genera including *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes, Firmicutes,* and *Proteobacteria*. Also, there were significant correlations between a group of metal-resistance genes and among the bacterial genera with metal concentrations in soil. Due to prolonged exposure to high metal concentrations, majority of the microflora that were found sensitive and unable to tolerate high doses of metals became extinct, while certain community members with different functional roles such as denitrification and metal resistance adapted and survived to form the basis for the emergence of other new community. Another important marker that indicates normal soil functionality is the soil microbial biomass that shows changes in soil properties due to complex environmental modifications (Hornick and Buschmann 2018; Rai et al. 2018). Yuan et al. (2015) reported a negative correlation between microbial viability and extended exposure to Pb. Similarly, Yao et al. (2017) studied the biological attributes of heavy metal-contaminated soils and established relationships between environmental variables and community composition. The microbes that were already tolerant became more competitive and thus were more in number. In a similar study, reduction in bacterial community, fungi, and actinobacteria in a heavy metal poisoned site located in one of the municipality of Brazil under the influence of a Votorantim Metal Company was reported by Dos Santos et al. 2016. Apart from composition and density, HMs have also been found to negatively affect the soil enzymes activity like cellulase, alkaline phosphatase, invertase, arylsulfatase, dehydrogenase, β-glucosidase, etc. (Burges et al. 2015).

#### 13.1.3.3 Heavy Metal Toxicity to Physiological Processes of Microbes: A Brief Account

In general, high concentrations of heavy metals or metals above certain threshold level cause discrete and apparent injuries to microbial cells due to oxidative stress, protein dysfunction, or membrane damage (Olaniran et al. 2013). Essentially, metal ions have variable targets within microbial cells and hence affect various microbial activities (Table 13.1).

#### 13.1.3.4 HMs-Induced Phytotoxicity and Physicochemical Changes in Plants

Although plants possess several strategies to offset metal toxicity but beyond certain limits such mechanisms often become ineffective and hence the survival of plant is compromised (Clemens and Ma 2016) (Table 13.2). The toxicity of heavy metals to plants varies with plant genotypes, metals species and its concentration, and soil characteristics (Topcuoğlu 2016).

## 13.2 Plant Defence Mechanisms Against Heavy Metal

### *13.2.1 Antioxidant Defence System*

Plants have developed a number of strategies to overcome the adverse impacts imposed by heavy metals. Heavy metal toxicity also leads to the over production of ROS, and in turn causes peroxidation of many vital cell constituents. In this way, plants have an efficient defence system comprising of a set of enzymatic and non-enzymatic antioxidants.

**Table 13.1** Effect of heavy metals on microbial growth and activities

| Metal | Effects on microorganisms | Reference |
|---|---|---|
| Cadmium | Damage nucleic acid, denature protein, inhibit cell division and transcription, inhibit C and N mineralization, oxidative damage | Thomas and Benov (2018) |
| Chromium | Elongation of lag phase, inhibit growth and oxygen uptake, damage DNA | Thorgersen et al. (2017), Fathima and Rao (2018) |
| Copper | Disrupt cellular function, inhibit growth and enzyme activities, oxidative stress | Warnes and Keevil (2011), Saphier et al. (2018), Águila-Clares et al. (2018) |
| Mercury | Decrease population size, denature protein, disrupt cell membrane, inhibit enzyme function | Mahbub et al. (2017), LaVoie and Summers (2018) |
| Lead | Growth inhibition, denature nucleic acid and protein, inhibit enzymes activities and transcription, membrane damage | Adam et al. (2014) |
| Nickel | Reduce lipid content, disrupt cell membrane, inhibit enzyme activities, oxidative stress | Gupta and Karthikeyan (2016) |
| Silver | Cell lysis, inhibit cell transduction and growth | Westersund (2018), Choi et al. (2018) |
| Zinc | Decrease biomass, inhibit growth, DNA damage, membrane destruction | Ishida (2018) |

**Table 13.2** Heavy metals affecting plant health

| Metal | Effects on plants | Reference |
|---|---|---|
| Chromium | Chlorosis, delayed, senescence, wilting, biochemical lesions, reduced biosynthesis germination, stunted growth, oxidative stress | Kabir (2016), Anjum et al. (2017) |
| Copper | Chlorosis, oxidative stress, retarded growth | Li et al. (2018) |
| Mercury | Affect photosynthesis and antioxidative system, enhance lipid peroxidation, induced genotoxic effect, inhibit plant growth, nutrient uptake and homeostasis, oxidative stress | Mishra et al. (2016) |
| Lead | Reduce growth, affect photosynthesis chlorosis, inhibit enzyme activities and seed germination, oxidative stress | Venkatachalam et al. (2017), Silva et al. (2017); Ahmad et al. (2018) |
| Nickel | Inhibit growth, decrease chlorophyll content, inhibit enzyme activities and growth, reduced nutrient uptake | Mir et al. (2018) |
| Cadmium | Affect growth, decrease chlorophyll content, inhibit growth, oxidative stress | Andresen and Küpper (2013), Shanying et al. (2017) |
| Zinc | Affect photosynthesis, inhibit growth rate, reduce chlorophyll content, germination rate and plant biomass | Dotaniya et al. (2018) |

A wide variety of antioxidants that include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione-s-transferase (GST) may efficiently convert superoxide radicals into $H_2O_2$ and subsequently $H_2O$ and $O_{2,}$ whereas low molecular weight non-enzymatic antioxidants like proline, ascorbic acid, and glutathione may directly detoxify ROS. These two groups of antioxidants may professionally quench a wide range of toxic oxygen derivatives and prevent the cells from oxidative stress. Depending upon their localization at different compartments of the cell, their quenching mechanism also differs and acts in an organized manner. For example, SODs are a group of metalloenzymes that convert superoxide radical (SOR, $O_2^{\bullet -}$) into hydrogen peroxide, whereas CAT, guaiacol peroxidase (GPX), and a variety of general PODs are involved in breakdown of $H_2O_2$ (Gautam et al. 2018).

### 13.2.2 Cellular Homeostasis

Proline has been considered as one of the most important osmoticum found in the cellular system exposed to metal, water and saline stress, etc. Under different stresses including HM stress, proline accumulates in cytosol and helps in several ways: (1) maintain intracellular redox homeostasis potential, (2) protects enzymes, (3) sustains 3-D structure of proteins and vital organelles including cell membrane, and (4) reduces the risk of peroxidation of lipids and proteins (Aslam et al. 2017). In addition, proline prevents the disruption of membranes by forming clusters with $H_2O$ molecules and stabilizes their structures (Slama et al. 2015). Under stress, proline provides variable benefits to plants such as (1) it chelates heavy metals in the cytoplasm (Sharma and Dietz 2006), (2) regulates the water potential which is often impaired by heavy metals (Kholodova et al. 2011), (3) maintains osmotic adjustment through cellular homeostasis and reduces metal uptake (Szabados and Savoure 2010). For instance, Hayat et al. (2013) showed that the exogenous application of proline alleviated the damaging effects of Cd in plants such as down regulation of water potential and thereby enhanced the growth and photosynthesis.

### 13.2.3 Role of Genes in Metal Uptake and Their Transportation

A number of genes are expressed differentially after the exposure to heavy metal stress which activates specific enzymes to overcome the negative impact of stress. For instance, genetically modified tobacco callus showed more resistance to methyl mercury ($CH_3Hg^+$) than the wild-type. MerB enzyme, a product of merB gene was found to dissociate $CH_3Hg^+$ to less toxic $Hg^{2+}$ which accumulates as Hg-polyP complex in tobacco cells (Nagata et al. 2010). Mostly, the detoxification/sequestration process occurs in plant vacuoles and involves several transporters, namely ABC, CDF, HMA, and NRAMP (Singh et al. 2011). Moreover in another study, over expression of AtPCS1 and CePCS genes enhanced the remediation efficiency of tobacco plants under As and Cd stress by causing increase in phytochelatins level (Wojas et al. 2008). Besides, introduction of a gene encoding moth bean D1-pyrroline-5-carboxylate synthetase (P5CS) that initiates proline synthesis in green microalga *Chlamydomonas reinhardti* assists to increase HM tolerance. Accumulation of more than 80% higher proline level than the wild-type cells caused a genetically modified microalga grow more rapidly under higher Cd concentrations (Hassinen et al. 2009). The mechanism of heavy metal detoxification in hyperaccumulators that protects themselves by the overexpression of genes of reduced glutathione (GSH), cysteine, and o-acetylserine resulting in increase in the antioxidant activities (Anjum et al. 2014). Some genes are exclusively expressed in hyper accumulator phenotypes such as HMA4 gene under heavy metal stress. Similar to this, other studies have revealed that upstream regulation of salicylic acid, *NgSAT,* etc. results in increased serine acetyltransferase activity and higher GSH level and resulted in tolerance to Ni, Co, Zn, and to a small extent Cd (Freeman et al. 2004, 2005; Freeman and Salt 2007).

## 13.3 Metal Detoxification Approaches

### *13.3.1 Physico-Chemical Remediation of Heavy Metals*

Taking into the account of the issues of metal toxicity, many techniques have been employed for the removal and/or recovery of heavy metals from polluted environments. Some established conventional procedures for heavy metal removal and/or recovery from solution include adsorption processes, chemical precipitation, electrochemical techniques, chemical oxidation or reduction reactions, ion exchange, evaporative recovery, reverse osmosis, and sludge filtration (Chen and Li 2010). However, these techniques have certain limitations as they are expensive, sometimes impractical, and not specific for metal-binding properties. Furthermore, high reagent requirement, generation of toxic waste, and unpredictable nature of metals are some of the disadvantages associated with these methods. Majority of these methods are ineffective when metal concentration in the solution is less than 100 mg/L (Ahluwalia and Goyal 2007). Separation by physical and chemical techniques is also challenging due to high solubility of heavy metal salts in solution. Thus, there is a need to develop inexpensive and suitable techniques which could be applicable under metal stressed conditions.

### *13.3.2 Biological Approaches to Combat Heavy Metal Stress*

Bioremediation is an environment friendly innovative technique for the removal and recovery of heavy metal from the polluted areas. It involves living organisms (e.g. algae, bacteria, fungi, or plants) and/or their associated activities that reduce and/or recover heavy metal pollutants into less hazardous forms. It has been employed for the removal of heavy metals from contaminated wastewaters and soils. This method is considered a viable and appealing alternative to physical and chemical techniques since it involves the use of inexpensive microorganisms and provides long-term environmental benefits (Emenike et al. 2018). These organisms help to detoxify hazardous components in the environment. The bioremediation process can function naturally or can be improved through the addition of electron acceptors, nutrients, or other factors.

#### 13.3.2.1 Microbial Resistance Strategies Towards Heavy Metals

The rhizospheric microbes play important role in HM detoxification in contaminated soils. According to Pires et al. (2017) the predominant bacterial populations in HM contaminated sites belong to Firmicutes, Proteobacteria, and Actinobacteria and the most common genera are *Bacillus*, *Pseudomonas*, and *Arthrobacter*. In order to survive under metal stress conditions, bacteria have developed certain strategies to regulate the intracellular levels of HMs. Microbial resistance to heavy metals occurs via acquisition of specific resistance systems such as efflux and uptake and extracellular precipitation. In a study, Karthik et al. (2017) isolated rhizobacterial strain AR6 from the rhizosphere of *Phaseolus vulgaris* which showed high Cr (VI) tolerance and multifarious plant growth promoting traits. Also, it was observed that the detoxification of toxic Cr (VI) occurred directly by enzymatic reduction to less toxic Cr (III) by chromate reductase or indirectly by making complexes with metabolites (Karthik et al. 2017). Different chromate reductases (e.g. ChrR, YieF, NemA, and LpDH) have been identified that are located either in cytoplasm or membrane bound in a bacterial cell (Huang et al. 2016). Similarly, other PGPR with improved metal remediation efficiency have been found to facilitate the growth of plants under adverse toxic conditions (Gopalakrishnan et al. 2018). Apart from traditional PGPR, both symbiotic and free living rhizobia have also been found capable of detoxifying HM and consequently upgrading the quality of contaminated soils (Checcucci et al. 2017; Rangel et al. 2017). Summarily, the metal removal mechanism can be grouped into five categories (1) extracellular precipitation, (2) intracellular accumulation, (3) oxidation and reduction reactions, (4) methylation and demethylation, and (5) extracellular binding and complexation (Ojuederie and Babalola 2017) as presented in Table 13.3.

#### 13.3.2.2 Metal Extrusion Strategies

Mostly the resistance mechanisms in bacteria known till date are encoded on plasmids and transposons and consequently have high probability of gene transfer or spontaneous mutation that help bacteria to acquire resistance against heavy metals. For example, in gram-negative bacteria (e.g. *Ralstonia eutropha*), a czc system is found responsible for the resistance to Cd, Zn, and Co. The czc-genes

**Table 13.3** Strategies adopted by metal-tolerant bacteria to overcome metal stress

| Mechanism | Organism | Description and effectiveness | References |
|---|---|---|---|
| Bioaccumulation and biosorption | *Sinorhizobium* sp. | Improved growth and nodulation in *M. sativa* under cd, cu, Pb, and Zn stress | Zribi et al. (2012) |
| Bioaccumulation | *Delftia* sp. B9 | Intracellular dissolution of cd<br>Reduce cd accumulation in rice grain | Liu et al. (2018) |
| Biotransformation and bioaccumulation | *Micrococcus* KUMAs 15 | Arsenite oxidation and accumulation | Paul et al. (2018) |
| Biosorption | *Bacillus* sp. MC3B-22 and *Microbacterium* MC3B-10 | EPS mediated sorption of $Cd^{2+}$ | Camacho-Chab et al. (2018) |
| Bioreduction | *Bacillus subtilis* MA13 | Reduction of Cr(VI) via Cr reductases enhanced growth and photosynthetic pigments of soybean | Wani et al. (2018) |

encode the cation-proton antiporter (CzcABC) that exports Cd, Zn, and Co. Similarly, ncc system found in *Alcaligenes xylosoxidans* displayed resistance to Ni, Cd, and Co. On the contrary, Cd resistance mechanism in *Staphylococcus*, *Bacillus,* or *Listeria* operates through Cd-efflux ATPase. Two most well studied Cu resistance systems (cop) are observed in *P.syringae* pv. tomato and pco in *E.coli*. The cop genes encode for different Cu-binding proteins, which sequester Cu in the periplasm or in the outer membrane. However, the pco system acts through an ion-dependent Cu antiporter (Kunito et al. 1997). Naturally occurring PGPR also show resistance to zinc which is mostly through efflux system, for example, a P-type ATPase efflux system transports Zn ions across the cytoplasmic membrane via ATP hydrolysis (Beard et al. 1997), while RND-driven transporter system moves Zn across the cell wall of gram-negative bacteria through a proton gradient (Nies 1999). Likewise, Ni resistance is inducible and depends on energy-dependent efflux system driven by chemiosmotic proton-antiporter system (Taghavi et al. 2001).

#### 13.3.2.3 Biotransformation

Numerous microorganisms have ability to reduce/transform a wide variety of multivalent metals that pose major threat to the environment. Though, various PGPR strains possessing metal reducing ability have been identified (Mallick et al. 2018), reduction of chromium only by PGPR will be discussed in the following section.

Among different forms of chromium, the hexavalent chromium is the more toxic and carcinogenic owing to its high solubility in water, rapid permeability through biological membranes, and subsequent interaction with intracellular proteins and nucleic acids (Kamaludeen et al. 2003). Among various forms of Cr, Cr (III) does not migrate freely in natural systems because it tends to precipitate as Cr (III) minerals or is removed by adsorption. Hence, reduction of toxic Cr (VI) to Cr (III) is a useful approach to remediate Cr (VI) affected environments (Thatoi and Pradhan 2017). In this regard, numerous chromium resistant PGPR like *Pseudomonas* sp., *Bacillus, Stenotrophomonas*, *Serratia*, *Arthrobacter,* and rhizobia have been identified and applied in Cr (VI) contaminated soils (Baldiris et al. 2018; Dong et al. 2018). Detoxification of chromium by microbes may occur directly or indirectly and can be affected by pH, incubation period, chromate concentration, and types of microbes (aerobic/anaerobic) involved (Narayani and Shetty 2013). In the direct mode, the microbes absorb chromium and then enzymatically (chromium reductases) reduce it (Mala et al. 2015). While in the indirect mode, metabolic products (reductants or oxidants) of the microbes in soil, such as $H_2S$, chemically reduce chromium by redox reactions. Jin et al. (2017) observed that the removal of Cr (VI) by *Acinetobacter* strain WB-1 was due to surface immobilization along with intracellular and extracellular reduction. Enzymatic reduction of Cr (VI) to Cr (III) usually is accomplished by chromium reductases and occurs both anaerobically (Masaki et al. 2015) as well aerobically (He et al. 2015) and sometimes involves chemical reactions associated with compounds such as nucleotides, sugars, amino acids, vitamins, organic acids or glutathione.

#### 13.3.2.4 Bioaccumulation

Bioaccumulation strategy involves the uptake of metal ions by an organism either directly following exposure to a contaminated medium or indirectly by consumption of contaminants. Intracellular accumulation of toxic metals is an energy-dependent transport system which depends on (1) intrinsic properties, (2) physiological and genetic adaptation, (3) metal speciation, availability, and toxicity (Sinha et al. 2013). Once taken up, toxic metals pass through biological membranes via carrier mediated transport, endocytosis, ion pumps, ion channels, complex permeation, and lipid permeation (Adriano 2017). Permeabilization of cell membranes to toxic elements can lead to further exposure of intracellular metal-binding sites resulting in enhanced passive accumulation. Several methods have been used for detecting the accumulation and localization of metals inside the bacterial cells. For example, using TEM, Podder and Majumder (2018) found that the growth and bioaccumulation of arsenic ions by *Corynebacterium glutamicum* MTCC 2745 varied with pH, inoculum size, contact time, temperature, and concentrations of peptone and As. In another study, AAS analysis of the culture products from *B. amyloliquefaciens* treated with Cr (VI) for 45 h showed the distribution of Cr(III) in pellet and culture supernatant in the range of $37.4 \pm 1.7$ and $62.6 \pm 3.4$ mg $L^{-1}$, respectively (Das et al. 2014). In SEM images, the Cr (VI) treated bacterial pellets looked rough, coagulated, and porous, whereas the untreated pellets appeared smooth, regular, and non-porous. Also, TEM–EDX study of the bacterial precipitates under Cr (VI) treatment had nanometric range of intracellular Cr (III). Bioaccumulation process has several advantages like it is a metabolically active process of living organisms that works through adsorption, intracellular accumulation, and bioprecipitation. However, bioaccumulation also has limitation because it is applied on live cells only. The living cells however have the potential of recombination or mutant formation which can change morphological and physiological features of the strain. Besides, high concentration of applied or already present metals significantly damage the surface of living cells and may lead to partial loss in cell-binding abilities and consequently release of accumulated metals back into solution (Kadukova and Vircikova 2005).

#### 13.3.2.5 Biosorption

Biosorption of heavy metals by certain living or dead microbial biomass is a very effective solution to remediate even very dilute aqueous solutions (Dadrasnia et al. 2015). Biosorption consists of several mechanisms, such as ion exchange, adsorption, chelation, and diffusion through cell walls and membranes, which differs and depends on the species used, the origin and processing of biomass, and the chemistry of solution. Biosorption in fact is a non-enzymatic process wherein pollutants are adsorbed onto the cell surface (Sulaymon et al. 2012). The uptake of metal could be active or passive (Vijayaraghavan and Yun 2008) and both may occur independently or simultaneously. Of these, passive process is relatively nonspecific (Volesky 2007) and does not involve cellular metabolism. Here, metal binds to poly ionic cell walls through ion exchange. This process is not affected by physical conditions such as pH and ionic strength. It is a reversible and fairly rapid process requiring only 5–10 min. For complete biosorption of heavy metals. The active process is slow and depends on cellular metabolism and therefore is influenced by uncouplers, metabolic inhibitors, and temperature. In the active process, the metal complexes with specific proteins like metallothioneins which is found in vacuole. For example, biosorption capacity of live and dead cells of a novel *Bacillus* strain for chromium showed that both live and dead biomass followed the monolayer biosorption on the active surface sites. Scanning electron microscopy and FTIR indicated significant influence on the morphological features of the dead cells during biosorption of chromium. Approximately 92% and 70% desorption efficiencies were achieved using dead and live cells, respectively (Dadrasnia et al. 2015). However, whatever may be the mode of metal uptake, the adsorption occurs due to the nonspecific binding of ionic species to cell surface associated or extracellular polysaccharides and proteins (Cristani et al. 2012) of different bacterial cell organization, bacterial cell walls, and envelopes (Puyen et al. 2012). Among bacteria, the cell walls of gram-positive bacteria in general bind larger quantities of toxic metals than the envelopes of the gram-negative bacteria (Silver and Phung 1996). FTIR studies have revealed that various functional moieties such as hydroxyl, amino, carboxylate, phosphoryl, etc. were present on the surface of bacterial cell which participated in metal binding and hence assisted biosorption process (Patil and Unnikrishnan 2017).

## 13.4 Role of Bioactive Molecules Secreted by PGPR in HM Removal

Many PGPR colonizing plants have been found to play significant roles in mobilization or immobilization of heavy metals and consequently reducing the availability and/or toxicity of metals to plants (Table 13.4). However, this metal accumulating ability and plant colonizing potential of rhizospheric bacteria together could be of practical importance in alleviating metal toxicity when bioinoculated plants are grown in metal-contaminated soils (Kidd et al. 2017). Fatnassi et al. (2015) reported that co-inoculation of plants, treated with 1 mM Cu and 2 mM Cu (1) increased the dry weight as compared with Cu-treated and uninoculated plants, (2) decreased Cu uptake in the roots, (3) increased copper tolerance status of *Vicia faba* compared to uninoculated plants exposed to Cu stress.

In a similar study, Mohamed and Almaroai (2017) found that the phosphate solubilizer *Bacillus* sp., *Azotobacter* sp., and *Pseudomonas* sp. produced a substantial amount of IAA both in the absence and presence of heavy metals. Besides these merits, they significantly decreased the uptake of heavy metals in corn plants grown under metal stress. Similarly, Subrahmanyam et al. (2018) reported that green gram, inoculated by *Enterobacter* sp. C1D had significantly better length and dry biomass of shoot and root and chlorophyll content when grown in the presence of Cr (VI). Confocal laser scanning microscopy (CLSM) of the roots showed heavy bacterial loads on root surface specifically at the root tip and the point of root hair/lateral root formation. Moreover, the elevated IAA levels and ACC deaminase activity enabled *Enterobacter* sp. C1D to enhance green gram production in Cr (VI)-amended soils.

## 13.5 Role of EPS as Biosorbents in Heavy Metal Removal

Extracellular polysaccharides, the complex biomolecules are consisted of proteins, polysaccharides, uronic acid, humic-like substances, nucleic acid, lipids, and glycoproteins and surround the bacterial cells (Sheng et al. 2010). Metal sorption by EPS is believed to be an important self-protection strategy of microbial cells against toxic substances (Bhunia et al. 2018). The ionic nature of metal, its size, and charge density regulate its interaction with negatively charged EPS (Gupta and Diwan 2017). Since EPS are usually the first barrier of microbial cells that directly contact and interact with metals, they are of vital importance not because they protect the interior microbial cells but also play important role in remediation of metal-contaminated environments (Ayangbenro and Babalola 2018). Recently, numerous PGPR found to secrete EPS are involved in metal removal, for example, EPS of *B. subtilis* and *P. putida* strains significantly enhanced their Cu (II) adsorption capacity (Fang et al. 2013). The complex and diverse EPS properties make it usually difficult to understand the adsorption behavior of microbial biomass. In recent times, FTIR has frequently been used to obtain structural information on metal-binding

**Table 13.4** Role of metal-tolerant PGPR secreting bioactive molecules in phytoremediation process

| Bacteria | Bioactive molecules | Role of PGPR | References |
|---|---|---|---|
| *Pseudomonas and rhizobium sullae* | IAA, siderophores, P solubilization | Increased growth of Sulla coronaria under cd stress, enhanced antioxidant response and cd accumulation in roots | Chiboub et al. (2018) |
| *Bacillus* | IAA, Ni, ACC deaminase | Increased plant growth and facilitated Ni accumulation | Akhtar et al. (2018) |
| *Mesorhizobium ciceri* | $N_2$ fixation | Enhanced growth of chickpea and Cr concentration in roots favoring phytostabilization | Velez et al. (2017) |
| *Gordonia alkanivorans, Cupriavidus necator, and Sporosarcina luteola* | EPS, IAA, NH3, N2 fixation | Increased phytoextraction of As and Hg in B. juncea and L. albus | Franchi et al. (2017) |
| *Pseudomonas sp.* | Auxin, siderophore, P solubilization, ACC deaminase | Enhanced plant growth and increased nodule biomass Influenced phytostabilization | Soussou et al. (2017) |
| *Bacillus sp.* | Zn solubilization | Zn mobilization and accumulation, yield enhancement in soybean and wheat | Khande et al. (2017) |
| *Enterobacter* | Siderophore, IAA | Increased Fe uptake, immobilized $Cd^{2+}$ in rhizosphere, influenced phytostabilization of $Cd^{2+}$ | Chen et al. (2017) |
| *Burkholderia cepacia* | IAA, Siderophore | Enhanced growth of Brassica rapa, Zn uptake | Kang et al. (2017) |
| *Bradyrhizobium liaoningensis* | – | Increased Ni and Fe uptake in Pongamia pinnata from V Fe magnetite mine failing site | Yu et al. (2017) |
| *Mesorhizobium* | IAA, Siderophore, ACC, TCP solubilization | Enhanced multi element tolerance in Leucaena leucocephala | Rangel et al. (2017) |
| *Variovorax paradoxus* |  | Increased biomass of plant and Ni uptake in roots and shoots | Durand et al. (2016) |
| *Brevibacterium casei* | NH3, ACC, IAA, HCN | Increased biomass, enhanced cd, Zn, cu accumulation in shoots | Plociniczak et al. (2016) |
| *Bacillus sp.* | Zn solubilization, P and K solubilization, biocontrol | Enhanced Zn translocation | Shakeel et al. (2015) |
| *P. aeruginosa* | Siderophore | As accumulation in all plant | Jeong et al. (2014) |

complex associated with various functional groups of EPS. Hence, the chemical state and the IR absorption spectrum of EPS functional groups change sensitively upon binding with metals. The FTIR spectral band in the region between 4000 and

400 $cm^{-1}$usually shows the major characteristic bands of various bonds present in functional groups of EPS. For example, the $Cu^{2+}$ complexation by carboxyl functional group of EPS can be clearly reflected by the change in peak intensity of FTIR spectral band at both 1400 $cm^{-1}$ (associated with the stretching vibration of $COO^{2-}$ bond from carboxylic group and deformation vibration of -OH from alcohols and phenol groups) and 1080 $cm^{-1}$ (attributed to the stretching vibration of -OH group) (Mohite et al. 2018). An interesting feature called *rescuing* was highlighted wherein EPS secreting (metal-tolerant) strains displayed a protective effect towards non-EPS secreting sensitive strain in co-culture experiments (Nocelli et al. 2016). Similarly, the non-symbiotic $N_2$ fixers such as *A. chroococcum* secreted EPS and formed complexes with Pb and Hg ions in pH dependent manner. At lower pH (between 4 and 5), the maximum adsorption was 40–47% of initial metal ion concentration in solution (Rasulov et al. 2013).

## 13.6 Role of Metallothioneins and Phytochelatins

Metallothioneins (MTs) are cysteine-rich, heavy metal-binding protein molecules synthesized due to mRNA translation (Guo et al. 2013) and play a crucial role in uptake, detoxification, and accumulation of metal. MTs are gene-encoded polypeptides. They show greater affinity for metals such as Cd, Cu, Zn, and As by cellular sequestration, homeostasis of intracellular metal ions as well as modification of metal transport. In addition to detoxification of heavy metals, plant MTs also play an important role in cell growth and proliferation, repair of plasma membrane, repair of damaged DNA, scavenge ROS, and maintenance of redox level (Emamverdian et al. 2015). Naik et al. (2012) explored the role of bacterial MTs in Pb-resistant bacterial isolates: *Salmonella choleraesuis* strain 4A, *Proteus penneri* strain GM10, *Bacillus subtilis* strain GM02, *Pseudomonas aeruginosa* strain 4EA, *Proteus penneri* strain GM03, and *Providencia rettgeri* strain GM04 which were quarantined from soil, polluted with car battery waste from Goa, India. All the isolates except *P. aeruginosa* strain 4EA had plasmids. Both bacterial MTs and intracellular bioaccumulation of Pb in *S.choleraesuis* strain 4A and *P.penneri* strain GM10 were responsible for the Pb resistance. Similarly Li et al. (2015) found that expression of PcPCS1gene from bean pear (*Pyrus calleryana* Dcne.) was induced after Cd and Cu treatments and *E.coli* with over-expressed PcPCS1 had enhanced tolerance to Cd, Cu, Na, and Hg. *E.coli* cells transformed with pPcPCS1 survived in solid M9 medium containing 2 mM $Cd^{2+}$,4 mM $Cu^{2+}$, 4.5%(w/v)$Na^{+}$, or200 μM$Hg^{2+}$. Moreover, the growth curve showed that1.5 mM $Cd^{2+}$, 2.5 mM$Cu^{2+}$, 3.5% (w/v)$Na^{+}$, and100 μM$Hg^{2+}$had no effect on growth of *E.coli* cells transformed with pPcPCS1. Also, the content of PCs and accumulation of Cd, Cu, Na, and Hg ions were enhanced in the recombinant *E.coli* strain Rosetta™(DE3).

Phytochelatins (PCs) are short-chain thiol-rich repetitions of peptides synthesized from sulfur-rich glutathione by the enzyme phytochelatin synthase which defend plants against environmental stresses such as salinity, drought, herbicide, and heavy metals (Emamverdian et al. 2015). They are used as biomarkers for an early detection of heavy metal stress in plants (Saba et al. 2013). Metallothioneins have much affinity for a wide range of metals such as Cu, Zn, Cd, and As by cellular sequestration, homeostasis of intracellular metal ions as well as adjustment of metal transport. Apart from detoxification of heavy metals, plant MTs also play a role in maintenance of the redox level, repair of plasma membrane, cell proliferation, and its growth, repair of damaged DNA, and scavenge ROS (Emamverdian et al. 2015). Glutathione (GSH), a precursor of phytochelatin, has been reported to play a vital role in metal detoxification (Hasanuzzaman et al. 2017) and in protecting plant cells from other environmental stresses including intrinsic oxidative stress reactions.

### *13.6.1 Phytoremediation Strategies*

Some of the strategies mediated by plants often termed as phytoremediation include:

1. Phytoextraction (phytoaccumulation): Uptake of the contaminant by plant roots from the environment and its translocation into harvestable plant biomass.
2. Rhizofiltration: Use of plant roots to absorb or adsorb contaminants present in solution form in the surrounding of root zone.
3. Phytostabilization: Reduction of mobility and bioavailability of pollutants in environment either by physical or chemical effects.
4. Phytodegradation (phytotransformation): Phytodegradation is the use of plants and microorganisms to uptake, metabolize, and degrade the contaminant. In this approach, plant roots are used in association with microorganisms to detoxify soil contaminated with organic compounds. It is also known as phytotransformation. Some plants are able to decontaminate soil, sludge, sediment, and ground and surface water by producing various enzymes. This approach involves organic compounds, including herbicides, insecticides, chlorinated solvents, and inorganic contaminants (Pivetz 2001).
5. Rhizodegradation: The breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of root zone is called rhizodegradation. This process uses microorganisms to consume and digest organic substances for nutrition and energy. Natural substances released by the plant roots, sugars, alcohols, and acids contain organic carbon that provides food for soil microorganisms and establishes a dense root mass that takes up large quantities of water. This process is used for the removal of organic substance (contaminants) in soil medium (Moreno et al. 2008).
6. Phytovolatilization: Phytovolatilization is the use of green plants to extract volatile contaminants, such as Hg and Se, from the polluted soils and to ascend them into the air from their foliage (Karami and Shamsuddin 2010).

Phytoremediation as a technique is inexpensive since it involves plants which can be grown and monitored easily (Saraswat and Rai 2012). Moreover, the recovery and re-use of valuable products of this method are easy, because it uses natural biological

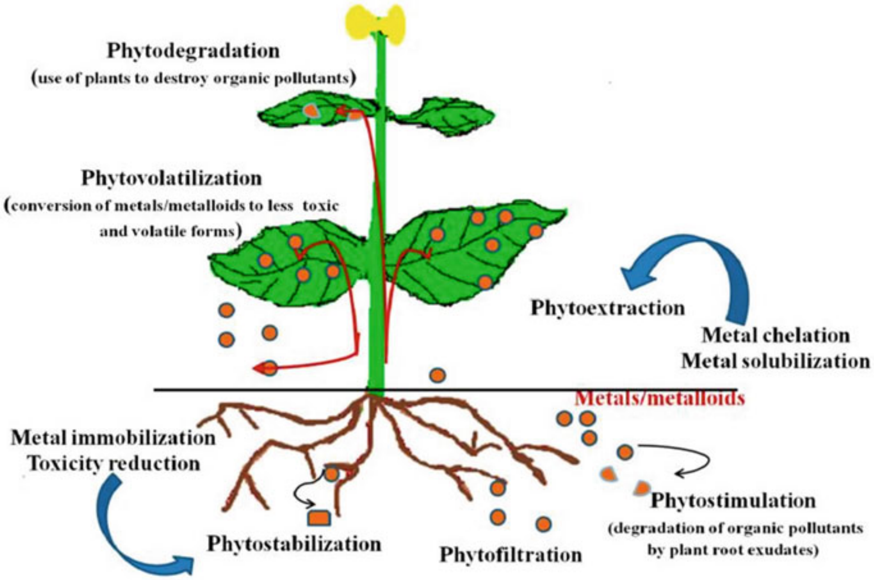

**Fig. 13.1** Strategies employed for HM removal using plants. Adapted from Ojuederie and Babalola (2017)

materials. Additionally, plants can be modified for any target characteristics, and the original state of the environment could be restored.

It is relatively a recent technology and perceived as cost-effective, efficient, novel, eco-friendly, and solar-driven technology with good public acceptance. Phytoremediation is an area of active current research (Fig. 13.1). New efficient metal hyperaccumulators are being explored for their application in phytoremediation and phytomining.

Plant species for phytoremediation are selected based on their root depth, the nature of the contaminants and the soil, and regional climate. The root depth directly impacts the depth of soil that can be remediated. It varies greatly among different types of plants, and can also vary significantly for one species depending on local conditions such soil structure, depth of a hard pan, soil fertility, cropping pressure, contaminant concentration, or other conditions. The cleaning depths are approximately <3 feet for grasses, <10 feet for shrubs, and <20 feet for deep rooting trees. The nature of on-site contaminants is a principal factor in the selection of a plant for phytoremediation (Sharma and Reddy 2004). Grasses are the most commonly evaluated plants used for phytoremediation (Shu et al. 2002). They are preferably used for phytoremediation because as compared to trees and shrubs, herbaceous plants, especially grasses, rapid growth, large amount of biomass, strong resistance, effective stabilization to soils, and ability to remediate different types of soils (Elekes 2014). They are pioneers and usually adapted to adverse conditions such as low soil nutrient content, stress environment, and shallow soils (Malik et al. 2010). The large surface area of fibrous roots of grasses and their intensive penetration of soil reduces leaching, runoff, and erosion via stabilization of soil and offers advantages for phytoremediation. Molecular tools are being used to better understand the mechanisms of metal uptake, translocation, sequestration, and tolerance in plants.

### *13.6.2 PGPR: Assisted Phytoremediation*

Degraded soils, following the harmful effects of emissions from non-ferrous metal smelters, are usually arid from organic material and devoid of proper microflora. Therefore, the poor condition of the soil environment makes it impossible to carry out an effective biological remediation of the degraded area. For this purpose, some organic substances, such as sewage sludge, are used to support the process of phytoremediation, because they are a source of biogenic elements and soil microorganisms (Kacprzak et al. 2014). Among the disadvantages, phytoremediation is a lengthy process and is affected greatly by the changing environmental conditions. Thus, the ability of plants to remove/sequester metals in contaminated sites can be improved by applying PGPR with various phytoremediation methods. When single or mixture of inoculants are applied along with phytoremediation process, PGPR affect the mobility and availability of metals to plants by releasing numerous chelating substances, acidification, phosphate solubilization, and redox changes (Zoomi et al. 2017). Chen et al. (2018a) reported that successful in situ phytoremediation depends on beneficial interactions between roots and microbes and plant cultivation. Soil amendment increases microbial diversity and restructures microbial communities. Rhizo-compartmentalization through selection of a specific core root microbiome by the metal-tolerant plant *H. cannabinus* with Enterobacteriaceae, Pseudomonadaceae, and Comamonadaceae included a large number of metal-tolerant and plant growth-promoting bacteria. The root-associated microbial community formed niche-assembled patterns and predominantly had *Proteobacteria*, *Actinobacteria,* and *Chloroflexi* under metal-contaminated conditions.

Złoch et al. (2017) evaluated the role of three metallotolerant siderophore-producing *Streptomyces* sp. B1–B3 strains in the phytoremediation of heavy metals using *S. dasyclados*. The bacterial inoculation significantly stimulated biomass and reduced oxidative stress. Moreover, the bacteria affected the speciation of heavy metals and finally their mobility, thereby enhancing the uptake and bioaccumulation of Zn, Cd, and Pb in the biomass. The best capacity for phytoextraction was noted for strain B1, which had the highest siderophore secretion ability. Five metal resistant PGPR (*Ralstonia eutropha* 1C2, *Chryseobacterium humi* ECP37, *Pseudomonas fluorescens* S3X, *Rhizobium radiobacter* EC1B, and *Pseudomonas reactans* EDP28) were investigated by Moreira et al. (2016) for their in vitro growth promoting traits and for their ability to induce growth of maize seedlings exposed to Zn and Cd. They showed that some bacteria only enhanced PGP traits when exposed to metals. The bacterial strains ECP37 and EDP28 were most efficient in improving seedling growth with increasing metal concentrations, followed by S3X. When inoculated in maize grown in mine soil, these strains also outperformed the others by increasing shoot biomass and elongation, metal accumulation, and by decreasing it in roots. The most evident effect of doubling the inoculum size was the increase in Cd accumulation, which was of 17% and 31% in roots and shoots, respectively. Other effects included a slight reduction in shoots' biomass (13%) and a general decrease in P content.

*Sinorhizobium meliloti* CCNWSX0020 was used to assess its effect on *Medicago sativa* seedlings under Cu stress (Chen et al. 2018b). This rhizobium inoculation alleviated Cu-induced growth inhibition, regulating antioxidant enzyme activities and increased nitrogen concentration in *M. sativa* seedlings. Moreover, the total amount of Cu uptake in inoculated plants was significantly increased compared with non-inoculated plants, and the increase in the roots was much higher than that in the shoots, thus decreasing the transfer coefficient and promoting Cu phytostabilization.

Bianucci et al. (2018) in a recent investigation observed no changes in growth variables (shoot and root dry weight) of soybean plants inoculated with *Bradyrhizobium* sp. grown in As (V) contaminated soils. Regarding As uptake by plants, metalloid accumulation followed the same distribution pattern among strains. Furthermore, at 6 μM As (V), *Bradyrhizobium* inoculated soybean revealed a significantly lower translocation factor (TF) in comparison to other inoculated strains and promoted As phytostabilization. At the highest As (V) concentration, only *B. diazoefficiens* USDA110 was able to nodulate the legume; however, a significant decrease in the number and dry weight of nodules and N content was observed. Similarly, Mallick et al. (2018) reported that two As-resistant halophilic bacterial strains *Kocuria flava* AB402 and *Bacillus vietnamensis* AB403 from mangrove rhizosphere of Sundarban could tolerate 35 mM and 20 mM of arsenite, respectively. Also, As had a variable impact on EPS synthesis, biofilm formation, and root association ability of both the bacterial strains. When used as inoculum they promoted the growth of rice seedlings by decreasing As uptake and accumulation in plants. Gupta et al. (2018) isolated Cr resistant plant growth promoting *Pseudomonas* sp. (strain CPSB21) from the tannery effluent contaminated agricultural soils and evaluated the plant growth promoting activities, oxidative stress tolerance, and $Cr^{6+}$ bioremediation potential. Assessment of different plant growth promoting traits, such as P solubilization, IAA production, siderophores, ammonia, and HCN production, revealed that the strain CPSB21 could serve as an efficient plant growth promoter under laboratory conditions. Further, the plant growth, pigment content, N and P uptake, and Fe accumulation were reduced when sunflower and tomato were grown in Cr (VI) amended soils. However, inoculation of strain CPSB21 alleviated the $Cr^{6+}$ toxicity and enhanced the plant growth parameters and nutrient uptake. Apart from these, Cr toxicity had varied response on oxidative stress tolerance at graded $Cr^{6+}$concentration on both plants, and increase in SOD and CAT activity and reduction in MDA were observed following inoculation of strain CPSB21. Additionally, inoculation of CPSB21 enhanced the uptake of $Cr^{6+}$ in sunflower, while no substantial increment was observed in inoculated tomato plants.

In order to select the PGPR for phytoremediation of heavy metal contamination, Roman-Ponce et al. (2017) isolated bacterial strains from the rhizosphere of two endemic plants, *Prosopis laevigata* and *Sphaeralcea angustifolia,* grown in a heavy metal-contaminated zone in Mexico. These rhizobacterial strains were characterized for the growth at different pH and salinity, extracellular enzyme production, solubilization of phosphate, heavy metal resistance, and plant growth-promoting (PGP) traits, including production of siderophores and IAA. Overall, the rhizobacteria showed multiple PGP traits. These rhizobacteria were also resistant to high levels of heavy metals including As as a metalloid (up to 480 mmol $L^{-1}$ As(V), 24 mmol $L^{-1}$ Pb(II), 21 mmol $L^{-1}$ Cu(II), and 4.5 mmol $L^{-1}$ Zn(II)). Inoculation of *Brassica nigra* seeds with *Microbacterium* sp. CE3R2, *Microbacterium* sp. NE1R5, *Curtobacterium* sp. NM1R1, and *Microbacterium* sp. NM3E9 facilitated the root development and significantly improved the seed germination and root growth in the presence of 2.2 mmol $L^{-1}$Zn (II).

Additionally, siderophores released by PGPR including legume nodulating rhizobia into the rhizosphere serve as an Fe source for plants (Ivanov et al. 2012) and therefore help to fulfil the Fe deficiency of plants in Fe limiting soils. Considering this, it is generally suggested to use PGPR in soils deficient in Fe. To substantiate this, metal resistant PGPR such as *P. putida* strains and *P. fluorescens* strains (showing IAA, siderophores and ACC deaminase production) were reported to show significant improvement in growth attributes of inoculated canola and barley plants even when grown with various concentrations of $CdCl_2$ and $PbNO_3{}^{2-}$. Furthermore, the translocation factor indicated that inoculated canola and barley had abilities of Cd and Pb phytoextraction in soil contaminated with respective metal. Conclusively, the enhancement in the inoculated canola and barley plants occurred due to the protection against the inhibitory effects of Cd and Pb by PGPR in addition to their ability to provide IAA, siderophore, and ACCD to the developing plants (Yancheshmeh et al. 2011). Nayak et al. (2018) used *B. cereus* (T1B3) strain able to produce various bioactive compounds such as ACC deaminase, indole acetic acid, and siderophores, nitrogen fixation, and P solubilization. Removal capacity (mg $L^{-1}$) of T1B3 strain was 82% for $Cr^{+6}$ (100), 92% for Fe (100), 67% for Mn (50), 36% for Zn (50), 31% for Cd (30), 25% for Cu (30), and 43% for Ni (50) during the active growth cycle in HM-amended soil.

Ma et al. (2015) isolated a PGPB strain SC2b from the rhizosphere of *Sedum plumbizincicola* grown in Pb/zinc (Zn) mine soils and characterized as *Bacillus* sp. using partial 16S ribosomal DNA sequencing analysis. Strain SC2b exhibited high levels of resistance to Cd (300 mg/L), Zn (730 mg/L), and Pb (1400 mg/L). Besides possessing various PGP features such as secretion of ACC deaminase, utilization of 1-aminocyclopropane-1-carboxylate, solubilization of P, and production of IAA and siderophore, the strain mobilized high concentration of heavy metals from the soils and exhibited different biosorption capacity toward the tested metal ions. Under pot trial, this metal resistant PGPB SC2b elevated the shoot and root biomass and leaf chlorophyll content of *S. Plumbizincicola* besides enhanced Cd and

Zn uptake through metal mobilization or plant-microbial mediated changes in chemical or biological soil properties.

A metal resistant bacterium *Enterobacter ludwigii* isolated from the rhizosphere of Kair "*Capparis decidua*" by Singh et al. (2018) was screened for its phytoextraction ability under gradient metal stress conditions. Among the PGP traits, isolate showed the production of ACC deaminase, produced IAA, and solubilized the inorganic P. The isolate was resistant to Zn, Ni, Cu, and Cd. Furthermore, inoculation of the test isolate significantly increased various growth parameters of wheat plants and also improved the photosynthetic pigments. In addition, inoculation resulted in significant increase in the Zn content in wheat plants under metal stress. Bacterial application significantly increased various compatible solutes such as proline content, total soluble sugar and decreased the malondialdehyde (MDA) content as compared to control, illustrating its protective effect under metal induced oxidative stress in wheat plants.

## 13.7 Conclusion

The toxic effect of heavy metals may result from the accumulation of HMs over time in vital parts of humans, plants as well as microbes. The direct and indirect mode of deleterious effect of HMs in plants results due to several reasons such as reduction in dry biomass, alteration in chlorophyll content and at molecular level due to over generation of ROS, damage to essential macromolecules, and thus constrain crop productivity. Undoubtedly the PGPR-assisted HM phytoremediation used in native or engineered forms is reported to have greater remediation potential but their impact on ecosystems needs to be elucidated before commercialization. Various steps of regulatory networks via plant-associated microbes including the synergistic action of plants and microbes and their mechanism for metal mobilization, transformation, and detoxification should be investigated for unravelling the dynamics of plant–microbe–metal interactions in the soils. Further monitoring and managing microbial heavy metal remediation require the characterization of the fate and behavior of the compounds of interest in the environment.

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# Chapter 14
# ROS Signaling Under Oxidative Stress in Plants

**Deepesh Bhatt, Saurabh C. Saxena, and Sandeep Arora**

**Abstract** Plants being sessile have to withstand an array of adverse environmental challenges throughout their life span. The chances of survival of a plant, under sub-optimal growth conditions, depend on the way they perceive and transmit environmental signals. Plants transmit these signals and initiate appropriate response with the help of an array of secondary messenger molecules which help them to adapt towards the changing environment. Secondary messengers are among the most versatile molecules or intermediates that include calcium messenger systems, reactive nitrogen oxide species along with the most prevailing reactive oxygen species. Examples of these signaling molecules include superoxide radical, hydrogen peroxide, nitric oxide, and hydroxyl radicals. One of the most intriguing facts about cellular metabolism is that some of the secondary messengers, involved in transducing signals are also part of potentially damaging molecules that can destroy membrane integrity and negatively affect the redox balance of the cell. During cellular metabolism, minimal generation of ROS takes place constantly, though its levels are kept under strict control.

Abiotic as well as biotic stresses lead to the production of reactive oxygen species. These reactive oxygen species are responsible for the activation of cellular defence network and causing cellular damage due to oxidative stress. There is a fine line separating these two seemingly antagonistic roles of ROS. The role of ROS as secondary messenger molecules is intricately linked with the downstream signaling and transcriptional regulation during oxidative stress in the plants. A delicate equilibrium between the role of ROS as a secondary messenger and as causative agents for oxidative damage determines the growth characteristics of a plant under

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stress. Several enzymatic as well as non-enzymatic antioxidants present in the cells act as agents for effective scavenging of ROS, produced during different kinds of stressful conditions. The current chapter underlines the dynamics of ROS production and their role as a secondary messenger as well as inducer of oxidative damage.

## 14.1 Introduction

All the living organisms usually come across a combination of many abiotic stresses in their natural habitats. Plants routinely face sudden and extreme changes in environmental conditions but being sessile in nature, they cannot move away in order to weaken the ill effect of those stresses. It is a well known fact that environmental stress affects the plant growth and its development in a pessimistic way. Plant exhibits several defence responses to mitigate the deleterious effects of the stresses. During the adverse conditions, plant activates various cellular processes for the synthesis of signaling molecules that trigger the protective mechanisms to protect the plant. Under such extreme conditions, plants shift their physiological and biochemical eminence through molecular shifting or molecular rearrangement which is said to impart protection to the plants (Formentin et al. 2018; Ferrer et al. 2018). These adverse conditions may range from cold, drought, heat, salinity, heavy metal, UV radiation, and attack of pathogens. In various cell organelles, such unfavourable surroundings lead to generation of Reactive Oxygen Species (ROS) which interact with various macromolecules and cause severe damage to proteins, DNA, and lipids which subsequently affect normal functioning of the cell that ultimately put the plant in the oxidative damage condition (Tripathi et al. 2017; Banerjee et al. 2018). ROS are produced due to reduction of molecular oxygen by high energy exposure or electron transfer reactions. Sequential transfer of one, two or three electrons to oxygen leads to the formation of a superoxide radical ($O_2^{\bullet-}$), or a hydroxyl radical ($^{\bullet}OH$), super oxide anions ($O_2^-$), Hydrogen peroxide ($H_2O_2$), and singlet oxygen ($^1O_2$), respectively (Mittler 2017; Waszczak et al. 2018). ROS may further be generated via involvement of RBOH (respiratory burst oxidase homologs) and few similar classes of oxidases and peroxidases, via interconnected pathways (Suzuki et al. 2011; Gilroy et al. 2016; Mignolet-Spruyt et al. 2016). Plants produce them during metabolic activities in different cellular compartments or through leakage of electrons from electron transport chain in chloroplast and mitochondria where their increased concentration leads to peroxidation of lipids, damage to nucleic acid, oxidation of proteins, activation of programmed cell death (PCD) which ultimately leads to cell death (Sharma et al. 2012). When concentration of ROS, via any sort of cellular or biochemical perturbation exceeds the defence mechanism, the cell is said to be in state of oxidative stress and ion toxicity.

In spite of destructive activities, ROS are also demarcated as important secondary messengers in various cellular processes during stressful conditions (Desikan et al. 2001). There exists a dynamic balance between ROS production and scavenging which ultimately decides the impact of ROS in the cell. Due to their dual role, plant cell has to take extra cautions to control the concentration of ROS inside the cell in order to avoid any lethal injury. ROS generation (metabolically or for signaling purposes) or ROS scavenging is a stimulated process which occurs in most of the cellular compartments simultaneously. To control the concentration of ROS, plant has to detoxify or scavenge surplus ROS by means of its efficient antioxidative system which consists of enzymatic and non-enzymatic antioxidants (Noctor and Foyer 1998). In non-enzymatic category, phenols, ascorbate, carotenoid, glutathione, and tocopherols act as potent antioxidants within the cell, whereas superoxide dismutase (SOD) and enzymes of ascorbate-glutathione (AsA-GSH) cycle such as Ascorbate peroxidase (APX), Dehydroascorbate reductase (DHAR), Monodehydroascorbate reductase (MDHAR), Catalase (CAT), Guaiacol peroxidase (GPX), and Glutathione reductase (GR) serve as a part of enzymatic antioxidant system (Noctor and Foyer 1998). Many researchers have reported the increased concentration of these enzymatic and non-enzymatic antioxidant molecules during adverse environmental conditions (Bhatt et al. 2011).

In addition, ROS produced during stressful conditions are recognized by the sensors which further translate the signal to nucleus via a relay of redox reactions. Signal once reaches nucleus, initiates the translation of new proteins and modification in the existing proteins through generation of stress related transcription factors and their binding to corresponding cis-acting elements in the nucleus (Foyer and Noctor 2016). TFs have one DNA-binding domain that assists its binding to cis regulatory element (CREs) which activates or deactivates the gene expression and allow TFs to work as molecular switch. Minimal levels of ROS can be sensed by NADPH oxidase, also termed as respiratory burst homolog (RBOH) which gets activated through calcium mediated signaling leading to its own phosphorylation to further generate ROS (Gilroy et al. 2016). ROS can further be recognized by ROS recognizing receptors, like Mitogen Activated Protein Kinases (MAPK), phosphatases, and several categories of transcription factors. Through genomic and transcriptomic studies, it has been revealed that expression levels of MAP Kinases and Transcription factors (TFs) alter in response to stress. Therefore, plant shows a high level regulatory mechanism consisting of TFs, CREs that control the expression of stress related genes.

### *14.1.1 ROS and Its Types*

Plants are known to synthesize ROS even under seemingly normal environmental conditions which are unwanted by-product of various metabolic processes in the cell. These free radicals or reactive molecules are produced through stepwise reduction of oxygen. In various organelles of plant cell such as chloroplasts, mitochondria, peroxisomes, production of ROS takes place where 1% of total oxygen absorbed by the plant is consumed. These ROS have dual role as secondary messenger in small quantities as well as deleterious for cell when produced in excess.

We have summarized generation of various types of ROS stepwise and their site of synthesis in detail.

1. Singlet oxygen ($^1O_2$)

Molecular Oxygen in its ground state has two electrons in its outer shell which has parallel spin. This makes molecular oxygen as an inactive molecule which does not react with organic molecule unless gets activated. There are two different activation mechanisms of oxygen: first, absorption of energy to reverse the spin of one of the unpaired electron and stepwise reduction. In the former mechanism, $^1O_2$ is formed while in later $O_2^{\bullet -}$, $H_2O_2$, and •OH are synthesized via reduction of molecular oxygen. Singlet oxygen is a typical ROS which is not formed through reduction of oxygen or electron transfer process rather it is produced through the reaction of oxygen with triplet form of chlorophyll in the antenna system of plant cell.

$$\mathrm{Chl} \rightarrow 3\mathrm{Chl}$$

$$3\mathrm{Chl} + 3\mathrm{O_2} \rightarrow \mathrm{Chl} + \ ^1\mathrm{O_2}$$

Environmental hardship favours deficiency of carbon dioxide ($CO_2$) inside the cell which is responsible for the formation of singlet oxygen. This can cause severe harm to both the photosystems (PS I & PS II). It can alter the metabolism and cause serious damage to protein, nucleic acid, and lipids in spite having the short life of around three microseconds (Krieger-Liszkay et al. 2008).

2. Superoxide radical ($O^{\bullet -\ 2}$)

When oxygen absorbs sufficient energy and reverses the spin of one of the electrons, it allows oxygen to participate in divalent reduction reaction where simultaneous transfer of two electrons occurs (Apel and Hirt 2004). There is a spin restriction in molecular oxygen, following that oxygen cannot take four electrons at a time to produce $H_2O$. Therefore it accepts electrons sequentially, due to which reduction of $O_2$ leads to production of stable intermediates in a stepwise manner (Halliwell and Gutteridge 1984). The primary ROS is $O_2^{\bullet}$in the cell which starts cascade of reaction producing secondary ROS either directly or through enzyme driven processes that further depend upon the site of ROS formation (Valko et al. 2005). This radical is moderately reactive with a short half-life of 2–4 microseconds. It itself is not so deleterious for the cell but it gets transformed into $OH^{\bullet}$ and $^1O_2$ which are more reactive and can damage the cell, largely causing membrane lipid peroxidation (Halliwell 2006).

3. Hydrogen peroxide ($H_2O_2$)

$O_2^{\bullet}$ can be further reduced to $H_2O_2$ through non-enzymatic dismutation or SOD catalysed dismutation. Major source of $H_2O_2$ generation is electron transport chain of mitochondria, endoplasmic reticulum, chloroplast, and photorespiration and β oxidation of fatty acid in the plant cell. $H_2O_2$ is moderately active molecule and can readily cross cellular membranes and can cause oxidative damage far from the site of its synthesis. It is the only molecule which can pass through aquaporins in the membranes so it has gained wide spread attention as a crucial ROS and as regular signaling molecule over other ROS species (Bienert et al. 2007).

4. Hydroxyl radical ($OH^{\bullet}$)

Among all the ROS family members, hydroxyl radical is the most reactive and toxic ROS known till now. It is formed through Fenton Reaction between $H_2O_2$ and $O_2^{\bullet}$ catalysed by transition metals like $Fe^{2+}$& $Fe^{3+}$ at neutral pH.

$$H_2O_2 + O_2{}^{\bullet -} \rightarrow OH^- + O_2 + OH^{\bullet}$$

Once formed, it can damage cellular components by lipid peroxidation and invariably causes membrane damage. Interestingly, there is no enzymatic defence system against this ROS, so it can lead to cell death when accumulated in high concentration (Pinto et al. 2003).

### 14.1.2 Sites of ROS Generation

ROS are produced under normal conditions as well as in stressful environmental conditions at several locations inside the cell such as mitochondria, cell wall, endoplasmic reticulum, chloroplast, peroxisome depending upon the availability of light. In dark, major site of production is mitochondria, while in light, chloroplast and peroxisomes are major site of production of ROS in the cell (Choudhury et al. 2013).

1. Mitochondria

Mitochondria are the major site for the production of ROS like $H_2O_2$ and $O_2^{\bullet}$ (Navrot et al. 2007). Mitochondrial electron transport chain (ETC) is majorly responsible for reduction of oxygen through energized electrons which form ROS. Complex I and II are the major counter parts of mitochondrial ETC, which are responsible for the production of ROS (Moller et al. 2007; Noctor et al. 2007). Complex I or NADH dehydrogenase directly reduces oxygen within its flavoprotein region. When in complex I, there is a limited concentration of $NAD^+$ substrate; the electron flow becomes reversed from complex II to complex I. This process is responsible for increasing ROS production in complex I and it is regulated by ATP hydrolysis. Complex III or Ubiquinone-cytochrome region is also responsible for production of $O_2^{\bullet}$. Many enzymes which are present in mitochondria can produce ROS as well. Some enzymes donate electrons to ETC and reduce oxygen and form the ROS indirectly, e.g. 1-galactono-$\gamma$ lactone dehydrogenase (GAL), while some enzymes directly participate in the process of ROS formation like aconitase (Andreyev et al. 2005; Rasmusson et al. 2008).

2. Cell wall

Cell wall acts as a potent site for generation of ROS like $OH^{\bullet}$, $O^{\bullet -\ 2}$, $H_2O_2$, and $^1O_2$ during adverse environmental conditions. Cell wall contains an enzyme lipoxygenase (LOX) which is responsible for hyper oxidation of poly unsaturated fatty acid (PUFA), which further acts as an active site for ROS generation. Diamine oxidase situated in cell wall uses diamines or polyamines to produce ROS during stress conditions. During biotic stress, when a pathogen attacks the cell then the lignin precursors cross link extensively with each other through $H_2O_2$ mediated pathway which in turn makes cell wall strong and rigid to inhibit the entry of the pathogen inside the cell (Sharma et al. 2012).

3. Endoplasmic Reticulum

Cytochrome P450 (Cyt $P_{450}$) in endoplasmic reticulum is responsible for ROS generation through NADPH-mediated Electron Transport System (Mittler 2002). Cyt $P_{450}$ reacts with organic substrate (Rh) and further get reduced by a flavoprotein resulting into a free radical intermediate (Cyt P450 $R^{-}$). Immediately after production of this intermediate, it reacts with triplet oxygen ($_3O^2$) and oxygenated complex is formed (Cyt P450-ROO−). During stress condition, this oxygenated complex is converted into Cyt P450-Rh and $O^{\bullet -\ 2}$ as a by-product.

4. Chloroplast

It is a well-organized organ made up of thylakoid membranes which contains light capturing and well efficient light harvesting machinery (Pfannschmidt 2003). The major source of ROS production in the chloroplast is core light harvesting system, i.e. Photosystems, PSI & PSII. During abiotic conditions like drought, salinity, cold, and heat, etc., the mehler reaction gets activated and water level gets limited and $CO_2$ concentration along with increased light results into the formation of $O^{\bullet -\ 2}$

$$2O_2 + 2\text{Fd (reduced)} \rightarrow 2O\bullet - 2 + 2\ \text{Fd (oxidized)}$$

Afterward at PSI, resulted $2O^{\bullet -\ 2}$ gets converted into $H_2O_2$ through a membrane bound Cu/Zn SOD (Miller et al. 2010). Other sources of electron leakage in PSI are 2Fe-2S and 4Fe-4S clusters. In PSII system, leakage of electrons occurs through $Q^A$ and $Q^B$ electron acceptor which results into the production of $2O^{\bullet -\ 2}$, which further converts itself into a more toxic form like $OH^{\bullet}$ via Fenton reaction with $H_2O_2$ as an intermediate product. Chloroplast is also involved in oxidative stress induced programmed cell death as revealed in the study performed in transgenic tobacco where expression of anti-apoptotic Bcl-2 was studied (Chen and Dickman 2004). In this way, chloroplast acts as a potent site of ROS generation.

5. Peroxisomes

These are single membrane bound organelles and probably act as a major site for generation of $H_2O_2$ as they have an oxidative type of metabolism. Many metabolic processes like β-oxidation of fatty acids, glycolate oxidase reaction, the disproportionation of $O_2^{\bullet -}$, and the enzymatic reaction of flavin oxidases are responsible for the production of $H_2O_2$ (Baker and Graham 2013). Along with $H_2O_2$, $O_2^{\bullet -}$ radicals are also generated in peroxisome. In the peroxisomal matrix, Xanthine oxidase is situated which is responsible for conversion of anthine and hypoxanthine into uric acid which generates $O^{\bullet -}$ $^2$ as a by-product. Second site of $O^{\bullet -}$ $^2$ is NADPH dependent small ETC which is localized in peroxisomal membrane. This small ETC is consisted of NADH and cyt b, and releases $O^{\bullet -}$ $^2$ into cytosol after utilizing $O_2$ as an electron acceptor.

In addition to these sites, three peroxisomal membrane polypeptides having molecular weight of 18, 29, and 32 kDa are also responsible for $O^{\bullet -}$ $^2$ generation. To reduce cytochrome c, 18 and 32 kDa membrane polypeptides use NADH as the electron donor while 29 kDa protein uses NADPH as an electron donor in the reaction.

## 14.2 Antioxidative Systems in Plants: ROS Mitigation

Stress faced by plants usually falls in two broad categories: one is abiotic stress that is caused via non-living environmental factors like water deficit, salinity, high or low altitude, UV radiation, high wind velocity, etc., while the other category is biotic stress where living organisms are responsible for imposing stressful conditions namely caused by pathogen, bacteria, and fungi, parasites, animals, etc. Repercussions of any type of stress leads to an overall change in the internal stress regulatory machinery and subsequent generation of ROS, if ROS generation exceeds the level of mitigation, then it leads to a condition termed as oxidative stress which triggers cell toxicity. Furthermore, ROS production is linked to stomatal closure and dehydration, therefore disturbing the osmotic homeostasis of the plant Fig. 14.1.

### *14.2.1 Antioxidative Defence*

The antioxidative defence system consists of two parts of defence machinery, one is enzymatic and other one is non enzymatic. This ROS defence system overall protects the plant from the hazardous effects of abiotic and biotic stresses. Various enzymes such as Catalase (CAT), Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Dehydroascorbate reductase (DHAR), Monodehydroascorbate reductase (MDHAR), Guaiacol peroxidase (GPX), and Glutathione reductase (GR) make strong enzymatic antioxidative defence system in the plants to reduce ROS level and mitigate the effect of oxidative stress (Fig. 14.1). It also maintains the reduced glutathione (GSH) pool to balance redox state of a plant cell under stress (Gill and Tuteja 2010; Nahar et al. 2015). In addition, non-enzymatic antioxidants like

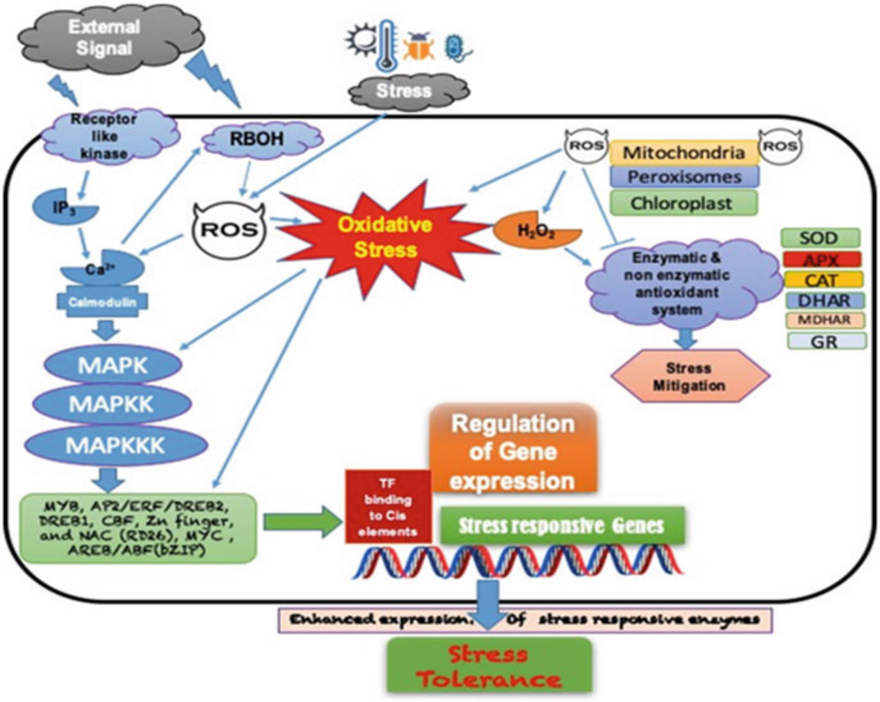

**Fig. 14.1** Drought stress tolerance: an insight to resistance mechanism and adaptation in plants

ascorbic acid and GSH also play an important role in ROS detoxification and they also act as photo protectant (Khan et al. 2012).

## 14.3 Enzymatic Antioxidative Defence System

Plant cell has got a very efficient and dynamic antioxidative defence system which comprises of various enzymes such as Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Catalase (CAT), Dehydroascorbate reductase (DHAR), Monodehydroascorbate reductase (MDHAR), Guaiacol peroxidase (GPX), and Glutathione reductase (GR).

### *14.3.1 Superoxide Dismutase (SOD)*

This enzyme makes a first line of defence against oxidative stress conditions. It belongs to the family of metalloenzymes which are universally found in all the aerobic cells. It catalyses the conversion of $O^{\bullet-\ 2}$ by dismutating it into $O_2$ and $H_2O_2$. Various studies have emphasized their role under stress and studies further indicated the upregulation of these enzymes during stress conditions (Boguszewska and Zagdańska 2012).

$$O^{\bullet -2} + O^{\bullet -2} + 2H^+ \rightarrow 2H_2O_2 + O_2$$

SOD can be classified into three main classes depending upon the type of metal it binds with, Mn SOD (found in mitochondria), Cu/Zn SOD (found in peroxisome, cytosol, and chloroplast), and Fe SOD (found in chloroplast).

### *14.3.2 Ascorbate Peroxidase (APX)*

APX is a vital part of Ascorbate-Glutathione cycle. It performs the same role as that of catalase, while catalase scavenges $H_2O_2$ in peroxisome, APX scavenges $H_2O_2$ in cytosol and chloroplast. It uses ascorbic acid as a reducing agent and reduces $H_2O_2$ to $H_2O$ and DHA

$$H_2O_2 + AA \rightarrow 2\ H_2O + DHA$$

APX is present in isoforms and depending upon the locations and amino acids, it can be found in cytosol, mitochondria, peroxisome, and chloroplast (Sharma and Dubey 2004). APX has a better affinity towards $H_2O_2$ than CAT and it is widely distributed inside the cell, so it effectively scavenges $H_2O_2$ at the time of prolonged stress conditions.

### *14.3.3 Catalase (CAT)*

It is a tetrameric heme containing enzyme and shows high affinity towards $H_2O_2$. It does not have greater affinity towards organic peroxide (R-O-O-R) and does not need any reducing equivalent; this property makes it unique among all antioxidant enzymes. Catalase has a very high turnover rate ($6 \times 10^6$ molecules of $H_2O_2$ to $H_2O$ and $O_2$ $\text{min}^{-1}$). It is found predominantly in peroxisomes as these are the prime site of $H_2O_2$ production due to various metabolic activities like photorespiration, purine catabolism, fatty acid oxidation, and oxidative stress (Mittler 2002). According to some recent studies, CAT is also found in other cell organelles like cytosol, mitochondria, chloroplast but CAT activity in these organelles is questionable (Mhamdi et al. 2010). Angiosperm plants are known to have three types of CAT gene; CAT1 gene is expressed in pollens and seeds, while CAT2 genes are reported in photosynthetic parts of the plant and CAT3 gene is expressed in vascular tissues and leaves. Catalase enzyme is known to scavenge $H_2O_2$ in thermodynamically efficient way during adverse conditions.

$$H_2O_2 \rightarrow H_2O + (1/2)\ O_2$$

### 14.3.4 Dehydroascorbate Reductase (DHAR)

It uses reduced Glutathione (GSH) as an electron donor and reduces dehydroascorbate (DHA) to Ascorbic Acid (AA) (Eltayeb et al. 2007). It maintains the redox state of the cell by regulating ascorbic acid concentration in apoplast and symplast and thus regenerates ascorbic acid pool inside the cell (Chen and Gallie 2006). It is majorly found in seeds, root, and green and non-green shoots.

$$\mathrm{DHA} + 2\mathrm{GSH} \rightarrow \mathrm{AA} + \mathrm{GSSG}$$

### 14.3.5 Monodehydroascorbate Reductase (MDHAR)

It uses NADPH as a reducing agent for regenerating ascorbic acid from MDHA which has a very short lifespan and therefore regulates AA pool inside the cell. It has several forms which are found in different cell organelles such as peroxisomes, cytosols, mitochondria, chloroplast, and glyoxysomes.

$$\mathrm{MDHA} + \mathrm{NADPH} \rightarrow \mathrm{AA} + \mathrm{NADP}^+$$

It is localized in peroxisomes and mitochondria along with APX as it supplies AA to APX by maintaining its pool inside the cell, which further oxidizes AA and scavenges $H_2O_2$ in the process (Mittler 2002).

### 14.3.6 Glutathione Reductase (GR)

It is a flavoprotein oxidoreductase and reduces GSSG to GSH by using NADPH as a reducing agent. Further MDHA and DHA regenerate ascorbic acid by using reduced Glutathione (GSH), which itself gets converted into oxidized form, i.e. GSSG. It is majorly situated in chloroplast with small presence in cytosol and mitochondria. It is an integral component of ASC-GSH cycle and maintains high ratio of GSH/GSSG by formation of disulphide bond in Glutathione disulphide (Asada 1999). Reduced

Glutathione (GSH) is a low molecular weight compound which acts as a reducing agent to prevent the oxidation of thiol group and further reacts with potent ROS like $^1O_2$ and $OH^•$.

$$\mathrm{GSSG} + \mathrm{NADPH} \rightarrow 2\mathrm{GSH} + \mathrm{NADP}^+$$

### 14.3.7 Guaiacol Peroxidase (GPX)

This enzyme contains heme group and throws out excess $H_2O_2$ in both normal and stressed conditions. It utilizes $H_2O_2$ during degradation of Indole Acetic Acid (IAA) at the time of stress and plays important role in lignin biosynthesis. It uses guaiacol and pyrogallol as electron donor (Asada 1999). It is active intracellularly as well as extracellularly so it is recognized as a key enzyme for the removal of $H_2O_2$.

$$H_2O_2 + \mathrm{GSH} \rightarrow H_2O + \mathrm{GSSG}$$

Apart from enzymatic protectants similar protective strategy employed by plants for stress mitigation is making use of osmolytes which are nontoxic, highly polar, and low molecular weight compounds and help to maintain membrane integrity by stabilizing protein function of plant under stress (Saxena et al. 2013; Slama et al. 2015). Proline, sucrose, trehalose, glycine betaine are few examples of osmolytes whose levels is frequently known to elevate under stressful conditions (Slama et al. 2015; Masood et al. 2016; Per et al. 2017). Another large group of compounds like amino acid, sugar, and polyhydric alcohols also acts as osmoprotectants and ROS scavengers under oxidative stress.

ROS can further be recognized by various ROS sensors that translate the signal to nucleus via a relay of redox reactions for translation of new proteins via activating downstream transcription factors and upregulating numerous stress-responsive genes via binding to corresponding cis acting elements present in their promotor region thereby imparting stress mitigation.

## 14.4 ROS Mediated Downstream Signaling and Transcriptional Regulation

Unfavourable conditions during life cycle of a plant compels plant cell to cope with harsh environmental conditions. Therefore, plants have evolved key signaling enzymes/molecules that can perceive external environmental stimuli and transduce them across membranes and cytoplasm which result in an interaction with regulatory elements present in the genome, thus eliciting an appropriate adaptive response for protection and stress mitigation. This intricate mechanism of signal transduction is mediated via alterations in the levels of indigenous ROS molecules. In normal course of homeostasis, ROS are countered by various enzymatic or non-enzymatic antioxidants, however, under oxidative stress these may further be perceived by various downstream receptors which help transmitting the signal for eliciting the appropriate response thereafter. This signal transmittance is mediated via alteration key intermediates namely $Ca^{2+}$, $Ca^{2+}$ binding protein mainly calmodulins, G protein activation and alteration in phospholipid signaling. Among all forms of ROS, $H_2O_2$ is supposed to be the most common form leading to the generation of calcium intermediates that modulate kinases like CDPKs and CBLs-CIPKs which are finally responsible for activating RBOH (respiratory burst oxidase homologues). RBOHs are membrane bound proteins having an oxidase domain, localized extracellularly and found responsible for generation of superoxide radical while an N-terminal calcium-binding domain, responsible for phosphorylation and contains EF hands, is localized towards the cytoplasmic side. (Ogasawara et al. 2008; Takeda et al. 2008). ROS usually have short life span therefore both sensing machinery and transducing mechanism need to be quick, efficient, and robust. Several intercellular and intracellular signaling cascades are activated as soon as ROS is generated and sensed and lead to a concomitant alteration in several downstream partners. The mechanisms that mostly prevail for ROS sensing are namely protein kinase and transcription factor based sensing and protein phosphatase inhibition based sensing which has been discussed in detail in the later part of the topic. These protein kinases are known to be the largest group of functional enzymes present in eukaryotes. Reports indicate that the plant genome consists of serine/threonine and histidine type protein kinase, however, in addition to above mentioned kinases the animal genome consists of Tyrosine kinase (Liang and Zhou 2018).

According to the plant diversity, protein kinases are categorized into four major clusters namely receptor-like kinases, Mitogen Activated Protein kinases, Calmodulin-like domain protein kinases, and Calcium dependent kinases. Apart from these, few less characterized kinases like SNF1/AMPK, PDK1, HKs, DGKs are also reported which have specific role in stress signaling (Chakradhar et al. 2019). Calmodulin-like domain protein kinases (CDPKs) and receptor-like kinases (RLKs) are most specific to plants but absent in other eukaryotic forms. Gauthier et al. (2011) reported two ROS sensors in *Arabidopsis thaliana*, namely LRR (leucine-rich repeat) and (RLKs) cysteine-rich receptor-like kinases. Domains of both these receptors are located extracellularly and known to modify while sensing ROS thus were found helpful in timely mitigation of oxidative stress. Further, authors reported that knockout lines for both these receptors were found susceptible against oxidative stress which signified their utility. Similarly COLD 1 is reported to be a sensor for cold stress and known to interact with some integral proteins thus regulating calcium gates in *Oryza sativa* (Zhu 2016). A sudden change in temperature following fluctuation in environmental conditions is found responsible for altering the fluidity of the membrane that is recognized by integral proteins namely receptor-like kinases (RLKs). Another kinase having an important role in ROS receptivity is OXI 1 (an oxidative signal inducible 1 kinase) and a serine/threonine protein kinase is known to trigger mitogen activated proteins and downstream transcription factors. MAPK cascade involves MAPKK kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK where each one is activated via phosphorylation of adjacent kinase thus transferring the signal that further relays stress mediated responses downstream (Fig. 14.1). Apart from enzymes like kinases, other intermediates that play a key role in modulation of gene/protein expression and protective osmolytes are the stress inducible transcription factors (TF). TFs possess a DNA-binding domain and a transcriptional activation/repression domain that facilitate its binding to cis regulatory elements (CREs) present in the promoter region of numerous stress related genes, which allow activation/deactivation of those genes thus allowing TFs to serve as a molecular switch. Transcription factors, the key factors against oxidative stress tolerance, are mostly characterized predominantly into following subfamilies viz. MYB, AP2/ERF, bZIP, Zn finger, and NAC (Riechmann et al. 2000).

MYB transcription factors bears a conserved MYB repeats (R) towards N-terminus to perform DNA-binding and protein–protein interactions and a variable region towards C-terminal responsible for the regulatory activity present in the protein. According to Chen et al. (2015), *GbMYB5* gene was able to impart drought tolerance in cotton species as well as in transgenic tobacco by regulating the genes related to ROS scavenging namely genes for polyamine biosynthesis, late embryogenic abundant proteins LEA, and other genes responsible for drought response like RD26. Another class of transcription factors belonging to a subfamily APETALA2/ethylene response element-binding factors (AP2/ERF) type is majorly responsible for promoting plant development. It is also involved in providing stress tolerance against both biotic and abiotic stress. (Xu et al. 2011; Sharoni et al. 2010). AP2/ERF class has further been characterized into four subfamilies viz. AP2 (Apetala 2), RAV (related to ABI3/VP1), DREB (dehydration-responsive element-binding protein), and ERF (Rashid et al. 2012; Sharoni et al. 2010). Next class of TF (bZIP type transcription factors) that belongs to basic leucine zipper family, bears conserved N terminal domain was responsible for DNA binding and a C terminal leucine-rich motif was responsible for protein dimerization (Wang et al. 2015). This class of transcription factors is also known to interact with phytohormone ABA for providing drought tolerance in Rice and Arabidopsis. Studies have indicated that over expression of OsbZIP23, OsbZIP46, OsbZIP16 in rice and TabZIP60 in Arabidopsis was able to impart an enhanced tolerance against stresses like drought, salinity, and freezing as well as increased sensitivity to ABA (Zhang et al. 2015). Next class belongs to Zinc-finger proteins (ZFPs) which are consisted of 23 different subfamilies. Most characterized class among this family is WRKY transcription factor, which has a proven role in biotic and abiotic stresses alleviation (Li et al. 2014). This protection is said to be mediated via maintaining equilibrium between ROS generation and scavenging process (Baek et al. 2015). This fine-tuning helps in maintaining the levels of metabolites namely proline, chlorophyll, soluble sugars, as well as ABA (Wang et al. 2016a, b). Zinc-finger structure is diversified on the basis of presence of C and H residues which are present in secondary structure of these transcription factors. On the basis of C and H repeats these DNA-binding Zn fingers are classified into nine diverse groups: C2H2, C8, C6, C3HC4, C2HC, C2HC5, C4, C4HC3, and C3H (Luo et al. 2012; Gupta et al. 2012). Next type (NAC gene family) is the largest gene family and has most diverse role from development to leaf senescence, and from developing lateral roots to stress acclimation (Nuruzzaman et al. 2013). Majorly NAC gene family is named by the presence NAC domain that is formed collectively by NAM (for no apical meristem), ATAF 1 and 2 (Arabidopsis transcription activation factor), and CUC2 (for cup-shaped cotyledon). This family consists of a DNA-binding conserved N terminal nuclear localizing DNA-binding domain and a variable C terminal region responsible for regulating transcription (Olsen et al. 2005). Several researchers highlighted the important role of NAC variant's viz. OsNAC2/6, OsNAC10, SNAC1 genes/transcription factors, when over expressed in rice under stress, conferred enhanced stress tolerance, and enhanced grain yield, respectively. (Hu et al. 2006). Thus it could be concluded that as plants routinely face severe stressful regimes so they synchronize ROS level via activating/repressing several stress inducible transcription factors, protein kinases, and phosphatases. These factors work in tandem to regulate the expression of stress-responsive genes either positively or negatively for mitigating the lethal effect of oxidative stress.

## 14.5 Conclusion

Environmental stress namely biotic and abiotic, inadvertently disturbs the internal homeostasis of plants resulting in compromised growth and development, thus affecting the final yield potential. These stressful conditions lead to overproduction of ROS which are highly reactive molecules and alter the membrane properties via lipid peroxidation. In addition, it also makes irreversible damages to nucleic acids, lipids, and proteins thus hampering its proper functioning which is termed as oxidative stress. Plant possesses an efficient antioxidative defence system that counters the deleterious effect of ROS and oxidative stress with the help of enzymatic and non-enzymatic antioxidative defence pathways. Interestingly at lower level, ROS is also known to function as secondary messenger having several receptors for downstream relay of stress signal. ROS influences various proteins and enzymes namely protein kinases, which are categorized as receptor-like kinases, Mitogen Activated Protein kinases, Calmodulin-like domain protein kinases, and calcium dependent kinases. ROS also regulates levels of various stress inducible genes and transcription factors for stress abatement. While functioning as signaling molecule, a cross-talk exists between ROS and various regulatory genes/transcription factors, namely MYB, WRKY, DREB/ERF, and NAC which imply that stress tolerance is a complex interplay and regulated by an intricate regulatory mechanism. As every year we lose a significant portion of our crop yield due to these stresses therefore in order to achieve global food security and to feed burgeoning human population it becomes imperative to further decipher the role of ROS and its types, and signaling for stress mitigation and its complex interplay with defence related pathways in plants.

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# Chapter 15
# Bacterial Antagonists Effective Against Soil Borne and Foliar Pathogens

**Stanzin Idong and Anil Kumar Sharma**

**Abstract** Diseases affect annual and perennial woody plants severely, hence lead to major food losses and damage to natural ecosystem. Management of plant diseases is urgently required to revive economic and ecological losses. Chemical pesticides are heavily exploited on large scale to overcome the hurdle of these losses. But as the world is facing the problems of human health hazard and ground water pollution due to injudicious use of agrochemicals (especially pesticides) in agriculture and public sector therefore a large group of agriculture scientists is looking forward to develop ecofriendly, harmless, and sustainable system of bioinoculants, in the form of antagonistic bacteria. Bacterial antagonist acts as potential and effective biocontrol agent for most of the plant diseases occurring by soil borne pathogens or air borne pathogens. Plant pathogens can be suppressed with the induction of bacterial antagonist as they produce a variety of secondary metabolites with different biological functions such as inhibitors, antimicrobial, and organic matter degrading enzymes. These bacteria also activate defense mechanisms of plants against various diseases.

## 15.1 Introduction

Microorganisms are generally found associated with the plant rhizosphere, which is considered as a hot spot of microbial diversity (Santoyo et al. 2016). These microorganisms are involved in competition among themselves which could be synergistic or antagonistic with plants. On the basis of the location on plants these bacteria can be categorized as epiphytic and endophytic where they live sharing numerous characteristics for host plant growth promotion. Depending on the physiochemical properties of soil, per gram of soil contains several hundred million of bacteria

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(Toppo and Naik 2015). Different rhizospheric bacteria have been reported to possess antagonistic activities against plant fungal and bacterial plant pathogens such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis*, *B. amyloliquefaciens*, *Trichoderma virens*, *Burkholderia cepacia*, and *Gliocladium* spp. (Kloepper et al. 1989).

Plant growth promoting rhizobacteria (PGPR) constitute a group of microorganisms, which is used as biofertilizer and found to promote plant growth by increasing nutrient availability, phytostimulation, and biocontrol properties (Pandey and Malviya 2014). Population of microbes can increase or decrease depending on the changes in soil condition, i.e., moisture, temperature, or substrates like carbon. Bacteria/fungi generally release different antibiotics to suppress the growth of other microbes in the soil. The root exudates in the rhizospheric region have numerous essential nutrients and growth factors to support the growth of important bacteria. The wide effect of rhizosphere is usually observed with bacteria rather than Actinomycetes and fungi. Groups of gram negative, rod shaped, and nonsporulating bacteria which respond to root exudates are dominant bacteria (*Pseudomonas*, *Agrobacterium*).

The bacteria isolated from rhizosphere soils are beneficial to the plants by directly having an effect on nitrogen fixation, solubilization of nutrients, production of siderophores, β 1,3-glucanase, antibiotics, fluorescent pigments, hydrolytic enzymes, and stimulation of phytoalexin or flavonoids like compounds. Genus like *Azotobacter*, *Actinomycetes*, *Bacillus*, *Azospirillum*, and *Pseudomonas* produce auxin that stimulate plant growth. It has been estimated that 80% bacteria isolated from rhizosphere can produce plant growth regulators (Patten and Glick 1996). These strains must be competent, able to survive and colonize in the rhizospheric soil.

In agriculture many phytopathogenic fungi, bacteria, and viruses affect the crops and lead to develop different diseases, poor crop health, and ultimately yield loss. To overcome the losses pesticides are invariably used in agriculture practices. However population is growing at a faster pace and in order to feed huge population the chemicals are being used blindly and un-judiciously on large scale. Unfortunately the use of agrochemicals causes environmental pollution and health problems in human. Thus the alternative of costly agrochemicals is the antagonistic microorganisms that are found in association with host plants.

## 15.2 Bacterial Antagonists Effective Against Soil Borne Pathogens

Tripathi and Johti (2002) studied some potential bacterial isolates from the rhizosphere of pea and wheat against maize sheath blight caused by *Rhizoctonia solani*. Few isolates were effective against multiple diseases and some were effective against only one pathogen showing their diversity. Tiwari and Thrimurthy (2007)

**Table 15.1** Bacterial antagonist effective against different soil borne diseases

| Crop | Antagonist | Disease | Pathogen | References |
|---|---|---|---|---|
| Tomato | *Bacillus spp.2P2* | Collar rot | *Sclerotium rolfsii* | Sahu et al. (2019) |
| Rice | *Bacillus amyloliquefaciens* spp. | Sheath blight | *Rhizoctonia solani* | Abbas et al. (2019) |
| Faba bean | *Rahnella aquatilis B16C* | Root rot | *Fusarium solani* | Bahroun et al. (2018) |
| Tomato | *Bacillus amyloliquefaciens FBZ24* | Wilt | *Fusarium oxysporum f. sp.lycopersici* | Elanchezhiyan et al. (2018) |
| Cucumber | *Paenibacillus polymyxa* | Wilt | *Fusarium oxysporum sp. cucumerinum* | Du et al. (2017) |
| Apple | *Bacillus amyloliquefaciens GB1* | Valsa Canker | *Valsa mali* | Zhang et al. (2015) |
| Rapeseed | *Bacillus subtilis EDR4* | Sclerotinia rot | *Sclerotinia sclerotiorum* | Chen et al. (2014) |
| Rice | *Bacillus subtilis MBI 600* | Sheath blight | *Rhizoctonia solani* | Kumar et al. (2012) |
| Tomato | *Bacillus subtilis BSF 4* | Southern blight | *Sclerotium rolfsii* | De Curtis et al. (2010) |
| Banana | *Pseudomonas*, *Burkholderia* | Fusarium wilt | *Fusarium oxysporum f. sp. cubense* | Fishal et al. (2010) |
| Cotton | *Pseudomonas* spp. | Verticillium spp. | *Verticillium dahlia* | Erdogan and Benlioglu (2010) |

isolated 21 isolates of *Pseudomonas fluorescens* from the rhizosphere of maize, rice, wheat, and chickpea from Raipur and Bastar region, where seven isolates showed bright fluorescence and also showed positive response of siderophore production. Among the isolates, PFR 1 and PFR 2 were significantly superior in increasing shoot and root length of plants. In vitro evaluation has confirmed the antagonistic ability of the two isolates against *Pyricularia grisea* and *Rhizoctonia solani* in dual culture test.

More than 10,000 species of fungi can cause disease in plants (Agrios 2005). A common fungal pathogen in agriculture crops is *Rhizoctonia* spp. existing in soil and attacks more than 200 species of crops. *Fusarium* has long been known for causing wilts in many species and considered as an important plant pathogen (Toppo and Naik 2015). Other important plant diseases are root rot, wilt, and sheath blight for which many antagonistic bacteria have been isolated and tested. Various *Pseudomonas sp.*, evaluated for antifungal activity against soil borne fungal plant pathogen, showed reduction in mycelium growth of *Fusarium oxysporum* (Table 15.1).

## 15.3 Bacterial Antagonists Effective Against Foliar Pathogens

Different biocontrol agents isolated so far are mainly found responsible for the management of soil borne pathogens as destruction due to the pathogen is more severe than foliar pathogens. The effective control of *A. alternata* of citrus, litchi, and muskmelon was observed with *Bacillus subtilis* (Jiang et al. 2001). In this study, rhizobacterial isolates were tested against the growth of *Alternaria brassicae* and mustard plants. Mates et al. (2019) reported that out of 29 isolates, *Bacillus velezensis* GF267 was considered to be a multi-site antagonist and controlled tomato bacterial spot. When *Bacillus velezensis* GF267 was applied on soil it increased the activity of peroxidase and polyphenoloxidase, amount of chlorophyll content, and reduced disease intensity (Table 15.2).

## 15.4 Mechanism

Various mechanisms involved in the inhibition of plant pathogens include secretion of lytic enzymes, siderophores, antibiotics, and volatile organic compounds (VOCs) emitted from bacterial antagonists which influence the mycelial growth of the soil borne phytopathogenic fungus *Rhizoctonia solani* Kühn. The biological control of pathogens can result from a combination of mechanisms, including competition for nutrients and space, antibiotic production, and induced systemic resistance. Iron depletion by the *M. pulcherrima* in the medium inhibited the mycelial growth and conidial germination of *B. cinerea*, *A. alternata*, and *P. expansum*. *Pseudomonas* known to produce the antibiotic 2, 4-diacetylphloroglucinol (DAPG) may also induce host defenses. Additionally, DAPG-producing bacterial antagonists can aggressively colonize root (Heydari and Pessarakli 2010). 2-phenylethanol isolated from *K. apiculata* (an antifungal volatile) has inhibitory effect on green and blue mold of citrus (Liu et al. 2014). Exo-β-1, 3-glucanase secreted by *P. membranifaciens* had a role in the biocontrol activity against *B. cinerea* on grapevine (Masih and Paul 2002).

## 15.5 Conclusion

Antagonistic bacteria can be effective in controlling the plant diseases through different mechanisms. These bacteria not only reduce the disease symptoms of plants by pathogens but also enhance plant growth and development. Farmers, in general still rely on the use of synthetic fungicides to control plant diseases. However, the misuse of these chemicals may cause serious environmental and health problems. Microbial antagonists are potential agents that can be explored to provide

**Table 15.2** Bacterial antagonists effective against foliar diseases

| Crop | Antagonist | disease | Pathogen | References |
|---|---|---|---|---|
| Tomato | *Bacillus subtilis* | Early blight | *Alternaria solani* | Awan and Shoaib (2019) |
| Rice | *Phosphorus solubilizing bacteria (PSB)* | Bacterial leaf blight | *Xanthomonas oryzae pv. oryzae* | Rasul et al. (2019) |
| Tomato | *Bacillus velezensis GF 267* | Bacterial leaf spot | *Xanthomonas perforans*<br>*Xanthomonas gardneri* | Mates et al. (2019) |
| Rice | *Serratia nematodiphila CT-78* | Bacterial leaf blight | *Xanthomonas oryzae pv oryzae* | Khoa et al. (2016) |
| Grapevine | *Bacillus pumilus*<br>*Paenibacillus* spp. | Grapevine trunk disease | *Phaeomoniella chlamydospora* | Haidar et al. (2016) |
| Potato | *Bacillus subtilis* | Late blight | *Phytophthora infestans* | Kumbar et al. (2019) |
| Rice | *Bacillus amyloliquefaciens BS5* | Brown spot | *Helminthosporium oryzae* | Prabhukarthikeyan et al. (2019) |
| Tomato | *Pseudomonas fluorescence SF4c* | Bacterial spot | *Xanthomonas vesicatoria* | Principe et al. (2018) |
| Cotton | *Bacillus licheniformis OE-04* | Anthracnose | *Colletotrichum gossypii* | Nawaz et al. (2018) |
| Mustard | *Bacillus amyloliquefaciens + earthworm* | Leaf blight | *Alternaria brassicae* | Ayuke et al. (2017) |
| Zucchini | *Serratia marcescens* | Powdery mildew | *Podosphaera xanthii* | Tesfagiorgis et al. (2014) |
| Tomato | *Streptomyces griseoviridisK61* | Early blight | *Alternaria alternata* | Cuppels et al. (2013) |
| Tomato | *Streptomyces lydicus WYEC108+ Pseudomonas fluorescens A506* | Anthracnose | *Colletotrichum phomoides* | Cuppels et al. (2013) |
| Sweet orange | *Bacillus subtilis ACB-69* | Postbloom fruit drop | *Colletotrichum acutatum* | Kupper et al. (2012) |
| Chili | *Pseudomonas fluorescens* | Powdery mildew | *Leveillula taurica* | Anand et al. (2010) |

effective and safe means to manage plant diseases. Several microorganisms have been tested and proven to possess antagonistic properties against plant pathogenic fungi to develop sustainable agriculture. Control of plant disease is very crucial and use of biocontrol agents is an alternative approach against chemical pesticide. Further exploitation and work are required to carry out on antagonistic bacteria to produce a commercial biocontrol agent for controlling phytopathogens.

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