Harikesh Bahadur Singh
Anukool Vaishnav
R. Z. Sayyed *Editors*

# Antioxidants in Plant-Microbe Interaction

Springer

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# Preface

Antioxidants have numerous biological roles in plants due to their contribution to signaling pathway. These compounds can be defined as secondary metabolites and are produced besides the primary biosynthetic pathway and metabolic routes of chief biological compounds associated with growth and development. In plants, interactions of the antioxidants are mostly associated with defense mechanism and signaling, especially in oxidative stress produced during abiotic stress or pathogen attack. In addition, these antioxidants are also part of nutritional content in plant products, which increase nutraceutical property of any crop plant, i.e., medicinal, spices, and vegetables. Furthermore, these compounds are also released by plant exudates that are involved in interaction with other organisms in plant holobiont. Among the two types of antioxidants in higher plants, enzymatic antioxidants include catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), peroxiredoxins (Prx), superoxide dismutase (SOD), dehydroascorbate reductase (DAR), monodehydroascorbate reductase (MDAR), peroxidases (PODs), glutathione *S*-transferases (GSTs), glutathione reductase (GR), and thioredoxins (Trx). These enzymes are directly involved in reactive oxygen species (ROS) scavenging or ROS by-products. Nonenzymatic antioxidants, such as ascorbic acid (vitamin C, ASC), glutathione (GSH), tocopherols (vitamin E), and carotene, work in correlation with antioxidant enzymes to alleviate oxidative stress or directly involve in plant growth, development, hormone signaling, cell cycle, and defense response.

The microbiome of plant holobiont has the ability to control antioxidant production and signaling in plants and vice versa. The plant-associated microbes induce antioxidant levels in plants to cope with stress conditions. Simultaneously, plants release some specific types of antioxidants through their exudates that recruit beneficial microbes in the surroundings. The understanding of the biosynthesis, signaling, and function of antioxidant compounds in plants during stress conditions will allow us to harness their activities in plant-microbe interactions and other rhizospheric signaling as a means to increase or restore plant ecosystem productivity and to improve plant responses to a wide range of stress conditions. These antioxidants contribute to a wide range of natural product formulations for agricultural practices. In addition, antioxidants have huge commercial and industrial applications due to their wide bioactive potential and biological activities. The main focus of this book is to highlight the role and potential of various antioxidant compounds in plant-microbe ecosystem for the management of plant growth under biotic and abiotic stresses. Accordingly, we have incorporated five comprehensive parts focusing on the types and biosynthesis of antioxidants and their role in microbe-mediated plant growth promotion under stress conditions.

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|---|---|
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# Part I

# Introduction of Antioxidants in Plant-Microbe Interaction

# Antioxidants in Plant–Microbe Interaction

# 1

Ajay Kumar Singh, Shailesh Kumar, and Trisha Sinha

## Abstract

Plant's continuous interaction with varieties of microorganisms through its root has been an important topic of discussion since decades. The type of interaction, duration, and changes due to interaction vary according to the type of microorganism and host plant both. Some of the nonpathogenic or harmless microorganisms form symbiotic association with plant root with the exchange of plant root exudates containing nutrients for those. In return, this mutual understanding between symbiont and plant has gifted plant with lots of improved mechanisms to mitigate negative impacts resulted due to invasion of various microorganisms. Formation of reactive oxygen species (ROS) is a common and unavoidable process in all aerobic life forms, be it plant or animal. But the problem appears when larger amount of ROS formation takes place. The rate of ROS production in plant gets faster due to occurrence of various microorganisms. Pathogenic microorganisms effect plant negatively and promote production of ROS which creates toxicity. Plants, naturally, have answer to neutralize the ROS-generated toxicity through their inherent defense system but sometimes plants have to rely on some outer sources to get some extra strength to face the challenges due to pathogen attack. Symbionts have been reported to boost the natural defense system in plants through directly helping in more production and up-regulation of defense-related molecules like phytohormones, genes, etc. Needless to say, antioxidants are molecules that are continuously dedicated to minimize oxidative stress due to ROS generation and their toxicity. These antioxidants, viz. enzymes (catalase, superoxide dismutase, enzyme components of AsA-GSH cycle, etc.) and nonenzyme (tocopherols, carotenoids, glutathione,

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etc.) compounds help plant stand in stressful situation through thoroughly giving support to plant in providing defense. Changes due to pathogen attack in plant and how a plant response to those is still not revealed to its best. But it can be said that this complex relationship between pathogen and plant could be understood better with thorough study of plant's interaction with pathogens and plant's acquired as well as induced defensive mechanisms through recruitment of vast network of antioxidants.

**Keywords**

Plant pathogens · Symbionts · ROS · Oxidative stress · Enzymatic and nonenzymatic-natured antioxidants · Defensive mechanisms

## 1.1 Introduction

Plant root is constantly in contact with numerous kinds of microorganisms. Pathogenic microorganisms create an ambience unfavorable for plants, thus make plants adopt some defense mechanisms. Nonpathogenic microorganisms, in the contrary, do not create negative impacts and some of them develop symbiotic association with plant through colonization in root. These symbionts have some beneficial roles in plant. In all aerobic life forms, viz. plants and animals, the formation of cellular reactive oxygen species (ROS) as a by-product of their intrinsic metabolisms is an inevitable process (Foyer and Harbinson 1994; Heyno et al. 2011; Bailey-Serres and Mittler 2006; Sharma et al. 2012). Examples of some major ROS include superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), etc. (Torres 2010; Mendoza 2011). Naturally, ROS at lower concentration do not possess toxic effects. The production of these ROS gets faster when plants have to face several stresses caused by biotic and abiotic factors. Biotic stress in plants is caused by the occurrence of different kind of pathogens such as fungi, viruses, bacteria, and nematodes (Dangl and Jones 2001). These harmful pathogens develop adaptive mechanisms with the target of sustaining themselves within plant life. In plants, these pathogens interfere with the growth and physiology and leave impacts on plants through promoted production of ROS (Mellersh et al. 2002; Samsatly et al. 2018). Formation of these ROS in plants costs molecular oxygen ($O_2$) to get reduced upon exposure to high energy input reactions, viz. reaction of electron-transfer involving plasma membranes, mitochondria, and chloroplast (Del Río et al. 2006; Blokhina and Fagerstedt 2010) in a step-worthy manner (Sharma et al. 2012). Now, these ROS, when formed in ample amounts, create toxicity and thus seem to be harmful for plant life and processes (Ryter et al. 2007), and cause necrosis and death of plant (Pitzschke et al. 2006). The ROS, which are generated by the partial reduction of molecular oxygen, are highly reactive in nature and cause damage to the macromolecules such as nucleic acids, proteins, and lipids by denaturing those (Mendoza 2011). Detoxification of these reactive oxygen intermediates requires cells to recruit a vast network of antioxidants. The function of an antioxidant is to either prevent or slow down the ROS-generated oxygenation by donating an electron to the unstable ROS, thereby oxidizing itself in place of cellular macromolecules which are highly susceptible to ROS. Therefore, antioxidants serve as very crucial components in terms of developing defense against damage caused by oxidative stress resulted due to ROS formation in plant when invaded by microorganisms. The mechanisms plants implement in order to sense and understand the invasion by various microorganisms and thereafter carry out transduction process to carry this received information to the elicitors for developing appropriate defense system are not broadly revealed. The role of beneficial microorganisms is found here. These microorganisms strengthen plant defenses through inducing more production of antioxidants to cope up with any stress. In view of these, this chapter was planned to describe all possible mechanisms of plant–pathogen interaction and role of antioxidants in amelioration of ROS-generated effects on plants under biotic stresses.

## 1.2 Types of Plant Pathogens and Their Characteristics

There exist a number of plant pathogens which have separate level of host-interaction and separate functions. These differences in the level of host specificity of phytopathogens and their degree of interactions they hold with their host plant could be attributed to their mode of infection, and also surrounding environmental factors (Ziska and Runion 2007). The term host specificity is actually reserved for extreme cases when preference to host is restricted narrowly. Pathogens like *Xylella fastidiosa* are able to rely on and infect a broad range of host plants from several genera as well families (Hopkins 1989), whilst some other pathogens are capable of infecting a narrow range of host plants which are closely related or better known as host biotypes (Agrios 2005). Functionally, plant pathogens can be classified with various categories like necrotrophic, biotrophic, and hemibiotrophic. To obtain nutrients from host plant, necrotrophic pathogens have to kill the host cells first, so, plants rarely develop interactions with these pathogens to restrain their active cell metabolisms to be interfered. Examples of necrotrophic pathogens include gray mold fungus *Botrytis cinerea,* bacterial pathogen *Erwinia carotovora,* etc. On the other hand, biotrophic pathogens depend for nutrients on living cells only, thus plant–pathogen interaction in this case is long and complicated too (Schumann and D'Arcy 2006). Fungus *Blumeria graminis*, bacterial pathogen *Xanthomonas oryzae* are some of the biotrophic pathogens. Obligate (biotrophic) pathogens generally have more specific host requirements than facultative (necrotrophic) pathogens. Once plant gets infected by either necrotrophic or biotrophic pathogen, air temperatures and water potentials of host plant control the rate of colonization of the host tissues, production of new inoculum, and the expression of disease symptoms by the host as well (Colhoun 1979; Campbell and Madden 1990). In case of hemibiotrophic pathogen, the pathogen initially relies on living cells for nutrient requirement but kills cells at later stages of infection.

## 1.3 Changes Occurred in Plants When Interacted with Pathogens

Plants act as a shelter or host for a number of pathogens that include viruses, bacteria, fungus, and nematodes. Pathogens which intend to complete their vital processes of life within a host plant, exhibit negative impacts on plants. Plants at the first understand that it had been attacked by pathogens and then carry out several changes to fight back for survival against pathogens. To infect the plant, pathogens aim to develop diseases for which favorable environmental condition needs to be present. For survival on the host plant and inducing infection, pathogen carries out production of propagules and their germination which in turn are often controlled by air temperature and air moisture (Colhoun 1973, 1979). Environmental features, i.e., slightly warmer temperatures, frequent rainfall can be more favorable for resulting in more detrimental effects (Campbell and Madden 1990). Thus, a triangle is formed among plant, pathogen, and environment for occurrence of disease (Singh et al. 2018). Avirulent pathogens have been often observed to induce accumulation of ROS in a biphasic manner. Firstly, the transient phase witnesses slow ROS accumulation in a small quantity, followed by a larger accumulation in the second or continuous phase with the target of developing host plant's disease resistance (Torres et al. 2006; Heller and Tudzynski 2011). On the other side, virulent pathogens which cause cell damage in larger extent, lead the transient phase induction of ROS only (Bolwell et al. 2002), not giving the host plant greater scope to develop resistance to disease. Biotrophic pathogens respond to the oxidative burst and as well as able to minimize it, while necrotrophic pathogens intend to exploit the oxidative burst in plants for their sustaining (Heller and Tudzynski 2011). Therefore, disease progression in plants by the necrotrophic pathogens is completely up to their ability to manipulate or surpass ROS-generated plant defenses, and as they have great sensitivity to ROS, detoxification of ROS is also mandatory for them while targeting their host (Samsatly et al. 2018). Plants have already acquired an improved defense system through their antioxidants which plants use when attacked by several stresses (Ballhorn et al. 2009). This already-existing along with some induced defense responses of plants imposes stress on the pathogen, and provides needful signals (Shalaby and Horwitz 2015). A number of changes has been observed in plants afterwards, i.e., after disease development following the triangle formation. Various kinds of changes due to different pathogen attack in plants are discussed below.

### 1.3.1 Changes Due to Plant–Fungi Association

Proteomic studies have explored the plant–fungi interaction and changes due to this association. To develop pathogenicity, fungi deploy a number of proteins to be either up-regulated or and down-regulated (Murad et al. 2007). A group of proteins such as peroxidase, polygalacturonase, chitinase, and a subtilisin-like protease was found in pathogen-infected tomato plant by Houterman et al. (2007). Proteomic approaches like gel electrophoresis, MALDI-MS/MS have revealed the dynamic roles of these proteins like responding to stress and build defense, signal transduction, taking part in electron transport system, photosynthesis, cell wall degradation, and protection, etc. (Yajima and Kav 2006; Singh et al. 2018).

### 1.3.2 Changes Due to Plant–Bacteria Association

Bacteria acquired five pathways for creating pathogenicity by participating in translocation of the bacteria-secreted proteins into the host plant cell (Lee and Schneewind 2001). These all pathways are involved in dynamic functions, from secretion of multiple toxins like hemolysins, rhizobiocin by type-I and export of these secreted proteins, toxins for building virulence by type-II (Singh et al. 2018) to counteracting virulence through secretion of Avirulence (Avr) proteins, *Xanthomonas* outer protein (Xop), *Pseudomonas* outer proteins (Pop), etc. by Type III (Noel et al. 2001). Other pathways are involved in regulation of various proteins and genes.

### 1.3.3 Changes Due to Plant–Nematode Association

Pathogenic nematodes like *Globodera* spp., *Heterodera* spp., *Meloidogyne* spp., etc. bring several changes in plants (Chitwood 2003). Gene products like β-1,4 endoglucanase (cellulase) developed by *Globodera* spp., pectate lyase, and polygalacturonase by *Meloidogyne* spp. have been found as cell wall degraders. These nematodes also alter nutrient and water balance in plant, thus hamper plant overall growth (Curtis 2007).

### 1.3.4 Changes Due to Plant–Virus Association

Viruses themselves are not able to directly infect plants, for which they hire transmitting factors known as vectors for development of diseases in plants. Viruses also have both compatible and incompatible interactions with host plant. For initiation of any interaction, viruses at first take shelter in host proteins for completion of their replication and influence those host proteins act against the pathogen-induced infection (Mehta et al. 2008).

### 1.3.5 Steps of Pathogenicity Development

In order to infect plant and develop diseases, pathogens have to go through three following steps, viz. perception, signaling, and response. Perception which describes how pathogen and host recognize each other, may take place either directly or indirectly. For this, plant cells perceive information about the invader pathogen and then carry out the second step, i.e., signaling. For signaling the information, there is a chain of reactions that take place sequentially to ultimately take the information to the molecules recruited for showing responses. Third step, i.e., response occurs here and plant cells get ready to exhibit response mechanisms with the target of ameliorating the stress impacts. Constitutive defensive responses include cell wall modification, developing waxy cuticle layer, etc.; while the induced plant defenses include programmed cell death (PCD), phytoalexins secretion, secretion of pathogenesis-related proteins (PR-proteins), etc.

## 1.4 Types of ROS, Their Functions and Effects in Plants

Reactive oxygen species are a class of free radicals and ions of reactive nature, derived from the breakdown of oxygen molecule in presence of various stresses in plants. An estimated 1.0% of total $O_2$ consumed by plants has been reported to be directed towards formation of ROS (Asada and Takahashi 1987). Role of ROS has been confined in dynamic areas (Shetty et al. 2008). The effects and severity of different ROS on plants depend on their accumulation in plant cell. When available in low concentration, ROS possess some beneficial roles in signal transduction; in contrast to their ample availability leads to cell damage by various means (Sharma et al. 2012). As per some previous research works, ROS formed during oxidative burst (Kannojia et al. 2017) not only act as protectant against pathogen attack, but also promote signaling activity related to plant defenses (Van Breusegem et al. 2008; Mendoza 2011). Molecular oxygen ($O_2$) when stable is a harmless molecule. The presence of two unpaired electrons with parallel spin makes oxygen molecule inactive. To get activated, oxygen molecule has to either absorb sufficient energy to cause the spin reversal of one of the unpaired electrons for formation of singlet oxygen or carry out monovalent reductions step by step for formation of other ROS, viz. $O_2^{\bullet -}$, $H_2O_2$, and $OH^{\bullet}$ (Apel and Hirt 2004; Sharma et al. 2012).

After being formed, ROS take part in various activities in plant. In spite of all those negative roles described (Table 1.1), ROS molecules have some prominent roles in plant through inducing growth and development by various ways. Oxidative burst, resultant of ROS production, could be observed to leave a direct impact on phytopathogen or their induced defenses (Mendoza 2011; Sharma et al. 2012). These ROS form an important sensory system to enable host plant perceive chemical signals from their pathogens and then to translate the sense received into biochemical responses required for defense development (Hancock et al. 2001). These ROS have distinct association with hypersensitive response (HR), a localized response at the place of pathogen conducts programmed cell death with the intent of lowering the spread of infection by the pathogen and being signal molecules for further establishment of defenses (Mur et al. 2007).

**Table 1.1** Different reactive oxygen species and their specific functions under stress in plants

| Sl. No. | Reactive oxygen species | Function | Reference |
|---|---|---|---|
| 1. | Singlet oxygen ($^1O_2$) | It reacts with biological molecules like unsaturated fatty acids, protein, DNA, etc. to oxidize those | Foyer and Harbinson (1994), Wagner et al. (2004) |
| | | Leads light-induced destruction of activities of PS-II and further promotes cell death | Krieger-Liszkay et al. (2008) |
| 2. | Hydrogen peroxide ($H_2O_2$) | Relatively more stable than that of other ROS. It can diffuse through membrane located aquaporins and can also travel longer distances within the cell | Bienert et al. (2007), Mendoza (2011) |
| | | Acts as signal molecule at its low concentration under pathogen-induced stress in plants | Yan et al. (2007) |
| | | When at higher concentration, $H_2O_2$ conducts oxidization reaction over various key enzymes such as enzymes of Calvin cycle, Cu/Zn-SOD, and Fe-SOD; methionine residues (–$SCH_3$) or cysteine (–SH) | Leegood and Walker (1982) |
| | | Creates imbalances by altering the redox status of intracellular antioxidants like ascorbate and glutathione, which play important role in signal transduction | Foyer et al. (1997) |
| | | Promotes production of MDA which is harmful for plant | Radwan et al. (2010) |
| 3. | Hydroxyl radical ($OH^{\bullet}$) | Interaction of $OH^{\bullet}$ with biological molecules leads to cellular damages, i.e., membrane leakage, lipid peroxidation, denaturation of protein; and brings death to cell | Foyer et al. (1997), Pinto et al. (2003), Œlesak et al. (2007) |
| 4. | Superoxide anion ($O_2^{\bullet-}$) | Oxidizes iron and Sulphur, thus leads to inactivation of various enzymes | Gardner and Fridovich (1991) |

## 1.5 Beneficial Microorganisms and Their Significant Roles in Plant

Several beneficial bacteria like *Azospirillum brasilense*, *Paenibacillus alvei*, *Pseudomonas fluorescens,* etc. colonize plant root and help plant provide protection (Van Loon 2007). These bacteria have link to more secretion of growth-inducing phytohormones such as jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) which also take part in building a network of signal for recognition of the microbe and stress amelioration. *Trichoderma*-induced phytohormones indole acetic acid (IAA) and ET play key roles in plant development and increase in plant defenses through up-regulation of some beneficial genes for control of diseases in plant (Hermosa et al. 2012; Kannojia et al. 2017). These hormones' presence in plant apoplast has great importance in control of pathogens for their connection in development of the first stages of infection (Farvardin et al. 2020).

## 1.6 Scavenging ROS: Defense System in Plant

Plants are naturally able to produce ROS as well as to detoxify those. Plants ameliorate the negative impacts of various toxic ROS by directing their antioxidants already present in them to scavenge the ROS. Wide range of antioxidants serve the purpose of reduction of toxicity formed due to excess ROS production when plant gets invaded by microbes.

### 1.6.1 Antioxidants and Their Characteristics

Various studies reported that reactive oxygen species at their lower level are not toxic. But when the level of ROS in plant cell exceeds beyond the plants defense mechanisms present in plant itself, then the cell is said to be in the state of oxidative stress or oxidative burst (Sharma et al. 2012). The potential of ROS being damaging agent or signal carrier from cell to cell under stressful condition, completely depends on the ability of cell to maintain the balance between production of ROS and their scavenging. Signature of ROS, i.e., their localization, duration of action, and amplitude of signal transduction in response to stress is also depended on this harmony (Miller et al. 2008). Detoxification of unnecessary ROS becomes successful by provoking the efficient antioxidative system. Antioxidants are the compounds that mediate regulatory functions for reactive oxygen species to lead those avoid or minimize cell damage, and untimely death (Foyer and Noctor 2005; Caverzan et al. 2016). Antioxidants are broadly classified into two groups- one being enzymatic antioxidants including superoxide catalase (CAT), guaiacol peroxidase (GPX), dismutase (SOD), enzymes of ascorbate glutathione (AsA-GSH) cycle, viz. glutathione reductase (GR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) (Noctor and Foyer 1998; Helepciuca et al. 2014); and the other being nonenzymatic compounds comprising of phenolics, ascorbate (AsA), tocopherols, glutathione (GSH), carotenoids, proline, and betaine (Mendoza 2011; Sharma et al. 2012). Plants can tolerate the pathogen-induced stress impacts when these antioxidant properties are at higher level than the ROS to be scavenged by those (Chen et al. 2010).

### 1.6.2 Role of Different Antioxidants in Plant Defense

Plants are blessed with presence of a wide range of antioxidants in themselves. These antioxidants continuously show helping nature to plants when they are under stress

**Table 1.2** Enzymatic antioxidants and their function in plant defense

| Sl. No. | Antioxidant | Symbol | Function | Reference |
|---|---|---|---|---|
| 1. | Ascorbate peroxidase | APX | Scavenges peroxidase ($H_2O_2$) by regulating their signals | Patterson and Poulos (1995), Mendoza (2011) |
| 2. | Superoxide dismutase | SOD | Carries out dismutation of superoxide anion to $H_2O_2$ as the name suggests | Scandalios (1993), Mendoza (2011) |
| 3. | Catalase | CAT | Plays key role in reducing the $H_2O_2$ level present in peroxisome and detoxifies those | Mallick and Mohn (2000), Mendoza (2011), Sharma et al. (2012) |
| 4. | Peroxidase | POX | Acts as a scavenger of $H_2O_2$ | Shao et al. (2008), Minibayeva et al. (2009) |
| 5. | Glutathione reductase | GR | Maintenance of high cellular glutathione/glutathione disulfide (GSH/GSSG) ratio by inducing reduction reaction of glutathione disulfide (GSSG) to glutathione (GSH) which takes part in detoxification of $H_2O_2$ | Sharma et al. (2012) |
| 6. | Dehydroascorbate reductase | DHAR | Apoplastic AsA recycling | Rubio et al. (2009) |
| 7. | Monodehydroascorbate reductase | MDHAR | 1. Regeneration of ascorbate (AsA) from MDHA<br>2. Mediates photoreduction of dioxygen to superoxide anion in absence of the substrate MDHA | Miyake et al. (1998) |
| | | | Regulation of oxidative stress in mitochondria, cytosol, and chloroplast | Das and Roychoudhury (2014) |

due to biotic factors, i.e., pathogens. The major classification of antioxidants includes enzymatic and nonenzymatic compounds. All these have some significant but specific function in detoxification of various ROS formed from oxidative stress. Their role in plant under stress is described below (Tables 1.2 and 1.3, respectively).

**Table 1.3** Nonenzymatic antioxidants and their function in plant defense

| Sl. No. | Antioxidant | Function | Reference |
|---|---|---|---|
| 1. | Tocopherols | Scavenge a range of ROS like $^1O_2$, lipid peroxy radicals, oxygen free radicals, and singlet oxygen species | Diplock et al. (1989) |
| 2. | Ascorbate | Prevents macromolecules from getting damaged due to oxidative burst | Sharma et al. (2012) |
|  |  | Reduces toxicity generated by $H_2O_2$ and $O_2^{\bullet-}$ | Noctor and Foyer (1998), Pinto et al. (2003) |
| 3. | Glutathione | Free radical scavenger | Sharma et al. (2012) |
|  |  | Up-regulates stress-responsive genes, synthesizes proteins, takes part in signal transduction | Foyer et al. (1997) |
|  |  | Regenerates AsA through taking part in AsA-GSH cycle | Loewus (1988), Sharma et al. (2012) |
| 4. | Phenolic compounds | Protect membrane | Arora et al. (2000) |
| 5. | Carotenoids | Detoxify multiple ROS | Young (1991), Sharma et al. (2012) |
|  |  | Act as signal carriers | Li et al. (2008) |

#### 1.6.2.1 Antioxidant Compounds with Enzymatic Nature in Plant–Microbe Interaction

Plants possess various enzymatic compounds which have antioxidant properties. The enzymatic components located in various subcellular compartments enable plant to counteract the oxidative stress. Major roles of some broad enzymes in plant defense under biotic stresses are discussed in Table 1.2.

##### Catalase

Catalase is the single antioxidant that can operate scavenging of hydrogen peroxide ROS without the availability of any reductant. This heme-containing enzyme catalyzes dismutation reaction of two molecules of $H_2O_2$ into $H_2O$ and $O_2$ (Sharma et al. 2012). Known to all, catalase has the highest enzymatic activity that is 0.2–2% of the overall enzymatic activity (Vanacker et al. 1998). An increase of CAT in essential cell organelles during membrane damage, photorespiratory oxidation, β-oxidation of fatty acids, and degradation of various proteins has been reported in previous studies (Corpas et al. 2008).

##### Superoxide Dismutase

This enzyme is a primary regulator of oxidative damage due to excessive superoxide anions, as the name suggests. Superoxide dismutase increases resistance to plants during stress condition by continuously detoxifying the surplus of $O_2^{\bullet-}$ in various organelles like cytosol, apoplast, chloroplast, peroxisomes, and mitochondria (García et al. 2020). In plants, three different isozymes of SOD, viz. manganese

SOD (Mn-SOD), SOD copper/zinc SOD (Cu/Zn-SOD), and iron SOD (Fe-SOD) have been reported of all which being nuclear encoded are engaged to protect their respective cellular organelles by an amino terminal sequence (Bowler et al. 1992).

#### Guaiacol Peroxidase

Guaiacol peroxidase is also an enzyme that contains heme. The preferable function of this enzyme is to take part in oxidation of guaiacol and pyrogallol for which it spends $H_2O_2$ molecule. GPX brings resistance to biotic stresses in plant through participating in some crucial biosynthetic processes like modification of cell wall, biosynthesis of ethylene, healing of wound due to pathogens to name a few (Kobayashi et al. 1996).

#### Glutathione Reductase

Glutathione reductase (GR) being a part of ascorbate-glutathione cycle serves as an important antioxidant in various plants. It leads the reduction of glutathione disulfide (GSSG) to obtain GSH with the use of reducing agent NADPH and thus plays prime role in maintenance of GSH/GSSG status in cell. In chloroplast, these two products, viz. GSH and GR have been engaged in detoxification of the reactive oxygen species $H_2O_2$ (Sharma et al. 2012). Besides, mitochondria, cytosol, and peroxisomes also witness GR's involvement in ROS scavenging. Studies by Vanacker et al. (1998) revealed that promoted GR activity was observed in oat and barley apoplast when infected with *Blumeria graminis.*

#### Monodehydroascorbate Reductase

This enzyme is FAD enzyme that is involved in regeneration of AsA from the MDHA radical at the expense of NAD(P)H that is a well-known electron donor (Hossain and Asada 1985; Farvardin et al. 2020). This enzyme conducts quenching of superoxide ions in cellular compartments like cytosol, chloroplast, mitochondria, and peroxisome (Miyake et al. 1998).

#### Dehydroascorbate Reductase

It leads to reduction reaction of dehydroascorbate (DHA) to produce ascorbate (Asc). Reduced GSH serves as the electron donor in the reaction (Eltayeb et al. 2007). DHAR also takes part in plant processes like growth, development, etc. gifts plant improved defense in tackling stress situation by maintaining plant's internal homeostasis (Eltayeb et al. 2011; Ding et al. 2020).

### 1.6.2.2 Antioxidant Compounds with Nonenzymatic Nature in Plant–Microbe Interaction

Not only enzymes, but also plants have been naturally offered antioxidant-induced defense by some nonenzymatic compounds. These compounds strengthen plant's immunity by boosting its ability to counteract the negative impacts created due to excessive ROS generation in cell because of pathogen invasion. It has been observed that synthesis and production of nonenzymatic antioxidants get promoted when plant comes in contact with pathogens. The principal functions of some well-known nonenzymes with antioxidant properties in plant defense under biotic stresses are discussed in Table 1.3.

### Ascorbate

Ascorbate (AsA) is the most commonly found antioxidant with low molecular weight. Their key function in amelioration of ROS scavenging has been found in previous research works. Most important functions that it performs are perception of stress, maintenance of redox balance, oxidative stress mitigation under biotic stress (Farvardin et al. 2020), and protection of cell membranes by conducting reaction with superoxide anions and hydrogen peroxidases with the target of regeneration of tocopherol and other beneficial enzymes that add more resistance to plant (Zaefyzadeh et al. 2009). More to be added, this antioxidant also helps plants carrying out some crucial physiological processes such as growth, differentiation, and others in presence of stress due to pathogens (Sharma et al. 2012). The ambiguous functions of this antioxidant towards plant defenses make this a highly desirable antioxidant in stress condition in plant.

### Tocopherols

Tocopherols belong to a group of fat-lover (lipophilic) antioxidants and are of four typed- α, β, γ, and δ (Diplock et al. 1989). Inclusion of these compounds in the category of antioxidant relies on their ability in scavenging of lipid peroxy radicals, oxygen free radicals, and singlet oxygen species. Plants direct tocopherols in various works like protection of membrane structure by ameliorating oxidative stress in photosynthetic organ chloroplast. Thus, plants become able to keep their PS-II intact and undisturbed, and function normally under stress (Ivanov and Khorobrykh 2003).

### Carotenoids

Carotenoids also possess lipophilic characteristics. Dynamic functions of carotenoids have been reported in plants under stress condition. When plants get invaded by various pathogens, carotenoids in the form of antioxidants take over some responsibilities of preventing harmful changes in plants. Pathogen-induced oxidative damages by formation of excited chlorophyll molecules to react with membranes and promoted production of singlet oxygen at excited stages get prevented by inference of carotenoids and thus photosynthetic apparatus gets protected.

### Glutathione

Glutathiones convey their antioxidant properties to dynamic roles in various directions they play in plant life. Several bio-molecules such as lipids, proteins, DNA, etc. are in safe position under biotic stress due to glutathione's active participation in scavenging ROS. Glutathione gets successful in ROS scavenging through either of the two ways- by glutathiolation, i.e., the formation of adducts directly reacting with highly unstable and excited electrophiles or by donating proton in the availability of ROS to yield GSSG (Asada 1994).

### Phenolic Compounds

The extraordinary ability of acting as scavenger of ROS by phenolics compounds is to be credited to their electron donating nature. Phenolics compounds have been found playing major role in protection of membrane and macromolecules (Arora et al. 2000). Though no specific and distinct studies have been available regarding their potential role in biotic stress, but it could be said that electron donating capacity during oxidative damages would get them function in amelioration of stress in plant because of pathogen attacks and disease development (Table 1.4).

## 1.7 Knowledge Gap and Future Perspective

Association of various microorganisms with plant through colonization in its root is of age-old discussion. Still, the complex nature of this association and the internal changes that plant has to adopt in order to respond to a number of microorganisms with similar or different groups has lot more to be revealed. Impact of global climate change scenario of recent years on the plant–microbe interaction is not clearly known and how these climatic factors directly or indirectly influence the intensity or severity of this association. Internal defense mechanisms that plants hire, while struggling to keep pace the harmony between ROS generation and scavenging, have not fully been disclosed. So, future studies regarding this area are of utmost requirement for proper understanding of plant–microbe interaction and amelioration strategies that plants adopt in response to the interaction.

## 1.8 Conclusion

Plant shows very specific interaction with pathogen. The complex and dynamic interaction between plant and microorganism to be initiated needs application of sensing and carrying that sense to the ultimate receptor cells to identify the type of microorganism from the plant side. Reactive oxygen species which create oxidative burst also have signaling properties to an extent. Plants have already evolved multiple defense mechanisms against various stresses and apply those to get rid of the challenges due to stress factors. Plants when recognize the invader; implement their intrinsic defensive mechanisms, i.e., antioxidants to keep balances between ROS generation and ROS scavenging. The beneficial microbes help in boosting this defense through continuously supplying antioxidants to detoxify the ROS. To be noted, individual antioxidant has individual function too. Enzymatic antioxidants are specific to a specific ROS and thus plants have a wide range of antioxidant molecules, even nonenzymatic compounds also serve as antioxidant in need of plants. So, it can be concluded that best understanding of plant–microbe interaction, the interaction-induced effects in plant and plant's outrageous response towards tackling the stress due to invasion by microorganisms could be possible through revealing compounds with antioxidant properties and future studies regarding this.

**Table 1.4** Antioxidants-generated crop-specific defense mechanisms to biotic stress

| Sl. No. | Crop | Pathogen involved | Effect due to pathogenesis | Antioxidant | Mechanism of defense | Reference |
|---|---|---|---|---|---|---|
| 1. | *Hordeum vulgare* | *Bacillus graminis* | Rust | POD | Increased secretion of pathogenesis-related proteins and improved phytoalexin production | Boyd et al. (1994) |
| 2. | *Solanum tabacci* | Fungal pathogens | Leaf blight | CAT, APX | Induced HR | Mittler et al. (1999) |
| 3. | *Linum usitatissimum* | *Odium lini* | Powdery mildew disease | CAT, GPX | Disease resistance | Ashry and Mohamed (2012) |
| 4. | *Vicia faba* | *Yellow mosaic virus* | Leaf curling | CAT, POD, APX | Mitigating ROS toxicity | Radwan et al. (2010) |

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# Plant-Microbe Symbiosis led synthesis of Bioactive Compounds

# 2

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## Abstract

Endophytic bacteria reside within plant tissues having mutually symbiotic relationship. They are ubiquitous in nature and known to acclimatize in extreme environmental conditions. Plant-endophyte interaction also helps in signaling and bacterial communication. Apart from the mutual benefits, it imparts to plants it also produces plethora of bioactive compounds of medicinal potential. Secondary metabolites like alkaloids, polyketides, terpenoids, peptides, flavonoids, quinines, and steroids are instances of the array of compounds, the endophytic bacteria produce. These bioactive compounds are known to be effective as antimicrobial, anticancerous, antibiotic, antioxidant, antiviral, etc. With the global burden of increasing drug resistance against diseases and their side effects, natural resources such as endophytes need to be explored further to discover novel bioactive compounds. Furthermore, exploration and characterization of bacterial endophytes from diverse environment conditions producing novel bioactive compounds, have promising applications in medicine, agriculture, and veterinary sciences, enabling us to counter health challenges in ecofriendly manner.

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## 2.1 Introduction

Endophytes are described as microorganisms often bacteria, fungi, archaea, protists living within the plant tissue harmlessly without damaging the plant (Hardoim et al. 2015). The terminology "endophyte" is taken from the Greek words "endon" which means inside, and "phyton" which means plant. Endophytic bacteria were initially observed during 1948 in stem, root, and tuber of potato (Tervet and Hollis 1948; Sanford 1948; Lutman and Wheeler 1948). Endophytic bacteria could be facultative, i.e., it could grow within plant host, plant surface as well as in soil and can be cultured, or it could be obligate which require specific growth condition provided by plant host only and are unculturable (Christina et al. 2013). The diversity observed in the bacterial endophytic niche is tremendous. Several factors affect the endophytic niche presence inside the host which includes plant-microbe, microbe-microbe interactions, soil, biogeography as well as the favorability of the environment. Endophytic bacteria could be seen colonizing plants from various environmental conditions such as tropic, temperate, rainforests, aquatic, xerophytic, etc. showing coevolution in different niches (Lodewyckx et al. 2002). Endophytes include both gram-positive as well as gram-negative bacteria which range to diverse genera. Endophyte characterization from plants which has been surface sterilized shows its varied distribution within the host be it root, stem, leaves, seeds, tubers, etc. It is observed that bacterial densities vary drastically and decrease progressively from roots to stem and leaves owing to the huge microorganism pool present in the rhizosphere (Lamb et al. 1996; Fisher et al. 1992). Cellulolytic enzymes synthesized by endophytes facilitate the mobility and spread to aerial parts of the plants (Elbeltagy et al. 2001).

The relationship between the endophyte and the host plant is symbiotic in nature and it is beneficial to both the microorganism in terms of nutrients, shelter, protection from environment, and to the host plant it colonize. Endophytic bacteria promote biotic and abiotic stress management by supporting the growth and development of plants in several ways (White et al. 2019). Endophytic bacteria act as plant growth-promoting microorganisms by enhancing acquisition as well as improved cycling of nutrients and minerals such as nitrogen fixation, solubilizing inorganic phosphorous and its uptake by plants, and production of siderophore for iron uptake, etc. (Santoyo et al. 2016). They modulate plant development by producing several phytohormones such as auxins (IAA-indole 3 acetic acid), gibberellins, Abscisic acid, and cytokines which help plant cells during division, differentiation, and elongation. Phytohormones are responsible for overcoming stresses such as drought, high salt, and extreme temperatures. They also reduce ethylene levels in plants by producing 1-Aminocyclopropane-1-Carboxylate Deaminase (ACCD) (Mehabo Maela and Hope Serepa-Dlamini 2019). Organic acid metal complex is absorbed by utilizing high affinity metal ion transporters present in endophytes to absorb metal ions such as zinc, copper, magnesium which help them in nutrient uptake and promote growth (Warner and Lolkema 2002). There are indirect processes also ongoing simultaneously which enhance the growth and survival of plants, which includes microbe association with other pathogenic microbes. Endophytic bacteria produce several antimicrobial compounds in riposte the competition for space and nutrients. It is observed that via host defense gene upregulation, it induces systemic resistance against pathogens (Hardoim et al. 2015). Bacillus sp. and pseudomonas sp. are few examples of endophytic bacteria that synthesize antimicrobial and antifungal compounds which potentially inhibit phytopathogens by targeting membrane proteins, which induces nutrient leakage by phytopathogens (Ongena and Jacques 2008). Biotic and abiotic stress induces ROS generation by plants which are detrimental for plant proteins and nucleic acid, some endophytes can induce stress tolerance by upregulation of transcript of ROS-degrading genes which help protect them to reduce the level of ROS generated (Lata et al. 2018). Production of alkaloids by endophytes in aerial parts of plant reduces insect foraging and deter herbivory (Panaccione et al. 2014). They are also responsible for the degradation of environmental toxins (Van Aken et al. 2004).

Apart from synthesizing antimicrobial compounds for control of phytopathogens, endophytic bacteria also produce low-molecular weight compounds which are biologically active compounds having the potential to obstruct bacterial, fungal, protozoan, viral growth responsible for causing diseases (Ek-Ramos et al. 2019; Langner et al. 1816). With the increasing population, emergence of new viruses, increase in drug resistance, drug efflux, emergence of complex health issues, infectious diseases, and cancer, it has now become more important than ever to look into this untapped source of bioactive compounds that could act as antimicrobial, antifungal, antiviral, anticancerous agents (Christina et al. 2013; Lodewyckx et al. 2002; Strobel and Daisy 2003). Natural products such as primary and secondary metabolites produced by microorganisms, plants, and animals can act as bioactive compounds (Gunatilaka 2006). Natural products synthesized by plants in association with microorganisms have been known as traditional sources of drugs. In several instances, they have served as sources of lead molecules, which yielded many synthetic drugs (Strobel and Daisy 2003). Bilateral metabolite production is often observed as well as plant metabolite synthesis attuned by endophytes are more common than presumed (Brader et al. 2014). Although, the specific circumstances and molecular dependencies for production of certain metabolites are not precisely understood. The massive genomic revolution in conjunction with the steady advancement in analytic techniques such as HPLC, NMR, mass spectrometry allows as well as escalates the discovery process for such novel compounds. Secondary metabolites synthesized by endophytic bacteria may act as bioactive compounds with potential to serve agricultural, pharmaceutical, and industrial needs (Lodewyckx et al. 2002; Strobel and Daisy 2003; Ryan et al. 2008).

## 2.2 Mode of Entry, Establishment, and Transmission of Bacterial Endophyte

The entry and establishment of endophytes into plant host are an intricate process involving series of physical, biochemical, and mechanical events. Soil being the magnificent source of endophytic bacteria, hosts plethora of microorganisms that could invade plant tissues (Turner et al. 2013). Roots of plants are intended as access point as endophytes residing in the rhizosphere attach to rhizoplane looking for access to internal tissue (Paungfoo-Lonhienne et al. 2010; Rosenblueth and Martínez-Romero 2006).

Host plant secretes exudates in the rhizosphere which are abounding in substrates ranging from lipids, phenols, amino acid, carbohydrates, flavonoids, etc. (Bertin et al. 2003). Endophytic bacteria present in the soil, nearby the plant roots sense these chemotactic affinities and swim towards the root exudates (Badri and Vivanco 2009). Endophytic bacteria use various mechanisms such as chemotaxis and quorum sensing to colonize specific host plants (Begonia and Kremer 1994; von Bodman et al. 2003). Subsequently, the endophyte attaches to the root surface allowing prospective entry close to sites such as lateral root, root tip, root hair, and any gaps originated by wounds. Flagella, pili, fimbriae, or cell surface polysaccharides are bacterial structures which are decisive and crucial in bacterial-host plant surface interaction (Sauer et al. 2002; De Weert et al. 2002). The pili, flagella allow propeller movement and migration in response to chemical components secreted by roots. The exopolysaccharides (EPS), lipopolysaccharides produced by endophytes are responsible for initiation of effective host-symbiont specificity, regulating endophytic colonization in the early phase (Janczarek et al. 2015). Preferential site for entry includes root hairs, apical root meristem, root cracks in virtue of their thin surfaces which allows favorable entry (Kandel et al. 2017). To hydrolyze the external covering, endophytes often secrete lytic enzymes for instance cellulases, lysozyme, cell wall degrading enzymes, endoglucanases, etc. which facilitate the entry of the endophyte (Reinhold-Hurek et al. 2006). Endophytic colonization is conditional to several variables such as microbial strains, plant tissue type, host genotype, nutrient constrain, biotic and abiotic factors, UV light, adverse temperature, drought, etc. (Hardoim et al. 2015).

## 2.3 Transmission of Endophytic Bacteria

Acquisition of endophytic bacteria can be carried out from the rhizosphere (soil root interface) with each new generation referred as horizontal transmission, could be vertically transmitted from one generation to another through seeds or by mixed modes (Bright and Bulgheresi 2010) (Fig. 2.1).

Most plant species harbor endophytic bacteria which could be recovered from different parts of plants such as roots, stems, leaves, seeds, fruits, flowers, and other tissues, etc. (Frank et al. 2017). Bacterial endophytes are known to reside within cell walls, xylem vessels, apoplast, and other intercellular regions since they are rich

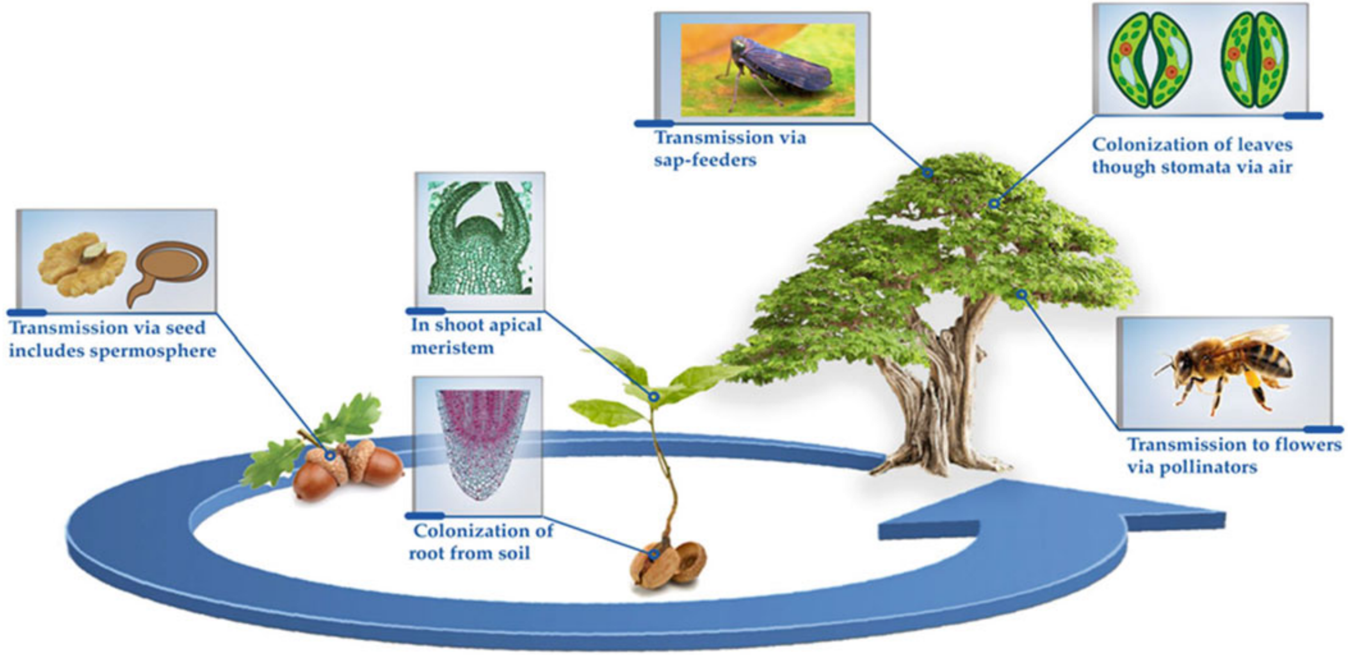

**Fig. 2.1** Routes for horizontal and vertical modes of transmission of endophytes

source of amino acids, carbohydrates, and inorganic nutrients (Bacon and Hinton 2006; Koskimäki et al. 2015). Ecological and evolutionary relationship between host and endophyte impacts the mode of transmission (Herre et al. 1999). Obligate endophytes prefer vertical transmission while facultative endophytes can be both vertically or horizontally transmitted, which allow plants to have greater endophyte diversity (Moran 2006; Wilkinson and Sherratt 2001).

Horizontal transmission of bacteria can be carried out by seeds, leaves, flowers, fruits dispersed in the external environment (Frank et al. 2017). Soil is regarded as a pivotal source of microbes and has plethora of endophytes that could inoculate the host. Seeds during germination imbibe water and release exudate which attracts endophytes from the rhizosphere and in the very early stage alter the bacterial composition of rhizosphere (Schiltz et al. 2015). These early relationships at the time of establishment potentially selected by the plants seed, are beneficial interms of nutrient acquisition, adaptation of biotic and abiotic stress (Nelson 2004). Plant roots attune the microbial selection through changes made in the pH, structure, and oxygen availability in the soil (Dennis et al. 2010). Chronological colonization was observed when *V. vinifera* was inoculated with Burkholderia sp. strain PsJN tagged with green fluorescent protein (GFP), where colonization initially started on root surfaces, followed by root internal tissues, subsequently in xylem vessels of internodes, finally in leaves, and young berries (Compant et al. 2005, 2008). Studies suggest that root microbiota can colonize all plant parts by utilizing xylem vascular system (Hurek et al. 1994; Chi et al. 2005). However, it is not necessary that all root bacteria will colonize the rest of the plant. Studies from *Nicotiana tabacum* show that some bacteria are exclusively present in roots, while other specialize in different part of roots (Saleem et al. 2016). Although not all microbes present in the phyllosphere are acquired through root, many are acquired via bioaerosols, these are tiny particles consisting of bacteria, fungi, viruses, or pollen, released in the atmosphere (Fröhlich-Nowoisky et al. 2016). Several bacteria which spread through bioaerosol are metabolically active and survive for a long time, this enables the global spread of bacterial species inhabiting several plant hosts (DeLeon-Rodriguez et al. 2013; Yamaguchi et al. 2012). Insect foraging puncture and expose the host, allowing bacterial colonization (Lòpez-Fernàndez et al. 2017). Flower surfaces such as petals, pistil, nectar, pollen, and fruits can also be bacterially colonized, originating from the rhizosphere, bacteria carried from air, rain, insects, birds, etc. (Aleklett et al. 2014).

Vertical transmission allows bacteria to pass on from one generation to another resulting in a faithful transfer of beneficial traits. They can be transferred via seeds and pollen grains, but they may not necessarily originate within the plant. Growing seeds may get colonized from parent plant; as vegetative parts of the plant allow movement of microbes through vascular connections to the endosperm, and can also colonize a seed via pollen (Truyens et al. 2015). Shoot apical meristem (SAM) also serves as a passage for microbes to colonize, as undifferentiated cells divide and develop into all kinds of postembryonic aerial tissues (Clark 1997). Pollen grains light in weight, are carried by the wind and are constantly exposed to the environment, wind, insects, and birds which could be responsible for horizontal colonization

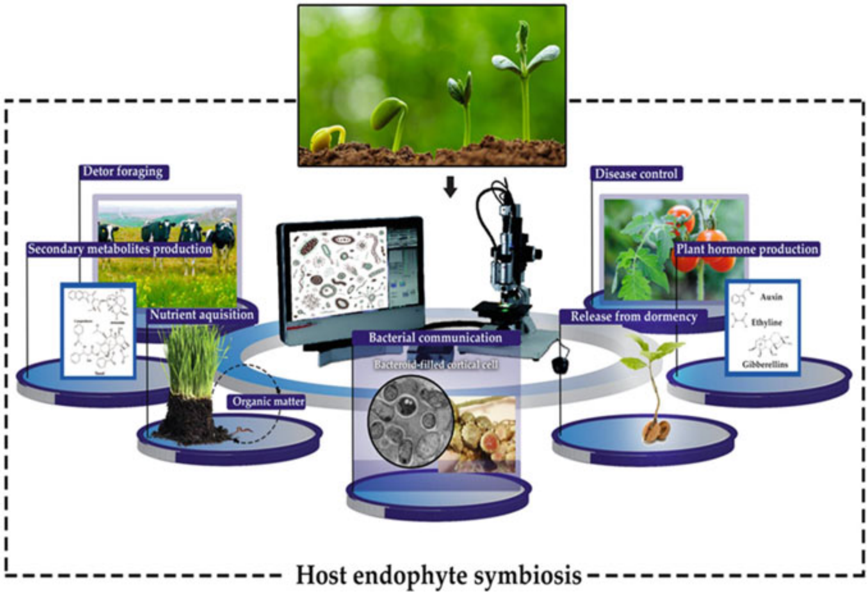

**Fig. 2.2** Benefits associated with host endophyte symbiosis helping the host plant in growth and development

of microbes. Although, if the bacteria present inside or on pollen surface originated from the parent plant, their transfer to seed and seedling would be considered as vertical transmission (Ambika Manirajan et al. 2016). Studies suggest that seed endophytes impart several benefits to the host which include seed preservation, production of cytokinin helping release from dormancy, preparation for germination by creating favorable environmental conditions such as supplying inorganic nutrients, fixing atmospheric nitrogen, etc. (Chee-Sanford et al. 2006; Shahzad et al. 2018). Removal of endophytes from seedlings of grasses shows shrinking of seedlings, loss of gravitropic response as well as reduced root hair formation (Verma et al. 2018). Seed endophytes are also known to have certain antifungal properties (Díaz Herrera et al. 2016). In a study where rice seed endophytes were removed revealed confined growth in comparison to control seedlings (Verma et al. 2017). The ubiquitous presence and the obligate symbiotic relationship of endophytes to their hosts are due to the benefits in plant fitness and growth and its aided ability to deal with biotic and abiotic stress (Santoyo et al. 2016) (Fig. 2.2).

## 2.4 Bioactive Compounds Synthesized by Endophytic Bacteria

Endophytes act as chemical synthesizers and are capable of producing low-molecular weight secondary metabolites. These secondary metabolites can act as bioactive compounds (Owen and Hundley 2005). Although these metabolites are not necessary for enhancing growth of an organism directly, they can act as barrier against phytopathogens and play a adaptive role as defense compound (Isah 2019). These compounds are also responsible for bacterial communication, signaling during ecological interactions, and environmental stresses. Endophytes fight against phytopathogens by acquiring resistance to plant invaders by synthesizing extracellular hydrolases which include cellulases, lipases, proteinases, and esterases (Tan and Zou 2001). Such compounds of biological interest can act as a source of novel drugs. Antimicrobial resistance is observed in agriculture, increasing health issues caused by drug resistant bacteria, cancer, parasitic infection, viral infection, fungal infection, etc., the requirement to explore untapped sources of bioactive molecules has increased (Christina et al. 2013; Ryan et al. 2008). The symbiotic association of plants with endophytes has emerged as a better source of secondary metabolites with medicinal potential in comparison to plants alone. Secondary metabolite production by endophytes associated with aromatic and medicinal plants may be widespread (Palanichamy et al. 2018). Secondary metabolites produced by endophytes include alkaloids, oligopeptides, terpenoids, polyketones, flavonoids, lipopeptides, phenols, steroids, and chlorinated compounds. Most of these isolated compounds are characterized to be bioactive in nature and are known to function as antibiotics, antimicrobial, immunosuppressants, anti-inflammatory, antioxidants, anticancer agents, antiviral, etc. (Gunatilaka 2006; Gouda et al. 2016). However, the synthesis of secondary metabolites suffers from low level of production and heterogeneous quality. Endophytic fungi and bacteria can be identified most reliably using genomic DNA isolation, 16S rRNA PCR-based amplification followed by sequencing. Identification of bioactive compounds and its efficacy require pure fungal or bacterial culture, but few instances suggest that endophytes when allowed to communicate with each other stimulate biological activity (Nair and Padmavathy 2014). With significant improvement and technical advancement in fractionation technique like High Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS)-based identification and quantification, Nuclear Magnetic Resonance (NMR)-based structure determination, isolation, and characterization of biologically active natural products have become more feasible (Salim et al. 2008).

Among all the bacterial endophytes studies for secondary metabolite production, Actinobacteria are reported to produce the most number of bioactive compounds. Actinobacteria are among the most prevalent phyla with high GC content in their DNA among all bacteria. Strains belonging to the phyla Actinobacteria, Proteobacteria, Bacteroidetes, and Firmicutes have the largest biosynthetic gene cluster responsible for synthesis of secondary metabolites that could be bioactive in nature. Actinobacteria have up to 26% of its genes putatively involved in the biosynthesis of secondary metabolites when compared to any other investigated phyla (13–1%) (Hardoim 2019). Endophytes are known to synthesize secondary metabolites alike their host plants. For instance, anticancer drugs such as taxol, camptothecin, podophyllotoxin are known to be co-produced by the endophytes as well as their hosts. It has been proposed that the co-production of natural products is the result of host-endophyte interaction (Heinig et al. 2013). While there could be possibility that "genetic recombination" of the endophyte with the host over an evolutionary time period (Tan and Zou 2001).

Alkaloids are produced by the decarboxylation of amino acids, such as histidine, tryptophan, lysine, proline, ornithine, and tyrosine (Tan and Zou 2001). Bioprotective alkaloids are found to be toxic to insects but not to mammals (Bush et al. 1997). The biosynthetic pathway responsible for oligopeptide generation is called nonribosomal peptide synthetases (NRPSs), the NPRS domain subtypes allow integration of proteinogenic amino acids, D-amino acids, fatty acids as their building blocks. NRPSs multienzyme complex permits complex chain generation as it consists of a series of modules, for amino acid recognition, activation, and bond formation (Fischbach and Walsh 2006). Polyketide synthases (PKS) are responsible for the synthesis of polyketides by condensation of several acetate units. They are multimodular enzyme complexes with three subtypes that are responsible for the large diversity seen in the natural products synthesized by PKS. The natural products synthesized by the endophytes possessing various biological activities are useful source for agricultural, veterinary, and pharmaceutical industry (Alvin et al. 2014).

## 2.5 Role of Bioactive Compounds

Endophytic bacteria exhibit complex interactions with their host. They adopt various mechanisms and gradually modulate the surrounding microenvironment that help them to adapt to that particular living environment (Nair and Padmavathy 2014). In order to maintain mutual symbiosis, endophytes themselves produce or prompt the host plant to produce an array of bioactive compounds that promote the growth of the host plant and enhance its endurance by improving the ability to withstand different stress conditions (White et al. 2019; Santoyo et al. 2016). Metabolites produced by these bacterial endophytes not only help in sustenance and growth of the host but also are of high biotechnological and pharmaceutical importance (Joseph and Mini Priya 2011; Parthasarathi 2012). However, very few studies have exploited the novel significance of these symbiotic microorganisms and their secondary metabolites in the health sector, in drug discovery and other developments in reference to well-being of the environment and human beings. Available literature suggests that bioactive compounds produced by bacterial endophytes have a wide range of pharmaceutical properties, for example, anticancer, cardiovascular, antihypertensive, antiglycemic, antithrombotic, antiatherogenic, and antidiabetic (Chang et al. 2013; Atanasov et al. 2015; Pastor-Villaescusa et al. 2015). Nowadays, bioactive compounds produced by bacterial endophytes are utilized as a preferred therapeutical alternative for the treatment of several diseases (Chang et al. 2013).

To date, various groups have isolated and identified about >300 bacterial endophytes belonging to genera *Streptomyces, Nocardiopsis, Brevibacterium, Microbacterium, Arthrobacter, Brachybacterium, Rhodococcus, Nocardioides,* and *Pseudonocardia* from tissues of *Dracaena cochinchinensis Lour*. Of these, 17 strains showed antimicrobial, antifungal, and cytotoxic activity tested against MCF-7 and Hep G2 cancer cell lines (Surjit and Rupa 2014; Salam et al. 2017). This section aims to highlight the role and importance of bacterial endophytes and their secondary metabolites, like antioxidant, antimicrobial, and anticancer properties.

### 2.5.1 Antioxidant

There is a growing interest to explore the compounds with strong ROS scavenging properties which can be utilized for the prevention and treatment of various ROS-associated diseases. Antioxidants have been implicated as potential candidate to serve the purpose. Bioactive compounds extracted from bacterial endophytes have been reported to act as a good source of natural antioxidants. Numerous parameters like reducing power, metal chelating ability, inhibition of lipid peroxidation; scavenging superoxide, hydroxyl, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals in vitro are utilized to evaluate antioxidant activity (Liu et al. 2012; Akinsanya et al. 2015; Zheng et al. 2016). Studies on exopolysaccharides (EPS) extracted from various plants and associated endophytes have shown promising antioxidant activity. EPS extracted from bacterial endophyte *Paenibacillus polymyxa* showed potent scavenging activity on superoxide and hydroxyl radicals (Valko et al. 2007). Akinsanya et al. (2015) have isolated at least 29 species of culturable bacterial endophytes from different tissues of *Aloe vera.* They have reported that the metabolites produced by these endophytes act as potent antioxidants with varying degrees of scavenging property, being highest in those belonging to genera *Pseudomonas, Macrococcus, and Enterobacter* (Akinsanya et al. 2015). In a similar study, Zheng et al. (2016) have demonstrated that EPS isolated from endophyte *Bacillus cereus* SZ-1 exhibits remarkable scavenging activity on DPPH, $O_2^-$ free radical, and $OH^-$ radicals tested on rat pheochromocytoma PC12 cells exposed to $H_2O_2$. Further, EPS was shown to increase the activity of antioxidant enzymes like glutathione and catalase in $H_2O_2$ exposed pheochromocytoma PC12 cells preincubated with EPS in vitro (Zheng et al. 2016). In another study, the antioxidant potential of exopolysaccharide EPS-1 derived from bacterial endophyte *Paenibacillus polymyxa* EJS-3 was demonstrated in D-galactose-induced aging mice model in vivo. It was reported that EPS-1 administration enhanced the total antioxidant status by increasing the activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Liu et al. 2010).

From the available data, it is evident that most of the studies in reference to the evaluation of the antioxidant potential of EPS have been carried out in vitro conditions and insufficient focus has been given to in vivo experiments till date. There are some basic differences in the affecting factors between in vitro and in vivo systems. In vitro systems are simple and easy to study, nevertheless, it is imperative to evaluate and validate the antioxidant potential of metabolites from endophytes in suitable animal models as far as its therapeutical and clinical significance is concerned.

### 2.5.2 Antimicrobial Compounds

In recent years, growing number of research has revealed that secondary metabolites and other compounds synthesized by bacterial endophytes could be considered as potential antimicrobial agents against broad spectrum of pathogenic microbes (Guo et al. 2008; Sette et al. 2006; Selim et al. 2011; Devaraju and Satish 2011). Alkaloids, flavonoids, lipophilic peptides, phenols, and terpenoids are some of the well-categorized secondary metabolites synthesized by endophytic bacteria, known to possess antimicrobial activity (Yu et al. 2010). These naturally occurring antimicrobial substances obtained from bacterial endophytes are now emerging as the drug of choice to overcome the increasing problem of drug resistance (Ferlay et al. 2010; Taechowisan et al. 2012). Bioactive compounds produced from bacterial endophytes isolated from *Tectonagrandis* L. and *Samaneasaman* Merr., were found effective against bacterial microbes *Escherichia coli, Bacillus subtilis, and Staphylococcus aureus*. Additionally, these metabolites were also found to effectively curb the growth of fungus *Candida albicans* in vitro (Chareprasert et al. 2006). Extracts from the TQR12-4 strain of endophytic bacteria *Streptomyces sp.* have been found to exhibit antimicrobial activity against pathogenic fungus and Gram-positive bacteria (Hong-Thao et al. 2016). Further, various strains of endophytic bacteria belonging to *Bacillus sp*. commonly found in the roots of wheat and rice, and in the leaves of capsicum plant, are known to produce thermostable exopolysaccharides EPS A, EPS B, and proteins E2 having antifungal property (Yu et al. 2010; Li et al. 2007). Interestingly, some bacterial endophytes have been reported to synthesize silver nanoparticles (AgNPs) and gold nanoparticles which are gaining significant attention in pharmaceutical research (Sunkar and Nachiyar 2012; Pissuwan et al. 2006). Strains of endophytic *Bacillus* sp. from *Adhatoda beddomei* and *Garcinia xanthochymus* are known to produce AgNPs (Sunkar and Nachiyar 2012; Pissuwan et al. 2006; Kitov et al. 2008). An increasing number of literature suggests that nanoparticles of endophytic origin have prominent antibacterial properties. Antiviral activity of these nanoparticles has also been shown to be effective against HSV-1, HIV-1, and hepatitis B virus (Baram-Pinto et al. 2009; Lu et al. 2008; Sun et al. 2005). Other than bioactive compounds discussed above, bacterial endophytes are the rich source of some novel antibiotics like ecomycin, kakadumycins, and pseudomycins (Christina et al. 2013). One such grass endophyte *Pseudomonas viridiflava*is known to synthesize ecomycins as a secondary metabolite, which are lipopeptide in nature, and have been implicated in the cure of infectious diseases of eye, skin, gut, and respiratory and urinary tract. Further, these lipopeptides are also effective against infectious fungal species such as *Cryptococcus neoformans* and *C. albicans (*Christina et al. 2013*)*. Species of the endophytic pseudomonads synthesize another class of antifungal substances, the pseudomycins (Harrison et al. 1991; Miller et al. 1998). Kakadumycin, extracted from culturable *Streptomyces sp.* NRRL30566 strain endophytic on *Grevillea pteridifolia* shows significant antibiotic activity against a broad range of Gram-positive bacteria (Castillo et al. 2003). *Streptomyces sp*. contribute to the production of almost 80% of the total antibiotics. Novobiocin analogs and cedarmycins are the two novel antibiotics produced by endophytic *Streptomyces sp*. Furthermore, alnumycin and munumbicin are the two newly identified antibiotics produced by endophytic *Streptomyces sp.* effective against various bacterial and fungal pathogens (Sathiyaseelan and Stella 2011; Thenmozhi and Kannabiran 2012). Strains of endophytic *Bacillus sp.* are one of the most extensively studied group for their antimicrobial activity. Endophytic

*B. subtilis* cenB has been reported to exhibit strong antifungal activity causing distortion in fungal morphology (Nongkhlaw and Joshi 2016). Recently, the intracellular and extracellular concentrates of the NCIB 3610 strain of endophytic *B. subtilis* demonstrated cytotoxic and antimicrobial properties. Moreover, 2,6-di-t-butyl-4-methyl phenol and; pentacosane, hexacosane, and 14-methylhexadecanoic methyl ester, respectively, are the major bioactive components of intracellular and extracellular fractions, respectively as determined by GC/MS analysis (Matloub et al. 2020). Crude or ethyl acetate extracts of bacterial endophytes *B. tequilensis* ALR-2, *B. aerophilus* ALR-8, *Chrysobacterium indologenes* ALR-13, and *Pseudomonas entomophila* ALR-12 isolated from *Aloe vera* have been shown to act as a potential antimicrobial agent against pathogens like *B. cereus* and *C. albicans (*Akinsanya et al. 2015*).*

### 2.5.3 Anticancerous

Currently, the most prevalent approaches in the treatment of cancer are radiation, surgery, biotherapy, and chemotherapy. Out of these, radiotherapy and chemotherapy are most common. These treatment strategies have several drawbacks like toxicity. Thus, the search for alternative approaches to treat cancer with least or no cytotoxic effects remains a critical objective for researchers to explore natural anticancer compounds. Bioactive compounds synthesized by endophytic bacteria as secondary metabolites could be implicated in cancer research as an alternative to hazardous chemotherapy in the treatment of cancer.

A number of metabolites obtained from endophytic bacteria have been analyzed for their anticancer activity both in vitro and in vivo *(*Firáková et al. 2007*)*. For example, lipophilic peptides produced by EML-CAP3 strain of endophytic bacteria isolated from *Capsicum annuum L.* were shown to regress angiogenesis and inhibit tumor progression in endothelial cells of human umbilical vein (Jung et al. 2015). Metabolites extracted from endophytic bacterium *Paenibacillus polymyxa* isolated from Ginseng leaves have shown potent anticancerous property (Gao et al. 2015). Several studies indicate the significance of EPS derived from endophytic bacteria as a potent antitumoral agent of high therapeutic value. EPS extracted from various strains of endophytic *Bacillus sp*. are reported to possess antitumoral property (Chen et al. 2013). The proliferation of MC-4 and SGC-7901 cells is reported to be inhibited by EPS obtained from endophytic *B. amyloliquefaciens (*Chen et al. 2013*)*. Antitumor activity of EPS produced from the culturable monocotyledon endophyte was analyzed both in vivo in H22 liver cancer in mice and in vitro on S-180 solid tumor. It showed its antitumor activity by interrupting the cell cycle and inhibition of cell division (Zhang et al. 2007). Endophytic *B. licheniformis, B. pseudomycoides, and Paenibacillus denitriformis* have been reported to synthesize L-asparaginase (Joshi and Kulkarni 2016), an enzyme that catalyzes the breakdown of L-asparagine (important for some neoplastic cells to function), owing to its anticancerous activity. The use of L-asparaginase has been implicated in the treatment of acute lymphoblastic leukemia as a part of multidrug chemotherapy and showed promising improvement in the majority of the patients (Piatkowska-Jakubas et al. 2008). Anticancerous activity of bioactive compounds extracted from various strains of endophytic *Streptomyces sp.* has been extensively worked out by various groups (Li et al. 2010; Qin et al. 2011; Yuan et al. 2015). 24-demethylbafilomycin C1 produced by endophytic *Streptomyces sp.* is reported to suppress B-cell acute lymphoblastic leukemia by promoting autophagy and apoptosis (Qin et al. 2011). Further, salaceyin produced by MS53 strain of endophytic *S. laceyi* isolated from *Ricicnus communis* has cytotoxic effects on human breast cancer cell line SKBR3 (Kim et al. 2006). Similarly, other metabolites from different strains of endophytic *Streptomyces sp.* are found to be cytotoxic against cancer cell lines (Taechowisan et al. 2017; Igarashi et al. 2006; Vu et al. 2018). Pterocidin from *S. hygroscopicus* TP-A0451 inhibits cancerous growth tested against cell lines NCI-H522, OVCAR-3, and SF539 (Qin et al. 2011; Igarashi et al. 2006) and *S. cavourensis* YBQ59 effectively inhibited the growth of human lung adenocarcinoma in vitro *(*Vu et al. 2018*)*. Furthermore, 4-arylcoumarins from endophytic *S. aureofaciens* CMUAc130 is reported to be cytotoxic on Lewis lung carcinoma in the murine model in vivo *(*Taechowisan et al. 2007*)*.

## 2.6 Conclusion

Endophytic bacteria are ubiquitous in nature and most plants examined are found to be harboring one or more species. Endophytic bacteria support plant growth by enhancing nutrient uptake and cycling, modulating the level of phytohormones, absorbing metal ions, increasing systemic resistance to pathogens, inducing stress tolerance, degrading environmental toxin, inhibiting the growth of pathogenic microbes, reduce insect foraging and deter herbivory, and managing biotic and abiotic stress. These potentially beneficial traits allow co-evolution of endophytes in different niches, be it obligatory or facultative. Endophytes act as chemical synthesizers and produce secondary metabolites known to inhibit phytopathogen as well as responsible for bacterial communication. Bilateral production of metabolites by host endophyte interaction produces biochemically active compounds which serve as a source for novel drugs, having potential in disease control. The advancement in techniques such as Next Generation Sequencing and omics approaches for mining biosynthetic gene cluster and metabolite identification in conjunction with analytical techniques such as HPLC and NMR for fractionation and structure elucidation have strengthened our capacity to isolate and characterize these metabolites. Molecular basis behind the biosynthesis of secondary metabolites allows us to comprehend the complexity as well as modulate the reaction for synthetic production of these compounds. Our increasing understanding and rigorous exploration of the untapped source of endophytes for these compounds will provide new avenues for drug discovery and design, ultimately benefitting agriculture, biotechnology, and pharmaceutical industry.

**Conflict of Interests** All the authors mutually declare that there is no conflict of interest.

Furthermore, this article does not contain any studies with human participants or animals performed by any of the authors during preparation.

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# Plant-Rhizobacteria Communications with the Antioxidant System

# 3

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## Abstract

Plants are provided by wide-ranging antioxidant systems that can elude the detrimental negative effect of oxidative stress and plant growth-promoting rhizobacteria (PGPR) have been claimed to improve the antioxidant defense systems in plants. The Plant-Rhizobacteria signaling is established in a highly sophisticated manner and is controlled by extensive specialized secretory metabolites and ends up in altered gene expression in one or both of the interacting partners. PGPRs affect the nitrogen fixation, solubilization of insoluble potassium, production of siderophores, auxin, zeatin, gibberellin, antibiotics, abscisic acid, volatile organic compounds, lytic enzymes, the system acquired resistance, and moreover. Among the various strategies used to enhance agriculture productivity, use of PGPR has been advocated as one of the most suitable

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strategies for sustaining the health of the soil, crop, and agro-ecosystem and research aimed at a better understanding of plant-rhizobacteria communications is of crucial importance.

**Keywords**

Antioxidant system · Plant-rhizobacteria signaling · Plant Growth

## 3.1 Introduction

### 3.1.1 Brief Overview of Plant Antioxidant System

Aerobic metabolism provides significant energy benefits to living organisms but contains by-products, such as reactive oxygen species (ROS), including singlet oxygen, superoxide radical, hydrogen peroxide, and hydroxyl radical. Oxidative stress is a natural physiological process, but if ROS overlaps detoxification system, an imbalance in cellular homeostasis can be caused (Lobo et al. 2010).

Plants are exposed to various environmental stressors. These environmental stressors can generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Hossain et al. 2015; Ramegowda and Senthil-Kumar 2015; Singh et al. 2017; Singh et al. 2016a, b; Kapoor et al. 2019; Laxa et al. 2019).

ROS can cause photo-oxidative damage of proteins, deterioration of lipids and nucleic acids, and ultimately death of a plant (Jha and Subramanian 2016; Demidchik 2015; Gill et al. 2015). However, ROS has been also considered as second messengers in several cellular processes that also involve tolerance to environmental stress, control cellular activity and regulation by modulating signaling pathways. Evidence suggests that ROS function as effector molecules in vital biological processes such as cell growth, proliferation, differentiation responses to a wide spectrum of external stimuli (Nath et al. 2016, 2017, 2018; Janků et al. 2019; Bobrovskikh et al. 2020). Thus, ROS in low concentrations act as signaling molecules and measure plant cell responses under stress conditions, while in high concentrations they are dangerous to cellular components.

All organisms have intrinsic cellular defenses to combat ROS, called antioxidants. Antioxidants work by keeping low levels of ROS in the cell, thereby preventing damage to the cell. In plant, the antioxidant system is composed of seven main classes of antioxidant enzymes, low-molecular antioxidants (e.g., ascorbate, glutathione, and their oxidized forms), and thioredoxin/glutaredoxin systems which can serve as reducing agents for antioxidant enzymes (Bobrovskikh et al. 2020).

A group of enzymes are composed of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX) that catalyze ROS decomposition. Other groups include monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR), which maintain the level of reduced forms of antioxidants (Fig. 3.1). Superoxides are neutralized in a dismutation reaction catalyzed by SOD, which produces hydrogen

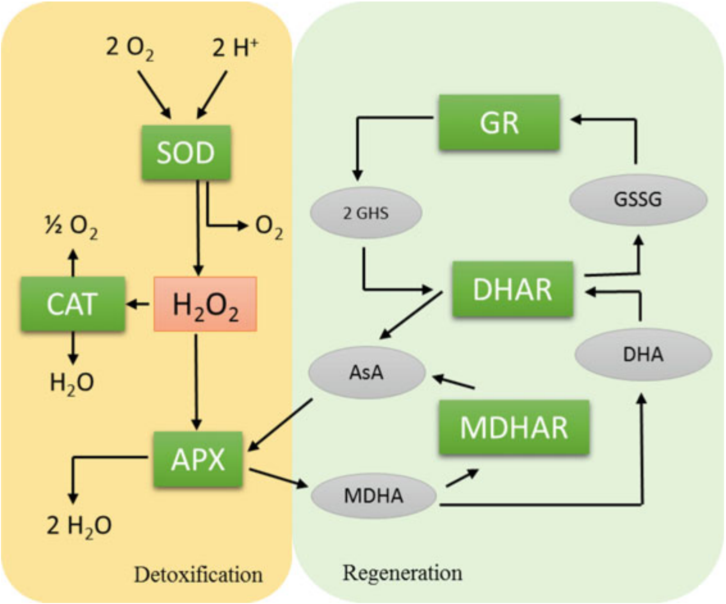

**Fig. 3.1** General pathway showing reactive oxygen species (ROS) and antioxidative defense system of plants, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), ascorbate (AsA), monodehydroascorbate (MDHA), dehydroascorbate (DHA), free glutathione (GSH), glutathione disulfide (GSSG). (Adapted from Kapoor et al. 2019)

peroxide ($H_2O_2$). Hydrogen peroxide can be neutralized in three ways: by CAT, by GPX with oxidation of reduced thioredoxins, and APX with AsA oxidation in monodehydroascorbate (MDHA). MDHA can nonenzymatically transform into ascorbate and dehydroascorbate (DHA) and can be restored by MDHAR, which can turn again into ascorbate under the reaction of DHAR with free glutathione (GSH) oxidation to oxidized glutathione-GSSG (Kapoor et al. 2019; Bobrovskikh et al. 2020).

Low-molecular antioxidants, such as thioredoxin and glutaredoxin protein classes, play an essential role in reducing oxidized forms of antioxidants in the cytosol, nucleus, peroxisomes, mitochondria, and chloroplasts (Noctor et al. 2018). Thioredoxins can act as electron donors for reducing (Bobrovskikh et al. 2020).

Nonenzymatic components of the antioxidant defense system include the main cellular redox buffers: ascorbate (AsA), glutathione (γ-glutamyl-cysteinyl-glycine, GSH), as well as tocopherol, carotenoids, and phenolic compounds. They interact with various cellular components and play a crucial role in the defense system and are enzymatic cofactors, influence plant growth and development, modulating processes of mitosis and cell elongation to senescence and cell death (Sharma et al. 2012). AsA is considered a powerful antioxidant due to its ability to donate electrons in a series of enzymatic and nonenzymatic reactions. AsA plays a key role in removing $H_2O_2$ by ascorbate-glutathione (AsA-GSH) cycle (Fig. 3.1) (Sharma et al. 2012). GSH works as an antioxidant in several ways. In addition to AsA-GSH cycle, it can react chemically with $O2^{\bullet -}$, •OH, $H_2O_2$ by scavenging these free radicals. It can also protect macromolecules (proteins, lipids, DNA), either by glutathionylation or acting as a proton donor in the presence of ROS or physical free radicals, yielding GSSG (Sharma et al. 2012). Carotenoids, cause thermal deactivation of $^1O_2$ by energy transfer reactions, besides, scavenging superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$) (Jin et al. 2015; Kang et al. 2017; Maoka 2019).

Tocopherol is effective in the elimination and extinction of several ROS in cooperation with ascorbate and glutathione. They protect biological membranes from lipid peroxidation. Tocopherols physically and chemically deactivate singlet oxygen in chloroplasts. Moreover, tocopherols can directly repair oxidative radicals by reacting with the alkyl radical (L•), alkoxy radicals (LO•), and lipid peroxyl radicals (LOO•) derived from the oxidation of polyunsaturated fatty acids. In these reactions, the tocopherols donate a hydrogen ion to the lipid radicals, followed by the formation of the tocopherol radicals, which can be reduced to tocopherols by the AsA-GSH cycle (Czarnocka and Karpiński 2018; Ma et al. 2020).

## 3.2 Brief Overview of Plant Growth-Promoting Rhizobacteria (PGPRs)

The close contact zone between soil particles and roots is called the rhizosphere (Vives-Peris et al. 2020), a region of the soil that is influenced by plant roots through the release of chemical substances and, consequently, abundant in microorganisms. It is one environment that can be used to enhance the growth of plants (Duarte et al. 2020) and assist in improving the soil quality (Pervaiz et al. 2020; Tian et al. 2020).

In the rhizosphere, the interaction between plant and microorganisms is more active, as plants release exudates that promote the growth of these species of microorganisms (Vieira et al. 2020). The rhizospheric region has stability regarding the physical, chemical, and biological aspects of soils; besides, microorganisms are influenced by the presence of growth factors, presence of nutrients in the soil, or inorganic substrates that favor the activation of microbial metabolism.

The most desired effect of the plant-microorganism interaction in the rhizosphere is the promotion of plant growth and the protection of plants against attack by pathogens. This is only possible because the microorganisms present in the rhizosphere not only take advantage of the created environment and the substances released by the roots but also produce several signaling molecules that help plants to tolerate biotic and abiotic stresses (Goswami and Deka 2020).

The rhizosphere is also the place where the weathering of rocks occurs and the dissolution of less soluble minerals (Finlay et al. 2020; Ribeiro 2018).

Research shows that plants and microorganisms (Vives-Peris et al. 2020) can change quantitatively and qualitatively, the composition of the rhizosphere as a function of stimuli. This information shows the possibility of modifying the composition of the microbiota and increases specific microorganisms such as plant growth promoters. Through management practices that favor the colonization of roots by such groups of microorganisms, one can increase crop yields, make agriculture more sustainable.

Bacteria that colonize the root system or other part of plants, multiply and compete with native microflora (Gosal et al. 2017) and as consequence have beneficial effects on plant growth are called plant growth-promoting rhizobacteria (PGPR) (Moncada et al. 2021).

Studies show that PGPR can have useful benefits for agriculture. The beneficial effects mainly involve the increase in physiological processes, such as water absorption (Enebe and Babalola 2018) and nutrients (Elhaissoufi et al. 2020), photosynthesis, stimulating plant growth and development (Ilangumaran and Smith 2017) through hormonal production (Goswami and Deka 2020), protection of plants against pathogens and pests (Ali et al. 2020), and in environmental mitigation (Zafar-ul-Hye et al. 2020).

The PGPR are present in the soil, especially in the rhizospheric region of plants and can engage various maintenance processes of the biological equilibrium of the soil microenvironments, as in cycling and availability of nutrients (Ju et al. 2020), the aggregation and weathering soil, as well as in the solubilization of minerals (Wang et al. 2017), assisting in environmental decontamination practices (Raklami et al. 2021).

The metabolic compounds consumed by microorganisms in the rhizosphere are organic compounds produced by photosynthesis in the leaves, translocated in a downward movement by the phloem and exuded by the roots of the plants (Williams and Vries 2020), so there is the formation of an environment rich in nutrients and growth factors very attractive to soil microorganisms, giving the rhizosphere soil unique chemical, physical, and biological characteristics, quite different from roots (Pervaiz et al. 2020).

The species of PGPR widely studied currently are *Pseudomonas fluorescens*, *Pseudomonas putida*, *Azospirillum brasiliense*, *Serratia marcescens*, *Bacillus subtilis*, *Bacillus megaterium*, *Rhizobium*, *Bradyrhizobium*, *Arthrobacter*, *Enterobacter*, *Azobacter*, among others. These microorganisms have been highlighted as fundamental to the plant ecosystem concerning the supply and availability of nutrients for growth such as nitrogen (Matse et al. 2019), phosphorus (Billah et al. 2020), potassium (Ashfaq et al. 2020), silicates (Adhikari et al. 2020), among others.

Among the cited species of PGPR, the genus *Pseudomonas spp*. is one of the main ones for its ability to suppress soil pathogens (Liu et al. 2018), for its occurrence naturally and in high populations, for being nutritionally versatile and having the ability to grow in a wide range of environmental conditions, in addition to producing a wide variety of antibiotics, siderophores (Abbaszadeh-Dahaji et al. 2020), and plant growth hormones.

The rhizobacteria have been tested in various cultures. The species *Bacillus xiamenensis* showed potential for use in the field as a biofertilizer and biopesticide to alleviate stress conditions and promote the growth of sugarcane (Amna et al. 2020). Besides, it showed the potential for suppression of several phytopathogens such as *Colletotrichum falcatum*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Pythium splendens* (Amna et al. 2020) by stimulating the production of extracellular compounds with fungicidal action (Haidar et al. 2016).

Strains of *Pseudomonas* spp. and *Bacillus* spp. also were effective in controlling diseases caused by *Fusarium oxysporum*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Colletotrichum falcatum*, *Aspergillus niger*, and *Aspergillus flavus*, in addition to promoting a stimulus for plant growth compared to uninoculated and control treatments (Ali et al. 2020).

*Bacillus velezensis* and *Bacillus mojavensis* have the potential to manage *Heterodera glycines* on soybean (Xiang et al. 2017). Similar to that observed in rice culture after the use of *Glutamicibacter* sp. which favored overcoming plants to salt stress (Ji et al. 2020).

*Pseudomonas simiae* increased salt tolerance in soy (Vaishnav et al. 2016). *Stenotrophomonas maltophilia* promotes growth and changes the biochemical parameters of wheat plants, providing tolerance to salt stress (Singh and Jha 2017). In corn, an increase in grain yield was observed when using inoculation with *Azospirillum brasilense* or *Pseudomonas fluorescens* individually or in a combination of both (Salvoa et al. 2018).

The study of these rhizobacteria has also been applied in the recovery of soils contaminated with heavy metals, such as *Pseudomonas putida* in areas with nickel (Kamran et al. 2016), *Enterobacter aerogenes* in areas contaminated by cadmium (Pramanik et al. 2018), *Azotobacter* sp. in areas with high levels of chromium and cadmium (Sobariua et al. 2017), and *Brevundimonas diminuta* for arsenic (Singh et al. 2016a, b).

The effects caused by PGPR on plants rely on the exudates liberated by the roots and the quality of the soil (Gosal et al. 2017), and can occur through direct and indirect mechanisms (Tariq et al. 2017).

The direct mechanisms are those affecting plant growth (Jeyanthi and Kanimozhi 2018), by providing nutrients (Paliwal et al. 2020), phytohormones (Kumari et al. 2019) such the gibberellins, auxins, cytokinin, and abscisic acid, biological nitrogen and phosphate solubilization (Kumar et al. 2019), and the indirect mechanisms such as inhibition of phytopathogens (Paliwal et al. 2020) through volatile hydrogen cyanide (HCN), siderophores, antibiotics, volatile metabolites, and ammonia (Kumar et al. 2019).

PGPRs can interact with plants via symbiotic, free-living saprophyte (Kumar et al. 2020), endophytic or associative form (Gosal et al. 2017). In symbiotic form, bacteria live within plants and exchange of metabolites occurs, and in free form, they live outside plant cells (Singh 2018; Nazir et al. 2018).

## 3.3 Plant-Rhizobacteria Signaling Mechanisms

The rhizosphere is an ecosystem inhabited by numerous organisms such as fungi, bacteria, arthropod, herbivores, and nematodes that live in association with healthy plants and form structured communities above and below ground plant organs (Bukhat et al. 2020; Backer et al. 2018; Rosier et al. 2018). Rhizosphere communication is an extremely specialized process and is controlled by different metabolites and exudates and ends up in altered gene expression in one or both of the interacting partners. This mutual communication results in an alteration in plant growth, inhibition of soil pathogens, nutrient availability, biofilm development, and accumulation of soil microbes (Bukhat et al. 2020; Rosier et al. 2018). Such alterations can probably be active simultaneously or sequentially at different stages of plant growth (Figueiredo et al. 2016). Figure 3.2 shows a summary of the direct and indirect effects of PGPRs on host plants.

The rhizosphere microbiota extends the capability of plants to adapt to the environment. It is believed that chemical exudates participate as signaling molecules (Venturi and Keel 2020).

Plant Growth-Promoting Rhizobacteria (PGPR) signaling mechanism happens primarily through quorum sensing (QS) signaling molecules that regulate phenotypes in microbes, production of hydrolytic enzymes and secondary metabolites, and through volatile organic compounds (VOCs), such as terpenoids, alkanes, alkenes, ketones, sulfur-containing compounds that provide the chemical interface and long-distance communication of microbial communities. These groups of molecules are involved in gene expression, hormonal signaling, and defense pathways (Bukhat et al. 2020; Backer et al. 2018; Rosier et al. 2018).

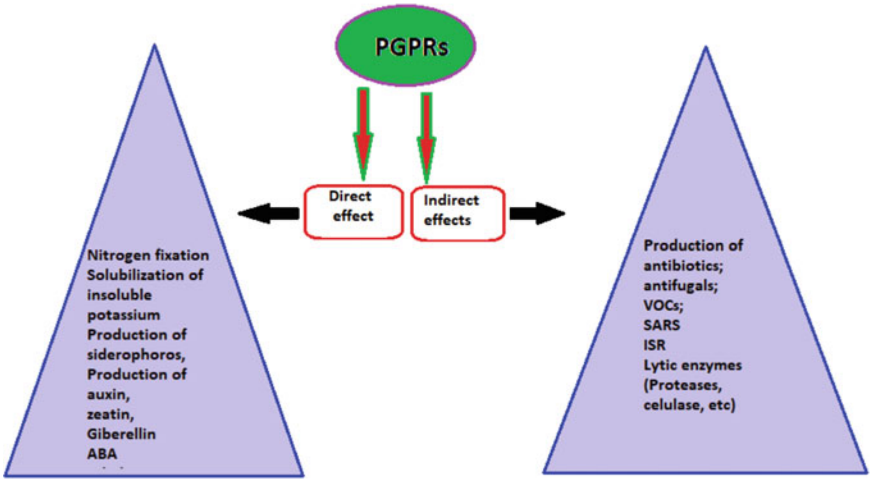

**Fig. 3.2** Direct and indirect mechanisms mediated by plant growth-promoting rhizobacteria (PGPR) with helpful effects on host plants

Rhizospheric region can be inhabited by parasitic, mutualistic, and commensal microorganisms. The root exudates and their chemical composition define the rhizosphere communities, as plants utilize these chemical combinations to select the soil microbial communities (Bukhat et al. 2020; Rosier et al. 2018). Root exudates can be composed of several low carbon molecules that act as precursors for the synthesis of phytohormones of PGPR, e.g., tryptophan is present in high concentration in root tips and acts as a precursor of indole-3-acetic acid (IAA). Furthermore, PGPR metabolize plant-ethylene using aminocyclopropane-1-carboxylic acid (ACC)-deaminase enzyme and regulate ethylene production in the plant under stress. PGPR produce also substances such as cytokinins (CK), auxins, gibberellins, jasmonic acid (JA), salicylic acid (SA), and abscisic acid that act similarly as those compounds produced by plants and significantly affect organ development, growth, hormonal signaling, and immune responses in plants (Bukhat et al. 2020; Backer et al. 2018; Rosier et al. 2018).

Flavonoids are the first communication signals that are exchanged during the development of symbiosis. These compounds are recognized by the rhizobial symbiont. For example, fungus improves the roots ability to access soil nutrients, particularly immobile phosphates, while the plant provides carbohydrates to fungus. Strigolactones and cutin monomers are also secreted from roots of plants and act as primary signaling molecules for the symbiosis of arbuscular mycorrhizal fungi (AMF). Many bacterial QS signaling molecules such as dipeptides, diketopiperazines affect auxin, ABA, and SA responsive expression of genes in plants (Bukhat et al. 2020; Rosier et al. 2018). Antimicrobials and VOCs such as pyocyanin and diacetylphloroglucinol produced by microbes stimulate systemic responses in plants through modulation of hormonal pathways. Other signaling mechanisms include the systemic acquired resistance (SAR) which involves the activation of pattern recognition genes and proteins and increased SA accumulation. Induced systemic resistance (ISR) is also activated by PGPRs and helps the plants in enhancing resistance against different pathogens and abiotic stress (Bukhat et al. 2020; Backer et al. 2018). According to Desbrosses et al. (2009), PGPRs induce the root hair elongation and auxin-dependent mechanism. Induced resistance by PGPRs has been also reported by Ryu et al. (2003)) in *Arabidopsis* and included brassinosteroid, IAA, salicylic acid, and gibberellins. Venturi and Keel (2020) point out that some beneficial compounds produced by plants include caryophyllenes, QS mimic compounds, and strigolactones. Figure 3.3 summarizes some of the PGPR-mediated signaling mechanisms.

## 3.4 Regulation of Antioxidant Defense System by PGPRs

PGPRs have been reported to reduce the toxicity of heavy metals in plants under polluted environments. According to Fatnassi et al. (2020), *Vicia faba* plants inoculated with Rhizobium, *Enterobacter clocae* and *Pseudomonas* sp. alleviated copper stress under hydroponic conditions. Similarly, plants inoculated with *Serratia* sp. and *Rhizobium* sp., under saline soils showed better performance by

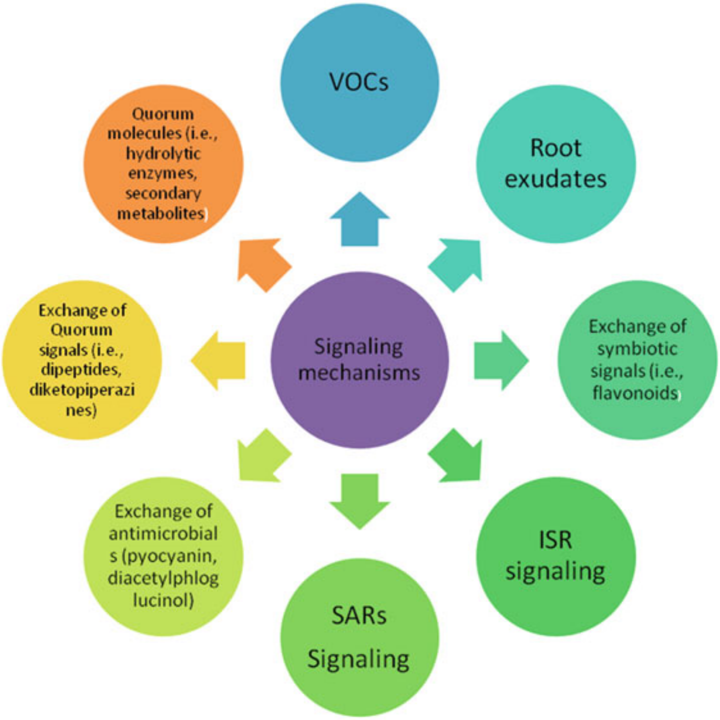

**Fig. 3.3** A summary of PGPR-mediated signaling mechanisms in plants

activation of ascorbate peroxidase (APX) and glutathione reductase (GR), and an improvement of photosynthesis, mineral content, and growth in lettuce plants (Han and Lee 2005). In research by Jha and Subramanian (2014), reported that PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. According to Azarmi et al. (2015), Inoculation with PGPR efficiently enhanced the concentrations of proline and the soluble sugars, whereas, reduced the hydrogen peroxide levels in the leaves and roots. The combined application of PGPR and Zn significantly increased the antioxidant enzyme activities and protein concentration in the pistachio seedling leaves and roots, especially at the higher salinity levels. As reported by Kang et al. (2014), Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus.* The PGPR-applied plants had reduced sodium ion concentration, while the potassium and phosphorus were abundantly present as compared to control under stress. Oxidative stress was mitigated by PGPR through reduced activities of catalase, peroxidase, polyphenol oxidase, and total polyphenol as compared to control. The control plants showed up-regulation of stress-responsive abscisic acid as compared to PGPR application, while salicylic acid and gibberellin were significantly higher in PGPR. Finally, Upadhyay et al. (2011a, b) reported that wheat coinoculated with PGPR strains, and under different salinity regimes, showed an increase in dry biomass, total soluble sugars, and proline content. Wheat sodium content was reduced under coinoculated conditions but not after single inoculation with either strain or in the control. The activity of antioxidant enzymes in wheat leaves decreased under salinity stress after PGPR coinoculation, suggesting that these PGPR species could be used to improve the stress in wheat plants. The activity of three antioxidant enzymes in wheat grown with both PGPR strains was also reduced, most notably that of catalase activity under salinity, when compared with the control treatments. The results reported in that research indicate that coinoculation with *B. subtilis* and *Arthrobacter* sp. could alleviate the adverse effects of soil salinity on wheat growth.

## 3.5 Main Defense Systems Involved in Plant-Rhizobacteria Communications

### 3.5.1 Benzoxazinoids (BXs)

Benzoxazinoids (BXs) are secondary metabolites (Fig. 3.4), found in Poaceae, and derived from indole-3-glycerol-phosphate. BXs have been studied extensively as important herbivore and pathogen resistance factors. BXs are reported to inhibit host recognition and virulence of the pathogenic *Agrobacterium tumefaciens* (Maresh et al. 2006). Hu et al. (2018) report that in maize plants, soil conditioning by exudation of BXs promoted an increase in the expression of defenses responsive to jasmonic acid (JA) and the suppression of the growth of herbivores (*Spodoptera frugiperda*). According to the authors, the suppression of herbivore growth in corn plants is associated with an increase in leaf concentrations of defensive phytohormones salicylic acid (SA) and jasmonic acid (JA), with a stronger expression of JA. Therefore, it is likely that BX-dependent changes in the soil microbiota will increase SA and JA signaling in the leaves and, thus, trigger JA-dependent defenses that subsequently induce greater resistance to herbivores. Induced systemic resistance (ISR) promotes JA signaling in several plant species (Pieterse 2001; De Vleesschauwer et al. 2008; Planchamp et al. 2015). Although ISR has traditionally been associated with a single rhizobacterium interacting with a plant, the authors believe that complex microbial communities accommodate the same characteristics and can elicit ISR.

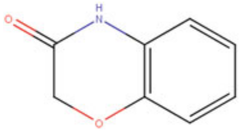

**Fig. 3.4** Chemical structure of 1,4-benzoxazin-3-one secondary metabolites. Its synthetic form has higher antimicrobial potency when compared to natural form

### 3.5.2 Microbe-Associated Molecular Patterns (MAMPs)

The rhizosphere is rich in molecular patterns associated with conserved microbes (MAMPs). In the past two decades, several MAMPs, such as flagellin, Tu-elongation factor (EF-Tu), cold shock protein (CSP), lipopolysaccharide (LPS), chitin, elicitin, and Nep1-like protein, have been characterized in several plant pathosystems along with its cognate pattern recognition receptors (PRRs) (Boutrot and Zipfel 2017). Plant roots can mount strong immune responses in the recognition of PRM-mediated MAMP, including callose deposition, camalexin biosynthesis, and activation of defense-related genes (Beck et al. 2014; Millet et al. 2010; Stringlis et al. 2018; Wyrsch et al. 2015).

Beneficial microbes have immunogenic MAMPs very similar to those of pathogens (Jacobs et al. 2011; Lopez-Gomez et al. 2012; Millet et al. 2010; Pel and Pieterse 2013; Stringlis et al. 2018). During initial contact with the roots, beneficial microbes are recognized by the plant's PRRs, activating immune signaling.

Immune activation of the root by beneficial microbes has been observed in many root-microbe associations. A study carried out by Libault et al. (2010), with *Bradyrhizobium japonicum*, in soybeans, reported the expression of defense-related genes in the early stage of infection in hair cells from soybean root. Liu et al. (2003) report that the arbuscular mycorrhizal fungus *Glomus versiforme* induces a substantial set of genes related to defense and stress during initial contact with Medicago truncatula. However, this induction appears to be restricted mainly to the early stages of these beneficial associations, suggesting an active interference of root immunity by beneficial microbes.

Several mechanisms by which beneficial microbes prevent activation of the plant's immune system have been described. According to Yu et al. (2019), some of them are Evasion of apoplastic recognition (Evolution of divergent MAMPs; Hiding excessive MAMPs), a Suppression of cytoplasmic immune signaling (Eliminating ROS burst; Targeting MAPK cascades; Modulation of hormonal signaling), and Interplay between immunity and symbiosis signaling.

### 3.5.3 Damage-Associated Molecular Patterns (DAMPs)

Are molecules arising from dead, damaged or stressed cells, e.g., cutin monomers, small peptides, and cell wall fragments? Plants similarly recognize these molecules as MAMPs and respond by activating defense signaling cascades. These plant defense responses are strictly regulated to minimize resource expenditure and fine-tune the signaling cascades. This crucial role is fulfilled by phytohormones like salicylic acid, jasmonic acid, and ethylene as essential signaling molecules for both local and systemic responses (Mhlongo et al. 2018).

### 3.5.4 *N*-Acyl-Homoserine Lactones (AHLs)

The exchange of signals between plants and rhizobacteria contributes to the activation of ISR. Small signaling molecules, for example, N-acyl-homoserine lactones (AHLs) from many Gram-negative bacteria, are used for their intrapopulation communication called quorum sensing (QS). Remarkably, plants can detect and respond to bacterial QS molecules. The detection of AHLs and systemic response is an essential aspect of the establishment of mutualistic relationships. Studies of plant responses to AHLs were first done in the model plant *Medicago truncatula*, where these molecules were found to affect extensive functions including cytoskeletal elements, transcriptional regulation, and responses to defense, stress, and hormones. Another study on the interaction between Serratia liquefaciens and tomato (*Solanum Lycopersicum*) provided also indications that QS molecules of rhizosphere bacteria influence plant defense responses. These results provided indications that AHLs play a role in the modulation of the plant immune system (Reyes et al. 2014).

### 3.5.5 Antibiotic-Producing Rhizobacteria

The production of antibiotics is considered one of the most powerful and studied biocontrol mechanisms for combating phytopathogens. Antibiotics constitute a wide and heterogeneous group of low-molecular weight chemical organic compounds that are produced by a wide variety of microorganisms. Antibiotics produced by PGPR include 2,4 Diacetyl phloroglucinol, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyoluteorin, pyrrolnitrin, oomycin A, viscosinamide, butyrolactones, kanosamine, zwittermycin-A, Perugini, rhamnolipids, cepaciamide A, ecomycins, pseudomonic acid, azomycin, antitumor antibiotics, cepafungins, and antiviral antibiotic karalicin. These antibiotics are known to possess antiviral, antimicrobial, insect and mammalian antifeedant, antihelminthic, phytotoxic, antioxidant, cytotoxic, antitumor, and plant growth-promoting activities (Martínez-Viveros et al. 2010).

### 3.5.6 Brassinosteroids

Brassinosteroids (BRs) are one group of phytohormone that regulates many common developmental processes throughout the plant life cycle are a unique class of polyhydroxylated steroidal phytohormones with important roles in regulating myriad physiological and developmental processes. BRs are also increasingly implicated in plant responses to pathogen attack. Exciting new developments connect BRs to a wide variety of defense-related pathways involved in innate immunity triggered by conserved microbial signatures, microbial-induced cell death, hormone signaling, oxidative metabolism, and secondary metabolite production (Bruyne et al. 2014).

## 3.6 Conclusion

Extensive research provided prompts us to state that PGPRs can affect plant growth by various direct and indirect mechanisms. These mechanisms can probably be active simultaneously or sequentially at different stages of plant growth. Plant-PGPR signaling mechanism occurs primarily through quorum sensing signaling molecules that regulate phenotypes in microbes, production of hydrolytic enzymes, and secondary metabolites. Another signaling mechanism includes volatile organic compounds. These molecules provide the chemical interface and long-distance communication of microbial communities. If achieving sustainability and maintaining long-term productivity without the use of agrochemicals are priorities in the soon future, sustainable food production must pay attention to prospect the use of PGPRs.

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# Association of Nonenzymatic Antioxidants in Plant Holobiont

# 4

Neha Sharma and R. S. Tomar

## Abstract

In plants, oxidative stress is caused by different factors such as salinity, pathogen colonization, herbicide activity, oxygen insufficiency, and other stress situation which are a part of abiotic and biotic factors. These factors directly affect biochemical and physiological pathways within individual plants and plant communities. Under oxidative stress condition, plants synthesize a verity of nonenzymatic antioxidant as defense mechanisms to protect themselves from the spectrum of harmful stress conditions. Aside enzymatic antioxidant, reactive oxygen species (ROS) is also prevented by a nonenzymatic antioxidant system including the low-molecular mass compounds which are produced by the host cell. It includes glutathione, ascorbic acid, α-tocopherol, carotenoids, flavonoids, and phenolic compounds. Endogenous and exogenous nonenzymatic antioxidants play an important role in plant health. Antioxidant is small organic molecules which has enzymatic activity. Antioxidants are complex system of overlapping activities and work synergistically to increase cellular defense. There is several reactive oxygen species (ROS) worked in unnecessary oxidation mechanism. Different reactive oxygen and nitrogen species (ROS and RNS) have activity to combat oxidative stress. Similarly, different reactive oxygen species are the targets of each antioxidant enzymes and provide defense in different environmental stress conditions. Antioxidants are reacting with reactive oxygen and nitrogen species which take part in oxidation reactions. An oxidation reaction increases the production of free radicals and induces the damage to various cell components such as lipid, DNA, proteins, and carbohydrates. It is

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wellknown, that antioxidants mediate assured some cellular functions such as defense mechanism against pathogens, redox signaling, and gene expression. Thus, the role of antioxidant enzymes is not only to remove oxidants completely, but also maintain them at an optimum level. The aim of this review will be limited to nonenzymatic antioxidant (exogenous and endogenous) induced by plant holobionts.

**Keywords**

Nonenzymatic antioxidants · Plant growth-promoting rhizobacteria (PGPR) · Reactive nitrogen and oxygen species (RNS and ROS)

## 4.1 Introduction

The term holobiont means interaction between two or more different species. It is a group of macroorganisms and eukaryotes as a host and related microorganisms which show functional interactions. It is also referred as single dynamic entity. The holobiome is another term to explain symbiotic interaction between host cell genome and associated genome of microorganisms (microbiome) (Guerrero et al. 2013).

Interaction between microorganisms (bacterial species) and plant cells is a common example of holobionts. Association of plant tissues with diverse group of microbial species is known as phytomicrobiome. Plant tissues help in composition and regulation of metabolic activity of associated microbial community. The connection between microbes and plants activities gives a broad range of profit to each other (Backer et al. 2018). Holobiont gives an original knowledge, adaptation, and selection processes (Zilber-Rosenberg and Rosenberg 2008).

Communication of rhizobacteria (PGPR) with tissues of host plant is a complex and mutually supporting relationship. It is connecting two species as well as comprises with abiotic and biotic factors of the rhizosphere region. In phytomicrobiomes, interaction takes place between microorganisms (bacterial and fungal) and main structure of plant including fruits, flowers, stems, roots, and leaves. Plant communities in the rhizospheric region increase the plant strength and soil fertility (Dutta and Podile 2010; Berg et al. 2016). The plant shows significant control over interactions of the rhizomicrobiome (Spallek et al. 2017).

PGPR is a kind of free-living soil bacteria. It is directly and indirectly involved to maintain the rooting and growth of plant (Mayak et al. 1999; Glick 1995). PGPR is a competitive microbial species over the other rhizosphere bacterial communities and it has positive results during inoculation of plant tissues.

PGPR commonly used two mechanisms, i.e., direct and indirect. In direct mechanism, PGPR directly affects plant growth through fixing of atmospheric nitrogen, conversion of insoluble phosphate into solubilizing form; affects the hormone secretion such as Indole acetic acid (IAA), Gibberellic acid (GAs), and Kinetins, which helps in regulation of ethylene. In indirect mechanisms, PGPR indirectly supports plant growth like antibiosis, induced systemic resistance (ISR), nutrient competition, parasitism, metabolite (hydrogen cyanide, siderophores) production suppressive to deleterious rhizobacteria (Glick 1995).

PGPR community is the effective tool for future agriculture and sustainable development of agriculture. PGPR bacterial communities belong to different genera like *Acetobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Derxia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Ochrobactrum*, *Pantoea*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Stenotrophomonas*, and *Zoogloea* (Babalola 2010).

## 4.2 Oxidative Stress and Nonenzymatic Antioxidant

PGPR is absorbed into the soil particles by simple ion exchange mechanism. The soil microorganism produces inorganic nutrients from organic resources which provide sufficient support to plant life and known as biologically fertile soil (Babalola 2010).

A number of researches have mentioned that synthesis of reactive oxygen species (ROS) like hydrogen peroxide ($H_2O_2$), superoxide radical ($O^{\cdot}$), and hydroxyl radical (OH) alter the activity of antioxidant enzymes. Antioxidants are activated in plant tissues under stress condition such as salinity (Shalata and Tal 1998; Bor et al. 2003). Imbalance between levels of antioxidant enzymes and free radical activity is used as oxidative stress and works as an indicator in plants (Mittler 2002). Reactive Oxygen Species (ROS) are involved in oxidative damage to large biomolecules such as protein and lipids. Some time oxidative stress condition leads to cell death. To protect against oxidative stress, plant cells produce both nonenzymatic and enzymatic antioxidants. Enzymatic antioxidant includes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) enzymes where as nonenzymatic antioxidants are ascorbate, tocopherol, and glutathione (Rio Del et al. 2003). Ascorbate peroxidase (APX) is a component of the scavenging reaction and catalyzes the reaction of ascorbic acid with $H_2O_2$ and restoration of ascorbic acid is catalyzed by glutathione reductase (GR). (Smirnoff 1993).

The activity and quantity of soil microorganisms are affected by a range of environmental factors, species, and age of plant. Plant growth-promoting rhizobacteria (PGPR) have been identified to play a necessary role in the plant development and metabolism and also promote growth of plants under some stressful conditions. In last two decades or so, various PGPR strains have been known to take part an important role in improving growth of plants and enhance plant biomass (Burd et al. 2000; Chaiharn et al. 2008; Adesemoye et al. 2009; Yang et al. 2009; Lugtenberg and Kamilova 2009; Bharti et al. 2013).

The nonenzymatic antioxidants such as amino acid, glutathione, α-tocopherol, carotenoids, phenolics, flavonoids are the other types of antioxidants. These not only protect different plant cell apparatus from damage but also play an essential role in plant growth and development also. It regulates the plant cellular process like cell elongation, mitosis, senescence, and apoptosis (De Pinto and De Gara 2004).

## 4.3 Types and Features of Plant Holobiont Community

The plant holobiont is comparatively well-studied, with focus on agricultural species such as legumes and grains. Plant holobiont includes bacteria, fungi, archaea, protists, and virus (Bulgarelli et al. 2013).

According to Vessey (2003), there are a variety of species of soil bacteria that increase diversity of microbial community associated with plant rhizosphere. They can grow in, on, or around the plant tissues. Gray and Smith (2005) have exposed that association of PGPR due to level of bacterial closeness to the root tissues. Rhizobacteria are frequently referred to as plant growth-promoting rhizobacteria, or PGPRs. The term PGPRs was first used by Joseph W. Kloepper in the late 1970s and has become frequently used since then in scientific literature.

PGPRs have different relationships with different species of host plants (Vessey 2003). The majority of rhizobacteria belong to gram-negative and gram-positive rods with cocci or pleomorphic (Bhattacharyya and Jha 2012). PGPR is divided into two types: Intracellular PGPR and extraellular PGPR (Gray and Smith 2005).

In present scenario, still not as much of attention has been given to the plant fungal microbiota, culture-independent community profiling exposed a diversity of fungi colony above on and underground tissues of plant. It primarily belonged to Ascomycota and Basidiomycota which are two major phyla. (Toju et al. 2013; Hardoim et al. 2015; Coince et al. 2014).

Alike to bacteria, communities of plant-associated fungi structure are not random and vary. It depends on type of soil, plant sub cellular structure, species of plant, or seasons. (Bulgarelli et al. 2013; Toju et al. 2014; Lê Van et al. 2017) (Table 4.1).

## 4.4 Role of Plant Growth-Promoting Rhizobacteria as Biocontrol Agent

PGPR works as a biocontrol agent. Microorganisms (bacteria and fungi) are responsible for a number of plant diseases but PGPR is able to manage plant diseases. Plant disease is suppressed due to synthesis of antifungal metabolites and systematic resistance provided by PGPR. For example: Biocontrol strain of PGPR such as *Pseudomonads* has been genetically modified to increase disease resistance in plant cells and promote growth of plant in different agricultural crops. Before sowing, seeds are coated with genetically modified inoculants of bacteria. After treatment bacterial inoculants coated seeds have enough rhizobacterial populations within the rhizosphere and produce beneficial effects on the agriculture crop (Vessey 2003).

Table 4.2 explains about a variety of rhizosphere microorganisms' species which are commonly established in the rhizospheric region of leguminous and nonleguminous crops. Plant growth was drastically improved when more than two PGPR species were applied together for treatment (Jha and Saraf 2012).

PGPRs increase growth of plants by two mechanisms: direct and indirect. The mechanisms are still not very well defined. When the plant pathogen or other rhizosphere microorganisms are absent, plant growth promotion is done by direct

**Table 4.1** Types of microholobionts: Following table explains about types, occurrence of microholobionts with examples

| S. No. | Types of microholobionts | Occurrence | Example | References |
|---|---|---|---|---|
| 1 | Extracellular PGPR (ePGPR) or Rhizospheric PGPR | Colonize on surface of the root, in the rhizosphere regions, on the region of rhizoplane, or in the extra cellular spaces between root cortex | *Azotobacter, Azospirillum*, *Agrobacterium*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Caulobacter*, *Chromobacterium*, *Erwinia*, Flavobacterium, Serratia Micrococcous, and Pseudomonas, etc. | Vessey (2003), Gray and Smith (2005), Figueiredo et al. (2011) |
| 2 | Intracellular PGPR (iPGPR) or endophytic PGPR | Located in complex nodular structures within the root cells or grow inside the host plant apoplastic space | *Allorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Rhizobium* | Vessey (2003), Gray and Smith (2005), Figueiredo et al. (2011) |
| 3 | Glomeromycota | Colonize within the plant tissues | *Arbuscular mycorrhizal fungi* | Begum et al. (2019) |
| 4 | Ascomycota | Located on root surface of plant | *Epichloë* species | Guerre (2015) |
| 5. | Trypanosomatidae (protist) | Located on root surface of plant | *Phytomonas* | Schwelm et al. (2018) |

mechanisms. While in indirect mechanisms, PGPR involves to decrease the destructive effects of plant pathogens on crop yield. It is reported that PGPRs directly boost the plant growth by different mechanisms:

1. Free atmospheric nitrogen fixation and transport to the plant.
2. Involve in construction of siderophores that chelate iron. Due to siderophores iron is available to the plant root.
3. Help in solubilization of minerals such as phosphorus. This also directly augmentates mineral.
4. Some PGPR are also reported which help in the synthesis of phytohormones such as auxins and cytokinins. PGPR also affects plant ethylene synthesis (Glick 1995; Gilck et al. 1999; Zakry et al. 2012; Bloemberg and Lugtenberg 2001).

**Table 4.2** Rhizosphere microorganism

| S. No. | Name of rhizosphere microorganism | Genetically modified species | Applications | References |
|---|---|---|---|---|
| 1. | *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Azospirillum*, and *Enterobacter* | *B. brevis*, *B. licheniformis*, *A. calcoaceticus* | The three genetically modified isolates have the capability to make IAA, solubilize inorganic phosphate, and produce ACC deaminase. They involve in formation of siderophores | Jha and Saraf (2012) |
| 2. | *Burkholderia*, *Alcaligenes*, *Arthrobacter*, *Azotobacter*, *Beijerinckia*, *Erwinia*, *Flavobacterium*, *Rhizobium*, and *Serratia* | – | They are used to improve the productivity of crop | Burd et al. (2000), Chaiharn et al. (2008), Yang et al. (2009), Bharti et al. (2013) |

## 4.5 Types of NonEnzymatic Exogenous and Endogenous Antioxidants

### 4.5.1 Glutathione

Glutathione is made up of three amino acids, i.e., γ-glutamyl-cysteinyl-glycine. It is a low-molecular weight thiol tripeptide, found in large quantities in approximately of all cellular components like cytosol, mitochondria, endoplasmic reticulum, chloroplasts, peroxisomes, vacuoles, and also found in the apoplast. Glutathione is involved in an ample range of cellular processes like:

- Growth, division, and differentiation of cell.
- Cell death or senescence.
- Detoxification of xenobiotics.
- Sulfate transportation regulation.
- Metabolites conjugation.
- Synthesis of nucleotides.
- Phytochelatins and proteins.
- Regulation of enzymatic activity.
- Work on genes involved in stress.

All above mechanisms of GSH are due to its high reducing potential (Mullineaux and Rausch 2005). Cysteine residue is sulphur-containing amino acid which has nucleophilic character. Central cysteine residue is a source of reducing power of glutathione. GSH also formed phytochelatins via phytochelatin synthase. GSH works as scavenges on different types of free radicals ($H_2O_2$, $O_2$, $OH^{\bullet}$, and $O^{\bullet-}{}_2$) and neutralizes their effects on biomolecules. It forms glutathiolated adducts and involves in protection of different biomolecules from reactive oxygen species. GSH also acts as powerful role in regenerating GSSG (Glutathione disulfide). GSSG is converted back to GSH, either by de novo synthesis or enzymatically. Therefore, the maintenance between GSSH and GSG is essential for redox state of the cell (Roy Choudhury et al. 2012a, b).

### 4.5.2 Ascorbic Acid

Ascorbic acid (AA) is a powerful and widely studied antioxidant compound. Ascorbic acid is able to donate electrons to different range of nonenzymatic and enzymatic reactions. In plant cells, ascorbic acid follows two pathways. Smirnoff-Wheeler pathway is major pathway in plant cells and catalyzed in the mitochondria by L-galactano-γ-lactone dehydrogenase enzyme, whereas other source of ascorbic acid is D-galacturonic acid. Ascorbic acid is not only single present in cell cytosol but also found in apoplast, where it worked in defense mechanism against reactive oxygen species (Barnes et al. 2002). Ascorbic acid also acts on metal-binding enzymes. AA is also protect and preserves the activities of metal-binding enzymes. At reducing state, ascorbic acid acts as cofactor for violaxanthin de-epoxidase and balances the excess excitation energy (Smirnoff 2000). It is also reported that ascorbic acid is also involved in preventing photooxidation of photo system II by pH-dependent modulation activity.

### 4.5.3 α-Tocopherol

α-tocopherol is lipophilic antioxidant. Tocopherols are known for their ability to work as potent scavengers of reactive oxygen species and free radicals. Scavenging activity is one of the essential components of biological membranes and central protectors. It protects membrane lipid of the chloroplasts by acting on superoxide ion and quenching its excess energy. It protects the structure and function of photo system II (PSII). Tocopherol also works as an effective free radical trap by interfering with the chain propagation in lipid peroxidation (Igamberdiev et al. 2004; Kiffin et al. 2006). Tocopherols have four isomeric forms (alpha, beta, gamma, and delta), among which α-isoform has shown the highest antioxidant potential. The tocopherols are synthesized only by photosynthetic organisms and thus only present in green tissues of plants. α-tocopherol is synthesized from γ-tocopherol by the enzyme γ- tocopherol-methyl-transferase (γ-TMT encoded by *VTE4*) (Igamberdiev et al. 2004; Holländer-Czytko et al. 2005).

### 4.5.4 Carotenoids

Carotenoid is a member of lipophilic antioxidants and belongs to molecules of antennae group. It absorbs light (450–570 nm) energy and transfers to the chlorophyll molecule. Chlorophyll molecule is present in the photosynthetic and nonphotosynthetic plant tissues. It is mainly located in plastids. Carotenoids are not only reported in plants tissues, but also found in some microorganisms. Carotenoids work on photosynthetic mechanism and show antioxidative activity by protecting in following four ways: (Agati et al. 2012).

A. It reacts with products of membrane lipid and involves in the end the chain reactions.
B. Helps in scavenging superoxide ion and releases by-product as heat.
C. Stop the development of superoxide ion with the help of chlorophyll molecule.
D. Removal of the surplus excitation energy through the xanthophylls molecule.

### 4.5.5 Phenolic Compounds

Phenolic compounds get considerable interests of the scientific society due to their antioxidant properties. They are present in plant parts and necessary for human diet. Phenolic compounds are made up of an aromatic ring which has one or more hydroxyl groups. These are found in a variety of range, simpler to complex high-molecular weight polymers. Phenolic compounds antioxidant activity is dependent on position and number of hydroxyl group and the nature of other substitution which is present on aromatic ring. The overall antioxidant activity is depended on the structure of phenolic compounds. Different agricultural products and food processing industries generate substantial quantities of phenolic-rich by-products, which could be valuable natural sources of antioxidants (Balasundrama et al. 2006).

### 4.5.6 Flavonoids

It is mainly present in different plant parts like leaves, pollen grain, and floral organs. It worked as secondary scavenger for reactive oxygen species. Due to excess excitation energy of reactive oxygen species, it is involved in the damage of photosynthetic apparatus. On the basis of structure, flavonoids can be characterized into four classes: flavones, flavonols, anthocyanins, and isoflavones. All flavonoids have variety of role like:

- Responsible for pigmentation in flowers and fruits.
- Provide color to seeds which are involved in plant fertility.
- Responsible for germination of pollens grains.
- Proving defense against pathogens associated to plants.

Flavonoids are also involved in scavenging of super oxide free radicals and lighten the damages occur on outer envelope of the chloroplast (Fini et al. 2011; Agati et al. 2012).

### 4.5.7 Proline

Proline is a good example of nonenzymatic antioxidant, which protects cells from damaging effect of reactive oxygen species. Proline is an osmolyte, hence it is also measured as a powerful antioxidant. Glutamic acid is a substrate for synthesis of proline via an intermediate pyrroline-5-carboxylate (P5C). The pathway of glutamic synthesis is catalyzed by pyrroline-5-carboxylate reductase (P5CR) and $ð^1$-pyrroline-5-carboxylate synthetase (P5CS). It is a good scavenger of hydroxyl ($OH^•$) and superoxide ($O_2$) ion and protects the damages of cell due to lipid peroxidation (LPO). For the duration of stress condition, proline concentration is increased in plant cell. This is due to either excess synthesis of proline or due to reduced proline degradation (Verbruggen and Hermans 2008).

## 4.6 Applications of PGPR

### 4.6.1 Role in Auxins Biosynthesis

Many PGPRs can induce production of auxins and show strong effects on growth as well as architecture of root. (Gupta et al. 2015; Jha and Saraf 2015; Ruzzi and Aroca 2015; Vacheron et al. 2013). Indole acetic acid (IAA) is the main example of auxin synthesized by PGPR. Auxins boost the interaction between plant and microbe (Ahemad and Kibret 2014; Afzal et al. 2015). Exogenous indole acetic acid activity is directly depended on the endogenous indole acetic acid levels in tissues of plants. Application of bacterial IAA may have positive, negative, or neutral effects on plant growth when an endogenous IAA concentration is optimum in plants (Spaepen and Vanderleyden 2011). Auxin-producing PGPR has shown changes at transcriptional level, mainly in defense, hormone, and genes related to cell wall (Spaepen et al. 2014). It increases biomass of root and decreases the density and size of stomata (Llorente et al. 2016).

### 4.6.2 Role in Cytokinin Biosynthesis

Phytohormones or plant hormones are naturally found organic molecules and influence many ways of growth and differentiation in plants. It also influences different stress mechanisms like biotic and abiotic (Davies 2010). Phytohormones are categorized into nine classes including cytokinins (CK), auxins, gibberellins (GA), ethylene (ET), abscisic acid (ABA), brassinosteroids (BR), jasmonates (JA), salicylates (SA), and strigolactones (SL) (Su et al. 2017).

Cytokinins (CKs) are isoprenoid-substituted adenine molecules. Isopentenyl transferases (IPTs) catalyze isoprenoid and synthesized various types of CKs including N6-(Δ2-isopentenyl)-adenine (iP), cis-zeatin (cZ), trans-zeatin (tZ), and dihydrozeatin (DZ). Plant physiology, growth, and development such as leaf senescence, flower and fruit development, seed germination, apical dominance, and plant-pathogen-interactions, etc. are directly affected by cytokinins (CKs). Trans-zeatin is one of the cytokinin which is found commonly in plants. CKs are metabolized and inactivated by two mechanisms. It is either through sugar molecule addition or degradation by cytokinin oxidases (CKXs). The activity and type of different CK molecules are depend on plant species, tissues, stages of development, and different environmental conditions. Cytokines are not only produced by plants tissues but are also produced by plant-associated microorganisms, microalgae, fungi, nematodes, phytoplasma, parasitic plants, and insects (Akhtar et al. 2020; Spallek et al. 2017; Dowd et al. 2017; Chanclud et al. 2016; Shanks et al. 2016; Ruzzi and Aroca 2015; Siddique et al. 2015). The CKs produced from above plant-associated micro organisms have balancing effect on growth of plant. It may attack on plant defense mechanism and increases virulence against disease (Spallek et al. 2018).

Direct role of cytokinin in stimulation and defense of plant cell have been documented in the early century 2010 but synthesis of cytokine phytohormone by plant- associated microbes is a new concept. It is recognized during signaling between different kingdoms. It is also reported during the defense mechanism between plants and associated pathogens. The CKs produced by microbes are interacting with plant hormone and related signaling pathways. It is a similar way mechanism as plant-derived CKs. It is integrated in plant signaling pathways and also induces the direct synthesis of phytoalexins. Thus, the bacterial and microalgal CKs provide a defense against pathogens as done by exogenous CKs (Akhtar et al. 2020). Production of cytokinin by PGPR is a new alternative pathway to increase the growth of plants and could be a good alternative to enhance the quality and yield crops of agricultural (De Garcia Salamone et al. 2005).

### 4.6.3 PGPR as Biofertilizer

Use of bacterial consortia in agriculture has many effects on yield of crops (Wu et al. 2009). For example, mixture of a bacteria (*B. amyloliquefaciens*) and fungus (*Trichoderma virens*) increases yields of tomato and corn (Akladious and Abbas 2012; Molla et al. 2012). It is reported that inoculation of plants with $N_2$-fixing bacteria (Azospirillum and Azobacter) allowed increase yield and quality of oil in sesame seeds (Shakeri et al. 2016) and improved growth of biofuel crops in switchgrass (Smith et al. 2015). PGPR have natural power to manage the soil contamination and it can be used for phytoremediation. Contaminated land space may be used to cultivate biofuel and energy crops (Weyens et al. 2009; Evangelou and Deram 2014).

Biological approach is an optional method for fighting against plant-associated pathogens (Harman 2000). Valuable rhizobacteria are able to produce antibiotics and other defense-related compounds which worked against pathogens associated with plant tissues. Antibiotic production is one of the alternative approaches for biocontrol mechanisms (Doumbou et al. 2001; Compant et al. 2005). But pathogens with time again develop resistance to the antibiotics, so that pathogens cannot be fully restricted in the long-term. Thus, there is always requirement of multiple controlling methods rather than unnecessary dependency on a single approach. PGPR is also able to generate antibiotics like polyketides, lipopeptides as well as produces some antifungal metabolites which are involved in suppression of the growth of plant pathogens (Prashar et al. 2013).

## 4.7 Future Outlook

The concept of plant holobiont provides a new viewpoint to understand the interaction between plants and microorgsanisms. In present scenario, the microholobionts may be seen as a supporting system or component which provides some supplementary genes to the host plant cell, which helps plants to adjust in the local environmental conditions. It is suggested by many studies in above content that host adapted microbes formed an environment around the plant cell which fight against pathogens. It also improves the nutritional and growth quality of agriculture crops. This chapter will open new doors to plan strategies for improving the effectiveness of biocontrol agents in natural way. Thus, PGPR community may prove to be an effective tool for sustainable development of agriculture in future.

## 4.8 Conclusion

PGPR or plant growth-promoting rhizobacteria, have numerous actions on plant growth promotion and control of other plant pathogens. It exhibits bioremediating properties by detoxifying pollutants present in soil like chemicals or pesticides, heavy metals, and controlling infection of phytopathogens as biopesticides. The effectiveness of a PGPR as holobionts is reported due to different nonenzymatic antioxidants present in it. In future, it is expected that PGPR is used to replace the chemical pesticides and fertilizers. It also overcomes the problem associated with synthetic growth regulators which have so many negative effects for agriculture crops. This review will be helpful in future research and help to understand the mechanisms of nonenzymatic antioxidant related to PGPR as well as mechanism of biocontrol agent.

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# Carotenoids and Flavonoids in Plant Stress Management

# 5

Antul Kumar, Sharon Nagpal, and Anuj Choudhary

**Abstract**

Globally escalating food demand and unpredictable global warming have threatened the humanity in jeopardy. Excessive use of commercial pesticides and chemical fertilizers is providing ease of handling, but their toxic nondegradable residues are known to exert negative impacts on the plants, microbes, and even soil health. The unpredictable climate change leads to enhanced incidence of abiotic and biotic stresses in plants. Several integral approaches of fungi, microbes, plant, and their derived metabolites are used to encounter the stresses for effective crop management. The secondary metabolites are proving their immense potential and have time and again being proven to cope up the unavoidable and unpredictable changes due to adverse environmental conditions. The chapter highlights the role of both microbial and plant-based flavonoids and carotenoids as key agents for managing biotic and abiotic stress tolerance in crop plants. Moreover, such feasible and efficient biological application using plant-based composition helps to mitigate the challenges and open new gates of sustainable agriculture.

**Keywords**

Carotenoids · Flavonoids · Metabolites · Stress · Tolerance

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## 5.1 Introduction

Plants as sessile organisms are constantly being affected by changes in their environmental conditions. The unfavorable or stress conditions have forced plants to change the functional aspect of powerful signaling sensors that guide them towards attaining optimal growth via manipulating their biophysical, biochemical, and molecular events (Ingle and Padole 2019; Taïbi et al. 2016; Enebe and Babalola 2018). Unpredictable and unavoidable climatic changes enforce abiotic stresses as the prime limiting factors among diverse stresses threatening agriculture and leading to decline in productivity both qualitatively and quantitatively (Grayson 2013; Nabavi et al. 2020). Majorly, diverse environmental constraints affect crop lands worldwide and only 3.5% of the global land area remains unaffected. The abiotic stresses, viz., salinity, floods, nutrient starvation, drought, heat, light intensity, cold/chilling, anaerobiosis, and acidic conditions are known to decline agricultural productivity worldwide (Meena et al. 2017). In this regard, the enormous chemical compounds have been produced in the plants which can be used as taxonomic markers and known as secondary metabolites (Bennett and Wallsgrove 1994; Tabatabaei and Ehsanzadeh 2016; Kaab et al. 2020). Secondary metabolites are attracting interest of the scientific communities owing to their indirect biological role in the growth and development of organisms. Secondary metabolites are versatile compounds often demonstrating in plant physiological processes as protectants against abiotic and biotic stresses in plants. These play a central role in various associations of plant–rhizobia, plant–microbe, soil microbes, and arbuscular mycorrhizal symbiosis and boost plant growth via the production of important biological agents (Del Valle et al. 2020).

In the last decades, the secondary metabolites are proving their immense potential in the plant stress physiology. The immobile nature of plants diverted the sieve of selection to utilize the secondary metabolites to overcome the stress constrains and help them to survive in the changing environments (Ingle and Padole 2019). Rhizospheric microbes form an interacting association with crop plants which ultimately regulates the nutrient supply to plants through varied direct mechanisms (Rob et al. 2020; Kaab et al. 2020). Studies on plant-based microbial associations at physiological, biochemical, and molecular levels recognized that microbial interactions directly affect plant responses under various stress conditions (Farrar et al. 2014). For deciphering the mysterious interaction among connections and mechanisms regarding the functional role at molecular levels with the resistance responses against abiotic stresses. The priming stimulates on antioxidant function have been found to compare with enhanced transcript levels of glutathione and ascorbate biosynthesis, enzymatic antioxidants that representing complex transcriptional alteration of the antioxidant components (Savvides et al. 2016). Different technological advancements also accelerated understanding of mutant technology, RNAi-mediated gene silencing, metabolite profiling gene editing systems, and proteomic analysis to identify capacious molecular communication that helped in enlightening our awareness of microbial-based mitigation approaches toward abiotic stresses tolerance in plants. New advances in Multiomics have appeared as integrated, holistic, and other analytical approaches for the analysis of one of the major dynamic and complex system of plants-microbial interactions that regulating the consequences progressed in the different plants to improve them to tolerate stress conditions (Luan et al. 2015).

## 5.2 Classical Example of Secondary Metabolites in Plant Resistance

Pigmentation is hallmark of microbial colonies rich in flavonoids and carotenoids content. These compounds protect microbes from various adverse conditions including drought, heat, cold, salinity, UV protection, and other damages (Rob et al. 2020). Carotenoids represent an important group of natural occurring pigments that are chiefly abundant in both photosynthetic (algae and plants, cyanobacteria) and nonphotosynthetic organisms (bacteria and fungi). These are synthesized within the chloroplasts of algae and plants naturally and the lycopene cyclization is major branch point in their biosynthesis. Lycopene, the product is converted by the activity of lycopene β-cyclase into β-carotene-like derivatives that plays significant roles against oxidative burst, photoprotection, salinity, drought, and salinity damages in higher plants (Paliwal et al. 2017). Quercetin is the aglycone form of ubiquitous flavonoid glycosides such as quercetin and rutin, rich in the citrus fruits, buckwheat tea, celery, broccoli, lettuce, tomatoes, *Ginkgo bioloba*, and onions (Table 5.1). They have high antioxidant potential hence fall under the category of effective antioxidants under the UV radiation, drought, salinity, cold, nitrogen deficiency, and heavy metal stress (di Ferdinando et al. 2012; Zhan et al. 2019). The apigenin is naturally occurring metabolite available in 4′, 5, 7-trihydroxyflavone form, and is found to be present in large quantities in celery, chamomile, vine spinach, oregano, and artichoke in the dried form (Table 5.1). They also have high antioxidant potential induced under the drought and UV radiation (Shukla and Gupta 2010). Fisetins are the ubiquitously present glycosylated compounds having powerful antioxidative potential (Gonçalves et al. 2018). Kaempferols are known to regulate several proteins and their functional activities such as cell growth and differentiation (Peer and Murphy 2006; Pei et al. 2020). Naringenin is widely distributed in the bergamot, tomatoes, citrus, and other fruits, which functions to interfere with the activity of the phenylpropanoid pathway enzyme, 4-coumarate: CoA ligase. It causes allelopathic impact on the growth and lessens the lignin deposition in plants (*Echinochloa oryzicola*, *Zea mays*, and *Oryza sativa*) (Deng et al. 2004). Chrysin is involved in the presymbiotic growth, i.e., spore germination, hyphal growth, branching patterns, and the formation of secondary spores, of the various arbuscular mycorrhizal fungi (*G. margarita, Gigaspora rosea*, *Glomus mosseae*, and *G. intraradices*) (Scervino et al. 2005). Apigeninidin is the anthocyanidin produced in the sorghum under the pathogen attack (Mizuno et al. 2014). Rutin is the ubiquitously present flavone enhancing the defense responses against the number of abiotic stresses in plants (Samanta et al. 2011). Catechins are the anthocyanins produced against the pathogen attack in the variety of plants (Barry et al. 2002).

**Table 5.1** Secondary metabolites involved in defense reactions

| No. | Metabolites | Class of secondary metabolites | Induced under type of stress | Plant source | Potential type | References |
|---|---|---|---|---|---|---|
| 1. | Quercetin | Aglycone form | UV radiation, cold, nitrogen deficiency, heavy metal stress, drought, and salinity stress | Ubiquitously in plant | High antioxidant potential | di Ferdinando et al. (2012) |
| 2. | Apigenin | Flavone | Drought and UV radiation | Ubiquitous | High antioxidant potential | Mekawy et al. (2018) |
| 3. | Genistein | Isoflavone | Salt stress | Currants, raisin, and legumes | Increased photosynthesis levels, nodulation, and nitrogen fixation | Gonçalves et al. (2018); Akcin and Yalcin (2016) |
| 4. | Fisetin | Flavonol | – | Ubiquitously in plant | Powerful antioxidative effects | Rashid et al. (2019) |
| 5. | Kaempferol | Flavonol | Oxidative damages and UV radiation | Ubiquitously in plant | Exhibits antioxidant property by inhibiting the AOX activity | Peer and Murphy (2006) |
| 6. | Naringenin | Flavanones | Heat stress and salinity | Citrus fruits, bergamot, and tomatoes | Allelopathic impact | Deng et al. (2004) |
| 7. | Diadzein | Isoflavone | Pathogen attack | Leguminous plants | Intermediate signal carriers, and defense responses particularly to pathogenic attacks | Yu et al. (2000) |
| 8. | Myricetin | Flavonols | – | Abundant in fruit, vegetables, tea, berries, and red wine | Allelopathic, antimicrobial, and antioxidant activities | Samanta et al. (2011) |
| 9. | Chrysin | Flavone | – | Abundantly present in many plant extracts, including *Propolis*, blue passion flower | Symbiotic growth | Scervino et al. (2005) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 10. | Apigeninidin | Anthocyanidin | Pathogens | Sorghum | – | Mizuno et al. (2014) |
| 11. | Rutin | Flavone | UV light, low temperature, and desiccation | Ubiquitous | Enhancing the defense system against environmental stresses | Samanta et al. (2011) |
| 12. | Catechin | Anthocyanins | Pathogen infection | Variety of plants | Represents the antimicrobial defenses | Barry et al. (2002) |
| 13. | Gallic acid | Simple phenolic acid | Salt and osmotic stresses | Rice | Promoting the antioxidant response | de Klerk et al. (2011) |
| 14. | Caffeic acid | Hydroxycinnamic acid derivatives | Soil salinity and other abiotic stresses | Legumes | Lignin synthesis, increasing cell wall thickness, and improving plants defense mechanism | Enebe and Babalola (2018) |
| 15. | Syringic acid | Simple phenolic acid | Osmotic stress | *Vicia faba* | Stomatal closure restoration induced by ABA | Purohit et al. (1991) |
| 16. | Vanillic acid | Simple phenolic acid | – | *Malus* | Enhances the rooting and inhibition of IAA decarboxylation | de Klerk et al. (2011) |
| 17. | Ferulic acid | Hydroxycinnamic acid derivatives | – | *Zea mays* and *Oryza sativa* | Increases the IAA oxidase activity in seedlings, gene overexpression involved in the biosynthesis of ethylene and jasmonic, inhibition of lateral roots and root hairs formation, and reversed ABA effect | Chi et al. (2013); Purohit et al. (1991) |

Gallic acid is the simple phenolic acid observed in the rice under salt and osmotic stress promoting the antioxidant responses (de Klerk et al. 2011).

Similarly, Syringic acid was observed in the *Vicia faba* under the osmotic stress involved in the stomatal closure to maintain the turgidity of plants (Purohit et al. 1991). Vanillic acid is a phenolic acid which was observed to enhance rooting in the *Malus* plant (de Klerk et al. 2011). Hydroxycinnamic acid derivatives such as ferulic acid and caffeic acid are involved in the defense responses. Caffeic acid was observed in the legume plants under the saline and abiotic stresses enhancing the cell wall thickening, lignifications, and inducing plant defense mechanisms (Enebe and Babalola 2018). Similarly, ferulic acid increases the IAA oxidase activity in seedlings, gene overexpression in the ethylene and jasmonic acid biosynthesis, and inhibition of lateral roots and root hair formation (Chi et al. 2013). The resistance provided to organisms mediated by secondary metabolites, viz., carotenoids may have ecological significance allowing organisms to colonize certain environments, for example, invasion of wounds and colonization of leaf surfaces by pigmented heterobasidiomycetous yeasts. In microbes, the knowledge of the mechanisms underlying the control of singlet oxygen species is based on few model organisms, viz., *Chlamydomonas*, *Rhodococcus sphaeroides*, *E. coli*, *Agrobacterium tumefaciens*, and *C. Crescentus*. The response to any kind of stress appears to be tightly controlled at the transcriptional and post-transcriptional levels. It appears that the response of the microbes to stress involves a complex network of regulatory factors, which will make it difficult to unravel their individual functions. Recent research on chemical priming has provided further knowledge of the complex mode of action of specific signaling molecules in plant stress tolerance.

## 5.3 Flavonoids and Carotenoids: New Contenders of Plant Resistance in Twenty-First Century

Flavonoids and carotenoids both of plant and microbe origin are well-known contenders providing tolerance response to higher plants in abiotic stresses (Fig. 5.1). Additionally, long-reported responses as protectors of damaging solar radiation both have been known for key roles as antioxidants by reducing the production of reactive oxygen species (ROS) after formation at primary response in stressed plant. Flavonoids are abundantly distributed in various cell organelles including chloroplast, nucleus, small vesicles endoplasmic reticulum, vacuole, cytosol, and extracellular space. Flavonoids such as flavones, flavonols, anthocyanins, and proanthocyanidins have been identified as antioxidants and function to protect plants from cellular damage under stress conditions (Clayton et al. 2018). The chloroplast-associated metabolites scavenge free radicals to maintain cellular redox. Kaempferol and Quercetin glycosides were reported abundant in chloroplasts during fungal attack, ultraviolet (UV) irradiation, salinity, drought, and function as protective antioxidants systems under stress conditions (Rob et al. 2020). According to Agati et al. (2009) flavonoids have long been accounted to be present in the cell vacuoles, epidermal cells wall, glandular, and nonsecretary trichome structures and hence have

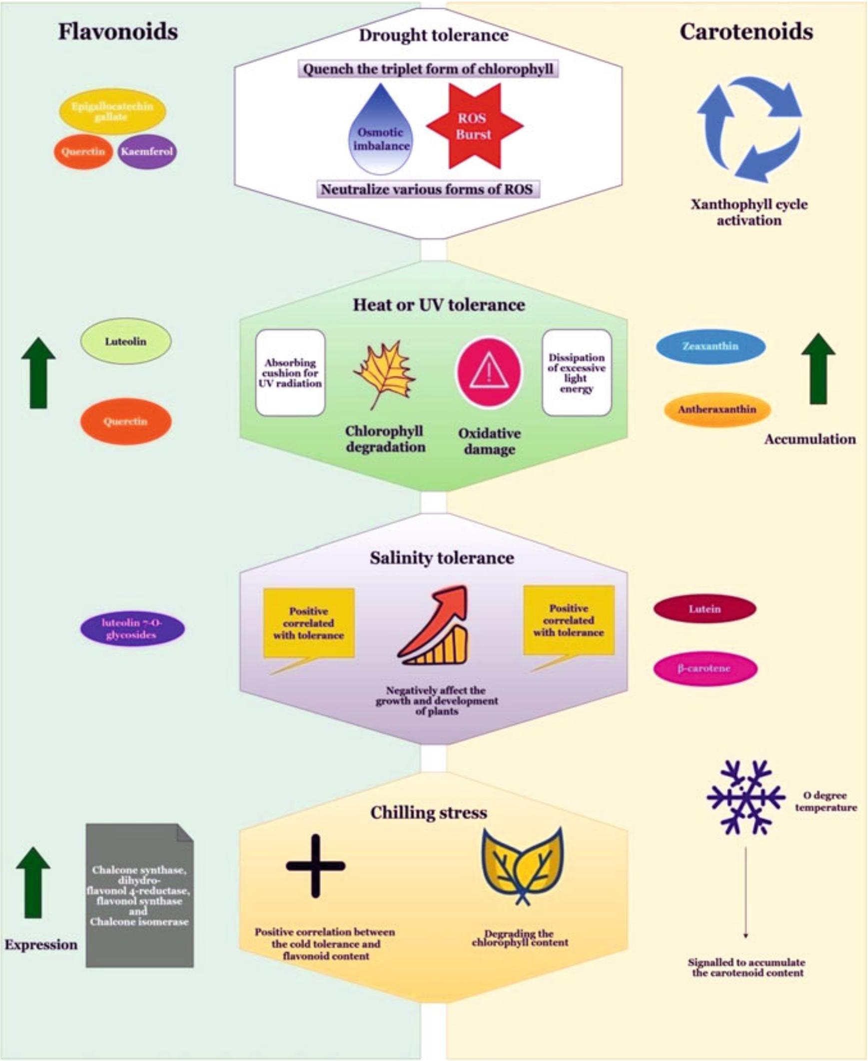

**Fig. 5.1** Carotenoids and flavonoids as defense agents

been expected mainly to have the role of mitigating short ranges of solar wavelengths. Although anthocyanins have been well known to store in the vacuoles (mesophyll cells) in most of the plant species, and strongly supported by in vitro studies (Agati and Tattini 2010). However, studies confined that flavonoids may help in ROS scavenging within or towards the production sites. Microalgae species belonging to genus *Chlorella, Dunaliella, Scenedesmus*, and *Haematococcus* are considered as the key producers of commercially vital carotenoids. Many microorganisms have been reported to produce secondary metabolites such as astaxanthin, lutein, and b-carotene, etc. (Table 5.2).

**Table 5.2** Diverse microbial strains as active producers of flavonoids and carotenoids

| Secondary metabolite | Producer strains | References |
|---|---|---|
| Quercetin | *Chryseobacterium balustinum*, *Arbuscular mycorrhiza* | Lattanzio et al. (2006) |
| Genistein | *Gibberella fujikuroi*, *Bradyrhizobium japonicum* | Dashti et al. (2014), Cesari et al. (2019) |
| Naringenin | *Bradyrhizobium* sp., *Azospirillum brasilense* | Boué et al. (2000) |
| Diadzein | *Gibberella fujikuroi* | Hamayun et al. (2017) |
| Syringic | *Arthrobacter* sp. | Cesari et al. (2019) |
| Vanillic | *Nitrinicola lacisaponesis* | Dashti et al. (2014) |
| Ferulic | *Halomonas* sp. | Passari et al. (2017) |
| Rutin | *Bacillus endophyticus*, *Pseudomonas aeruginosa* | Kousar et al. (2020) |
| Catechin | AMF (*Glomus mosseae*) and PGPR (*Bacillus pumilus*) | Bhutia et al. (2012), Dashti et al. (2014) |
| Gallic | *Pseudomonas mendocina* | Passari et al. (2017) |
| Caffeic | *Bacillus pumilus* | Kousar et al. (2020) |

Rutin accumulation was significantly lowered in salt susceptible species. High antioxidant ($O^{-2}$ and $H_2O_2$) contents were detected in both types of rutin-reduced transgenic plants under high salt stress. Moreover, exogenous application of rutin effectively enhances the ROS scavenging and ultimately gains the salinity tolerance. The environmental stresses have multiple and complicated effect on plant growth and development. The environmental stresses altered either incrementally or rapidly. Essentially plants recognize and respond differentially to biological signals appropriately with the required pace of survival (Takahashi and Shinozaki 2019). The sophisticated networks of physiological processes in plants arise due to involvement of diverse molecular and cellular level of regulatory mechanisms required for both short- and long-term responses varying from cellular to whole plant level (Rob et al. 2020). It has been accepted that flavonoids also control movements of phytohormones like auxin, and a nuclear allocation of chalcone isomerase (CHI) and chalcone synthase (CHS) is consistent with regulation employed by flavonoid compounds on the genes transcription mediated for development changes, facilitated via the auxin-transporting facilitator proteins (ATFP) (Agati and Tattini 2010).

## 5.4 Stress Tolerance Responses by Flavonoids and Carotenoids

### 5.4.1 Drought Tolerance

Under the drought conditions, the osmotic and ROS balance are disturbed which stressed the cellular metabolism and structure (Rao et al. 2020). Plants develop array of mechanisms such as compatible solute production, antioxidant production, and other entities to withstand the unavoidable stressed conditions. The concentration of ascorbic acid and α-tocopherol and epigallocatechin gallate (flavonoids) were higher in the *Cistus clusii* grown under the drought condition (Hernandez et al. 2004). In the *A. thaliana* seedlings, the total flavonoids and the quercetin, and kaemferol (flavanols) were higher in roots (Shojaie et al. 2016). Similarly, the *Jatropha* seedlings showed higher concentration of flavonoids under the drought conditions (Lama et al. 2016). Carotenoids have ability to neutralize various forms of ROS and can quench the triplet form of chlorophyll in the leaves (Ramel et al. 2012). Under the drought conditions, the carotenoid levels increased in the olive trees, beans, and alpine plants which were considered to be associated with the xanthophyll cycle activation (Doupis et al. 2013; Buchner et al. 2017; Schweiggert et al. 2017). The increase in the zeaxanthin content was also suggested to play important role in the different abiotic stress tolerance (Wu et al. 2015). However, it has been also reported that under moderate drought conditions, carotenoid content tends to decrease (Sudrajat et al. 2015). Applications of *Rhizobium* cultures enhance N-supply and fixation process in cowpea, soybean, red-clover, pea, and alfalfa crops. The symbiosis between *Bradyrhizobium* and *Rhizobium* sp. effectively supports the nutritional strategies, ensuring an efficient N-supply in legumes, pastures, and grasses (Nagpal et al. 2020; Kumawat et al. 2019). The N-fixation, nodulation number, and growth in *Medicago sativa* improved with *R. meliloti* strain along with increased yield under water- deficient environment. In another study, *Rhizobium* strains effectively enhanced the nodulation rate in *M. sativa*, *V. faba*, *P. sativum*, and *P. vulgaris*, whereas reports support the effectiveness of plant–microbe interactions to improve drought stress in legumes via degrading phosphorus (Rob et al. 2020).

### 5.4.2 Heat or UV Tolerance

UV radiations are naturally imparted on the plants along with the sunlight. To which plants have to sense and respond on the basis of damage caused which depends upon the repair and acclimation response. Flavonoids provide themselves as absorbing cushion for UV radiation nullifying the ROS- induced cellular damages. The activation of photoreceptors induces the transcription factors to trigger the transcription of flavanoid biosynthetic genes. It has been observed that UV-B photoreceptor mutants (uvr8-1) and *Arabidopsis* wild plant under continuous 4 h of low-dose UV radiation depict the activation of chalcone synthase stimulation (enzyme involved in flavanoid biosynthesis) (Jenkins 2013). In several plant species, the ratio of luteolin to apigenin and quercetin to kaempferol has been enhanced under the UV light (di Ferdinando et al. 2012). The quercetin and luteolin glycosides aid in reducing the ROS production by effectively chelating with Cu and Fe ions (Brown et al. 1998; Berli et al. 2010). In the pepper plant, the compounds for UV-B absorption and antioxidants such as carotenoids have been induced under the UV-B radiation exposure (León-Chan et al. 2017). Similarly, in the tobacco plant, the carotenoid content and antioxidant capacity have been intensified after the low and high UV-B exposure (Shen et al. 2017). Moreover, the antioxidants accumulated in apical epidermal layer of leaves and stems to protect from the injuries from internal tissues against UV-B radiation. In *A. thaliana*, the kaempferol synthesis is deficient in flavone (chalcone) isomerase mutant shows much sensitivity to UV radiation. The worthy role of flavonoids is not exceptional functional of UV-protection via inducing antioxidant molecules, signal molecules, secretion of defensive compounds, and allelochemicals during exposure of UV radiation in plant. Moreover, flavonoids that accumulate in upper epidermis layer specially absorb a large amount of light ranging from 280 to 340 nm wavelengths.

Xanthophyll cycle was activated during intensive light conditions and helps in biosynthesis of zeaxanthin and proteins. According to Couso et al. (2012), the cytochrome-dependent hydroxylases and carotenes enzymes were evaluated to be up-regulated that control the xanthophyll cycle during light stress. Accumulation of zeaxanthin and antheraxanthin, and corresponding decrease in violaxanthin amounts were correlated with high light intensity resistance. This interconversion of zeaxanthin and violaxanthin is associated to a promising photoprotective role in the photosynthetic machineries indulging in dissipation of excessive light energy when exposed to intensive light stress.

In algal species, carotenoid accumulation was reported to enhance with temperature that activates cellular response or antioxidant mechanism related to enhanced activity of biosynthetic enzyme, free oxygen radical formation, and damaging effects observed at elevated temperatures. The enhanced threshold levels and carotenoid content by two-folds were reported by Juneja et al. (2013) in *Haematococcus pluvialis* and *Chlorococcum* sp., with temperature rise from 20 to 30 °C. *Dunaliella salina* (Halophytic green alga) has been noted to accumulate huge concentration of β-carotene when exposed to excessive water salt, light, and nutrient amounts in extreme conditions (Llorente et al. 2017).

The effect of ultraviolet-B radiation affected the chlorophyll content by degrading them and induced the accumulation carotenoids in *Capsicum annuum* (León-Chan et al. 2017). Enhanced level of UV-B rapidly elevates carotenoid content compared with control plants and the production of those compounds. The increased carotenoids and ROS at high UV-B radiation were reported in *Nicotiana tabacum* L. leaves play important role in tolerating the oxidative damage in plant caused by high UV-B conditions (Shen et al. 2017).

### 5.4.3 Salinity Tolerance

Excessive accumulation of ROS is primary cause of salinity stress usually resulting in deleterious effects on plants (Kang et al. 2018). Flavonoids and carotenoids play an important protective role against oxidative burst and injuries caused by salt stress. In *Lupinus albus* seedling, the *Chryseobacterium balustinum* Aur9 elevated total nitrogen content, root surface, germination rate, aerial growth, N-absorption, and biological N-fixation. It also promoted the systematic induced responses and helped to overcome the salt stress in soybean and *Arabidopsis* species. Under high salinity condition, strains of *Sinorhizobium fredii* were used to enhance the nitrogen fixation rate via eliciting the nodule number in significant manner (Rodríguez-Navarro et al. 2002). In *Rhizobium tropici,* salt-tolerant wild-strain, the increased antioxidant enzymes activities including dehydroascorbate reductase and superoxide dismutase resulted in salinity tolerance and were correlated to enhanced antioxidant enzymes in nodules (Tejera et al. 2004). The various nitrogen-fixing bacteria tolerant to high concentration of NaCl are *Rhizobium meliloti*, *R. leguminosarum*, and rhizobia from *Vigna unguiculata* whereas *Rhizobium fredii*, *R. meliloti*, and *Sinorhizobium fredii* show regulatory mechanism for $K^+$ strictly but control $Mg^{2+}$ flux under salt stress condition. The *R. meliloti* derived from xerophytic tree species formed a symbiotic association with *M. sativa* and confirmed effective salt tolerance (Zahran 1999).

Several metabolic and physiological changes are affected by the salinity stress. On the basis of duration and intensity, the salinity stress can negatively affect the growth and development of plants. According to Zhao and Dixon (2009), a positive correlation was observed between the flavanoid biosynthesis level and the Glutathione-*S*-transferase content (role in flavonoids transportation to vacuole). Similarly, the biosynthesis of luteolin 7-*O*-glycosides was significantly enhanced under the salinity conditions (Agati et al. 2011). The seedling of *Solanum nigrum*, having lower dry biomass of leaves and roots along with the higher flavanoid contents (Abdallah et al. 2016). The accumulation of carotenoids such as β-carotene and lutein in the sweet potatoes were observed under the salinity stress. The IbZDS gene encoding β-Carotene desaturase is related with the carotenoid accumulation for tolerance whose expression induced for carotenoid biosynthesis under salinity condition (Li et al. 2017). In the *Solanum nigrum*, the expression of phytoene desaturases, zeta carotene desaturases, phytoene synthase, capsanthin/capsorubin synthase, and lycopene b-cyclase was down-regulated under the higher salt concentration in the salt sensitive *Capsicum* plants (Maurya et al. 2015).

According to Li et al. (2017), the expression of carotenoids can be up-regulated or down-regulated by overexpression or suppression of genes inducing activity of f-Carotene desaturases like enzymes and their biosynthetic pathways. The overexpression of IbZDS (*Ipommea batata*) gene is associated with ZDS, resulted in significant increases in β-carotene and lutein-related accumulation improved salinity tolerance sweet potatoes. The suppression of lycopene-cyclase (α-LCY) and lycopene β-cyclase (β-LCY) genes by RNA interference resulted in enhanced concentrations of total carotenoids, β-carotene, and ultimately stress tolerance cells of sweet potatoes (Kim et al. 2015). The findings were also supported by Kang et al. (2017) in sweet potatoes, where CHY-b suppression up-regulates the carotenoid level and enhances tolerance to stress. According to Jin et al. (2015), salinity stress tolerance observed to be associated with LCY-β and α-LCY genes in *Nicotiana tabacum* in which suppression of α-LCY and overexpression of LCY-β play role for enhanced salinity tolerance. The variations reported in species level depend upon the complexity in regulation of carotenoids. However, the transcriptional control regulating genes interaction that is all involved in regulating level of carotenes compounds during stress tolerance. The expression of phytoene synthase 2 and LCYB genes was associated with the enhanced level of lutein and carotene observed in *Solanum nigrum*. The expression of lycopene β-cyclase, zeta carotene desaturases, phytoene desaturases, phytoene synthase, and capsorubin/capsanthin synthase in capsicum sp. was down-regulated with increase in salinity level (Maurya et al. 2015; Ben Abdallah et al. 2016).

Elevated levels of copper induce the production of ROS and carotenoids formation; in *Vicia faba*, subjected to elevating the amount of copper were reported to be concentration-dependent. The increased carotenoid levels could be result of enhanced activity of nonenzymatic antioxidant system related to secondary metabolic compounds (Abdel-Latef and Abu-Alhmad 2013). In *Cicer arietinum*, cadmium (Cd) stress caused significant enhancement in level of carotenoids. The carotenoid synthesis lowered ROS damage induced by cadmium toxicity (Ahmad et al. 2016) whereas, phosphorus (P) or nitrogen (N) repletion was observed to elevate the carotenoid levels in algae and increased levels of thiobarbituric acid (TBA) and $H_2O_2$ reactive species along with decreased SOD activity (Moussa et al. 2017). In microalgae, Zhang et al. (2017) studied enhanced carotenoid accumulation in unfavorable conditions. The findings showed that nitrogen depletion facilitated the synthesis of carotenoids, compared to chlorophylls and demonstrated that carotenoids were highly sensitive to N stress. Comparatively declined chlorophyll content was observed with progressed nitrogen stress. These findings showed that the carotenoid content in *Chlorella vulgaris* varied over time with different salinity environmental conditions (Zhang et al. 2017).

### 5.4.4 Chilling Stress

The low temperature can affect the plant growth cycle with each stage from its germination to the maturity. Plants respond differentially based on their genetic potential to tolerate chilling stress. Many plant species either tropical or subtropical (rice, maize, and tobacco) fail to survive under chilling temperature; however, some overwintering cereals and *Arabidopsis* can continue to survive and grow (Liu et al. 2018). The increase in flavonoid content was noticed with the lowering in temperature reported in the epidermal cells of many plant species (Bilger et al. 2007). In the *A. thaliana* accessions, the positive correlation was recorded between the cold tolerance and the level of flavonoid content (Korn et al. 2008). Many biosynthetic genes such as chalcone synthase, dihydroflavonol 4-reductase, flavonol synthase, and chalcone isomerase for flavonoids were expressed higher in the cold tolerant plants (having higher anthocynin and quercetin derivative content) than the sensitive ones (Hannah et al. 2006). The higher flavonoids in the fruits of *Phoenix dactylifera* L. were reported at the 0 °C temperature condition along with the no any kind of chilling injury symptoms observed (El-Rayes 2009). The low temperature resulted in decline in chlorophyll content and signaled to accumulation of carotenoids in the *Capsicum annuum* (León-Chan et al. 2017).

## 5.5 Biotic Stress Tolerance: A Feasible and Efficient Biological Application

The microbial inoculants and their secondary metabolites are proved to be efficient agents and act as biofungicide, bioherbicides, and biopesticides. Microbial inoculants play important role as biocontrol approach could be driven by elevating phosphorus solubilization, improve biological nitrogen fixation, nutrient absorption at rhizospheric region, systemic acquired resistance, and production of synergetic secondary metabolites in low concentration provides toxicity to pathogens. Moreover, the toxic effect could be overcome by production of regenerative hormones (ethylene and auxin) at damage site. Several fungi and microbe inoculation of *Aspergillus niger*, *Penicillium citrinum*, *Aspergillus fumigatus*, *Penicillium funiculosum*, *Penicillium aurantiogriseum*, *Trichoderma koningii*, *Phytophthora infestans*, *Sporolactobacillus inulinus*, *Amphibacillus xylanus*, *Bacillus amyloliquefaciens*, *Microbacterium oleovorans* release inhibitory compounds against fungal pathogens (Nesci et al. 2005; Pereira et al. 2007; Etcheverry et al. 2009). Strains of *Bacillus* spp. secrete inhibitory compounds and used as biological controlling agent in wide range of fungal infections in plants. *Bacillus subtilis* was used to be control production of aflatoxin and *Aspergillus flavus* in field condition, whereas Pseudomonads and rhizobia are effectively known to suppress the growth of *Fusarium* and *Pythium* sp. (Antoun and Prévost 2005; Cepeda 2012; Alori and Babalola 2018). Moreover, both microbes and plants employed alternative mechanism in form of antibiotics or phytoalexins containing flavonols, carotenoids, alkaloids substance to control the plant pathogens. The compounds show antagonistic effect at rhizosphere and exhibit biocidal properties on soil-borne pathogens. The properties can be altered by formulation, cultivation, and application methods of PGPR under stress responses (Glick 2012, 2015; Raaijmakers and Mazzola 2012; Grobelak et al. 2015; Bhattacharyya et al. 2016; Olanrewaju et al. 2017). Certain classical examples for PGPR producing bactericidal effects are *Pseudomonas* spp. and *Bacillus* that have been identified to liberate aerugine, azomycin, bacilysin, butyrolactones, bacillaene, cepaciamide A, cepafungins, chlorotetain, 2,4-diacetyl phloroglucinol, fengycin, tas A, karalicin, phenazine-1-carboxylic acid, rhamnolipids, iturin, pseudomonic subtilin, subtilosin, sublancin, oomycin A, zwittermycin A, kanosamine, and pyrrolnitrin (Alori and Babalola 2018). The total phenols, carotenoids, and anthocyanin content observed to be enhanced in lettuce via exogenous application of inoculation containing *Glomus fasciculatum* and *Azotobacter chroococcum* (Baslam et al. 2011). Similarly, flavonoid content was reported to be increased with inoculation of *Glomus mosseae* and *G. fasciculatum* in lettuce (Baslam et al. 2011). Arbuscular mycorrhizal fungi (AMF) speed up antioxidant biosynthesis upto 75% significantly through rhizobacterial inoculation in soybean seedlings. The above findings are well supported by the inoculation studies of *Bacillus megaterium* and *Pseudomonas fluorescens* in catharanthus under stress conditions (Alori and Babalola 2018).

### 5.5.1 Fungal Tolerance

Different bacterial strains of PGPR including *Bacillus subtilis*, *Bacillus licheniformis*, *Chryseobacterium*, *Balustinum*, and *Pseudomonas* sp. when combined enhance significant level of resistance against soil-borne diseases in tomato and pepper under in vitro conditions. The genes isolated from *Lycium chinensis* such as carotene isomerase (LcCRTISO), phytoene desaturase (LcPDS), and β-carotene desaturase (LcZDS) via carotenoid biosynthesis are significantly enhanced making the tobacco plant salinity resistant (Lattanzio et al. 2006; Domenech et al. 2006). *Pseudomonas mendocina*, *Nitrinicola lacisaponensis*, *Bacillus pumilus*, *Halomonas* sp., and *Arthrobacter* sp., isolated from salinity conditions showed siderophore, indole acetic acid (IAA), ammonia production, P-solubilization, and other plant growth-effecting traits. Studies on bacterial inoculation of above isolates enhanced presence of flavonoid quercetin and phenolics compounds cinnamic, ferulic, gallic, vanillic, syringic, and caffeic, acids in the rhizosphere soil. The high concentration of phenolics, quercetin, and IAA in rhizosphere soil zone exhibited a cumulative synergistic function that supported elevated salinity stress tolerance in wheat (Venkidasamy et al. 2018; Pathan et al. 2020; Tiwari et al. 2011; Mahmoudi et al. 2019).

### 5.5.2 Bacterial Tolerance

*Bacillus cereus* (strain BS107) against *Xanthomonas axonopodis* pv. v*esicatoria* in pepper leaves shows induction of induced systematic response where, ISR-elicited responses of *Bacillus* spp. was also reported against root-knot nematodes, viral, bacterial, and fungal pathogens. Yang and Yen (2000) reported molecular evidences in favor of priming effect of Rhizobacterium strains on the up-regulation of ISR and defense genes in pepper plant.

### 5.5.3 Insect and Nematode Tolerance

The flavonoids and isoflavonoids are lethal to the insect pests by altering the behavior, growth, and development of target insects (War et al. 2013; Melo et al. 2017). The flavones 5-hydroxyisoderricin, 5-methoxyisoronchocarpin, and 7-methoxy-8- (3- methylbutadienyl)-flavanone are present in *Tephrosia villosa* (L.), *T. vogelii*, and *T. purpurea* (L.), respectively, which can be used to repell *Spodoptera exempta* and *S. littoralis* (Simmonds et al. 1990). The overexpression of transcriptional factors required for flavonoid production confers the resistance in *Arabidopsis* against the *Spodoptera frugiperda* (Johnson and Dowd 2004). The chickpea plant was showing antifeedent activity against the Helicoverpa armigera by mean of maackiain, judaicin, 2-methoxyjudaicin, and judaicin-7-*O*-glucoside isoflavonoid productions (Simmonds and Stevenson 2001). The alliarinoside, cyanopropenyl glycoside, and isovitexin-600-D-β-glucopyranoside interfere with the feeding activity of Pieris napi oleracea (native American butterfly) (Renwick et al. 2001). The overexpression of CCD1 leads to the β-ionone (carotenoid) in *Arabidopsis* plant increases the emission of β-ionone to prevent the damage induced by the insect attack (Wei et al. 2010).

## 5.6 Recent Advancement in Functional Aspect of Flavonoids and Carotenoids in Crop Improvement

Recently, many reports published on the functional aspect of flavonoids and carotenoids in term of stress tolerance in crop plants (Liu et al. 2015; Kudoyarova et al. 2019). The sulfoquinovosyl transferase enzyme induces glycosylation of flavonoid that elevates tolerance to osmotic stress in rice (Zhan et al. 2019). Application of soil – organic matter enhances the efficacy of secondary metabolites including flavonoids and microbes- based communication (Del Valle et al. 2020). Identification of allelochemicals and phytotoxic activity induce tolerance from *Schumannianthus dichotomus* observed by Rob et al. (2020). The overexpression of the cytochrome CYP82A3 gene derived from *Glycine max* improves drought and salinity tolerance and also proves a strong resistance against *Botrytis cinerea* and *Phytophthora parasitica* in transgenic tobacco (*Nicotiana benthamiana*) species (Yan et al. 2016; Tian et al. 2017). Rhizosphere acts as hotspot for allelochemicals, PGPR, and secondary metabolites production based on soil-plant –microbe interaction inducing cycling of nitrogen and carbon in soil (Del Valle et al. 2020). Enhanced level of Arbuscular mycorrhiza (AMF) induces production of root flavonoids, shows colonization in invasive tree species reported by Pei et al. (2020). Screening of tunisian plant extract formulation shows bioherbicide properties and in *Cynara cardunculus* (Kaab et al. 2020). In sweet potato, enhanced lutein and β-carotene level through suppression or down-regulation of the β-carotene hydroxylase gene and increase abiotic stress tolerance in transgenic stress tolerance (Li et al. 2017). Similarly, findings reported by Kea et al., showed enhanced β-carotene amount, lycopene β-cyclase gene (IbLCYB2), and suppression of lycopene ε-cyclase expression in sweet potatoes (Li et al. 2017; Kang et al. 2018). Multiple stress conditions impose more beneficial impacts on plants compared to that posed in presence of individual stress alone (Liu et al. 2015). Combination of stresses ultimately reduces the detrimental effect of each other thereby, increasing the probability of better survival of plants. The cumulative impact of drought and accumulation of ozone ($O_3$) in plants resulted in better tolerance. The combined affect was attributed to decreased values of stomatal conductance. Elevated concentration of reduced glutathione and ascorbic acid effectively scavenge ROS, thereby causing a considerable drop in the total ROS content. Several mechanisms highlighting the role of microbes in abiotic stress alleviation have been proposed (Kumar et al. 2019; Ke et al. 2019; Khan et al. 2020). Soil-inhabiting microbes belonging to genera *Achromobacter*, *Azospirillum*, *Variovorax*, *Bacillus*, *Enterobacter*, *Azotobacter*, *Aeromonas*, *Klebsiella*, and *Pseudomonas* have been shown to enhance plant growth even under unfavorable environmental conditions (Kaushal and Wani 2016; Sorty et al. 2016) (Fig. 5.2).

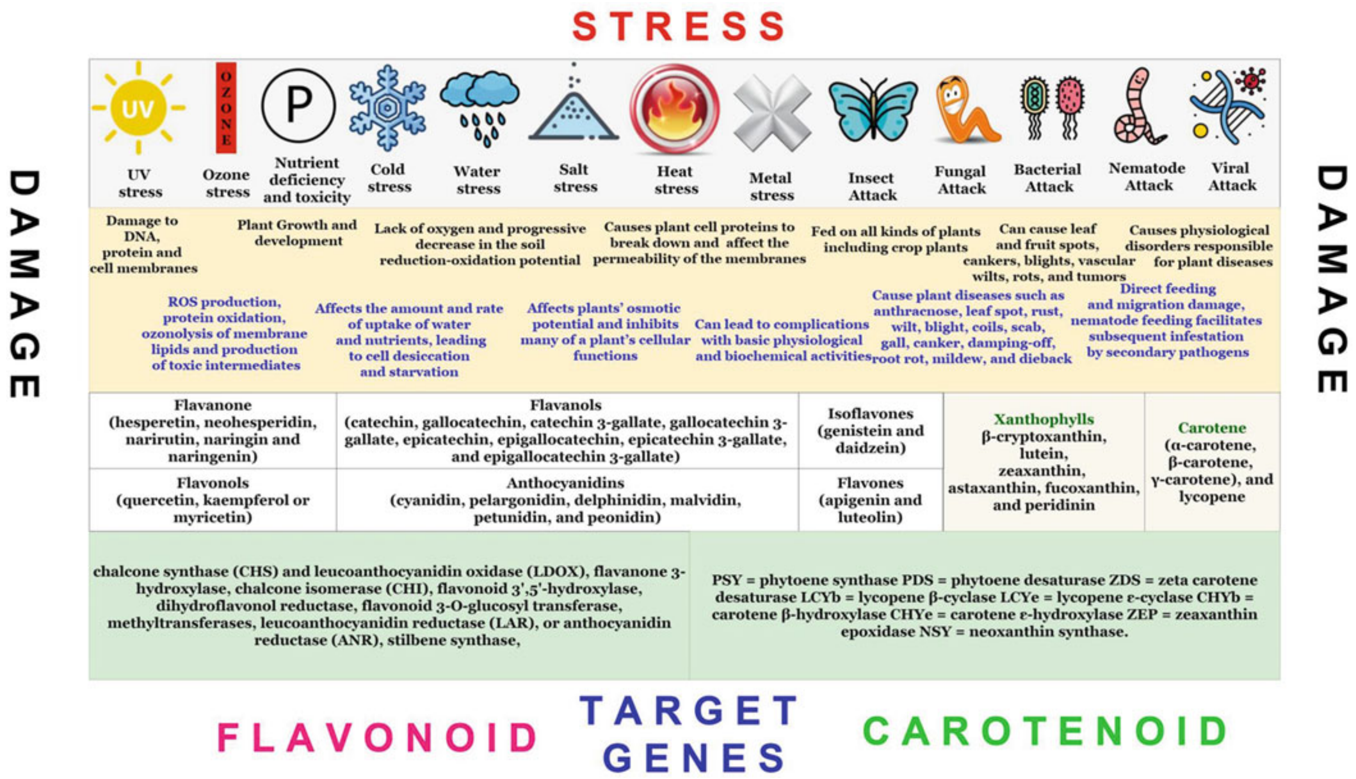

**Fig. 5.2** The defense mechanism mediated by microbial carotenoids and flavonoids to combat varied biotic and abiotic stresses in plants

## 5.7 Conclusion and Future Remarks

Presently, greatest interest is towards new prospective of microbial and their secondary products including carotenoids and flavonoids that can be used in plant stress tolerance and crop improvement under climate change scenario. Looking into application of crop production without compromising yield loss due to severity of biotic and abiotic using microbial-based inoculants become more efficient and effective. Moreover, the PGPR, microbial and plants-based metabolites having both biological as well as ecofriendly approaches in term of sustainable crop improvement, stress management, and promoting soil health are common. Enhanced flavonoids and carotenoids level in plants are significantly relayed to the elevated light-dependant oxidative damages and promoting antioxidant systems in plants. Morphological structures including specialized glandular trichomes and stomatal guard cells may be conveniently noted to investigate the functional-localization interaction of flavonoids in photoprotection systems. It is evident that high concentration of carotenoids and flavonoids in rhizosphere increases bacterial-mediated growth-promoting responses in root exudates and leaves confirmed growth and resistance in plants. These play a central role in various associations of plant–rhizobia, plant–microbe, soil microbes, and arbuscular mycorrhizal symbiosis and boost plant growth via the production of important biological agents. These microbial-based inoculations may lead to the identification of new signaling molecules and decipher the pathways responsible for triggering stress resistance in challenging environment.

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# Part II

# Role of Antioxidants in Microbe Mediated Abiotic Stress Alleviation

# PGPR: The Redeemer of Rice from Abiotic Stress

# 6

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## Abstract

Most of the world population relies on rice for their nutritional demand. But unfavorable environmental conditions, population explosion, anthropogenic activities like industrialization, and usage of chemical fertilizers adversely affect the soil structure, plant growth, and yield, which imposed a serious threat to food security worldwide. Abiotic stresses may cause detrimental effect on almost all features of plants like germination, growth, metabolism, gene regulation, and reproduction. Plant growth-promoting rhizobacteria (PGPR) can support as a redeemer of rice from various abiotic stresses promoting its growth and productivity. Diverse group of PGPR can offer multiple abiotic stress tolerance in rice, via direct and indirect mechanisms. Hence, understanding the challenges imposed by each abiotic stress and the mechanism of PGPR-mediated stress adaptation in rice plants becomes an essential one. Advent of multi-omics approach, genetic engineering, plant tissue culture, and seed priming techniques plays a key role in developing a potent PGPR as a sustainable remedy for mitigating abiotic stresses. However, certain pitfalls like establishment of PGPR under field conditions and economical production of PGPR in industrial scale have to be addressed before

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commercialization. On achieving this, PGPR strains may facilitate rice plants to adapt and grow efficiently under continued environmental changes.

**Keywords**

PGPR · Rice · Abiotic stress · Antioxidants · Phytohormones · Stress tolerance · Omics approach · Seed priming · Genetic engineering · Tissue culture

## 6.1 Introduction

Rice is one of the most vital food crops, which contributes to 20% of global calorific demand. But rice productivity could not compete with the increasing demand, due to environmental degradation, climatic conditions, and ever-growing population. Abiotic stress is the chief constraint for rice productivity and food quality, reducing average yields of the plants by 20–50% (Shrivastava and Kumar 2015). Abiotic stresses like salinity, temperature, drought, and heavy metals can adversely affect the growth and productivity of rice. Moreover, it can also favor the biotic stress and affects the plant growth (Chodak et al. 2015). Usually, farmers depend on agrochemicals to overcome the abiotic and biotic stresses. However, indiscriminate usage of chemical pesticides and fertilizers for agriculture purposes leads to decline in soil fertility and environmental pollution (Sharma and Chetani 2017). Hence, it becomes mandatory to discover a safer and efficient tool for addressing sustainable agriculture.

Application of plant growth-promoting rhizobacteria (PGPR) offers an ecologically safe and economically feasible alternate to minimize the harmful effects of agrochemicals. Several reports are available on the plant growth-promoting effects of microbes such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Variovorax*, *Klebsiella*, *Burkholderia*, *Azospirillum*, *Azotobacter*, and *Serratia* in mitigation of plants from various abiotic stresses (Arshad et al. 2008). In addition, several PGPR contribute to multiple abiotic stress tolerance in plants. This book chapter mainly focuses on the PGPR which demonstrate their potential to combat various abiotic stresses with special reference to rice plants. Challenges imposed by each abiotic stress in rice plants, mechanism of PGPR-mediated stress adaptation, role of omics, tissue culture, and genetic engineering techniques in mitigation of adverse conditions will be explained along with the pros and cons in field application and commercialization of PGPR.

## 6.2 Importance of Rice in Global Nutrition

Rice is used as a basic and major food grain throughout Asia, and its importance as a nutritive food has been increasing worldwide during several past decades. Other than Asia, rice farming is majorly concentrated by millions of people in Africa and Latin America to increase their economy as well as to meet their dietary requirements. Out of total calorific consumption, the rice uptake in Asia has reached the mean value of 40% in which most of the countries showed greater than 60% in their total calorific consumption. More than 60% of the protein was obtained through intake of rice-based diet in Myanmar and Bangladesh whereas in Philippines, Thailand, and Indonesia, this was recorded as 40%. Moreover, the GDP (Gross domestic product) of Bangladesh and Vietnam was dependent on rice production to the maximum of 25%. To manage the increasing need of rice, the net yield has to upturn 1% /year till 2020 (Rosegrant et al. 1995).

Besides this rice is a good source of several other growth factors including vitamins such as niacin, thiamine, and riboflavin. There are several research studies are focusing on the biofortification of rice with increased provitamin A and folate content to overcome the severe effects of malnutrition (Bhullar and Gruissem 2013). The vitamin content of three different varieties (Njavara, IR 64 Njavara and Jyothi) of rice was compared by Deepa et al. (2008), and their study revealed that IR 64 Njavara had higher thiamine (27–32%) content than other two varieties. This variety can be recommended to manage impaired neural function such as neuritis and general muscle weakness that are the results of thiamine deficiency. Other than vitamins, rice is also enriched with minerals like Zn and Fe to overcome the negative effects of malnutrition (Sperottoa et al. 2012). Comparatively the parboiling technique showed higher phosphorous and potassium content than the milled rice. The higher ash content (18%) was observed in parboiled rice than the milled rice. The other mineral elements such as calcium, zinc, and manganese were found lesser in parboiled rice than milled rice. All other minerals were present in the same range among the products of two different rice processing methods (Heinemann et al. 2005).

Aspartic acid and glutamic acid are the most predominant amino acids present in the rice, and trace amount of lysine was also observed (FAO 2004). Two different varieties of rice such brown rice and white rice were compared for their protein content. Among them, the former variety has higher protein content (14.6 g/100 g) than the later (7.3 g/100 g). Seki et al. (2005) reported that the fat level was also higher in brown rice (24.8 g/100 g) than the white rice (1.5 g/100 g). Even though nutritive value of rice exhibits a wide profile, it will not serve as a sole source of diet for growing population. It has to be supplemented with all other dietary food materials such as pulses, vegetables, meat, egg, etc., to meet the daily energy need of an individual.

Though rice has higher glycemic index and projected as one of the cause for Diabetes, there are varieties with different levels of GI. Based on the Glycemic index, the rice varieties were grouped into three major categories those have less than 55% of GI, the moderate GI of 55–69%, and the last with more than 70% GI. The glycemic load concept was introduced to include the quantity of rice consumed in a diet that gives clear picture of postprandial sugar. The diet with low GL is having $<10$, the medium GL diet ranged between 11 and 19, whereas the high GL diet has the recorded value of $>20$ (Wolever et al. 1991). Furthermore, this was also supported by the studies of International Rice Research Institute that specifies the GI was dependent on the variety of rice and the variation is mainly due to the composition of starch with wide range of amylose and amylopectin concentration. The rice varieties with greater amylose concentration had comparatively less GI (FAO 2001). However, another study reported that the detection of glycemic range of amylose content was not readily applied to find out the digestion rate of starch (Panlasigui et al. 1991).

The increased rice production is much useful to overcome hidden hunger and poverty crisis through affordable economically feasible rate for the poor people in Asia. This scenario leads to the farmers and researchers to produce diversified indigenous varieties of rice with improved traits and high nutritive value to increase the economic value of rice in the global market (Dawe 2000).

## 6.3 Effect of Climatic Change on Rice Cultivation and Yield

The growing industrialization and automobile usage resulted in global warming due to the continuous liberation of greenhouse gases like nitrous oxide, methane, and $CO_2$ that absorbs higher heat energy. Especially the increase in $CO_2$ concentration and its effect on global warming is studied widely for the past few years due to its role in considerable increase in earth's temperature. The natural way of sequestering $CO_2$ is happening through photosynthesis where the excess of carbon dioxide is fixed as carbohydrate. The increasing $CO_2$ concentration increases the rate of photosynthesis and ultimately results in increased leaf lamina, high dry weight, and increased yield (Kimball 1983).

The climatic adaptation of rice cultivars includes cool regions located in higher altitudes such as Nepal to hottest areas such as Egypt. At the same time, the higher yield with essential parameters was observed in temperate regions such as Asia and North Africa. The total production of rice throughout the entire world was 0.55 billion tonnes in 2007. The rice cultivation in Asia was highly affected by drastic changes in climatic factors including temperature, air, water availability, precipitation. Furthermore, the increasing $CO_2$ concentration is leading to the enhanced photosynthesis in the growing countries (Watanabe and Kume 2009). The higher $CO_2$ concentration increased the yield of rice up to 30% through enhanced photosynthetic rate (40%) than under normal $CO_2$ level whereas the high temperature reduced the yield by shortening the growing period (Erda et al. 2005).

The higher concentration of greenhouse gases other than $CO_2$ which are not utilized in photosynthesis will continue to increase the global temperature up to 0.5 °C within 20–24 years interval (Hansen et al. 1999). Photosynthesis is highly sensitive to temperature stress due to its impact on physiology of the plant that ultimately affects the growth, development of the plant, and flowering that result in poor yield. Though the greenhouse gas such as $CO_2$ is accelerating the growth of crop varieties, the global warming leads to increase in temperature and directly related to drought and poor precipitation. So, global warming leads to the poor yield and a crisis for food for the growing population (Santra et al. 2014). The increase in temperature more than 36 °C reduces the yield due to the temperature mediated poorly developed sterile inflorescence. The higher $CO_2$ concentration more than the

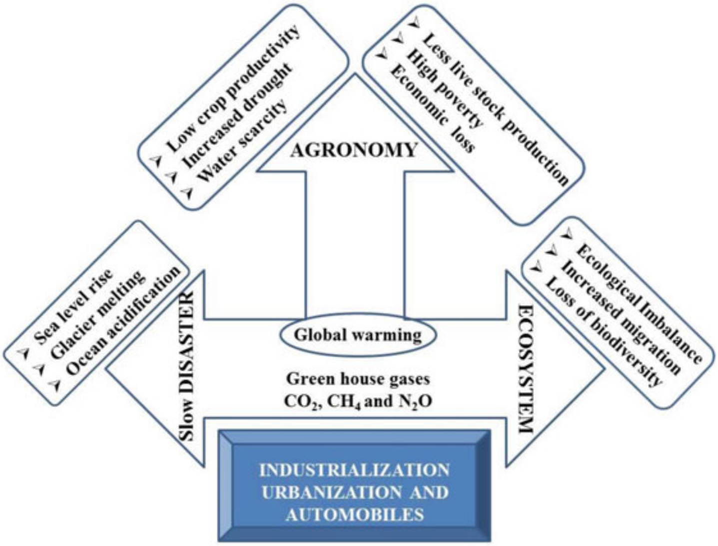

**Fig. 6.1** Impact of global warming induced climate change on the emerging human population and its impact on crop productivity, eco and socio-economic factors

optimum level also makes the plants sensitive to sterility caused by extreme temperature (Kim et al. 2001). Comparatively, the rice plants grown under high night temperature decrease the total yield of the rice plant up to 90% than the plants grown in a normal temperature exposure. This drastic decrease in the yield is caused by spikelet sterility and poor pollen development (Mohammed and Tarpley 2009). (Fig. 6.1)

The Intergovernmental Panel on Climate Change of United Nations (IPCC 2019) reported that the rise in sea level will become far higher than the predicted levels that may reach more than a meter (3.61 ft) before entering into the next century. Melting of glaciers may result in the sea level rise and thereby affects the water availability for irrigation. This will enhance the salinity of available fresh water bodies through flooding of coastal lines and agricultural fields (Richardson et al. 2009).

## 6.4 Population Explosion and Rice Productivity

The explosion of human population is a result of modern concepts such as profound medical treatments, industrialization, and automation which increased the survival capacity of humans and reduced the death rates (Gage and DeWitte 2009). The end of nineteenth century was marked by several revolutions in the human community that led to the increasing population and growing need for food crops. Green revolution emerged in the middle of twentieth century throughout the world for satisfying the global nutritional demand. Sudden decrease in the fertility rate was observed among human population of various countries due to the change in lifestyle and excessive usage of hazardous chemicals for industrial applications and agricultural practices. Nevertheless, the need for high-quality food was growing simultaneously due to high standard of living. The increase in per-capita consumption and population explosion are the major reasons for deforestation. The high input is needed in the fields of agriculture and plant biotechnology to increase the yield of major crop varieties such as rice, wheat, maize, millets, and pulses (Southgate 2009). Hence, the transition in developed and developing countries from higher to lower birth rate as well as higher to lower death rate led to a constancy of human population. This demographic transformation has a great impact in food production (Thompson 1929; Caldwell et al. 2006).

The chief source of energy was obtained through rice consumption by the growing population of Asia; more than 90% of rice production and consumption is carried over by the people of this biggest continent. Among the total agricultural area of Asia, more than 25% is utilized for rice cultivation and rice occupies more than 40% of total food grain production. Moreover, the agricultural production was increased up to 16% based on the gross value. The major amount of calories (31%) and protein (22%) were obtained by rice intake in Asia. The rice market has a considerable effect on the economy and poverty level of the people (IRRI 2008). The rice cultivation is a major source of employment and income in rural area that meets the entire food security of most of the nations such as India, China, Indonesia, Bangladesh, and African countries. The drastic change in human population through demographic transformation has a direct impact on rice production and consumption by controlling the availability of farmlands, farmers, and other workers. On the other hand, the high input needed for rice cultivation also affects the production cost, demand, and market value. The continuous research on the effects of demographic transformation on rice cultivation will be useful to produce the self-sufficient amount of rice grains for the growing population with reasonable cost. Furthermore, the export of rice to other countries will become easier when these primarily producing countries reach self-sufficiency (Bhandari and Mishra 2018).

## 6.5 Effect of Various Abiotic Stresses on Plants

Undergoing abiotic stress, the plant stress markers were regulated by PGPR. Surrounding stress conditions like salinity, infectious agent, drought, UV, high temperature, cold, and alkalinity were broadly scaled and revealed by plants. Beyond 30% crops have been deprivation due to initial source of abiotic stress. Important processes such as respiration, protein amalgam, and photosynthesis were turned down, and capitulated crops are restricted by vital abiotic stress called salinity (Ahmad and Prasad 2011). Increase in the salinity influences the nutrient availability and thereby reducing the plant growth and yield (Zahedi et al. 2012).

In acute instances, salinity stress shows oxidative stress in plants and hypersonic occurrences in foremost stages (Parvaiz et al. 2012). The peer group of reactive oxygen species (ROS) is managed by oxidative stress which is harmful to plants (Azooz et al. 2011). The ROS groups like hydroxyl radical and superoxide ions are affected by toxic substances targets the metabolism of plants. Nucleic acids, lipids, and protein biomolecules are destructed by highly precepting ROS (Apel and Hirt 2004). Well-organized antioxidant network has evolved to shield it from the calamity, which decreases the consequences of oxidative stress (Azooz et al. 2011).

Glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POX) are ROS forage enzymes (Apel and Hirt 2004), and isoenzymes in chloroplast and mitochondria have present in diverging cellular chambers. Solicitation of PGPR strain *B.cereus* AR156 on tomato appeared increased activity of CAT, POX, and SOD of abiotic stress in plant likely shows the intensify activation combined with PGPR (ChunJuan et al. 2012). PGPR increases the cell well strength by restoring the biochemical and physiological response of the host through induced systemic resistance mechanism. Rhizobacteria also enhances the production of safe guarding chemicals like peroxidases, pathogenesis interconnected proteins and chitinases to overcome the abiotic stress (Silva et al. 2004).

In order to salinity, another main abiotic stress marker enzyme gathered in plant tissue is L-proline amino acid. During salinity, another main abiotic stress marker gathered in plant tissue is L-proline. This assemblage improves the ability of hydroxyl radical scavenger, secures folded protein formation, maintains the cell membrane integrity in collaborative with phospholipids and mediates the distribution of nitrogen as well as energy supplements. Altogether the main play, i.e., osmotic balancing, was done by L-proline in plant. Foliaceous entreaty of diazotrophic *Klebsiella* sp improves crop relent and enhances yield on wet rice land against drought stress, high nutrient intake, and increased L-proline content. PGPR regulate the measure of plant stress markers below abiotic stresses were apparent (Razi and Sen 1996).

## 6.6 Physiological and Biomolecular Response of Rice Plants to Abiotic Stress

Various environmental components influence the microbial communities in the rhizosphere soil. The ecosystem includes biotic and abiotic components, firm to categorize them as different segments. Certain abiotic factors affect the rhizosphere microbial community; hereby complex investigations of particular effects of single factor were observed (Liu et al. 2000). To elucidate the structure of microbial group, soil pH is considered as major component (Lauber et al. 2009). By managing the chemical configurations of the soil groups, the soil pH is interconnected to accessible usage of nutrients for plants (Zhalnina et al. 2014). Nutrients like phosphorus, nitrogen, and carbon in countryside soil show major limitations to manufacture in a barren soil (Reich and Oleksyn 2004). The considerable effects on constituents of bacterial colony and plant production in soil were mainly raised by nitrogen enhancement (Turner et al. 1997).

Geographical elements such as altitude, latitude and longitude influences the disribution of vegetation and the variety. The advancement in development of plant and decreasing the humiliation capacity of microbes was achieved by reduced amount of N resulted by elevated level of $CO_2$ (Van Ginkel et al. 2000 and Hu et al. 2001), changes the soil structure. C inherence related to atmospheric $CO_2$ in soil is same as microbiome effects of abiotic factor of soil nutrients. The microbes in soil space scatter, hold on live, population of microbiome are restricted and minimize the moisture content by Global warming (Carson et al. 2010). When the soil temperature increases, it results in structure modification of the microbiome rhizosphere that is constructed by the plant in the soil. This effect was analyzed in vitro microbiological community by Zogg et al. 1997.

The detailed study used phospholipid profiles present in soils by subjecting the soil to a temperature range between 5 and 25 °C to monitor any residual prolonged changes. The study gave out a valid inside about the changes observed in the microbial community. The results turned out that the increase in the soil temperature was more associated with the climate change that occurred globally. This climate change altered the organic matter decomposition process which has a direct impact on the bio availability of carbon content in the soil. In further to investigate the effect of high temperature, Mosier et al. (2015) experimented with expression of protein on a microbial colony present in an acidic mine drainage using tandem mass tag technology-based proteomics. From the above observations, it is very clear that the application of PGPR helps the plants to ameliorate extreme drought stress. This could be achieved by proper studies of root parameters and perform functional structural modeling. The effect of high temperature creates heat stress effects on various visible and hidden growth parameters such as delayed seed germination, inhibition growth, leaf agedness, and apoptosis (Wahid et al. 2007). This drought stress is the product of a variety of reactive oxygen (ROS). Some prominent ROS are hydrogen peroxide, super oxide radical. These ROS affect the plant growth through the damaging lipids and other macromolecules (Mittler 2002).

When considering the biochemical characterization, the antibiotic sensitivity profile of the selected strains was analyzed to identify the optimal root characterization that would aid in making the plants counter drought stress. These identified root traits could be used to counter inhibiting of shoot growth by incorporating in breeding programs. This allows the plants to perform osmotic adjustment. The Relative water content (RWC) that could be observed in plant leaves acts as a critical indicator to qualify and quantify the metabolic activities in plant tissues by evaluating the plant water status. This RWC helps on osmotic adjustment which is a cellular level adaptation to make plants drought tolerant via active accumulation of organic and also inorganic compatible solutes. Using these observations and molecular characterization, the desired bacterial genomic DNA was identified and isolated. It was further amplified using polymerase chain reaction with various universal primers to be purified and sequenced using GenBank accession numbers. This 165rDNA gene sequence was evaluated with globally available sequences such as EMBL and DJB database through NCBI servers (Han and Lee 2005).

## 6.7 PGPR as a Tool for Sustainable Agriculture

Plants exist in a symbiotic relationship with microorganisms in soil. The free-living microorganisms in soil inhabit the rhizosphere region and have diverse effects on the host plant (Raza et al. 2016), and hence they are known as Plant Growth-Promoting Rhizobacteria (PGPR). Akhtar et al. (2012) reported that PGPR tend to defend the plant health in an eco-friendly approach. PGPR and its relationship with host plant are commercially applied, as it has scientific applications in agriculture (Gonzalez et al. 2015). Gray and Smith (2005) reported that extensive studies on PGPR have been done in oat, canola, soy, potato, maize, peas, tomato, lentil, barley, wheat, radicchio, and cucumber.

PGPR inhabit plant roots and improve the growth of plants by special mechanisms (Fig. 6.2), such as phosphate solubilization (Ahemad and Khan 2012), nitrogen fixing ability (Glick 2012), indole-3-acetic acid (IAA) production, siderophore synthesis (Jahanian et al. 2012), 1-amino-cyclopropane-1-carboxylate (ACC) deaminase, hydrogen cyanide production (Liu et al. 2016), degradation of environmental pollutants, production of phytohormones, and antibiotics or lytic enzymes (Xie et al. 2016).

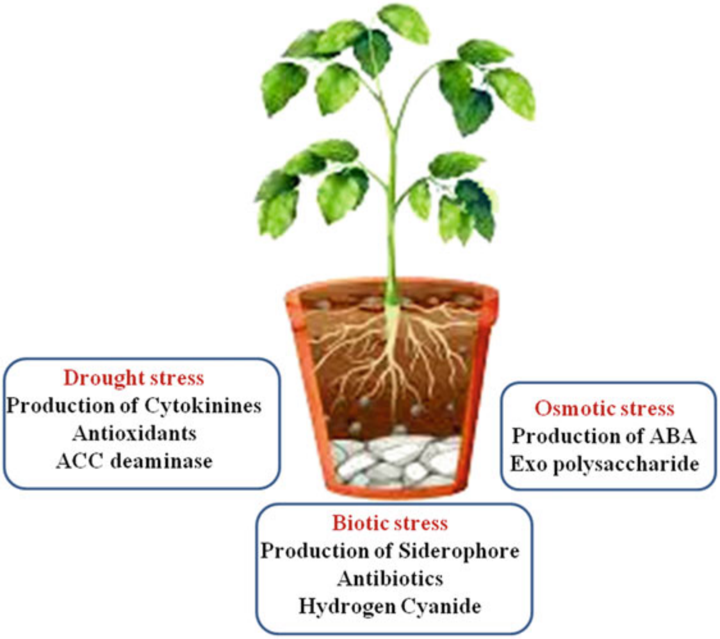

**Fig. 6.2** Mechanism of PGPR in mitigation of abiotic and biotic stresses

## 6.8 Diverse Forms of PGPR

PGPR can be classified into two types, namely extracellular plant growth-promoting rhizobacteria (ePGPR) and intracellular plant growth-promoting rhizobacteria (iPGPR) (Viveros et al. 2010). ePGPR colonize the rhizospheric region or in the spaces between the cells of the root cortex, whereas iPGPR reside inside the specialized root nodular structures. The endophytic microbes included as ePGPR are *Azotobacter*, *Serratia*, *Azospirillum*, *Bacillus*, *Caulobacter*, *Chromobacterium*, *Agrobacterium*, *Erwinia*, *Flavobacterium*, *Arthrobacter*, *Micrococcus*, *Pseudomonas*, and *Burkholderia*. The bacterial genera belonging to iPGPR include *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* which can fix atmospheric nitrogen in plants (Bhattacharyya and Jha 2012).

## 6.9 Role of PGPR as a Plant Growth Enhancer

PGPR improve the growth of the plant by direct and indirect mechanisms. These mechanisms comprise nitrogen fixation, neutralizing biotic and abiotic stress, production of volatile organic compounds (VOCs), and enzymes to avoid disease. However, the approach of different types of PGPR varies based on the host plant (Garcia et al. 2015). They are also inclined by various biotic factors (genotype of the plant, developmental stages of the plant, defensive mechanism of the plant and microbial community) and abiotic factors (composition and management of the soil, climatic conditions) (Vacheron et al. 2013).

PGPR act as plant growth enhancers, as they have the affinity to augment the ease of use and absorption of nutrients (Kumar 2016). Plants absorb nitrogen from soil in the form of nitrate and ammonium, which are vital for plant growth. Nitrate is the main form of available nitrogen in aerobic soils where nitrification occurs and is absorbed by the plant (Xu et al. 2012). Some PGPR have the capability for phosphate solubilization, resulting in an improved amount of phosphate ions available in the soil (Paredes and Lebeis 2016).

Biological nitrogen fixation is an astonishing process that accounts for nearly two-thirds of the nitrogen fixed globally. This biological process is performed either by symbiotic or by non-symbiotic exchanges between plants and microbes (Shridhar 2012). Inoculating an amalgamation of rhizobacterial species into soil enhances root nodule formation. Nitrogen fixation is performed by *nif* gene. Nitrogen fixing gene along with other structural genes is concerned in activating the iron protein, donating electrons, biosynthesizing the iron molybdenum cofactor, and regulatory genes necessary for the synthesis and activity of the enzyme (Reed et al. 2011).

Plant growth regulators at low concentrations ($<1$ mM) promote the growth of plants (Damam et al. 2016). Common groups of Plant growth regulators include gibberellins, cytokinins, abscisic acid, ethylene, and auxins. Plant growth regulators promote the plant growth, aids in proliferation of lateral roots, root hairs with a successive augment in nutrient and water uptake (Sureshbabu et al. 2016).

Siderophores are small organic molecules produced by microorganisms under iron-limiting conditions. Siderophores enhance iron uptake capacity (Saha et al. 2016). *Pseudomonas* sp., as PGPR, utilizes the siderophores produced by other microbes present in the rhizosphere for satisfying their ions requirement. A potent siderophore, such as the ferric-siderophore complex, plays an imperative role in iron uptake by plants in the existence of metals, such as nickel and cadmium (Beneduzi et al. 2012). As PGPR can generate siderophores, they are the most vital asset providing the plant with the requisite quantity of iron. Research concerning the capability of siderophores to enhance iron uptake capacity of plants is very limited, and significant research is further required in this perspective.

Indirect mechanisms engage the process through which PGPR avert or neutralize the deleterious effects of pathogens on plants by producing substances that increase natural resistance of the host (Singh and Jha 2015). This mechanism can also be defined as a process that helps plants grow dynamically under abiotic stress or protect plants from pathogens (Akhgar et al. 2014). The involvement of PGPR in this system includes production of hydrolytic enzymes such as chitinases, cellulases, and proteases. Further, PGPR also involve in production of various antibiotics in reaction to phytopathogen, induction of systematic resistance against pests, production of siderophores, VOCs, EPSs, etc. (Gupta et al. 2014).

## 6.10 PGPR-Mediated Mitigation of Abiotic Stresses

A wide variety of abiotic and biotic factors affect the growth and rice productivity worldwide (Fig. 6.3). The most important abiotic stresses include drought, salinity, heavy metals, and temperature. These abiotic stresses may lead to reduced crop yield and act as an obstacle for cultivation of crop in certain regions and make them unfavorable. The magnitude of biotic stress is greatly influenced by the abiotic

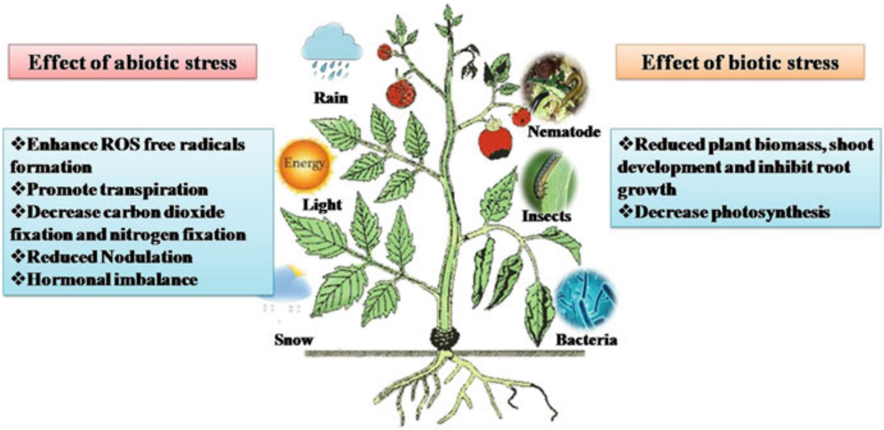

**Fig. 6.3** Influence of different biotic and abiotic stresses on plant growth

stress. Altogether these stress conditions make a wide impact on plant growth, physiology, biochemical contents, and gene expression (Chodak et al. 2015). Moreover, abiotic stress can also lead to the loss of soil microbiota. Replenishing the plant growth-promoting rhizobacteria (PGPR) in the abiotic stressed environment could serve as an eco-friendly, effective strategy to overcome the environmental instability and to achieve the agricultural sustainability.

### 6.10.1 Impact of Drought on Rice Productivity

Water stress is a vital part in agricultural sector as a notable share of farmland. Of the roughly 40 million hectares of harvested rice area in India, only about 60% is irrigated (AIREA 2015). Rest is precariously dependent upon rainfall and hence susceptible to drought. Droughts have noticeable consequences in terms of yield reductions, particularly if droughts happen during key stages in the rice growth cycle in which plant growth is predominantly susceptible to water requirements.

Drought is a major limiting factor for rice production as it causes crop failure (Mahajan and Tuteja 2005) leading to a decline of food availability across the world. A deficient in water content may cause morphological, biochemical, and physiological injuries on plants affecting various significant cellular processes (Farooq et al. 2009). Among the most lethal effects of drought are damages on the photosynthetic apparatus in chloroplast (Chaves et al. 2009) and oxidative injuries on proteins, membrane lipids, and other cellular components (Zlatev and Lidon 2012). In addition, water deficit can reduce the size of crops, delay flowering, and decline grain yield and quality (Farooq et al. 2009). These negative effects are often coupled with decrease in the microbial activity of the soil (Rousk et al. 2013) and in the amount of macro and micronutrients offered to plants (Hu et al. 2007).

Drought resistance is an intricate attribute comprised of several mechanisms such as drought avoidance, drought tolerance, and drought recovery (Fang and Xiong 2015). Interactions with environment considerably affect resistance to drought as well (Hu and Xiong 2014). Therefore, the molecular mechanism of rice drought resistance and its evolutionary process in rice remain unexplored.

It is assumed that a balance exists between drought resistance and productivity (Fletcher et al. 2015). At the physiological scale, a plant always inhibits its photosynthetic ability and many other activities during drought to ensure enhanced survival. However, these acclimation responses may delay growth and decrease the productivity of a plant (Pandey and Shukla 2015).

At the individual scale, elite irrigated rice varieties bred for elevated productivity and high-quality are very sensitive to drought (Luo 2010). These annotations specify a potential trade-off between drought resistance and productivity. If the trade-off exists, it will considerably shape the adaptive evolution of drought resistance in rice and must be overcome in breeding for drought-resistant cultivars (Vikram et al. 2015). However, knowledge is still restricted about this genomic trade-off and its influences on the evolution of drought resistance in rice.

There are two rice ecotypes observed in agroecosystems with contrasting soil–water conditions, which allows them to be differentiated in drought resistance. Upland rice is cultivated in rain-fed uncontrolled fields and accumulated genetic variance during its adaptation to drought-prone mountainous areas resulting in enhanced drought resistance (Bernier et al. 2008). However, upland rice generally exhibits poor yield (IRRI 1975; Xia et al. 2014). In contrast, lowland rice is commonly grown in fields with water maintenance and irrigation facilities. Lowland rice encounters relatively lower drought risk during its domestication compared to upland rice (Bernier et al. 2008).

Based on the field performance evaluated under drought conditions, upland rice recognized considerably higher drought resistance than lowland rice based on drought resistance traits. For example, it possessed a higher ratio of deep rooting, lower rate of water loss in excised leaves, higher relative water content under drought, higher relative fecundity, and higher relative grain weight. In contrast, lowland rice exhibited better performance in growth, development, and productivity under well-watered and drip-irrigated conditions, which was represented through more panicles, better biomass, and higher grain yield.

#### 6.10.1.1 Mechanism of PGPR-Mediated Drought Resistance

Rhizosphere microbes having inherent potential to adapt and survive under drought situation can promote the plant growth under water-limiting conditions. These PGPR strains promote the drought resistance in plants by various mechanisms viz. induced systemic resistance, phytohormone synthesis, production of exopolysaccharides, and biosynthesis of ACC deaminase (Goswami et al. 2013). The phytohormones secreted by PGPR like ABA and IAA enhance the shoot and root development in rice under water-deficit conditions. ABA regulates the amelioration of drought-related stress by stimulating hydraulic conductivity and activating genes involved in drought tolerance. Saleena et al. (2002) documented the growth-promoting activity of *Azospirillum* sp. on rice plants cultivated under drought and saline environment. Belimov et al. (2001) reported that PGPR producing ACC deaminase enzyme can stimulate plant growth by converting ACC (ethylene precursor) into ammonia and F-ketobutyrate thereby preventing excessive production of ethylene when subjected to adverse climatic conditions. Naseem and Bano (2014) observed that the inoculation of PGPR elevated the production of sugar, amino acids, water content, and proline which increases the water potential and biomass of the plant. Treatment involving *Psueudomonas putida* ameliorates stress caused by drought condition in plants by increasing the chlorophyll and biomass content (Kang et al. 2014). PGPR could be applied in combination with mycorrhizae for ameliorating the deleterious effect of drought.

### 6.10.2 Challenges Imposed by Salinity Stress

Salinity stress is the important abiotic stress that suppresses plant development and productivity worldwide. About 7% of land area in the world is affected by the soil salinity (Szabolcs 1994). Inappropriate irrigation of cultivable land may result in the increase of secondary salinity up to 20% (Al-Maskri et al. 2010). Accumulation of charged ions such as sodium, potassium, calcium, nitrate, and chloride ions in soil as a result of weathering of rocks and very low rainfall may contribute for soil salinity (Shrivastava and Kumar 2015). Almost all features in the plant viz. seed germination, plant growth, nutrient and water uptake, photosynthetic efficiency, reproductive development, productivity, ecological balance, etc., have been influenced by the soil salinity (Netondo et al. 2004). Major impact of saline soil in plants includes oxidative stress, osmotic stress, nutrient deficiency, and ion toxicity (Shrivastava and Kumar 2015). Ions such as chlorine, boron, and sodium have detrimental effect on plants. Increased level of sodium ion in the plant cell wall may induce osmotic stress which results in death of the cell (Munns 2002). Saline nature of the soil can affect microsporogenesis, elongation of stamen, ovule development, and embryo formation in crops and enhances programmed cell death (Ashraf 2004). Moreover, it also inhibits nodulation and thereby reducing the nitrogen fixation process.

#### 6.10.2.1 Mechanism of PGPR-Assisted Osmotolerance

Plant growth-promoting rhizobacteria (PGPR) can serve as an efficient strategy to mitigate the salinity stress on plants. It alleviates the stress and enhances plant development by indirect and direct mechanisms. Among which, direct mechanism involves nitrogen fixation, siderophore formation, phytohormones production, and mobilization of nutrients (Hayat et al. 2010). Whereas, indirect mechanism includes inhibition of infection by various plant pathogens. PGPR produce ACC deaminase enzyme, facilitate the conversion of ACC into alphaketobutyrate and ammonia, and also suppress ethylene production by the plants. It also enhances root nodule formation in the plants by regulating the synthesis of rhizobitoxine enzyme under saline stressed condition (Vijayan et al. 2013). Exopolysaccharide produced by the PGPR acts as a barrier which binds with the cations and helps the plant to alleviate salinity stress (Kasim et al. 2016).

A wide variety of rhizobacteria have exhibited PGPR traits that help to overcome the salinity stress. Bano and Fatima (2009) reported the efficiency of *Pseudomonas* and *Rhizobium* in promoting plant growth under saline condition. During salt stress conditions, superoxide dismutase and lipid peroxidation activity in the rice plant was found to be reduced by the inoculation of *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* (Jha and Subramanian 2014). They also enhanced the production of glycine betaine in the rice plant and make them salinity tolerant (Jha et al. 2011). *Bacillus subtilis* and *Bacillus pumilus* facilitate the plant growth in saline soil by solubilizing the phosphate and by producing HCN, IAA, ammonia (Damodaran et al. 2013). *Bacillus amyloliquefaciens* produces phytohormones like auxins, ABA, and induces salinity resistance in rice (Shahzad et al. 2017). Mitigation of saline stress in rice plants mediated by *Bacillus amyloliquefaciens* involves regulation and suppression of 14 different genes in the rice plant (Nautiyal et al. 2013).

### 6.10.3 Implications of Heavy Metals on Growth of Rice Plants

Urbanization, industrialization, and other anthropogenic activities resulted in the accumulation of heavy metals in soil. Heavy metals like Al, Cr, As, Cu, Hg, Pb, Se, Hg, Zn, and Mn are non-degradable and toxigenic in nature that can affect plant as well as human health. By the consumption of rice and other grains cultivated in heavy metal contaminated soil, they got into the humans and may lead to various health risks. Heavy metal also imposes a harmful effect on plant growth, assimilation of water and other nutrients, physiology, metabolism, and senescence. It affects the plant growth by damaging cell membrane integrity and by inhibiting the production of plant growth hormones (Chibuike and Obiora 2014). As a result of this, plants cultivated in heavy metal contaminated region exhibit stunted growth, low biomass, chlorosis, browning of root, and ultimately death. Assimilation of heavy metals by the plants enhances the production of reactive oxygen species which results in cell death. Therefore, for enhancing crop productivity, conserving the ecosystem and to ensure the environmental sustainability, removal of heavy metals via an eco-friendly approach, becomes mandatory.

#### 6.10.3.1 Mechanism of Heavy Metal Sequestration by PGPR

Plant growth-promoting rhizobacteria have the potency to enhance the plant growth amidst of stress imposed by various heavy metals. The mitigation of heavy metal toxicity by PGPR involves various mechanisms like sequestration by exopolysaccharide, detoxification, volatilization, efflux of heavy metals, blocking the metal entry into the cells, and complex formation. Moreover, PGPR enhance the production of various plant growth regulators like ACC deaminase and IAA and suppress the ethylene production (Glick 2010). PGPR promote the plant growth by solubilizing phosphorous, fixing atmospheric nitrogen, mobilizing the essential nutrients, and by producing siderophores (Verma et al. 2013)

Zhang et al. (2015) suggested that the Actinobacteria and proteobacteria could be used for bioremoval of As, Mn, and Pb from contaminated soil. Reduction in the Cu phytotoxicity was noticed when the crop plants were treated with PGPR (Fatnassi et al. 2015). *Klebsiella sp.* and *Micrococcus sp.* were found to mobilize cadmium found in the contaminated soil and promote shoot and root elongation of the plants cultivated in that soil (Prapagdee et al. 2013). Bacteria such as *Azospirillum brasilense* and *Bradyrhizobium japonicum* incorporate arsenic in the biomass and enhance the plant development under metal stressed condition (Armendariz et al. 2015).

Phytoremediation is an upcoming strategy which involves usage of plants for removing heavy metals present in the soil. Microorganisms are being employed for improving the efficacy of phytoremediation. In addition, microbes can also be used an indicator of heavy metal contamination (Chen et al. 2014). Some of the microbes possess the ability to degrade the metal pollutant, while some microbes transform the highly toxic form of metal ion into a less toxic form (Babu et al. 2013). For instance, *Pseudomonas sp.* detoxifies the heavy metals by biotransformation of cadmium-citrate and Fe (III)–Zinc complex (Qian et al. 2012). Siderophores produced by the bacteria chelate other metals along with ferric ion and help in phytoremediation (Saha et al. 2016). Hence, PGPR assist the plants to ameliorate heavy metal stress and promote their growth.

### 6.10.4 Role of Temperature Stress in Phytoretardation of Rice Plants

Both high-temperature and low-temperature are considered as one of the major abiotic stress factors which affects the plant growth and yield. It affects almost all the physiological processes in the plant like photosynthesis, cell division, transpiration, membrane potential, and overall growth of the plant. Extreme heat makes the cell membrane more fluidic, while extremely low temperature makes them highly rigid. Elevated temperature enhanced the ROS production which may result in oxidative damage of the cell (Zinn et al. 2010). In case of low temperature, visible impairment like necrosis, chlorosis, and wilting will be observed in plants (Ruelland and Zachowski 2010). Fluctuation in temperature may alter the calcium concentration within the plant cell (Knight et al. 1998). It may also change the structure of plastids and thylakoid membrane and phosphorylate the mitochondrial as well as thylakoid proteins (Ruelland and Zachowski 2010).

#### 6.10.4.1 Mechanism of Thermic Stress Adaptation Influenced by PGPR

Plant growth-promoting microbes can be applied to alleviate adverse effect of thermic stress in plant. Ubiquitous nature of microbes allows them to act as a potential tool for promoting plant growth under varying thermal conditions. Psychrophilic bacteria such as *Arthrobacter nicotianae, Brevundimonas terrae,* and *Pseudomonas cedrina* exhibited plant growth enhancing activity under extreme cold condition (Lavakusha et al. 2014). Several reports are available on plant growth-promoting efficiency and antimicrobial activity of psychrophilic bacteria (Meena et al. 2015; Javani et al. 2015). Similarly, thermotolerant bacteria capable of producing organic acids for converting phosphate from insoluble to soluble form have been well documented (Chang and Yang 2009).

To cope up the temperature fluctuation and oxidative damage of plant cell membrane at high temperature, plants produce their own osmolytes, polyphenols, and other secondary metabolites which make them tolerant to varying temperature (Cheruiyot et al. 2007). Plants are also capable of producing antioxidants such as glutathione to prevent the cells from deleterious effect of ROS. Bacteria *Paecilomyces formosus* enhance the growth of plants and exhibited plant growth-promoting traits under elevated temperature conditions. Similar to the bacteria, endophytic fungi and other symbiotic fungi like *Curvularia protuberate* increased the heat tolerating capability of plants and influence its growth (Hubbard et al. 2014; Redman et al. 2002).

### 6.10.5 Impact of Other Abiotic Stresses on Rice Plants and PGPR-Mediated Adaptation

Soil fertility is the prime factor which influences the crop productivity. Salinity and drought may cause unavailability of nutrients reducing crop yield. Mobility of elements like phosphorous, iron, copper, zinc, and potassium is very limited in soil. Inoculation of *Bacillus polymyxa* and *Pseudomonas alcaligenes* in nutrient-depleted soil enhanced the mobility of nutrients such as nitrogen, phosphorous, and potassium, and enhanced the plant growth (Egamberdiyeva 2007). Under iron-limiting conditions, siderophores produced by PGPR sequester ferric ions from the soil and facilitate its uptake by the plants (Compant et al. 2005). Nitrogen-fixing bacteria serve as a major source of inorganic nitrate for amino acid biosynthesis. Phosphate solubilizers like *Pseudomonas*, *Bacillus*, and *Rhizobium* are efficient in mobilizing the phosphate and are widely applied as phosphate fertilizer (Rivas et al. 2006). Exopolysaccharide produced by certain PGPR strains serves as a cementing material that binds soil particles together which is essential for enhancing soil structure and water holding potential.

### 6.10.6 Cross-Protection Against Abiotic and Biotic Stresses

Plants produce a wide variety of organic compounds to mitigate from abiotic stress. These compounds may also provide resistance against other types of stresses which are non-specific and so called cross-protection. For instance, glycine betaine synthesized by the plants during drought conditions may also contribute to tolerate other abiotic stresses like salinity and frost. Similarly, inoculation of PGPR to combat resistance against one abiotic stress can have significant influence in overcoming other types of stresses. Induced systemic resistance in the plants can be achieved by inoculating a non-pathogenic rhizobacteria to create resistance against a potent phytopathogen (Walters and Fountaine 2009). Barriuso et al. (2008) documented that *Bacillus sp.* elicit induced systemic resistance to the plants and this primed physiological condition helps them to ameliorate various abiotic stresses. Gene expression profile of *Arabidopsis thaliana* inoculated with a non-pathogen, pathogenic strain, and exposure to drought stress revealed that the genes involved in the regulation of plant resistance against various biotic and abiotic stresses are co-regulated (Timmusk and Wagner 1999). Comparable results were noticed by Vannini et al. (2004), where transcription factor for mitigation of cold provides resistance to salinity, UV radiation, drought, ozone, and other phytopathogenic microbes. In contrast to this, Xiong and Yang (2003) reported that the suppression of mitogen-activated protein kinase gene increased tolerance to bacterial and fungal pathogens while significantly reduced tolerance against abiotic stresses.

## 6.11 Influence of PGPR on Plant Antioxidative Mechanism

Higher activity of ROS-Scavenging enzymes is induced by PGPR inoculation in plant. Usually, PGPR colonize in the root system of the inoculated plants grown under various stress condition which was identified with the help of the soil attached to the roots and tubers of the plant collected after the process of harvesting. The abiotic stress treatments like drought, salt, and heavy metal were given to PGPR inoculated plants and control plants. As a result, abiotic stress influences the antioxidant capacity in PGPR-inoculated plants and produced less amount of hydrogen peroxide when compared with the control plants (Gururani et al. 2013).

### 6.11.1 ROS in Abiotic Stress Amelioration

Plants undergo various environmental stresses throughout their life cycle. Normal plant growth and environmental stress were maintained by Reactive oxygen species (ROS). Environmental factors such as heat, cold, toxicity, organic pollutants (OPs), and pathogens could influence the generation of ROS in plant cell which acts as a signaling molecules and trigger signal transduction pathways in response to those stresses. But, ROS causes irreversible cellular damage due to their strong oxidative properties, which promote alterations in plant morphological structures that enhance resistance. Due to the presence of interconvertible ROS, it is very difficult to differentiate the events of cytotoxic and signaling events that are influenced by a specific ROS production. Altogether, stress-induced ROS have to appear rapidly when the plant influenced by abiotic stress and it should be cautious that when the stress response disappears, ROS production should also get stopped which protects the plant from alterations in their morphological structure in future (Honglin et al. 2019).

### 6.11.2 Source of ROS During Abiotic Stress

All form of life exists in oxidizing environment where oxygen supports aerobic life with great energy output. The molecule which sustains in aerobic environment can act as a lethal contaminant in mildly reduced cellular environment through endless formation of ROS. The term Reactive Oxygen Species (ROS) comprise of ions or small molecules consisting of oxygen ions or free radicals of inorganic or organic forms. Abiotic stress conditions like drought, heat, or effect of salinity in plant growth result in the reduction of agricultural production worldwide. These reductions may occur due to climate change and shortage of fresh water supply as well as the concurrent occurrence of different abiotic stresses (Mittler and Blumwald 2010). In order to deal with the abiotic stress, plants have evolved a specific mechanism by the activation of a stress-responsive genes so as to increase the tolerance level to the environmental stresses by the production of Reactive oxygen species (ROS), including hydrogen peroxide ($H_2O_2$), superoxide radical ($O_2^{\bullet -}$), hydroxyl radical (OH•), and singlet oxygen ($^1O_2$), etc., resulting from excitation or incomplete reduction of molecular oxygen which are considered to be signaling molecules that regulate plant development, biotic and abiotic stress responses (Jun and Chan 2015).

### 6.11.3 Protein Modification Induced by ROS

ROS productions were helpful in tolerating the abiotic stress but if it is produced in enormous amount, then it may lead to protein denaturation, modification in protein which initiates the process of apoptosis that eventually leads to the cell death. Soumen (2012) reported about the ability of ROS in oxidizing the redox-sensitive proteins through redox-sensitive molecules like glutathione or thioredoxins which control the cellular redox state. Redox-sensitive proteins function through downstream signaling components like kinases, phosphatases, and transcription factors. In certain cases, ROS directly oxidize the target proteins, like peroxyredoxins and thioredoxins. Indeed, most of the redox regulation of gene expression is facilitated by a family of protein disulphide oxidoreductases like thioredoxins, peroxyredoxins, glutaredoxins, and protein disulphide isomerases. Thioredoxins are small (approximately 12 kDa) protein with S=S reducing activity. They have been oxidized directly by ROS or indirectly by peroxyredoxins (thioredoxin peroxidase). Soumen (2012) through his study concluded that thioredoxins and other similar proteins act as enzymatic mediators of the regulatory effects of ROS at transcriptional levels.

### 6.11.4 Interaction of ROS with Stress Hormones

Reactive oxygen species (ROS) are produced within several plant organelles, particularly in the organelles like chloroplasts, mitochondria, and peroxisomes that act as cellular messengers and redox regulators of several plant biological processes. Signaling pathways regulate plant responses to developmental clues toward abiotic and biotic stress factors. They have highlighted the role of phytohormones and redox signaling, where multiple signaling cascades converge. The integration of multiple signals allows the plant to fine-tune its response to particular conditions. In this regard, growing evidence shows that the generation of ROS is one of the most common plant responses to different stresses, representing a point at which various signaling pathways come together to modulate the plant response to environmental cues. Integral pathway protein's redox regulation process provides a rapid and simple mechanism for the regulating the plant development and defense pathways. MAPK pathways are common and versatile signaling components which are present downstream to secondary messengers and hormones which play an important role in plant responses to react to various stress hormones (Soumen 2012).

### 6.11.5 Role of ROS in Systemic Signaling

Raja et al. (2017) stated that the reactive oxygen species (ROS) are astonishingly versatile in sophisticated network of signaling pathways in plants and act as core regulator of cell physiology and cellular responses to the environment. ROS are continuously produced endogenously in plants as an unavoidable consequence of redox cascades of aerobic metabolism. The molecular language associated with ROS-mediated signal transduction, is found to be one of the early stress response in the acclamatory performance of the plant. They may even act as "second messenger" modulating the activities of specific proteins or expression of genes by changing redox balance of the cell. The network of redox signals orchestrates metabolism for regulating energy production to utilization, interfering with primary signaling agents (hormones) to respond to changing environmental cues at every stage of plant development. The oxidative lipid peroxidation products and the resulting generated products thereof (associated with stress and senescence) also represent "biological signals," which do not require preceding activation of genes. Unlike ROS-induced expression of genes, these lipid peroxidation products produce nonspecific response to a large variety of environmental stresses. The present review explores the specific and nonspecific signaling language of reactive oxygen species in plant acclamatory defense processes, controlled cell death, and development. Special emphasis is given to ROS and redox-regulated gene expression and the role of redox-sensitive proteins in signal transduction event. It also describes the emerging complexity of apparently contradictory roles that ROS play in cellular physiology to ascertain their position in the life of the plant.

ROS communicate with other signaling molecules and being part of the signaling network may control responses both downstream and upstream of ROS. The facts discussed in the review indicate that we are now going through the initial steps in understanding how oxidants/ROS modulate signal transduction pathway with or without activating genes. So, in spite of tremendous development in our understanding of ROS biology, the exact nature of ROS-signaling network largely remains obscured. Molecular Genetic studies in addition to unique physiological approaches will be required to ascertain the position of ROS in the signal transduction pathways and also to understand how these short-lived endogenous signaling compounds are perceived and transduced to specific and nonspecific responses necessary for survival of plants. This will ultimately help us to screen better performing plants under environmental stress for breeding program.

## 6.12 Addressing Abiotic Stress Using "Omic" Approach

Understanding the plant–microbe interaction and their molecular signals involved in various physiological and biochemical pathways is essential for crop improvement techniques (Schenk et al. 2012). Application of multi-omic approach is highly useful to decode the changes in plant physiology, metabolism, and genetics. Advancement in bioinformatics accelerates the research outcomes based on omics approach.

Metagenomics and metaproteomics widened the knowledge about various microbial communities found in a specific habitat like rhizosphere (DeCastro et al. 2013).

Plant breeding programs for ameliorating stress require large amount of genomic data. Genomic profile is also mandatory for crop improvement programs (Tomar et al. 2014). With an intention to understand the mechanism of abiotic stress resistance, genes and their regulatory elements associated with microbial communities should be unraveled. Ma et al. (2004) applied PCR-based molecular markers for expressed sequence tag and microsatellite to determine the accumulation of silicon in rice plant and to screen its ability to mitigate abiotic stresses. Gene silencing strategy involving RNAi helps to engineer abiotic and biotic stress tolerant varieties in plants (Kubicek et al. 2011; Viterbo et al. 2010). Estimation of microbial diversity in the rhizosphere region using high throughput metagenomics approach does not involve isolation, culturing of microorganisms (Chen and Pachter 2005). Gene contributing for salt tolerance in *E. coli*, crotonyl-CoA hydratase, was identified based on metagenomics data and is used to develop salt-resistant transgenic plants (Kapardar et al. 2010). Genes contribute for cold tolerance such as cold-shock proteins, pH homeostasis, and anti-freeze proteins were recognized from the metagenome of acid mine drainage and are used to alleviate cold stress in plants (Liljeqvist et al. 2015). Metagenomic approach also reveals the stress ameliorating ability of each bacterium found in the rhizosphere soil. Whereas, metaproteomics analysis in combination with metatranscriptomics reveals gene expression pattern and abundance of stress-related proteins (Turner et al. 2013). Transcriptomic profile generated by microarray technology and mRNA sequencing has been applied to study the plant-microbe interactions (Wang et al. 2016). Transcriptome profile of rapeseed and the symbiotic microorganism *Stenotrophomonas rhizophila* revealed the synthesis of a plant growth regulator protein, spermidine during abiotic stresses (Alavi et al. 2013). miRNAs in rice as well as in other plants regulate various physiological functions under abiotic stress conditions (Trindade et al. 2010). For instance, miR169 regulates the synthesis of nuclear transcription factor YA to mitigate drought and salinity stress in rice plants Zhao et al. (2009). miRNAs also provide information about various signaling pathways which are involved in stress response (Curaba et al. 2014). Expression of miRNA in rice under aluminum stress exhibited a complex response involving 16 different stress responses (Blokhina and Fagerstedt 2010).

Proteomics plays a crucial role in understanding the regulation of signal proteins which are involved in physiology, metabolism, and plant–microbe interactions during stress situations (Silva-Sanchez et al. 2015). Ability for producing siderophore, biocontrol potential, synthesis of plant growth-promoting substances, and versatile nature makes *Pseudomonas sp.,* a well-characterized strain (Sorty et al. 2016). With the aim to study the mechanism behind the stress tolerance, it is mandatory to reveal the molecular insights of PGPR by using proteomics as well as metabolomics approach. Metabolomics reflects the molecular pathway activated under a particular environmental condition (Bundy et al. 2005). Metabolomics approach paves a way for identifying the signals molecules that are produced by the plants to attract various microbiota (Micallef et al. 2009). Metabolic products from microorganisms influence plant growth either by direct or by indirect mechanism. For instance, synthesis of auxins, IAA, gibberellins, siderophores, and cytokinins by the microbes has a significant impact on plant growth (Robin et al. 2006). These cellular processes can also be quantified with high degree of accuracy by an emerging approach called quantitative metabolomics (Noack and Wiechert 2014). Plants and rhizosphere microbes communicate with each other by synthesizing certain root exudates and other signaling molecules (Peiffer et al. 2013). Hence, metabolic profiling of microbes and the plants will provide a clear insight on the influence of microbe to mitigate various abiotic stresses. To conclude, integration of data generated from multi-omic approach will resolve the mechanisms behind stress resistance in various crop plants.

## 6.13 Role of Plant Tissue Culture and Genetic Engineering to Combat Abiotic Stress in Rice

The physiological and chemical nature of crop has been affected by biotic and abiotic stresses resulting in hindrance of plant growth, development, and crop yield (Kazan 2015). Abiotic stresses that affect the yield in rice crops are heavy metal, drought, cold, heat, and salinity. The impact of abiotic stresses can be noticed in the rice during the stage of development (Feller and Vaseva 2014; Paul and Roychoudhury 2019). Globally, drought is considered as a severe abiotic stress in rice that affects the growth and yield every year (Nasim et al. 2018). Salinity is the second most abiotic stress affecting the crop competence (Roychoudhury and Chakraborty 2013; Mariani and Ferrante 2017). The decrease in plant productivity due to these abiotic and biotic stresses is likely to be around 50% that results in a significant economic loss (Ishaq and Memon 2017). In India, high temperature is considered to be the chief abiotic stress which affects rice crops (Tian et al. 2012). In order to overcome this issue, tissue culture and genetic engineering is one of the excellent techniques to produce crops that are resistant and tolerant to various abiotic stresses.

Plant tissue culture technique helps in production of desirable agronomic character with biotic and abiotic stress tolerance. Stroud et al. (2013) succeeded in developing desirable agronomic traits in rice plants by tissue culture technique and reported that the regenerated rice plants exhibited consistent epigenomic traits with unique pattern of DNA methylation.

Transformation experiments in rice plants are usually carried out by gene gun method, Agrobacterium-mediated approach, and protoplast fusion technique. Rice plants expressing desirable characters can be produced by genetic engineering technique. For instance, genetically modified heat-tolerant rice varieties can be produced by changing the expression of heat shock protein (HSPs) genes, enhancing osmolytes production, altering membrane permeability, and by promoting the synthesis of enzymes which are involved in detoxification of cells (Wahid et al. 2007). Scafaro et al. (2018) developed thermic-resistant rice variety by transforming genes involved in synthesis of Rubisco activase enzyme and reported the enhanced growth, yield of recombinant plants under heat stressed conditions. Kumar et al. (2018) emphasized the importance of genetic engineering and antisense technology for getting a clear insight on the biological role and regulation of HSPs.

Ma et al. (2017) applied RNA interference technology to prove the role of OVATE family proteins (OsOFP6) in influencing growth and development of rice plants under cold and drought stresses. Similarly, Zhang et al. (2016) reported another gene called CTB4a, which is involved in development of cold resistance in rice plants. Role of other genes such as qCTB1 and qCTB7, which confer for cold resistance, has been well documented by Saito et al. (2010) and Zhou et al. (2009), respectively. Xu et al. (2011) reported the enhanced cold resistance in GM rice plants expressing C-repeat binding factor (CBF) gene, ZmCBF3. Major QTL gene namely qLTG3-1, which is involved in seed germination of rice, was identified by Fujino et al. (2008).

Recombinant rice plants expressing dehydration responsive element binding protein (DREB) like AtDREB1/OsDREB1 genes revealed tolerance to multiple stress factors such as salinity, drought, and low temperature. Zhang et al. (2009) identified another cold-tolerance provoking DREB gene, OsDREB1D. Similar to the above-mentioned gene transformations, various other genes involved in mitigation of various abiotic stresses have been successfully cloned and expressed in GM rice plants like OsCOIN (Liu et al. 2007), AlSAP (Ben-Saad et al. 2012), OsMYB2 (Yang et al. 2014), TERF2/LeERF2 (Zhang et al. 2010), OsPRP3 (Gothandam et al. 2009), and DaCBF4 (Byun et al. 2018) to ameliorate abiotic stresses like cold, salinity, and drought, etc. Advancement in molecular techniques like RNA sequencing, microarrays, protein–protein interaction, and proteomics approach leads to the recognition of several stress-related genes in rice plants (Chandran and Jung 2014) for further genetic engineering experiments.

## 6.14 Application of Seed Priming Technique to Overcome Adverse Stress Conditions

Seed priming is a cost-effective and indispensable tool for generation of stress-resistant plants. In this technique, the plants are trained physiologically to respond various abiotic stresses rapidly. It helps to enhance the growth and productivity of plants under adverse environmental conditions (Paparella et al. 2015). Primed seeds usually exhibit high seedling vigor and germination rate. Priming of seeds can be done by various processes viz. chemical priming, hormonal priming, osmopriming, redox priming, hydropriming, and nutrient priming in order to overcome the unfavorable climatic conditions (Paparella et al. 2015).

Hydropriming is the technique widely used to mitigate drought stress in rice plants by enhancing its growth and crop productivity (Mahajan et al. 2011). Primed seeds revealed the presence of high soluble protein and proline content under drought stressed conditions (Yuan-Yuan et al. 2010). Kalhori et al. (2018) reported that the primed rice seeds alter the catalase and ascorbate peroxidase activity and thereby helps the plant to withstand under water-deficit environment.

Polyethylene glycol-mediated chemical priming revealed only a limited efficiency in overcoming drought stress (Goswami et al. 2013). Seed priming activity mediated by salicylic acid (100 ppm) resulted in increase of seed dry weight, reduction in germination time and enhanced shoot, root development in rice plants during drought (Shatpathy et al. 2018) and cold stress situations (Hussain et al. 2016). Similarly, seed priming activity of ascorbic acid (200 ppm) under extreme heat stressed condition on paddy was well documented by Kata et al. (2014).

Osmopriming using $CaCl_2$ influences the growth, establishment, quality, and yield of rice by increasing flavonoids, antioxidants, and polyphenols under water-deficit (Hussain et al. 2017) and saline conditions (Rehman et al. 2012). In saline environment, Seed germination rate, dry weight, and length of the seedling can be enhanced by priming the seeds using $CaCl_2$ and KCl as chemical mediators (Afzal et al. 2012). Similarly, vinegar, $KNO_3$, and mannitol can also contribute rice plants to overcome salt stress (Theerakulpisut et al. 2017). Such type of osmopriming involving mixed salts elevated the activity of root dehydrogenase, p-amylase, shoot catalase, and α-amylase in high-salt environment. Hydrogen peroxide-mediated seed priming contributes tolerance to a wide variety of stresses like drought, salt, chilling, and thermic stresses (Uchida et al. 2002).

Mitigation of abiotic stresses by seed priming involves various mechanisms like DNA repair, activation of related genes, enzymes involved in various biochemical mechanisms and metabolism, mobilization of nutrients, etc. Usage of appropriate priming agents for different crops and corresponding stress conditions has a great impact in sustainable mitigation of abiotic stresses.

## 6.15 Opportunities and Challenges in Commercialization of PGPR

Abiotic stresses and nutritional requirements of ever-increasing population have now become a major threat to crop production globally. It necessitates strengthening of agriculture by using a sustainable approach in order to supply nutrients, provoke various plant pathogens, develop stress resistance as well as to promote growth and yield of the crop plants (Salme et al. 2017). Although PGPR have been proven to be a suitable alternate to conventional chemical fertilizers and pesticides, successful application and commercialization of PGPR depend on

- thorough understanding of growth-promoting mechanism of PGPR using inter-disciplinary research,
- commercial scale production of PGPR using low cost raw materials,
- optimizing the formulation and mode of application of PGPR,
- marketing and legalization of product,
- educating farmers reading handling of PGPR as biofertilizer (Bushra et al. 2017).

One of the major limitations in commercialization of PGPR is its sensitivity to prevailing environmental conditions and poor shelf life which ultimately results in inconsistent performance of PGPR under field trials. A clear insight on the PGPR-mediated plant protection mechanism may help to overcome this inconsistency issue. Based on its long-term viability in soil, spore forming *Bacillus* sp. is considered to be a highly suitable strain for commercial production of PGPR rather than *Pseudomonas* sp. But knowledge on the interaction of PGPR with indigenous field microflora is mandatory for determining its sustainability. Adaptability and viability of PGPR can also be enhanced by genetic engineering techniques.

Framing international regulatory guidance for assessment of potential risk and efficacy of PGPR is also needed before marketing. Moreover, it is often difficult for the companies to patent the process involved in industrial production of PGPR as biostimulant. Also, challenges in the harmonization of legislation regarding commercialization of PGPR have to be addressed. Upon overcoming all these technical, scientific, and legislative issues, commercial production and application of PGPR are really a boon for mitigating abiotic stresses on plants.

## 6.16 Future Prospects

Application of PGPR well exhibited their potential to mitigate environmental stress, improving soil health, enhancing plant growth, productivity, and to overcome food security issues. Further research is essential to formulate and commercialize efficient microbial consortia for mitigating the adverse effect of abiotic stress on plant growth and yield. It is foremost important to screen and identify potent bacterial strain that provides cross-resistance to multiple stress factors. Knowledge on mechanism of stress resistance, signaling molecules, and induced system resistance has to be widened by the applying multi-omics approach. During field application, a wide range of factors including soil properties, plant variety, agricultural practices, indigenous microbial community, mode of application, and prevailing environmental conditions may influence the survival of PGRP. Hence, field testing is mandatory for scrutinizing the capability of PGPR strain to adapt and establish itself in natural environment. Production of PGRP in industrial scale should be made economical by utilizing low-cost carrier molecules, cheap growth substrates, and by formulating a simple manufacturing procedure. Other pitfalls in the commercial production and practical application of PGPR have to be addressed.

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# Impact of PGPR on Plant Health and Antioxidant Enzymes Under Water Stress Conditions

# 7

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## Abstract

Climatic changes lead to various abiotic stresses around the world, causing global food insecurity. Among all the abiotic stresses, water stress is one of the chief constraints for plant health. Water stress causes oxidative stress by producing excessive reactive oxygen species (ROS), thereby affecting the physiological process which leads to the death of plant cells. So there is a need to improve the water stress ability of the plant in stressed conditions. Modern conventional method such as drought-tolerant crops could be adopted in drought prone areas but their implementation is time-consuming and prudent. Therefore, microbiological communities such as plant growth-promoting rhizobacteria (PGPR) have numerous eco-friendly bacteria associated with roots are gaining awareness by enhancing the stress tolerance ability of plants in abiotic stresses. PGPR act as biofertilizer and mitigate water deficiency by influencing the antioxidative enzyme activity and removing the ROS. Their role as bio-inoculants improves the water holding capacity and decreases the effect of water stress in arid areas with low water accessibility. This chapter deals with the usage of PGPR to boost plant water stress tolerance and agricultural sustainability.

## Keywords

PGPR · Biofertilizer · Water stress · Plant health · ROS · Antioxidative enzymes

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## 7.1 Introduction

The population of the world will surpass 9.1 billion by 2050 (Carvalho 2006), increasing the demand for food by more than twofolds (Green et al. 2005). However, green revolution has dramatically increased food production in 50 years, but the stress is predicted to cause severe growth problems in plants for above 50% of arable land (Vinocur and Altman 2005; Kasim et al. 2013). Water stress is an increasing threat to food production in arid and semi-arid areas (Neupane and Guo 2019). Additionally, water stress also increases the requirement for irrigation, which consumes 70% of global water. This will raise the water consumption by 10% due to the change in climate by 2050 (Shiklomanov and Rodda 2003). Hence, water-conserving strategies are used to increase the productivity of water in water-limited areas (Pereira et al. 2002). The term stress refers to any unfavorable condition which affects the growth, development and metabolism of the plant caused by various natural factors (Lichtenthaler 1998).

Various stresses associated with plants are heat, cold, salt, alkalinity, and acidity (Shao et al. 2005; Glombitza et al. 2004), but the water stress has been regarded as most important in plant research. It is highly destructive abiotic stress and has affected global food security as its intensity has been amplified over the past decades. The duration of water stress ranges from average to short, to exceedingly harsh and extended, restricting the yield (Bottner et al. 1995).

The soil is occupied by numerous species of microbes containing prokaryotes and a small number of eukaryotes per gram of soil (Lesueur et al. 2016). These microbes provide nutrients to plants by recycling minerals and carbon and protecting the quality of soil (Morgan et al. 2005; Leake et al. 2006). The greater part of soil microbes consists of bacteria, which form an association with roots that may be beneficial, harmful, or neutral to the plants (Bhattacharyya and Jha 2012). The beneficial microbes such as PGPR (free-living bacteria), also known as Plant Growth-Promoting Rhizobacteria, are a miscellaneous set of bacteria that generate a range of metabolites and hormones influencing the acquisition of nutrients, altering the level of hormones, and improving the harmful effect of biotic and abiotic stresses (Ahemad and Kibret 2014; Ngumbi and Kloepper 2016). PGPR word was foremost used in the late 1970s for the fluorescent *Pseudomonas* strains for enhancing the yield by 500% through the production of iron-chelating siderophores and withdrawing iron from native bacterial pathogens (Kloepper et al. 1980). Plant growth-promoting bacteria have received global attention for increasing productivity and tolerating biotic and abiotic stresses (Mayak et al. 2004; Marulanda et al. 2009; Yang et al. 2009). The use of PGPR in managing abiotic stress, for example drought, is recently gaining importance (Yang et al. 2009; Dimpka et al. 2009; Grover et al. 2010). Thus, in these years, efforts have been made to harness these root-colonizing beneficial microbes to withstand climate change (Yang et al. 2009; Nadeem et al. 2014). The mechanism involved in the response of the plant to various abiotic and biotic factors has been extensively studied (Quartacci et al. 2000; Sgherri et al. 2000) which comprises of numerous physiological, biochemical, and molecular pathways and metabolic processes such as nutrient and water relation, protein metabolism, carbohydrate metabolism, hormones metabolism, and antioxidant defense (Huang et al. 2014). Thus these rhizobacteria are exploited as bio inoculants to increase the production of the crop under stressful conditions.

Its application is used for over 100 years. Still, from the last three decades, its application has gained importance as its commercial products are available in the market (Babalola and Glick 2012), but it has to be fully exploited. This chapter discussed the employment of PGPR bioinoculant in combating the oxidative stress in crops and their use in water deficit region for sustainable agricultural production.

## 7.2 Effect of Water Stress on Plants

Water stress as multifaceted stress affects the plant, cell organs, and subcellular compartment level (Choluj et al. 2004; Rahdari et al. 2012). Water stress is one of the most significant environmental aspects and the main reason for limiting the quality production in crops (Chandler and Bartels 2003). The water stress affects the plant growth at every stage leading to changes at the morphological and molecular level (Farooq et al. 2009). The first effect seen is reduced germination (Kaya et al. 2006). Water stress also reduces the seedling germination and development in wheat and sunflower (Kaya et al. 2006; Nezhadahmadi et al. 2013). Water stress interferes with the normal functioning of the plant by affecting the plant water potential (Hsiao 2000), which changes the morphological and physiological behavior of the plant (Rahdari and Hoseini 2012). It is a complex chemical-physical phenomenon that also interferes with the structure of small and large biomolecules viz. proteins, carbohydrates, fatty acids, hormones, nutrients, and ions (Dhanda et al. 2004; Chaves et al. 2003). The soil water accessibility to plants helps in the plant growth (Song et al. 2009). Growth of several crops such as wheat (Rampino et al. 2006), rice (Lafitte et al. 2007), maize (Kamara et al. 2003), and barley (Samarah 2005) is reduced due to water stress. Common growth parameters like fresh weight and water content are also affected by the drought condition (Jaleel et al. 2009). Moreover, it also affects the transport and availability of nutrients as water is responsible for carrying nutrients to the soil. Hence it declines the diffusion of nutrients and water-soluble nutrients mass flow like Ca, Mg, Si, $NO^{3-}$, and $SO_4^{2-}$(Barber 1995; Selvakumar et al. 2012). Free radicals are produced that affect the antioxidative defense and Reactive Oxygen Species (ROS) like hydrogen peroxide, hydroxyl radical, and superoxide radical creating oxidative stress. The higher concentration of ROS causes injury at various levels (Smirnoff 1993), such as initiation of lipid peroxidation, deterioration of lipids, protein, and nucleic acid (Sgherri et al. 2000; Hendry 2005; Nair et al. 2008). The reduction in the chlorophyll content in bean (Beinsan et al. 2003), *Paulownia imperialis* (Astorga and Melendez 2010), and *Carthamus tinctorius* (Siddiqi et al. 2009) is due to photooxidation under water stress (Anjum et al. 2011; Rahdari et al. 2012). Water stress also lowers the uptake of nitrate in the soil, affecting biochemical activities such nitrogen reductase (Caravaca et al. 2005). Plant growth is also inhibited through various mechanisms by ethylene biosynthesis (Ali et al. 2014). Plants exposed to water stress form a range of reactive oxygen species (ROS) through reaction during the process of photosynthesis, transpiration, and dark respiration, which damages the cells (Taylor et al. 2003). These are toxic to cells when combined with vital molecules like proteins, fats, and nucleic acid leading to denaturation of protein, peroxidation of lipid, and mutation of DNA (Quiles and Lopez 2004). Water stress also causes a negative impact on the cell membrane and cellular organelles such as chloroplasts and mitochondria (Candan and Tarhan 2003), which leads to the leakage of cellular content (Karabal et al. 2003). Deficiency of water at the plant or cellular level occurs when transpiration surpasses the uptake of water, causing a decrease in the cell volume, relative water content, and cell turgor (Lawlor and Cornic 2002). Deficiency in cellular water is caused by drought and other stresses, i.e., salinity, high and low temperature (Bray 1997; Song et al. 2009). Water stress also influences the various physiological activities, including leaf temperature, water potential, relative water content, transpiration rate, and stomatal conductance (Machado and Paulsen 2001). It also affects the efficiency of photosynthesis occurred by the leaf senescence of premature leaves, damaged photosynthetic machinery, and reduction in leaf expansion (Wahid and Rasul 2005). The reduction of photosynthesis in the water stress conditions is due to the fall in Rubisco activity (Bota et al. 2004). Hence, water stress negatively affects the quality and quantity of plant growth. To alleviate the water stress, plants have developed several mechanisms such as morphological adaptations, water resources development, osmotic adjustment, antioxidant systems that reduce the detrimental effect of reactive oxygen species (ROS) associated with drought by inducing a range of stress-responsive genes and proteins (Farooq et al. 2009).

In plants' various physiological processes, such as photosynthesis and respiration, ROS releases as by product in small quantities (Apel and Hirt 2004). But stress conditions alter the normal homeostasis of cells releasing free radicals, and the higher concentration of ROS are toxic. Water stress in plant generates the reactive oxygen species (ROS) with hydrogen peroxide ($H_2O_2$), hydroxyl radicals (OH), alkoxy radicals (RO), superoxide anion radicals ($O_2^-$), and singlet oxygen ($^1O_2$) (Munné-Bosch and Peñuelas 2003; Helena and Carvalho 2008). These ROS are very active reacting with protein, lipids, and deoxyribonucleic acid, creating oxidative damage slowing the plant metabolism and normal functioning of a cell, ultimately causing death (Foyer and Fletcher 2001; Mittler 2002; Farooq et al. 2009; Hasanuzzaman et al. 2014) (Fig. 7.1).

## 7.3 Oxidative Stress in Crop Plants Under Water Stress

Several studies observed the overproduction of ROS by the drought, creating oxidative stress in various species of plants. The degree of drought severity relies on the capacity of the genotype to deal with oxidative stress. Abideen et al. (2020) observed a 22% enhancement in the (malondialdehyde) MDA content when *Phragmites karka* is grown under drought conditions in a plastic tube for 35 days retaining 40% water holding capacity. Similarly, higher MDA content was also observed by Campos et al. (2019) after 20 days in *Coffea arabica*. Saha et al. (2018) found an

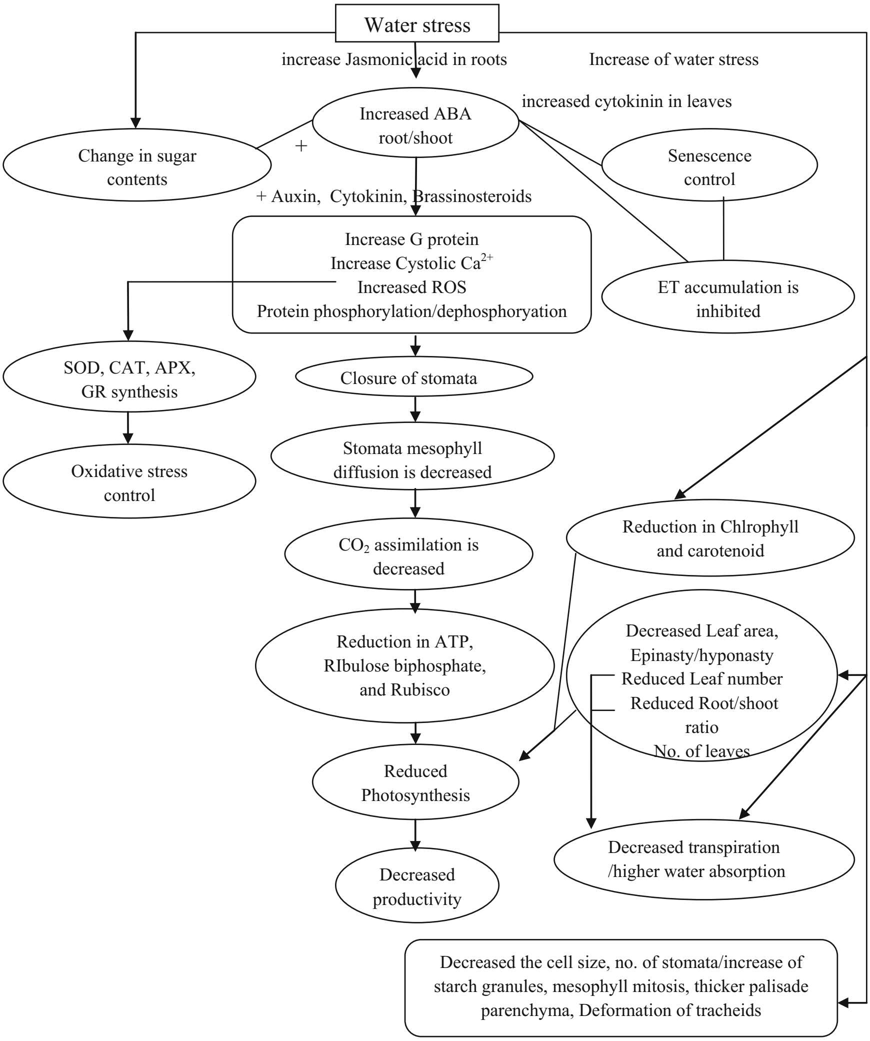

**Fig. 7.1** Effect of water stress on the physiology of plant

increase in $O_2$, $H_2H_2$, and MDA content in rice plants when not watered for 8 days creating drought stress as compared to control. Finger millets (*Eleusine coracana* L. Gaertn.) grown in 75% water stress for 3 weeks lead to an increase in the EL (electrolytic leakage) and $H_2O_2$ content (Satish et al. 2018). Malhotra et al. (2017) showed the augmentation of MDA content and 39% EL due to the withdrawal of irrigation in tomato plants for 6 days. *Solanum lypopersicum* L. cv. Login 935 exposed to water stress of 60% field capacity for 20 days leads to an increase in $O_2$ (75%), $H_2O_2$ (37%), and MDA content (83%) (Rady et al. 2020) Filippou et al. (2011) also recorded enhancement in the MDA and $H_2O_2$ content under water stress conditions in *M. truncatula* which were reversed by rewatering. Two genotypes of *Phaseolus vulgaris* viz. Bn-16 and Bn-150 were compared under water stress for 14 days with 50% FC showing that Bn-16 contained two times grated production of MDA as compared to Bn-150 (Kusvuran and Dasgan 2017). It also contained a higher amount of $O_2$, $H_2O_2$, and OH content. Wheat grown in a water-deficient condition of 70% FC showed a higher amount of $H_2O_2$, EL, and TBARS content (Abbas et al. 2018). Khan et al. (2017) evaluated the consequence of water deficiency at 30% FC in *B. napus* cv. Bulbul-98 found a considerable enhancement in the EL and $H_2O_2$ content. Nxele et al. (2017) recorded a 113% boost in the $H_2O_2$ and MDA (94 and 98%) in leaves and roots in *S. bicolor* cv. Sugargraze grown in water scarcity for 16 days.

## 7.4 The Antioxidant Defense Under Water Stress

Production of ROS species due to drought stress promotes oxidative stress in the biological systems (Reddy et al. 2004; Samarah et al. 2006). Excessive production of ROS leads to enhanced peroxidation of lipid and thereby damaging the lipids, protein, and DNA (Pompelli et al. 2010). Still, ROS also activates the stress reaction and defense pathway (Pitzschke et al. 2006). Hence, it is important to control the level of ROS by coordinating the production and scavenging system of ROS to control the oxidative injury and at the same time altering the events of signals (Stajner et al. 1997). The degree and quantity of increase of antioxidant system are extremely variable under drought stress. It mainly depends on the species, cultivar (Manivannan et al. 2007a, b), metabolic or developmental state of the plant, intensity, and stress duration. In stress situations, enhancement in the activity of foliar antioxidants is also observed (Pastori et al. 2000; Sharp and LeNoble 2002). The plant usually defends against the production of ROS by enhancing the components of defense systems (Ahmad et al. 2010a, b). Research has been done on the detoxifying enzyme activity of plants when exposed to drought stress (Djibril et al. 2005; Manivannan et al. 2008). Cellular damage to the cell by ROS is overcome by developing a complex non-enzymatic and enzymatic antioxidant defense system providing deleterious effect and accumulation of ROS during water stress (Miller et al. 2010; Helena and Carvalho 2008; Simova-Stoilova et al. 2008). Enzymatic components comprise catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), and glutathione reductase (GR). Non-enzymatic component comprises reduced glutathione, cysteine, and ascorbic acid (Gong et al. 2005; Kaushal and Wani 2015). Thus, drought-tolerant plants develop an effective system for scavenging of ROS (Apel and Hirt 2004; Huang et al. 2014). These scavenging enzymes and ROS have been widely studied (Helena and Carvalho 2008; Farooq et al. 2009; Gill and Tuteja 2010; Hasanuzzaman et al. 2014).

### 7.4.1 Peroxidase

Peroxidase (POX) helps in mediating the signaling of ROS in the chloroplast as well its accumulation in the nucleus, chloroplasts, mitochondria, and peroxisomes (Vaahtera et al. 2014; Mignolet-Spruyt et al. 2016). They are generally chloroplastic enzymes and help in scavenging $H_2O_2$ produced through the action of superoxide dismutase (Chaitanya et al. 2002). It also helps in promoting the ROS-scavenging system such as POD, APX, CAT, and SOD (Suzuki et al. 2011). It plays an essential part in scavenging by coordinating with SOD (Farooq et al. 2009).

### 7.4.2 Superoxide Dismutase

Superoxide Dismutase (SOD) is the frontline defense system located in all the aerobic individuals and the main antioxidative enzyme in the subcellular section affected by ROS-assisted oxidative stress. It enhances the plants' tolerance t the stress by catalyzing ${O_2}^{2-}$ to $H_2O_2$ and $O_2$ (Mittler 2002). It protects from oxygen toxicity by regulating the concentration of anionic radical of superoxide (Nordberg and Arner 2001). Its overproduction enhances stress tolerance (Pastori and Foyer 2002) and is important in cellular defense against reactive oxygen species and reduces the OH radical causing membrane, protein, and DNA damage (Kage et al. 2004).

### 7.4.3 Catalase

Catalase (CAT) helps eliminate $H_2O_2$ produced during the metabolic process harming the cell function (Gaspar et al. 2002), converting them into $H_2O$ and $O_2$. The $H_2O_2$ present in the cytosol is scavenged by the peroxisomes proliferation during the stress (Lopez-Huertas et al. 2000; Kusaka et al. 2005) formed through oxidase and involved in the catabolism of purines, β-fatty acids' β-oxidation, and photorespiration (Polidoros and Scandalios 1999).

### 7.4.4 Ascorbate Peroxidase

Ascorbate Peroxidase (APX) is a vital antioxidant enzyme for detoxification of $H_2O_2$ and reduction of ascorbate. It reduces $H_2O_2$ to $H_2O$ in the ascorbate-glutathione cycle using ascorbate as an electron donor (Lawlor and Cornic 2002) and the water-water cycle.

### 7.4.5 Glutathione Reductase, Glutathione Peroxidase, and Glutathione S-Transferase

Glutathione reductase (GR) is a vital enzyme in the cycle of ascorbic acid – glutathione, which toxifies ROS's harmful effect. It is present in the chloroplast and a little quantity in the cytosol (Edwards et al. 1990; Creissen et al. 1994).

Glutathione Peroxidase (GPX) protects the cells from oxidative damage by using glutathione for reducing the $H_2O_2$ and also lipid and organic hydroperoxide (Noctor et al. 2002).

Glutathione S-Transferase (GST) class is big and multifaceted, having various functions. One of the functions is to help in the plant response to biotic and abiotic factors (Dixon et al. 2010).

The antioxidant mechanism of defense extensively studied uses ascorbate-glutathione pathway (also called as Halliwell-Asada cycle) catalyzed by four sets of enzymes, namely Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) (Fazeli et al. 2007) used in the detoxification of ROS. The activity of all these enzymes is increased in drought stress (Jaleel et al. 2007a, b). Ascorbate peroxidase oxidizes ascorbate to scavenge Hydrogen peroxide ($H_2O_2$). It also oxidizes ascorbate to mono-dehydroascorbate, which is later transformed back to ascorbate (ASA) by mono-dehydroascorbate reductase (MDHAR). The escaped mono-dehydrogenase from this cycling is rapidly changed to dehydroascorbate (DHA). Afterward, this is changed back to ascorbate through dehydroascorbate reductase (DHAR). It uses reduced glutathione (GSSG), which is restored to its oxidized form, i.e., glutathione disulfide (GSSG) by glutathione reductase (GR) (Murshed et al. 2008). These enzymes form a main antioxidant system where SOD catalyzes the dismutation of $O_2$ to $H_2O_2$ and $O_{2,}$ and CAT and POD scavenge $H_2O_2$ (Egert and Tevini 2002) (Fig. 7.2).

## 7.5 PGPR-Mediated Water Stress Tolerance

Some influential members of bacteria present in the rhizobacteriome are *Pseudomonas*, *Bacillus*, *Azospirillum*, *Serratia*, *Acinetobacter*, *Arthrobacter*, *Bradyrhizobium*, *Rhizobium*, *Agrobacterium*, *Enterobacter*, *Achromobacter*, *Azotobacter*, *Phyllobacterium,* etc. (Naylor and Coleman-Derr 2018). PGPR have received attention due to their ability to withstand abiotic and biotic stress conditions (Saravanakumar et al. 2007). Water stress affects the growth and productivity of the crops, mainly in arid and semi-arid areas. The various mechanism by which PGPR induce water stress tolerance in plants includes change in root morphology, phytohormonal activity, EPS production, ACC deaminase activity, volatile compounds, Accumulation of osmolytes, and antioxidant defense. Inoculation with PGPR can lead to an improvement in tolerance to water stress (Figueiredo et al. 2008) through the production of cytokinins, IAA, ACC deaminase, and antioxidants. There occurs a considerable relationship between drought stress and antioxidant enzymes, but PGPR inoculation lessens the harmful effect of water stress on the

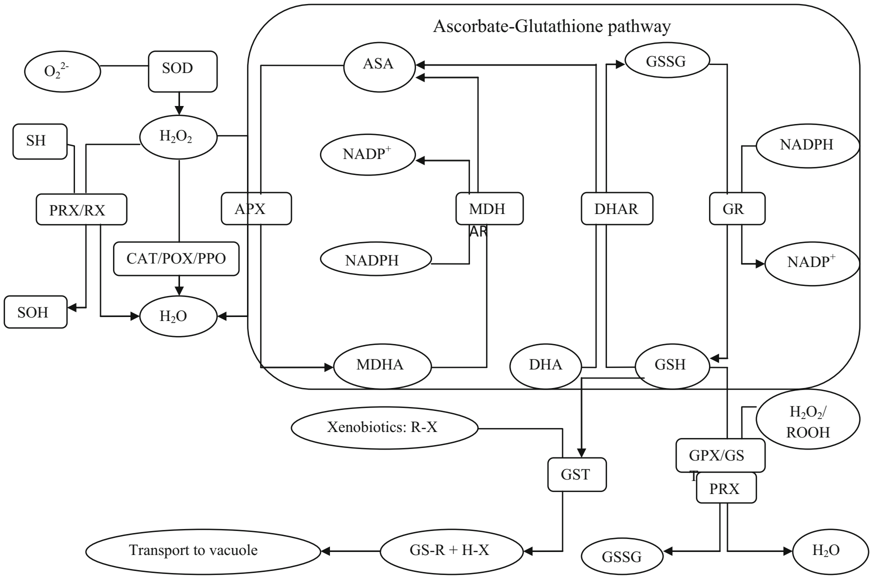

**Fig. 7.2** Antioxidant mechanism of antioxidant defense system (-SH: thiolate, -SOH: sulfenic acid, NADPH: nicotinamide adenine dinucleotide phosphate, ROOH: hydroperoxidase, TRX: thioredoxin; X, sulfate, nitrate or halide gp, $O_2^{2-}$: superoxide anion)

antioxidant enzymes activity (Han and Lee 2005). Many authors have recommended the role of PGPR in reducing the oxidative damage caused by abiotic stresses by manipulating the antioxidant enzymes in various crops (Sandhya et al. 2010; Saravanakumar et al. 2011). Activities of enzymes lead to the alleviation of oxidative damage, but at an elevated level of ROS, it causes harmful effects. So, it is essential to retain a balance between the production of ROS and the removal of the production of free radicals. It can be achieved by inoculating PGPR in the plants, and the inoculated plants were seen to have a higher survival rate as compared to non-inoculated plants.

The enzymatic activity reduces the oxidative damage but at a high level of ROS, its deleterious effect was observed (Halliwell 2006). Therefore, it is necessary to retain the balance between the production of ROS and eradication of the formation of free radicals (Miller et al. 2010). Inoculation of PGPR to the plants helps in increasing the survival rate by checking the oxidative damage as compared to the plants that were not inoculated with PGPR.

## 7.6 Role of PGPR in Enhancing the Oxidative Enzymes

Experiments are done to study the activity of antioxidant enzymes and their role in the scavenging system in drought stress mediated by plant growth-promoting bacteria (PGPR) (Table 7.1). The study illustrated an increment in the level of antioxidant enzymes by their application. They lead to the accumulation of antioxidant enzymes such as CAT and POX resulting in a decrease in oxidative injury. Selected PGPR strains result in the overproduction of these ROS-scavenging enzymes, which reduces the overproduction of ROS, giving drought tolerance. Induction of oxidative enzymes is directly correlated to the extent of drought of drought tolerance (Contour-Ansel et al. 2006; Guo et al. 2006).

The ROS-scavenging enzymes play an important in the PGPR-mediated water stress tolerance. In water-deficient conditions, the application of PGPR increases water stress tolerance by altering the antioxidative enzymes (Gusain et al. 2015). It is also helpful for the growth of tomato and pepper and providing resistance to water stress (Aroca and Ruiz-Lozano 2009). Modulating antioxidative and glyoxalase enzymes in mustard can induce resistance to the oxidative stress caused by drought (Mohammad et al. 2013). Dong et al. (2013) observed the ultrastructure of cucumber cells under drought stress suggesting that damage can be avoided by stabilizing the cell structure and reducing the peroxidation of lipid by improved activity of antioxidant enzymes and metabolites.

Water stress-tolerant PGP *Pseudomonas* spp.. such as *P. putida*, *P. syringae*, *P. montelli*, *P. stutzeri*, and *P. entomophila* when inoculated in maize plants demonstrated lesser activity of antioxidant enzymes in contrast to uninoculated plants (Sandhya et al. 2010). In a similar way, *Bacillus* spp. inoculation provides tolerance to water stress in the maize plant by reducing antioxidative enzyme activity, namely APX and Glutathione peroxidase (GPX) (Vardharajula et al. 2011). *Pseudomonas* spp. applied under water stress considerably improved the activity CAT enzyme under the field in basil plants. Similarly, when consortia of microbes like *Pseudomonas* spp., *A. brasilense,* and *Bacillus lentus* were applied, the activity of APX and GPX was highest (Heidari and Golpayegani 2011). Maize plants inoculated with EPS-producing bacteria decreased APX, CAT, and GPX enzyme activity providing stress tolerance in plants (Naseem and Bano 2014). Under water stress conditions, indigenous PGPR *Bacillus thuringiensis* promote growth and drought tolerance in the plants *Layandula dentata* and *Salvia officinalis,* decreasing the stomatal conductance and activity of glutathione reductase (GR) and ascorbate peroxidase (APX) (Armada et al. 2014). Using consortia of PGPR (*P. synxantha* R81, *P. jessenii* R62, and *A. nitrogua-jacolicus* strain YB3 and YB5) in rice cultivars (*Oryza sativa* L.) (*Oryza sativa* L.) such as IR-64 (drought-sensitive) and Sahbhagi (drought tolerant) increased the growth of the plant and induce the stress-related enzymes like CAT, SOD, APX, peroxidase (POD), malondialdehyde (MDA), and lower levels of $H_2O_2$ under water stress in comparison with control (Gusain et al. 2015). Potato plants treated with PGPR strains such as *Bacillus firmus* str. 40 and *Bacillus pumilus* str. DH11 increased the ROS-scavenging enzymes such as catalase, peroxidase, and ascorbate. Significant increases in the scavenging enzymes, as well as CAT, SOD, and APX, were also reported by Gururani et al. (2013). In PGPR-treated plants, the activity of CAT was found 1.8 times higher under drought stress as compared to non-inoculated plants. The main reason advocated for the water stress tolerance in potato plants treated with PGPR causes the elevation of ROS-scavenging enzymes. An enhancement in the

**Table 7.1** Effect of certain PGPR on the antioxidant activity of various crops

| Plant species | PGPR strains | Effect on antioxidant activity | Reference |
|---|---|---|---|
| Rice | *Pseudomonas jessenii*, *P. synxantha*, *Azospirillum nitroguajacolicus* | Increased the CAT, SOD, POD, and APX, reduced MDA and $H_2O_2$ level | Gusain et al. (2015) |
| Wheat | *Pseudomonas* spp. | Controls the oxidative damage | Chandra et al. (2018) |
| Wheat | *Bacillus amyloliquefaciens* 5113, *A. brasilense* N040 | Increases the antioxidant enzymes | Kasim et al. (2013) |
| Maize | EPS-producing bacteria | CAT, APX, and GPX activity is reduced | Naseem and Bano (2014) |
| Maize | *Bacillus* spp. | Increase CAT activity | Vardharajula et al. (2011) |
| Green gram | *B. subtilis* EPB2 2, EPB5 and EPB 31, *P. fluorescens* strain Pf1 | Improved stress-related enzymes | Saravanakumar et al. (2011) |
| Green gram | *Pseudomonas* spp. GGRJ21 | CAT, SOD, and POX activity is enhanced | Sarma and Saikia (2014) |
| Chickpea | *P. putida* MTCC5279 (RA) | Increased CAT, GST, and APX | Tiwari et al. (2016) |
| Cucumber | *B. cereus*, *B. subtilis* and *Serratia* spp. | Enhanced CAT | Wang et al. (2012) |
| *Ocimum basilicum* L. | *Bacillus lentus*, *A. brasilense* and *Pseudomonas* spp. | Increased CAT, GPA, and APX | Heidari and Golpayegani (2011) |
| *Lavandula dentate* and *Salvia officinalis* | *B. thuringiensis* | Increased GR and APX activity | Armada et al. (2014) |

CAT activity with the treatment with *Bacillus subtilis* EPB and *Pseudomonas fluorescens* Pf1 was observed in the green gram plants (Saravanakumar et al. 2011). Tolerance to drought and CAT production are found associated in wheat (Kasim et al. 2013), maize (Sandhya et al. 2010; Sarma and Saikia 2014; Vardharajula et al. 2011), and cucumber plants (Wang et al. 2012). Inoculation with *Pseudomonas mendocina* along with *Glomus intraradices* or *G. mossae* increases the CAT level and reduces the oxidative stress induced by drought in lettuce (*Lactuca sativa* L.) (Kohler et al. 2010)

## 7.7 Challenges and Future Prospects of Using PGPR for Bioinoculation

The benefits of PGPR as bioinoculant include the plant growth promotion by mobilization and synthesis of nutrients as well as growth-promoting substances, including induced systemic resistance (ISR). Besides enhancing plant nutrients, they also help in restoring the fertility of the soil in an environment-friendly way. In addition, they alleviate environmental stress like drought in plants (Olanrewaju et al. 2017; Maxton et al. 2018).

Even though there are many benefits of these microbes, but some shortcoming is there which prevent their effective exploitation and commercialization. PGPR performance is effective under in vitro conditions. Still, they are not found sustainable under the field due to their exposure to the natural environment as they are giving inconsistent results. Microbe efficacy is affected by the fluctuation in soil structure, texture, pH, and composition of minerals, creating a disadvantage for their use on a large scale.

Hence, PGPR should be tested under pot conditions using unsterilized and sterilized soil to establish their real efficacy before ultimate testing in the field under water-stressed conditions with natural microbes. This will help to determine the effectiveness of PGPR to colonize the roots effectively and compete with the well-established indigenous microbes.

The extreme heat also affects the efficacy of the PGPR, excluding those forming endospores and producing biofilms. High temperature reduces the bacterial population as inoculums of bacteria do not have shielding support. To formulate the effective bioinoculant, a required quantity of bacteria with a physical shield should be applied to decrease the introduced PGPR (Zambrano-Moreno et al. 2016).

The strains of PGPR need to be tested for two or more plant growth-promoting factors in stressed conditions. The strains are effective when used alone but using one or more strains in a consortium is more effective. However, some strains showed incompatibility when used in the consortium and gave excellent results in single strains. Thus, greenhouse experiments should be conducted before bacterization in the field.

There is a need for an appropriate carrier that would be cheap, rich in organic matter, non-toxic, 50% water holding capacity, and easily accessible used for the bioformulation of PGPR. Different carriers such as farmyard manure, charcoal, peat, press mud, vermiculite, lignite, and soil mixture are suggested as a suitable carrier (Anubrata 2014).

The quality of the biofertilizer should be assured before officially registering for commercial use.

The farmers should be made aware of this technology to harness the benefit from the PGPR.

PGPR is exploited as a bioinoculant for the healthy growth of the plant under unfavorable environmental conditions. But they are occasionally incapable to endure in harsh environmental surroundings, and the activity and development of PGPR are hampered by extreme pH, salinity, temperature, drought, and heavy metal pollution.

Under field conditions, the efficacy was also reduced due to the short shelf life applied in the field. Therefore, PGPR strains with longer-shelf lives should be developed for the sustainable production of crops in drylands.

PGPR provide stress tolerance to many host crops, including monocotyledonous, dicotyledonous, and vegetable crops (Mayak et al. 2004; Sandhya et al. 2009; Kasim et al. 2013) for more than one abiotic and biotic stress tolerance (Mayak et al. 2004; Coleman-Derr and Tringe 2014).

To boost plants' performance under water stress, there is a need to develop a genetically engineered transgenic plant that is drought tolerant and reduces the negative effect of drought on crop and productivity (Barrow et al. 2008; Eisenstein 2013). But it is time-consuming, labor intensive, and there can be loss of required traits from the gene pool of the host, and it is easier to modify a bacterium as compared to the complex higher organism. Breeding provides resistance to a single crop and cannot be transferred to other crop systems (Ashraf 2010; Eisenstein 2013; Phillippot et al. 2013). Additionally, the response of consumers to genetically modified crop products differs in various countries; therefore, success is not guaranteed (Fedoroff et al. 2010). Hence as an alternative to individual genetically engineered crops, a single, non-specific genus such as *Azospirillum* can be used as genetically engineered inoculants for several crops under water stress. PGPR strain effective at low-inoculum dose, and different environmental conditions can be developed by genetic engineering. Drought-tolerant novel genes can be identified by using the recent advances done in fields of biotechnology, microbiology, molecular biology, and bioinformatics. Indigenous species of PGPR should be isolated from the water-stressed soils through micro biotechnology, and rapidly selected effective strains based on their stress could be used for dryland crops. Carrier-based inocula of microbes are a new avenue developed through the application of bionanotechnology. Using nanoformulations of PGPR enhances their stability to heat, desiccation, and UV inactivation.

## 7.8 Conclusion

Water stress is a major menace and severe environmental constraints for sustainable agriculture. It causes ROS buildup in plants, which leads to oxidative damage affecting the plant's health. This results in a decrease in growth of plant and crop production caused by damaged physiological and biochemical affecting the farmers' income. Thus an effective, inexpensive, and eco-friendly strategy of using beneficial PGPR not only promotes the plant health but also provides protection from the environmental stress mitigating the effect of drought. They tend to elicit the RIDER mechanism which plays a significant role to counteract osmotic and oxidative damage. PGPR increase the osmolytes production and antioxidant defense system, reducing the harmful effect of ROS on plants. To alleviate the drought stress, introduction of drought-tolerant varieties is crucial but it is an overlong process . Hence the use of PGPR draws the attention of farmer to use microbes in the areas which are drought prone. The identification, cloning, and functional characterization of stress-tolerant genes in PGPR strains could be further harnessed for their use in dryland agriculture.

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# Rhizospheric Plant–Microbe Interactions Releasing Antioxidants and Phytostimulating Compounds in Polluted Agroecosystems

# 8

Kriti Sengupta and Siddhartha Pal

## Abstract

Rhizomicrobiome consists of plant growth-promoting rhizobacteria, arbuscular mycorrhizal fungi, soil protozoa and other microorganisms associated with plant roots for several benefits. The rhizosphere biology has been focused mainly on exploring plant–microbial interactions which stimulate growth and stress response in plants, especially in polluted lands. Several microcosm-based studies suggested that rhizospheric microorganisms are able to bioremediate heavy metals or organic pollutants and improve plant resistance in pollutant-impacted agrofields. Antioxidants and several phytohormones released due to plant–microbe interactions in polluted rhizosphere can trigger the defence system of plants. The present chapter describes the role of rhizomicrobiome and plant–microbe associations involved in antioxidants and phytostimulation mechanism in polluted environments. Integrated multi-omics and bioinformatics approaches to understand plant–microbe associations have been discussed here, which is an emerging research interest in developing next-generation agricultural process aided with rhizosphere bioengineering.

## Keywords

Rhizomicrobiome · Antioxidants · Phytohormones · Plant–microbe interaction · Rhizospheric meta-omics · Rhizoremediation

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## 8.1 Introduction

Frequent soil pollution with chemical fertilizers, heavy metals, pesticides, herbicides, petroleum hydrocarbons and other organic pollutants created enormous threats to crop health in several agroecosystems by altering soil pH, salinity and water activity (Sengupta et al. 2015). According to the Food and Agricultural Organization (FAO) report, more than 96% of global agricultural land has been affected by environmental constraints (Meena et al. 2017). Due to these agroecological disturbances, crop production has been affected in terms of quality and quantity loss (Cramer et al. 2011). Plants possess intrinsic metabolic pathways to produce antioxidants or phytohormones to combat with rapid adversity and fluctuations in environmental conditions (Simontacchi et al. 2015). More importantly, plant-associated microbiome supports the plant by releasing phytostimulants and degrading pollutants which enable the plants to survive under environmental stresses (Ahemad and Kibret 2014; Gkorezis et al. 2016; Kotoky et al. 2018). Rhizosphere microorganisms are found to be promising in biofertilizer application which not only improves plant health but also helps in detoxifying the soil contaminants (Kotoky et al. 2018; Oberai and Khanna 2018). The major research on rhizosphere biology relies on the interesting associations between plant and rhizospheric microbes for plants and microbes' mutual benefits and balancing of soil nutrients (Ahemad and Kibret 2014). However, triggering of several antioxidants and phytostimulating compounds in plant–microbe interactions is governed by multiple biotic and abiotic factors (Oberai and Khanna 2018). Interestingly, plant roots secrete necessary phytochemicals and stimulants which cause a selective enrichment of rhizomicrobiome (Lu et al. 2011). However, it is not well understood whether these microbes are interacting with specific plants either in positive or in a negative relation as there is significant diversity in microbial population which differs from plant to plant. The rhizomicrobiome, a mixed microbial population of plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal (AM) fungi and soil protists, all together plays roles in secreting phytohormones, antioxidants and pathogen-controlling agents (Fig. 8.1).

It has been evident from several studies that these microbes of rhizosphere have been evolved to degrade or tolerate heavy metals or organic pollutants and thus also create a detoxified environment for plants (Guo et al. 2019). The utmost critical aspect in defending stress in plants is to study molecular machinery and its networks functioning within stress conditions. The elucidation of metabolic pathways and their regulatory genes overexpressed during plant–microbe interactions have been important in understanding the molecular machinery of stress response. Documentation of multigenic characters related to stress responses and investigation of relevant marker genes are in current focuses of stress extenuation strategies. Other approaches that have been implemented for the mitigation of abiotic stresses in plants include the use of several biomolecules from plant and microbial sources (Meena et al. 2017). The ability of a microorganism to build an interaction with a plant is influenced by several elements including the environment, microbial genes expression and regulatory proteins and its interactome partners. Currently, a huge

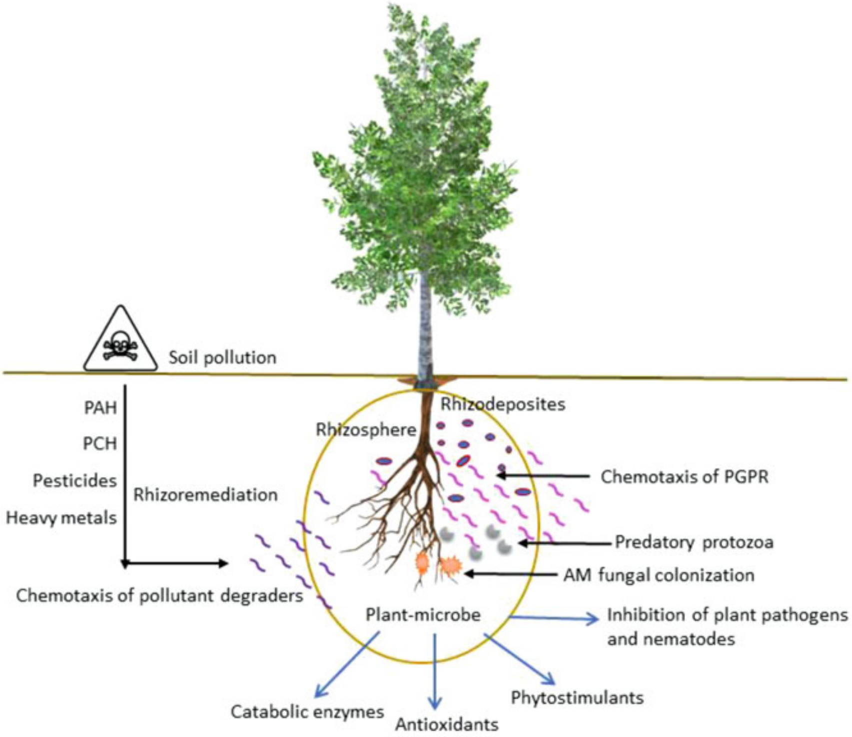

**Fig. 8.1** Representation of rhizoremediation in polluted soil aided with several plant–microbe interactions involves in plant stress resistance; process includes sequential release of rhizodeposits, chemotactic movements of PGPR, migration of pollutant degraders to roots rhizodeposits, colonization of roots with AM fungi and grazing of pathogens by soil protozoa

amount of genome sequence data is available and researchers are now motivated to explore plant–microbe relationship based on genome information (Martínez-García et al. 2016). In recent years, multi-omics methods have established itself as a complete and integrated strategy for investigation of microbial interactions with plants (Meena et al. 2017; Kotoky et al. 2018). To address the present scenario of research on rhizomicrobiome and their association with plants specially in polluted lands, this chapter focuses on the diversity of rhizomicrobiome in polluted soil, several plant–microbe associations and microbe-facilitated plant antioxidants mechanism in stress conditions. This chapter also summarizes the previous experiments of microcosm studies and trending multi-omics approaches which require more attentions to understand plant–microbe associations in pollutant-impacted lands.

## 8.2 Rhizomicrobiome of Polluted Ecosystems

Rhizosphere is a microenvironment that harbours complex microbial community structure where plant root symbiotic rhizobacteria, mycorrhizal fungi, predatory protozoa and other free-living microorganisms play crucial roles to maintain the biogeochemical balance of soil. Unfortunately, several anthropogenic activities and rapid industrialization have introduced pollutants into agricultural lands such as petroleum hydrocarbons, pesticides and heavy metals. Such soil pollutions have changed the dynamics of plant–microbe interactions which is unusual in non-polluted or less toxified soil (Guo et al. 2019). It has been previously reported that plant growth promotion has been improved under contaminated conditions when rhizosphere was inoculated with PGPR with biodegradation abilities (Sengupta et al. 2015; Correa-García et al. 2018; Kotoky et al. 2018; Roy et al. 2020). Several PGPRs identified in contaminated soil with multiple activities related to heavy metal tolerance and pollutant detoxification are enlisted in Table 8.1.

Rhizosphere manipulation and engineering require the understanding of diversity of cultivable microorganism and their metabolic functions in toxic environment (Haichar et al. 2008). Several rhizobacterial species are root symbionts such as *Bradyrhizobium*, *Frankia*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* while others are free-living nitrogen fixers such as *Acetobacter*, *Azotobacter*, *Azospirillum*, *Azoarcus* and *Herbaspirillum* (Benidire et al. 2017; Kumar and Dubey 2020). Siderophore-producing certain PGPR groups, phosphate solubilizers and fungi increase the accessibility of minerals and microelements (Fe, P, Cu, Zn) to plants (Hashem et al. 2017). These rhizobacteria such as *Actinomyces*, *Bacillus*, *Pseudomonas* and *Streptomyces* produce antibiotic compounds like bacteriocins, phenazine, hydrogen cyanide, oligomycin and are also documented as potential biocontrol agents (Calvo et al. 2014; Zope et al. 2019). Most importantly, application of PGPR strain as phytostimulants, biopesticides and in rhizoremediation is the popular methods for secure crop management. In this context, *Bacillus licheniformis*, *Bacillus pumilus* and *Bacillus subtilis*, known as “Bacillus-based products,” were commercialized earlier (Ongena and Jacques 2008). The soil bacterium *Klebsiella* sp. with 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, inoculated to a bunch grass species (Festuca arundinacea), showed rhizoremediation of petroleum hydrocarbons and phytostimulating activities (Liu et al. 2014). Another study of co-inoculating PGPR strain of *Acinetobacter* sp. with common oat (*Avena sativa*) performed by Xun et al. (2015) showed dry mass gain, increased stem height and high rate of hydrocarbon degradation in polluted soil. Several metagenomic studies revealed that the rhizoremediation of petroleum-impacted soil microbiome is rich in hydrocarbon-degrading bacteria which belonged to major families like *Actinomycetaceae*, *Alcaligenaceae*, *Alteromonadaceae*, *Burkholderiaceae*, *Bradyrhizobiaceae*, *Caulobacteraceae*, *Rhizobiaceae*, *Rhodospirillaceae* and *Solirubrobacteraceae* (Pagé et al. 2015). Another important microbe that dominates in the rhizosphere is AM fungi which are responsible in altering the microbial communities of contaminated rhizosphere (Xun et al. 2015). The role of AM fungi in plant health promotion and positive interactions with PGPR (special emphasis on

**Table 8.1** List of plant-associated PGPR, their plant growth-promoting activities and tolerance to xenobiotic pollutants and heavy metals

| PGPR strains | Phytostimulants/ antioxidants | Xenobiotic tolerance | Heavy metal tolerance | References |
|---|---|---|---|---|
| *Azotobacter chroococcum*, *Bacillus megaterium*, *Bacillus mucilaginosus* | $N_2$ fixation | NR | Cd | Wu et al. (2006) |
| *Azomonas*, *Bacillus*, *Xanthomonas*, *Pseudomonas* | IAA production | NR | Cd | Sheng and Xia (2006) |
| *Bacillus* | IAA, siderophore and phosphate solubilization | 4-Nitrophenol | Zn, Pb, Ni and Cr | Sengupta et al. (2015) |
| *Bacillus weihenstephanensis* | IAA production and phosphate solubilization | NR | Ni, Cu, Zn | Rajkumar et al. (2008) |
| *Bacillus edaphicus* | ACC deaminase, IAA and siderophore | NR | Pb | Sheng et al. (2008) |
| *Bradyrhizobium* | ACC deaminase, IAA production, $N_2$fixation and phosphate solubilization | Herbicides-quizalofop-p-ethyl and clodinafop) | NR | Ahemad and Khan (2011) |
| *Psychrobacter* | CAT, peroxidase and siderophore production | NR | Ni | Ma et al. (2011) |
| *Rhizobium*, *Pseudomonas*, *Stenotrophomonas* | Siderophore, IAA, phosphate solubilization | *n*-hexadecane, diesel oil | NR | Pawlik et al. (2017) |
| *Pseudomonas aeruginosa* | IAA and siderophore production | PAHs, naphthalene, pyrene | NR | Wu et al. (2018) |
| *Pseudomonas* | ACC deaminase | Benzene, Toluene, Ethylbenzene, Xylene | As, Cu | Imperato et al. 2019 |
| *Pseudomonas*, *Pantoea*, *Burkholderia* | ACC deaminase | Alkane degradation | NR | Pawlik et al. (2017) |
| *Pseudomonas* | Phosphate solubilization, IAA and siderophore production | NR | Ni | Tank and Saraf (2009) |
| *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Ralstonia metallidurans* | Siderophore production | NR | Cr and Pb | Braud et al. (2009) |

(continued)

**Table 8.1** (continued)

| PGPR strains | Phytostimulants/ antioxidants | Xenobiotic tolerance | Heavy metal tolerance | References |
|---|---|---|---|---|
| *Brevibacillus* | IAA production | NR | Cd, Zn | Vivas et al. (2005) |
| *Pseudomonas*, *Rhizobium sullae* | APX, SOD and CAT activities | NR | Cd | Chiboub et al. (2018) |
| *Rhizobium* | SOD, CAT and APX | NR | Cu | Fatnassi et al. (2015) |
| *Spartina maritima* | COX and AOX pathways | NR | Cu, Pb and Zn | Mesa-Marín et al. (2018) |
| *Variovorax paradoxus* | ACC deaminase, IAA and Siderophore production | NR | Cd | Jiang et al. (2012) |

*ACC* 1-aminocyclopropane-1-carboxylate; *AOX* alternative oxidase, *APX* ascorbate peroxidase, *IAA* indole acetic acid, *CAT* catalase, *COX* cytochrome oxidase, *SOD* superoxide dismutase, *NR* not reported, *PAHs* polycyclic aromatic hydrocarbons

tripartite plant-bacteria-fungi associations) has been discussed in detail in Sect. 8.7 in this chapter. The AM fungi, *Glomus mosseae,* was experimented to improve rhizoremediation of petroleum hydrocarbons and showed higher anthracene removal in pot with contaminated soil as compared to non-polluted control pot (Joner et al. 2001). Other fungi of the genera *Penicillium*, *Aspergillus* and *Rhizopus* have been studied broadly for the detoxification of heavy metals from polluted agroecosystems (Deshmukh et al. 2016).

Interestingly, several organic compounds released from plant roots like terpenes, lignin-derived components and flavonoids found in the rhizosphere are analogous to many contaminants (Singer et al. 2003; Hartmann et al. 2009). Gao et al. (2011) and Ling et al. (2013) showed reduced polyhydrochlorinated compounds (PHCs) concentration in presence of maximum concentration of root exudates which indicated and established a negative correlation between concentration of root exudate and petroleum hydrocarbons. This result was supported by the nature of root exudate which was generally phenolic such as caffeic acid and protocatechuate which is connected with bacterial degradation pathway intermediates of polycyclic aromatic hydrocarbons (PAHs) and PHCs (Ely and Smets 2017). Actually, the plant rhizospheres are commonly enriched in actively expressed microbial genes responsible for degradation of organic pollutants, irrespective of contaminants presence or absence (Yergeau et al. 2014). Guo et al. (2017) demonstrated that supplementation of maize and soya bean root exudate into petroleum-contaminated soil increased PAH degradation initially but did not worked for longer period in absence of the plants. They have also shown that interaction of *Mycobacterium* sp. with root exudate enhanced PAH removal by shifting the structure and diversity of soil bacterial community. The PGPR co-inoculation and microcosm studies to reduce the contamination in pollutant-impacted soil have been discussed in Sect. 8.9 in this chapter.

## 8.3 Antioxidants and Phytostimulating Compounds

Soil pollution due to toxic heavy metals, pesticides or herbicides often increases the soil salinity and decreases the water availability which creates stress conditions for plants (Ahemad and Kibret 2014). Under such stress, deregulated metabolism of plants induces the generation of reactive nitrogen species (RNS) and reactive oxygen species (ROS) which also other way round triggers the stress response pathways to prevent cellular damages in plants (Laxa et al. 2019). RNS-mediated stress response leads to production of several enzymes as antioxidants such as nitrate reductase, xanthine oxidoreductase and nitrosoglutathione reductase (GSNO), whereas the ROS detoxification system of plants is mediated by ferredoxin, glutathione, redox thioredoxin, NADPH-thioredoxin reductase and glutaredoxins and peroxiredoxins (Finkel 2001; Liebthal et al. 2018). Among antioxidant enzymes, ascorbate peroxidase, catalase, dehydroascorbate reductase, glutathione peroxidase, glutathione-S-transferase, monodehydroascorbate reductase, protein disulphide isomerase and superoxide dismutase have been reported to be upregulated as drought stress response in wheat, rice, maize, cotton, stiff brome, date palm and pea plants (Laxa et al. 2019). Helepciuc et al. (2014) showed that plant-bacteria association increased the number of isoforms of superoxide dismutase and catalase enzymes which indicated that bacteria activate plant enzymatic antioxidant systems. Several other studies related to enhanced peroxidase activity in plant–microbe interactions suggested that microbial associations increase the defence capacity of plants (Mittler 2002; Gkorezis et al. 2016; Kumar and Dubey 2020). Additionally, pollutant-resistant plants and their associated microorganisms are reported to produce reduced glutathione (GSH) which play an important role in chelating heavy metals, detoxification of pollutants and protecting plants from oxidative damage in contaminated soils (Seth et al. 2012). Previous studies suggested that several microbial enzyme systems have been successfully engineered in plant tissue which enable phytoremediation of xenobiotic compounds (Katerova and Miteva 2010; Kotoky et al. 2018). Those transgenic plants were observed to tolerate and biodegrade organophosphate pesticides and chlorinated phenols by exhibiting increased activity of glutathione-S-transferase (GST) and P450-dependent monooxygenase.

Plant–microbe association not only triggers the antioxidant enzyme defence system but also responsible for the exudating several phytohormones (auxins) or phytostimulating chemicals (siderophore). Numerous PGPR release auxins (IAA) as their major metabolites like ethylene, cytokinin, indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and methylthiozeatin. Inoculation of plant roots or seeds with auxin-producing bacteria leads to the development of profuse root system (Martínez-Morales et al. 2003; Spaepen et al. 2007). Several bacterial genera such as *Arthrobacter*, *Azotobacter*, *Bacillus*, *Flavobacter*, *Pseudomonas* and *Rhizobium* are involved in producing significant amount of phytohormones when associated with plant roots (Calvo et al. 2014; Sengupta et al. 2015). Among several phytostimulants, siderophores are the most important compounds which are of low molecular weight, mainly chelate iron and transfer it into the cell. Iron is an essential microelement used by bacteria as well as plants and it is an obligatory requirement for their metabolism. However, iron is unavailable in the soil for direct assimilation by plant or microbes (Dhungana and Crumbliss 2001). Microbial siderophores are broadly classified into two groups, one is hydroxamate and other catechol types. Both types of siderophores bind with ferric ion ($Fe^{3+}$) in the rhizosphere and competently control the invasion of fungal pathogens. Suppressions of the pathogens are possible due to iron scarcity and subsequent growth inhibition with reduced DNA synthesis, changes in cell morphology and retarded sporulation. Other than these, several exudates of PGPR help to promote plant growth by releasing phytostimulants (e.g. phytohormones, organic acids, siderophores), fixing atmospheric nitrogen and solubilizing inorganic phosphate (Ahemad and Kibret 2014). Additionally, they carry out bioremediation by detoxifying pollutants like petroleum, heavy metals and pesticides. The association of plant-PGPR could be further amended for biofertilizer use with the optimization of soil conditions like pH, salinity and water activity.

## 8.4 Plant–Microbe Interactions in Pesticide Pollution

Past few decades of extensive applications of chemical fertilizers, pesticides, nematicides, fungicides and herbicides into agricultural lands not only affected the soil salinity but also had a negative impact on beneficial rhizospheric microbial community (Oberai and Khanna 2018). The ecological stability of the soil microorganisms has been compromised which mostly affected the rhizospheric competence for beneficial plant interacting bacteria. It has been observed by researchers that microorganism isolated from pesticide-contaminated soil showed pesticide-degrading or tolerance properties (Roy et al. 2020). Such strains are able to acclimatize in the micro-niches of pesticide-impacted soil and exhibit their plant growth-promoting activities and antagonistic effect to plant pathogens. On the other hand, application of exogenic PGPR into the pesticide polluted soil has been failed due to lack of pesticide degradation or tolerance abilities of such PGPR as a consequence of their reduced survivability in toxic microenvironment (Sengupta et al. 2015). As per the survey of literature, many rhizospheric bacteria were experimented either as ex-situ in small pots or in-situ in agrofields. This suggested the ability of bacteria with dual ability of pesticide degradation and plant growth promotion useful for pesticide-resistant plants. Major bacterial genera in such applications were recognized as *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Rhizobium*, *Pseudomonas* and *Xanthomonas* (Wu et al. 2006; Sheng and Xia 2006; Ahemad and Khan 2011). Application of such PGPRs into a pesticide polluted site increases the potential of plants that grow there to hold heavy metals and to recycle nutrients, sustain soil fertility, detoxify pesticidal chemicals and control plant pathogens.

PGPR also reduces the toxicity of heavy metals by altering their bioavailability to plants (Ahemad and Khan 2011). In exchange, plants supply the microorganisms with free amino acids, vitamins, polysaccharides, proteins and hormones which are vital sources of their nutrition. Thus, the rhizosphere acts as reservoir of such nutrients secreted from plant roots and attract beneficial bacteria (Babalola 2010). A special prominence of several species of endospore-forming *Bacillus* genus which subsist in stress environments (Sengupta et al. 2015), thus PGP properties of *Bacillus* strains isolated from pesticide-contaminated soil is of additional advantage in using it in contaminated agricultural soil for its bioremediation. Reports available in last 10 years indicated free-living PGP bacteria playing pivotal role in plant morphogenesis processes such as root elongation and lateral root formation that is directly linked with increased plant growth (Ranjitha 2015). Auxins synthesized by free-living rhizosphere bacteria regulate stimulation of root system development and plant growth promotion. Phosphate solubilization, siderophore activity and ammonia production are important activity to promote the plant growth in various ways (Ahemad and Kibret 2014). Biodegradation active rhizobacterial population are also highly capable of colonizing the rhizosphere like other PGPR community (Oberai and Khanna 2018). These microorganisms sometimes show specific migration or positive chemotactic movements towards the root and they consecutively spread during emergence and proliferative growth of plant roots. Since pesticide pollution generally alters soil pH, salinity, moisture contents, metal precipitations and nutrient bioavailability which not only determine the composition of rhizobacterial community but also their longer survival in toxic niches. Moreover, composition of rhizobacterial community and their ecological interactions with the stressful micro-environment actually governs their successful colonization on plant roots.

## 8.5 Plant Root-Associated Microbes in Heavy Metal Polluted Soil

Several agroecosystems have been reported to be highly contaminated with heavy metals along with toxic pesticides (or hydrolytic intermediates of pesticides, herbicides, fungicides, etc.) which prevent the beneficial plant-bacteria interactions (Kotoky et al. 2018; Roy et al. 2020). As discussed in previous section, due to continuous practice of agrochemicals usages, the salinity and infertility of the soils have increased. All these have a long-term negative impact on the functional community structure of PGPR and constantly affecting crop yield (Ahemad and Khan 2011). The haphazard discharge of heavy metals into the soil and waters has long-term effects on the plant health as they cannot be broken down to non-toxic forms except some cases of microbial detoxification. However, some metals are vital for plant growth while on the other hand some exhibit toxicity even at very low concentrations such as As, Cd, Cr, Cu, Pb, Hg, Ni and Zn. It has been observed that plants have assorted ability in taking and accumulating metals in various parts (Guo et al. 2019). Plants absorb heavy metals from the subsurface of the soil with their roots. Therefore, there is a necessity to look for novel consortium of PGPR which not only promotes the plant growth but also withstands stresses imposed by heavy metal remains in the soil for longer period. Remarkably, most bacteria augmented in the metal-impacted rhizosphere belonged to the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria*. Investigators have repetitively verified that *Proteobacteria* may be the greatest metal-tolerant microorganisms present in metal-contaminated soils (Sheik et al. 2012). Their metabolic and ecological functions have been described as tolerable to the extreme toxic environment and to detoxify heavy metals (Li et al. 2015). For example, *Acidobacteria* and *Bacteroidetes* are degraders of organic compounds in nutrient-deficient environments (Rawat et al. 2012). Sengupta et al. (2015) explained that a *Bacillus* strain isolated from pesticide-contaminated soil was halotolerant and showed tolerance to four typical heavy metals (As, Cd, Cr and Pb) that has been reported to be incorporated into the soil as co-contaminant along with pesticides and herbicides. Several root-associated *Rhizobiales* group of bacteria which symbiotically associate with plant root to fix nitrogen are able to detoxify arsenic and help in reducing its toxicity within the rhizosphere (Watson et al. 2017). Some of the root symbiont PGPR such as *Bradyrhizobium* and *Rhizobium* were studied extensively for their application in heavy metal-polluted fields due to their ability to produce ACC deaminase and IAA, nitrogen fixation along with heavy metal tolerant property. However, many free-living PGPRs such as *Azomonas*, *Azotobacter*, *Bacillus*, *Brevibacillus*, *Pseudomonas*, *Ralstonia*, *Variovorax* and *Xanthomonas* were able to show up ACC deaminase, IAA, siderophore, phosphate solubilization, nitrogen fixation along with Cd, Cr, Ni, Pb and Zn tolerance (Table 8.2).

Certain bioengineered plant root associative rhizobacteria were used to remediate the heavy metal contamination of rhizosphere (Wu et al. 2006; Dixit et al. 2015). Wu et al. (2006) have cloned and successfully expressed a resistant plasmid in *Pseudomonas putida* strain and demonstrated the capability of the strain to improve plant root health in high level of cadmium polluted soils. These microorganisms of rhizomicrobiome are able to detoxify heavy metals in soil and show adaptability in the toxic environment and plant health promotion.

## 8.6 Rhizospheric Interactions in Resistant Plants of Petroleum-Impacted Soil

Soil polluted with petroleum, diesel and its derived products is seen to be less fertile in comparison with garden soil or other vegetative soil. Generally, toxicity of petroleum-derived products towards vegetations includes inhibition of seed germination, photosynthesis and other plant growth factors (Gkorezis et al. 2016). Mostly, vegetated soil contains high concentration of organic compounds like flavonoids, terpenes and lignin-derived compounds which are structurally similar to petroleum-derived PAH or PHC. Such kinds of “rhizodeposits” often induce and attract PAH-degrading microorganisms followed by their colonization on plant roots. Once plant–microbe association is established, it helps to improve plant tolerance towards PAHs or PHCs (Lu et al. 2011). For example, Toyama et al. (2011) have

**Table 8.2** List of some bioinformatics tools used to study factors associated with plant–microbe interaction and relevant multi-omics analyses

| Sl. No. | Database/software/online tool | Applications | Website address | Reference |
|---|---|---|---|---|
| 1 | PIFAR (Plant-bacteria Interaction FActors Resource) | Database maintains comprehensive information on gene products in plant-bacterial interactions and helps researchers to identify these products in input genome sequences | http://bacterial–virulence–factors.cbgp.upm.es/PIFAR | Martínez-García et al. (2016) |
| 2. | Plant-Associated Microbe Genomics Initiatives | Online platform for analysis and storage of data for the genomes of plant-associated microbes | https://www.apsnet.org/edcenter/apsnetfeatures/Pages/MicrobeGenomics.aspx | The American Phytopathological Society (APS, USA) |
| 3 | MG-RAST | Processing, analysing, sharing and disseminating metagenomic datasets | http://metagenomics.anl.gov/ | Keegan et al. (2016) |
| 4 | MOTHUR | Data trimming and filtering, diversity analysis, and visualization | http://www.mothur.org/ | Schloss et al. (2009) |
| 5 | MetaQuast | Quality assessment tool for metagenome assemblies | http://bioinf.spbau.ru/metaquast | Mikheenko et al. (2016) |
| 6 | QIIME | Data trimming and filtering, diversity analysis, and visualization | http://qiime.org/ | Kuczynski et al. (2011) |
| 7 | MetagenomeSeq | Analysis of differential abundance of 16S rRNA gene in metaprofiling data. | http://bioconductor.org/packages/release/bioc/html/metagenomeSeq.html | Paulson et al. (2013) |
| 8 | IMG/M v.5.0: Integrated Microbial Genomes and microbiomes | Functional annotation, phylogenetic distribution of genes and comparative metagenomics analysis | https://img.jgi.doe.gov/cgi-bin/m/main.cgi | Chen et al. (2019) |
| 9 | MetaBAT | Metagenome sequence binning | https://bitbucket.org/berkeleylab/metabat | Kang et al. (2015) |
| 10 | deFUME | Processing, annotation and visualization of functional metagenomics sequencing data | https://github.com/EvdH0/deFUME | Van Der Helm et al. (2015) |

(continued)

**Table 8.2** (continued)

| Sl. No. | Database/software/online tool | Applications | Website address | Reference |
|---|---|---|---|---|
| 11 | DADA2 R package | Analysis and visualization of metagenome sequencing data | https://benjjneb.github.io/dada2/index.html | Callahan et al. (2016) |
| 12 | SAMSA2 | Annotation of metatranscriptome sequence reads, a stand-alone metatranscriptome analysis pipeline | https://github.com/transcript/samsa2 | Westreich et al. (2018) |
| 13 | MetaTrans: | Open-source pipeline for metatranscriptomics analysis | http://www.metatrans.org/ | Martinez et al. (2016) |
| 14 | MOSCA | Automated pipeline for integrated metagenomics and metatranscriptomics data analysis | https://github.com/iquasere/MOSCA | Sequeira et al. (2019) |
| 15 | MetaGOmics | Metaproteome analysis tool | https://www.yeastrc.org/metagomics | Riffle et al. (2017) |
| 16 | MetaLab | Integrated data-processing pipeline for metaproteomics | https://imetalab.ca/ | Cheng et al. (2017) |
| 17 | MetaProteomeAnalyzer | Software for metaproteomics data analysis and interpretation | https://code.google.com/p/meta-proteome-analyzer | Muth et al. (2015) |
| 18 | MetaboAnalyst | Web-based analytical pipeline for high-throughput metabolomics studies | https://www.metaboanalyst.ca/ | Chong et al. (2019) |

shown the direct relationship of increased phenolic compounds present in rhizodeposits with high rate of benzopyrene degradation in rhizosphere of common reed species (*Phragmites australis*). This result was further strengthened by the PHC degradation pattern among rhizospheric bacteria, observed by Phillips et al. (2012). The study described that acetate or alanine helps the plant–microbe association and thus enhanced the degradation process while saccharides like glucose, sucrose, trehalose, xylose, mannose reduced the degradation capacity of rhizospheric bacteria. This result suggested that simple sugars do not facilitate the growth of PAH or PHC-mineralizing microorganism by omitting the selective pressure. On the other hand, phenolic compounds suppress the growth of other bacteria which thrive only on simple sugars and create a selective environment for the growth of degrading microorganisms. Another example of such enhanced biodegradation study by suitable plant–microbe interactions was performed by Lu et al. (2011). It has been reported that the associations of bacterial strains of genera *Flavobacterium*, *Pseudomonas* and *Rhodococcus* with two common plants, Alfalfa and Indian mustard, effectively carried out the removal of diesel oil from polluted soil. The maximum oil reduction was observed nearly up to 70% by specific plant-bacteria association. Major enzymatic activities such as catalase and polyphenol oxidase activities were increased in soil and consecutively enhanced microbial populations in the rhizosphere which ultimately also stimulated the important bacteria-plant association. Several bacterial genera have been stated to be involved in petroleum degradation metabolic pathways such as *Acinetobacter*, *Haemophilus*, *Pseudomonas*, *Paenibacillus*, *Mycobacterium*, *Rhodococcus*, *Ralstonia* and *Stenotrophomonas* (Tyagi et al. 2011). Although the presence of catabolic genes in such bacteria is responsible for degradation process, the role of plant is also significant in the process. It has been reported already that microbial activity in rhizospheric soil is ten to thousand times better in comparison with bulk soil. Also, efficient microbial reduction of hydrocarbon is lower in absence of plants (Gaskin and Bentham 2010). The role of plant can also be defined by the morphology and physiochemical properties of root zone. For example, plant with shallow root system may not encounter the polluted microenvironment. The primary functional retort of plants to PAH or PHC in soil depends on concentration and chemical nature of pollutants such as its solubility, volatility and polarity.

## 8.7 Tripartite Interaction of Plant-Bacteria-Fungi

A three-partner relationship of plant, bacteria and fungi has been proven as the most effective in removing toxic pollutants from rhizosphere (Jambon et al. 2018). One such primary study by Nie et al. (2011) has shown that overall soil nutrients and microbial activity were enhanced by addition of AM fungi into petroleum-polluted soils. The colonization of AM fungi helped in increased absorption of nutrients and dissolved organic nitrogen by root system of plants. It has also been observed that AM association change the ecophysiology of pollutant consuming bacteria with increased potentiality of biodegradation. Additionally, greater abundance of bacterial catabolic genes of petroleum degradation pathways such as alkane monooxygenase, xylene monooxygenase and naphthalene dioxygenase has been found in tripartite association of plant root, bacteria and AM (Nie et al. 2011). Moreover, rhizospheric fungi have a tendency to spread easily through their penetrating hyphae into soil matrix and facilitate the grasp of degrading bacteria towards pollutants. Also, non-motile PGPR can enter into root tissue of the symbiotic plant by adhering to the fungal hyphae (Minerdi et al. 2002; Jambon et al. 2018).

Among all plant-associated fungi, mycorrhizae are able to colonize root with their profound mycelial structure. Mainly, they are classified as two groups, one is ectomycorrhizae and other is endomycorrhizae. One of the special characters of ectomycorrhizae is to develop a Hartig net which is composed of intercellular hyphae on the surface of roots. Some of the major genera of ectomycorrhizae are *Ascomycota*, *Basidiomycota* and *Zygomycota* which are found to be associated with 3% of vascular plants (Barman et al. 2016). Another group, Endomycorrhizae, can penetrate and cause invasive intracellular association with higher plants. They are subgrouped into arbuscular or ericoid, among which arbuscular mycorrhizae associations are well known. For example, *Glomeromycota*, the most abundant phylum of arbuscular mycorrhizae associates with 80% of total vascular plant species (Barman et al. 2016). One of the advantages of AM fungi implication in phytoremediation is that they are having finely branched hyphal system (arbuscules) which also improve the plant nutrient absorbing capacity in pollutant-impacted soils. In the association of endosymbiont *Glomeromycota*, bacterial population is more inside the spores (Minerdi et al. 2002). Such bacterial associations have been reported with AM fungi such as *Glomeribacter* (obligatory endosymbionts) and *Burkholderia* (non-obligatory endosymbiotic) of AM fungus *Gigaspora* (Minerdi et al. 2002). Some strains of genus *Pseudomonas* have been co-inoculated with mycorrhiza and found to associate with stimulated mycelial growth of fungi in the rhizosphere along with increased level of auxin production by bacteria (Navarro-Ródenas et al. 2016). These rhizospheric bacteria are known as "mycorrhiza helper bacteria" which encourage the colonization of mycorrhiza to root system. Another association of AM fungi and leguminous rhizobacteria has been involved in enhanced bacterial $N_2$ fixation and phosphate solubilization into the rhizosphere (Nadeem et al. 2014). Application of plant-fungi-bacteria associations in phytoremediation of petroleum products has been observed by introducing consortium of bacterial species of *Rhodococcus* and fungus *Aspergillus*/*Penicillium* which helped in removing anthracene, pyrene and phenanthrene in toxified soil (Kim and Lee 2007). Bioremediation of petroleum hydrocarbons has been performed by co-inoculating *Vibrio* sp. (*Proteobacteria*) and *Cunninghamella* (Mucor) into polluted mangrove soil sediments (Li and Li 2011). Diesel removal has also been implemented with introducing mixed microbial cultures of *Trametes* (*Basidiomycota*) and bacterial genera such as *Acinetobacter*, *Alcaligenes*, *Chryseobacterium*, *Gordonia*, *Pseudomonas*, *Serratia* and *Stenotrophomonas* (Zanaroli et al. 2010). This mixed consortium reduced 90% of diesel within 10 days of incubation period which was not achieved by either fungi or bacteria alone. Besides this, AM fungi are reported as good partners with plant and bacteria for positive interactions and thus this tripartite relationship should be more explored specially in polluted agroecosystems.

## 8.8 Beneficial Soil Protozoa

Other than plant-associated bacteria and fungi, soil protozoa have a great influence on plant health, inhibiting root pathogens and decomposing cycle of soil. Soil protozoa also determine the functioning of other plant–microbe interactions (Weidner et al. 2017). For instance, Weidner et al. (2017) showed a plant growth promotion of a wheat variety by co-inoculation of amoeba *Acanthamoeba* with beneficial *Pseudomonas* into the rhizosphere and found to inhibit the growth of a plant pathogen (*Pythium ultimum*). Protozoa play an important role in maintaining the ecophysiology of soil by grazing upon microfauna and thus also regulate the growth of other rhizobacteria. Since, plant exudate supports the growth of huge rhizobacterial population, resulting in increase of biomass in the soil. Hence, protozoa have been assigned as grazers which consume such large biomass and release nutrients from the consumed microfauna which is termed as “microbial loop” (Bonkowski 2004). Some protists acting as biological controlling agent show antagonistic effect against plant pathogenic bacteria. Protozoan ingestion of bacterial genera mainly *Escherichia*, *Enterobacter*, *Bacillus* and *Micrococcus* helps in maintaining soil equilibrium. However, predation of bacteria by protozoa affects the biodegradation of soil pollutants by reducing the microbial population which mineralize the pollutant and decrease the rate of its removal from the soil. So, complete dynamics of protozoan relationships with other rhizospheric microorganisms are complicated and are not fully understood yet. Several factors such as initial cell density of degrading microorganism, prey-predator relationship, size and shape of the cell and soil properties of the microenvironment would be important for studying the effect of protozoan predation on biodegradation rates.

## 8.9 Microcosm Studies

Microcosm studies were performed to check the effectiveness of co-inoculation of PGPR strains and AM fungi along with plant seeds, based on whether the plant growth promotion and decontamination of pollutants occur simultaneously or not. Previously, Gurska et al. (2009) carried out a three-year field study where PGPR inoculation had provided dual effects, reduction of petroleum hydrocarbon as well as enhanced plant growth. Another important study by Asghar et al. (2017) showed the application of ACC deaminase-producing *Bacillus subtilis* strain to increase the root-shoot length and reduction of petroleum hydrocarbon up to 43% within 60 days of inoculation with *Zea mays* growing in crude oil-contaminated soil. Taghavi et al. (2005) showed that inoculation of two *Burkholderia* sp. strains with plasmid-harbouring toluene-degrading genes improved plant health and enhanced toluene biodegradation simultaneously. Some successful inoculation of PGPR strains of

*Pseudomonas* with a plasmid encoding hydrocarbon catabolic genes was monitored for increased degradation process. Several pot experiments showed higher abundance of PAH-degrading genes in phenanthrene-contaminated soil with plantation of ryegrass (genus *Lolium*) as compared to control (non-planted soil). The plants stimulated the bacterial degradation with high expression of PAH ring cleavage dioxygenase genes (Guo et al. 2017). This plant stimulated the biodegradation activities of *Actinobacteria* and members of *Caulobacterales*, *Pseudomonadales* and *Xhantomonadales*. Lumactud et al. (2016) observed that root-associated *Actinobacteria* with hydrocarbon-degrading genes were responsible for plant resistance of hop clover (*Trifolium*), yarrow (*Achillea millefolium*), orchard grass (*Dactylis*) and Canadian goldenrod (*Solidago*) in heavily polluted soil. Other root-associated alkane degraders such as *Rhodococcus*, *Rhizobium* and *Stenotrophomonas* were capable of utilizing n-hexadecane when inoculated with bird's-foot trefoil (*Lotus corniculatus*) and evening primrose (*Oenothera biennis*) in petroleum-impacted soil (Pawlik et al. 2017). Pot experiments with AM fungi were also performed where mycorrhiza *Glomus mosseae* was detected as degrader of PAH in polluted soil (Joner et al. 2001). Therefore, plant-fungus association has been proven as the most efficient interaction to protect plants from various stress-induced damages in polluted habitats.

## 8.10 Multi-Omics Approaches in Rhizomicrobiome Interaction Studies

The microbiome of rhizosphere was greatly understudied till several multi-omics methods have been implemented to explore the majority of microbial community (Kotoky et al. 2018). Study of complex microbiome of rhizosphere is challenging due to multiple molecular responses at genes, proteins or metabolites level which could be easier with implication of meta-omics techniques. Applications of high-throughput sequencing data integrated with advanced bioinformatical analyses have been implemented to understand the inter-connected responses among different microbial population and plants in polluted rhizosphere. For these applications, different meta-omics approaches such as metagenomics, metatranscriptomics, metaproteomics and metabolomics have been proven promising to decipher the functional microbial diversity and their possible molecular response mechanisms (Castro et al. 2013). Functional metagenomics analyses have described novel metabolic pathways for biodegradation of PAH or aromatic compounds by recognizing different groups of catabolic genes such as dioxygenases from different bacterial genera. In this way, study of rhizospheric soil microbial diversity represents the occurrence of functional genes and allows to integrate microbial phylogeny with the functional diversity of microorganisms. Also, huge data set of metagenomes provides the insights into important plant–microbe interactions involved in rhizoremediation of PAH or other pollutants (Kotoky et al. 2018). Thus, metagenomics not only provides the information regarding unculturable microbial diversity of rhizosphere but also offers direct access to the genetic content of rhizomicrobiome and functional potential of entire community of a particular habitat (Martín et al. 2006). It has been evident from several metagenome sequencing that uncultivable microorganisms are commonly responsible for pollutant removal in any polluted site. Another meta-omics technique, metatranscriptomics, is useful to track the genetic expression level of contaminated rhizomicrobiome and helps to investigate the change in microbiome functional gene expression profile in presence of contaminants. Some of the studies related to transcript profiling have been helpful to decipher the metabolic responses such as Kantar et al. (2011) showed the regulatory microRNA (miRNA) involved in superoxide dismutase activity and its role in abiotic stress response while Lima et al. (2011) found miRNA responsible for aluminium stress response in plants. Yergeau et al. (2014) investigated the transcript profiles of petroleum-contaminated rhizosphere of *Salix* sp. (willow) which was enriched with PAH-degrading genes. Another powerful tool is metaproteomics which has been used to check the response related to protein expression profile and protein-protein interactions in plant–microbe relations (Meena et al. 2017). Metaproteome analysis deals with the total protein profiling present in given environmental sample and thus directly reflects the phenotypic traits and physiological metabolic network of microorganisms dwelling in that microenvironment. Proteomic analyses to understand stress responses in crops have been executed in several common plant species such as *Hordeum* (barley), *Lycopersicon* (tomato), *Oryza* (rice), *Phaseolus* (bean), *Pisum* (Pea), *Solanum* (potato), *Triticum* (wheat) and *Zea* (maize) (Liu et al. 2015). In recent years, several databases and bioinformatics tools are available for analysis of meta-omics data to decipher plant–microbe interactions. Such multi-omics investigations are helpful in providing a deep insight into the regulatory systems of plant–microbe associations by recognizing numerous signal proteins responsible for stress response mechanisms (Meena et al. 2017; Kotoky et al. 2018). Therefore, a comparative metatranscriptomics or metaproteomics analyses of plant-associated microbe in stressed versus non-stressed condition could be helpful to identify protein targets and metabolic networks.

## 8.11 Conclusions

This chapter has been focused mainly on the rhizomicrobiome potential to improve pollutant resistance capacity and growth of plants. Several plant–microbe associations involved in antioxidant defence system and release of phytostimulating compounds in contaminated rhizosphere have been discussed here with examples of relevant experiments. Summarization of all relevant findings would provide an insight into rhizoremediation mechanism and better understanding for further rhizosphere genetic engineering. A very few reports are available which directly relate the synergistic biodegradation or detoxification system of plant–microbe relation in polluted soil with heavy metals, petroleum and pesticides. The plant-bacteria-fungi, a tripartite association, has a key role in rhizoremediation which has been studied through several microcosm studies but yet to be explored at molecular and gene expression level. This chapter also highlights updated information regarding current multi-omics techniques and bioinformatics tools which have been recently implemented to find novel metabolic pathways in rhizospheres.

## 8.12 Future Aspects

Future research in rhizosphere biology would be based on the progress of biotechnological methods to intensify our knowledge of rhizosphere ecology. The combination of rhizospheric microorganisms having potential of plant growth promotion with capacity to withstand stresses imposed by heavy metals and toxic xenobiotics could be useful to build smart biofertilizers. This futuristic biofertilizer would be in great demand for sustainable agriculture of next generations. It has been proved now that plant–microbe associations are beneficial for rhizoremediation of pollutants and should be considered for application in contaminated soil to enhance plant resistance capability. A deeper understanding of such complicated plant–microbe ecology requires microcosm studies entwined with meta-omics (metagenomics, metatranscriptomics and metaproteomics) approaches. Other multi-omics techniques such as metabolomics (cellular metabolites), phenomics (phenotypic changes) and interactomics (protein-protein interactions) could be considered in such application-based studies. However, the success of such bioremediation strategy would be based on the understanding of inter-related multiple biotic and abiotic factors.

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# Role of Antioxidant in Plant- and Microbe-Based Remediation of Metal Stress

# 9

Arun Pratap Singh, Balendu Shekher Giri, Anuradha Singh, and Preeti Chaturvedi

## Abstract

Heavy metals (HMs) are toxic, non-biodegradable elements, which causes oxidative stress in plant and microbes. Oxidative stress generates reactive oxygen species (ROS) that damage the cells of plants and microbes. Plant and microbes evolved a biological mechanism to protect themselves from reactive oxygen species. Antioxidants are the molecules that neutralize the effect of reactive oxygen species (ROS). Antioxidant defense system contains enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx), ascorbate peroxidase (APx), etc. Glutathione (GSH), ascorbic acid (AsA), phenolic acid, thiols, proline, etc., are non-enzymatic antioxidants. Bioremediation of heavy metals through phytoremediation and/or microbial remediation is eco-friendly approaches. Phytoremediation refers to the technique in which the use of the plant to remediate the contaminant from the contaminated sites. Microbial remediation involves the microorganisms to remediates the pollutants from the environment. Antioxidants play an important role in tolerance against heavy metal stress and provide the potential to plant and microbe to bio-remediates heavy metals. It this chapter, we explain the role of antioxidants in the remediation of heavy metals through phytoremediation or microbial remediation.

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**Keywords**

Antioxidants · Reactive oxygen species · Bioremediation · Heavy metals · Phytoremediation

## 9.1 Introduction

The natural and anthropogenic activities contribute to non-biodegradable pollution such as heavy metals pollution that are a major concern for environmental health and safety. Heavy metals (HMs) are those elements characterized by relatively high densities ($>5$ g cm$^{-3}$) and are toxic to living beings at low concentrations (Alaraidh et al. 2018). Anthropological activities or natural processes such as mining, pesticides, metal industries, mineral fertilizers, and others caused heavy metal pollution in the environment in the present time (Bhadur and Fulekar 2012). HMs are non-biodegradable pollutants, accumulated in tissues cause deleterious effects on living beings through a different mechanism. Due to heavy metal toxicity, oxidative stress generates ROS like $H_2O_2$, $OH^-$, singlet oxygen ($^1O_2$), superoxide radical ($O^{2-}$), in plant cells (Rajkumar et al. 2012). HMs induce oxidative damage in plants, develop ROS which alters enzymatic activity, DNA damage, membrane permeability, respiratory and photosynthesis processes induce plant senescence, and leakage of ions (Quartacci et al. 2001; Monferran et al. 2009). Various bioremediation technologies such as phytoremediation, mycoremediation, and microbial remediation are applied to deal with heavy metals pollution. In a biological system, antioxidant defense systems are present to neutralize the effect of reactive oxygen species caused by oxidative stress. Antioxidant provides defense against the toxic effect of heavy metals and other pollutants that cause oxidative stress in a living being. Antioxidant defense systems present in cells can be enzymatic and non-enzymatic, develop against oxidative damage, and are those that prevent ROS occurrence and capture, block, free radicals that are formed in cells (Cheeseman and Slater 1993). The biological antioxidant (present at a lower concentration) refers to any compound that can either prevent or delay the oxidation of the substrate (Halliwell and Gutteridge 2015). The main feature of antioxidants is reversing the effect of free radicals (Prakash et al. 2012). In the environment, physical, chemical, and biological methods are used for the remediation of heavy metals. Bioremediation is involved in the biological mechanism of plant and microorganism to improve environments contaminated with heavy metals, which is a profit-making and eco-friendly method (Ojuederie and Babalola 2017).

Phytoremediation and microbial remediation is an efficient strategy for the removal of environmental pollution as well as sustainable to the environment. Phytoremediation is an alternative method as an environment friendly, profit-making to cope with the kind of pollutants from soil, water, and plant tolerant to pollutants require for this process (Wang et al. 2012). Phytoremediation includes several processes, namely phytostabilization, phytoextraction, and rhizofiltration, and accumulation of toxic compounds by plants (Jasrotia et al. 2017; Sarwar et al. 2017; da Silva et al. 2018). Due to the toxic effect of heavy metals, ROS accumulated in the cell cause disruption of cellular activity. To alleviate their deleterious effects and scavenge reactive oxygen species, plants have developed an enzymatic and non-enzymatic mechanism that protects from oxidative damage (Goswami and Das 2016). Hence, studying the antioxidant defense system in the phyto-accumulator plant may reveal the phytoremediation potential of such a plant. Antioxidants can be categorized into enzymatic antioxidants such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) and non-enzymatic antioxidants including ascorbic acid (AsA) and glutathione (GSH) which prevent cells against $O^{2-}$ and $H_2O_2$ (Halliwell and Gutteringe 2006). In plants, the ascorbate-glutathione pathway consists of the enzymes monodehydroascorbate reductase, ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase, and glutathione and ascorbate is a very efficient system to remove lipid peroxides and hydrogen peroxide ($H_2O_2$) (Foyer and Shigeoka 2011). Microorganisms induced different enzymatic and non-enzymatic antioxidants to alleviate the oxidative stress caused through HMs and lessen the radicals' formations in plant cells under metal stress (Khanna et al. 2018). In wheat plants under Zn stress, *Pseudomonas aeruginosa* modulates the activity of enzymatic antioxidants such as CAT, POD, and SOD which scavenge ROS to prevent from $H_2O_2$ and malondialdehyde (MDA) level (Islam et al. 2014a, b). *Pseudomonas aeruginosa* up-regulated SOD, APX, CAT, and POD levels, whereas *Solanum nigrum* alleviated oxidative stress generated under stress (Shi et al. 2016). The remediation of HMs has been carried out using phytoremediation, mycoremediation, or microbial remediation or a combination of these techniques. Recent findings have reported the use of Genetically modified bacteria for Arsenic remediation (Mateos et al. 2017).

This chapter focuses on the importance of antioxidants in the plants and microbial defense system in the phytoremediation and bioremediation of heavy metals. Antioxidants play a crucial role in scavenging ROS generating during the oxidative stress of toxic compounds. Plant and microorganisms used different biological mechanisms for bioremediation purposes and able to tolerate with the help of an anti-oxidative defense system and accumulate and detoxification of heavy metals.

## 9.2 Classification of Antioxidant and Its Applications

An antioxidant is an enzyme or molecule capable of inhibiting or preventing the oxidation of other molecules (Fig. 9.1). Antioxidants may be able to donate or accepting electron (s) to neutralize free radicals (Lü et al. 2010). Antioxidants can protect the cells against oxidative stress through different mechanisms (Aziz et al. 2019). Antioxidants can be classified into enzymatic peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), etc., and non-enzymatic antioxidants ascorbic acid (AsA), thiols, glutathione (GSH), proline, and carotenoids on the bases of their activity. The production of POD, SOD, CAT,

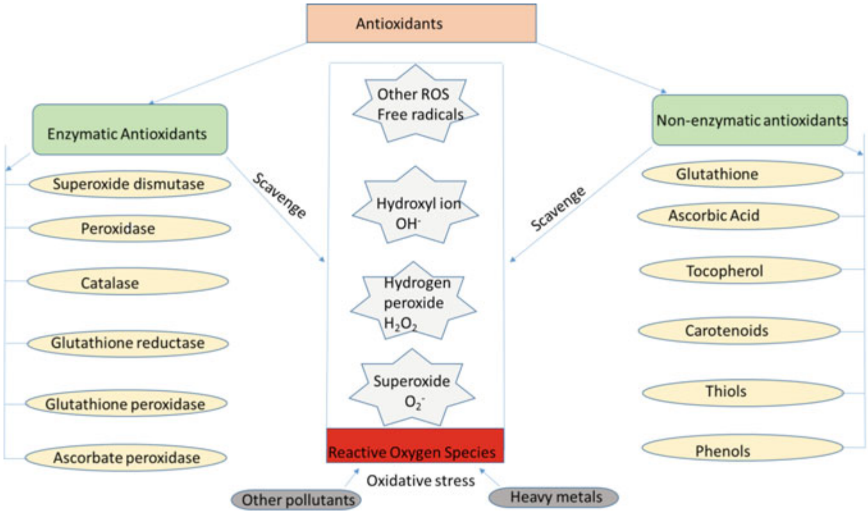

**Fig. 9.1** Schematics diagram show enzymatic antioxidants and non-enzymatic antioxidants and their role in scavenging ROS, generating due to oxidative stress under Heavy metals and other pollutants

AsA, GSH, and carotenoids protects against oxidative stress (Hall 2002; Caregnato et al. 2008). The enzyme antioxidant glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) are an essential unit of the antioxidant defense system, and they are responsible for scavenging free radical (Butnariu and Grozea 2012). Antioxidant enzymes are the crucial substances of the protection mechanisms, preventing ROS via transferring ROS into relatively stable compounds (Pandey and Rizvi 2010). Among non-enzymatic antioxidants tocopherol, glutathione and ascorbate play a major role in the defense mechanism of a plant to prevent oxidative stress (Mittler et al. 2004; Scandalios 2005; Halliwell 2006).

### 9.2.1 Antioxidant Enzymes and Its Applications

The application of antioxidant enzyme is to detoxify the deleterious effect of HM pollution in plants and microbes. Antioxidant provides potential to plant to tolerance against heavy metals toxicity. Antioxidant enzymes play a crucial role to alleviate metal stress during the bioremediation of heavy metals. The tolerance mechanisms of plant for heavy metal toxicity such as to alleviate the ROS effect through antioxidant enzyme can be assessed for phytoremedial potential of the plant (Bhadur and Fulekar 2012). The Plant possesses a complex system of enzymatic antioxidants; antioxidant enzymes are important substances that provide defense against oxidative stress and alleviate the toxic effect of oxidative stress (Bano and Ashfaq 2013). CAT, SOD, POD, APx, and GRx get activated in ROS detoxification (Gratao et al. 2008; Roychoudhury et al. 2012). SOD catalyzes the dismutation of superoxide ion ($O^{2-}$)

into either hydrogen peroxide ($H_2O_2$) or molecular oxygen ($O_2$) (Rusin et al. 2020). Catalase enzyme involved in the reduction of $H_2O_2$ to $H_2O$. Glutathione peroxidase (GPx), using glutathione as an essential cofactor to catalyze the reduction of lipid hydroperoxide, organic hydroperoxides, and $H_2O_2$ to $H_2O$ or corresponding alcohols (Kieliszek and Błażejak 2013; Pisoschi and Pop 2015). SOD-specific activity increases in leaves of *Medicago sativa* plants grown in the presence of sludge (Martí et al. 2009). Five different SOD isoenzymes, such as Mn-SOD, Fe-SOD, and three Cu-, Zn-SODs, were detected in leave extracts of the alfalfa plant (McKersie et al. 1993). The first line of defense toward metal stress is generally SOD enzyme. The breakdown of $H_2O_2$ into $H_2O$ and $O_2$ in plant cell becomes oxidative stress which is protected by catalase enzyme (Chelikani et al. 2004). The POD, CAT, and APX encoding gene showed a significant increase in mRNA expression levels were observed in response to Pb, Cd, and Cr (Alaraidh et al. 2018). Goswami and Das (2016) observed that under Cu stress, SOD activity in root tissues was higher than that of leaves tissues in *C. officinalis.*

### 9.2.2 Non-Enzymatic Antioxidant and Its Applications

Various non-enzymatic antioxidants are involved in ROS-scavenging pathways, and HMs detoxification, produced in plants upon heavy metal exposure, antioxidants like phenolics, and non-protein thiol have a role in Cd detoxification (Mishra et al. 2014). Plant uses non-enzymatic antioxidants like glutathione (reduced form) to scavenge ROS generating during oxidative stress (Noctor and Foyer 1998; Chou et al. 2011). A small amount of glutathione presents in the fully oxidized form (GSSG), and glutathione is normally found in reduced form (GSH) (Pocsi et al. 2004). Reduced GSH (contain cysteine residue) is one of the important thiol compounds that alleviate HMs stress and protect plants (Deng et al. 2010; Sun et al. 2014; Mahawar et al. 2018). Glutathione functions as a non-enzymatic antioxidant by ROS scavenging in cells, as well as a cofactor for various enzymes, such as glutathione reductase, glutathione transferase, and glutathione peroxidase (Sun 2010; Skowyra 2014). Glutathione is a key antioxidant in HMs tolerance. Glutathione is also important for the synthesis of phytochelatins that are important in HMs detoxification (Jozefczak et al. 2012). An increase in proline level can play a crucial role against metal stress, most likely reduced loss due to oxidation and the effect of rise in metabolism (Dash and Panda 2001). *Potamogeton pectinatus L.* and *Potamogeton crispus L.* grown under Pb, Cr, Cu, and Zn stress showed increased non-enzymatic activity of proline and cysteine under Pb and Cr stress (Upadhyay et al. 2014). N-acetylcysteine can alleviate HMs stress and improved the growth of the wheat by coordinated induction of antioxidant defense system (Colak et al. 2019).

## 9.3 Effect of Metal Stress on Living Being

The contamination of heavy metal in soil, water, and air through anthropogenic activities causes harmful effect on a living being. Heavy metals such as cadmium, arsenic, chromium, lead, and mercury cause toxic effects (various disease) in human as well as plants and animals. The non-biodegradable nature of heavy metals is responsible for their poor elimination from tissues (Ayangbenro and Babalola 2017). The best-known indirect effects of heavy metals include elevated levels of ROS such as hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2•^-$), alkoxyl (RO•), hydroxyl radical (•OH), and development of oxidative stress (Colak et al. 2019). The ROS detoxification process in plants through the antioxidant defense system is important for protection against oxidative stress (Apel and Hirt 2004). The degree of toxicity of heavy metal is determined by absorbed dosage as well as the duration of exposure by the organism (Ojuederie and Babalola 2017). In humans, heavy metals like Pb, Hg, and As drastically affect the nervous system and kidney leading to mental disorders along with abdominal cramps, anemia, diarrhea, and headache (Sharma et al. 2014). The excessive exposure to Pb causes lead poisoning/intoxication. The exposure of human beings to the mercury may lead to nervous and renal disorder (Azimi and Moghaddam 2013). Various microbial processes such as enzymatic activity, respiration, and denitrification and hence retard bioremediation processes due to heavy metal toxicity (Zhuang et al. 2007; Sobolev and Begonia 2008). Heavy metals reduce the microbial populations that cause a shift in the structure of microbial communities (Saxena et al. 2019). It affects disrupting the cell membranes, morphology, and microbial growth by altering the nucleic acid (DNA and RNA) structure, metabolism, causing lipid peroxidation and inhibiting enzyme activity, protein denaturation, and cause functional disturbance (Fashola et al. 2016). The HMs toxicity varies in plants, depending on metal concentration, plant species, specific metal involved, the oxidation state of metal, and pH and composition of soil (Nagajyoti et al. 2010). To study the effects of stress on plants, cell membrane stability has been determined. The effect of accumulation of HMs in plant tissue is on growth inhibition and development, which is related to cell division (Kumar and Rai 2007). An accumulation of HMs in soil and aquatic environments can induce adverse toxic effects on plants, such as biomass decrease, growth inhibition, deficiency of nutrient uptake, and photosynthesis disturbance (Gavrilescu 2004; Pavel et al. 2013). In *Brassica napus* decline in seedling growth and seed germination due to the toxic effect of Cadmium (Cd) (Irfan et al. 2014) and enzyme activity inhibit in *Brassica juncea* (Bashir et al. 2015). Lead stress caused disturbed in metabolic function and inhibited plant growth in *Brassica oleracea* (Ashraf et al. 2011; Theriappan et al. 2011).

## 9.4 Role of Antioxidant Under Metal Stress

Heavy metals tolerant plant and microbes which possess antioxidant defense system (ADS) can be used for remediation of heavy metals through phytoremediation and microbial remediation techniques. Non-enzymatic activity of proline and cysteine and antioxidant enzymes (guaiacol peroxidase, superoxide dismutase, and ascorbate peroxidase) increased particularly under lead and chromium stress (Upadhyay et al. 2014). Due to the metal stress, the effect of increased proline level, an increase in plant metabolism and prevent to oxidative damage (Dash and Panda 2001). The antioxidant defenses of *Arabidopsis thaliana* to the Heavy metals altered by subtle change in glutathione (Sobrino-Plata et al. 2014). Plant produced sufficient amount of antioxidants such as phenolics, flavonoids, and polyphenolics, to prevent the oxidative damage (Garhwal 2010). The toxicity of heavy metals causes the formation of ROS thereby decreasing the antioxidant defense systems which protect cells (Ojuederie and Babalola 2017). To remove the oxidative stress produced by ROS, an active antioxidant defense system is found naturally in plants (Skórzyńska-Polit et al. 2010). Uraguchi et al. (2006) observed the increased activity of CAT, SOD, and GRx in *Avena strigose* under Cadmium stress. Enzyme participated in ascorbate-glutathione, and SOD, POD, and parallel to total homoglutathione showed increased activity allowing the plant to tolerate HMs and hydrocarbons stress (Martí et al. 2009). The non-enzymatic antioxidants, such as AsA, GSH, phenolic compounds, carotenoids, and tocopherol, are best known for their important role to chelate/bind HMs and/or scavenge the ROS in plant cells (Maleki et al. 2017). Th antioxidant defense system (ADS) includes enzymatic and non-enzymatic antioxidants prevent the cell from the toxic effect of ROS which caused by oxidative stress.

## 9.5 Role of Antioxidant System in Phytoremediation

The process of phytoremediation involves the use of plant to remediate hazardous materials from environment and applied to remediate contaminants present in water, soil, and air (Yanqun et al. 2005). The detoxification/decontamination processes through plants are commonly known as phytoremediation (Fig. 9.2). Phytoremediation includes several processes, namely phytostabilization, phytoextraction, and rhizofiltration, and accumulation of toxic compounds by plants (Jasrotia et al. 2017; Sarwar et al. 2017). Phytoremediation includes phytoextraction that involves the use of hyperaccumulators plants to detoxification of HMs from contaminated soil by concentrating them in plant tissue (Rajkumar et al. 2012). Phyto-stabilization is a process in which plant should have low mobility of HMs from root to shoots and broad plant root system (Islam et al. 2013). The success of phytoremediation as a means of HMs eradication from the polluted site using plants, depending upon the bioavailability of the metal impurity, the level of contaminated soil, as well as the accumulation of HMs as plant biomass (Tak et al. 2013). *Arabidopsis thaliana* and *Pteris vittata* have been widely utilized in the remediation of Arsenic polluted soil (Huang et al. 2016). The use of microorganisms can

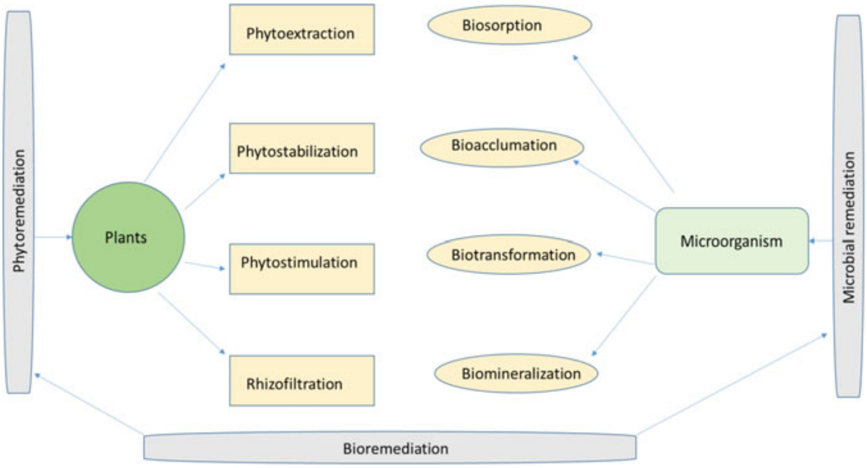

**Fig. 9.2** Bioremediation techniques remove pollutant from environments includes phytoremediation and microbial remediation, phytoremediation involves, phytoextraction, phytostabilization, phytostimulation, and rhizofiltration while microbial remediation through biosorption, bioaccumulation, biotransformation, and biomineralization

stimulate the phytoremediation process to provide tolerance against HMs stress, by altering the level of phytohormone, upregulation of antioxidant enzymes, modulation of protein related to defense, and modulation of metal transporters (Gallego et al. 2012). The application of plants alone or in combination with PGPB is an efficient method for the phytoremediation, prevention, and control of heavy metals (Saxena et al. 2019) (Table 9.1). Microorganism enhances plant survival and HMs stress, and stimulating the activity of reactive oxygen species-scavenging pathways and maintains homeostasis of ROS (Khanna et al. 2018). The tolerance mechanism in plant against oxidative stress induced by antioxidant enzymes that help in reduce the oxidation of molecules and inhibit the process of ROS formation and oxidative chain reaction (Bhadur and Fulekar 2012). Plants have developed an efficient antioxidant defense system by which ROS is scavenged by antioxidant enzymes such as GRx, POD, SOD, and CAT (Joseph and Jini 2010). Lead toxicity caused oxidative damage in plants, and the antioxidant enzymes include GRx, SOD, and POD play an important role in alleviating oxidative stress in plants (Verma and Dubey 2003). The mechanism of the ascorbate-glutathione cycle involved in controlling the cellular oxidation-reduction status especially due to HMs stress (Cuypers et al. 2000; Smeets et al. 2005). Akinyemi et al. (2017) suggested that non-enzymatic antioxidants like phenolic compounds and GSH have a crucial role in the cadmium detoxification process. Glutathione reductase, SOD, CAT, GSH, AsA, tocopherols, alkaloids, etc., have prevented the effect of oxidative damage of ROS (Rastgoo et al. 2011; Singh et al. 2016). To study the role of the antioxidant defense system provides a better understating of optimizing the efficient process of phytoremediation and selection of most appropriate plants.

**Table 9.1** Effect of heavy metals on plant and response of antioxidants under metal stress

| Order | Heavy metal | Plant | Antioxidant | Effects | References |
|---|---|---|---|---|---|
| 1 | Cd | *Zea Mays* | Total phenolic, GSH, and nitric oxide (NO) | Decrease in GSH, NO, and total phenolic, and nitric oxide (NO) levels at the highest concentration | Akinyemi et al. (2017) |
| 2 | Cd, Cr, and Pb | *Trigonella foenum-graecum* | (CAT, POD, and APx) | The high antioxidant capacity of the plant indicates high tolerance to these oxidative stresses | Alaraidh et al. (2018) |
| 3 | Hg | *Abelmoschus esculentus* L. | SOD, AP, GRx, and CAT | Rise the activity of SOD, APx, and GRx but reduce the activity of CAT | Hameed et al. (2011) |
| 4 | Cu, Pb | *Onobrychisvicifolia* | SOD, CAT, and GPx | SOD, CAT, and GPx activities increased in the leaves | Beladi et al. (2011) |
| 5 | Cu, Mn, Pb, and Zn | *Cicer arietinum* | Proline, total phenolic and flavonoid | Proline accumulation increase antioxidative response and indicating induced stress. | Bhagyawant et al. (2019) |
| 6 | Cu, Hg, Cd and Pb, | *Triticum aestivum* | Phenolic acids (PAs), *N*-acetylcysteine | Induction of the phenolic compound and the antioxidant defense system induced in HMs stressed plant | Colak et al. (2019) |
| 7 | As | *Salvinia molesta* | SOD, CAT, POD, APx | Antioxidant defenses system alleviate AsIII toxicity in the floating leaves | da Silva et al. (2018) |
| 8 | Cu | *Calandula officinalis* | SOD,CAT, and GPx. | Increase in antioxidant enzymes reflected stress and mitigate ROS in Cu stressed plant | Goswami and Das (2016) |
| 9 | Cd | *Triticum aestivum* L. | GST, POD, and CAT | Inoculated with rhizobacterial strains activities were upraised in metal stress plants | Hassan et al. (2016) |
| 10 | Cu | *Medicago Lupulina* | SOD, CAT, APx, and GRx | GRx activities as an antioxidant defense mechanism and inoculated plants showed stimulation in SOD, CAT, APx | Kong et al. (2015) |
| 11 | As | *Azolla caroliniana* | Anthocyanin and thiol SOD, POD, CAT | Non-enzymatic antioxidants are main factor responsible for the high as tolerance | Leão et al. (2017) |
| 12 | Cd | *Medicago truncatula* | SOD, CAT | Enhanced antioxidative and ascorbate–glutathione-related metabolism enzymes (APx and MDAR) | Rahoui et al. (2017) |

(continued)

**Table 9.1** (continued)

| Order | Heavy metal | Plant | Antioxidant | Effects | References |
|---|---|---|---|---|---|
| 13 | Oil refinery sludge (HMs) | *Medicago sativa* | SOD, GSH | Antioxidant enzyme and homoglutathione, allowing showed significant activity increases, and provide tolerance to plants | Martí et al. (2009) |
| 14 | Cd and Hg | *Arabidopsis thaliana* | GSH | Glutathione (GSH) may be required for adequate metal tolerance | Sobrino-Plata et al. (2014) |
| 15 | Cu, Cr, Pb, and Zn | *Potamogeton pectinatus* L. and *Potamogeton crispus* L. | SOD, APx, and guaiacol peroxidase proline and cysteine | Antioxidant enzymes activities increased particularly under Cr and Pb stress. | Upadhyay et al. (2014) |
| 16 | Cd | *Solanumnigrum* | Glutathione | Express higher GSH activities in metal stressed plants | Wan et al. (2012) |

## 9.6 Role of Antioxidant System in Microbial Remediation

The microbial remediation of heavy metals depends on microbial interaction with heavy metals and different factor such as microbial community, concentration, and toxicity of heavy metals. Microbes perform the oxidation, reduction, precipitation, and absorption of HMs in the soil (Su 2014). Microbial remediation includes bioaccumulation, biotransformation, biosorption, and biomineralization mechanism employed by microbes involved in the remediation of contaminated sites, and biosorption is the key process of microbes involved in metal sequestration (Ayangbenro and Babalola 2017). In modern technology, microorganisms are used to perform the function of bioremediation to remediate heavy metals. Multi-metal resistance *Paenibacillus* sp. isolated from *Trida xprocumbens* can be utilized as an appropriate candidate for the bioremediation from heavy metals (Govarthanan et al. 2016). Mechanism of tolerance in bacteria, by which they uptake and transform, mobilize and immobilize heavy metals. Bacteria employed the mechanisms are exclusion, physical sequestration, detoxification, and complexation to alleviate the toxicity of HMs (ul Hassan et al. 2017). Microorganism interacts with HMs through extracellular polymeric reactions with transformation, intracellular accumulation, cell wall-associated metals, production of siderophore, immobilization, or mobilization (Ahluwalia and Goyal 2007). The toxic character of hazardous waste influences the survival of microbes in incompatible environments resulting in the reduction of specific microbes in the environment and has led to evolved mechanisms by microbes that prevent to them by HMs contamination (Förstner and Wittmann 2012). Antioxidants enzymes neutralize the ROS and repair damage biomolecules (Poljsak et al. 2010). Superoxide dismutase mainly catalyzes the reaction of superoxide anion to hydrogen peroxide and oxygen. Catalase is responsible for the conversion of hydrogen peroxide to $H_2O$ and $O_2$, thereby alleviate $H_2O_2$-induced oxidative stress (Medvedeva et al. 2017). *Bacillus* sp. improved antioxidant defense system in *Triticum aestivum* under Copper stress through increased activities of APX, POD, dehydroascorbate reductase (DHAR), and SOD by reduction of superoxide radicals and hydrogen peroxide in plants (Wang et al. 2013). The robust antioxidant defense system, based on the redox couples MSH/Mrx-1 and Trx/TrxR, suggests the potential of *Corynebacterium glutamicum* for bioremediation purposes (Mateos et al. 2017). Antioxidant defense system enhanced the tolerance capability of microorganisms against oxidative stress, hence increase in the potential of bioremediation of contaminants such as heavy metals.

## 9.7 Conclusion and Future Prospective

This chapter summarized the role of antioxidant defense system which are scavenging of ROS and reduce the oxidative stress under heavy metal. It also focused on the detoxification or decontamination of heavy metals through phytoremediation and microbial remediation. Heavy metal contamination in the environment and its related toxicity in living beings is a major concern for environment. The toxicity of heavy metal causes oxidative damage of organisms. Organism evolved the antioxidant defense system to protect against oxidative damage. Antioxidant enzyme plays a crucial role during the stress induced by HMs or uptake of HMS in the phytoremediation and microbial remediation process. Antioxidant provides potential to Plant and microorganism to remediate HMs in the environments. Antioxidant plays a crucial role in bioremediation process to alleviate the toxic effect of HMs. Plants possess a best-known antioxidant defense mechanism to reduce and neutralize the free radicals. The defensive biological mechanisms of Plants and microorganisms help to survive under HMs stress and remediate the metals from the environment.

However, future research is based on role of antioxidant and mechanism found in plant and microbes involved in remediation of pollutant in contaminated site. In bioremediation approaches, Detailed study is required at cellular and molecular level for comprehension the role of antioxidant. Characterization of antioxidants incriminate in oxidative stress management will involve in upcoming work.

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# Amelioration of Drought Stress Through PGPR-Mediated Regulation of Antioxidant Defensive Machinery

# 10

Indranil Singh and Shuchi Kaushik

**Abstract**

The world is witnessing a major decline in crop productivity with a simultaneous rise in population and food demands. The global climatic change, irrational use of pesticides, fertilizer, biotic and abiotic stress are equally responsible for the decline of yield. The quest for novel, sustainable, and eco-friendly approaches has been the primary concern. Abiotic stress like drought results in the overproduction of reactive oxygen species (ROS). That, in turn, results in oxidative stress through its toxic and reactive nature towards DNA, lipids, proteins, and carbohydrates. Mitochondria and chloroplast are the sources of ROS generation that could be free radical or non-radical. Over a period of time plant has evolved its own effective antioxidant pathways producing isoenzyme like peroxidase, superoxide dismutase, catalase with scavenging potential for various radical ions. But there are scenarios where a plant's inherent capabilities to combat oxidative stress are just not enough. In the last few decades, microbial interactions with plants have emerged as a tool of great significance. Implementation of plant growth-promoting rhizobacteria (PGPR) showed significant alleviation in crop productivity. Recently, PGPR has also been shown to trigger phytohormone production along with enzymatic and nonenzymatic antioxidant pathways to mitigate ROS and oxidative stress. This review aimed to present recent advances in making the most of PGPR-mediated antioxidant production and its potential in abiotic stress-mediated drought management.

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**Keywords**

PGPR · Drought stress · Antioxidant enzyme · Osmolytes

## 10.1 Introduction

Exposure to different environmental stress is a major cause behind the declining productivity of crops. Every year, a huge fortune of crops is either being lost due to drought, degrading soil quality, salinity, extreme temperature, or pathogenic infections. The pressure generated on agricultural lands by these biotic and abiotic stress is being further added on by the ever-increasing population. In the upcoming three decades, it is expected to reach the mark of nine billion, an approximate 60% increase in food demands (Van Kernebeek et al. 2016; Muller et al. 2017). Increasing demands, loss of land, soil quality degradation, climatic change, and cultivation shift have made farmers depending more and more on chemical fertilizers and pesticides. On the other hand, unrestrained use of pesticides and fertilizers in nonbiological stress situations possesses a huge disadvantage in a long run (Jewell et al. 2010). These include but not, in any case, limited to fouling of ecosystem, the addition of contaminant, toxic pollutants, reduction in yield, and expedition of environmental deterioration. In order to meet the increasing food demands along with the intent to conserve the environmental demands some novel and innovative agriculture practices are required (Majeed et al. 2017).

Recent climatic change is anticipated to increase the severity and frequency of drought, making it major abiotic stress which crops might face in the near future. Depending upon various characteristics like dehydration, soil water content, field capacity, moisture content, holding capacity, water potential, and length of applied stress one can classify drought into the severe, moderate, and mild stage. Though these factors tend to differ with species, and drought being the result of crosstalk between these multiple players that have their own limits, it is hard to find an absolute value of dehydration defining transitions. Recently, relative water content is used to have a broader image of drought severity (Laxa et al. 2019).

Agriculture crops grown in the arid and semiarid regions are expected to see a decline in productivity and growth. Almost half of the arable land could also face critical growth issues by 2050 (Vinocur and Altman 2005). The anticipated mechanisms that undergo in plants include changes in nutrient homeostasis, disturbance of water potential, photosynthetic assimilation, and shift in metabolic processes like nitrate reductase by reducing nitrate uptake (Caravaca et al. 2005; Heffernan 2013). Drought causes a decrease in chlorophyll content in bean (Beinsan et al. 2003), *Carthamus tinctorius* (Siddiqi et al. 2009), *Paulownia imperialis* (Astorga and Melendez 2010). Accentuated levels of phytohormone like ethylene could be seen in some cases while other work also reported a negative effect on subcellular components of cells (Ali et al. 2014; Rahdari et al. 2012).

Drought has been reported to affect the growth through a decrease in fresh weight along with water content in wheat (Rampino et al. 2006), maize (Kamara et al.

2003), barley (Samarah 2005), and rice (Lafitte et al. 2007). Inhibited growth reduces the root length resulting in dampened absorption of water-soluble nutrients like Mg, Si, Ca, and sulfate (Selvakumar et al. 2012). Hence, in order to meet the current demand, effective mitigation of stress aftermath and tolerating the drastic outcomes of drought became a major issue. Different strategies have been adopted for abatement of stresses with the likes of genetic modifications, resources management, shifting of cropping patterns, etc. Though most of them are cost-intensive, they come at huge additional prices in terms of labor, initial setup, and management. At the same time, adaptation of plants to stressful conditions requiring microbial association and advanced technologies promises a cost-effective way to improve crop yield, reach eco-friendly and sustainable agriculture practices (Etesami and Maheswari 2018).

## 10.2 PGPR at a Glance

Rhizosphere harbors millions of microbes forming a complex and rich ecological community around the roots of plants. These kinds of microbe–plant interfaces have been reported to heavily favor plant growth by metabolite-induced activation of different pathways (Qiao et al. 2017). Depending upon mechanism, metabolite, and induced response, the interaction could result in being neutral, deleterious, or favorable in nature (Singh 2018). This symbiotic relationship of microbes and plant is possible because of root secretion system. It provides a source of energy in terms of secretion rich in amino acids, carbohydrates, vitamins, sterols, phenolics, fatty acids, etc. (Uren 2007). From the consortia of microorganisms present in the rhizosphere, rhizobacteria are the ones holding the key position. These rhizobacteria are also referred to as plant growth-promoting rhizobacteria (PGPR). PGPR-mediated benefits come from a wide number of mechanisms that include but not, in any case, limited to the production of phytohormones, siderophores, inhibitory compounds, lytic enzymes, nutrient improvement, nitrogen fixation, resistance to pathogens (Singh et al. 2015; Shameer and Prasad 2018; Gouda et al. 2018; Pérez-de-Luque et al. 2017). PGPR can enhance plant growth by phosphate solubilization (Otieno et al. 2015), mobilization and increase in nutrient uptake (Jacoby et al. 2017), nitrogen fixation (Stokstad 2016), heavy metal remediation (Ma et al. 2016), addition of several growth-promoting substances (Glick 2014), increase resistance to disease with enhancement in immunity (Rahman et al. 2018).

## 10.3 Complex Signaling Network

Recent technological development has made it easier to understand phenomena happening at the molecular level, from the recognition of stimulus to translation into expression and even further down to structural and metabolite change (Sewelam et al. 2016). This understanding lately helped us to know numerous components with the potential to initiate signaling, interconnectivity, and overlap among different

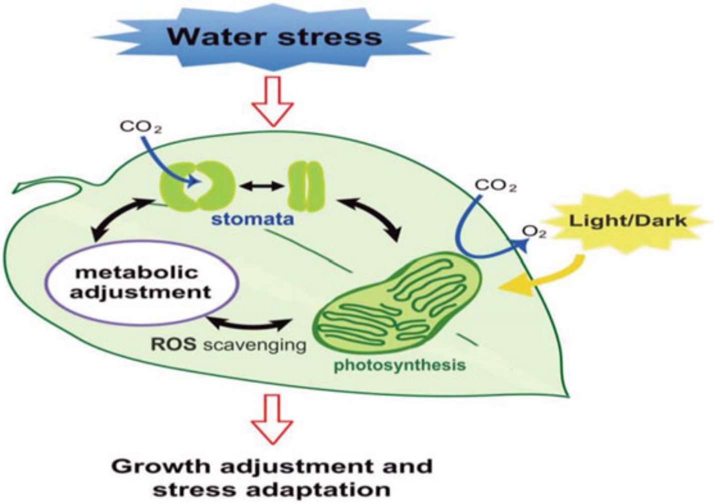

**Fig. 10.1** Illustration of physiological and biochemical adaptations of plant to adjust and adapt to water stress (Adapted from open access mini review on Response of plants to water stress by Osakabe et al. 2014)

signaling pathways, posttranslational and transcriptional modification, changes at the spatiotemporal level, the role of metabolite, and compartmentalization (Krasensky and Jonak 2012). A recent finding has made it evident that the signaling pathway is not linear, rather a complicated set of events with significant overlap as depicted in Fig. 10.1 which indicates that in case of water stress condition the photosynthetic machinery is also affected and generation of reactive oxygen scavengers signals the cells to undergo metabolic adjustments in order to show adaptive response for survival. There are numerous sensors that can perceive primary signal like stress and result in activation of numerous secondary signals like calcium or phytohormone that can interact between themselves to give adequate expression and protection (Knight and Knight 2001; Xiong et al. 2002).

## 10.4 ROS Chemistry

Environmental perturbations result in the activation of different protective mechanisms in plants through appropriate gene expression and activation of the suitable signaling pathway. Generally, the biotic and abiotic stress involves the rapid generation of RNS (reactive nitrogen species) and ROS (reactive oxygen species), abrogating the critical balance between the production and scavenging maintained during normal development and growth. These changes fuel the plant's stress response along with the activation of the enzymatic and nonenzymatic antioxidant systems to counteract the abrupt increase in oxidative species (Gill and Tuteja 2010). During evolution, frequent and persistent interaction with stress resulted in acclimation through alteration in metabolism (Zhang et al. 2014), cell wall (Lü et al. 2013), antioxidant system (He et al. 2017), change in the ratio of shoot–root (Silva et al. 2012), and osmotic adjustment (An et al. 2013). Lately, drought resistance is seen as the function of the plant's antioxidant capacity and is held centrally in the abatement of dehydration and acute drought stress (Laxa et al. 2019).

ROS are produced as a consequence of several metabolic pathways and are kept in check through the antioxidant system. Cellular compartments like mitochondria, chloroplast, and peroxisomes are reported as the localization center for these reactive species. The check and balance system is generally found to be abrogated in response to biotic and abiotic stress. Accumulation of ROS results in oxidative stress with the potential to damage DNA, protein, lipids, carbohydrates besides being lethal to plants. Depending upon localization, equilibrium status, ROS can act as a signaling, protective, or damaging factor. ROS can influence the transcription program through anterograde and retrograde signaling pathways (Gill and Tuteja 2010; Choudhury et al. 2013; Hossain et al. 2015; Kleine and Leister 2016).

Oxygen produced in chloroplast can accept electron while passing through photosystem and result in the generation of reactive species that are capable of independent existence and have an unpaired electron in the outer orbit. These chloroplasts are storehouse of polyunsaturated fatty acids, and photosensitizer favors bioenergetic lifestyle of the photosynthesizing organism. This lifestyle keeps later at a higher probability of having high oxidative damage. Although atmospheric oxygen is comparably nonreactive, it is used in the production of energy and respiratory metabolism, along with the demands of $O_2$ as a final electron receptor makes it more vulnerable for generation and accumulation of ROS. Besides, free radical productions are exaggerated post abiotic and biotic stress that causes excitation of $O_2$ resulting in the generation of singlet oxygen or by transfer of 1, 2, or 3 electrons to $O_2$ to form $O_2^-$, $H_2O_2$, $OH^-$. Mittler (2002) has stated that besides the electron transport chain and photosynthetic process, amine oxidase, photorespiration, peroxidase, NADPH oxidase can also result in the generation of ROS. Detailed descriptions of the above have been already covered in previous reviews (Møller et al. 2007; Baxter et al. 2014; Mittler 2002; Wrzaczek et al. 2013).

## 10.5 ROS Damaging Effect

Plants introduction to abiotic stress like drought, extreme temperature, salinity, nutrient deficiency results in the productions of various ROS. In response to it, plants induce the defense system to counter the deleterious effect of these ROS accumulation through scavenging. However, the defense system can quench only a certain amount of produced ROS, beyond that lies $O_2$ toxicity, the formation of $H_2O_2$, singlet oxygen, $O_2^-$, and OH. (Akter et al. 2015; Miller et al. 2009). These products in turn target the crucial and important players of the cells like mitochondrial DNA, membrane lipids, nucleus DNA, and other high molecular molecules (Tuteja et al. 2001).

Though the genome is considerably stable, the accumulation of different stress has the potential to cause damage resulting in genotoxic stress. Lack of repair mechanism and chromatin organization in the mitochondrial DNA further makes it more susceptible to oxidative damage when compared to the nuclear DNA. The spontaneous or directed nature of this DNA damage is still a matter of debate and needs further evidence to establish its mode of action. Though some literature argued about the hotspot region in the genome for the caused mutation, no considerable data has been reported on particular gene susceptibility or corelation to the ROS-mediated damage, to the best of our knowledge. ROS has been reported to modify the DNA through various ways that include but not, in any case, is limited to direct oxidation of DNA, cytosine methylation resulting in the alteration of gene expression, $^1O_2$ mediated attack on guanine, $OH^·$ mediated damage to pyrimidine and purine along with the backbone of DNA. These damages could further be classified into base modification, for example, alkylation, oxidation, base deletion, single and double-strand break, cross-linking, or dimerization. The further downstream effect includes an error in replication, genomic instability, loss or gain of function, destruction of the cell membrane, reduction in protein synthesis, and dysregulation of photosynthesis pathway leading to arrest of growth are few amongst many (Halliwell and Gutteridge 1999; Wiseman and Halliwell 1996; Tuteja et al. 2001; Britt 1999; Cooke et al. 2003).

Protein oxidation is another very common effect that is observed in the case of ROS accumulation. It is generally seen as the covalent modification in side chains of amino acids either due to ROS accumulation or through other indirect byproducts produced in the process of oxidative stress. Though this protein carbonylation was generally considered to be irreversible with exception of few amino acids containing sulfur. The oxidation of amino acid results in the release of the carbonyl group that in turn makes them susceptible to proteolytic attacks. Carbonylation has been suggested to be the result of many stresses. This modification could see the extraction of H atom from cysteine, hence forming a thiyl radical that in turn dimerizes with another thiyl radical through disulfide bridges; additionally, methionine sulfide production is also seen as the addition of oxygen on methionine residue, or side-chain modification in lysine and threonine resulting into aminoadipic semialdehyde or aminoketobutyrate (Ghezzi and Bonetto 2003; Job et al. 2005; Shringarpure and Davies 2002; Hancock et al. 2006; Sadanandom et al. 2000).

Polyunsaturated fatty acids (PUFAs), a major constituent of fatty acids that are present in the plant membrane, are also vulnerable to the accumulation of ROS. PUFAs generally undergo peroxidation in the presence of ROS leading to the formation of lipid hydroperoxides. As a result, membrane damage could be seen along with the damage to protein, leakiness, and reduction in the fluidity of the membrane (Mueller 2004). This does not only affect the normal cellular process but it in turn exaggerates the ROS accumulation through the production of lipid-derived radicals (Montillet et al. 2005; Møller et al. 2007).

## 10.6 ROS and RNS in Drought

Drought stress response generally begins with closure of stomata to regulate loss of water. But the continual photosynthesis depletes the intracellular stock of $CO_2$, which in turn stimulates RuBP (ribulose-1,5-biphosphate) mediated oxygenation resulting in $H_2O_2$ production. $CO_2$ depletion also reduces the level of $NADP^+$ through a reduction in the rate of oxidation of NADPH from the Calvin–Benson cycle, which ultimately results in a reduction of electron transport, accumulation of electron, and increase in oxygen reduction forming hydrogen peroxide ($H_2O_2$) and superoxide anion ($O_2^-$).The depleted pool of NADPH in chloroplast activates malate dehydrogenase and opens the valve for the transportation of accumulated electron to the mitochondrion electron transport chain and cytosol. To counteract the increased inflow of electrons to ETC, various *aox* genes and alternative oxidase are reported to be upregulated in order to maintain the balance. Besides this, it is also reported to see upregulation of ATP-sensitive potassium gateway, inhibition of respiratory functions, and stimulation of PUCPs (plant uncoupling proteins) as a response to ROS and drought-mediated changes (Noctor et al. 2002; Pastore et al. 2001; Bartoli et al. 2005; Selinski et al. 2018; Cruz de Carvalho 2008; Barreto et al. 2017).

Besides ROS, RNS are also produced as the repercussion of the drought stress. Nitric oxide has been already proven to be an essential signaling molecule involved in germination, stress management, hormone regulation, and development. While the mechanism of production through NO synthase is not conserved from animal to plant, there have been many other reported mechanisms like nitrite and nitrate NO reductase, production as a byproduct of electron transport chain, xanthine oxidoreductase, through associated enzymatic proteins of NO (Corpas et al. 2008, 2009; Gupta et al. 2011). NO bioactive concentration has been found to be a function of three major factors: (a) concentration of nitroso glutathione (GSNO), (b) GSNO reductase activity, and (c) interactions with hemoglobin (Dı́az et al. 2003; Igamberdiev et al. 2010; Chamizo-Ampudia et al. 2017).

A threefold increase in NO uptake has been reported post 24 h. of osmotic stress of 20 mmol/L mannitol (Cai et al. 2015). Similar enhancement of NO uptake was seen in rice post concealment of irrigation for 9 days (Xiong et al. 2012). While in trees like *Ailanthus altissima* it took around 14 days post concealment of irrigation to significant enhancement of NO uptake (Filippou et al. 2014). The difference in time span seen in different studies could be the potential result of many detrimental factors like (a) time taken to accumulate similar stress level, (b) sensitivity towards drought, (c) developmental stages, (d) ratio of mature and developing cell present in the leaf lamina, (e) the extent of antioxidant response and APX activity, (f) activation of regulatory photoprotective mechanism, (g) SOD (superoxide dismutase) activity, and others like (h) carotenoid metabolism, redox process, photosynthesis, etc. (Tholakalabavi et al. 1994; Moustaka et al. 2015; Polle et al. 2001; Li et al. 2018).

## 10.7 Antioxidant Defense Machinery

During environmental perturbations, the production of reactive oxygen species and free radicals are generally upregulated and so is their accumulation. ROS being capable of causing oxidative damage, impairing normal functioning, are often counteracted with activation of the antioxidant systems. Antioxidant defense machinery consists of both enzymatic and nonenzymatic approaches that help in the reduction of ROS accumulation and lessen the damage caused by oxidative stress (Miller et al. 2010). Enzymatic antioxidants include superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione s transferase, and glutathione peroxidase, while nonenzymatic includes water-soluble like glutathione (GSH), ascorbic acid (AA), and lipid-soluble like tocopherols and carotenoids. (For an extensive review on plant antioxidant defense system see Ahmad et al. 2010; Gill and Tuteja 2010.)

Superoxide dismutase (SOD) is a metalloenzyme arguably considered as the first responder to ROS accumulation. It is associated with all the major cellular compartment sensitive to ROS and to the aerobic organism. SOD works on the principle of converting $O_2-\bullet$ to $H_2O_2$. SOD can be categorized into various subgroups on the basis of the metal ion they possess in their active centers like iron (FeSOD), manganese (MnSOD), and copper and zinc (Cu/Zn SOD) (Kim et al. 1996). Over the years there has been numerous literature showing the critical role and upregulation of SOD post abiotic stress and SOD-mediated activation of other enzymes of the antioxidant defense machinery (McKersie et al. 1999; Shalata et al. 2001; Ahmad et al. 2012; Tuna et al. 2008).

Ascorbate peroxidase (APX): APX that consists of five different isoforms and has an important function as the scavenger of $H_2O_2$ and also in the ascorbate–glutathione cycles where it employs ASH as the electron donor (Kangasjärvi et al. 2008). APX has been widely covered by earlier literature for its important role in the defense system of plants. Noctor and Foyer (1998) in their work have described different isoforms of APX based on their attributes that consist of either the membrane-bound microsomal or thylakoid or soluble form in cytosolic, stromal, and apoplastic enzymes. Prior literature has established APX crucial role in the defense system and higher affinity toward $H_2O_2$ when compared to the POD and CAT. Overexpression of tylAPX resulted in an increase in resistance to stress while suppression made plants more prone to oxidative stress (Zhang et al. 2008; Davletova et al. 2005; Giacomelli et al. 2007).

Catalases (CAT): Another important antioxidant enzyme from the plant's defense machinery that facilitates the scavenging of $H_2O_2$ by converting them to $O_2$ and $H_2O$ (Ben Amor et al. 2005). It is a tetrameric heme-containing enzyme that is generally localized in peroxisomes. Different isoforms of catalase have been reported in the literature, some directly dismutate $H_2O_2$ while others choose to regulate its concentration by oxidizing substrates like ethanol, formic acid, methanol, and ethanol. Willekens et al. (1994) in their work classified catalase into the three subtypes, based on their function and localization. Class 1 catalase was found in photosynthetic tissue, class 2 in vascular tissue, and class 3 in young plants and seeds. Catalases are indispensable in their role of $H_2O_2$ removal that is generated as the result of ongoing processes like purine catabolism, photorespiration, and fatty acid degradation (Vital et al. 2008). An increase of catalase in cotton, tea, tobacco, and alfalfa nodule is accepted as the possible trait adaptation that helps towards the efficient management of the oxidative stress either through reducing the extent of damage or through the reduction of damage like photorespiratory loss (Hediye Sekmen et al. 2007; Vital et al. 2008; Upadhyaya et al. 2008; Zhang et al. 2008).

Glutathione peroxidase (GPX): Family of GPX isoenzyme is known to provide protection to the plant from oxidative stress through reduction of the level of $H_2O_2$ or through the detoxification of the toxic hydroperoxides or by-products of lipid peroxidation (Dixon et al. 1998). Post introduction of oxidative stress, Leisinger et al. (2001) found upregulation of the *Gpxh* gene. And it was highly correlated with the conferring of resistance to singlet oxygen and abiotic stress in different transgenic plants.

## 10.8 PGPR-Mediated Alteration in Antioxidant Defense System

Chiappero et al. (2019) demonstrated that the *Mentha piperita* under severe to moderate drought stress showed amplification of PX and SOD activity when inoculated with *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens*. Wang et al. (2012a, b) showed that systemic tolerance to drought could be achieved by SOD amplification in *Cucumis sativus* L. through the application of *Serratia* sp., *Bacillus subtilis*, and *Bacillus cereus* consortia. *B. safensis* and *O. pseudogregnonense* were further shown by Chakraborty et al. (2013) to be crucial in maintaining the high level of SOD and CAT in different varieties of *Triticum aestivum*. Addition of PGPR was crucial in the maintenance of the elevated level of antioxidants while the decline is seen in case of one not being treated with PGPR. They also observe the accumulation of additional small antioxidants like ascorbate and the carotenoids. Gusain et al. (2015) found that inoculation of PGPR consortia like *Pseudomonas jessenii* and *Pseudomonas synxantha* can enhance various antioxidant enzymes like CAT, SOD, APX, and POD, hence conferring characteristic trait-like drought resistance to *Oryza sativa* L.

Khan et al. (2019) reported yet another addition of combinational treatment with PGPR and PGR (plant growth regulator) on *Cicer arietinum* L. They used the *Bacillus thuringiensis*, *Bacillus subtilis*, and *Bacillus megaterium* consortia along with salicylic acid and putrescine showed a significant increase in their response to drought stress. They have also observed an upregulation of antioxidant enzymes like CAT, SOD, POD, and APOX along with other potential contributors like an increase in proline content and lipid peroxidation (Table 10.1).

**Table 10.1** Plant species and their associated PGPR with their antioxidant enzymes

| Plant species | PGPR | Antioxidant | Reference |
|---|---|---|---|
| *Lactuca sativa* L. cv | *Pseudomonas mendocina*<br>*Glomus mosseae*<br>*Glomus intraradices* | POX, SOD, and CAT (their activity varies in moderate and severe stress) | Kohler et al. (2008) |
| *Beta vulgaris* | *Azotobacter chroococcum* | SOD | Štajner et al. (1997) |
| *Hyoscyamus niger* | *Pseudomonas putida*; *Pseudomonas fluorescens* | SOD and POX | Ghorbanpour et al. (2013) |
| *Solanum tuberosum* | *Bacillus pumilus*; *Bacillus firmus* | SOD, DHAR, GR, APX, and AT | Gururani et al. (2013) |
| *Vigna radiata* | *Pseudomonas fluorescens*; *Bacillus subtilis* | CAT, POX | Saravanakumar et al. (2011) |
| *Zea mays* L. | *Bacillus licheniformis*, *Bacillus thuringiensis*, *Paenibacillus favisporus*, *Bacillus subtilis* | APX, CAT, and GPX | Vardharajula et al. (2011) |
| *Triticum aestivum* L. | *Bacillus amyloliquefaciens*; *Azospirillum brasilense* | APX, MDHAR, DHAR, and GR | Kasim et al. (2013) |
| *Vigna radiata* | *Pseudomonas aeruginosa* | SOD, POX, and CAT | Sarma and Saikia (2014) |
| *Zea mays* L. | *Pseudomonas* spp. (*P. entomophila*; *P. stutzeri*; *P. putida*; *P. syringae*; and *P. monteilli*) | GPX, CAT, and APX | Sandhya et al. (2010) |
| *Zea mays* | *Ochrobactrum* sp. | APX, GPX, PPO | Mishra et al. (2020) |
| *Mentha pulegium* L. | *Azotobacter chroococcum*; *Azospirillum brasilense* | GPX, SOD, and CAT | Asghari et al. (2020) |
| *Triticum aestivum* L. | *Bacillus subtilis* | POX, SOD, and CAT | Sood et al. (2020) |
| *Helianthus annus* | *Planomicrobium chinense*; *Bacillus cereus* + salicylic acid | APOX, CAT, and SOD | Khan et al. (2018) |
| *Cupressus arizonica* G. | *Pseudomonas fluorescens*; *Rhizophagus irregularis*; *Funneliformis mosseae* | APX, GPX, SOD, and CAT | Aalipour et al. (2020) |
| *Lycopersicon esculatum*; *Capsicum annuum* | *Rhizophagus intraradices*, *Rhizophagus fasciculatum*, *Burkholderia seminalis* | CAT and GPX | Tallapragada et al. (2016) |
| *Mentha piperita* | *Pseudomonas fluorescens*; *Bacillus amyloliquefaciens* | PX; SOD | Chiappero et al. (2019) |
| *Pelargonium graveolens* (L.) Herit. | *Glomus mosseae*, *Glomus intraradices* | GPX, APX, and CAT | Amiri et al. (2015) |
| *Linum usitatissimum* L. | *Funneliformis mosseae* or *Rhizophagus intraradices*; *Pseudomonas putida* | GPX, SOD, APX, and CAT | Rahimzadeh and Pirzad (2017) |

(continued)

**Table 10.1** (continued)

| Plant species | PGPR | Antioxidant | Reference |
|---|---|---|---|
| *Oryza sativa* L. | *Pseudomonas* strains | PX, CAT | Sen and Chandrasekhar (2015) |
| *Solanum tuberosum* L. | *Bacillus subtilis* | CAT, POD, and SOD | Batool et al. (2020) |
| *Triticum aestivum* L. | *Bacillus lentus*, *Bacillus subtilis* and *Bacillus cereus* | SOD, CAT, and GR | Annapurna et al. (2019) |
| *Oryza sativa* L. | *Pseudomonas fluorescence*, *Pseudomonas jessenii*, *Pseudomonas synxantha*, *Bacillus cereus*, *Arthrobacter nitroguajacolicus* | APX, SOD, CAT, POD | Gusain et al. (2015) |
| *Triticum aestivum* L. | *Stenotrophomonas maltophilia* | SOD, CAT, POX | Singh and Jha (2017) |
| *Lavandula dentata* and *Salvia officinalis* | *Enterobacter* sp., *Bacillus thuringiensis*, and *Bacillus* sp. | GR and APX | Armada et al. (2014) |
| *Abelmoschus esculentus* (L.) | *Pseudomonas fluorescens* | SOD, CAT, APX, and GPX | Pravisya et al. (2019) |
| *Calotropis procera* | *Rhizophagus irregularis*, *Pseudomonas putida* | SOD, CAT, APX | Bahmani et al. (2018) |
| *Oryza sativa* L. | *Bacillus haynesii*; *Bacillus licheniformis*; *Bacillus paralicheniformis*; *Bacillus licheniformis* | SOD, CAT, and GPOX | Joshi et al. (2020) |
| *Ziziphus jujuba* | *Pseudomonas lini*; *Serratia plymuthica* | SOD and POD | Zhang et al. (2020) |
| *Cucumis sativus* | *Bacillus methylotrophicus* | SOD, CAT, GPX, and APX | Hou et al. (2018) |
| *Ocimum basilicum* L. | *Azotobacter chrocoocum*, *Pseudomonas fluorescens*, *Pseudomonas mendocina* Palleroni and *Azospirillum lipoferum* | PX, PPO, and CAT | Agami et al. (2016)) |
| *Cicer arietinum* L. | *Pseudomonas putida* | CAT, APX, and GST | Tiwari et al. (2016) |
| *Zea mays* L. | *Bacillus licheniformis* | CAT | Akhtar et al. (2020) |
| *Lavandula dentata* | *Bacillus thuringiensis* | SOD, CAT, and APX | Armada et al. (2016) |
| *Triticum aestivum* L. | *Burkholderia phytofirmans* PsJN | CAT and GR | Naveed et al. (2014) |
| *Cucumis sativus* | *Burkholdera cepacia*, *Promicro monospora* sp., *Acinetobacter calcoaceticus* | PPO, PX, and CAT | Kang et al. (2014) |

(continued)

**Table 10.1** (continued)

| Plant species | PGPR | Antioxidant | Reference |
|---|---|---|---|
| *Ocimumbasilicum* L. | *Pseudomonades* sp., *Bacillus lentus*, *Azospirillum brasilens* | CAT, GPX, and APX | Heidari and Golpayegani (2012) |
| *Lycopersicon esculentum* | *Bacillus cereus* | SOD, PX, and CAT | Wang et al. (2012a, b) |
| *Triticum aestivum* L. | *Azotobacter chrocoocum*, *Pseudomonas putida* | CAT, PPO, POD | Khalilzadeh et al. (2016) |
| *Zea mays* L. | *Bacillus megaterium* | SOD, POD, CAT, and APX | Li et al. (2019) |
| *Capsicum annum* L.; *Solanaceae* | *Pseudomonas aeruginosa* and *Bacillus amyloliquefaciens* | SOD, POD, and CAT | Gupta et al. (2019) |

*APX* ascorbate peroxidase, *CAT* catalase, *GPX* glutathione peroxidase, *SOD* superoxide dismutase, *APX* ascorbate peroxidase, *GPX* guaiacol peroxidase, *PPO* polyphenol oxidase, *PX* peroxidase, *GPOX* guaiacol peroxidase

## 10.9 Conclusion

Use of PGPR is nowadays gaining popularity as it proves to be very effective biofertilizer and biocontrol agent (Babalola 2010). The soil is rich in beneficial microbes; the need of the hour is to make use of this hidden wealth for sustainable agriculture through organic farming which pays much focus on food security, preservation of nutrients, and environmental protection. The field crops are in constant threat from a number of factors including biotic as well abiotic stress. The present scenario in which climate change is unpredictable, plants frequently suffer from high degree of abiotic stress which not only hampers the growth of crops and but also causes a huge setback in yield. To mitigate the harmful effects of these stressful conditions, plants have developed various complex mechanisms involving plant associated bacteria. The studies involving use of PGPR in ameliorating drought stress in time-sensitive and cost-effective manner support the large-scale production and commercialization of these inoculants. The signaling network of extracellular compounds released in response to environmental stress stimuli and the regulatory pathways modulated to tolerate the stress need to be explored in the whole process of plant–microbe interaction. The potential application of PGPR to help plants deal with stress in agricultural fields seems vastly large and effective and a lot has to be utilized yet.

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# Part III

# Role of Antioxidants in Microbe Mediate Biotic Stress Alleviation

# 11

# Potential of Plant Growth-Promoting Microbes in Disease Reduction by Influencing the Antioxidant Enzymes of Medicinal and Spice Plants

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## Abstract

Rhizosphere is the main sink of plant growth-promoting microbes (PGPMs), where they colonize profusely; promote plant growth, biomass production, and yield; and activate the defense system of host by direct and indirect mechanisms. They are capable to multiply at different ecological niches of roots and modify root functioning by improving the mineral and water acquisition. The rhizospheric mircobiome may have neutral, detrimental, or positive effect on plant health. Currently, plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) are commonly used as biopesticides against many pathogenic diseases of agricultural crops including medicinal and spice crops. The influence of PGPM interaction with medicinal and spices herbs enhances the antioxidant defense associated with non-enzymatic and enzymatic reactions that protect the plant against herbivory and pathogen attack. Therefore, impact of pathogenic stress on medicinal and spice crops can minimize by utilizing the antagonistic potential of PGPMs instead of chemical fertilizers, owing to their features such as environmentally safe, rapid multiplication rate, broad spectrum of mechanism, and high compatibility over other rhizobacteria.

This chapter discusses the novelty of PGPMs in rhizosphere and their antagonistic potential in the reduction of pathogens stress of many medicinal and spice crops by various modes of action particularly antioxidant defense mechanisms. The chapter also highlights the progress of morphological as well as physiological characteristics of plants on PGPM inoculation.

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**Keywords**

PGPMs · Rhizosphere · Antioxidant enzymes · Medicinal and spice plants · Pathogen

## 11.1 Introduction

Plant-derived medicines are the richest source of various bioactive compounds, which have been used intensively in traditional healthcare system across the world for centuries (Toussaint et al. 2007; Kandari et al. 2012). With the increase of population pressure, preferences of herbal medicine have become popularized due to the increased awareness of toxic effects, cost, and resistance development to synthetic chemical and indiscriminate usage of antibiotics in the treatments of many diseases (Saganuwan 2010; Nema et al. 2013). Medicinal plants play vital role to maintain the economy of the country or support the local farmers in earning money through export and import services. World population around 70–80% of developing countries primarily depend on health conservations by endorsing the herbal medicines because of relatively minor side effects, lesser expensive, and globally competitive (Kamboj 2000). Therefore, medicinal plant cultivation is being focused by using both modern and traditional practices world widely. However, the pest and pathogen attack is the major hampering problem in the quality of growth and development of medicines-spice herbs. Additionally, excessive use of pesticides may degrade the quality of medicines-spice products and its residue causes environment pollution. Thus, development of new or modified traditional technologies for cultivation of medicines-spice plants is necessary. Herbal medicine-spice is the premise bowl of natural substances which are using intensively to maintain the physiological functioning of living cells, alleviate illness, and can promote health. Hence, they are believed to be better for patient acceptability and compatibility (Table 11.1).

Antioxidant is one of the vital plant metabolites that are capable to encounter the oxygen free radical and have antiproliferative properties to protect plant against herbivory, pathogen, and abiotic stress (Wink and Schimmer 2010; Briskin 2000; Wu et al. 2007). Considering potential role of antioxidants in food, pharmaceutical industry, and human health, thus herbal medicines-spice plants of broad range of naturally occurring antioxidants properties are classified on the basis of composition its physical, chemical nature, and site of action (Table 11.2). Antioxidants act as scavenger of oxygen free radical (RFR) by donating electrons during oxidation or reduction (redox) reaction. It produced non-toxic oxidized products of metal ions or other compounds to repair cells by iron-transporting protein system (Brewer 2011). Herbaceous medicines-spices (HMS) have been identified as source of various biochemical compounds, among them many of which are potent to have antioxidant potential (Dragland et al. 2003). Moreover, antioxidant and redox signaling of HMS plants are capable to activate essential defense at cellular level against abiotic and

**Table 11.1** PGPM association with Medicinal-spices plants enhances phytochemicals

| S. No. | Medicine-spices herbs | PGPMs | Phytochemicals | References |
|---|---|---|---|---|
| 1 | *Anethum graveolens* L. (dill) | *Pseudomonas putida* | Carvone, limonene | Tajpoor et al. (2013) |
| 2 | *Ocimum basilicum* (common basil) | *Bacillus subtilis* | Terpineol, eugenol | Banchio et al. (2009) |
| 3 | *Origanum majorana* L. (marjoram) | *Pseudomonas fluorescens*<br>*Bradyrhizobium* sp. | Terpinen-4-ol, cissabinene hydrate, transsabinene hydrate, α-terpineol | Banchio et al. (2008) |
| 4 | *Salvia miltiorrhiza* Bunge (red sage) | *Bacillus cereus* | Diterpenoid, tanshinones | Wu et al. (2007) |
| 5 | *Stevia rebaudiana* Bert. (sweet leaf) | *Burkholderia gladioli, Enterobacter aerogenes, Serratia marcescens* | Stevioside, rebaudioside-A contents | Gupta et al. (2011) |
| 6 | *Tagetes minuta* (Mexican marigold) | *Pseudomonas fluorescens, Azospirillum brasilense* | Essential, phenolic contents | Cappellari et al. (2013) |
| 7 | *Withania somnifera* (Indian ginseng) | *Azospirillum, Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus megaterium* | Withaferin A | Rajasekar and Elango (2011) |
| 8 | *Curcuma longa* L. (turmeric) | *AMF of Glomus, Gigaspora and Acaulospora* sp. | Curcumin, phenolic content, 145.23% higher flavonoids | Dutta and Neog (2016) |
| 9 | *Trigonella foenum-graecum* L. (Fenugreek) | *Sinorhizobium meliloti* and *P. fluorescens* | Nicotinic acid and trigonelline<br>Rhaponticin antioxidant lymphatic cleansing<br>Isovitexin (antioxidant potential) | Sharghi et al. (2018), Wani and Kumar (2016) |
| 10 | *Cinnamomum verum L.* (Cinnamon) | *Trichoderma asperellum, T. harzianum, T. hamatum, T. koningiopsis* | Procyanidin, cinnamic acid, and cinnamaldehyde (antioxidants) | Tanaka et al. (2008), Hoyos et al. (2020) |
| 11 | *Zingiber officinale L.* (Ginger) | *Aspergillus terreus* | Gingerol, Shogaol<br>Zerumbone (antioxidant and anti-inflammatory) | Uzma and Chowdappa (2018) |

(continued)

**Table 11.1** (continued)

| S. No. | Medicine-spices herbs | PGPMs | Phytochemicals | References |
|---|---|---|---|---|
| 12 | *Allium sativum* L. (Garlic) | *Saccharomyces cerevisiae*, *Bacillus pumilus* | Phenols, flavonoid, terpenoid, steroid glucoside, volatile oil, tannins, allicin | Cavalcanti et al. (2020) |
| 13 | *Origanum vulgare* L. Oregano | *Bacillus megaterium, Bacillus subtilis, Paenibacillus polymyxa, Pseudomonas putida and Pseudomonas fluorescens* | Thymol, carvacrol, flavonoids | Gutiérrez-Grijalva et al. (2017); Kutlu et al. (2019) |
| 14 | *Rosmarinus Officinalis* L. (Rosemary) | *P. fluorescens* | Rosmarinic acid, derivatives, phenolic diterpenes, and falvones | Kasmaei et al. (2019) |
| 15 | *Thymus vulgaris* L. (thyme) | *Azotobacter chrococcum ML1, Bacillus circulans* ML2, *and Bacillus megaterium* ML3 | Phenols, thymol, flavonoids, p-cymene | Zaghloul et al. (2016) |
| 16 | *Myristica fragrans* L. (nutmeg) | *Bacillus* strains | Lignans, phenylpropenoid, terpenoid acids, diphenylalkanes, phenolic acid, sabinene | Marthin et al. (2020) |
| 17 | *Catharanthus roseus L.* (periwinkle*)* | *Pseudomonas fluorescens* | Enhance antioxidant enzymes like APX, SOD, CAT, POX, and PPO | Jaleel et al. (2010) |
| 18 | *Anethum graveolens* L. (Dill) | *Glomus macrocarpum, Glomus fasciculatum* | Limonene, α-phellandrene | Kapoor et al. (2002) |
| 19 | *Lavandula angustifolia L.* (Lavender) | *Glomus lamellosum* | Essential oil | Karagiannidis et al. (2012) |
| 20 | *Ocimum basilicum* (common basil) | *Glomus mosseae* | Essential oil | Copetta et al. (2006) |
| 21 | *Salvia officinalis (common sage)* | *Glomus intraradices* | Essential oil, bornyl acetate, 1,8-cineole, α-β-thujones | Geneva et al. (2010) |
| 22 | *Arabidopsis thaliana* L. (Thale cress) | *Erwinia carotovora* | Camalexin, indole glucosinolates | Namdeo (2007) |

(continued)

**Table 11.1** (continued)

| S. No. | Medicine-spices herbs | PGPMs | Phytochemicals | References |
|---|---|---|---|---|
| 23 | *Catharanthus roseus* L. (Periwinkle) | *Trichoderma viride* | Ajmalicine | Namdeo (2007) |

biotic constrains. HMS represents a valuable source in strengthening human cells by reducing the oxidative tension.

A rhizosphere, rhizoplane, and phylloplane are specialized ecological niches where diversity of microbes colonized intensively. Simultaneously, these microbes may also interact with the plant independently, synergistically, or antagonistically. Consequence leads to beneficial, harmful, or neutral response in plants (Hiltner 1904). Rhizosphere is a narrow zone associated with plants roots and surrounding soil, mainly influenced by secretion of root exudates which serve as wide sources of organic nutrients (mainly protein and sugars) to enhance the microbial colonization in that zone in comparison with rhizoplane (Smith and Read 1997; Morgan et al. 2005). There are ample reports on plant's root exudates that play an active role to regulate the symbiotic and protective relationship between the beneficial rhizosphere microbes and plants (Jones et al. 2003; Barriuso et al. 2008; Lugtenberg and Kamilova 2009; Dilfuza 2015).

## 11.2 Plant Growth-Promoting Microbes (PGPMs)

Rhizosphere of plant is a repository of plant growth-promoting microbes (PGPMs) or biocontrol agents particularly fungi and bacteria which are effective to promote the plant growth by enhancing the innate immunity of plants against invading pathogens (Hyakumachi 1994; Whipps 2001; Thakore 2006; Weller 2007; Murali and Amruthesh 2015). Rhizobacteria can also promote seed germination and enhance plant vigor, yield, procurement of nutrients, and resistance against abiotic and biotic stresses (Egamberdieva et al. 2013; Parmar et al. 2017). PGPMs providing protection in medicine and spice plants against bacterial, fungal, nematodes, and viral soil-borne diseases by owing different mechanisms: antibiotics, phytohormones, siderophores synthesis, competition for nutrients and space, production of lytic enzymes are well documented by researches of Raaijmakers et al. (2009), Mishra et al. (2010), Egamberdieva and Lugtenberg (2014). The rhizosphere microbes especially a varied variety of fungi and bacteria are found to improve medicinal, spice, and aromatic plants' values and quality by modifying the plant physiological as well as biochemical processes such as increase in the nitrogen fixation, nutrient uptake, and production of secondary metabolites (Shaikh and Mokat 2018). Root-associated bacteria which belong to several genera including *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Cellulomonas*, *Clostridium*, *Enterobacter*, *Flavobacterium*, *Micrococcus*,

**Table 11.2** Antioxidant compounds of medicine-spice plants

| S. No. | Plants | Plant Parts | Antioxidant compounds | Mode of action | References |
|---|---|---|---|---|---|
| 1 | *Rosmarinus officinalis* (Rosemary) | Leaf | Isoprenoid quinones, Carnosol, carnosic acid, rosmanol, rosmadial, diterpenes (epirosmanol, isorosmanol, rosmaridiphenol, rosmariquinone, rosmarinic acid) | Antimicrobial and antioxidant properties, lipid antioxidant, and metal chelator | Milda (2019),<br>Nieto et al. (2018) |
| 2 | *Salvia officinalis* L. (Sage) | Whole plant | Carnosol, carnosic acid,, rosmadial, and methyl and ethyl esters of rosmarinic acid | Scavenger of free radical | Pavic et al. (2019) |
| 3 | *Origanum vulgaris* (Oregano) | Leaf | Rosmarinic acid, protocatechuic acid, caffeic acid, dighyrokaempherol, carvacrol, thymol, dihydroquercetin, phenyl propionic acid | Scavenger of free radical | Embuscado (2015),<br>Milda (2019) |
| 4 | *Thymus vulgaris* L. (Thyme) | Leaf | Thymol, phenols, caffeic acid, gallic acid acid, rosmarinic acid, phenolic diterpenes, flavonoids | Scavenger of free radical | Roby et al. (2013),<br>Embuscado (2015) |
| 5 | *Zingiber officinale* (Ginger) | Rhizome | Gingerols, shogaols, paradols, zingerone, and essential oils | Free radical scavenger | Mao et al. (2019) |
| 6 | *Curcuma longa* L. (Turmeric) | Rhizome | Curcuminoid (curcumin I,II,III; bisdemethoxycurcumin, and demethoxycurcumin) | Antioxidant, antimicrobial, anticancerous, neuroprotective | Ahmad et al. (2020),<br>Kumar et al. (2018) |
| 7 | *Piper nigrum* L. (Black pepper) | Fruits /berries | Kaempferol, rhamnetin, quercetin, piperlongumine piperic acid, pellitorine pellitorine, piperettine, piperamide piperolein B, and kusunokinin | Antioxidant of $SO^{-1}$, $H_2O_2$, NO, anticancerous, antidiabetic, neuroprotective, antimicrobial, anti-inflammatory | Takooree et al. (2019) |
| 8 | *Capsicum frutescence* L. (Chili pepper) | Fruits /berries | Capsaicin, capsaicinol | Antimicrobial and anti-carcinogenic and other medicinal spices, antioxidant values | Saleh et al. (2018) |

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|---|---|---|---|---|---|
| 9 | *Majorana hortensis* (Marjoram) | Leaf | β-carotene, β-sitosterol, eugenol, caffeic acid, carvacrol, linalool-acetate hydroquinone, terpinen-4-ol, rosmarinic acid | Scavenger of free radical ions | Embuscado (2015) |
| 10 | *Cumimum cymimum* (cumin) | Seed | Cuminal, linalool, γ-terpinene, carotol, pinocarveol | Scavenger of free radical ions | Yashin et al. (2017) |
| 11 | *Aloe vera* (Aloe) | Shoots, leaves grayish green sharp-edged coated forms rosette | Arachidonic acid, $\gamma$-linolenic acid, triterpenoid, $\beta$-carotene, folic acid, choline, α-tocopherol, catalase, superoxide dismutase, carboxylase, glutathione peroxidase | Antimicrobial, antioxidant, anti-tumor wound healing properties, strong immune system | Hes et al. (2019), Surjushe et al. (2008) |
| 12 | *Cinnamomum verum* (Cinnamon) | Bark | Limonene, eugenol, terpineol, catechins, linalool, methyleugenol, benzaldehyde, safrole, proanthocyanidins, tannins | Free radical scavenger, chelator | AllwynSundarRaj et al. (2014), Gruenwald et al. (2010) |
| 13 | *Allium sativum* (Garlic) | Leaf/bulb | Allicin, phenols, flavonoids | Antioxidant property, inhibit the cancer of stomach, liver, colon | Ghasemi et al. (2015) |
| 14 | *Elettaria cardamomum* (Cardamon) | Fruits | Terpinolene, limonene, quercetin, caffeic acid, kaempferol, 1,8-cineol, myrcene, luteolin, caffeic acid, pelargonidin, quercetin, kaempferol | Phenolics and antioxidant and free radical scavenger properties | Yashin et al. (2017), AllwynSundarRaj et al. (2014) |
| 15 | *Emblica officinalis/ Phyllanthus emblica* (Amla) | Fruits | Glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes and anthraquinones, gallic acid, ellagic acid, chebulinic acid Emblicanin-A, chebulagic acid, quercetin, corilagin, methyl gallate, luteolin | Antioxidant and free radical scavenging properties | Hasan et al. (2016), Nath and Dhivya (2019) |
| 16 | *Rauwolfia serpentina* Sarpagandha | Stem | Phenolic acid, alkaloids, indole, phytosterol, fatty acid, ketone, pyrimidine, and dicarboxylic acid | Antioxidant and free radical scavenger | Harisaranraj et al. (2009) |

(continued)

**Table 11.2** (continued)

| S. No. | Plants | Plant Parts | Antioxidant compounds | Mode of action | References |
|---|---|---|---|---|---|
| 17 | *Trigonella foenum-graceum* (fenugreek/ Methi) | Leaf and seed | Alkaloids, flavonoids, fibers, saponins, steroidal saponins, vitamins, and minerals, nitrogen compounds | Antioxidant and free radical scavenger | Wojdylo et al. (2007), Gupta and Prakash (2009), Wani and Kumar (2016) |
| 18 | *Coriandrum sativum* (Coriander) | Stem, leaf, seed | Cumene, linalool, borneol, pinene, terpinene, geraniol, terpineol, quercetin, caffeic acid, kaempferol, n-coumaric and vanillic acids, rutin, tocopherols, pyrogallol | Radical scavenging and antioxidant activity | AllwynSundarRaj et al. (2014) |
| 19 | *Ocimum sanctum* (Basil/ Tulsi) | Leaf | Eugenol, quercetin, rutin, apigenin, kaempferol, anthocyanins, limonene, geraniol, carvacrol, menthol, safrole, tannins, p-coumaric acids | Radical scavenging and antioxidant activity | Yashin et al. (2017) |
| 20 | *Anethum graveolens* L. (Dill) | Seed, root, leaf | d-limonene, terpenoid, d-carvone, glycosides, tannins, α-phellandrene, eugenol, anethole, flavonoids, phenolic acids, coumarins, triterpenes, umbelliferones, kaempferol, myricetin, quercetin, catechins, and isorhamnetin | Antioxidants and scavenger free radical activity | Jana and Shekhawat (2010), AllwynSundarRaj et al. (2014) |

*paenibacillus*, *Pseudomonas*, *Rhizobium*, *Sinorhizobium*, and *Serratia* are enhancer of plant growth and productivity (Rajasekar and Elango 2011; Egamberdieva et al. 2013). The interaction between beneficial microorganisms and rhizosphere of medicinal-spices plants is very crucial due to their complex mechanisms such as promoting plant growth and yield; improving metabolites secretion of phytohormones, production of siderophore and antibiotics; increasing the availability of phosphate for medicinal-spice plants; and providing protection to plants against pests and pathogen (Raja et al. 2006; Bafana and Lohiya 2013). Presence of PGPR species in the rhizosphere makes the entire plant resistant to pest and pathogen attack and strengthens the plants by lignifiying cell wall or triggering the induce systemic (ISR) and systemic acquired resistance (SAR) (Figueiredo et al. 2010). Thus, there needs a commercialization of several PGPMs inoculant for agricultural crops including medicinal and spice crops for yield improvement. PGPR associated with medicinal plants can either be used as biofertilizers and biological control agents in diminishing the disease density and improving growth and yield traits of plants (Tenuta 2003; Vasudha et al. 2013).

Rhizosphere fungi colonize around the root system and offer plant growth-promoting (PGP) traits called PGP fungi (Hyakumachi 1994). Plant growth-promoting fungi (PGPF) naturally survive as saprophytes which constitute non-pathogenic diverse genera belonging to class Ascomycetes, Basidiomycetes, and Oomycetes (Hossain et al. 2017a, b). PGPF employs a variety of mechanisms including mineral acquisition, phytohormones production, secretion of volatile organic compounds, and cell wall lytic enzymes. PGPF enzymes increase nutrient uptake that strengthens plant against abiotic and abiotic stresses and increases the standing stability of plant. PGPF also improves seed germination rate, plant vigor, flowering, and photosynthesis in host plants, apart from inducing defense system in plants against diseases (Murali et al. 2013; Hossain et al. 2017a, b; Muslim et al. 2019). On pathogen infection, PGPF triggered induced systemic resistance (ISR) primarily by modifying cell wall deposition of defense metabolites, such as lignin, callose, and phenol (Muslim et al. 2019; Nawrocka et al. 2018; Lee et al. 2013; Zhu et al. 2019). It has been reported that PGPF is also known as activator and enhancer of antioxidant bioaccumulation of defense-related enzymes in plants (e.g., phenylalanine ammonia-lyase (PAL), peroxidases (POX), and chitinase, β-1,3 glucanase) which are directly signaling to immune-defense response and alter the metabolic pathway to protect the plant against invading phytopathogens (Hossain et al. 2017a, b; Sindhu et al. 2018; Zhou et al. 2018; Liu et al. 2019). Defense-related enzymes such as PAL, LOX, POX, PPO, and chitinase play a vital role to strengthen plant defense via lignin deposition in cell wall and synthesis of phenolic compounds which combat against invading pathogens (Hu et al. 2017; Basavaraj et al. 2019). According to studies of Hassan et al. (2014), Murali and Amruthesh (2015), Zhou et al. (2018), PGPF treatment causes higher activities of PAL and POX enzymes in pathogen-inoculated seedlings of cucumber, chili, pearl millet, and *Salvia* sp. compared to control plants, as acting directly to defense the host plants against pathogen attack. Thus, the beneficial effects of both PGPF and PGPR on agricultural and non-agricultural crops have increased the attention of researchers to focus more on the application and commercialization of plant growth-promoting microbes (PGRMs).

The aim of this chapter was to describe the novelty of PGPMs to propose the various mechanisms in medicinal-spices plants against diseases as well as in the improvement of plant health and productivity. This chapter discusses (1) the brief introduction related to potential role of PGPMs in enhancing medicinal and spices plant growth and productivity by offering various mechanism; (2) next, the interaction between beneficial microbes and medicine-spice plants modulates physical and chemical characteristics of the plant against biotic and abiotic constrains; (3) PGPM inoculant boosts the herbaceous medicines-spice plant health by induce systemic resistance (ISR) or triggering the antioxidant-defense immune machinery in plants against biotic and abiotic stresses; and (4) final sections of the chapter describe future prospect, mainly emphasizing the application and exploration of commercialized PGPMs (especially fungi and bacteria) based on technology of twenty-first-century agricultural programs for the improvement of medicinal spices crop quality and yield.

## 11.3 Interaction of PGPMs with Medicinal-Spice Plant's Rhizosphere

### 11.3.1 PGPM Diversity Promotes Plant Health

The plant rhizosphere is defined as a region where an extensive variety of organic compounds available. Plant root secretion attracted microbial community (Hiltner 1904; Brimecombe et al. 2001; Compant et al. 2010). Each plant root exudate may specific to group of microbes present in soil. Root exudates are the composition of water soluble sucrose, organic and amino acids, hormones, vitamins, phenols, and other mineral nutrients (Nicholas 2007). A specific content of root exudates may harbor specific reservoir niche of microbes in rhizosphere of plants. Thus, root exudate plays key role in modification or differentiation phytomicrobiomes (Marschner et al. 2004; Doornbos et al. 2012). Plant rhizospheric interactions with microorganisms are highly predisposed by EDAPHIC factors (McCully 1999). The rhizosphere-inhabiting microorganisms developed the competition with existing microbes for water, nutrients, and space and lead to the evolution of micro-environments in natural ecosystems (Schloter et al. 2003). Karthikeyan et al. (2008) evidenced that medicinal plants *Ocimum sanctum* L., *Coleus forskohlii* Briq., *Catharanthus roseus*, (L.) G. Don and *Aloe vera* increase microbial population in zone of rhizosphere than non-rhizosphere. Vessey (2003) stated that rhizosphere of plants has flourished the distinct group of PGPR species, mainly prefers to grow in, on, or around plant tissues to promote plant growth and development. Since few decades, PGPF has been studied including genera of *Trichoderma*, *Penicillium*, *Phoma*, and *Fusarium* (Hyakumachi 1994). Furthermore, plant mycorrhizosphere stimulated the mycorrhizal colonization by modifying the plant root morphology and metabolic functions (Johansson et al. 2004). The arbuscular mycorrhizal (AM) fungi form mutual symbiosis with >80% of all plant species which especially increases the availability of phosphorous ions to plants from soil through fungal hyphae (Jasper et al. 1989; Smith and Read 2008). Many researchers have been reported that mycorrhizal colonization around medicinal plants play a vital role to modify the soil texture and to increasing soil fertility, thereby plant growth and productivity improved in varied range of agroecosystems of medicine-spice plants (Smith and Read 2008). Among all types of mycorrhizas such as Arbuscular mycorrhizal, ectomycorrhiza, ectendomycorrhiza, ericoid, orchid, arbutoid, and monotropoid mycorrhizal and Arbuscular mycorrhizal fungi (AMF) are known widely to show beneficial symbiotic relationship with diversity of medicinal plants (Kumar et al. 2010; Zeng et al. 2013). *Agathosma betulina* (Berg.) Pillans a medicinal sclerophyll has increased in growth by colonizing with microsymbiont of Cryptococcus laurentii a soil yeast which enhance plant nutrient-availability (Cloete et al. 2010). Mycorrhizal colonization of *Poncirus trifoliata*, *Piper longum*, *Salvia officinalis*, and *Plectranthus amboinicus* (medicinal plants) enhances phytochemical accumulation, shoot height, and root biomass (Wang et al. 2006; Rajeshkumar et al. 2008; Geneva et al. 2010; Gogoi and Singh 2011). Rani Beula et al. (2016) validated and identified the rhizosphere microbial diversity of medicinal plant *Alpinia galangal* on the basis of its cultural, morphological, and biochemical studies. Among these *Bacillus* sp., *Aspergillus* sp., and *Streptomyces* sp. were recorded in higher percentage of distribution. The plant-endophytic microbial associations are also found to improve health as well as protect plant by different mechanisms against microbial pathogenesis (Malhadas et al. 2017). Hassan (2017) isolated and identified the plant growth-promoting (PGP) bacterial and fungal endophytes, e.g., *Bacillus cereus, Bacillus subtilis, Penicillium chrysogenum,* and *Penicillium crustosum* of medicinal plants *Teucrium polium* to produce indole acetic acid (IAA) and ammonia, enzymes, antimicrobial metabolites and mobilized insoluble phosphate to plants.

Apart from the rhizosphere and rhizoplane colonization of PGPMs, some prefer to colonize the internal tissues of plants called endophytes and some colonize plant superficially known to epiphytes. Endophytic bacteria and fungi are living in plant tissues and are capable to induce ISR and SAR of plants. PGPR and PGPF colonized the medicinal plant's roots and protect plant from various soil-borne pathogens by employing various mechanisms, some commonly observed are (1) increased nutrient availability to host plants; (2) nitrogen fixation; (3) competition for nutrients acquisition and space sharing with pathogenic microbes; (4) activation of host plant's defense system that altered anatomical structure and root morphology; (5) producing antibiotics, siderophores, hydrogen cyanide against from pests and pathogenic diseases of plant; (6) balance phytohormone production (Medeiros et al. 2005; Van Loon 2007; Keel and Maurhofer 2009; Yang et al. 2012; Egamberdieva et al. 2013; Egamberdieva and Lugtenberg 2014; Hameed et al. 2014; Hossain et al. 2017a, b). These mechanisms allow plant to adopt well under varied stresses of biotic and abiotic environment. Martinez-Viveros et al. (2010) PGPR degree of association with plant roots are classified as extracellular-PGPR survives in rhizosphere, rhizoplane, or spaces between the cells of root cortex and intracellular-PGPR generally found inside the cells root nodules (Fig. 11.1). El-Deeb et al. (2013)

isolated the endophytic bacteria, viz., *Bacillus* species, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus licheniformis*, *Micrococcus luteus*, *Paenibacillus* sp., *Pseudomonas* sp., and *Acinetobacter calcoaceticus* from *Plectranthus tenuiflorus* medicinal plant. Li et al. (2013) isolated the endophytic bacterial diversity from three different medicinal plants of *Codonopsis pilosula*, *Ephedra sinica,* and *Lamiophlomis rotata* which have shown great variations. Kharwar et al. (2010) also identified endophytes and epiphytes out of 33 fungal species from healthy leaf segments of *Eucalyptus citriodora* (have medicinal value). Among them, nine fungi were confirmed as *Alternaria alternata*, *Aspergillus fumigatus*, *A. terreus*, *Cladosporium cladosporioides*, *Drechslera rostrata*, *Humicola grisea*, *Nigrospora oryzae*, *Penicillium cristata*, and *Pestalotia* sp. Rana et al. (2020) also reported that out of 478 fungal isolates, 279 were epiphytic (reside on host plant surface) while only 199 were endophytic (living inside the roots) of medicinal plants. Endophytic fungi identified, namely *Acremonium*, *Alternaria*, *Apiospora*, *Aspergillus*, *Aureobasidium*, *Bartalinia*, *Cephalosporium*, *Chaetomium*, *Chloridium*, *Choanephora*, *Colletotrichum*, *Cryptosporiopsis*, *Emericella*, *Eupenicillium*, *Eutypella*, *Hypoxylon*, *Penicillium*, *Fusarium*, *Paecilomyces*, *Pestalotiopsis*, *Pseudomassari*, *Quercina*, *Talaromyces*, and *Trichoderma*. Similarly, Caruso et al. (2020) isolated endophytes such as *Acremonium*, *Ampelomyces*, *Bipolaris*, *Botryosphaeria*, *Calonectria, Cercospora, Coniochaeta, cylindrocarpon*, *Epicoccum*, *Exserohilum*, *Memnoniella*, *Paecilomyces*, *Periconia*, *Podospora*, *Pezicula*, *Pyrenophora*, *Scopulariopsis*, *Seiridium*, and *Xylaria* were found to be associated with plants of family Asteraceae which include food crop, oil crop, and medicinal and aromatic host plants. Fungal species diversity of endophytes is higher than epiphytes. Both epiphytic and endophytic microorganisms offer good plant health and provide protection to plants (Andrews and Harris 2000). Provision of plant-beneficial microbial interactions in terms of plant biomass improvement can be distinguished into two categories. (1) direct association of microorganisms and plants is able to increase the mineral nutrient solubilization and acquisition to plant, (2) indirect interaction between microorganisms and plant reduces the soil biotic factors (growth or activity of soil-borne phytopathogens) and abiotic factors (include environment stress such as intense light, UV, temperature low and high, saline soils, drought, and herbicides) that indirectly affects the plant growth and yield (Huber and Watson 1974; Evelin et al. 2009) as displayed in Fig. 11.1. Mineral nutrients are the main components of plants which regulate metabolic activity and increases resistance in plant against virulence pathogens (Huber and Haneklaus 2007). So, adequate quantity of nutrition intake induces greater tolerance in plants or resist against several diseases (Khaliel et al. 2011; Sullivan 2019). More than 24 genera which belong to non-pathogenic group of the rhizobacteria have been identified till now. Kloepper and Schroth (1978) first demonstrated the plant growth-promoting rhizobacteria control plant diseases and enhance the plant growth and yield by offering broad spectrum of mechanisms. Arun et al. (2012) proved that isolates of medicinal plant *Cassia occidentalis* can be used as biofertilizers and biocontrol agents in agroecosystems (Smith and Read 2008). Oregano (*Origanum vulgare* L.) root was colonized by bacterial strains of *Stenotrophomonas maltophilia*

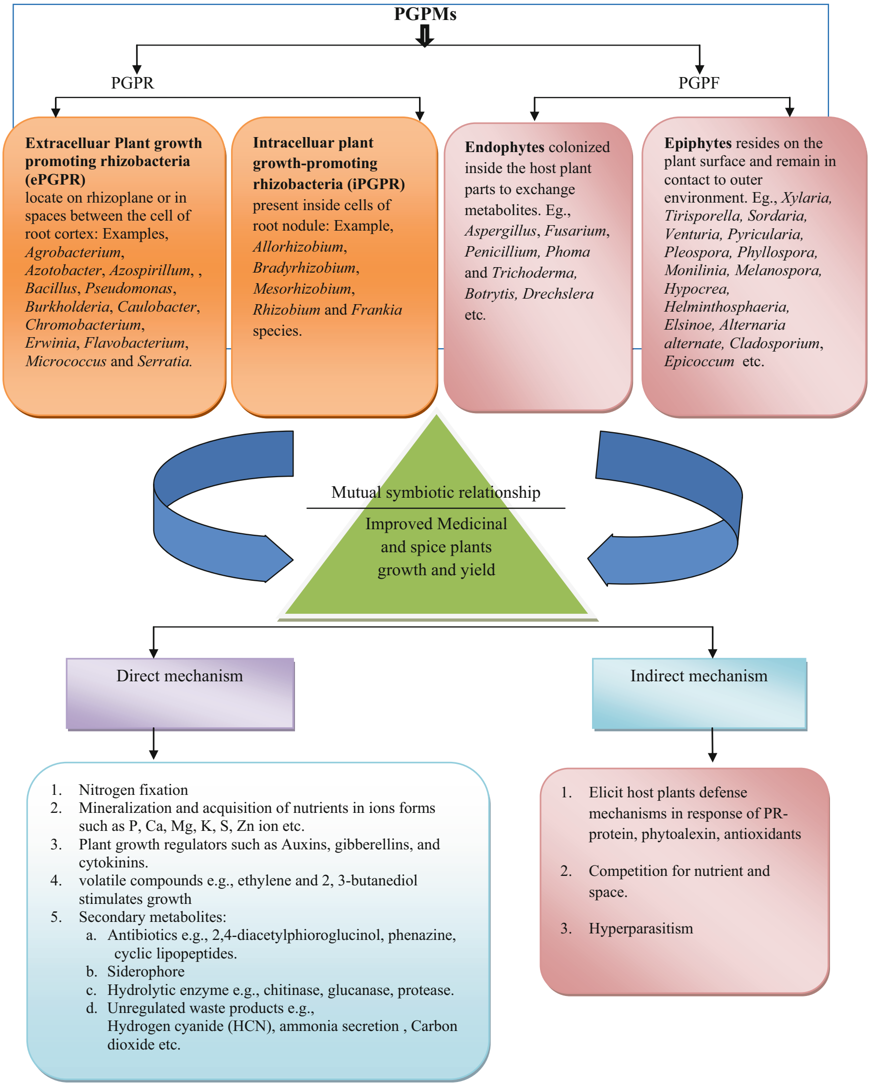

**Fig. 11.1** Potential of PGPMs on plant growth and yield elevation

and *Rhodococcus* sp. (Bafana and Lohiya 2013). Koberl et al. (2011) reported that medicinal plants cultivated in Egypt, e.g., *Matricaria chamomilla* L., *Calendula officinalis* L., and *Solanum distichum Ochrobactrum* and *Rhodococcus* were found to be associated with *Ochrobactrum* and *Rhodococcus*. Zhao et al. (2013) identified the microbial diversity association with some medicinal plants and distinguished in total 7 genera named as *Myxococcus*, *Corallococcus*, *cystobacter*, *Archangium*, *Stigmatella*, *Chondromyces,* and *pyxidicoccus*. Gupta et al. (2011) examined potential of *Burkholderia gladioli*, *Enterobacter aerogenes,* and *Serratia marcescens* bacteria in phosphate solubilization and can be used as biofertilizer. Lopez-Fuentes et al. (2012) identified 103 bacteria belonging to genera *Acinetobacter, Agrobacterium, Enterobacter, Pseudomonas, Sphingobium, Stenotrophomonas, Pantoea, and Serratia* from *Hypericum silenoides* Juss rhizosphere. Kumar et al. (2012) determined the plant growth-promoting effect and biotechnological potential of 123 bacterial strains associated with medicinal plant *Ajuga bracteosa* rhizosphere. Raichand et al. (2011) described diversity in gram-negative bacteria which were isolated from medicinal plants *Nerium indicum* (*Chuvanna arali*). They also observed that the rhizosphere of *Fritillaria thunbergii* medicinal plant harbors a distinct group of bacterial population like Proteobacteria, Acidobacteria, Actinobacteria, and Bacteroidetes (Shi et al. 2011). Nimnoi et al. (2011) stated that bacterial diversity varied among medicinal plants. Suryadevara and Ponmurugan (2012) studied that the diazotrophic bacterial inoculant of *Pseudomonas* and *Bacillus* sp., (in 1:1 ratio) was significant in increasing the soil microbial diversity as well as enhancing the rhizome yield by 21%, plant height by 5%, and rhizome weight by 60% over control. Boominathan and Sivakumaar (2012) determined the efficiency of *Pseudomonas fluorescens* and *Bacillus megaterium* strains in improving the curcumin pigmentation in rhizome and curcumin have strong antioxidant potential (Funk et al. 2006). Kumar et al. (2014) reported that turmeric rhizome inoculation with *Azotobacter chroococcum* enhances plant biomass, productivity, and biochemical constituents of rhizome. Kumar et al. (2016) also indicated that *Pseudomonas flourescens* inoculation in plants increases yields and curcumin content in turmeric rhizome. Qi et al. (2013) isolated a unique group of bacteria from the rhizosphere of *Rumex patientia* a wild medicinal plants, i.e., classified as *Proteobacterium* (43.37%), *Bacteroidetes* (13.25%), *Acidobacteria* (10.84%), unclassified bacteria (9.64%), *Gemmatimonadetes* (7.23%), *Verrucomicrobia* (4.82%), *Planctomycetes* (4.82%), Actinobacteria (3.61%), Firmicutes (1.20%), and Chloroflexi (1.20%). Zhang et al. (2011) identified a novel actinobacterial strain named CPCC 201356(T) belong to family Geodermatophilaceae, from medicinal plant *Astragalus membranaceus* rhizosphere soil. Muley et al. (2009) stated that *Calendula officinalis* (marigold) have good antioxidant, anti-inflammatory, and anticancer properties.

Mycorrhizal fungi colonization has also been identified as growth and yield promoter in many crops because it is strong to proliferate and to access nutrients in or around plant root system. Arbuscular mycorrhizal (AM) inoculants improved diversity index and functionality of microbes in rhizosphere of *Atractylodes lancea* medicinal plant (Guo et al. 2006). Rhizosphere of *Abutilon indicum, Argemone mexicana, Aloe vera, Amaranthus polygamus,* and *Achyranthes aspera* (medicinal and aromatic plants) is associated with a variety of fungal diversity (Srivastava and Kumar 2013). Yang et al. (2011) identified and isolated five genera of AM fungi from the rhizospheric soil of medicinal plants, namely *Withania coagulans, Mitragyna parvifolia,* and *Leptadenia reticulata*. Thombre et al. (2016) also identified and isolated 11 species of fungi from rhizosphere of *Santalum album.* Out of these ten species found to belong class hyphomycetes, viz. *Aspergillus fumigatus*, *A. niger*, *A. funiculosus*, *A. flavus*, *A. restrictus*, *A. terricola*, *A. flavipes*, *A. terreus*, *Fusarium oxysporum*, *Penicillium* spp. and one species *is Mycelia sterilia* that belongs to Basidiomycetes. Fungal variety of 104 was found to be in association with the rhizosphere zone of three different medicinal plants namely basil *(Ocimum basilicum*), peppermint *(Mentha piperita*), and *(Aloe vera*) (Ahmed et al. 2018). PGPR strains, e.g., Azotobacter, *Azospirillum, Pseudomonas*, and AM fungi stimulate shoot and root growth; increases availability of nitrogen, phosphorus, potassium, and photosynthetic pigment in *C. officinalis* (Hosseinzadah et al. 2011). Over few decades, utilization and reliability on biological control have been increased extensively for the management of various plant pathogens and also increase sustainability in agriculture crop management practices (Weller 2007). Therefore, current research enlightens to use biological fertilizers with innovative and improved biotechnologies in the cultivation of medicines-spice plants, which is found efficient in increasing bioactive compounds accumulation in medicinal plants (Rajasekar and Elango 2011; Bharti et al. 2013; Teixeira da Silva and Egamberdieva 2013). Therefore, many countries have engaged to cultivate medicines-spice plants on large scale commercially across the globe (Fabricant and Farnsworth 2001).

### 11.3.2 PGPMs Suppress the Abiotic Stress and Biotic Stress in Medicine-Spice Plants

Successful cultivation of medicine-spice plants is affected by biotic and abiotic factors, (Juliani et al. 2006). The interaction between plants and beneficial microbes promotes yield improvement and disease protection (Raja et al. 2006). There are ample of literatures available to describe the potential role of plant-microbes association in increasing soil nutrient availability to plants (Welbaum et al. 2004). The inoculation of *Streptomyces pactum* (Act12) in ginseng plant causes high yield (Zhang et al. 2013). Mansoor et al. (2007) reported that *P. aeruginosa* in soil rhizosphere of medicinal plant (*Launaea nudicaulis*) reduced the maximum disease severity of *Macrophomina phaseolina* in mung bean roots. Ushamalini et al. (2006) reported that *Trichoderma viride* was effective to show early expression and quantitative enhancement of defense enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase in turmeric plant against *Pythium aphanidermatum*. Murugappan et al. (2013) determined the endophytic strain of *Bacillus pumilus* in medicinal plant *Ocimum sanctum* that can be used as a biocontrol control agents in plant growth promotion. *Fusarium chlamydosporum* (Frag. & Cif.) and *Ralstonia solanacearum* (Smith) causing complex root diseases (rot and wilt) in *Coleus forskohlii* a medicinal plant by producing toxic forskolin compound. Thus, bacterial pathogen restricted by using PPGR especially in *Ocimum sanctum* (Singh et al. 2013). The rhizosphere microbes of three medicinal plants (*Matricaria chamomilla* L., *Calendula officinalis* L., and *Solanum distichum* Schumach. & Thonn.) had shown high colonization rate of Gram-positive bacteria to suppress pathogens (Koeberl et al. 2013).

## 11.4 Mechanism of PGPM-Mediated Disease Reduction in Medicine-Spices Plants by Enhancing the Enzymatic and Non-Enzymatic Activity of Antioxidants

### 11.4.1 Antioxidant Defense Activity of Medicinal-Spices Plants

The medicine-spice herbs are vast repository of natural antioxidants compounds, can neutralize the oxygen free radicals species (OFRS), or reduce the other molecules by gaining electron from oxygen free radicals. So, simultaneous reduction and oxidation of molecules takes place at same site called redox reaction or also called as oxidation inhibitor (Pokorny and Korczak 2001). Thus, antioxidant defense is activated by oxygen free radical (OFR) because its molecular state is relatively non-reactive. In living cells, $O_2$ generates excited states of oxygen free radicals during metabolism. Reactive oxygen species (ROS) disturbs normal cellular activity such as DNA replication, protein denaturation, lipid peroxidation in membrane and its severity can cause cell death. During day time, ROS is produced by chloroplasts and peroxisomes. While in dark, mitochondria serve as main producers of ROS. Generally, regeneration of ROS is seen virtually in all intracellular organelles, plasma membrane, and in cytoplasm.

Accordingly, Halliwell and Gutteridge (2007) describe the antioxidant mode of action as comprise: (1) ROS production is suppressed by inhibiting enzymes or chelating elements, (2) antioxidant defense scavenges ROS. Natural antioxidant has protected the primary biochemical functions of the plant cells. Major antioxidant nutrients are classified on the basis of biochemical and molecular features into (i) enzymatic (endogenous origin) peptide defense mechanisms include catalases, peroxidases, superoxide dismutases, glutathione, and other proteins; (ii) non-enzymatic (exogenous origin) phenolic defense compounds include vitamin E, flavonoids, phenolic acids, nitrogen compounds (e.g., alkaloids, amino acids, amines, carotenoids, and chlorophyll) as presented in Fig. 11.2 (Sujogya 2012; Apel and Hirt 2004). Both types of antioxidants not only provide protection to plant cells from oxidative stress, but also induce defense response against the abiotic and biotic constrains (Racchi 2013). Moreover, consumption of antioxidant-rich foods offers great protection and increase immunity in human body against oxidative stress, diabetes, cardiovascular, and cancerous diseases. Therefore, medicinal and spices plant-derived antioxidants, represent a valuable source and gain enormous popularity in the improvement of human health. These antioxidants are abundant in polyphenol compounds (e.g., phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamin E and C) (Baiano and del Nobile 2015; Manach et al. 2004). Generally, antioxidant-rich nutrient shows anti-inflammatory, anti-bacterial, anti-viral, anti-aging, and anti-cancerous effects (Peng et al. 2014; Manach et al. 2004; Zheng et al. 2016). Accordingly, several techniques are used for the extraction of bio-antioxidants from medicinal and spice plants, e.g., Trolox equivalence antioxidant capacity (TEAC) assay, ferric ion-reducing antioxidant power (FRAP) assay, oxygen radical absorbance capacity (ORAC) assay, inhibiting the oxidation of

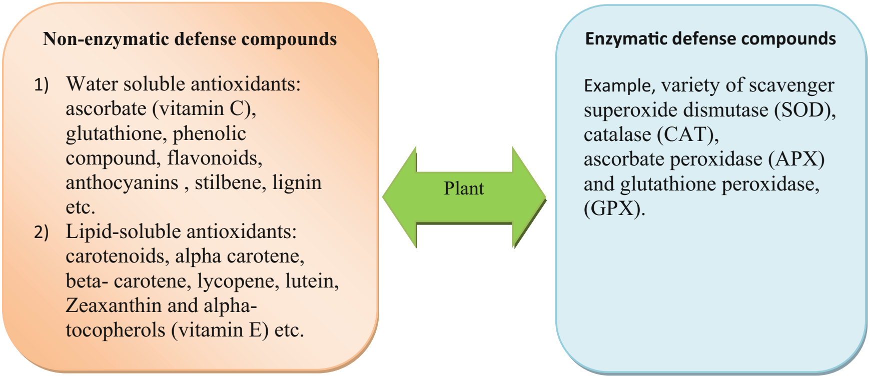

**Fig. 11.2** Antioxidant defense response in plants

low-density lipoprotein (LDL) assay, and cellular antioxidant activity assay, (Xu et al. 2017). PGPF induced both enzymatic antioxidants (e.g., peroxidase, catalase, superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, glutathione S-transferase, and guaiacol peroxidase) and non-enzymatic antioxidants (e.g., ascorbic acid, reduced glutathione, oxidized glutathione) to enhance the innate the immunity in plants (Ahmad et al. 2015). Jaleel et al. (2010) studied the phytohormones play vital role in increasing the antioxidant enzymatic activity of Ascorbate peroxidase (APX, EC: 1.11.1.11), Superoxide dismutase (SOD, EC: 1.15.1.1), Catalase (CAT, EC: 1.11.1.6), Peroxidase (POX, EC 1.11.1.7), and polyphenol oxidase (PPO, Ec 1.10.3.1) in *Catharanthus roseus* plants. In addition, *Glomus intraradices* either alone or in combination with *G. mosseae* increased the total phenolic content in *Cynara cardunculus* (Ceccarelli et al. 2010). *Bupleurum chinense*, *Ginkgo biloba*, and *Astragalus membranaceus* produce high antioxidant phenolic compound (Meng and He 2011). In sweet basil shoots, rosmarinic acid proved antioxidant activity (Toussaint et al. 2007). *Origanum vulgare* a perennial medicinal aromatic plant is rich in phenolic antioxidants. Frankel (2012) and Brewer (2011) determined the antioxidant activity of bioactive compounds such as phenolic compounds (gallic, protocatechuic, caffeic, and rosmarinic acids), phenolic diterpenes (carnosol, carnosic acid, rosmanol, and rosmadial), flavonoids (quercetin, catechin, naringenin, kaempferol, epicatechin, gallate, epigallocatechin gallate, and rutin), volatile oils (eugenol, carvacrol, thymol, menthol, safrole, 1,8-cineole, $\alpha$-terpineol, p-cymene, cinnamaldehyde, myristicin, and piperine), and phenylpropanoids (thymol, eugenol, carvacrol, p-cymene) in herbs and spices. Seleem and Mohamed (2014) identified the natural antioxidant properties of aromatic and medicinal plants, e.g., garlic, coriander, sumac, fennel, marjoram, thyme, and cardamom. Darughe et al. (2012) and Patel et al. (2013) examined the essential oil in coriander a medicine-spice herb that has antioxidant property. AM fungi were found to enhance the morphological, physiological, and biochemical parameters of *Sesbania sesban* under salt stress. AM fungi inoculation in plants improves the activity of enzymatic antioxidant, e.g., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) as well as non-enzymatic antioxidants, e.g., ascorbic acid and glutathione up to great extent against salinity (Abduallah et al. 2015).

### 11.4.2 Induce Systemic Resistance and Systemic Acquired Resistance of Medicines-Spices Plants

Disease in plants is the result of association of susceptible host plant and virulent pathogen. The plant metabolic machinery is able to sense the secretion of bio-molecules by invading pathogen termed as pathogen-associated molecular patterns (PAMPs). Once PAMPs onset, plant defense response triggered the plant immunity (PTI) and effector-triggered immunity (ETI) to inactivate penetrating pathogens. Thus, this triggered immunity strengthens the plant and hampers further infection proliferation (Chisholm et al. 2006). Bacteria-III type protein secretion system (TTSS) directly carried the effector protein into the plant cell.

Chemical, physical agents, or non-pathogenic rhizobacteria involved to activate induce defense system in host plants. So, plant can resist damage caused by various pest and pathogens. An inducer defines as flagella, lipopolysaccharides, siderophores, VOCs, and other components of non-pathogenic rhizobacteria mediate signaling to trigger defense response (Van Loon et al. 1998; Lugtenberg and Kamilova 2009). The ISR is known by inoculants of non-pathogenic rhizobacteria, whereas SAR is activated by other agents (Van Loon et al. 1998). Monteiro et al. (2005) reported that *Bacillus* strains produced lipopeptides against *Xcc*. Ongena et al. (2007) believed that lipopeptides are stimulant of ISR in plants and are able to increase the defense activity in plants. SAR or ISR does not show complete resistance in pathogens, such as viruses, bacteria, and fungi, but provides significant protection to plants against broad range of pathogens for a long interval (Bakker et al. 2007). The advantage of PGPR in rhizosphere induces resistance in plants against the diseases by leading biochemical and physiological changes. ISR also observe in response of pathogenesis-related (PR) proteins (Mpiga et al. 1997; Zdor and Anderson 1992). Plants are also capable to produce wide variety of enzymes in inducing defense. Peroxidase and PPO act as catalysts in the formation of lignin, while PAL, chitinases, lipoxygenases, and glucanases involved in the formation of phytoalexins (Belinky et al. 2003; Figueiredo et al. 2010). Thus, there is need to select PGPR indigenously to promote plant growth and develop resistance against pests and pathogens. The interaction between microbes and plants regulates mutual beneficial activity in plant-growth enhancement. Rhizomicrobiome benefits the plant growth by increasing nutrient acquisition (Backer et al. 2018). Vinayarani et al. (2019) confirmed that 30 isolates of fungi produce defense-related enzymes such as peroxidase (PO), phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and PR-protein β-1,3 glucanase as well as higher accumulation of phenolic compounds in PGPF pre-treated turmeric plants against *Pythium aphanidermatum* (Edson) Fitzp of turmeric. Thus, PGPR and PGPF play dual roles to induce defense mechanisms (ISR and SAR) and promote growth of plants against the various exposed pathogens such as fungi, bacteria, virus, and insect. ISR is mediated in response of Jasmonic acid (JA) and Ethylene (ET) while SAR expresses defense in relation to pathogenesis-related proteins (PR-proteins) against phytopathogen (Mandal 2019).

## 11.5 Future Prospects of PGPR in Disease Reduction by PGPR

Medicinal and spices plants serve as the main source of antioxidants nutrients; some are commonly known which have antioxidant properties like Ascorbic acid, flavonoids, carotenoids, and tocopherols. These antioxidants are responsible to induce plant growth and development against the environmental constrains and to promote human health. Therefore, these medicinal and spices plants are natural origin of antioxidants that attracts researcher to cultivate medicinal and spices plants on large scale at commercial level, but prevailing biotic and abiotic stress affects crop quality and quantity. As we have discussed, the PGPMs play crucial role in plant health improvement and suppression of pathogenic constrains by employing multiple mechanisms in plants to defense them against the environmental stress. So, there is need to isolate the diversity of PGPMs from rhizosphere or rhizoplane of plants belonging to medicinal and spices family. Then, morphological and molecular characteristic defines characteristics of bacterial genera, which will further use to test their impacts on plants health. Further, PGPMs formulation was commercialization as biocontrol agents and biofertilizers on large scale in sustainable agricultural crop health improvement. Generally, organic carriers include peat, talc, lignite, kaolinite, pyrophyllite, zeolite, montmorillonite, alginate, press mud; sawdust and vermiculite are effective in preparing PGPM formulations. Potential of PGPMs formulation product at commercially level should be viable in long shelf life, packaging, transport, non-cost-effective, storage at varied environment conditions. The formulations should not pose any toxicity biodiversity and safe to environment. The formulated products can be delivered through different methods of applications such as seed treatment, seed priming, soil application, foliar application, root dip, sett treatment in sugarcane, sucker treatment in banana. Vidhyasekaran and Muthamilan (1995) reported that talc-based and peat-based formulations of PGPR can be stable up to 240 days and highly effective to induce the systemic resistance in crop plants against diseases. Bora et al. (2004) confirmed that talc-based formulations of *P. putida* strain remain active for 6 months. Nakkeeran et al. (2005) reported the shelf life of *P. chlororaphis* (PA23) and *B. subtilis* (CBE4) in peat carriers was retained for more than 6 months. In future, major focus would be emphasized on novelty of PGPMs in the enhancement of plant antioxidants because its ability to maintain life of plants and animals. Thus, major concerns would be related to cultivate the medicinal and spices plant which is valuable for antioxidant compounds as well as determine the association of PGPM diversity with these plant rhizosphere niches.

## 11.6 Conclusion

As the economic importance of the herbaceous medicinal and spices plants throughout the world, these plants are affected greatly by various abiotic and biotic factors. On the other hand, the medicinal and spice plant's rhizosphere is associated with wide variety of microbes. Beneficial microbes are found to improve the plant health and suppress the phytopathogenic diseases in medicinal and spices plants. Beneficial potential of PGPMs on crop plants is found in many ways. This will open up the opportunities to manage the medicine-spice plant diseases as well as improve the quality and quantity of plants in progress of sustainable agriculture system. PGPMs benefit the plants by conveying various mechanisms, most commonly used in increasing the nutrient uptake, disease resistance, and flourish the quality of secondary metabolite compounds particularly antioxidant compounds of plant. Therefore, the positive impact of phyto-microbiome association with medicine-spice plants grasps the interest in research field to understand and identify the genetic and functional diversity between the microbial communities. Hence, multifunctional PGPM-based commercial formulations used as biocontrol agents and biofertilizers in agricultural crop management practices, which would give more focus to minimize synthetic fertilizers and agrochemical residual impact on the environment and biodiversity. Although several researches have been proved the efficiency of PGPF and PGPR on plants for few decades, broad spectrum use of these products is in continue till now. Farmers are still lacking knowledge of awareness or benefits of good quality of biofertilizers and biopesticides as well as its mode of application in crop field area. The availability of bio-products to end users especially farmers should be ensured that it is of good quality with providing proper knowledge of PGPMs application on particular crops. A successful commercialization of any good biological agents' formulation depends on longer shelf life, easy to use, and increases its demand in market as well as to finale handlers. Currently, global interest would need to focus on identification and isolation of medicinal and spices plants derived antioxidant compounds which have may utilize in pharmacological industry also. Thus, this chapter explores the knowledge of PGPMs to enhance the essential constituents of natural antioxidant in herbaceous medicine-spice plants as well as its consumption by human as organic nutrients would reduce the negative impact of oxygen free radicals on both human and plant health.

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# Antioxidants in Spices: A Review of the Antioxidant Components and Properties of Some Common African Spices and Their Role in Human Nutrition and Plant–Microbe Interactions

# 12

Oluwatoyin Bolanle Oluwole and Oluwaseun Ademuyiwa

## Abstract

Across several civilisations of the world, spices have played a very important role. They are used not only for their culinary benefits but also for their medicinal values. In Africa as well, spices are special part of the cuisine and also a huge part of the traditional medicine system of the continent. Oxidative stress has been implicated in the pathophysiology of several diseases such as hypertension, diabetes and ageing. Spices have been touted as rich sources of dietary natural antioxidants after vegetables and fruits. Some notable spices which are indigenous to Africa include *Tamarindus indica, Trachyspermum ammi* and *Piper guineense*. These spices possess important bioactive components responsible for their biological activities. Some of these compounds are Capsaicin (*Capsicum annuum*), Piperine (*Piper guineense*) and Carvacrol (*Origanum syriacum*). These compounds have been reported to possess biological activities ranging from anticancer, cardioprotective, anti-inflammatory and antineurodegenerative. They have also been reported to be instrumental in plant–microbe interactions. These review attempts to look into some indigenous African spices, their bioactive antioxidant components and biological activities and their role in plant–microbe interactions.

**Keywords**

Sesquiterpene · Eugenol · Carvacrol · Piperine · Capsaicinoids

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## 12.1 Introduction

Spices have been used for centuries as they play significant roles in ancient civilisations (Erhenhi et al. 2016). In fact, as far back as 2000 BC, spices have been an important part of local South Asia and Middle Eastern dishes (Tapsell et al. 2006). They have been cultivated as far back as 3000 BC and used for their health benefits as far back as 5000 BC (Singletary 2016). Apart from the culinary appeal of spices, they have also been used since ancient times for their food preservation qualities and also for their health-promoting benefits (Sachan et al. 2018). Several of the common spices used have their origin in Asia (Green et al. 2012); however, with the advent of international travel and trade, most of these spices have become globally available and are used across several cultures and regions of the world.

Spices are often called adjuncts or food accessories as they confer taste and aroma on food and also stimulate appetite, give visual appeal to food and also enable the flow of gastric juice and are used in minute quantities in food without being the main food ingredients (FAO 2010). Concisely, spices are defined as dried seeds, roots, flowers, fruits or bark of plants which are utilised in minute quantities in order to give flavour, colour and taste to food or to serve as preservatives (Sachan et al. 2018).

Although the words “herbs” and “spices” are used interchangeably, herbs refer exclusively to green leafy parts of plants. These are different from vegetables as they do not make a complete food but are rather added in minute amount to confer taste and aroma on food. Spices, however, are obtained from every other parts of plants apart from leaves such as flower bud (cloves), bark (cassia, cardamom), fruit (*Capsicum* spp.), dried berry (allspice), root (horseradish), rhizome (turmeric, ginger) and seed (*Piper* spp., anise) (Green et al. 2012).

Apart from their property as food adjuncts due to ability to confer taste and aroma on food, spices are favoured due to their ability to act as carminative, antioxidants and also their ability to improve gastrointestinal movement, thus aiding digestion (Takeda et al. 2008). Spices are also functional foods as they have been demonstrated to play beneficial role in well-being and longevity beyond the basic nutritional requirement (Lobo et al. 2010).

Antioxidants are molecules which prevent, delay or reverse oxidative damage to target molecules (Yadav et al. 2016). They inhibit oxidative process even in minute amount. Since several metabolic activities in the body produce free radicals which an imbalance in the body’s production and elimination of these free radicals could prove harmful to the body’s homeostasis, antioxidants as a result are the body’s first line of defence against free radical damages (Santos-Sanchez et al. 2019). This imbalance has been implicated in several pathological diseased conditions ranging from cancer to cardiovascular diseases to neurodegenerative diseases (Santos-Sanchez et al. 2019). Some antioxidants are endogenous to the body, for example, catalase, superoxide dismutase and glutathione peroxidase; however, several important antioxidants are obtained from dietary sources such as fruits, vegetables, tea and spices (Yadav et al. 2016). While these endogenous antioxidant systems help to maintain homeostasis by maintaining balance in production and elimination of free radicals/reactive oxygen species/reactive nitrogen species, also dietary antioxidants have been associated with improving free radical/antioxidant homeostasis thus implicated in reduced risk of cancer, cardiovascular diseases and ageing (Salehi et al. 2018b).

Apart from fruits and vegetables, spices are another very important dietary source of exogenous antioxidants. They are rich source of antioxidant vitamins, minerals and polyphenols.

## 12.2 African Spices

Spices are important components of several African dishes. In fact, it is said that the cultivation and use of spices originated from Egypt and then moved through the Middle East before spreading through the Mediterranean and then to Europe. It later spread to India, China, Indonesia and then to America in the seventeenth century (Fasoyiro 2015). Spices contribute to national security in sub-Saharan Africa and have become a source of foreign exchange in the continent (Fasoyiro 2015). Several countries in Africa are known for their spice trade. For example, Ethiopia is the ninth spice producing nation in the world (FAO 2011), Zanzibar, an island in Tanzania; East Africa is referred to as the spice island (Akyoo and Lazaro 2007), while Nigeria is the second ginger-producing nation in the world after India (Mazza et al. 2019).

Some herbs and spices which are indigenous to Africa and are important component of several cooking traditions across the continent include *Trachyspermum ammi* (ajwain), *Piper guineense* (West Africa Black Pepper), *Aframomum melegueta* (Grains of Paradise), *Parkia biglobosa* (African Locust Beans) and *Tamarindus indica* (tamarind). Apart from the spices which are indigenous to Africa, the advent of globalization and international trade have ensured that other exotic spices which originally are not native to the region have been domesticated and have become popular in the region, example of such is *Syzygium aromaticum* (cloves) which originated from Indonesia and *Capsicum annum* (red chilli pepper) which originated from Mexico (Van Wyk 2013).

Several African countries also have some specialised spice blends which are used traditionally in their cooking. For example, “Berbere” is an Ethiopian spice blend of several spices which include onions, garlic, ginger, fenugreek, black cardamom, cumin, nutmeg and cinnamon. Another East African spice blend is “Mitmita” which contains chilli pepper, cardamom seeds, cloves and salt (Peethambaran et al. 2016). In North Africa, “Harissa” paste is a commonly used spice blend made from red chilli pepper, garlic, red bell pepper, coriander seed, caraway seed, lemon, tomatoes, olive oil and salt (R’him et al. 2010). The Moroccan “Ras El Hanout” is a blend of green cardamom pods, cloves, bay leaves, cinnamon, nutmeg, coriander seed, allspice berries, cumin seed, black peppercorns, fenugreek seeds, anise seeds, ginger, paprika, turmeric and red chilli pepper (Van Wyk 2013). In West Africa, the very popular “Suya” spice blend is a blend of roasted peanuts, garlic powder, onion powder, paprika, red chilli pepper, ginger powder and salt (Egharevba and Gamaniel 2017).

Apart from their use as flavourants and colourants in food, spices have been used traditionally in Africa for various therapeutic, biological and industrial purposes. In ancient times, the Egyptians embalm their dead with spices. The oils extracted from some of these spices are used in perfumery and as insect repellents (Fasoyiro 2015). Several of these spices (*Aframomum melegueta* (Grains of Paradise), *Piper guineense* (West African Black Pepper), *Zingiber officinale* (Ginger), *Allium ascalonicum* (Shallot), *Ocimum basilicum* (Sweet basil), *Allium sativum* (Garlic) and *Eugenia caryophyllata* (Clove)) are used traditionally across several African cultures to prepare soup for post-partum women as an aid to uterine contraction (Adepoju and Oluremi 2013). Also, *Piper guineense* has been used as antiemetic, antihelminthic and also as treatment for rheumatism and stomach ache (Erhenhi et al. 2016). Another African spice which has been utilised traditionally for its therapeutic purpose is tamarind. It has been used as an anti-inflammatory, antidiuretic, laxative and carminative. Spices also act in synergy with other food ingredients to enhance the health-promoting properties of other foods (Mann 2011). African spices like all other spices are also rich source of antioxidant vitamins, minerals, polyphenols and other antioxidant compounds. They can also enhance the antioxidant potential of other food ingredients.

## 12.3 North Africa

It has been reported that the use of spices originated from the Mediterranean region, specifically Egypt, and these spices still remain a major part of the cooking traditions in this region of Africa. These spices have also been used for their therapeutic properties as well. Some indigenous North African spices are *Origanum syriacum* (Syrian oregano), *Cuminum cyminum* (cumin) and *Pimpinella anisum* (Anise).

**Origanum syriacum***:* This spice originates from the East Mediterranean region of Egypt, Sinai Peninsula, Lebanon, Jordan and Syria. It is the plant which bible scholars believe is the biblical hyssop, and thus, it is commonly referred to as bible hyssop or Syrian oregano. It is also called Za'atar in the Arab world. It is a perennial herbaceous plant of the family *Lamiaceae* (Farhat et al. 2012). It has been used since ancient times in Arab folk medicine as an analgesic, antihelmintic, carminative, antiparasitic, expectorant, remedy for tooth and joint aches and also as a relieve for bronchitis and gastrointestinal problems. It has also been used to relieve dysmenorrhoea, colic or cough (Chishti et al. 2013).

Although most research work done on the plant has been on its essential oil, its crude extract has been reported to contain several phenolic compounds which are very strong natural antioxidants. These compounds include rosmarinic acid (which is peculiar to plants of the lamiaceae family), catechol, rutin, quercetin, apigenin, luteolin and caffeic acid. These compounds have been demonstrated to possess antioxidant activity which is responsible for their anticancer, antineurodegenerative, cardioprotective, antidiabetic and antimicrobial effects (Afify et al. 2014). Ayesh et al. (2014) reported the cytotoxic effect of the ethanolic extract of *Origanum syriacum* on human leukaemia THP-1 cells. The methanolic extract has also been reported to inhibit the proliferation of breast adenocarcinoma and human cervical adenocarcinoma cell lines (Al-Kalaldeh et al. 2010; El-Desouky et al. 2009). Phytochemical analysis of these extract revealed the majority of the constituents to be flavonoids (El-Desouky et al. 2009).

The essential oil of *Origanum syriacum* has been acclaimed to possess numerous biological activities. It is considered to be one of the top essential oils in the world. This essential oil is a combination of several triterpenoids; however, the major components of the essential oil of *Origanum syriacum* are carvacrol (78.4%) (a phenolic oil which is responsible for its pungent flavour), thymol (17.9%), thymoquinone (2.5%) and β-caryophyllene (0.8%) (Farhat et al. 2012). These components have been reported to possess antioxidant activities. Carvacrol inhibits the synthesis of inflammatory cytokines and prostanoids, thereby useful in the treatment of gastric ulcer (Silver et al. 2012). The antioxidant property of this compound also makes it a very effective antiageing agent. Thymoquinone is another bioactive compound in the essential oil of *Origanum syriacum* which has been reported to possess anticancer, anticonvulsant, analgesic and antioxidant properties. It also inhibits angiogenesis, thus protecting the liver, heart and kidney against damage (Torres et al. 2010; Zein et al. 2012). Studies have also shown the strong antioxidant effect of essential oil of *Origanum syriacum* in inhibiting lipid peroxidation and protein oxidation in processed chicken meat patties and cooked chicken meat, thereby maintaining the quality and stability of the products (Al-Hijazeen 2018, 2019). Thymol and Carvacrol have also been reported to possess antimicrobial activity against gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and fungi such as *Aspergillus niger* and *Aspergillus flavus* with thymol being more effective than carvacrol as an antimicrobial agent (Al-Mariri et al. 2019).

The use of essential oil of *Origanum syriacum* has been found to be safe with no adverse effect; however, consumption above 200 mg/kg body weight could result in food not being palatable as a study in Wistar rat has demonstrated (Rychen et al. 2017). Also, according to FAO/WHO (2008), the $LD_{50}$ of the essential oil of *Origanum syriacum* is 2790 mg, $LD_{50}$ of thymol is 980 mg and $LD_{50}$ of carvacrol is 810 mg.

***Cuminum cyminum* (Cumin):** Cumin is an herbaceous, annual, flowering plant of the family *Apiaceae*. It is native to the region of East Mediterranean to East India. It thrives in hot and arid lands; however, it has found its way to warm regions of Europe such as Spain, Greece and Turkey. It is used traditionally in several Middle Eastern, Indian Mexican and Cuban cuisines. It is used to flavour dishes such like tacos, curries and enchilada. It is very rich in antioxidant vitamins and minerals (Nadeem and Riaz 2012). Cumin was used traditionally in ancient Egyptian civilisation to preserve their dead in a process called mummification. The seed extract is been used therapeutically in several ancient medical systems. For example, it has been used in Indian Ayurveda medicine to treat dyspepsia and chronic diarrhoea (Tabasun et al. 2018). It has also been used in traditional Chinese medicine to treat hypertension, blood hyperviscosity, arteriosclerosis and high cholesterol (Fang et al. 2018). The characteristic flavour and aroma of cumin are due to the presence of cuminaldehyde in the essential oil of cumin (Khan et al. 2017). It has also been used traditionally as anticonvulsant, anti-inflammatory, carminative, anti-spasmodic, diuretic and remedy for toothaches, jaundice, flatulence and indigestion. The essential oil is used as flavourant for condiments, desserts and alcoholic beverages. It is also used as a fragrance in cosmetics industries in the production of creams, lotions and perfumes (Singh et al. 2017).

The cumin seeds consist majorly of aldehyde (60%) with fats, amino acids, flavonoids and glycosides making up 22% and volatile oil making up 2–5%. The major component of its yellow-coloured essential oil is cuminaldehyde (Singh et al. 2017). Cumin seeds contain phenolic acids (gallic acid, cinnamic acid, salicylic acid), flavonoids (rutin, coumarin, quercetin) and several diterpenes which are strong antioxidants inhibiting lipid peroxidation and protein oxidation (Gallo et al. 2010). The essential oil of cumin contains β-pinene, γ-terpinene, cuminaldehyde and ρ-cymene with cuminaldehyde and ρ-cymene being the major component. The antioxidant activities of these compounds have been documented (Singh et al. 2017). Cuminaldehyde has been demonstrated to be effective in treating the symptoms of Parkinson's disease as it inhibits the fibrillation of alpha-synuclein (α-SN) (Morshedi et al. 2015) and also inhibits tyrosinase which in turn prevents the oxidation of L-3,4-hydroxyphenylalanine (L-DOPA) (Singh et al. 2017). Cuminaldehyde has also been demonstrated to possess antidiabetic effect as it is able to inhibit α-glucosidase almost as well as the therapeutic drug acarbose. It has also been demonstrated to inhibit lipid peroxidation in the liver of rats by scavenging hydroxyl and peroxyl radicals (Nadeem and Riaz 2012). Also, the chemopreventive, antibacterial, antiplatelet and antifungal activities of cuminaldehyde have been reported.

Cuminaldehyde and another constituent of the essential oil of cumin and thymol have been reportedly used to treat skin disorders as they help to detoxify the skin via the excretion of toxic substances from the body (Singh et al. 2017). Also, the anticholesterolemic and antiobesity effects of cumin have been associated with its phytosterols which include β-sitosterol, δ5-avenasterol and δ7-avenasterol. These phytosterols have also been demonstrated to possess radical scavenging activities (Zare et al. 2014).

However, cumin should be taken with caution as toxicity could lead to dermatitis, respiratory reaction and hypoglycaemia. Also, people taking drugs like antidiabetic, painkillers, antibiotics, anticancer and oestrogens should use cumin with caution. The $LD_{50}$ of the essential oil of cumin is 0.59 ml/kg body weight, while the seed powder can be used at a single dose of 300–500 mg (Al-Snafi 2016).

***Pimpinella anisum* (Anise):** *Pimpinella anisum* is an annual herbaceous plant of the family Apiaceae or umbelliferae indigenous to the East Mediterranean region of Egypt, Sinai Peninsula and Iran. It has, however, been domesticated and is commercially produced in regions of South Asia, Europe, Russia and North Africa. It is commonly called anise or aniseed and is one of the most commonly used spices in the world (Kucukkurt et al. 2009).

It has been used traditionally in Asian and Mediterranean folk medicine as remedy for cough, bronchitis, asthma, kidney stones and other inflammatory diseases. It has also been used as an anaesthetic, carminative, antispasmodic and for stimulating lactation in nursing mothers (Zheljazkov 2013). The fruit of anise is also used traditionally in Bulgarian culture in baking and liquor production, while the essential oil is used as a flavouring agent in pharmaceutical, cosmetics, candy and perfume-making industries (Tonutti and Liddle 2010). Several recent studies have demonstrated the hepatoprotective, gastroprotective, antiepileptic, anticancer and analgesic agent (Acimovic et al. 2015). The seeds have also been used as diuretic, disinfectant and antidepressant (Shojaii and Fard 2012).

Essential oil makes up about 1.5–5.0% of anise seed, and studies have reported that the essential oil of anise seed contains the compounds *trans*-anethole which is the major bioactive component and makes up about 85% of the oil. It is responsible for the distinctive aroma and taste of anise seed. It also contains eugenol, methyl chavicol, anisaldehyde, estragole (Gulcin et al. 2003; Tabanca et al. 2005) and γ-himachalene (Acimovic et al. 2015). Other compounds which are present in the essential oil of anise in concentration above 0.06% are α-cuparene, *cis*-anethole and β-bisabolene (Ozcan and Chalchat 2006).

A study by Kucukkurt et al. (2009) reported that the supplementation of aniseed in the diet of laying quails help to improve antioxidant activity by increasing the production of glutathione while also reducing lipid peroxidation. In another report, consumption of anise was found to aid digestion, prevent fatty acid oxidation, act as antioxidant and also increase the utilisation of nutrients (Al-Shammari et al. 2017).

Studies on the extract of anise seeds have reported the presence of polyphenols such as quercetin, rutin, luteolin, isoorientin, isovitexin, catechin syrinic acid, chlorogenic acid, ρ-coumaric acid, coumarin, cinnamic acid, chrisin, kaempferol and naringenin all of which have been reported to possess strong antioxidant activities which are responsible for their cardioprotective, neuroprotective, hepatoprotective, antidiabetic and chemopreventive effects (Shojaii and Fard 2012; Rebey et al. 2019).

Reports have demonstrated the cerebroprotective, neuroprotective and anticonvulsant effect of the compound *trans*-anethole. It is a substrate used for the synthesis of several anticonvulsant drugs such as chloral and pentobarbital (Karimzadeh et al. 2012). Anethole has also been proven to possess anti-inflammatory, anticancer and antimicrobial effect. It has been reported to inhibit the growth of the bacteria *Salmonella enterica, Pseudomonas aeruginosa* and *Bacillus subtilis* and the fungi *Candida* spp., *Trichophytum* spp., *Microsporum* spp. and *Geotrichum* spp. The aqueous, ethanol and methanol extracts of the seed have also been demonstrated to possess antimicrobial effect as well; however, the essential oil is a more powerful antimicrobial agent than the extracts due to the higher concentration of anethole in the oil (Kosalec et al. 2005; Azadeh et al. 2016). Asadollahpoor et al. (2017) also reported the ability of *trans*-anethole to protect against hepatic liver damage in nonalcoholic fatty liver disease. Anethole and eugenol (another component in the essential oil of anise) have been demonstrated to possess immunomodulatory effect by stimulating cell-mediated immune response. Eugenol also inhibits leucocyte chemotaxis in vitro (Al-Omari et al. 2018). Anise oil has also been demonstrated to possess antidiabetic, hypolipidemic and antioxidant effect in male albino rats (Helal et al. 2019). It has also been reported to help in the relief of dysmenorrhoea and menopause symptoms (Anwar 2017).

However, when consuming anethole, its effect on hormonal balance should be taken into consideration as it could lead to low sperm count in males. It should also be taken with caution in young children, pregnant and breast-feeding women. In fact, it is not recommended for children under the age of 12. It also has blood-thinning effect and thus should be taken with caution when on anticoagulants. The $LD_{50}$ of anise oil is 2.7 g/kg body weight, while anethole can be taken at an acceptable dose of 0.2 mg/kg body weight (EMA 2013) (Fig. 12.1).

## 12.4 East Africa

East African cuisines have been influenced by Omani, Yemeni and Indian Settlers who brought with them their spices. These spices have now been domesticated in this region alongside the spices which are indigenous to this region of Africa such that East Africa is the biggest Spice exporting region in Africa with Ethiopia and Tanzania being major players in the world spice trade industry (McCann 2009; Van Wyk 2013). Some of the spices for which East Africa is famous include *Trachyspermum ammi* (Ajwain), *Elettaria cardamomum* (Cardamom) and *Tamarindus indica* (Tamarind).

***Trachyspermum ammi* (Ajwain):** Trachyspermum ammi is an annual herbaceous plant of the family Apiaceae. It is commonly called “ajwain” or “ajowan”, bishop’s weed, carom or Ethiopian cumin (Nisar et al. 2019). It is commonly grown in the arid and semi-arid region of the world. It is indigenous to Ethiopia, Egypt, while it is also wildly cultivated in Iran, India, Pakistan, Afghanistan and some parts of Europe (Zarshenas et al. 2014).

Traditionally, the seed of “ajwain” was useful for treatment of neuronal and cerebral disorders such palsy, paralysis and tremor. It was also used to treat eye and ear infections. It has also been used as an antitussive and also to treat respiratory infections. Fruits were also administered for treatment of gastrointestinal disorders such as nausea, reflux, vomiting, loss of appetite and abdominal cramps. It was also used widely as a carminative, diuretic, antihelmintic, galactagogue and also as aphrodisiac (Zarshenas et al. 2014). It is also used in Ayurveda medicine to relieve colic pains, asthma, piles dyspepsia, flatulence, acute pharyngitis, sore throat and common cold (Chauhan et al. 2012). It has also been used as a flavourant in food, pharmaceutical and cosmetic industries.

**Fig. 12.1** Some North African Spices (Source: Gardenia.net 2015; Singh et al. 2017)

“Ajwain” is very rich in vitamins and minerals, containing appreciable amounts of riboflavin, nicotinic acid, thiamine, ascorbic acid, carotene, calcium, manganese, phosphorus, zinc, iron, chromium, cobalt, copper and iodine (Dwivedi et al. 2017). It also contains abundance of secondary metabolites such as flavonoids, phenolic compounds, coumarins, steroids, alkaloids, tannins and terpenoids (Mostafavi and

Pezhhanfar 2015). Essential oil makes up 2.5–5% of the seeds; this essential oil is majorly responsible for the pungent aroma and taste of ajwain. The major component of the essential oil of ajwain is thymol which makes up about 40–50% of the brown-coloured oil. Other components of the essential oil of ajwain which are the nonthymol part are generally called thymene, and it consists of carvacrol, γ-terpinene, ρ-cymene, α-pinene, β-pinene, α-terpinene, styrene, limonene, fenchyl alcohol, ethylene methacrylate, heptadecane, diethyl phthalate, terpinene-4-ol, δ-3-carene and β-phyllanderene (Nisar et al. 2019).

It has been demonstrated that the alcoholic extract of ajwain seeds has analgesic and antinociceptic effect, so does the essential oil. This could be due majorly to the presence of thymol which has also been reported to possess analgesic and antinociceptic effects (Zarshenas et al. 2014). The essential oil of ajwain is a strong antioxidant with better peroxide scavenging activity than ascorbic acid with increasing concentration. It also possesses better ferric reducing antioxidant power than ascorbic acid with increasing concentration (Chatterjee et al. 2013).

Thymol and carvacrol have also been reported to possess antibacterial activity against *Helicobacter pylori, Staphylococcus aureus, Escherichia coli, Bacillus pumilus* and *Bordetella bronchiseptica* (Zarshenas et al. 2014). Thymol has also been reported to possess antifungal activity against *Candida* spp.*, Aspergillus niger* and *Curvularia ovoidea* (Khan and Jameel 2018). Also, thymol and γ-terpinene have been demonstrated to possess antiulcerogenic activity due to their antioxidant, anti-inflammatory and vasorelaxant activities (Eftekhari et al. 2019). Thymol has also been reported to prevent oxidative damage to DNA due to its free radical scavenging activity, thus responsible for its chemopreventive activity (Goswami and Chatterjee 2014). Thymol has also been demonstrated to possess antiplatelet and immunomodulatory activities via several antioxidative activities (nitric oxide scavenging activity, inhibition of nuclear factor kappa B (NF-κB)) which prevent oxidative damage to biological membranes (Ranjbaran et al. 2019). The antioxidant and antiaflatoxigenic activities of thymol and carvacrol have also been implicated in the food preservation potential of the essential oil of ajwain (Kedia et al. 2015).

ρ-Cymene, another important component of the essential oil of ajwain, has been demonstrated to possess antioxidant, anticancer, anti-inflammatory, antinociceptic and antimicrobial activities (Marchese et al. 2017). Although ajwain is relatively safe for consumption, it has been reported that over consumption could lead to teratogenicity which could explain its traditional use as an abortificant. The LD50 of the essential oil of ajwain is 2294 mg, thymol is 980 mg and carvacrol is 810 mg, and ρ-cymene is 1695 mg (Vazirian et al. 2018).

***Tamarindus indica* (Tamarind):** *Tamarindus indica* L. is a fruit tree belonging to the family *Fabaceae*. It is native to tropical Africa but also widely cultivated in other tropical continents/regions of the world (Menezes et al. 2016). It is widely cultivated in Ethiopia, Cameroon, Uganda, Central African Republic and Guinea, and it also grows in the wild in Nigeria (Naeem et al. 2017). It has been used traditionally to treat inflammation, sore throat, stomach disorders and rheumatism. Furthermore, the plant has been used as treatment for several other disease conditions such as dysentery, diarrhoea, respiratory diseases, constipation, worm infestation, malaria, gonorrhoea, diseases of the eye and aphrodisiac (Komakech et al. 2019). The fruit pulp of tamarind has a characteristic sweet acidic taste due to the presence of tartaric acid and reducing sugars. The pulp is used as spice in Indian and African dishes for seasoning foods, confectionaries, sauces, juices and other beverages, while the leaves and flowers can be eaten as vegetables and are prepared in a variety of dishes (Abubakar et al. 2010). It is also added to the traditional Northern Nigerian breakfast called pap or "kunun tsamiya" (Rao and Mathew 2012).

The tamarind pulp which is the part used as spice contains tartaric acid (8–18%), reducing sugars (25–40% of which 70% is glucose and 30% is fructose). It is also rich in vitamins and minerals like niacin, riboflavin, thiamine, potassium, iron, zinc, calcium, phosphorus and copper with little amount of vitamins C and A. The pulp also contains other organic acids like citric acid, malic acid, succinic acid and formic acid. This high amount of organic acids makes it one of the most acidic fruits known. It is also rich in amino acids, invert sugars, pectin, fats, pyrazines and thiazoles (Naeem et al. 2017). The pulp is also rich in phytosterols (β-sitosterol and stigmasterol) and the bitter principle tamaridine (Ferreira 2019). The pulp has been reported to be an antihypertensive agent, as a remedy for sore throat, cure for malaria fever, to relieve the effect of sunstroke and as an aid for gastrointestinal disorders (Zohrameena et al. 2017). The bitter principle tamaridine has been demonstrated to possess antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas savastanoi* and antifungal activity against *Candida albicans* and *Aspergillus niger.* Tartaric acid which is responsible for the acidic taste of the pulp has been reported to be a good laxative and also responsible for its antimalarial effect (Ferreira 2019). The hypolipidemic effect of the pulp has been associated with the presence of β-sitosterol and stigmasterol. These compounds have also been implicated in the ability of the pulp to inhibit atherosclerosis and also reduce blood pressure, although the presence of significant amount of potassium in the pulp is also a factor in its blood pressure-lowering effect. The pulp has also been reported to possess strong antioxidant activity which is associated with the presence of these earlier discussed phytonutrients as well as other phenolic compounds like procyanidins and catechins (Ferreira 2019). The phytosterols have also been reported to possess analgesic and anti-inflammatory activities. The hepatoprotective activity of the pulp has also been reported (Zohrameena et al. 2017). The presence of phytosterols has also been implicated in the antiobesity activity of the aqueous extract of the pulp (Iskandar et al. 2017).

Tamarind pulp is practically nontoxic and considered safe for consumption with an $LD_{50}$ above 5000 mg/kg body weight (Iskandar et al. 2017).

***Elettaria cardamomum* (Cardamom):** *Elettaria cardamomum* is a perennial herbaceous plant of the family *Zingiberaceae*. It is native to Tanzania in East Africa and widely cultivated in Guatemala, Mexico, Sri Lanka, India, Indonesia and Nepal (Ashokkumar et al. 2019). It is well known as "queen of spices", and it is commonly called small cardamom, true cardamom or green cardamom. It the third most expensive spice in the world after saffron and vanilla (Sharma et al. 2011).

Cardamom has been used traditionally for centuries in the management of asthma. It has also been used to treat cataracts, diarrhoea, nausea, teeth and gum infections and also to manage kidney, digestive and cardiac disorders (Ashokkumar et al. 2019). It has also been used for centuries as spice in traditional dishes like curry, coffee, cakes and bread. It is also used in the food industries as flavouring agent for alcoholic and nonalcoholic beverages, candies, meat and meat products and frozen desserts (Singh et al. 2018). Cardamom is very rich in vitamins and minerals with appreciable amounts of ascorbic acid, tocopherols, thiamine, riboflavin, niacin, manganese, magnesium, potassium, sodium, zinc, copper, calcium and iron.

Cardamom contains about 6–14% essential oil and has been used in pharmaceutical and nutraceutical industries (Hamzaa and Osman 2012). The components of the essential oil of cardamom include 1,8-cineole, α-terpineol, linalyl acetate, sabinene, nerolidol, linalool, α-pinene and α-terpinyl acetate (Yashin et al. 2017). The characteristic aroma of cardamom is due to the combination of 1,8-cineole (eucalyptol) and α-terpinyl acetate which are its most abundant components. The difference in aroma across species and varieties is due to the differences in the percentage composition of 1,8-cineole and α-terpinyl acetate. These essential oil components have been reported to possess antioxidant, antidiabetic, antiviral, antifungal, anti-inflammatory and gastroprotective abilities (Ashokkumar et al. 2019). Also, extracts of cardamom seeds and pods have been reported to contain other components such as anthocyanins, flavonoids (catechins, myricetin, quercetin and kaempferol), carotenoids (lutein and β-carotene) and little alkaloids. These components have also been reported to have potential benefit in the management of cardiovascular, lungs, kidney and pulmonary disorders (Vaidya and Rathod 2014).

The flavonoids, phenolic compounds and components of the essential oil of cardamom make the extract and the essential oil very good antioxidants and thus are useful in food preservation. The use of either the seed extract or the essential oil of cardamom in food preservation has been reported to exhibit antibacterial, antifungal and antioxidant activity, thereby preventing food spoilage (Singh et al. 2018). The presence of flavonoids and phenols has been implicated in the antimutagenic activity of cardamom due to their ability to scavenge free radicals and thus prevents oxidative damage to biomolecules like DNA, proteins and fatty acids (Saeed et al. 2014).

1,8-Cineole (eucalyptol) and α-terpinyl acetate have reported to be majorly responsible for the antimicrobial activity of cardamom essential oil. The essential oil has been reported to inhibit the growth of *Staphylococcus aureus, Salmonella typhi. Candida albicans, Streptococcus mutans* and *Aspergillus terreus* (Abdullah et al. 2017). The antiulcerogenic activity of the essential oil and petroleum ether extract of cardamom in ethanol and aspirin-induced gastric ulcer has also been reported (Farah et al. 2005). Eucalyptol has also been reported to possess anti-inflammatory and immunomodulatory activities. Another study also reported the antiscabies activity of essential oil of cardamom. This activity has been associated to the activities of 1,8-cineole, α-terpineol and γ-terpinene. 1,8-Cineole stimulates the activity of antioxidant enzymes superoxide dismutase and glutathione-*S*-transferase which helps protect against Sarcoptes scabiei mites. It also has insecticidal activity. α-Terpineol and γ-terpinene also possess insecticidal activity which could be responsible for their antiscabies potential (Sharma et al. 2020).

The essential oil of cardamom has also been reported to increase the level of glutathione in the body. Furthermore, the extract has been reported to possess anti-inflammatory, analgesic, antilipid peroxidation, antiplatelet aggregation and antispasmodic activities. It also possesses anticonvulsant and antidepressant activities (Sharma et al. 2011). Cardamom essential oil is quite safe with an $LD_{50}$ 5000 mg/kg body weight (Iskandar et al. 2017) (Fig. 12.2).

**Fig. 12.2** Some East African Spices (Source: Shameem 2016; Tramil.net 2017; Chauhan 2019)

## 12.5 West Africa

Spices have been part of West African cuisines for centuries. Apart from their culinary uses, they also have acclaimed importance in the traditional medicinal practices of the region. Some spices are native to the region; however, with the advent of globalization and influence of colonialism, several exotic spices have been domesticated and are now widely cultivated in the region as well. Some native West African spices are *Aframomum melegueta* (Grains of Paradise), *Piper guineense* (West African black pepper) and *Parkia biglobosa* (African Locust Beans).

***Aframomum melegueta* (Grains of Paradise):** *Aframomum melegueta* is a perennial herbaceous plant of the family Zingiberaceae (ginger family). It is widely cultivated in West Africa especially in Nigeria, Ghana, Cote d'ivoire, Togo and Liberia. It is commonly called grains of paradise or guinea pepper with the local names "ataare" in Yoruba, "ose-oji" in Igbo, "chitta" in Hausa and "fam wisa "in Ghana (Onoja et al. 2014). It is one of the most important plant in African ethnomedicine. It is used in divination and has been traditionally used to treat rheumatism, fever, constipation, snake bite, worm infestation and diarrhoea and gastrointestinal disorder (Onoja et al. 2014). The leaf has been reported to be used in the treatment of measles. The seed extract has also been reported to possess antiviral activity particularly against HIV. It has also been demonstrated to possess hepatoprotective, antimicrobial, anti-inflammatory, antioxidant and antiviral activities (Lawal et al. 2017).

The seed extract has reported to contain 6-paradol as the major constituent which is responsible for its pungent, peppery aroma. Other constituents include 6-gingerol, 8-gingerol, 6-gingeredione, zingiberone, methyl-6-gingerol, *cis*-isoelemicin, β-bisabolene, aromadendrene, geraniol, α-guaiene and *trans*-β-farnesene (Lawal et al. 2017; Osuntokun 2020). The entire plant is made of essential oil with the leaf oil containing myrtenyl acetate and isolimonene as its major constituents; the stem oil is made up majorly of caryophyllene oxide, myrtenyl acetate, β-eudesmene and β-caryophyllene. Also, the oil from the root contains myrtenyl acetate and pinocarvyl acetate as its major constituents, while the seed oil is largely made up of humulene, β-caryophyllene and their epoxides (Owokotomo et al. 2014).

6-Paradol and 6-shagoal have been reported to be responsible for the antimicrobial activity of *A. melegueta*. Reports have shown that 6-paradol and 6-shagoal were able to inhibit the growth of *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Serratia marcescens* (Lawal et al. 2017). Also, gingerol and 6-paradol possess anti-inflammatory activity as they inhibit prostaglandins and leukotrienes synthesis. (Kokou et al. 2013). Also, 6-paradol has been reported to possess anticancer activity. It was able to induce apoptosis in human pro-myelocytic leukaemia (HL-60) cells. It has also been demonstrated to inhibit growth of tumour on the skin (Osuntokun 2020). The ability of *A. melegueta* to protect against neurodegenerative diseases has also been reported as it inhibits the enzyme acetylcholine esterase (Adefegha and Oboh 2012a). Also, it was able to protect against neurotoxicity induced by monosodium glutamate in rat brain by scavenging nitric oxide, increasing glutathione levels, increasing the activities of antioxidant enzymes catalase and superoxide dismutase while also inhibiting the activities of acetylcholine esterase and monoamine oxidase (Fasakin et al. 2017). This neuroprotective activity could be due to gingerols that have been found to inhibit or prevent the progression of Alzheimer's disease in rat model by inhibiting neuroinflammation while also decreasing β-amyloid deposits (El-Halawy et al. 2017). Its ability to inhibit the activities of the enzymes α-amylase and α-glucosidase has been reported, thereby reducing blood glucose and consequently producing an antidiabetic effect (Adefegha and Oboh 2012b). Mohammed et al. (2017) traced the antidiabetic activity of *A. melegueta* to the presence of 6-gingerols and oleanolic acid in the extract. Another report also demonstrated the hepatoprotective activity of *A. melegueta* as it was able to protect against hepatic damage by carbon tetrachloride ($CCl_4$) by scavenging trichloromethyl, a metabolite of $CCl_4$ which induces liver damage. It also inhibits inflammation which occurs as a result of liver injury by inhibiting the synthesis of C-reactive protein, cyclooxygenase-2 enzyme and prostaglandins formation (Kokou et al. 2013). A recent study has also demonstrated the efficacy of methanolic seed and leaf extract of *A. melegueta* in treating anaemia as it is able to increase haemoglobin levels and platelet count in anaemic rats (Omoboyowa et al. 2017). Also, the ability of the plant to increase body's energy metabolism via activation of brown adipose tissues has been employed in the management of obesity (Sugita et al. 2013).

Although according to Food and Drug Administration (FDA), *A. melegueta* is considered relatively safe for consumption with minimal or no side effect with the seed oil having an $LD_{50}$ of 273.86 mg/kg body weight (Akpanabiatu et al. 2013), a study showed that it causes loss of pregnancy especially during the first trimester in Sprague Dawley rats and therefore should be used with caution during pregnancy (Inegbenebor et al. 2009).

***Piper guineense* (West African Black Pepper):** *Piper guineense* is a tropical West Africa spice plant of the family *Piperaceae.* It is a species of piper which is native to West Africa. It is commonly referred to as West African Black Pepper and locally called Ashanti pepper in Ghana, "Uziza" in Igbo and "Iyere" in Yoruba. It is also referred to as Benin pepper and false cubeb (Balogun et al. 2016). The fruits and leaves of *P. guineense* have been used in traditional medicinal practices across West Africa. It has been used in the Nigerian traditional medicine landscape as a relieve for stomach discomfort due to excess gas. It has also been used to treat rheumatism, syphilis and respiratory infections (Uhegbu et al. 2015). It has also been reportedly used in Southern Nigeria to treat infertility and pain and also as an aphrodisiac (Oyemitan et al. 2014). In the Yoruba herbal medicinal practice, it is used in conjunction with other herbs to make a potherb for the treatment and management of neurological and neurodegenerative diseases (Elufioye et al. 2012).

It is a spice of note in West African cuisine, especially Nigeria and Ghana and use to flavour soups, stew and meat. In South Eastern Nigeria, it is one of the ingredients used to prepare soup for post-partum women to help aid uterine contraction. It is also used as a preservative in food and food products. Its essential oil has also been employed for soap and perfume making (Uhegbu et al. 2015).

The plant is rich in nutrients and is a rich source of vitamins and minerals like ascorbic acid, tocopherols, thiamine, riboflavin, niacin, manganese, calcium, magnesium, potassium, sodium, zinc, iron, copper and chromium (Imo et al. 2018). Phytochemical analysis of extracts of the plants has also identified the presence of alkaloids, saponins, tannins, cardiac glycosides and flavonoids (Balogun et al. 2016). The seed of the plant is also rich source of essential oil containing about 2.5–7% essential oil and vary in component from one region to the other. However, some of the components of the essential oil which have been identified are 1,8-cineole, myristicin, safrole, elemicin, α-pinene, β-pinene, D-limonene and caryophyllene (Oyemitan et al. 2014). *P. guineense* like all other plants from the genus piper contain the alkaloid piperine which is responsible for their distinctive peppery aroma and taste.

The extracts of the plant be it leaves of seeds have been reported to possess several biological activities. For example, the aqueous extract of the seed has been reported to protect against oxidative damage to liver by increasing the activity of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase (Uhegbu et al. 2015). The methanolic extract of the seed also was able to reverse or prevent hepatic injury caused by $CCl_4$ by scavenging free radicals, increasing the activity of antioxidant enzymes, preventing the accumulation of lipids and overall maintaining the integrity of the cell membrane of hepatocytes (Oyinloye et al. 2017). The leaf extract has also been reported to possess antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans* and *Saccharomyces cerevisiae* (Anyanwu and Nwosu 2013). Other biological activities of the extracts include hypolipidemic, antitumour, immunomodulatory, antiparasitic, antianaemic, anxiolytic and antiatherosclerotic effects (Balogun et al. 2016).

The essential oil of the plant has also been reported to possess several biological activities. The antioxidant activity of the essential oil has been attributed partly to the presence of α-pinene and 1,8-cineole which have strong ferric reducing antioxidant power. The essential oil has also exhibited the ability to inhibit starch metabolising enzymes α-amylase and α-glucosidase. It also inhibited angiotensin converting enzyme 1 and therefore could be a possible therapeutic approach for the management of diabetes mellitus and hypertension (Oboh et al. 2013). The anti-inflammatory and analgesic effects of the essential oil have also been reported (Oyemitan et al. 2014). In another study, the effect of the essential oil of *P. guineense* on the central nervous system was elucidated. It was reported to act as sedative, anticonvulsant, anaesthetic, acetylcholine esterase inhibitor, antidepressant and monoamine oxidase inhibitor. All these activities have been reported to be due to the synergistic and additive effects of the various components of the oil such as D-limonene, linalool and β-sesquiphellandrene (Oyemitan et al. 2015).

Piperine which is a major distinctive bioactive component of *P. guineense* has been isolated and studied extensively. A study elucidated the activity of piperine in preventing hepatic and neurotoxicity caused by microcystic-LR in mice via its antioxidant and anti-inflammatory activities. However, when combined with thymoquinone, it produced better therapeutic effect (Abdel-Daim et al. 2019). Other studies have also reported the antidiabetic, immunomodulatory, antiinflammatory, antihypertensive, chemopreventive and neuroprotective effect of piperine (Stojanovic-Radic et al. 2019).

Another component of *P. guineense* piperamide has also been reported to possess antimicrobial activity against *Sarcina* sp.*, Staphylococcus aureus* and *Bacillus subtilis* (Mgbeahuruike et al. 2018).

*P .guineense* is quite safe for consumption with the essential oil having an $LD_{50}$ of 1265 mg/kg body weight oral and 693 mg/kg body weight when administered intraperitoneally (Oyemitan et al. 2014), while the seed extract has an $LD_{50}$ of 2000 mg/kg body weight (Kabiru et al. 2016).

***Parkia biglobosa* (African Locust Beans):** *Parkia biglobosa* is a perennial, deciduous tree plant of the family Mimosaceae. It is native to Nigeria, Ghana and some other West African countries. It can also be found in Southern and East Africa (Sackey and Kwaw 2013). It is commonly called African Locust Beans. It is locally called "Iru" in Yoruba and "Dawadawa" in Ghana and Hausa. Different parts of the plants have been used in folk medicine to treat ailments like hypertension, dermatosis and haemorrhages. In Northern Nigerian folk medicine, the stem back is commonly used to treat diabetes mellitus (Dluya et al. 2015). The plant is used across West Africa in their folk medicine practices. It is used in Benin to treat cardiovascular diseases, infectious diseases and rheumatism. In Burkina Faso, it is used to treat oral infections and sores. In Ghana and Cote d'ivoire, it is used to treat malaria and stomach ache. In Mali, it is used to treat urinary tract infections and malaria. In Togo, it is used to treat haemorrhoids, diarrhoea and cardiovascular diseases. In Nigeria, it is used to treat diarrhoea and inflammation in the North, hypertension, infertility, stroke, skin lesions and eye infection in the South-West and also used to treat hypertension in the South-East (Alinde et al. 2014). Several recent reports have also demonstrated the antidiarrheal, antibacterial, anti-inflammatory, analgesic anticancer and antihypertensive effect of the plants (Alinde et al. 2014).

The seeds are usually fermented (as fermentation reportedly enhanced the nutritional status of the seeds) and used as seasoning/flavouring agent in soups and stews in West African cuisine (Sackey and Kwaw 2013). The plant is a rich source of nutrients such as reducing sugars, free amino acids, tocopherols, fatty acids like, arachidic acid, linoleic acid, palmitic acid and stearic acid. It is also rich in thiamine, riboflavin, magnesium, sodium, potassium, zinc, calcium, copper and iron (Daramola 2014). Phytochemical analysis has reported the presence of alkaloids, saponins, cardiac glycosides, sterols, tannins, terpenes and resins (Alinde et al. 2014).

The fermented seeds which are commonly used as spice in West African cuisines were found to produce better growth and feed utilisation when supplemented in the diet of *Clarias gariepinus* due to its rich protein and amino acids content (Michael and Matthias 2020). It has also been suggested the antioxidant activity of *P. biglobosa* could be due to the presence of free amino acids, peptides, oxidised lipid-amino acid reaction products, phenolic compounds and tocopherols (Daramola 2014). The cardioprotective and hypolipidemic effects of the fermented seeds have also been reported. It increases HDL-cholesterol concentration while reducing LDL-cholesterol and triglyceride concentration in included in the diet. The high calcium and potassium content and the presence of flavonoids and cardiac glycosides of the seeds have been implicated in its antihypertensive and cardioprotective activities (Ognatan et al. 2011). It also antioxidant and hypolipidemic activities when supplemented in the diet of rats induced with hyperlipidemia using tyloxapol (Ayo-Lawal et al. 2014). The fermented seeds have also been reported to produce antidiabetic effect in alloxan-induced diabetic rats (Builders 2014).

Although it is the seeds of the plant which is used as spice, various studies have associated a lot of biological and therapeutic activities to its stem bark, roots and leaves. For example, the stem bark has been reported to possess antimicrobial activity (Abioye et al. 2013). The leaves have also been reported to possess neuroprotective, antioxidant, cardioprotective and antihypertensive effects (Komolafe et al. 2014; Komolafe et al. 2017a, b). Fermented *Parkia biglobosa* seed is safe for consumption, and no toxicity has yet been identified (Fig. 12.3).

## 12.6 Southern Africa

Very little evidence is available for the use of spices in Southern African dishes in ancient times as spices are quite rare to the southern African landscape. However, the settling of other groups of people (Khoi, Bantu, Indian, Dutch, Malay) in the region has greatly influenced the cooking traditions of the region. For example, the famous Cape cuisine is influenced by the Dutch and Malay settlers of the region (Asowata-Ayodele et al. 2016; Van Wyk 2013). However, some plants which were later recognised as spices have been used for centuries for their medicinal benefits. Also, global travel and immigration have meant that some exotic species have also been introduced into the region. Some of the spices used in Southern Africa are *Anethum graveolens* (Dill), *Capsicum annum* (Chilli pepper) and *Carissa edulis* (Natal-plum).

***Carissa edulis* (Natal-Plum):** *Carissa edulis* is a perennial shrub belonging to the family Apocynaceae. It is native to tropical and subtropical regions of the world. In Southern Africa, it is found in countries like South Africa, Lesotho, Zimbabwe, Eswatini, Namibia and Zimbabwe. It is commonly called natal-plum or num-num, while locally it is called “noem-noem” in Afrikaans and “cizaki” in Hausa (Bester 2014). Apart from being used as a flavourant and preservative, the fruit is eaten as snack as it has a delicious flavour. It is also used to make jellies, jams and soups. It has been used traditionally to treat chest pain and as an antiviral agent (Asowata-Ayodele et al. 2016). It has also been reported to be used to treat

*Aframomum melegueta*

*Piper guineense*

*Parkia biglobosa*

**Fig. 12.3** Some West African Spices (Source: Wikiwand 2021; Patil 2017; Senckenberg.de 2021)

rheumatism, epilepsy, hernia, sickle cell anaemia, gonorrhoea, syphilis, toothache and worm infestation (Yadang et al. 2019). It was also used as part of an ancient Swazi ceremony to increase courage and ferocity of black bull, when Swazi warriors are tested by killing the bull with bare hands (Bester 2014). Scientific reports have reported the use of *C. edulis* as antiplasmodial, anticonvulsant, diuretic, antiviral, analgesic, anti-inflammatory, cardioprotective, hepatoprotective, antitumour and antidiabetic agent (Nantango et al. 2018).

Phytochemical analysis has confirmed the presence of flavonoids (anthocyanins which are characteristic of the fruits red and purple colour, lupeol), sterols, phenolic compounds (ursolic acid, oleanolic acid), sesquiterpenes, lignans and triterpenes (β-amyrin, carandinol) (Kaunda and Zhang 2017).

The fruit extract has been reported to possess antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis* and *Schleichera oleosa* (Ibrahim et al. 2010; Toobpeng et al. 2017).

The fruit extract also displayed cytotoxic activity in breast adenocarcinoma, lung cancer, cervical carcinoma and hepatocellular carcinoma cell lines (Souilem et al. 2019). Its considerable flavonoid content has also been implicated in its antinociceptic activity in rats (Gitahi et al. 2015). A molecular docking study done using some compounds isolated from Carissa carandas showed that the compounds carandinol, carissone, lupeol and ursolic acid which are also present in *C. edulis* were able to inhibit the HIV-1 reverse transcriptase and HIV-1 protease enzymes and thus can be exploited as possible therapeutic alternative for HIV treatment and management (Singh et al. 2019). The sesquiterpenes (6β-carissanol, 2α-carissanol, dehydrocarissone, cryptomeridiol, carissone and β-eudesmol) present in *C. edulis* have been reported to possess antimicrobial, antimalarial, anticancer and anti-inflammatory effects. Also, the lignans (secoisolariciresinol, carinol, (−)olivil and nortrachelogenin) which have been isolated from *C. edulis* have been reported to possess antitumour, antiallergic, antiviral and antimitotic effects (Al-Youssef and Hassan 2014).

*C. edulis* is safe for consumption as the fruit can be eaten as snack and used to make jams and jellies (Asowata-Ayodele et al. 2016).

***Capsicum annuum* (Chilli Pepper):** *Capsicum annuum* has been in use as far back as when civilisation began. It is a perennial shrub of the family *Solanaceae*. Its use in human diet has been traced to as far back as 7500 BC. Its origin has been traced to the Americas as Native Americans have been cultivating *Capsicum annuum* since between 5200 and 3400 BC. It was distributed to other continents of the world by the Spanish and Portuguese traders (Nadeem et al. 2011). It has been used traditionally in folk medicine as relieve for gastric ulcer, toothache, diabetes and rheumatism. Recent reports have also elucidated the antioxidant, antiobesity, hypolipidemic, anti-inflammatory and blood glucose-lowering effect of *C. annuum* (Badia et al. 2017). *Capsicum annuum* is a rich source of vitamins and minerals like selenium, iron, calcium, phosphorus, sodium, potassium, copper, niacin, thiamine and ascorbic acid (Salehi et al. 2018a).

The strong pungent taste of *C. annuum* is due to the group of alkaloids called Capsaicinoids. These capsaicinoids possess chemical structure similar to Piperine (in *Piper* sp.) and Zingerone (in ginger). The most abundant of the capsaicinoids is capsaicin; others are dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin (Badia et al. 2017). Apart from the pungent principle capsaicin, other components of *C. annuum* include flavonoids (3-O-ramnosilquercetin, 7-O-glucosilluteolin), phenolic compounds of the cinnamic derivative group and carotenoids (Badia et al. 2017). Other compounds which have been identified in *C. annuum* are gallic acid and the stilbene, resveratrol (Medina-Juarez et al. 2012).

While the pungent taste and burning sensation on mucous membrane are due to capsaicinoids, the colours of *Capsicum annum* are due to the carotenoids present in the fruits. The carotenoids capsanthin and capsorubin are responsible for the red colour, while the yellow colour is due to zeaxanthin, luteolin and β-cryptoxanthin (Salehi et al. 2018a).

Extracts obtained from the plant were found to possess strong antioxidant activity as the capsaicinoids, carotenoids, flavonoids and resveratrol which are components of the extract are reported to be strong antioxidants; thus, additive and synergistic effects of these components are expected (Medina-Juarez et al. 2012).

The plant has been reported to be used for food preservation due to its antimicrobial activity. Earlier report attributes the antimicrobial activity of *C. annuum* majorly to capsaicin and dihydrocapsaicin; however, a recent report linked its antimicrobial effect to the synergistic activity of these two capsaicinoids and chrysoeriol, with chrysoeriol showing a better antimicrobial activity than capsaicin and dihydrocapsaicin when they were all tested singly (Salehi et al. 2018a).

Capsaicin has also been demonstrated to possess antioxidant activity comparable to butylhydroxyanisole (BHA). Also, sinapoyl and feruloyl glycosides in *C. annuum* have been reported to possess stronger antioxidant effect than capsaicin (Materska and Perucka 2005). Capsaicin has also been reported to possess anticancer, antirheumatoid and antiosteoarthritic activities (Saleh et al. 2018). Capsaicin was reportedly able to inhibit the proliferation of cancer cells in colon cancer by inhibiting the synthesis of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 10 (IL-10) and interferon gamma (IFN-γ) (Imran et al. 2018). Capsaicin is also able to initiate apoptosis in prostatic benign hyperplasia, thus exhibiting antitumour activity (Badia et al. 2017). Capsaicin has also been implicated in the hepatoprotective activity of *C. annuum*. It has been reported to stimulate the activities of antioxidant enzymes, scavenge free radicals and inhibit active caspase-3 (Hassan et al. 2012). The antiulcerogenic activity of capsaicin has also been reported which is partly due to its ability to inhibit the growth of *Helicobacter pylori*, a bacterium responsible for peptic ulcer in man (Badia et al. 2017). Capsaicin has also been reported to protect against neurotoxicity caused by glutamate. It decreases the synthesis of reactive oxygen species (ROS) in the brain while also inhibiting neuronal death caused by apoptosis. It also inhibits the synthesis of inflammatory cytokines (TNF-α and IL-1β) in the brain. It also inhibits lipid peroxidation in brain cells (Imran et al. 2018). Another report also showed that capsaicin when combined with soyflavones induced hair growth in guinea pigs (Imran et al. 2018). Other biological activities associated with capsaicin include hypoglycaemic, hypolipidemic, antiplatelet aggregation and cardioprotective activities (Saleh et al. 2018).

Dihydrocapsaicin, another capsaicinoid, has also been reported to possess antimicrobial activity against *Bacillus cereus, Bacillus subtilis, Clostridium sporogenes, Streptococcus pyogenes* and *Clostridium tetani* Al-Snafi 2015. Dihydrocapsaicin and capsaicin also inhibit lipid peroxidation in erythrocyte membranes. They also protect against radiation from X-rays (Hassan et al. 2012).

Although so many biological activities have been associated with the capsaicinoids, the huge biological benefits associated with *C. annuum* are not only due to them. Some reports showed that some species of capsicum with more flavonoids than capsaicinoids had better antioxidative potential than species with more capsaicinoids (Chavez-Mendoza et al. 2015). The carotenoids capsanthin, capsorubin and cryptocapsin possess very strong free radical scavenging activity, so also does the flavonoids luteolin and quercetin it contains (Nadeem et al. 2011).

Despite all these wonderful biological functions, *C. annuum* should be consumed cautiously as some reports have shown that overconsumption of could lead to impairment in learning and memory in rats due to the neurotoxic effect of capsaicin when consumed in large quantities over a long period of time (Nmaju et al. 2017). Also, some other studies have reported that overconsumption could lead to cancer even though pure capsaicin in itself is not carcinogenic. This could be due to Aflatoxin as the plant is easily infected by this carcinogenic agent, so this has been suggested to be the reason for the carcinogenicity observed in overconsumption. Another reason could be overconsumption above normal human consumption level as it has been observed that capsaicin is a weak mutagenic agent (Saleh et al. 2018). It should also be consumed cautiously in children below the age of 2 and pregnant women as capsaicin causes burning sensation in the stomach (Olatunji and Afolayan 2018). The $LD_{50}$ of *C. annuum* extract is 932.44 mg/kg body weight while that of capsaicin is 34.07 mg/kg in mice (Nmaju et al. 2017).

***Anethum graveolens* (Dill):** *Anethum graveolens* commonly called Dill is an aromatic herb of the family *Umbelliferaceae/Apiaceae*. It is native to the Mediterranean region and West Asia. However, it is now widely cultivated in India, Pakistan, Africa, China, USA, Canada, Turkey and Uzbekistan (Chahal et al. 2017). It is locally called “Dille” in South Africa. It is used by some indigenous community in South Africa to treat erectile dysfunction and ulcer while also being a good seasoning agent and preservative in their culinary preparations (Asowata-Ayodele et al. 2016). The seeds are used as flavouring agent due to their strong spicy aroma and used to flavour soups, salads, pickles, sauces and tea (Babri et al. 2012). In Ayurvedic medicine, the seeds are used to treat bladder inflammation, sleep disorder and hepatic diseases (Chahal et al. 2017). In the seventeenth century, it was used as a brain tonic in Europe. It is also used to stimulate lactation in nursing mothers while also been used as an antiemetic. The essential oil has also been reported to possess cardioprotective and hypolipidemic effects (Chahal et al. 2017). It was also used in Mediterranean culture to treat indigestion and flatulence (Haidari et al. 2020). The seed has also been reported to possess anticonvulsant, antispasmodic and wound-healing activities (Naseri et al. 2012).

The essential oil of *A. graveolens* contains carvone, D-limonene, α-phellandrene, dillapiole, dihydrocarvone and linalool as its major constituents (Singh 2012). The seed is a rich source of flavonoids, phenolic compounds and terpenoids.

Aqueous extract of the seed was reported to possess strong antioxidant activity which is comparable to Trolox. The antioxidant activity has been linked to the presence of anethole, D-limonene, carvone, apiole and polyphenols which are present in the extract (El-Mansouri et al. 2016). Another report demonstrated the antidiabetic and hypolipidemic effects of *A. graveolens.* Supplementation of the seed powder into the diet in patients with type 2 diabetes mellitus caused reduction in LDL and total cholesterol levels in the serum while increasing HDL level. Supplementation of 1.5 g/day for 6 weeks was able to reduce fasting blood glucose significantly. It was suggested that the ability of flavonoids, ascorbic acid and carotenoids which are some of the bioactive components identified in the seed powder to repair β-cells of the pancreas which in turn increases insulin secretion is one possible mechanism for the antidiabetic activity of *A. graveolens* (Haidari et al. 2020). *A. graveolens* also reduced the formation of advance glycation end products. It has also been reported to activate peroxisome proliferation-activated receptor alpha (PPAR-α), thereby normalising lipid profile in obese mice with diabetes mellitus. It could also upregulate the expression of genes involved in fatty acid oxidation. Quercetin, another flavonoid component of *A. graveolens,* suppresses the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis. All these various activities of the different components of *A. graveolens* are possible mechanisms in which *A. graveolens* produces its antidiabetic and hypolipidemic effects (Goodarzi et al. 2016).

D-Limone and carvone inhibit the synthesis of inflammatory cytokines and have been implicated in the analgesic and antinociceptive activities of alcoholic extract of *A. graveolens* (Rezaee-Asl et al. 2013). The antimicrobial activity of the seed extract against *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Escherichia coli, Yersinia enterocolitis* and *Salmonella typhi* has been reported. The essential oil also showed antifungal activity against *Penicillium islandicans* and *Aspergillus flavus*. The antimicrobial effect of D-limonene and carvone against *Aspergillus niger, Saccharomyces cerevisiae* and *Candida albicans* has been reported (Dhiman et al. 2017). The gastroprotective effect of the essential oil and seed extract of *A. graveolens* has also been documented. The essential oil had an antispasmodic effect on the intestine of rabbits, the seed extract protects the gastric mucosa from mucosa lesions caused by oral administration of hydrochloric acid in mice, and it also moderately inhibited the growth of *Helicobacter pylori,* thus protecting against peptic ulcer (Dhiman et al. 2017).

In a recent report, the boiled seed of *A. graveolens* when consumed was able to reduce anxiety during labour and reduce duration of labour while also increasing dilatation and effacement. This is as a result of the ability *A. graveolens* to inhibit biochemical changes which occur as result of stress which is indirectly linked with its antioxidant activity (Hetmatzadeh et al. 2020). The seed extract also had hepatoprotective effect in paracetamol-induced hepatotoxicity in rats. It increases the level of antioxidant enzymes while also acting as a free radical scavenger (Ramadan et al. 2013).

Carvone and D-limonene have been linked to the anticancer effect of *A. graveolens* due to their proven cytotoxic activity. The essential oil had antiproliferative and cytotoxic effect in human hepatocarcinoma cell lines. The methanolic seed extract has also been reported to inhibit the proliferation of cancer cells in mouse leukaemia, mouse skin melanoma and human cervical cancer cell lines (Al-Sheddi et al. 2019).

*A. graveolens* extract when used in combination with *Oryza sativa* (rice) extract caused a reduction in brain infarction and decreases levels of pro-inflammatory cytokines (interleukin 6 (IL-6) and nuclear factor kappa B (NF-κB)) in the brain while increasing the activity of antioxidant enzymes and the expression of endothelial nitric oxide synthase (eNOS) in the brain, thereby causing the improvement in neurological deficit in animal model of cerebral ischemia (Jintanaporn et al. 2019).

*Anethum graveolens* is quite safe for consumption but in rare cases could lead to allergic reaction such as throat swelling, urticarial, vomiting and diarrhoea. It is not recommended for use during pregnancy. The average daily dose for the seed is 3 g, while the essential oil is 0.1–0.3 g (Al-Snafi 2014) (Fig. 12.4).

## 12.7 The Role of Antioxidants in Plant–Microbe Interactions

Microorganisms impact so much on plant growth, productivity and quality. As a result, the study of the interactions between plants and microbes is very important as these interactions have implications on ecology and food security; some interactions between plants and microbes are beneficial, while some are detrimental (George et al. 2016). Although these interactions are not visible to the naked eyes, almost all parts or organs of a plant are involved either directly or indirectly in these interactions. These interactions could also be symbiotic in nature where the plants serve as habitat for the microbes, while the microbes in turn secrete compounds that could help plant growth or make it more resistant to stress be it biotic or abiotic or even act as a means of defence against microorganisms that are parasitic to the plants (Schirawski and Perlin 2018). Some beneficial plant–microbe interactions involve interactions between plants and nitrogen-fixing bacteria or mycorrhizal fungi, while interaction of plants with pathogenic bacteria or fungi is considered detrimental (George et al. 2016).

Plants interact with microbes mostly via their roots, and since plants are largely immobile, their roots continuously synthesise, accumulate and secretes several compounds into the soil. These compounds are referred to as root exudates (Ho et al. 2017). It has been reported that these root exudates are usually responsible for the types of the bacterial community in the soil surrounding the plant (Lareen et al. 2016); thus, different plant species has its own specific bacterial community in its rhizosphere. Also, any change in the composition of these microbes can impact on the performance of the plant (Bever 2003). Some of the compounds which form the root exudates are antioxidants such as flavonoids and phenolic acids such as

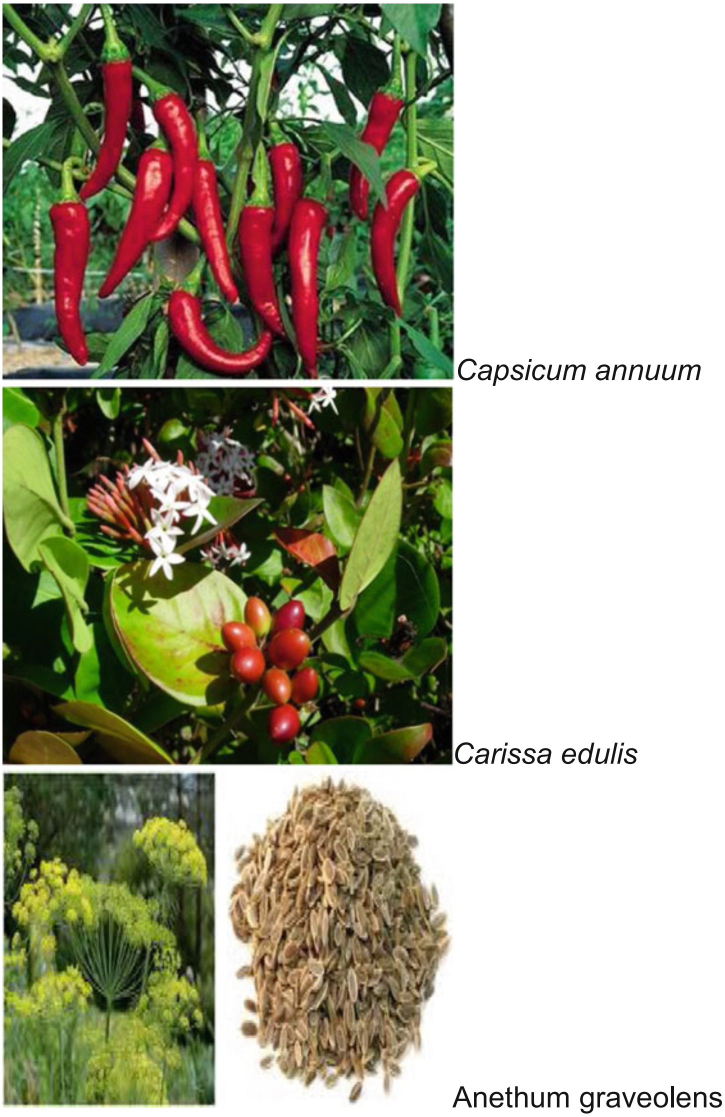

**Fig. 12.4** Some Southern African Spices (Source: Exercise.com 2020; Comboni Missionaries Ireland 2021; Goodarzi et al. 2016)

hydroxybenzoic acid and ρ-coumaric acid. Some reports have associated the resistance of some species or cultivars of plants to pathogens to the levels of these exudates; that is, the higher the level of exudates, the more resistant the species or cultivar is to the pathogens. This therefore implies that these exudates regulate the resistant mechanism observed (Li et al. 2013).

- **Flavonoids and its roles in plant–microbe interactions:** Flavonoids are plants' secondary metabolites which are distributed universally throughout all plants. They carry out several functions in plants which are very necessary for plants survival such as giving flowers their colours, transport of the plant hormone, auxin, inhibition of some plant processes and protection of plants against ultraviolet radiation (Khalid et al. 2019). Due to the diversity in their structures, flavonoids have been found to perform several diverse activities in plants. One of such key roles is that they help plants to develop a beneficial *Rhizobium* symbiotic relationship between plants and microbes in legumes and also help to improve the biomass of plants (Buer et al. 2010). They also protect plants against abiotic stress such as salt, heat, drought and ultraviolet radiation and biotic stress such as attack by pathogens or herbivores. Their ability to scavenge reactive oxygen species helps to maintain the balance between oxidative and reductive status inside the plant cells (Khalid et al. 2019). Flavonoids via root exudations have been found to acts as signalling molecule in response to several environmental stresses in plants be it biotic (beneficial symbiotic organisms or harmful pathogens) or abiotic (drought, temperature or nitrogen). Infection of plants by microorganisms can generate reactive oxygen species which could be harmful to plants as they could cause oxidative damage to plant biomolecules such as nucleic acids, proteins and lipids; however, flavonoids help to quench these reactive oxygen species (Kanazawa et al. 2012). The ability of flavonoids to protect plants from pathogenic organisms has been linked to their antioxidant properties (Raks et al. 2017). Some flavonoids work to protect against pathogens via transport of flavonoids to site of infection, thereby generating a hypersensitivity reaction. Some could also act by inhibiting enzymes of the pathogens especially the ones that digest the plant cell wall via activity of metal chelators either directly or indirectly (Khalid et al. 2019). For example, quercetin, a flavonoid which is common to so many plants including spices such as oregano and *Capsicum* spp., helps to resist the activity of plant pathogens through the suppression of ATPase activity of DNA gyrase (Babii et al. 2016). Kaempferol is another flavonoids which provides protection against fungi toxicity to plants (Monazzah et al. 2016).

  Apart from protection against harmful microorganisms, flavonoids also participate in plant–microbes symbiotic relationship. In the rhizosphere region of plant roots, flavonoids help to stimulate spore germination and enhance the expression of nod genes from symbiont and rhizobia chemoattraction. Some flavonoids have been found to play a role in nodule meristem formation. These root nodules serve as home to some beneficial bacteria like *Azorhizobium, Mesorhizobium, Bradyrhizobium* and *Sinorhizobium*. These bacteria then help in nitrogen fixation where atmospheric oxygen is converted to ammonia to be acted upon by other organisms to subsequently form nitrates which are source of nutrients to plants (Singla and Garg 2017). Also, the metabolic activities of these symbiotic microbes can alter the structure and composition of some plant flavonoids (Weston and Mathesius 2014). For example, the activity of the fungus hyphal in the plant *Medicago truncatula* has been implicated in the enhanced production of the flavonoid coumestrol in the plant which has been reported to be beneficial to the plant's defence system and also as a medicinal component to humans (Truong et al. 2015).

Some flavonoids exudates have also been reported to modulate some genes in Rhizobium which are responsible for the synthesis of some biomolecules such as exopolysaccharides and proteins, thereby assisting in the metabolic processes of these microbes. Also, the activity of flavonoid root exudates can act in signal transduction which in turn causes a cascade of reactions in these beneficial microbes such as rhizobium. These reactions in turn lead to different beneficial activities which include nitrogen fixation and growth modulation via regulation of the synthesis of the hormone auxin. The activity of flavonoid root exudates also helps to attract the beneficial microbe mycorrhizal fungi which help to protect host plants from pathogens (Hassan and Mathesius 2012).

- **Phenolics and their roles in plant–microbe interactions**: Phenolic root exudates have also been reported to help attract beneficial soil-borne microorganisms which will positively affect the microbial community of the soil around the plant. These phenolic exudates also possess the ability to protect plants from microbial attack (Badri et al. 2013). An example of such phenolic root exudate is canavanine. Canavanine helps to attract the beneficial fungi community arbuscular mycorrhizal which help to protect plants from adverse effect resulting from drastic change in soil pH and drought while also helping to maintain the nutrient content of the soil (Ho et al. 2017). Mycorrhizal fungi also aid plants in absorption of nutrients such as iron from the soil (Pii et al. 2015). Interaction between plants and microbes especially those of *Rhizobia* spp. and *Frankia* spp. have been found to help promote plant growth directly or indirectly by producing and aiding in the absorption of compounds necessary for plant growth from the soil. They also modulate the levels of plant hormones and also help to control pathogenic organisms and pests (Glick 2012). Also, phenolic compounds of the cinnamic acid derivatives have been reported to help plant to resist fungal attack (Lanoue et al. 2010).
- **Capsaicinoids and Ethylene in *Capsicum* spp. and their roles in plant–microbe interactions:** Capsaicinoids present in *Capsicum* spp. have been reported to act as mediators in plant–microbe interactions. Apart from their activity in protecting plant against mammalian predators, they also act as antifungal agents, thus protecting plants from infestation by fungi (Barchenger and Bosland 2016). Capsaicinoids are allelochemicals which have been reported to influence the root, shoot and germination of *Capsicum* spp. or when applied exogenously to other seeds could affect these parameters as well (Barchenger and Bosland 2016). Capsaicin protects plants from bacterial and fungal infestation by inhibiting energy production via inhibition of oxidative phosphorylation in these pathogenic organisms (Adams et al. 2020).

  Another important compound in *Capsicum* spp. is ethylene which has been reported to help in upregulation of the expression of some transcription factors XLOC_021142 and XLOC_02182 which when adequately expressed help to provide resistance against infection by *Phytophthora capsici*, a fungus which is responsible for root and collar rot disease in *Capsicum annuum* (Bagheri et al. 2020).

- **Piperine and its role in plant–microbe interactions:** Tetrahydropyridine alkaloids of which piperine is one have been reported to interact with beneficial microbes in the plant rhizosphere such as *Pseudomonas koreensis* and *Pseudomonas fluorescens* such that these microbes help to secrete plant hormones such as indole acetic acid and gibberellic acid which stimulate plant growth. They also produce antimicrobial compounds such as pyoluteorin, pyrrolnitrin, lipopeptide and hydrogen cyanide which protect plants from microbial attack. The activity of these microbes could also help to suppress disease. For instance, *Pseudomonas fluorescens* produces phenazine-1-carboxylic acid which helps to suppress the activity of the fungi *Gaeumannomyces graminis (*Lozano et al. 2019).

  Piperine has also been reported to inhibit aflatoxin production in the fungus *Aspergillus flavus*. This fungus has been reported to infest several plants including *Piper* spp. Piperine acts by downregulating the expression of the entire AFB1 gene clusters which is responsible for the expression of the mycotoxin aflatoxin B1 (Caceres et al. 2017)
- **Terpenoids and their roles in plant–microbe interaction:** Some root exudates are terpenoids, and they have been reported to protect plants from fungal and bacterial attack. Volatile terpenoids such as limonene, carvone, carvacrol, linalool, thymol, terpineol, myrcene and pinene which are commonly found in volatile oils of spices such as *Pimpinella anisum, Elettaria cardamomum* and *Origanum syriacus* have been reported to possess antifungal activity against aflatoxin-producing *Aspergillus flavus,* thus protecting the plant against activity of the fungus (Loi et al. 2020). Also, cinnamaldehyde from cinnamon has been reported to possess antifungal activity, thus protecting the plant against infestation by *Aspergillus* spp. via inhibition of the synthesis of ergosterol, an important component of the fungal cell membrane, thereby causing damaging morphological alteration on the fungal cell membrane (Khorasani et al. 2017).

  Carvacrol, a major component of the essential oil of oregano, has been reported to act against plant pathogens be it soil-borne pathogens, foliar pathogens, plant-parasitic nematodes and post-harvest pathogens. It has been reported to protect plants against pathogens such as *Phytophthora capsici, Xanthomonas perforans* and *Rhizoctonia solani.* It has also been reported to possess antifungal effect against *Aspergillus niger* (Liu et al. 2019).

  Thymol, another antioxidant component which is common to the essential oil of some spices such as *Thymus vulgaris* and *Origanum syriacus* when applies exogenously to some plants have been found to act as protection against bacterial activity while also helping to promote growth (Kumari et al. 2018).

## 12.8 Conclusion

Spices are rich source of antioxidant components, and Africa is home to numerous spices which have been discovered to possess several biological activities. The antioxidant components present in these African spices range from the alkaloid groups, flavonoids, phenolic compounds and terpenoids. All the spices examined possess numerous bioactive constituents and possess proven antioxidant and other biological activities. They have been demonstrated to possess anticancer, antitumour, cardioprotective, antiageing and neurodegenerative effects. Also, some of these antioxidant components are very important in plant–microbe interactions which are of utmost importance in plant ecology and food security.

Consumption within acceptable limit is safe with numerous beneficial qualities accruable from the use in local dishes and cuisines. These bioactive compounds can be further employed for new therapeutic approach in the management of several pathological conditions. They can also be processed into functional food products which will confer health benefit on the consumers.

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# Impact of Plant Growth-Promoting Microbes (PGPM) in Plant Disease Management by Inducing Non-enzymatic Antioxidants

Somenath Das and Arpan Mukherjee

## Abstract

Among different biological factors, plant disease is one of the most effective problems for considerable loss of crop production in current time. The sustainable way for crop production and plant disease management is the use of beneficial microbes. Beneficial soil microbes surrounding the rhizosphere of the host plant can protect them from plant pathogen and also stimulate further development of plants. Different species of plant growth-promoting microbes (PGPM) are being well-reported by scientists to manage different diseases of plants and improvement in crop productivity. PGPM helps host plant to induce their growth as well as suppress the disease incidence. Interaction of PGPM with plant pathogens in the rhizosphere regions modulates innate immune responses in the host plant which efficiently suppresses the pathogenic infections. It has been well studied that PGPM helps host plant to protect them from disease by producing different enzymes, metabolites, plant antioxidants and inducing plants immunity. In addition to antioxidants of enzymatic origin, the non-enzymatic antioxidants, viz. glutathione (GSSG/GSH), ascorbic acid, tocopherol, phenolic constituents, riboflavin, carotenoids, and thiamine, play outstanding role against oxidative stress as well as a number of plant disease occurrences. Hence, this article has been discussed critically to provide an updated account describing the active participation of PGPM in improvement of plant defense as well as disease management through the production of different non-enzymatic antioxidant and develop a green horizon to maximize their practical application in sustainable agriculture.

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**Keywords**

Rhizosphere · Plant growth-promoting microbes (PGPM) · Plant disease · Non-enzymatic antioxidants · Biocontrol

## 13.1 Introduction

Different environmental stresses such as drought, heat, high salinity, metal toxicity, ozone levels, UV radiation, and infection of different pathogens have modulatory effects on growth and development of plants (Ratkevicius et al. 2003; Peltier et al. 2006). Among the biotic and abiotic stress, biotic stresses have played prime role in reduction of the productivity of plants and influence morphological, physiological, biochemical, and molecular patterns. In addition to biotic stress, the abiotic stress such as water deficiency, increasing salinity, highly variable temperatures, and the stress by oxidation of toxic free radicals further compromises the plant cellular components leading to declining plant survivability (Waśkiewicz et al. 2014).

Oxidative stress has been recognized as a matter of great concern during biotic as well as abiotic stress culminating in production of reactive oxygen species like superoxide ion, hydrogen peroxides, and hydroxyl radicals which have detrimental effects on plant survivability. During the stress condition, plants exhibit an array of defense mechanisms with special effects on some enzymatic antioxidants (peroxidases, catalase, α-tocopherol, β-carotene, polyphenol, carotenoids, and glutathione) involving the absorption of superoxide radicals. The accumulation of these important biomolecular compounds has played a critical role in the osmotic adjustment and helps to adjust the water loss and maintain the ion toxicity (Ehsanpour and Amini 2003). Most importantly, after pathogenic infection, some important antioxidant molecules of non-enzymatic origin such as phenolic contents, glutathione, ascorbic acid, and lipophilic antioxidants (α-tocopherol and β-carotene) are activated in plants to mitigate the oxidative stress.

Plant growth-promoting microbes (PGPM) and other beneficial symbiotic microorganisms, especially bacteria and fungi, have important role to develop an emerging strategy by inducing plant growth in stress conditions. The strategy may include root and rhizosphere colonization, altering the metabolites and production of enzymatic and non-enzymatic plant beneficial compounds (Vessey 2003; Mukherjee et al. 2019, 2020a).

Till now, a lot of mechanisms are available to overcome the plant disease by PGPM and fungi-mediated production of lytic enzyme, siderophore, HCN, induction of defense-related protein, and the production of enzymatic antioxidant in the plant to alleviate the disease occurrence (Mukherjee et al. 2020b; Waśkiewicz et al. 2014; Das et al. 2020). However, the impact of PGPM on non-enzymatic antioxidant to manage the disease occurrence and proliferation has not been properly demonstrated. Hence, in the present article, an effort has been made to describe updated account of PGPM-induced cellular antioxidant molecules which are of non-enzymatic origin, especially tocopherols, carotenoids, ascorbic acid, glutathione, phenolic compounds, riboflavin, and thiamine with special emphasis to their disease management potentiality.

## 13.2 Why Non-enzymatic Antioxidants?

In addition to antioxidants of enzymatic origin, the non-enzymatic biomolecules of antioxidants, viz. ascorbic acid, tocopherol, carotenoids, glutathione, and phenolic compounds, are major scavenger of free radicals and maintain the oxidative level in plant cell (Panda 2012). The antioxidants of non-enzymatic origins have been engaged in donating the electrons for many enzymatic reactions reducing the level of hydrogen peroxide and play prominent role in disease management (Jaleel et al. 2009). They act as major driver for rationale plant resistance and subjected to oxidation–reduction efforts. These non-enzymatic antioxidant components are able to inhibit cellular lipid peroxidation and regulate membrane stability. Infection of plant growth-promoting rhizobacteria improves the content of non-enzymatic antioxidants leading to enhancement in ascorbate peroxidase and superoxide dismutase activity. Therefore, the association of plant growth-promoting microbes provides a new horizon to maximize the improvement of crop species and act as a coordinator for tolerance mechanisms. Brief descriptions of different non-enzymatic antioxidants are presented in the section below.

### 13.2.1 Ascorbic Acid

Ascorbic acid is prime regulatory molecule in the eukaryotes potentially involved in free radical scavenging reactions. Ascorbate can found in either reduced (ascorbic acid) or oxidized forms (mono- and dehydroascorbic acid). Modulatory effects on ratio of oxidized and reduced form of ascorbic acid further influence plant resistance to the oxidative stress conditions (Zechmann 2011). Ascorbate also helps in different biological processes like photosynthesis, photoprotection, cell wall development, and plant growth (Wolucka and Van Montagu 2003). Ascorbate has also played important role in the biosynthesis of some of the key factors in plants like anthocyanins, ethylene, gibberellins, and hydroxyproline (Mellidou et al. 2012). In addition to its major function as potent biomolecule, ascorbic acid (AsA) is recognized as first sequence marker to defense a number of toxic free radicals and protection of healthy plant from different environmental factors as well as pathogen attack. Ascorbic acid interacts with the glutathione (GSH), and the AsA-GSH complex is able to mitigate the stress by enhancement in activity of the nonexpressor proteins suppressing the pathogenic infection, regulation in transcription, cell wall rigidity, and some of defense–hormonal signaling pathway. Ascorbic acid helps in induction of resistance component to protect the plant from pathogens by elicitor-mediated interactions of some important defense-related components such as β-aminobutyric acid, methyl ester, jasmonic acid, and extracellular polysaccharides (Boubakri 2017). Reports on several plant growth-promoting bacteria such as

*Acetobacter*, *Pseudomonas, Gluconobacter*, *Rhizobium* spp., and *B. megaterium* are for conversion of different components such as D-sorbitol, 2-keto-L-gulonic acid, D-glucose, and L-sorbose to L-ascorbic acid in some of important metabolic cycle (Bremus et al. 2006) which is directly linked with the plant growth and help in protection from various stress. Ascorbic acid is the major scavenger of singlet oxygen, hydroxyl, and oxide ions and helps in regeneration of tocopherol from tocopheroxyl radical which plays active role in membrane protection. Ascorbic acid also works as cofactor of violaxanthin de-epoxidase and reduces the excess excitation energy, minimizing the damage occurred by the oxidative process (Smirnoff and Wheeler 2000). Recent report suggested the incorporation of ascorbic acid in photosynthetic reactions for reduction of $H_2O_2$ through major electron carrier and a great modulator for plant defenses. In AsA-deficient *Arabidopsis* plants, different defense genes are activated which specially encode the pathogenesis-related proteins. Moreover, the infection of plant growth-promoting bacteria has specific role in the synthesis of salicylic acid exerting systemic acquired resistance in plants (Pastori et al. 2003; Barth et al. 2004). Higher level of AsA in combination with glutathione may contribute to alleviation of RNA virus infection in *Arabidopsis* spp. (Wang et al. 2011). Recent investigation of Khanna et al. (2019) suggested the elevation of cellular AsA by infection of plant growth-promoting bacteria with resultant enhancement of defense in *Lycopersicum esculentum* against *Meloidogyne incognita* infection.

### 13.2.2 Tocopherols

Tocopherols are lipid-soluble non-enzymatic antioxidants that are only produced by some photosynthetic organisms including higher plants, algae, and cyanobacteria (Quadrana et al. 2013). Tocopherol has different group like alpha-, beta-, gamma-, and delta-tocopherol. Tocopherol β- and δ are not very much abundant in the plant species. Among all the tocopherol, α-tocopherol is abundant in different plant and shows superior biological activity due to three methyl groups in the molecular stoichiometric structure (Szarka et al. 2012). Tocopherol acts as an antioxidant and is associated with the diminution of lipid peroxidation in membrane and helps in the scavenging of ROS (reactive oxygen species) (Munné-Bosch 2005; Kruk and Trebst 2008). Synthesis of tocopherol is regulated by different stress of plant hormones such as jasmonic acid (JA), salicylic acid (SA), and abscisic acid (AbA) (Szarka et al. 2012). Several reports suggested the synthesis of tocopherol during the unfavorable environmental stress conditions such as drought, heavy metals, salinity, and high light intensity (Ledford and Niyogi 2005; Tounekti et al. 2011). Tocopherols have modulatory effects in quenching of ROS and eliminate the toxic radical species of polyunsaturated fatty acids (PUFA) leading to termination of lipid peroxidation (Caretto et al. 2009). Recent study of Caretto et al. (2009) displayed the participation of tocopherol in the intracellular signaling mechanisms in plants. Wu and Tang (2004) reported that one molecule of α-tocopherol molecule can neutralize 120 singlet oxygen molecules. It also works in premature termination of recycling of PUFA radicals during lipid peroxidation (Hare et al. 1998). Moreover, α-Tocopherols can able to quench and scavenge different ROS generated through lipid oxidation and stabilize membranes and increase the signal transduction process in plant (Kruk et al. 2005; Noctor 2006). Fritsche et al. (2017) described the activation of tocopherol biosynthesis genes in higher plants upon activation of stress. Infection of plant growth-promoting bacteria has played potent role in increment of cellular tocopherol, and the greater content of tocopherol could be correlated with ascorbate synthesis and accumulations (Canellas et al. 2019).

### 13.2.3 Glutathione

Glutathione is an abundantly found nonprotein low-molecular-weight thiol component and acts as non-enzymatic antioxidant and crucial metabolites in all living aerobic organisms (Ramírez et al. 2013; Gullner et al. 2017). Glutathione is mainly localized in the plant cytosol, mitochondria, endoplasmic reticulum (ER), vacuoles, chloroplasts, peroxisomes, and the apoplast (Noctor and Foyer 1998). Reports on antioxidant activity of glutathione by affecting several key mechanisms including direct scavenging of cytotoxic free radicals such as hydroxyl, singlet oxygen, hydrogen peroxides, and superoxides have been demonstrated. The prime antioxidant potency of glutathione is completely dependent on modulation of glutathione–ascorbic acid cycle (Millar et al. 2003). Glutathione mainly occurs in two forms, viz. oxidized (GSSG) and reduced (GSH). Variation in two forms of glutathione maintains the redox state and signaling pathway (Foyer and Noctor 2005). Ding et al. (2009) reported the role of glutathione in oxidative stress management in tobacco via transgenic line. Hence, both the oxidized and reduced glutathione play vital role in reactive oxygen species-mediated plant disease management. Mukherjee et al. (2020c) recently reported the *Saccharomyces cerevisiae* (yeast)-based plant growth-promoting microbe with prominent inhibiting potentiality of pathogen interactions and postharvest disease management. Investigation of Cartieaux et al. (2003) demonstrated the glutathione *S*-transferase-mediated changes in RNA transcript level of *Arabidopsis* plants after infection with *Pseudomonas thivervalensis* (strain nMLG45) which developed resistant against the virulent pathogen of *Pseudomonas syringae* pv. in Tomato.

### 13.2.4 Carotenoids

Carotenoids are the pigment compounds which are of common occurrence in both plants and microorganisms. Plant carotenoids belong to the isoprenoid-derived compounds, and their synthesis belongs to plastid-mediated 2-*C*-methyl-ᴅ-erythritol 4-phosphate and cytosol-dependent mevalonic acid pathways. More than 600 different types of carotenoids are found in nature. Most important functions of the plant carotenoids are in the photosynthesis, photomorphogenesis and plant growth and development, plastid biogenesis, flowering, and fruit growth (Wurbs et al. 2007;

Giuliano et al. 2008). Carotenoids are important for the primary and secondary metabolisms of plants; it required for photosynthesis and helps in pigment determination in different crop plants (Botella-Pavía and Rodríguez-Concepción 2006). Carotenoid and chlorophyll ratio suggested and indicted the seed tolerance to different stress factors (Smolikova et al. 2011). The antioxidant property of the carotenoids arises due to conjugation of double bonds in structure (Mortensen et al. 2001) to delocalize unpaired electrons and quench the effect of hydroxyl, peroxyl, singlet oxygen, and superoxide radicals.

### 13.2.5 Phenolic Components

Among different antioxidants, phenols are one of the important non-enzymatic antioxidant which help in elimination of radical species and act as metal chelator. Phenolics, viz. hydroxycinnamate, lignin, ester, tannins, and flavonoids, are the products of secondary metabolites with well-known antioxidant properties. Significant $H_2O_2$ scavenging activity of peroxidase has also been correlated with reduced ascorbic acid and phenolics (Schroeter et al. 2002). Alvarez (2000) reported the role of salicylic acid to induce the phenolic constituents and stress tolerance. The high antioxidant activity may also due to higher phenolic components and anthocyanin content. The stable nature of phenoxy radical intermediate can easily terminate the chain reaction catalyzed by lipid peroxidation and rapid donation of free hydrogen atom to radicals. Table 13.1 presents different non-enzymatic components having prominent role in plant defense during various pathogenic infections.

### 13.2.6 Riboflavin and Thiamine

Riboflavin participates in plant defense mechanisms by neutralizing several toxic free radicals and maintains the pathogenic infections. Involvement of riboflavin as antioxidant and antiperoxidation helps in disease management by modulation of hypersensitive reactions culminating into death of pathogen. Jianling et al. (2002) demonstrated that the exogenous application of riboflavin helps in plant growth promotion. Zhang et al. (2009) demonstrated the riboflavin-mediated priming of NPR-1 (regulator for systemic acquired resistance) and critical maintenance of cellular burst of $H_2O_2$ in *Arabidopsis*. Taheri and Tarighi (2010) reported rice resistant against the infection of *Rhizoctonia solani* via riboflavin-dependent modulation in jasmonate and phenylpropanoid pathway. Induction of resistance in soybean plant against charcoal rot disease (causal organism: *Macrophomina phaseolina*) by riboflavin and thiamine has been reported by Abdel-Monaim (2011). Hamada and Jonsson (2013) reported thiamine-mediated alleviation of aphid (*Rhopalosiphum padi*) infestation in *Hordeum vulgare* and *Pisum sativum*. Boubakri et al. (2013) illustrated the role of riboflavin to develop resistant in grapevine (*Vitis vinifera*) against the infection of *Plasmopara viticola* by modulation in defense responses. Mahmoud et al. (2020) reported the induction of riboflavin

**Table 13.1** Some important non-enzymatic components important for plant defense during pathogenic infections

| Non-enzymatic components | Test plant | Pathogen infection | References |
|---|---|---|---|
| Riboflavin | *Arabidopsis thaliana* | *Peronospora parasitica* | Dong and Beer (2000) |
| | | *Pseudomonas syringae* | Zhang et al. (2009) |
| | | *Alternaria alternata* | Zhang et al. (2009) |
| | *Nicotiana tabacum* cv. NC89 | Tobacco mosaic virus (TMV) | Liu et al. (2010) |
| | *Oryza sativa* | *Rhizoctonia solani* | Taheri and Tarighi (2010) |
| | Chikpea | *Fusarium oxysporum* | Saikia et al. (2006) |
| | *Phaseolus mungo* | *Botrytis cinerea* | Azami-Sardooei et al. (2010) |
| | *Solanum lycopersicum* | | |
| | *Glycine max* | *Macrophomina phaseolina* | Abdel-Monaim (2011) |
| | *Vitis vinifera* | *Plasmopara viticola* | Boubakri et al. (2013) |
| Thiamine | *Oryza sativa* | *Xanthomonas oryzae* | Ahn et al. (2005), Bahuguna et al. (2012) |
| | | Sheath blight | |
| | *Lagenaria* spp. | *Colletotrichum lagenarium* | |
| | | *Sphaerotheca fuliginea* | |
| | *Arabidopsis thaliana* | *Pseudomonas syringae* | |
| | *Nicotiana tabacum* | Pepper mild mottle virus | |
| | *Glycine max* | *Macrophomina phaseolina* | Abdel-Monaim (2011) |
| | Barley | Aphids | Hamada and Jonsson (2013) |
| | Pea | | |
| | *Vitis vinifera* | *Plasmopara viticola* | Boubakri et al. (2013) |
| Ascorbic acid | *Arabidopsis thaliana* (Experimental plant) | *Pseudomonas syringae* pv. *maculicola* ES4326 and *Peronospora parasitica* pv. *Noco* | Pastori et al. (2003) |

which has greatly influenced the disease occurrence in tomato plant by Tobacco mosaic virus (TMV).

## 13.3 Signaling Mechanism of Non-enzymatic Antioxidants for Disease Management

Plants are exposed to variety of pathogens which create harsh environment for cell growth and proliferation. The non-enzymatic antioxidant components easily provide hydrogen and electrons to scavenge free radicals, thereby reducing the risk of disease proliferation. Two different pathways of interaction, viz. compatible and incompatible signaling, confer resistance against viral and bacterial infections. The physiological responses of stressed plants after pathogen infection significantly changed the cellular photosynthetic pigments, proteins, carbohydrate, and energy metabolism pathways leading to alteration in signal transduction (Radwan et al. 2010). Recently, Sofy et al. (2019) reported the improvement in disease management of cucumber plants after infection with cucumber mosaic cucumo virus by modulation of non-enzymatic antioxidants such as AsA, phenols, and glutathione. Different species of plant growth-promoting rhizobacteria help in promotion of nitrogen fixation, phosphate solubilization, and production of auxin and cytokinin in infected plants, a potential prerequisite for effective management of disease (Ryu et al. 2003). It was reported that systemic acquired resistance (SAR) induced by plant growth-promoting microbes follows the special pathway of endogenous salicylic acid production with resultant upregulation of complex sets of genes. Hoffland et al. (1996) demonstrated the enhancement in non-enzymatic antioxidant status by *Pseudomonas fluorescence* infection having inhibitory effects against fusarium wilt disease. Ethylene, salicylic acid, and jasmonic acid played a key role in modulation of cellular phenolic, carotenoids, ascorbic acid, and glutathione level. The application of mixture of plant growth-promoting microbes can develop systemic resistance against anthracnose disease in cucumber (Wei et al. 1996). Mixed consortia of *Pseudomonas aeruginosa* and *Trichoderma harzianum* effectively controlled the *Sclerotinia* rot of cauliflower by inducing the total phenolic constituents and antioxidant activity (Ram et al. 2019). Ali et al. (2006) reported methyl jasmonate and salicylic acid-mediated induction of cellular ascorbate and glutathione in *Panax ginseng*, which has displayed active participation for a number of stress-related disease management. In agreement with the earlier study, both the methyl ascorbate and glutathione have a prominent role in sequestration of $H_2O_2$ toxicity and lipid hydroperoxides such as malondialdehyde (MDA). Phenolic components have regulatory role as intraspecific signaling for induction of PGPM-mediated pathogenesis-related protein formation in plants with concomitant decrement of disease proliferation (Compant et al. 2005). Figure 13.1 represents signaling pathway for modulation of non-enzymatic antioxidants by plant growth-promoting microbe (PGPM).

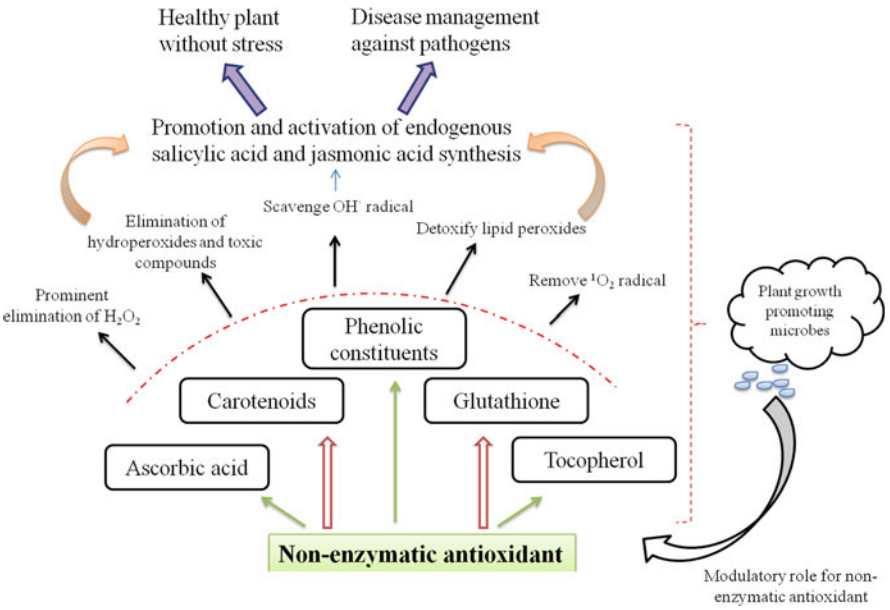

**Fig. 13.1** Possible signaling pathway for modulation of non-enzymatic antioxidants by plant growth-promoting microbe (PGPM)

## 13.4 Conclusion

Biotic factors especially the pathogenic infection and occurrence of plant disease are a burning issue for declining in food crops around the world. Exposure of variable stress during pathogenic infection leads to the production of superoxide radicals ($O_2^{\bullet -}$), hydroxyl ($^{\bullet}OH$), reactive oxygen species (ROS), and peroxyl ($ROO^{\bullet}$) in plants. To prevent the negative effect of toxic free radical components and to ensure plant survivability, non-enzymatic antioxidants in plant cell possess a prominent role with immense significance. Among all the non-enzymatic molecules, tocopherols, carotenoids, glutathione, ascorbic acid, and phenolic constituents are described as the most important and well-studied components. Beside the genomic, proteomic, and transcriptomic approaches, the application of plant growth-promoting microbe (PGPM) has added a new dimension in disease management with concurrent role in modulation of non-enzymatic antioxidants in cell. A very little information is available in the aspect of non-enzymatic antioxidant production to cope the pathogenic infection, but the production of crops through application of PGPM is well established.

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# Antioxidants as Modulators of Plant Defence Against Soilborne Fungal Pathogens upon Microbial Interaction

# 14

Sushree Suparna Mahapatra

## Abstract

Antioxidants are the compounds which have the ability to appease active oxygen species (AOS) and protect the plant tissues from destruction without converting their own forms. The antioxidant enzymes catalyse the process of the deformation of the toxic ions. Antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione (GSH), glutathione reductase (GR) and glutathione *S*-transferase are produced in several stress conditions and act as defence molecules to combat the stress. SOD converts superoxide molecules into hydrogen peroxide which directly inhibits penetration of the pathogen invasion at the epidermis. These antioxidants are known to induce the systemic acquired resistance (SAR) to resist the invasion of the pathogen. They are also involved in signal transduction and in turn limit the pathogen infection. Antioxidants are produced during both biotic and abiotic stresses which may trigger the hypersensitive response (HR) in the host tissue as defence against the adverse condition. The increase in the antioxidant level in response to the pathogen invasion subsequently may result in increased tolerance to the development of necrosis.

## Keywords

Hypersensitive response · SAR · Biotic stress · Pathogen · Infection · Toxic ions

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## 14.1 Introduction

The term antioxidant can be referred to as a compound which is capable of quenching Active Oxygen Species (AOS) without converting itself to a destructive radical and the antioxidant enzymes either catalyse the reactions or are directly involved in processing of AOS (Nishikimi and Yagi 1996). Phenolics are the phytochemicals, which are produced in plants in response to several biotic or abiotic stresses (Briskin 2000). The extent of accumulation of the phenolics depends on the host and disease reaction. Phenolics are accumulated extensively in case of resistant varieties after pathogen's attack which proves itself as an yardstick for resistance (Chérif et al. 1992).

Various microbial interactions in the soil are the deciding factors for suppression of soilborne diseases. Interactions like antibiosis and mycoparasitism have direct deleterious effect on the pathogens, and some have indirect effect on microbial populations including pathogens, when quite active nonpathogenic microorganisms intensively exploit trophic or spatial resources. Soilborne pathogens can adversely affect the plant system either by directly penetrating the plant and causing diseases like rot, wilt and damping-off or by infecting the aerial parts through air or water splashes.

## 14.2 Antioxidants in Resistance and Signalling

Antioxidants are the compounds which have the ability to appease active oxygen species (AOS) and protect the plant tissues from destruction without converting their own forms. Certain antioxidant enzymes are Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Monodehydroascorbate reductase (MBR), Dehydroascorbate reductase, Glutathione reductase, Catalase, Glutathione peroxidase, Guaiacol-type peroxidases and Glutathione *S*-transferases. According to Bowler et al. (1992), SOD rapidly converts the superoxide produced in the different compartments of the plant cells into Hydrogen peroxide, whereas Catalase (CAT) are involved in conversion $H_2O_2$ to water and molecular oxygen (Willekens et al. 1995). APX uses two molecules of ascorbate to reduce hydrogen peroxide to water along with the generation of two molecules of monodehydroascorbate. It is also reported that transgenic tobacco plants with elevated levels of manganese superoxide dismutase in their chloroplasts exhibited enhanced tolerance to parquet (Tsang et al. 1991). Tseng and Tsai (2007) showed that SOD and CAT inoculated plants expressing both SOD and APX or GR are able to rapidly scavenge $O_2$ and $H_2O_2$ at the site of generation, as well as prevent the formation of hydroxyl radicals, the most toxic ROS, prior to their interaction with target molecules in transgenic Chinese cabbage plants expressing both Cu/Zn SOD and/or CAT in chloroplasts under the control of the rbcS promoter indicating enhanced tolerance to oxidative stress induced by $SO_2$ and high salt levels. Mellersh et al. (2002) suggested the role for $H_2O_2$ in directly inhibiting pathogen penetration at the epidermis during symptomless (type I) nonhost resistance to powdery mildews in cowpea and cucurbits

**Table 14.1** List of antioxidants and their site of cellular localization

| Sl. no. | Enzymatic antioxidant | Cellular localization | Nonenzymatic antioxidant | Cellular localization |
|---|---|---|---|---|
| 1. | Superoxide dismutase (SOD) | Chloroplasts, cytosol, mitochondria, peroxisomes, apoplasts | Vitamin E (α-tocopherols) | Membranes |
| 2. | Dehydroascorbate reductase (DHAR) | | Glutathione (GSH) | Chloroplasts, cytosol, mitochondria, peroxisomes, apoplast |
| 3. | Glutathione peroxidase (GPX) | Cytosol | Carotenoids (Car) | |
| 4. | Catalase (CAT) | Peroxisomes | Flavonoids | |
| 5. | Glutathione *S*-transferase (GST) | | Nonprotein amino acids | |
| 6. | Ascorbate peroxidase (APX) | Chloroplasts, cytosol, mitochondria, peroxisomes, apoplasts | Ascorbic acid (AsA) | Chloroplasts, cytosol, mitochondria, peroxisomes, apoplast |
| 7. | Guaiacol peroxidase (GOPX) | Cell wall, vacuole, cytosol, and extracellular space | | |
| 8. | Glutathione reductase (GR) | Mitochondria, cytoplasm | | |
| 9. | Monodehydroascorbate reductase (MDHAR) | Cytosol, chloroplast | | |

against *Vigna unguiculata* and *Erysiphe cichoracearum,* respectively. Sharma et al. (2018), found a strong negative correlation between defence components such as ROS, MDA, SOD, SA and the progression of spot blotch, which implies that these signalling pathways are important regulators in plant resistance against fungal infection (Table 14.1).

SOD gives the first line of defence for plants and plays a crucial role in plant survival under stress, making them stress-tolerant (Ahmad et al. 2010), and is involved in dismutation of $O_2$ to $H_2O_2$ which is required to activate cell death.

Catalase dismutates hydrogen peroxide produced by peroxisomes. It has been seen that the gene responsible for encoding CAT makes the plant resistant to various stresses. Ascorbate peroxidase (APX) plays a great role in foraging ROS and defending cells. It scavenges hydrogen peroxide in ascorbate glutathione (ASH/GSH) cycle.

GR (flavoprotein), GST and GPX are involved in defence against ROS in plants and involved in conjugation reaction between the substrates of xenobiotics and GSH. GST in the plant body is to detoxify those compounds which are involved in damaging DNA, RNA or proteins. GSH acts as a reducing agent and involved in reduction of dehydroascorbate (DHA) into ascorbate (AsA) which is catalysed by the enzyme dehydroascorbate reductase (Foyer and Mullineaux 1998).

## 14.3 Plant–Microbe Interaction

### 14.3.1 Soilborne Fungi Impact and Severity

Soilborne pathogens cause significant economic losses in agricultural production all over the world. These species can survive for many years in the absence of a host plant by forming persistent structures such as microsclerotia, sclerotia, chlamydospores or oospores. Consequently, soilborne diseases are particularly difficult to predict, detect, diagnose and successfully control. Plant diseases caused by *Rhizoctonia* spp., *Fusarium* spp., *Verticillium* spp., *Sclerotinia* spp., *Pythium* spp. and *Phytophthora* spp. affect a number of important crops, including wheat, cotton, vegetables and temperate fruits. The symptoms of soilborne disease, caused by different pathogens, are very similar. They include root rot, root blackening, wilt, yellowing, stunting or seedling damping-off, bark cracking and twig or branch dieback. Consequently, these pathogens are particularly difficult to predict, detect, diagnose and control (Åström and Gerhardson 1988).

Soilborne pathogens can penetrate the host plant directly from the soil, which may result into characteristic diseases like seed rot, damping-off, root rot or wilt diseases, although some can also infect aerial plant parts by means of airborne or waterborne spores. The soilborne fungi survive in soil by producing resistant propagative structures that allow them to survive in the soil. These structures can persist in soil and are protected by thick walls against the influence of various adverse elements like saturation and desiccation, freezing and thawing, microbial predation and other destructive elements. The rigid structures can be both sexual and asexual in nature. Most of the pathogens produce resistant structures which are asexual in nature like chlamydospores, hyphal aggregates, rigid conidia, sclerotia and rhizomorph. Pathogens like *Pythium* produce sexual structures like thick-walled sexual oospores. A persistent structure with the hyphal characteristics of radial growth and physiological continuity, such as a rhizomorph, will extend through the soil, increasing the territory of one genotype for potentially many years. These structures which are small, individually separate propagules like the chlamydospores and sclerotia, occupy small, differentiated territories and can be rearranged spatially when soil is cultivated or irrigated, but may remain stationary in uncultivated native soils. Excess moisture and soil pH act as drive force for the growth of the soilborne pathogens like *Phytophthora, Rhizoctonia, Fusarium, Verticillium* and *Armillaria. Phytophthora* causes late blight, whereas *Armillaria* and *Rhizoctonia* cause root; *Verticillium* and *Fusarium* are known to cause wilt. These pathogens are prevalent in the Northwest pacific. The life cycles of various soilborne pathogens may vary significantly at different stages of their growth. So, it is necessary to mark the conducive environmental factors required for their growth and development. This

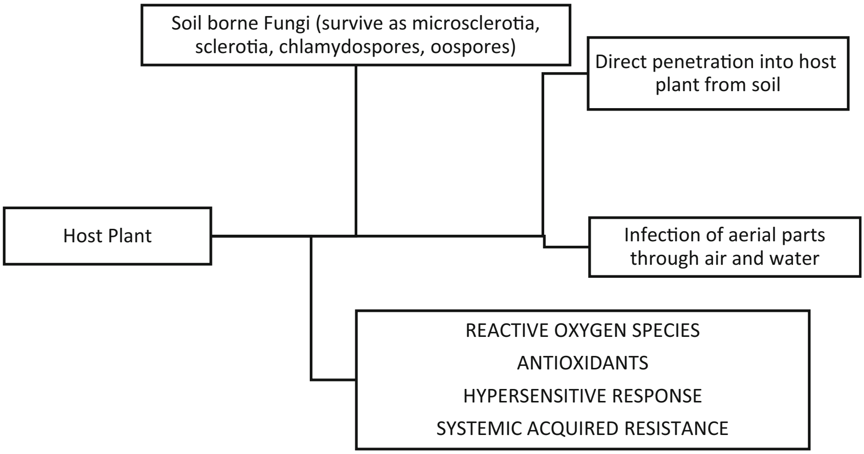

**Fig. 14.1** Response of host plant against soilborne fungi

helps us in arranging for various management measures to overcome the deteriorating effect of the pathogens on the soil as well as the plants. Hence, the weather and soil conditions can be manipulated as preventive measures and destroy the rigid pathogenic structures.

Legume crops are more specifically susceptible to invasion by different fungal pathogens like *Fusarium oxysporum* f. sp. *ciceri*, *Ascochyta rabiei*, *Botrytis cinerea*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Mazur et al. 2004). Among these diseases, losses due to Fusarium wilt alone range around 10–15% each year which makes it alarming. In the years of severe epidemics, crop losses have even gone as high as 60–70% (Chand and Khirbat 2009) (Fig. 14.1).

### 14.3.2 Production of Antioxidant as a Result of Pathogenesis by Soilborne Fungi

Fountain et al. (2016), observed that different isolates of *Aspergillus flavus* showed differences in oxidative stress tolerance which could be correlated with their aflatoxin production capabilities. They also found coexpression of Aflatoxin biosynthetic genes and antioxidant enzyme genes with fungal biomass under stress. This led to the conclusion that secondary metabolites might be produced as part of coordinated oxidative stress responses in *A. flavus* along with antioxidant enzyme gene expression and developmental regulation.

Several studies have also been performed stretching on the importance the role of antioxidants on the growth of *Aspergillus* and the production of aflatoxin. Phenolic compounds like caffeic acid and tannic acid which are derived from tree nuts have been found to inhibit aflatoxin production in *A. flavus* (Mahoney et al. 2010). Other synthetic phenolic compounds such as butylated hydroxyanisole (BHA) and propylparaben (PP) have also been proved to have a similar effect on the pathogen growth and pathogenicity in a definite medium pH and water activity (Nesci et al. 2003; Passone et al. 2005). Treatment with BHA has also been seen to inhibit sclerotial differentiation in *A. flavus*. Antioxidant enzyme activity and capacity in host plant tissue usually correlates with reduced *A. flavus* growth and aflatoxin production in buckwheat (Chitarrini et al. 2014).

Recent studies have shown that reactive oxygen species (ROS) and their reactive products like peroxidized lipids such as oxylipins are a requisite for the production of aflatoxin which can lead to stimulation aflatoxin production when applied in vitro (Jayashree and Subramanyam 2000).

According to Palomares-Rius et al. (2011), several proteins were identified in chickpea genotypes as oxidoreductase which were produced in response to the invading pathogen causing *Ascochyta* blight. These enzymes basically are involved in the reaction to stressful conditions. They either act as catalysts in hydroxylation steps in the biosynthesis of antimicrobial and antioxidant secondary metabolites such as isoflavonoid phytoalexins in chickpeas (as is the case for cytochromes P450, acting as antioxidant enzymes (as for QOR and NDH, or assist in the folding of defence proteins. Production of these enzymes is basically modulated by the host signalling system which imparts resistance against the pathogen invasion.

According to Saad et al. (2014), antioxidants like salicylic acid, citric acid, benzoic acid and ascorbic acid when treated on *Alternaria solani* and *Fusarium solani* in combination with a group of bio fungicides in vitro condition showed inhibitory effect of the growth of the fungi (Table 14.2).

## 14.4 Homeostasis of Production of Antioxidants and Regulation of ROS Levels

Various injuries on plant activate the antioxidant system of the host plant as a response to the adverse situation. This includes production of reactive oxygen species which are associated with the oxidative damage at the cellular level. Plants produce ROIs as signalling molecules in order to control adverse processes like programmed cell death, abiotic stress responses, pathogen defence and systemic signalling. These signalling systems are in turn the warriors produced in the plant system against the damage caused by the invasion foreign elements precisely the pathogens to protect itself at both cellular and subcellular levels. These ROS can lead to lip peroxidation which further can cause localized damage to the membrane, inactivate the enzyme system and cause membrane lipid peroxidation and disruption of DNA strands. This nature of the antioxidant system may sometime lead to adverse effect on the cellular components and, however, have significant role in imparting resistance against the pathogens. The plant system has advantageously ROS scavenging system to neutralize the adverse implications caused on the cellular components of the plant. The active oxygen species have a great role to play in the plant defence system.

**Table 14.2** List of soilborne fungi and their hosts causing changes in the antioxidant-level imparting defence

| Pathogen | Crop | Antioxidant(s) | Response | Reference |
|---|---|---|---|---|
| *Fusarium* sp. | Flax | Enhanced level of glutathione due to increase in methionine and cysteine biosynthesis | Protection through resistance | Czuj et al. (2009) |
| *Botrytis cinerea* | Tomato | Decrease in GSH content | Facilitation of penetration of necrotrophic phytopathogens and spread of necrotic lesions | Kuźniak and Skłodowska (1999) |
| *Phytophthora nicotianae* | Betelvine | Enhanced activities of POX, phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and lipoxygenase (LOX) (in response to Rhizobacterial strain) | Increased resistance against the pathogen | Diallo et al. (2011) |
| *Sclerotinia sclerotiorum* | Pea | PAL, POX, PPO and SOD | Increased tolerance against the fungus in response to the microbial consortium | Jain et al. (2012) |
| *Sclerotium rolfsii* | Chickpea | Increased activity of SOD and POX | Increased tolerance against the disease-causing microbes when treated with consortium of beneficial microbes | Singh et al. (2013) |

ROS act as toxic component against the soilborne pathogens directly penetrating into the host system. They lead to localized cell death and result into hypersensitive response (HR) which protects the host tissue from further spread of the infection or activate salicylic acid components for systemic acquired resistance (SAR). These elements try to activate the cell wall components to suppress the pathogen invasion by cross-linking the bonds of lignin and protein. Active oxygen species indirectly induce the plant defence by activation and modulation of plant resistance genes by acting as secondary messengers in signalling system.

ROS, like superoxide and $H_2O_2$, are produced by plant cells as secondary messengers in many processes associated with plant growth and development (Foreman et al. 2003). Plant cells produce ROS as second messengers in many processes associated with plant growth and development (Foreman et al. 2003). Doke et al. (1983), stated that bursts of superoxide at the plasma membrane are one of the major ways in which plants transmit information concerning changes in the environment.

Although ROS deeply induce plant defence against various stress conditions, but excessive production, these elements may have deleterious effect on the plant system. Excessive enhancement in the ROS level can be damaging by inducing oxidative stress which leads to cell death, so the concentration needs to be neutralized to keep it under control. This can be maintained by appropriate antioxidant production to make the balance optimal. They majorly act as effective ROS scavengers. Chloroplasts, mitochondria and peroxisomes play key marked role in imparting defence to plants against ROS by the producing of several antioxidants both enzymatic and nonenzymatic to scavenge the excess ROS (Gill and Tuteja 2010).

## 14.5 Elicitation of Antioxidants by Rhizobium

*Rhizobium* is known to play a great role in the control of soilborne plant pathogenic fungi such as *Fusarium, Rhizoctonia, Sclerotium* and *Macrophomina*. Rhizobia have antagonistic effect on the fungal growth and thus inhibit and limit the pathogenesis process. Inoculation of Rhizobia tends to produce several pathogen inhibiting elements like hydrocyanic acid (HCN), antibiotics, phenolics, antioxidants, antioxidant producing enzymes and siderophores. These factors cumulatively lead to the activation of defence-related genes and in turn induce systemic resistance and impart resistance against the invading pathogens. Rhizobial strains are inoculated in plants through seed bacterization or seed priming which results in elicitation and accumulation of phenolic compounds, isoflavonoid phytoalexins and activation of enzymes like L-phenylalanine ammonia-lyase, chalcone synthase, peroxidase and polyphenol oxidase which are involved in phenylpropanoid and isoflavonoid pathways and enhance the defence mechanism of the plant against pathogenic attack (Dutta et al. 2008). Rhizobia treatment basically restricts soilborne pathogenic infection through activation of antioxidant-dependent signalling pathways. According to Arfaoui et al. (2007), treatment of chickpea seeds with Rhizobium isolate (PchDMS) before sowing with resulted in significant increase in the levels of peroxidases, total phenolics, polyphenol oxidases, isoflavonoids in general and formononetin and biochanin in specific before the infection of Fusarium oxysporum and reported induction of resistance against the fungus.

## 14.6 Conclusion

Soilborne pathogens being a major group of loss causing pathogens need to be managed adequately to diminish the loss. The host uses its own metabolism by producing antioxidants in order to fight against the invading pathogen. So, identification of resistant cultivar is necessary for safeguard the crop against the economic loss. The cultivars can be categorized on the basis of the activity of the antioxidants. Plant defence can also be manipulated by using organic fertilizers which enhance the level of antioxidants in the host system that in turn make the plant resistant to the disease.

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# 15

# A Promising Approach of Managing Seed-Borne Pathogens Through Plant Growth-Promoting Microbes

Rohit Sam Ajee and Shuchi Kaushik

## Abstract

Rapid increase in the global population is directly responsible for the increase in the overall food requirement worldwide. As it stands a report by the Food and Agriculture Organization (of the UN) states that in view of the remarkable development that the Food industry has undergone, the rate of food production will continue to exceed the rate of increase in population. However, the rate of increase in food production is expected to decline from 2.2% per annum to 1.5%. The report also highlights the role of livestock and nonagricultural sources of food in its estimates. Inevitably, such a growth will affect the environment and cause severe damage to it. To ensure that the rate of production of food keeps abreast of the increase in population, the agricultural sector will have to rely on insecticides, pesticides, herbicides, fertilizers, and other manmade substances which have distressing effects on the environment.

Keeping the facts mentioned above in mind, researchers have turned their attention to nature and any alternatives it may provide. Plant Growth-Promoting Microbes (PGPMs) are an interesting class of organisms that could prove useful in enhancing rate of production of crops. PGPMs include a variety of fungi, bacteria, and other microbes. These organisms are able to enhance plant growth by inducing stress tolerance, improving nutrient amassing properties, enhancing the antioxidant systems, inducing resistance to a variety of diseases, and much more. Interestingly, there are several strains of microbes that naturally form symbiotic associations with several plants and aid in their growth and development. The current challenge is to find a way to augment the natural system and

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commercialize it for global use. The review article will cover a range of topics focusing on the importance of antioxidants in such Plant–Microbe interactions.

**Keywords**

Agricultural sources · Disease resistance · Fertilizers · Plant–microbe interactions · Stress tolerance

## 15.1 Introduction

Food plays a pivotal role in the development of the human race. It is the fuel for our bodies and is an absolute necessity for progress. One would think that the rapid increase in the populations worldwide would put tremendous pressure on the Food Industry. While it is true that we may be struggling to meet food requirements, reports show that the current rate of increase in food production actually exceeds the rate of increase in population. However, expecting the current scenario to continue is folly. Although synthetic additives and supplements help meet production demands, they do so at enormous risk to the environment.

Agrochemicals contaminate groundwater sources, are blown away from fields onto other plants causing damage, bioaccumulated within nontarget organisms causing health issues, remain in the biosphere to produce harmful derivatives and by-products, and are generally very resistant to degradation. Repeated and unchecked usages of fertilizers and other agrochemicals impact the soil quality and are directly responsible for Soil Pollution. Subsequently, the leftover chemicals leach into the soil and find their way to groundwater sources which contaminate them and are responsible for Water Pollution. Runoff from farm lands may reach the atmosphere where certain compounds interact with water vapor to form Acid Rains which severely damage life and property.

The presence of various chemical compounds in the air, water, soil, and food we consume is particularly troubling. They may cause diseases, deformations, or other medical conditions due to their accumulation and biomagnification. Chemical runoffs not just affect human life. Beneficial microbes present in the soil, nontarget plants, animals, and insects are all affected and impaired by it. These and other equally disturbing reasons make Agrochemicals and Commercial farming techniques an unsustainable method of meeting our requirements (Naik et al. 2019).

On the other hand, leaving crops to grow on their own without any external aid is unwise as well. Most crop plants are susceptible to a variety of pathogens and insects that hamper production. Additionally, stress caused by salinity, presence of heavy metals, water deficiency, and lack of appropriate nutrients highlights the need external intervention. Plant Growth-Promoting Microbes are a suitable solution to this problem. Not only will these organisms aid the growth of crops in different ways, but also they do so in a natural and sustainable manner. PGPMs are capable of enhancing plant stress tolerance, increasing nutrient acquisition and uptake rates, activating additional defense systems against pathogens and diseases, and inducing photosynthesis.

## 15.2 Plant Growth-Promoting Microbes

PGPMs are classically defined as a group of organism, fungi, bacteria, and actinomycetes that induce or promote the growth and development of plants. They do so by performing one or more activities which have been briefly mentioned in the following section.

Microbes which are capable of solubilizing phosphorus and making it available to crop plants are quite important as Indian Soils show a marked lack of Phosphorus. Phosphate Solubilizing Microbes (PSMs) are predominantly part of the *Pseudomonas* and *Bacillus* species in the case of bacteria and *Aspergillus* and *Penicillium* in the case of fungi. Additionally, several other bacteria, fungi, actinomycetes, and algae have exhibited potential Phosphate Solubilizing activity. Interestingly enough, Actinomycetes that exhibit Phosphate solubilization are observed to have the ability to survive radical environments making them better suited for crops on arid and frigid agriculture (Sharma et al. 2013).

Just as stress factors affect us, drought, salinity, excessive heat or cold, water logging, and other external factors all affect plant growth development, usually in an adverse manner. Drought conditions create an imbalance in the rate of water uptake and evaporation in plants. This leads to decreased water availability for the plants which causes increased competition among them, thereby decreasing overall yield. Plants require optimal amounts of light, water, and temperature for profitable growth. When these factors fluctuate beyond a certain range plant metabolism, photosynthesis, flowering, and fruit development are all hampered. Excessive soil salinity also has similar adverse effects on plant growth. Other than a small percent of the total plant population, most plants cannot grow well in the excessive presence of salt and usually wilt and die under such conditions (Fig. 15.1).

## 15.3 Benefits of Plant Growth-Promoting Microbes (PGPMs)

On a basic level, the role of PGPMs in plant growth and development can be divided into direct and indirect effects. Direct effects comprise mechanisms that physically support plant growth such as atmospheric Nitrogen fixation into the soil, solubilization of Phosphorus minerals, inducing the increased production of plant growth hormones, secondary metabolites and enzymes required for optimal growth, and increasing tolerance to biotic and abiotic stress factors, thereby making the crops more tolerant, on the other hand indirect mechanisms by which PGPMs benefit plants are mainly through prevention of pathogenic diseases by eliminating the harmful microbes and activating plant defense systems to help ward of diseases (Kumari et al. 2019).

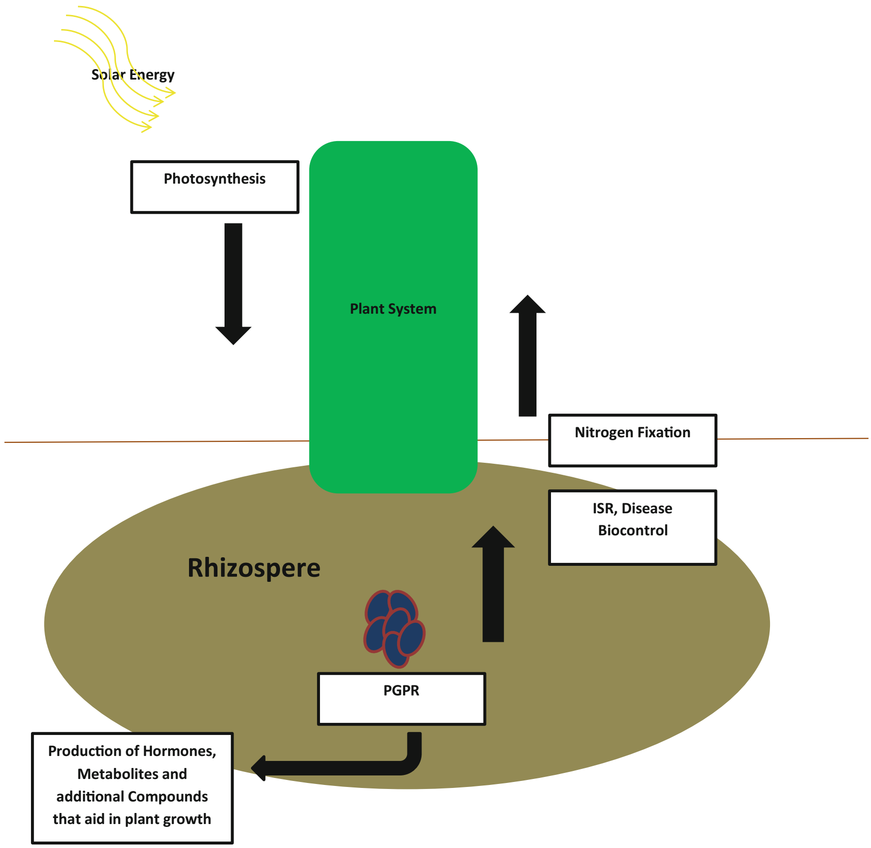

**Fig. 15.1** Plant–microbe interaction

The different Direct and Indirect Mechanisms via which PGPM promotes plant growth and productivity are discussed below.

### 15.3.1 Abiotic Stress Tolerance

Although it is possible to control and regulate most physical parameters to ensure optimal crop productivity, variations could increase the vulnerability of crops to pathogenic attacks. This is where PGPMs could step in as they are more than capable of preventing pathogenic attacks, while bolstering plant growth in adverse conditions.

#### 15.3.1.1 Drought

A study conducted by Marulanda et al. (2009) showed that *Pseudomonas putida*, *Pseudomonas* sp., and *Bacillus megaterium* were all able to enhance plant growth under conditions of drought. They do so by increasing the production of Indole-3-Acetic Acid (IAA) and accumulating Proline. The paper theorizes that the increased production of IAA could be responsible for the enhancement in root growth which helps in increasing the water absorption and retention capacity, thereby reducing the severity of drought conditions. PGPM can also step in during pathogenic attacks and provide protection against them. According to a study conducted by Shoebtiz et al. (2009), certain microbes isolated from the rhizosphere of *Lolium perenne* were shown to possess noticeable biocontrol properties. Additionally, the isolate was shown to bolster growth as the fresh root weight of samples inoculated with the isolate was shown to have increased by 50%. The isolate also promoted the solubilization of the phosphorus present in the mineral form.

#### 15.3.1.2 Salinity

Among the different abiotic stress factors which hamper crop production, salinity of the soil caused by the presence of excessive ions of Sodium, Calcium, Chloride, etc., is one of the most concerning. A large percent of the agricultural lands worldwide, 20%, are excessively saline. Additionally, these saline lands are increasing on a global scale at a rate of 10% per year. Such patches of land repress plant growth resulting in suboptimal yields (Shrivastava and Kumar 2015). Soil salinity causes ion toxicity in the plants, reduces the availability of essential nutrients and minerals, induces osmotic and oxidative stress on the crops, reduces water absorption and photosynthetic ability, etc. Excessive accumulation of the different ions may even lead to cellular dysfunction and death. Different strategies already exist to combat the problem known as soil salinity in crops. One can reduce the salt content of the soil by leaching it out, using better irrigation practices which do not contribute to soil salinity, using salt-tolerant plants, etc. However, these solutions are restricted in their availability and are not very economical in financial terms. PGPMs are a cost-effective and efficient solution to this problem. They have various beneficial effects which have been mentioned above and can also help in controlling the problems caused by salinity (Dodd and Perez-Alfocea 2012). While the term ISR is used to denote the activation of plant defense systems against pathogens by beneficial microbes, IST or Induced Systemic Tolerance was the term coined to refer to the tolerance induced by PGPM. PGPMs induce a range of interlinked effects that collectively reduce the impact of salinity. They may assist in plant–water uptake and retention by accumulating osmolytes in the roots of the plant, alter ion transporter expression to reduce the uptake of ions like $Na^+$ and $Cl^-$, increase nutrient uptake by upregulating the root transporters, prevent water loss, alter phytohormone production, etc.

#### 15.3.1.3 Heavy Metals

The rapid industrial expansion worldwide has also caused a rapid increase in the amount of pollutants being dumped into our environment. Among these, heavy metals pose a significant risk to all living beings. Although these substances are present in the earth's crust naturally, accumulation of these compounds via human activities may have serious implications. Certain heavy metals are required by plants in very low doses. An increase in the concentration of these substances is extremely damaging living organisms as they break down internal systems and cause poisoning. Oxidative stress in plants, caused by excessive levels of the same, can cause cellular destruction and ultimately death (Singh et al. 2011; Tchounwou et al. 2012). Several types of PGPMs are capable of enabling plant growth in soils with high heavy metal concentrations. Various mechanisms exist for the same, such as production of enzymes that degrade and breakdown the heavy metals, effluxing the compounds, promoting the production of plant growth promoters, phytoremediation using suitable microbes, and metal complexation. Phytoremediation refers to the process of removing or degrading contaminants from the environment by using living organisms like plants and microbes. Reducing heavy metal concentration in the soil falls neatly into this category, and several studies have been carried out to identify potential Bioremediation agents. The phytoremediation effects of a few different bacterial strains were explored in a study conducted by Vigliotta et al. (2016). The report confirmed that bacterial strains belonging to *Bacillus, Lysinibacillus,* and *Pseudomonas* were obtained in the rhizosphere of maize plants grown on heavy metal contaminated soil, thereby confirming their tolerance toward them. The study also reaffirmed the claim that PGPMs are capable of mitigating the stress caused by heavy metal pollution. Accumulation of the compounds within the microbes is another commonly observed approach in PGPM to reduce heavy metal stress. *Thiobacillus thiooxidans, Pseudomonas putida, Glomus fasciculatum,* and *Pseudomonas putida* are few microbes capable of this (Mani et al. 2016).

A more comprehensive study by Zhang et al. (2015) highlights the various types of microbes involved the remediation process. Siderophore production is another key process by which heavy metal induces stress can be mitigated. These are small organic compounds that are capable of binding to and transporting iron which are secreted by various microorganisms. Bacteria that are capable of producing Siderophores are sometimes termed as Siderophore-producing Bacteria (SPB). Their ability to chelate to metallic compounds does not stop at Iron. Siderophores have been observed to chelate to heavy metals such as $Al^{3+}$, $Zn^{2+}$, and $Cd^{2+}$. Złoch et al. (2016) showed in their report that bacterial strains belonging to *Streptomyces* showed efficient Siderophore synthesis. The study also suggests that these organisms are capable of increasing the production of required Siderophores under the influence of heavy metals. Increased $Cd^{2+}$ levels were shown to be responsible for the enhance Siderophore production by the different *Streptomyces* strains used for the study.

#### 15.3.1.4 Temperature

Fluctuations in the temperature are a common weather phenomenon. Over the past few decades, we have observed an increase in the global temperature. It does not end there; many researchers speculate that human activities will continue to cause such noticeable temperature changes. The effects of these changes can be seen all around us, in the melting of the Polar glaciers, and the record high temperatures experienced worldwide. Living organisms, especially plants, are also impacted by such changes. As the temperature fluctuates, different plant functions like transpiration and photosynthesis are affected. Different plant components like enzymes and organelles are also influenced by such changes resulting in stunted growth and development. The impact of high-temperature conditions is directly observed on the final yields of the crops. This is due to the reduction in the reproductive efficiency of the crop plants (Alam et al. 2017). The same is true for lower temperature conditions. Most living organisms, crop plants included, are only able to survive in a narrow optimal temperature range. One can reduce the impact of extreme weather on crop plants by inoculating them with resistant PGPM. Adding thermotolerant microbes with plant growth-promoting properties is a cheap and effective means to combat the same. In a study conducted by Meena et al. (2015), four PGPR strains were successfully isolated from the root nodules of pea plants. All the four strains were shown to tolerate low-temperature conditions while also promoting plant growth by solubilizing phosphate and producing IAA.

### 15.3.2 Biotic Stress Tolerance

Plant growth and development is not affected just by abiotic factors. Biotic factors, namely the different microbes, plants, insects, and animals that interact with crops, also play a deciding role in their growth. Even in nature, complex webs of interaction exist among various plants, insects, microbes, and animals. These ‘webs’ are mostly beneficial and are responsible for promoting overall growth and are essential for survival in many cases. Such symbiotic relationships can be utilized in agriculture to enhance crop production and yields. Although some plants are able to resist infection against certain pathogenic microbes, not all crops can display such defense mechanisms and may need outside assistance (Table 15.1).

### 15.3.3 Promotion of Growth

It is also possible to create a consortium of different microbes that enhance plant growth and development. Studies conducted by Sivasakthi et al. (2014) and Hayat et al. (2010) outline the basic benefits that PGPMs provide to agricultural crops in lieu of synthetic additives. Not only do the microbes enhance plant growth by stimulating production of various substances and increasing the availability of nutrients, but also they avert pathogenic infections either by eliminating the microbes themselves or by activating plant systems that eradicate them. Naik et al. (2019) use the term EM (effective microbes) to refer to microbes that can increase yields and be beneficial for agricultural use. These effective microbes can be conveniently classified into Phototrophic Bacteria, Lactic Acid Bacteria, Fermentative Fungi, and Actinomycetes. A mixture of microbes falling into these categories may be applied to fields to bolster plant growth and development. The various microbes falling into the different categories mentioned above all exhibit some advantageous trait like suppressing pathogen growth, mineral solubilization,

**Table 15.1** Stress tolerance mediated by PGPM

| S. no. | Microorganism | Crop | Function | Reference |
|---|---|---|---|---|
| 1 | *Pseudomonas putida* MTCC5279 | *Cicer arietinum* L. | Inducing the activation of stress–response genes, ROS defense systems, etc. | Tiwari et al. (2016) |
| 2 | *Azospirillum brasilense* sp. 245 | *Arabidopsis* | Decreased stomatal activity which helps in preserving water levels and accumulation of proline and other osmolytes | Cohen et al. (2015) |
| 3 | *Thermomyces lanuginosus* | Cucumber plant | Maintains photosystem II activity under heat stress and reduced rate of transpiration, and accumulation of various metabolites that alleviate heat stress | Ali et al. (2018) |
| 4 | *Hartmannibacter diazotrophicus* E19 | Barley | Mediates salinity stress via ACC deaminase activity, enhanced root and shoot production, etc. | Suarez et al. (2015) |
| 5 | *Piriformospora indica* | Barley | Increased tolerance to root diseases and salt stress and enhanced biomass production under saline conditions as compared to untreated plants and greater antioxidants activity | Waller et al. (2005) |
| 6 | *Trichoderma* spp. 582PDA4 | Wheat | Increased plant growth under heavy metal and saline conditions, expressed ACC deaminase activity, etc. | Ripa et al. (2019) |
| 7 | *Acinetobacter* spp. and *Pseudomonas* sp. | Barley and Oats | ACC deaminase activity lowered ethylene levels and production of hormones to enhance plant growth | Chang et al. (2014) |
| 8 | *Azospirillum* | Lettuce seeds | Enhanced biomass production and greater antioxidant activity under saline conditions | Fasciglione et al. (2015) |
| 9 | *Thiobacillus thiooxidans* and *Pseudomonas putida* | *Gladiolus grandiflorus* L. | Enhance heavy metal tolerance by accumulating Cd and Pb and promote overall plant growth | Mani et al. (2016) |
| 10 | *Claroideoglomus claroideum* and *Funneliformis mosseae* | Marigold | Heavy metal tolerance by enhancing antioxidant activity and accumulation of metabolites for stability | Hristozkova et al. (2016) |
| 11 | *Pseudomonas brassicacearum* and *Rhizobium leguminosarum* | *Brassica juncea* | Heavy metal tolerance by phytoremediation, heavy metal chelation, etc. | Adedrian et al. (2015) |

enhancing nutrient uptake, activating plant defense systems, and promoting phytohormone production.

### 15.3.4 Nitrogen Fixation

Ready availability of Nitrogen is an important consideration for achieving maximum crop productivity. Nitrogen naturally present in the soil does not fulfill the crop requirements, and plants are unable to fix atmospheric Nitrogen directly for use. Hence, additional sources, Nitrogenous fertilizers, need to be made available. It is possible to reduce the dependence on Nitrogenous fertilizers by incorporating symbiotic bacteria that exhibit the biological Nitrogen fixation process. Although legumes are the crops that pop into mind when thinking of symbiotic, Nitrogen-fixing bacteria, there are various PGPRs that are able to bond with other crops as well. The plant may associate with the symbiotic bacteria either through endosymbiosis and endophytic association or interact with free-living nitrogen-fixing bacteria. The first is the most intimate form of relation, while the third is the simplest (Mus et al. 2016). The plants return the favor by providing sugars, essential amino acids, vitamins, and other organic substances to the symbiotic bacteria in the form of exudates. Typically, root exudates are the primary source of energy for the PGPR, and it also helps in attracting the desirable microbes to the rhizosphere through chemotaxis.

When the symbiotic bonds are formed among the bacteria and the crop, major changes are observed in the metabolic pathways of both organisms. The host plant ensures that a steady supply of carbon is made available to the PGPR, while changes are made (either internally or forced by the host plant) in bacterial nitrogen metabolism pathways such that the fixed nitrogen is excreted and not just fixed internally. In addition to carbon, in endosymbiotic relations, the host plant ensures that the bacteria have access to phosphorus, iron, sulfur, and other necessary metallic and nonmetallic compounds.

The process of Nitrogen fixation is governed by the highly conserved enzyme known as nitrogenase. The complex process involves the catalytic conversion of atmospheric nitrogen to ammonia. Nodule formation is a key process for the establishing a symbiotic relation between the two and involves host–bacteria communication and is facilitated by the Nodulation genes (Franche et al. 2009). The genes and the Nod factors are important in creating and maintaining the bond as it is necessary for the formation of root nodules as well as bacterial occupation of the nodule (Gage 2004).

### 15.3.5 Phosphate Solubilization

Phosphorus is another essential macronutrient required for optimal plant growth which has limited availability naturally. The geochemical cycling of the element reduces its availability to plants, and as such, external sources of phosphorus need to be provided to compensate. A greener approach to the process can be considered by using bacteria, fungi, and other soil microbes which are capable of solubilizing it from the soil into usable forms. Bacteria belonging to *Pseudomonas* and *Bacillus* and Fungi belonging to *Aspergillus* and *Penicillium* are shown to have notable phosphate solubilizing properties (Mehta et al. 2019).

Phosphate Solubilizing Bacteria (PSB) can be of the free-living type that exist in the root–rhizosphere region or may form endophytic connections with the host. Microbes with phosphate solubilizing properties play an important role in the biogeochemical cycle of the element. They solubilize the inorganic and organic, bound forms of phosphorus, and make it available for plant use. The process is carried out by several different ways by the microbes. Secreting extracellular enzymes, mineral dissolving compounds, etc., are some of the means by which phosphate is made available (Walia et al. 2017). Microbial compounds like siderophores and exopolysaccharides are suspected to play key roles in the process.

### 15.3.6 Phytohormone Production

Another way by which PGPM is able to enhance plant growth is by synthesizing and making available certain Plant Hormones or Phytohormones (Table 15.2). These microbe synthesized hormones that help in a wide range of plant physiological and

**Table 15.2** Plant growth-promoting activities mediated by PGPM

| | Microorganism | Crop | Function | Reference |
|---|---|---|---|---|
| 1 | *Aspergillus* sp. and *Penicillium* sp. | Diverse plant species | Efficient phosphate solubilizing property | Elias et al. (2016) |
| 2 | *Paenibacillus polymyxa* | *Zea mays* | Phosphate solubilization under abiotic stress conditions, biocontrol activity against several fungal species, etc. | Din et al. (2020) |
| 3 | *Enterobacter* sp. ITCB-09 | Habanero pepper | Phosphate solubilization, siderophore production, synthesis of phosphate solubilizing enzymes, etc. | Mendoza-Arroy et al. (2020) |
| 4 | *Pseudomonas maltophilia* | Sugarcane | Biological nitrogen fixation, exhibited siderophore production, and some amount of ACC deaminase activity | Xing et al. (2016) |
| 5 | *Pseudomonas* sp. K1 | Rice | Enhanced phytohormone production, nitrogen fixation, and increased yield | Mirza et al. (2006) |
| 6 | *Rhizophagus irregularis* | In vitro study | Production of phytohormones (cytokinin, auxin, gibberellin) | Pons et al. (2020) |
| 7 | *Azospirillum* | Diverse plant species | Production of phytohormones (cytokinin, auxin, gibberellin, ethylene, abscisic acid) and other plant growth regulators like (nitric oxide, polyamines) | Cassan et al. (2014) |
| 8 | *Nocardiopsis* | Mandarin | Production of phytohormones (auxins) and potential phosphate solubilizing activity | Shutsrirung et al. (2013) |

metabolic activities. Auxins, Gibberellins, and Cytokinins are common phytohormones produced by several PGPMs. Studies have observed that overall plant growth is bolstered to an extent by the presence of microbial phytohormones or due to the induction of hormone production by microbes (Cassan et al. 2014). Studies have also demonstrated that fungal strains too have this property and are capable of producing plant phytohormones (Pons et al. 2020; Tiwari et al. 2020).

### 15.3.7 Biocontrol Agents

Different microbes exhibit different modes of action to prevent pathogenic infestations and infections. While some prevent the growth of pathogenic microbes, others degrade or break down the harmful components produced by them. Bacteria like *P. fluorescens* produce certain compounds like 2,4-diacetyl phloroglucinol which inhibits fungal growth and development. Others like *Pseudomonas stutzer* produce compounds that lyse the pathogenic cells directly. Yet others induce systemic resistance in the host plants and activate innate defense systems of the plant to protect them from pathogens. Such activations of the plants defense systems are referred to as Induced Systemic Resistance (IRS). Microbes that naturally exist in the fields already help in the biocontrol process. However, PGPM can be incorporated into agricultural practices to inhibit bacterial, viral, fungal, and nematode diseases. Assimilating PGPMs into integrated pest management programs is a step in the sustainable direction for agriculture.

Of the different microbes used as potential PGPR, bacteria belonging to the *Pseudomonas* genus is one of the most widely known for their range of beneficial effects and actions. They have shown effective pathogenic repression in cereals, pulses, different fruits and vegetables, cottons, and mushrooms (David et al. 2018). *Pseudomonas* has a variety of factors that make them one of the most dominant PGPM. They are capable of rapidly growing and attaining critical mass and colonizing the rhizosphere and interior of the plant roots and are able to adjust themselves to the external conditions with relative ease. They are also able to compete and survive in the presence of other microbial colonies and also produce a large amount and variety of active compounds that assist in the plant growth and development process.

Certain species like *P. putida* strain B2017 were shown not to produce any toxic compounds or metabolites. Instead, they expressed their biocontrol activity by producing other compounds like surfactants, siderophores, and pyoverdine. As these strains lack any harmful or antagonistic agents, they are able to express biocontrol activity without negatively affecting the crop plants in any way (Pich et al. 2020). Additionally, the harmful impact they may have on the surrounding environment, nontarget organisms, and humans (the ultimate consumers of the crops) is negligible.

Several studies have been conducted into the potential biocontrol activity that the different strains of *Pseudomonas* exhibit. *Pseudomonas chlororaphis* ToZa7 was studied for their antipathogenic activity, particularly as an antifungal agent in tomato plants. The study identified that ToZa7 was able to upregulate three genes involved in the defense system. ToZa7 was made available to the tomato plant along with *Clonostachys rosea* IK726. The combination of the two microbial strains induced a significant positive effect on the defense systems of the tomato plant (Kamou et al. 2020). Another study highlights the possible biocontrol activity of a *Pseudomonas fulva* strain. The *Pseudomonas* strain, known as HARBPS9.1 in the study, was found to consistently exhibit antifungal activity in maize crops. The microbe was isolated from the rhizosphere of the maize crops in study and exhibited significant activity against the fungal pathogens. HARBPS9.1 exhibited noticeable bioprotection of the maize plants that were grown in the presence of *F. graminearum* and *F. culmorum* pathogens. Not only did the *Pseudomonas* stain inhibit fungal growth, but also they enhanced the growth of the infected seeds (Adeniji et al. 2020).

In another study, microbes isolated from the rhizosphere of the medical plant *Valeriana wallichii* were subjected to biocontrol tests to identify suitable PGPMs. The study concluded that out of the twelve isolated strains, *P. aeruginosa* showed the greatest resistance toward the fungi *F. oxysporum, Alternaria alternata,* and *Aspergillus flavus.* Using *P. aeruginosa* as a biocontrol agent in agricultural contexts has a dual role. Not only can they help in preventing fungal pathogens from harming the crops, but also they produce metabolites, compounds, and hormones that actively assist in plant growth and development. Such a dual role is particularly useful for farmers that cannot invest large sums of money into farming. Using PGPMs serves as a sustainable and reliable way to reduce pathogenic infestations while also bolstering crop yield. *P. aeruginosa* can be used as a nontoxic, eco-friendly, and budget alternative to conventional farming techniques (Chandra et al. 2020).

On average, using PGPM in place of chemical supplements to protect crops from pathogens is not only cost-effective in the longer run but also protects the environment to a greater extent. The amount of resources (labor, fossil fuels, machinery, transportation) required to create chemical agents are much higher when compared to creating a microbial consortia that do the same task in an eco-friendly manner. Using PGPM for biocontrol, and other crop-related activities, has the added benefit of only affecting the target organism. Nontarget organisms and the surrounding environment are relatively unaffected by the presence of microbes in the soil. PGPM does this by the production of various metabolites, compounds, and hormones, like antibiotics, hydrogen cyanide, phenazines, siderophores, and volatile compounds directly. Indirectly, they compete with the pathogenic microbes for nutrients and induce the activation of plant defense systems (Naamala and Smith 2020).

### 15.3.8 Seed Biopriming

Seed priming is an agricultural technique that is used to allow metabolic activity to occur within the seeds without the emergence of the radicle. By controlling the temperature and water levels, it is possible to keep the seed in a state where the pregermination metabolic activities occur, thereby increasing the chance that these primed seeds will germinate successfully when planted into the soil (Raj and Sheeja 2019). The process of seed priming has been in practice since ancient times, as far back as the Greek civilization. Farmer understood early on that not all seeds germinated into plants and that by priming them one could increase the chances of germination as well as the yield. Various seed priming techniques exist such as the following:

1. **Hydropriming:** Seeds are simply soaked in water under well-defined conditions.
2. **Halopriming:** Seeds are soaked in solutions containing inorganic salts.
3. **Osmopriming:** The seeds are soaked in solutions containing osmotic fluids like glycerol or PEG.
4. **Solid Matrix Priming:** The seeds are embedded in an insoluble matrix which provides the appropriate water levels and other conditions to allow for priming.
5. **Biopriming:** In this method, seed priming involves using PGPM in the liquid in which the seeds are submerged to allow the microbes to colonize and proliferate within the seeds.
6. **Hormonal Priming:** This method involves using hormones to enhance seed priming and growth.

Of the different seed priming methods mentioned above, seed biopriming is the most interesting technique. In addition to increasing yields and efficiency of germination of the seeds, the method also protects the seeds from seed-borne diseases. Additionally, it has been observed that plants that germinate from primed seeds use water more efficiently, are able to better compete with weeds, show enhanced nutrient usage, and have greater stress tolerance.

When it comes to the application of the PGPM inoculum to crops, we have many different methods such as direct application of the inoculum to the soil, dipping the root of the desired plants in the consortia, carrier-based inoculation, etc. Inoculation of the beneficial bacteria onto seeds prior to germination (seed biopriming) is accepted as the most efficient and time-saving method of application. When a seed is suspended in a solution containing PGPM, the microbes are able to colonize the seeds and acclimatize to the conditions (Mahmood et al. 2016). The process of soaking also activates the metabolic processes within the seeds. After they have soaked for a calculated time period, these seeds are removed from the solution and dried which kick-starts the germination process. However, the procedure ensures that germination does not result in plumule and radicle emergence. Only the internal physiological activities such as cellular growth and development are carried out, allowing the seeds to be better prepared for the actual plant growth.

It has also been observed that the seeds that are bioprimed show uniformity in their germination stage. Better yet, the primed seeds have also shown to exhibit enhanced stress tolerance, in the case of both biotic and abiotic stresses. One can even deliver the desired active compound/PGPM into the seeds to express a specific effect. For example, if your crops are negatively affected by weeds, introducing herbicides (in the form natural compounds synthesized by the PGPM) in the seeds during the biopriming stage would increase the ability of these crops to compete with the weeds. All of these effects indirectly add up to the plant disease resistance as well. The presence of the PGPM in addition to enhanced plant tolerance makes bioprimed plants more capable of withstanding diseases and pathogenic infestations. All in all, the process of biopriming seeds has various benefits and as such is an extremely efficient and economically viable method of improving crop yield (Bisen et al. 2015).

In an experimental study carried out by Deshmukh et al. to determine the effect of seed biopriming on *Alternaria* leaf spots, it was observed that bioprimed seeds showed a greater ability to manage and tolerate the disease as compared to hydroprimed and untreated seeds (Deshmukh 2019). Seeds biopriming was done using six different PGPMs: *T. viride, T. harzianum, T. fasciculatum P. fluorescens-I, P. fluorescens-II,* and *P. Aeruginosa.* The *Alternaria* leaf spot disease is a common fungal disease that affects a wide range of host plants. In Green gram, this disease is a seed-borne type and as such causes a great loss by decreasing seed viability and germination ability. The seeds that do germinate produce lower and/or subpar yields. Treating the seed with the PGPM inoculation had a significant beneficial effect in all cases. The study concluded after a 2-year study period that *T. harzianum, T. viride,* and *P. aeruginosa* all showed a remarkable decrease in the incidence of the disease and also increased plan production. Lucas et al. demonstrated the ability of *Pseudomonas fluorescens* Aur 6 and *Chryseobacterium balustinum* Aur 9 in controlling the incidence of blast disease in rice plants over a 2-year experimental study (Lucas et al. 2009). The two microbes are well-characterized PGPM and are used individually and in combined forms to control disease incidence. A number of mechanisms have been proposed to explain the ability of the organisms to prevent disease induction. The most widely accepted one is the induction of systemic plant resistance. However, production of antifungal compounds, production of fungal cell wall degrading enzymes, competition for limited resources, etc., are all valid proposals as well. Although both *Pseudomonas* and *Chryseobacterium* were able to produce noticeable results, it was observed that leaves treated with a combination of the two showed the greatest ability to tolerate the blast disease. They also concluded that seed biopriming was of great importance as inoculation with even a single PGPM agent decreased disease incidence by up to 50%.

*Trichoderma asperellum* was used in another study to bioprime pea seeds (Singh et al. 2016). *Trichoderma* is a well-characterized fungal agent that is commonly found in plant rhizosphere. The microbe not only promotes plant growth and development, but also actively prevents disease incidence. The fungus is reported to increase root and shoot length by enhancing plant nutrient uptake and inducing the production of various plant growth-promoting hormones and increase nitrogen and phosphate utilization by solubilizing the compounds into forms that plants can use. The study reported a direct correlation between seed biopriming with *Trichoderma asperellum* and increases in root length, shoot length, number of leaves, and plant wet and dry weight when compared to untreated seeds.

Yet another study reported the effects of biopriming maize seeds with *Pseudomonas geniculate. P. geniculate* was shown to exhibit satisfactory plant growth-promoting properties in earlier studies. Of particular interest is the fact that

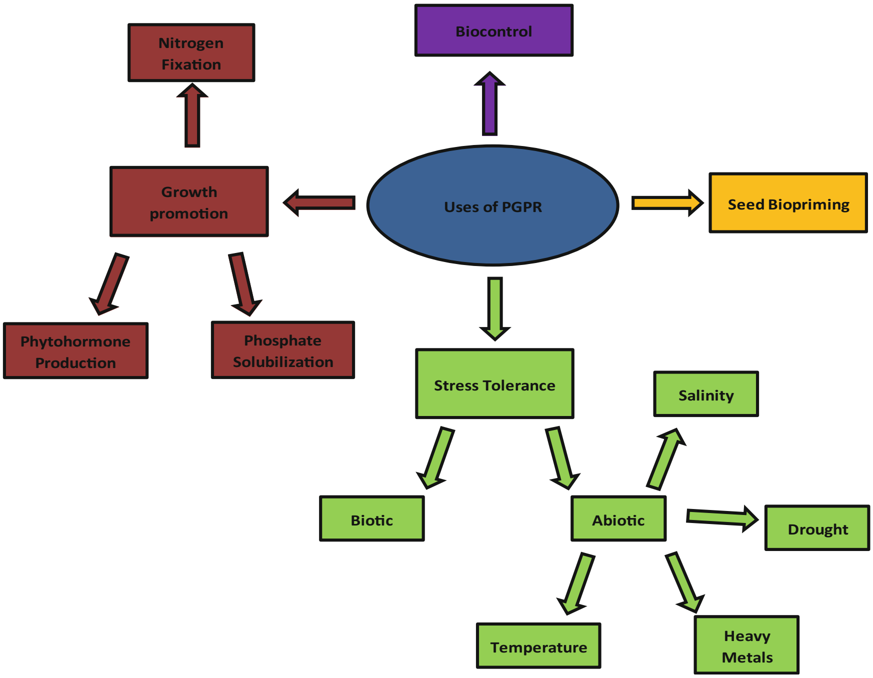

**Fig. 15.2** Different benefits of PGPM

*P. geniculate* also confers stress tolerance to agricultural crops. The study was able to determine that under saline conditions, maize growth was beneficially affected by *P. geniculate* (Singh et al. 2020*)*. Biopriming the maize seeds allowed them to better survive the toxic effects of high salt conditions. When exposed to high saline conditions, plants produce antioxidant enzymes in order to protect themselves. The study observed that plants frown from *P. geniculate* bioprimed seeds showed an increased production of antioxidant enzymes. Not only were the levels of catalase (CAT), peroxidase (POx), and superoxide dismutase (SOD) enzymes found to be elevated in treated plants, they also showed significantly greater antioxidant activity. Additionally, *P. geniculate* was shown to maintain internal cell pressure by increasing the proline and internal sugar content and regulating the uptake of different ions to allow for optimal plant growth (Fig. 15.2).

## 15.4 Mechanisms of Action of PGPM

Now that we have an idea as to the beneficial effects that certain microbes are able to induce in plants, let us take a look at how they achieve this. Although much still remains to be learned about the functioning of PGPM and their effects, we do have a basic understanding of most of their activities.

### 15.4.1 Mechanism of Stress Tolerance

As discussed previously, biotic and abiotic forms of stress are one of the leading causes in the reduction of plant growth and overall yield. Drought, saline soil conditions, heavy metal poisoning, excessive temperatures, and other biotic factors of stress all negatively impact plant development.

Of the different abiotic stress factors, most consider high salt condition as the greatest limiting factor in terms of agricultural productions. A study by Ruan et al. (2010) states that nearly 10% of the global agricultural lands are affected by high saline conditions. Excessive irrigation and use of synthetic agricultural supplements are the leading cause for human-induced soil salinity. High salt conditions have a variety of detrimental effects on plant physiology like production of ROS, ion toxicity, inhibition of germination, excessive ethylene production, and induced osmotic stress. Certain PGPMs are able to balance the saline conditions and allow for normal plant growth (Salwan et al. 2019). It has been observed that microbes which exhibit 1-aminocyclopropane-1-carboxylate deaminase (ACCD) are able to adapt plant roots to high salt conditions. ACCD is able to reduce salt stress by regulating ethylene levels. The deaminase is capable of degrading ACC, which is an ethylene precursor, to prevent Ethylene formation. Additionally, these microbes are also capable of regulating the production of plant growth-promoting hormones to enhance growth. Certain PGPMs are also shown to be able to regulate the levels of ions entering the plant. These microbes may enhance the activity of ion transporters to eject these ions or may even induce the formation of a biofilm over the plant surface to restrict the import of ions (Dodd and Perez-Alfocea 2012). Another way in which microbes alleviate salt stress is by accumulating beneficial osmolytes within the cells to maintain cell turgor pressure. Activation of antioxidant systems and production of antioxidant enzymes is yet another way in which salt stress can be alleviated. Microbial activity has shown to trigger various enzymatic antioxidant systems within plants which are beneficial to it (Helepciuc et al. 2014).

Similarly, under drought conditions, PGPMs confer similar benefits to the host plants in order to tolerate the adverse conditions. Several studies report that inoculation with PGPR resulted in an increase in the levels of phytohormones, especially auxins like IAA, which helped to combat the drought stress. An increase in the level of IAA allowed plants to express enhance metabolic activity and nutritional uptake. A similar increase in Gibberellins and ABA was also observed in the host plants. According to one review, the increased ABA levels caused due to *Phyllobacterium brassicacearum* resulted in a decrease in the water transpiration rates in *Arabidopsis (*Vurukonda et al. 2016*)*. Several strains of bacteria are also able to mediate changes in the root cell membranes of plants, thereby allowing them to survive drought conditions. They also activate various antioxidant systems and enzymes in plants that protect them from the water deficiency induce stress. As explained in the case of Saline stress tolerance, plants that exhibit ACCD activity are able to alleviate the stress caused by the accumulation of ethylene. As ethylene regulates overall plant growth and inhibition and acts to inhibit shoot and root growth under conditions of stress, degradation of the ACC precursor allows for optimal plant growth under stress conditions (Glick 2005). Accumulation of osmolytes is another way by which plants are able to survive under conditions of stress. PGPMs are able to secrete and enhance the accumulation of such osmolytes like proline, trehalose, and choline. Accumulation of proline helps the plant as the compound protects the cells and proteins from the drought stress and maintains cell water status. Trehalose acts as an osmolyte by providing stability to dehydrated cell membranes and plant enzymes. The synthesis of this disaccharide is induced by the action of various microbes like *Rhizobium etli (*Suarez et al. 2008*), A. brasilense (*Salazar et al. 2009*), and Stenotrophomonas rhizophila (*Alavi et al. 2013*)*. Several PGPMs are also able to produce Exopolysaccharides (EPS) which are able to alleviate stress conditions to a great extent.

And finally, these beneficial microbes help in the activation of various Antioxidant systems to combat the increased generation of ROS due to drought and other unfavorable environmental conditions. The presence of ROS hampers the normal plant functioning as these compounds cause oxidative damage to cells, proteins, DNA, and lipids (Gill and Tuteja 2010). Plants have various antioxidant systems in place to combat these deleterious effects caused by ROS. Several enzymatic and nonenzymatic antioxidants can be found in the plant systems. Inoculation of crops with PGPM has the added benefit that these microbes are able to enhance the production and activity of the antioxidants. The same has been observed in the case of Basil plants inoculated with different bacterial strains (Heidari and Golpayegani 2012). However, under certain situations, the PGPM may also decrease the activity of the antioxidants. When inoculated with *B. thuringiensis,* the host plant *L. dentate* showed a significant decrease in the ascorbate peroxidase (APX) and glutathione reductase (GR) enzymatic antioxidants (Armada et al. 2014). This decrease highlights an energy saving process that the bacterial inoculated plant exhibits. The energy saved is then used for vital, life-sustaining activities under stress condition indicating that these plants are better adapted to stress. Lower antioxidant activity directly correlates to lower ROS levels, indicating better plant health and physiology under stress conditions in plants inoculated with PGPM.

In addition to regulating the harmful effects of the ROS generated on the plant physiology, the antioxidants produced may assist in plant–PGPM communication. The antioxidants can be used to transmit stress signals via the oxidant–antioxidant interaction allowing communication between the host crop and the symbiotic PGPM. This allows the host organism to better differentiate between harmful pathogens and beneficial PGPM (Hamilton et al. 2012). Fungal symbiotes have been shown to not only enhance the production of antioxidants within the hosts, but also produce their own antioxidants to aid in stress tolerance.

Biological Remediation is the key process by which PGPMs are able to enhance plant growth under heavy metal conditions. Not only do the microbes help in cleaning up the soil of the unwanted contaminants, but also they bolster plant growth and development. A variety of remediation mechanisms like sorption, bioaccumulation, and enzymatic oxidation heavy metal efflux are all options that PGPM have when it comes to remove the heavy metals. All of these are possible because many microbes have developed a resistance to such conditions and also have internal metabolic pathways that help in the conversion of these complex compounds into relatively simpler forms (Mustapha and Halimoon 2015). Of the different microorganisms, bacteria occupy the forefront when it comes to heave metal biotransformation. Bacteria reduce the heavy metal stress load on plants by absorbing the heavy metals and accumulating them into their cell walls, precipitating them, detoxifying them via oxidation–reduction reactions, storing them within extracellular capsules, etc. (Zubair et al. 2016) After Bacteria, Fungi occupies the number two position when it comes to bioremediation. They are able to efficiently sorb (either by absorbtion or adsorbtion) and hold these compounds in compartments within their cell walls. Fungal symbiotes also exhibit many of the mechanisms expressed by Bacteria during heavy metal transformation. Storage, Transport, and Sequestration are the commonly observed mechanisms by which fungal PGPMs alleviate heavy metal stress in crops plants (Tiwari and Lata 2018).

### 15.4.2 Mechanisms of Disease Suppression/Biocontrol

Plant Growth-Promoting Microbes express antagonistic activities against various phytopathogens through a variety of modes. They are capable of suppressing plant pathogen activity both directly, via physiological activities exhibited by themselves, or indirectly by prompting or inducing a response in the host plant. Competition for resources and root surface area is the greatest of the direct mechanism exhibited by PGPM. The beneficial microbes reach the root surface using their motile cell extensions. The plants also produce certain compounds in their root exudates which help in attracting the beneficial microbes. PGPMs are suitably adapted to identify and target the specific chemoattractants that their host plant exudes. In addition to serving as chemical signals for calling the attention of various PGPM, the root exudate also acts as the major nutritional source for symbiotic microbes (Rovira 1965).

The second direct mechanism by which PGPM protects plants for phytopathogens is by the production of various secondary metabolites, antibiotics, active compounds, and hormones which prevent pathogenic growth. Siderophores are a class of microbial compounds produced to acquire iron from the soil. The bioactivity of bacterial siderophores is greater than fungal siderophores making them better equipped to acquire iron. This starves the fungal counterparts and reduces the viability of fungal species, thereby decreasing rates of fungal infections (Pandian 2013). Siderophores have the added benefit that they are also able to efficiently accumulate toxic metals, thereby alleviating plant heavy metal stress. Several bacteria are also able to synthesize different classes of antibiotics which also aid in the process of biocontrol. The production of antibiotics is controlled to some degree by the growth and development of the host plant. Only after the plant has reached a certain maturity do the PGPM secret antibiotics, in response to changes in the root exudates (Piccard et al. 2000). Of particular interest is the fact that the beneficial microbes are able to synthesize a variety of antibiotics, thus allowing them to suppress a wide range of pathogenic growth under diverse conditions. Several microbes are also capable of producing lytic enzymes that are used to directly attack the pathogenic cells and damaging them. Production of extracellular lytic enzymes like chitinases, laminarinases, proteases, and glucanases is all essential for preventing phytopathogen infections. Another promising mechanism that several PGPMs exhibit is the detoxification of virulence factors produced by the microbes (Qureshi et al. 2016). In some cases, the beneficial microbes have been observed to block the activity of several virulence genes, thereby reducing the pathogenic activity of phytopathogens. They do so by preventing pathogenic quorum-sensing capacity. Since the pathogens are unable to communicate, they are unable to effectively turn on the key virulence factor gene cascades which severely restrict the degree of the infection.

Indirect mechanisms of action include inducing systemic plant resistance by activating internal defense mechanisms. Induced Systemic Resistance (ISR) is very similar to Systemic Acquired Resistance (SAR), which can be described as disease tolerance and resistance depicted by the plant after an earlier exposure to pathogenic organisms. ISR works in a similar manner; the beneficial microbes help to activate overall plant defense systems making them more disease tolerant. On a broader scale, ISR is the induction of resistance to pathogenic diseases due to the activity of microbes or their secretions (Kuc 2001). The process involves the activation of various in-built systems and processes to enhance plant immunity. This results in the production and accumulation of various compounds. The nature of these compounds varies greatly; some may be inherently antagonistic compounds, while others may simply hamper pathogenic infections. The basic difference between the two is that SAR is activated due to exposure to pathogens in response to the formation of disease symptoms, while ISR is induced due to the presence of PGPM and does not require any symptoms to manifest. On a technical scale, the activity of different hormones has been linked to the regulation and induction of the two mechanisms. SAR is thought to be dependent on the presence of Salicylic Acid and activation of Pathogenesis-Related Genes (PR), while ISR is Salicylic Acid independent and does not involve the activation of the PR genes (Jain and Khurana 2018).

Both endophytic and free-living bacteria have been shown to be able to induce ISR in host plants. Similarly, many fungal strains have also been shown to induce ISR while also promoting plant growth. However, the mechanism of induction of ISR by fungal microbes is yet to be clearly defined (Hossain and Sultana 2020). Several studies indicate that the plant hormones Jasmonate and Ethylene play a key role in regulating the ISR activity mediated by the PGPM. However, the PGPMs do not increase the biosynthesis of these hormones; rather, they increase plant sensitivity to these hormones. While a few microbes that induce ISR cause the production and accumulation of PR proteins, most PGPM regulates the accumulation of compounds like phytoalexins, polyphenol oxidase, and peroxidase (Compant et al. 2005). The induction of ISR is host plants causes them to enter a defense mode and is referred to as priming. Primed plants are more sensitive to pathogenic attacks and are able to activate cellular defenses faster. Such primed plants are always ready to activate their defense systems in response to pathogenic threats and are much more disease-tolerant than unprimed plants. In addition to entering a stage of enhanced cellular activity, the plant cells themselves undergo certain changes in order prepare for the ensuing invasion. There is an increase in the accumulation of phenolic compounds in the plant cell walls which strengthens it. Certain plants are also able to express enhanced stomatal closure when the pathogenic route of infection is via the stomatal pores. All these structural barriers, in addition to the primed state of the plant, increase the overall fitness of the crop (Pieterse et al. 2014).

## 15.5 Conclusion and Future Prospects

Although PGPM can help in so many ways, there are some limitations to their use. For starters, the beneficial microbes present naturally in the soil will not be able to induce the desired amount of activity when it comes to crop plants. For them to express optimal activity, they will have to be isolated from the soil or the rhizosphere and cultured in vitro to obtain the appropriate amount of microbial culture. These laboratory-cultured microbes can then be applied to plants or their seeds to obtain results. Additionally, we have yet to come up with a commercial means to synthesize and transport created cultures over long distances. There is also the added problem that the in vitro culturing of microbes may cause certain mutations which could drastically change the effects of the microbes, meaning a strict quality control phase needs to be set in place. However, the greatest limitation to the widespread use of PGPM in agriculture is the lack of knowledge regarding it among farmers. This manifests as a low demand for the cultured PGPM, which in turn results in lower profits for agrocorporations that produce them. Awareness regarding the sustainable nature and the long-term benefits that the PGPMs yield needs to be created among farmers.

PGPMs have come a long way and still need refinement and adjustment to be used to their max potential. Public knowledge and funding into the field could reveal more about the organisms and mechanisms involved in the sustainable practice and make it more available and widespread. The key to producing enough crop food to feed the entire world without exhausting all our resources lies in sustainable agriculture. And PGPMs can play a pivotal role in the same, if used properly.

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# Antioxidant Potential of Plant Growth-Promoting Rhizobacteria (PGPR) in Agricultural Crops Infected with Root-Knot Nematodes

# 16

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## Abstract

Rhizosphere comprises inimitable environs, acting as a battleground for PGPR to form an origin of enriched source of unique functionalities for plants. PGPR are closely bound to roots in order to nurture them and protect them from pathogens specifically Root-knot Nematodes (RKNs) through direct and indirect manner. RKNs are mainly endoparasitic to cause dreadful diseases in plants, especially economically important crops. The traditional use of chemical agents for nematode control in agricultural yields has been adversely affecting the soil microbiota as well as physiochemical attributes of soil. Contrastingly, PGPR forms symbiotic associations alongside plants and act as biofertilizers, bioprotectors, and sustainable means for agricultural practices. PGPR are observed to improve nutrient acquisition and release various secondary metabolites like phenols, enzymes, siderophores, hydrogen cyanide (HCN), phytohormones, and hydrolytic enzymes to boost the antioxidant potential of plants damaged by RKNs. Moreover, PGPR also synthesize various antimicrobial agents or toxins in order to directly attack the invading RKNs for activating induced systemic resistance mechanism of plants and enhance antioxidative properties within them. Therefore, all these mechanisms are of paramount importance as they are well-documented biocontrol agents in the field of agronomy. Here, in this chapter,

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we will discuss the role of PGPR and their interaction along with plant roots within rhizosphere to form RKN resistance. In general, we have elaborated the mechanistic role of PGPR in mediating antioxidant capacity of plants against nematode infestations. Ultimately, we have recapitulated the role of PGPR to be used as alternatives to chemical fertilizers for sustainable agriculture.

**Keywords**

Biocontrol · PGPR · Plant disease · Nematodes · Antioxidant

## 16.1 Introduction

Root-knot nematodes (RKNs) are predominant soil populace that deteriorate many agricultural crops. Approximately, $US 157 billion of annual crop damage has been observed only due to RKNs (Singh et al. 2015a). RKNs being highly destructive pests show unique nature, owing to which they are quite intricate to manage and identify, typically because they share similar appearance with many other pathogenic diseases. An abundant of nematode species have been explored till date that cause severe agricultural damage and the lifestyle of each species deploys specific parasitic behavior. For instance, many of the species display migratory behavior, while other might show sedentary lifestyle along with endoparasitic or exoparasitic nature. They might invade the host and form extensive network within cells, or they could just wander through soils and damage the root morphology (Gheysen et al. 2000). Therefore, this feeding mechanism deteriorates plant roots and dwindles their capability to absorb water as well as minerals from the soil. Typically, RKN damage is revealed through stunted plant biomass, deformed root system and swelling and bushy appearance roots. Moreover, RKN damage also enhances the incidence of infection of other plant pathogens that may also enter the plant roots to cause intense damage. One of the most economically significant nematode categories of nematodes belonging to sedentary lifestyle is RKN, *Meloidogyne* spp., followed by cyst nematodes, migratory endoparasitic nematodes, and burrowing nematodes, respectively (Jones et al. 2013). It is worth noting that RKNs possess extremely specialized structures for feeding mechanism, known as stylet that enables the penetration of cells within plant tissues (Kikuchi et al. 2017). Apart from this, they release a chain of effector molecules within host cells via stylet to mediate the formation of a specific feeding constriction termed as syncytia or giant cells for cyst as well as RKNs, respectively (Mitchum et al. 2013). Alongside this, these characteristic feeding sites are filled with cytoplasm, large nuclei, vacuole, and other organelles that play a vital role in nutrient requirement for nematode development (Golinowski et al. 1996). However, the release of specific molecules or compounds is escorted by a broad alteration of genetic patterns of RKN-parasitized plant cells and roots along with the action of genes specific to defense responses, cell wall modulation, and other biochemical and metabolic pathways (Barcala et al. 2010). Basically, there are two main pathways such as shikimate and phenylpropanoid pathway in plants associated with plant metabolic interactions among nematodes (Castaneda-Alvarez and Aballay 2016).

RKN management is mostly dependent on chemical agents since decades, and these are usually applied on crops directly. These chemicals are very noxious and contaminate the environment very badly along with its negative effects toward human population. To illustrate, methyl bromide has been debasing the environment since many years and therefore, has been banned from the commercial market (Meadows 2013). Looking into all these scenarios, an alternate strategy is need of the hour that could be more beneficial in all aspects. In this regard, environment-friendly biocontrol agents would give a best possible alternative to minimize the RKN proliferation from the soils (Beneduzi et al. 2012). Plant Growth-Promoting Rhizobacteria (PGPR) is the most feasible agent that also promotes plant growth through solubilizing nutrients, secreting plant hormones, and various metabolites (Santoro et al. 2011). Moreover, the PGPR inhibit the pathogenic organisms within soils, regarding which ample literature have been reported so far (Noreen et al. 2015). PGPR and RKNs share the same habitat within rhizosphere and affect the functional processes of one another along with the plant activities they colonize with (Singh et al. 2017). Plethora of PGPR curb the RKN population within soils by different modes of action and improve plant growth as well as yield. Various species such as *Bacillus, Pseudomonas, Serratia, Burkholderia, and Streptococcus* have been studied for its antagonistic potential (Wei et al. 2014; Chen et al. 2015; Almaghrabi et al. 2013). Investigations pertaining to rhizobacteria against RKNs have been increased since recent past due to their divergence in physiology, structural behavior among nematode species, life cycle, environmental aspect, as well as edaphic factors. Further, these aspects could also escalate their importance toward commercialization of these strains.

A wide range of compounds released by PGPR are functionally important for their nematicidal activity (Castaneda-Alvarez and Aballay 2016). Although their biological composition may also make nematodes susceptible toward PGPR antagonistic behavior. The substantial RKN composition comprises collagens, lipids, chitins, proteins, etc., during various stages of development. Strikingly, numerous rhizobacterial metabolites possessing nematicidal activity are classified into enzymes that may impede the external structures of nematodes at different developmental stages. Moreover, they also secrete metabolic products toxic toward RKNs and hinder their proliferation within soils. One of the most important enzymes is proteases that directly attack on infective RKNs and lead to severe cuticle damage (Huang et al. 2010). Further, collagenases and chitinases also alter the cuticular membranes and egg hatching and cause their degradation (Chen et al. 2015). Furthermore, many metabolic products such as hydrogen cyanide (HCN) and 2,4-diacetylphloroglucinol are secreted by several genera like *Pseudomonas* (Meyer et al. 2009). There are many studies conducted such as *Pasteuria penetrans* which is one of the most effective microbe that inhibits *M. incognita* infestation in tomato and cucumber (Kokalis-Burelle 2015), while *Bacillus nematocida* suppress RKNs by releasing proteases to degrade their cuticle (Niu et al. 2006). Along with this, *B. thuringiensis* release Cry proteins against the noxious pathogens (Bravo et al.

2007). All these findings depicted that virulent factors in the form of metabolites are pivotal for biocontrol activities. And these secondary metabolites also act as ecological engineers to compete against pathogens and form symbiotic relationship among useful organisms. All these metabolic functions of PGPR trigger the antioxidant potential of plants under RKN incidence of infection. Here in this chapter, we have mainly focused on the PGPR-mediated biocontrol of RKN infection in plants and promote their antioxidative capacities to counteract the adverse effects of nematode infestations. We have discussed the elaborative role of antioxidative defense system, toxins, metabolites, siderophores, and phenolic compounds against RKN infection. Despite advancement in acquiring these stances, we provide a perspective to commercialize PGPR for RKN suppression in the fields and promote sustainable agriculture.

## 16.2 Impact of Root-Knot Nematode (RKNs) on Plants

RKNs (*Meloidogyne*) spp. impose highly destructive threat toward agricultural crop production. Over most of the tropical nematode species (*Meloidogyne* spp.), *M. incognita, M. javanica, and M. arenaria* are observed as the most rapidly spreading pests globally (Bebber et al. 2014). The source of nutrition in root nematodes is the profitable relationship among the host cells which lead to the formation of feeding or giant cells. The feeding cells induced by stylet secretions of RKNs are formed by morphological and physiological modifications of root cells results in multinucleate, enlarged, and active feeding sites. After the establishment of permanent feeding sites, the active juvenile undergoes three molts and become adults, and the females lose ability to move and remain sedentary, producing large egg masses, while males migrate out from the plant when the food supply diminishes (Abad et al. 2003). So the formation of galls inhibits or limits the ability to absorb and transport water and nutrients toward the plant. This condition drastically affects the plants and causes their permanent wilting. Followed by this, when the infection takes place at the early stage of plant's development, nematodes can directly kill the host plant, and at mature stages, the incidence of worse situations prevails that can impede the metabolic and developmental activities of plants. Later, this situation may undergo complete mortality of the host plant as well as hinder the soil properties by extensive proliferation of RKNs within soils, respectively. The ill effects of nematodes on plants during different stages of life cycle have been presented in Fig. 16.1.

## 16.3 Management of RKN Infection

A broad spectrum of management strategies has been adopted to protect the agricultural crops from the attack of various *Meloidogyne* spp. and other categories of nematodes. As revealed by the earlier studies, there were traditional methods utilized to get rid of these irritable pests. Therefore, without gaining much information about

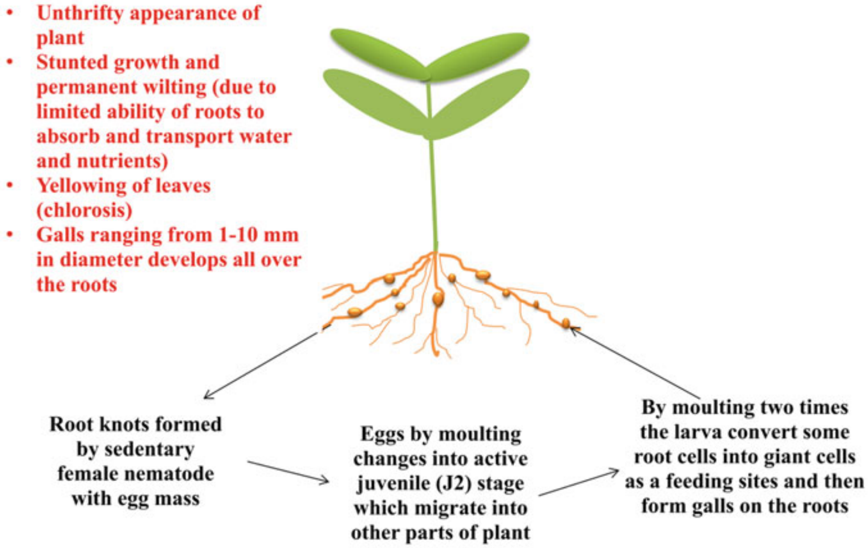

**Fig. 16.1** Impact of nematodes toward plant growth and development

the other aspects about these methods, main focus was laid on eliminating these dreadful biotic factors for effective food security and environmental health. Due to less advancement in the research and technology, many conventional methods were adopted to safeguard the crops from RKNs and their adverse impacts. Under following sections, we have discussed about management of RKNs.

### 16.3.1 Cultural Control

In cultural control practices, crop rotation and cover cropping are effective strategies utilized for integrated pest management that has been proved to be favorable for farmers to reduce the root nematode infection. Rotational cultivation of nonhost cultivars decreases the soil nematode levels. But the effectiveness of this crop rotation method is diminished by some *Meloidogyne* spp. The cultivation of corn as a rotational crop reduces the effect of northern root-knot nematode (*M. hapla*). The cover crops *Mucuna pruriens* L. and *Crotalaria spectabilis* belong to fabaceae are multiresistant to three root-knot nematodes spp., i.e., *Meloidogyne arenaria, M. incognita*, *M. javanica,* respectively (Osei et al. 2010). Nematodes cannot move to another field if a cover crop is not a suitable host to the nematodes as they are able to move only to a short distance by their own (Gill and Mcsorley 2011). Some cover crops are Sorghum-Sudangrass, cowpea, sunn hemp, and marigolds. However, this method is quite intricate and may sometimes give pseudoresults. Moreover, it is also time-consuming and may not always be proved beneficial.

### 16.3.2 Plant Extracts

Plant extracts contain many compounds which exhibit nematicidal properties. The extracts of *Azadirachta indica*, *Tagetes erecta*, *Withania somnifera,* and *Eucalyptus citriodora* were reported to show nematicidal activity against many *Meloidogyne* spp. (Khan et al. 2008). The leaf extracts of *Hunteria umbellata* and *Mallotus oppositifolius* decrease the egg hatch and larval development of root-knot nematode and also enhance the cashew seedlings growth (Okeniyi et al. 2010). Ethanol extracts from *Azadirachta indica* leaves, *Capsicum annuum* fruits, and *Zingiber officinale* rhizomes significantly increase the plant height, fruit quality, and weight of infected tomatoes (Bawa et al. 2014).

### 16.3.3 Nematode-Resistant Varieties

Nematode-resistant plants and rootstocks have the ability to massively contribute toward reduction in the incidence of nematode infestations. Through phenotypic screenings and genetic analysis, the crops have been selected artificially many years for the enhancement of their disease-resistant properties. To exemplify, forage sorghum, a most useful crop found in tropical and subtropical areas, could be planted at any time in the year, is easy to grow and produced in high biomass within 2–3 months, and is highly resistant to nematode infection. Most of the breeders are focused on a single dominant gene (*Mi* gene) that provides resistance to various vegetable crops mostly in tomato cultivars. It was reported that *Mi2* and *Mi8* genes in tomato cultivars and the *Me* and *Ni* genes from pepper are effective against *Meloidogyne* spp. resistance (Mitkowski and Abawi 2003). But *Mi* gene is more sensitive to temperature, so it has been shown that infected resistant tomato varieties may lose their resistance at temperature above 26 °C. Upon damage, plants synthesize and then secrete some secondary metabolites that are involved in various biotic and abiotic stresses. It has been reported that one member of soybean terpene synthase family gene, *GmAFS,* revealed a nematicidal role to protect plants against extreme infections (Yadav et al. 2006). Researchers have been continuously working hard to identify the genes with resistance properties so as to incorporate them to develop highly resistance crops to overcome and combat the RKN infections. Moreover, various transcriptomic studies reveal that plant breeding could be most method for this problem, but it requires high skills, laboratory environment and are not feasible on economic terms. Also, it is quite laborious task, but still is better than the use of chemical nematicides that pose serious threat toward humans as well as environment.

### 16.3.4 Chemical Control

Chemical means to control the RKN infection is the most convenient method of all types to eliminate the RKNs from agricultural fields. Majorly, three types of chemicals have been practiced so far to control nematode infection, namely fumigant nematicides, nonfumigant nematicides, and multipurpose fumigants (Noling 2012). The fumigant nematicides enter into the soil particles through pore spaces by diffusion in the gaseous form. The application of fumigants depends on the soil temperature, moisture, texture, and the amount of undecomposed organic matter. Due to phytotoxic nature, fumigants are applied at least 3 weeks before crops are planted (Noling 2014). A number of organophosphate and oxime carbamate are nonfumigant nematicides are also utilized whose action is nemastatic rather than nematoxic. They temporarily inactivate the nematodes by preventing their egg hatching, limiting feeding and developmental process. Followed by this, their action dissipates after a short period of time. So, nonvolatile nematicides can be useful only for annual vegetable crops, seed germination, and transplant. Multipurpose fumigants can be useful for the crops of high value as they are generally more expensive. Methyl bromide is widely used multipurpose fumigant due to its broad spectrum of activity on nematodes, fungi, and other pests. It is an excellent nematicide and is used in the sole nematode management in vegetable production system in Florida over last 30 years (Noling 2012). Three alkyl halides, E BD, DBCP, and 1,3-D, are active ingredients primarily used to control the nematode infection. Although the efficiency of these chemical agents is skyrocketing, they have serious threat toward environment. They have been primarily involved in waning off food safety and standards and may enter into the food chain with an utmost ease. Moreover, they can also instigate the infections of other phytopathogens and can make pathogens resistant to these chemical agents. Henceforth, all these methods have some drawbacks that make them unsuitable for use and trigger the urgency for an alternative, feasible, and more eco-friendly system to counteract the nematode infection from plants. For this purpose, PGPR could serve as the best alternatives to control and manage plant pathogens specifically RKNs. In coming sections, we will discuss about the role of PGPR in managing RKNs and the mechanism associated along with them.

## 16.4 PGPR During Biotic Stresses

Plants are sessile beings that are continuously exposed to biotic stresses including pathogens and pest and are intimidating quality and yield of crop. These pathogens and pests include wide array of phytopathogens such as bacteria, fungi, viruses, and other organisms. This pathogenic microorganism generally abates or deteriorates the plant tissue and results in decline in productivity of crops ranging from 25% to 100% (Choudhary and Sindhu 2015). The utilization of naturally available PGPR as biological control agents has been flourished as a means to tackle the concerns associated with agrochemical approaches. Employment of favorable soil bacteria, i.e., the PGPRs, helps in reducing the damage caused by varied plant pathogens. The use of various strategies to combat pathogen infestation includes antibiotic synthesis, secondary metabolite production, secretion of iron-rich chelating compounds, production of defense enzymes, and stimulation of systemic tolerance in plants, i.e., induced systemic resistance (ISR) (Glick et al. 1999). The PGPR endorse and replenish growth of infected plants and subsequently elevate plants resistance to various diseases caused by phytopathogens, which might indirectly result in stimulation of growth (Beneduzi et al. 2012). The criteria of selection of defense approach are directly dependent upon the ability to elevate disease resistance and low cost of expenditure. Resistance to biotic stress stimulated by certain agents entails specific cost due to employment of resources of defensive components (Bakker et al. 2013). In retaliation to ISR, various physiological and metabolic responses are altered which further result in augmentation in biosynthesis of plant protection components and chemicals. These chemicals provide resistance to specific pathogen. The PGPR forms a first line of protection in reprisal to pathogen infestation; these include (a) sequestration and elevated accumulation of pathogenesis-related proteins (PR proteins), (b) elevation in strength of cortical and epidermal layers of plant cells under stress, phenomena observed in *Pisum sativum* and *Solanum lycopersicum* with *B. pumilus* strains, i.e., SE34 (Benhamou et al. 1998), and in *Solanum lycopersicum* with *P. fluorescens* strains, i.e., WCS417r (Duijff et al. 1997), and (c) accumulation of specific chemicals such as phenylalanine ammonia-lyase (PAL), phytoalexins, and chalcone synthase (Mariutto et al. 2011). The nonpathogenic rhizobacteria have been affirmed to restrain the sternness and prevalence of pathogen infestation by employing various resistance strategies which are termed as ISR (Ramamoorthy et al. 2001). The plant part which is not affected by pathogenic infestation becomes more tolerant to pathogen attack, nematode infestation, and insect assault via Rhizobacteria-mediated ISR. This Rhizobacteria-mediated ISR is similar to the induced systemic acquired resistance (SAR) (Van Loon et al. 1998; Pozo and Azcón-Aguilar 2007). Both ISR and SAR have been well documented to enhance defense of plants via stimulation of plethora of signaling cascades. ISR is directly dependent upon ethylene and jasmonic acid signaling cascades and has no visible symptoms (Van der Ent et al. 2009; de Vleesschauwer and Höfte 2009). SAR and ISR simultaneously defend plants and induce resistance to pathogen infestation (Van Wees et al. 2000). Various observations by research groups reveal that ISR in comparison to SAR provides significantly less protection and resistance than acquired by SAR. Furthermore, a high degree of reliance on the genotype of plant is observed in the generation of ISR (Bloemberg and Lugtenberg 2001). Figure 16.2 depicts role of PGPR in combating biotic stresses.

Salicylic acid stimulates expression of certain specific defense-associated genes termed as pathogenesis-related proteins (PRs). The elevation resistance of plants as a characteristic of enhanced SAR is co-related to augmentation in sequestration of PRs (Van Loon 2007). In contrast, ISR can be stimulated in plants that do not accumulate high levels of salicylic acid; these plants are NahG mutants. In *Arabidopsis* plants, enhanced accumulation of salicylic acid and triggered expression of PR genes are directly linked to ISR cascade (Pieterse et al. 1996). The signaling pathway of SA entails activator protein, i.e., NPR1 or NIM1 which has a function in the terminal section of SAR pathway (Van Loon et al. 1998). The overexpression of these NPR1 gene elevates the tolerance and resistance of plants to pathogen infestation (Friedrich et al. 2001). NPR1 has been affirmed to modulate the defense responses which are

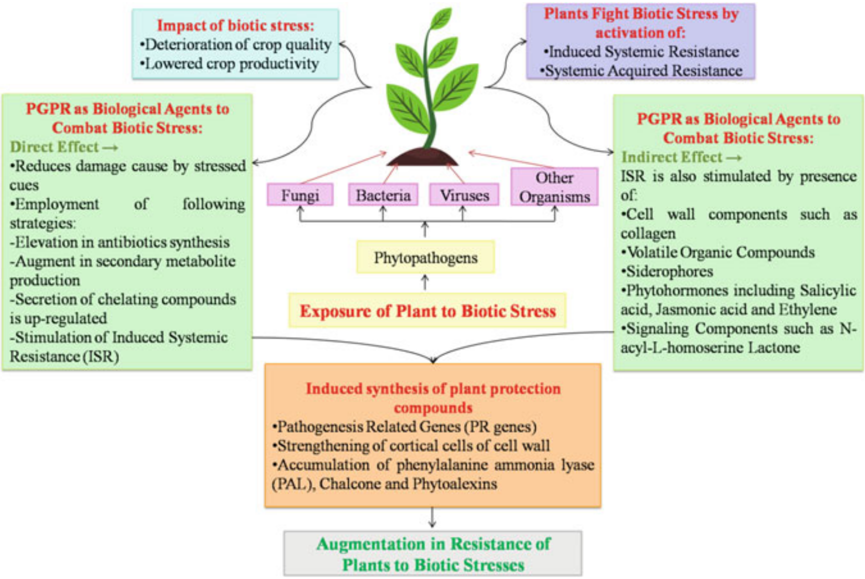

**Fig. 16.2** Role of PGPR in combating biotic stress

mediated via several signaling cascades which have functions not dependent upon PR genes, which further indicates that ISR and SAR pathways congregate at the last past of signaling cascade (Van Loon et al. 1998). It was reported by Pieterse et al. (2000) that the rhizobacterial *P. fluorescens* strain WCS417r in *Arabidopsis* plants revealed that WCS417r-regulated ISR pathway works independently of SA and is directly dependent upon NPR1 expression and involves jasmonic acid and ethylene signaling cascades. In addition to this, ISR is induced via metabolites and specific characteristics of the bacterial strain employed (de Vleesschauwer and Höfte 2009). There exists wide array of bacterial strain features which are responsible for stimulating ISR; these include components of cell wall (Leeman et al. 1995), VOCs (Ryu et al. 2004); metabolites such as siderophores and phytohormones, including SA (Höfte and Bakker 2007; Ran et al. 2005), cellular ultrastructure specifically flagella (Meziane et al. 2005), fengycin lipopeptides and surfactins (Ongena et al. 2007), signaling components, such as *N*-acyl-L-homoserine lactone (AHL) (de Vleesschauwer and Höfte 2009) and phenols and polyphenols (Akram et al. 2013). Induction of ISR in retaliation to specific rhizobacteria has been elucidated in several plant species and has been affirmed to be dependent on the explicitness of the association between particular rhizobacteria and plant (Van Loon et al. 1998). The PGPR stimulates the acquired systemic tolerance by triggering several signaling pathways in plant system, including salicylic acid, ethylene, and jasmonic acid (Maurhofer et al. 1998). Stimulation of ISR by varied PGPR is directly dependent upon the various signaling cascade triggered by pathogen infestation. Wide range of rhizobacteria stimulates systemic resistance by concurrent activation

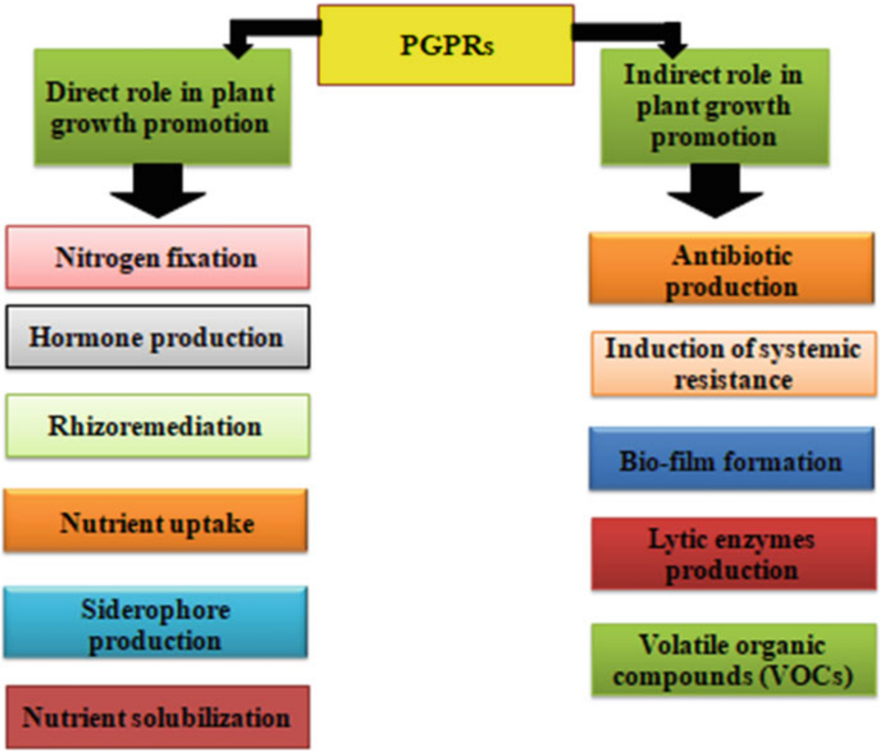

**Fig. 16.3** Schematic representation of PGPR-mediated plant growth under nematode stress

of other signaling cascades such as salicylic acid and ethylene/jasmonic acid pathways. The ISR induced by rhizobacterium *B. cereus* AR156 has been reported to be involved in both phytohormone cascades, viz. salicylic acid and jasmonic acid/ethylene and NPR1 signaling (Niu et al. 2011). As PGPR are active growth inducers during plant–pathogen associations. A series of mechanisms involving PGPR-mediated growth and development under biotic stressors are depicted in Fig. 16.3.

## 16.5 PGPR-Mediated RKN Management

Rhizobacteria have been affirmed to modulate growth and development of plants via varied mechanistic approaches, including solubilization of hormones, minerals, and certain other compounds (Santoro et al. 2011). As already affirmed that rhizobacteria aid in lowering the susceptibility of plants to pests and pathogens present in the vicinity of soil, there are wide range of reports which suggest imperative role of rhizobacteria in reduction of plant-parasitic nematode (PPN) infestations (Aballay et al. 2013; Noreen et al. 2015). Bacterial strains of bacteria, serratia, and pseudomonas have been revealed to have nematicidal activity (Almaghrabi et al. 2013;

Wei et al. 2014; Chen et al. 2015). Plethora of strategies have been employed to ensure rhizobacterial suppression of PPN activity, and the criteria of selection of a strategy are dependent upon: (a) varied nematode species and their structural and behavioral differences, (b) physiological variance of rhizobacteria and (c) environmental cues, plants, and soil attributes. Different metabolites and enzymes of rhizobacteria have been frequently related to the nematode suppression ability (Paiva et al. 2013; Castaneda-Alvarez et al. 2016). The structural variation and chemical composition of various classes of nematodes make PPN vulnerable to a wide range of fungal and bacterial pathogen antagonistic activities. Various nematode structural components which determine the rhizobacterial activity include lipids (both during mobile and stationary stages), collagens, and proteins such as chitin.

Diverse rhizobacterial compounds which are responsible for nematicidal activity are broadly classified into two, i.e., enzymes and metabolic by-products. The enzymes usually affect the peripheral structural components, and the metabolic by-products affect the organs of nematodes and eventually result in alteration in their behavior and may also cause modifications in plants recognition process employed to identify a parasite. One of the most notably studied and identified enzyme is protease which causes major deleterious impacts on the cuticle. This enzyme is evaluated at the free-living stage of infection cycle (Niu et al. 2007). Similarly, other enzymes such as collagenases and chitinases are both involved in damaging the cuticle of nematodes (Page et al. 2014). In addition to this, these enzymes have also been affirmed to cause damage to nematode eggs (Chen et al. 2015). The metabolic by-products include compounds such as hydrogen cyanide and 2,4-diacetylphloroglucinol both reported in genera Pseudomonas (Meyer et al. 2009). Additionally, more and more PGPRs have been recently identified and have been revealed to act as plant-parasite nematodes. They have been confirmed to augment the ability of plants to suppress the effects of nematode infestation on plants (Sidhu 2018). Plant growth enhancement is one of the most important strategies to combat nematode infestation and is regulated by synthesis of plant growth hormones such as gibberellins, auxins, and cytokinins. These growth regulators also aid in elevating plants nutrient balance by modulating solubilization of phosphorous and nitrogen fixation. Another mechanism involves augment in production of certain inhibitory components such as siderophores, antibiotics, cell wall rupturing enzymes, and antifungal metabolites. This indirect method of PGPR action is activated when the synthesis of plant growth hormones is reduced by enhancing the natural tolerance of the host by ISR (Sidhu 2018).

## 16.6 PGPR-Mediated Mechanisms to Enhance Antioxidant Potential of Plants During RKN Infection

The zone of rhizosphere is inhabited by a diverse range of PGPR (Schroth and Hancock 1982) which is associated with a group of free-living soil bacteria (Kumar et al. 2016). The rhizosphere is rich in organic carbon sources due to the accumulation of a variety of plant exudates that promote plant growth and increase the yield of

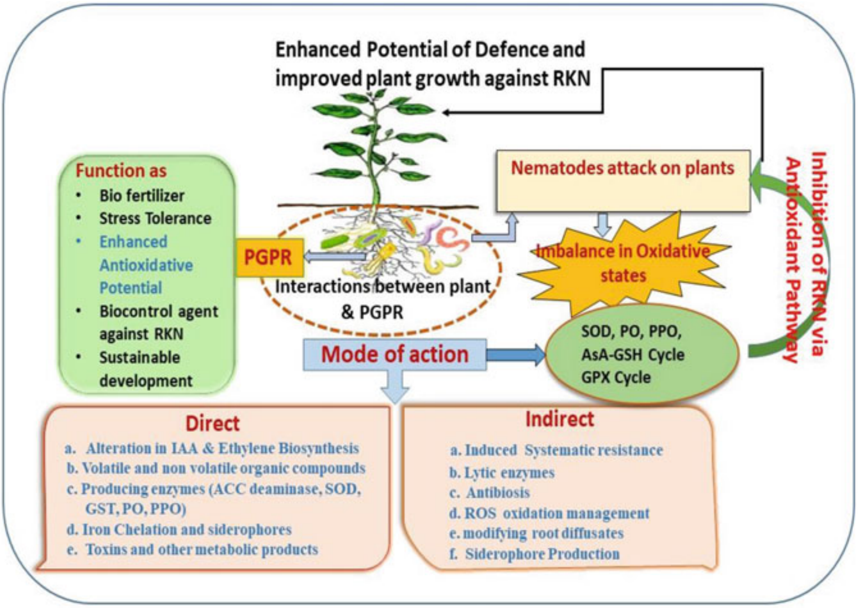

**Fig. 16.4** Diagrammatic representation of PGPR-mediated mechanisms to promote antioxidant potential of RKN-infected plants

agriculture crops (Kumar et al. 2016). Therefore, plants are occupied with organic-rich sphere such as sugars (glucose, xylose, maltose, and sucrose), amino acids (aspartic acid, glutamic acid, isoleucine, and leucine), organic acids (citric acid, malic acid, lactic acid, and succinic acid), and secondary metabolites (phenolic acids, flavonoids, enzymes, fatty acids, tannins, steroids, terpenoids, and alkaloids) (Campbell et al. 1990; Kaitaniemi and Honkanen 1996; Walker et al. 2003; de Weert et al. 2004; Rudrappa et al. 2008; Gray and Smith 2005). The commensalism of roots with PGPR can protect plants from soilborne pathogens and can support to acquire more nutrients and grow better by producing beneficial phytohormones (Lugtenberg and Kamilova 2009). PGPR can stimulate different plant defense mechanisms against pathogens (Lee et al. 2015) like *Pseudomonas* sp. and *Bacillus* sp. which are known as the dominant antagonists of plant pathogens in the rhizosphere (Mhatre et al. 2018). It has been observed that PGPR may induce defense mechanism in the rhizosphere to conquer parasitic nematodes. PGPR enhance plant growth due to specific traits via direct and indirect mechanisms, which involve enhancing plant physiology and resistance to different phytopathogens through various modes and actions (Liu et al. 2017; Beneduzi et al. 2012) (Fig. 16.4).

It was revealed that some important PGPR strains like *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, *Azotobacter*, *Gluconacetobacter*, *Azoarcus*, *Bradyrhizobium*, *Burkholderia*, and *Serratia* have been used as biocontrol agents and have a major role to inhibit pathogenic organisms by modulating antioxidant defense system (Abd El-Rahman et al. 2019; Korejo et al. 2019; Khanna et al.

2019b). The application of PGPR inoculants as biofertilizers helps in production of antibiotic compounds and generation of antifungal and antibacterial secondary metabolites (Kumar et al. 2015). These antibiotics are categorized into volatile and nonvolatile complexes. The volatile compounds include alcohols, aldehydes, ketones, sulfides, and hydrogen cyanide, whereas the nonvolatile antibiotics are categorized as polyketides, cyclic lipopeptide, amino polyols phenylpyrrole, and heterocyclic nitrogenous compound (Fernando et al. 2007). These antibiotic complexes have antimicrobial, insecticidal, phytotoxic, antioxidant, and cytotoxic properties which endorse plant growth (Ulloa-Ogaz et al. 2015; Fernando et al. 2007; Liu et al. 2017).

There are a number of mechanisms for nematode suppression such as enzymes (antioxidants and non-antioxidants), nutrient fixation, and producing volatile organic compounds (VOCs) to prevent disease. These process can be categorized manly in two major classes, viz. direct antagonism by producing enzymes, toxins, and other metabolic products and indirect methods that effect by regulating nematode behavior, modifying root diffusates, inducing repellent's production by host that adversely affects the host recognition and alteration of the nematode feeding site development at the root tissue, and inducing systemic resistance (Siddiqui and Mahmood 1999; El-Nagdi and Youssef 2004; Mhatre et al. 2018) (Fig. 16.2). Moreover, plants have also evolved some cellular strategies that involve the activation of various enzymatic antioxidants to combat against pathogen toxicity (Krishna et al. 2013). Many plants are known to produce small molecular antioxidants like phenolic compounds, ascorbate, glutathione, and tocopherols for cellular protection (Shohael et al. 2006; Margesin et al. 2007; Mhatre et al. 2018). Under normal conditions, there is regulation of the scavenging process and the production of both enzymes and antioxidants (Yordanova et al. 2004).

The PGPR environment and bacterial antagonistic activities in plants are possibly by specific parameters, such as (a) due to synthesis of hydrolytic enzymes (chitinases, glucanases, proteases and lipases) that can lyse pathogenic fungal cells (Maksimov et al. 2011; Mhatre et al. 2018), (b) by competition for nutrients and suitable colonization at the root surface (Dobereiner 1992; Patten and Glick 2002; Kamilova et al. 2005), (c) regulation of plant ethylene levels through the ACC deaminase enzyme, which modulate the level of ethylene in response to stress imposed by the infection (Glick et al. 2007; Van Loon 2007) (Fig. 16.4). It was studied in the literature that production of siderophores, bacteriocins, and broad-spectrum antibiotics also acts as antagonistic (Baker and Cook 1982; Riley and Wertz 2002). Siderophores, bacteriocins, and antibiotics are the most effective and accepted mechanisms of antagonist to prevent phytopathogenic proliferation (Maksimov et al. 2011; Sayyed et al. 2013; Jha and Subramanian 2014).

Along with this, Gupta et al. (2017a) hypothesized that these biocontrol agents had a stimulatory effect in defense mechanisms where they reduced the free radical production and strengthen their immune system. The mechanism associated with the reduced levels of oxidative stress markers is most likely due to elevation in the chain of resistance processes and antioxidative defense genes in plants along with their abilities to directly scavenge the ROS initiated during stressed conditions. In addition to this, Khanna et al. (2019a) revealed that the defense mechanism might be through the microbial resistance processes and expression of antioxidative defense genes in plants. Along with this, they also restrict their invasion inside the plants so as to balance the redox homeostasis within the plant inner zone. They also found triggered antioxidant levels in plants in the form of both enzymatic and nonenzymatic activities during nematode infection. Also, they are known to modulate the expression of plant metabolites by upregulating the enzymatic activities of secondary metabolites, e.g., phenols, sugars, amino acids, and organic acids (Li et al. 2019; Abd El-Rahman et al. 2019; Mhatre et al. 2018). The mechanism associated with stimulatory effects of microbes on antioxidative defense system is most probably due to upregulation of genes and protein transcripts of antioxidant molecules. And this further attributes to the adaptive responses of plants with protection from RKN infection. In the following section, a brief review of the literature related to involvement of defense molecules particularly PGPR-mediated enhanced antioxidant potential of plants has been discussed.

### 16.6.1 Antioxidative Enzymes

Antioxidant system modulation could reflect a defense response to the cellular damage activated by pathogen toxins (Singh and Upadhyay 2014). Plant–pathogen interactions are affected by peroxidases, and it interferes with the growth of plant cells (Passardi et al. 2004). Peroxidase in the plants is affected by special in vitro conditions, including limited space, metabolic waste products, limited exchange of gases, and medium nutritive substance content (Svabova et al. 2011). In recent decade, many researchers discovered improvement in the plant growth and resistance toward biotic components by manipulation of essential microbes (Avis et al. 2008; Singh et al. 2015b). Some PGPR strains in the rhizosphere, chiefly chitinolytic microbes have been established to produce enzymes, i.e., chitinases, β-1,3-glucanases, proteases, and lipases which can lyse fungal cells and are responsible for first line defense to the plants upon nematode attack by degrading chitin, the main constituent of eggshells (Ashoub and Amara 2010; Ramyabharathi et al. 2012; Gupta et al. 2017b). It was confirmed by various studies that PGPR also protect plants through induced systemic resistance and synthesizing quorum sensing molecules (e.g., acyl homoserine lactone) to regulate the activities of nematodes in the rhizosphere (Hartmann et al. 2014).

To control the level of ROS and to protect cells under biotic stress conditions, plant tissues contain several ROS scavenging enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), glutathione peroxidase, dehydroascorbate reductase (DHAR), and detoxifying lipid peroxidation products (glutathione *S*-transferase, and phospholipid hydroperoxide glutathione peroxidase) (Blokhina et al. 2003). Antioxidant system modulation could reflect a defense response to the cellular damage activated by pathogen toxins (Singh and Upadhyay 2014). Plant–pathogen interactions are affected by peroxidase, and it interferes with the growth of plant cells (Passardi et al. 2004). In the plants, expression of peroxidase is affected by distinct in vitro environments like restricted space, metabolic waste products, partial exchange of gases, and nutritive substances (Svabova et al. 2011). Increased activity of defense-related enzymes, i.e., polyphenol oxidase (PPO), phenyl ammonia-lyase (PAL), super oxide dismutase (SOD), peroxidase (PO) or polyphenol oxidase (PPO), has been elicited by biocontrol agent strains in different plants (Anita and Samiyappan 2012, b). It was confirmed that PO and PPO strengthen cell walls (lignification and suberization) at the frontier of infection and allow narrow spread of pathogens (Passardi et al. 2004). Earlier studies showed that application of bioarc in combination with nemastrol under in vitro conditions increased the activities of enzymes, i.e., PO and PPO in *M. incognita*-infected roots of sugar beet. However, PGPR enhanced plant growth by producing certain metabolites that control phytopathogenic agents (Meena et al. 2016; Gouda et al. 2018).

Microbes protect the plants by activating the defensive state of plants through mitigation of redox species produced in the host plant (Torres 2010; Heller and Tudzynski 2011). Plants naturally acquired antioxidant defense systems to combat the oxidative damage upon excessive ROS generation (Gill and Tuteja 2010). Additionally, PGPR also highlighted the intrinsic functions of the plants by maintaining the ROS levels through effective scavenging mechanisms in plants (Mehmood et al. 2018; Mhatre et al. 2018). The various biochemical studies shown that host plant progresses resistance against pests and pathogens, specifically plant-parasitic nematodes via activating or deactivating of certain enzymes involved in physiological and biochemical processes of plants (Li et al. 2019).The strains from genera *Pseudomonas* and *Burkholderia* have been used as the biological control agents for PPNs in *L. esculentum* (Khanna et al. 2019a, b). It was found that PGPR strains improved tomato plant resistance from PPN infection by dwindling the levels of oxidative stress markers in plants against nematode infection. These upregulated expression of antioxidants have been known to directly contribute toward free radical scavenging during nematode attack (Khanna et al. 2019a). Moreover, similar studies have been demonstrated that nematode-infested *B. monnieri* L. plants when inoculated with biocontrol agents (*Chitiniphilus* sp. and *Streptomyces* sp.) dropped lipid peroxidation, superoxide anions, and hydrogen peroxide levels (Gupta et al. 2017a). They hypothesized that these biocontrol agents had a stimulatory effect in defense mechanisms where they reduced the free radical's production and strengthening their immune system. The upregulation in the activities of enzymatic antioxidants has also been observed in *Lycopersicon esculentum* Mill plants manifested with nematode infection. Similar study had been reported in tomato and barley plants when infected with PPNs and showed enhanced activities of SOD, POD, CAT, and PPO (Sahebani and Hadavi 2009; Bajestani et al. 2019). Likewise, one more study in tomato plants infected with PPNs described elevated activities of SOD and POD after the inoculation of *Drechslerella dactyloides* and *Dactylaria brochopaga* due to up regulation of specific biomolecules and antioxidants (Singh et al. 2019). The protective role of peroxidase genes in *Ipomoea batatas* during root-knot nematode infection has also been reported by transcriptomic analysis (Sung et al. 2019).

Another investigation conducted by Moghbeli et al. (2017) highlighted that increase in antioxidative enzymes is comparative to resistance mechanisms established by plants which are directly interconnected to structural rigidity of plant tissues such as lignin synthesis in cell walls in order to prevent the nematode penetration in plants. Similarly, Korayem et al. (2012) showed that when sugar beet genotypes were infected with *M. incognita,* they indicated significant increase in the activity of catalase. The application of *P. putida* and *B. subtilis* both alone and in combination significantly enhanced the activity of antioxidant enzymes, viz. SOD and CAT against pathogenic infection. It was observed with enhanced activities of antioxidant enzymes of antagonistic bacteria in response to multiple pathogens (Singh and Gaur 2017). Some studies indicated that plants treated individually or with dual inoculation of AMF and PGPR displayed significantly improved plant growth by suppressing nematode infection. This is mainly attributable to the potent activity of defensive enzymes like peroxidase, polyphenyloxidase and superoxide dismutase respectively. Plants inoculated with *M. incognita/P. betavasculorum* or *R. solani* played a significant increase in the activities of SOD, CAT, PPO, and PAL (Khan and Siddiqui 2019). The antagonistic bacteria enhanced the activities of antioxidant enzymes as a defensive mechanism against multiple pathogens (Singh and Gaur 2017). The activation of antioxidant system in response to oxidative burst after pathogen infection has been proposed for conveying the signal transduction for downstream defense responses (Liu et al. 2017). The increase in the antioxidant enzymes activity in plants against nematode infection must be owing to higher glutathione pools which further activate a vital enzymes of GR cycle, i.e., DHAR and GPOX enzymes. Similarly, the level of nonenzymatic antioxidants glutathione, ascorbic acid, and tocopherol was enhanced via AsA-GSH cycle. It has been reported to activate the antioxidants as a defensive strategy in plants followed by GR and AsA-GSH cycle to maintain the redox state during plant–nematode interactions (Labudda 2018). Table 16.1 summarizes the impact of PGPR and role of antioxidative enzymes on different crops infected with Root-Knot Nematodes.

### 16.6.2 Volatile Compounds

PGPR either act directly for nutrient mobilization or they trigger the levels of phytohormones and siderophore formation at low ion availability. On the other hand, they act indirectly as inhibitors to eliminate various pathogens by modulating systemic resistance and root colonization and also by producing biocontrol agents like Volatile organic compounds (VOCs), organic acids, and other metabolic products (Bhattacharyya and Jha 2012). PGPR cohabit with nematodes in the rhizosphere as well as inside the root tissues. Interaction between these two effects their functioning and further changes the health of the host plants and rhizosphere (Singh et al. 2017). A variety of rhizobacterial inoculations show nematicidal activities at certain inhibitory concentrations. Secondary metabolites produced by

**Table 16.1** Antioxidant enzymes potential of PGPR against RKN infection in plants

| S. no. | Plant species | PGPR | Role of antioxidative enzymes | References |
|---|---|---|---|---|
| 1. | *Trigonella foenum-graecum*, *Lactuca sativa*, *Spinacia oleracea*, and *Daucus carota* | Rhizobacteria NRRL B-30488 | Induction of antioxidant level of various enzymes PPO, APX, CAT, and SOD to control oxidative damage | Nautiyal et al. (2008) |
| 2. | *Lycopersicum esculentum* | *Trichoderma harzianum* and *Serratia marcescens* | The enhanced activity of PPO and β-1,3-glucanase (GLUC) causes resistance toward nematode | Abd-Elgawad and Kabeil (2012) |
| 3. | *Oryza sativa* | *Pseudomonas fluorescens* | Increased activities of POD, PPO phenylalanine ammonia-lyase (PAL) and chitinase and induced systemic resistance against nematode infection | Anita and Samiyappan (2012, b) |
| 4. | *Lycopersicon esculentum* | *Arthrobotrys oligospora* | Higher activities of PAL, POD, and PPO for improved growth of plant against the RKN infection | Mostafanezhad et al. (2014) |
| 5. | *Lycopersicon esculentum* | *Pseudomonas fluorescens* | Inclined ROS scavenging enzyme activities for stimulating plant defense to plant challenged with *M. javanica* | Nikoo et al. (2014) |
| 6. | *Gossypium hirsutum* | *Bacillus subtilis* | Prevented from *Meloidogyne incognita* and *M. arenaria* induced ill effects by improving morphological and physiological characters | Prathap and Ranjitha (2015) |
| 7. | *Solanum lycopersicum* L. | *Pochonia chlamydosporia* | Resistance in tomato plants against *M. javanica* infection mainly mediated by the action of PPO and POD activities | De Medeiros et al. (2017) |
| 8. | *Vigna radiata* | *Pseudomonas aeruginosa, Bacillus subtilis* | Preventive measures from root-knot formation and higher enzyme activities | Ngumbi (2016) |
| 9. | *Solanum lycopersicum* cv. PT-3 | *Pseudomonas jessenii* and *Pseudomonas synxantha* | Activity of POD, PPO, and SOD increased abundantly with enhanced RKN resistance in plants | Sharma and Sharma (2017) |
| 10. | *Withania somnifera* | *Cellulosimicrobium cellulans*, *Flavobacterium johnsoniae*, *Chitiniphilus* sp. and *Streptomyces* sp. | Elevated antioxidant enzymes activities and highlighted chitinolytic function in response to *M. incognita* | Gupta et al. (2016) |

(continued)

**Table 16.1** (continued)

| S. no. | Plant species | PGPR | Role of antioxidative enzymes | References |
|---|---|---|---|---|
| 11. | *Bacopa monnieri* | *Chitiniphilus* sp. MTN22<br>*Streptomyces* sp. MTN14 | SOD activity and free radical scavenging activity were stimulated along with improved lipid peroxidation and root gall formation in *B. monnieri* which further strengthened the host to withstand the pressure generated by the biotic stress | Gupta et al. (2017b) |
| 12. | *Daucus carota* | *Purpureocillium lilacinus* and *Aspergillus niger* | Inhibited hatching of nematode eggs by promoting enzymatic degradation by disintegrating chitin | Nesha and Siddiqui (2017) |
| 13. | *Solanum lycopersicum* L. | *Streptomyces* sp. | PGPR showed drastic effect on the root to resist against pathogens by activating enzymes or by improving root zone microecology | Ma et al. (2017) |
| 14. | *Beta vulgaris* L. | *Bacillus megaterium* | POD and PPO enzyme activity enhanced better growth of plants | Mostafa et al. (2018) |
| 15. | *Pogostemon cablin* | *Pseudomonas putida* strain, BG2 and *Bacillus cereus* | CAT enzyme caused reduction in *Meloidogyne incognita* infection and promoted plant growth | Borah et al. (2018) |
| 16. | *Solanum lycopersicum* | *Drechslerella dactyloides* and *Dactylaria brochopaga* | PGPR established a potential to enhance site-specific accumulation and also induced activation of defense-related mediator molecules and enzymes against *M. incognita* | Singh et al. (2019) |
| 17. | *Solanum lycopersicum* | *Trichoderma harzianum, Pochonia chlamydosporia, Agrobacterium radiobacter, Bacillus subtilis, Streptomyces* spp. | The antioxidant activities of enzymes like CAT, APOX, endochitinase, and 24 glucanases-induced Systemic Acquired Resistance (SAR) in plants | Molinari and Leonetti (2019) |
| 18. | *Lycopersicon esculentum* | *Pseudomonas aeruginosa* and *Burkholderia gladioli* | Antioxidant potential of various defense enzymes like SOD, POD, CAT, GPOX, APOX, GST, GR, DHAR, and PPO control the proliferation and penetration of nematodes inside plants by altering the root exudations | Khanna et al. (2019a) |

these rhizobacteria can trigger a direct or indirect mechanism to act against the parasitic nematodes. PGPR including other microbes are well known to synthesize volatile organic and inorganic compounds (Audrain et al. 2015).

VOCs are mostly available as lipophilic liquids with a characteristic feature of having high vapor pressure. These compounds easily migrate through the membrane with little or no restrictions into the soil environment and further move to their destined targets through soil pores (Pichersky et al. 2006). These volatile compounds are active against the phytopathogens and act as biocontrol agents for soilborne pathogens (Gotor-vila et al. 2017; Rath et al. 2018). PGPRs like *Pseudomonas*, *Bacillus,* and *Arthrobacter* are considered to be the significantly important genera to produce VOCs. Rhizobacteria-related VOCs and their nematicidal activity are mostly observed in vitro experiments, as they are not easy to maintain under open field condition. Huang et al. (2010) found that in an experiment performed in sealed and compartmentalized Petri plates and pots *Bacillus megaterium* strain, YMF3.25 positively promoted plant growth and lowered *M. incognita* infection by synthesis of VOCs. Along with this, Yang et al. (2012) revealed that rhizobacteria *Lysinibacillus mangiferahumi* extracted from the soil in association with the mango rhizosphere and exhibited the enhanced production of VOCs with nematicidal properties against *M. incognita*. Another report revealed that incubation of five different bacterial strains in an independent manner with *M. incognita* or *C. elegans* showed a hindrance in the nematode movement within 24 h. Bioactive compounds involved in these processes are identified as butyl isovalerate, acetophenone, *S*-methyl thiobutyrate, ethyl 3,3-demethylacrylate, dimethyl disulfide, nonan-2 one, and 1-methoxy-4-methylbenzene (Xu et al. 2015). However, an investigation found that VOCs produced by *Paenibacillus polymyxa* KM2501-1 showed nematicidal activities against *Meloidogyne incognita*. In this experiment, second-stage juveniles (J2) of *M. incognita* are treated with biocontrol agents released from *P. polymyxa* KM2501-1 strain that resulted in 87.66% mortality of these J2 stage juveniles. This in vitro experimentation lowered the level of pathogenicity of tomato plants up to 82.61%. GC-MS studies revealed that 11 VOCs have been isolated from this KM2501-1 strain, out of which 8 VOCs act as nematicides against *M. incognita*. These VOCs work as honey traps, fumigants, attractants, and repellents. VOCs like furfural acetone and 2-decanol also act as attractants against *M. incognita* to further cause their mortality by fumigation (Cheng et al. 2017).

### 16.6.3 Phenolic Compounds

Phenolics are metabolic molecules that exhibit antioxidant activity to prevent pathogenic attack (Wijngaard et al. 2009; Jin and Mumper, 2010). These natural plant compounds arise biogenetically from phenylalanine and tyrosine in shikimate, phenylpropanoids and flavonoids pathways (Parween et al. 2019). Phenolic compounds are synthesized in plants as a response to physiological and environmental pressures such as pathogens attack, wounding, UV radiation, and toxic molecules (Diaz Napal et al. 2010; Kennedy and Wightman 2012). Mostly phenolic compounds are polyphenols which are characterized into various groups, i.e., simple phenols and phenolic acids, phenylpropanoids, flavonoids, tannins, and anthocyanins. The advancement in molecular analysis showed that secondary metabolites (phenols and organic acids) enhanced gene expression in infected plants due to presence of PGPR. The increase in the expression of genes encoding phenylalanine ammonia-lyase (PAL) and polyphenol oxidase enzymes in soybean plants was found in response to nematode attack. It is mainly attributed to PGPR-mediated activation of shikimate pathway that triggers the synthesis of many phenolic and metabolite compounds in plants as defense response (Gao et al. 2018). It was noticed that PAL is the entry point enzyme into phenylpropanoid metabolism which is involved in the production of phenolics and phytoalexins to establish defense against pathogens (Mariutto et al. 2011). Previous literature indicated that the disease suppression is correlated to the PAL enzyme activity and is also in accordance with the enhancement of the PGPR treated plants (Ali and McNear 2014; Basha et al. 2006; Sharma et al. 2007). Further, Singh et al. (2012a, b) and Borah et al. (2018) confirmed that the carbofuran treatment is more effective in overpowering nematode infection. The PAL is a vital enzyme in the phenylpropanoid pathway which catalyzes the nonoxidative deamination of phenylalanine to *trans*-cinnamate and directs the carbonflow to the various branches of the phenylpropanoid metabolism such as lignin and flavonoids, isoflavonoids, and anthocyanins (Starr et al. 2014).

Lignin biosynthesis from phenylalanine occurs via a series of side-chain modifications, ring hydroxylations, and O-methylations which lead to the production of monolignols (hydroxycinnamyl alcohols). These monolignols support structural and vascular integrity and provide resistance to plants against pathogens and play an important role in plant–nematode interactions. Some researchers reported that resistance toward nematodes is correlated with increased lignin deposition in the cell walls of resistant plants (Wuyts et al. 2006; Dhakshinamoorthy et al. 2014). Similarly, Veronico et al. (2018) revealed that susceptible tomato plants when treated with benzothiadiazole (BTH) causes reduction in penetration and development of the root-knot nematode *M. incognita* in response to higher lignin levels. Furthermore, lignification and total phenolic content of infected and uninfected plants were also studied in *Oryza sativa* associated with nematode infection and results illustrated that total phenols induced lignification in epidermal regions of plants, as a constitutive and inducible post penetration mechanism to provide resistance against nematode infection (Galeng-Lawilao et al. 2019). The increased levels of total phenolics and carotenoids in RKN resistance have also been reported by Galvez et al. (2019) in *C. annuum* to induce the resistance in plants. Another important phenolic constituent, i.e., flavonoids, a large class of secondary metabolites, is also found to induce protective response against pathogen attack like fungi (Christensen et al. 1998), bacteria (Shirley 1996), insects (Misra et al. 2010; Diaz Napal et al. 2010; Thoison et al. 2004), and nematode infections (Hutangura et al. 1999; Jones et al. 2007). Flavonol effect on PPRs is species-specific. Using similar concentrations of flavonols, kaempferol, quercetin, and myricetin repelled *M. incognita* and *R. similis* juveniles but not *Pratylenchus penetrans*, whereas the flavonols inhibited the motility of *M. incognita* juveniles, but not *R. similis* and *P. penetrans* juveniles, respectively (Wuyts et al. 2006). Recently, a study showed that isoflavonoids are elicited in huge amounts in *Medicago truncatula* in response to *M. javanica* infection where plants at the early production of isoflavonoids directed less severe infection (Chin et al. 2018).

Tannins are a group of water-soluble polyphenolic compounds that have the ability to precipitate proteins and other molecules ions (Jakobek 2015) and significantly reduced egg hatching of the root-knot nematode *M. javanica* in chestnut (Maistrello et al. 2010; D'Errico et al. 2018). The total phenols increased at the early stages of nematode infection, but declined at later stages reported in mungbean (Ahmed et al. 2009) and in tomato (El-Beltagi et al. 2012; Farahat et al. 2012). The enhancement in the levels of total phenols, flavonoid, and anthocyanin was examined in the plants treated with nematodes in response to the inoculum of PGPR, i.e., *P. aeruginosa* and *B. gladioli* (Khanna et al. 2019b). The content of anthocyanins was raised in *Arabidopsis thaliana* when infected with nematodes as observed by Labudda (2018), and their results confirmed that anthocyanin could act as an important antioxidant in protecting the plants from infection. It was also revealed by Nunes da Silva et al. (2019) that biosynthesis of phenolics was intensely increased with the fall of nematode population at specific site in nematode-infected *P. pinaster* plants. In another study, it was also found that caffeic acid, one of the phenolic compounds, affected the classification between the susceptible and resistant genotypes of *Solanum lycopersicum* in response to nematode activity (Afifah et al. 2019). In this line, Ohri and Pannu (2010) had also investigated α-resorcylic acid, ferulic acid, 3,4-dihydroxybenzoic acid, and caffeic acid phenolic compounds for nematicidal activity and found high mortality effect on nematodes. Hence, various studies validated that plant growth-promoting bacteria reduce nematodes severity by inducing plant systemic resistance (Ramamoorthy et al. 2001; Pieterse et al. 2014; Afifah et al. 2019; Khanna et al. 2019a, b). This induced resistance is attained by mechanical strengthening of cell wall such as cell wall thickening, callose deposition, and phenolic compound accumulation and by synthesis of several biochemical compounds which are upregulated in defense responses (Table 16.2).

### 16.6.4 Siderophores

Apart from $CO_2$, water, and $O_2$ living plants require different essential elements along with iron as a specific factor to carry out specific biological activities (Shelake et al. 2018). Being essential, it is required by living organisms to activate several biological processes such as photosynthesis, respiration, electron transport chain, and cofactors for many enzymes (Aguado-Santacruz et al. 2012). Iron exists mostly insoluble form under aerobic soil environment, so availability to living organisms is quite difficult. Thus, to overcome these complexities, PGPR have adopted mechanisms for chelation of iron ions with the help of organic compounds with a low molecular weight of 500–1500 or 200–2000 Da known as siderophores (Dell'mour et al. 2012; Goswami et al. 2016). These siderophores compete for the

**Table 16.2** Role of phenolic compounds under the influence of PGPR against RKN infection in plants

| S. no. | Plant species | PGPR | Role of antioxidative enzymes/phenolic compounds | References |
|---|---|---|---|---|
| 1. | *Solanum lycopersicum* | *Pseudomonas* spp. | Secondary metabolites including 2,4-diacetylphloroglucinol (DAPG) act as the inducing agents of systemic resistance in tomato roots | Siddiqui and Shaukat (2003) |
| 2. | Leguminous plants | *Rhizobium* | Flavonoids a polyphenolic compounds act as signaling molecule in plant–microbe interactions for improved growth | Mandal et al. (2010) |
| 3. | *Oryza sativa* | *Pseudomonas aeruginosa* | The higher accumulation of phenolic compounds in bacterized rice roots inhibited root galls | Anita and Samiyappan (2012, b) |
| 4. | *Lycopersicon esculentum* | *Arthrobotrys oligospora* | PAL plays a valuable role in stress alleviation against nematode-stressed seedlings | Mostafanezhad et al. (2014) |
| 5. | *Solanum lycopersicum* L. | *Pochonia chlamydosporia* | In contrast, PAL was detrimental for the colonization of pathogen and the induction of resistance | De Medeiros et al. (2017) |
| 6. | *Withania somnifera* | *Cellulosimicrobium cellulans*, *Flavobacterium johnsoniae*, *Chitiniphilus* sp. and *Streptomyces* sp. | Stimulation of phenylpropanoid pathway and phenolics accumulation effectively accomplish *M. incognita*-induced stress along with enhanced active molecules | Gupta et al. (2016) |
| 7. | *Solanum lycopersicum* cv. PT-3 | *Pseudomonas jessenii* strain R62 and *Pseudomonas synxantha* strain R81 | PGPR exhibited potent activity of phenolics by 28% of control | Sharma and Sharma (2017 |
| 8. | *Oryza sativa* | Resistant and partially resistant recombinant organisms | Higher total phenolic content was detected in infected plants of some RILs | Galeng-Lawilao et al. (2019) |
| 9. | *Beta vulgaris* | *Bacillus megaterium* | Rosmarinic acid (RA) and terpenoid compounds demonstrated nematicidal activity against plant-parasitic nematodes | Mostafa et al. (2018) |

| | | | | |
|---|---|---|---|---|
| 10. | *Pogostemon cablin* | *Pseudomonas putida strain* and *Bacillus cereus* | Phenylpropanoid pathway through overproduction of PAL enzyme and enhanced flavonoid synthesis involved in stress tolerance toward RKN | Borah et al. (2018) |
| 11. | *Solanum lycopersicum* | *Bacillus cereus* BCM2 | 2,4-Di-*tert*-butylphenol,3,3-dimethyloctane, and *n*-tridecane decreased the number of nematodes by 54.9% and 70.6%, respectively, the number of galls (by 53.7 and 52.4%), and the number of *M. incognita* in root tissues | Li et al. (2019) |
| 12. | *Bacopa monnieri* | *Streptomyces Chitiniphilus* sp. | Bacoside A content and total phenolic content enhanced that showed the efficacy of potent microbes in terms of chitinase activity to ameliorate biotic stress | Gupta et al. (2019) |
| 13. | *Solanum lycopersicum, Daucus carota* | *Bacillus firmus, Bacillus aryabhattai, Paenibacillus barcinonensis, Paenibacillus alvei and Bacillus cereus* | Secondary metabolites produced by different PGPR strains are responsible for the biocontrol activity and have potential as biological control agents of *M. incognita* on carrots and tomatoes, respectively | Viljoen et al. (2019) |
| 14. | *Capsicum annuum* | Rootstocks (C19, C25, and RT17) | Initiated the production of biochemical defense compounds to induce resistance against RKN | Galvez et al. (2019) |
| 15. | *Solanum lycopersicum* | *Drechslerella dactyloides* and *Dactylaria brochopaga* | Assisted growth promotion by activating phenylpropanoid pathway in root apoplast and initiated ISR to reduce *M. incognita* infection in plants | Singh et al. (2019) |
| 16. | *Lycopersicon esculentum* | *Pseudomonas aeruginosa* and *Burkholderia gladioli* | Total phenols, flavonoids, and anthocyanins play a valuable role in stress amelioration in nematode-stressed seedlings | Khanna et al. (2019a, b) |
| 17. | *Solanum lycopersicum, Daucus carota* | *Bacillus firmus*, *Bacillus aryabhattai*, *Paenibacillus barcinonensis*, *Paenibacillus alvei* and *Bacillus cereus* | Identified secondary metabolites in infected plants upregulated and showed better growth | Viljoen et al. (2019) |

(continued)

**Table 16.2** (continued)

| S. no. | Plant species | PGPR | Role of antioxidative enzymes/phenolic compounds | References |
|---|---|---|---|---|
| 18. | *Solanum lycopersicum* L. | Resistant tomato genotypes, GM2 and F1 | Caffeic acid is one of the phenolic compounds to induced ISR for amelioration of *M. incognita* infection in plants | Afifah, et al. (2019) |
| 19. | *Vigna unguiculata* | *Bacillus subtilis*, *B. pumilus* and *Pseudomonas fluorescens* | Phenolic compounds and soluble proteins significantly increased and enhanced resistance toward stress | Abd-El-Khair et al. (2019) |
| 20. | *Solanum lycopersicum* | Plant root exudates | Amount of phenols increased significantly and enriched tomato plants with better growth in response to the *M. incognita* | Yang et al. (2020) |

$Fe^{3+}$ ions (ferric ions) under low $Fe^{3+}$ ion availability. PGPR with siderophore producing property are gaining importance as they sequester $Fe^{3+}$ ions from the surroundings (Saha et al. 2016). Rhizobacterial-associated siderophores are mainly of four types such as phenol catecholates, carboxylate, pyridine, and hydroxamates based on iron-containing functional groups (Crowley 2006).

Nevertheless, microbial siderophores also improve the availability of iron and some other elements to promote the growth and developmental processes under stress conditions. *Phyllobacterium*-associated siderophores improve the size and quality of strawberries by enhancing iron availability (Flores-Félix et al. 2015). Moreover, genus *Pseudomonas* produces different siderophores; among them, Pyochelin (Pch) and pyoverdine (Pvd) are well-studied siderophores concerned with agricultural applications. Siderophores also compete with the phytopathogens for the sequestration of iron ions available for the growth and further kill the pathogens to reduce their number (Scavino and Pedraza 2013). Under low iron availability, pyochelin (Pch) is the first to be produced by the rhizobacterium, but at critically low iron levels, pyochelin gets converted into pyoverdine which shows a high affinity toward metal ions (Dumas et al. 2013). Pyoverdine, a yellowish-green pigment, is a well-known virulence factor of the genus *Pseudomonas* which is significantly required for quorum sensing communications, controls biofilm formation, and further stimulates the regulation of other virulence factors (Imperi et al. 2009) (Table 16.3).

### 16.6.5 Nonenzymatic Antioxidants

RKN after entering into the roots of plants fluctuates the water and mineral uptake (Jang et al. 2015). Oxidative burst and ROS production at site of infection are the immediate action of plants after the recognition of RKN (Saed-Moucheshi et al. 2014). Ascorbic acid, glutathione, and carotenoids are low-molecular-mass compounds, and tocopherol and carotene are lipophilic and nonenzymatic antioxidants which by modulation in the cell elongation, mitosis, and gene expression affects the growth and development of plants (Waśkiewicz et al. 2014). Stress faced by plants leads to the accumulation of ROS in plants, due to which the level of these antioxidants increases in order to fight the stressful conditions. This increase in the level of these antioxidants can be because of their participation in AsA-GSH cycle which includes scavenging different types of ROS produced under stress conditions (Foyer et al. 1994). This increase in the level of antioxidants can also be due to their role as redox buffers activated with the help of microorganisms and scavenging of superoxide and hydroxyl radicals which is further followed by reduction of hydrogen peroxide into water (Mollavali et al. 2016). Smirnoff and Wheeler (2000) had suggested that free radicals are directly attacked by ascorbic acid during the oxidative damage. The scavenging role of tocopherols was explained by Kesba and El-Beltagi (2012) and found that the process is localized within the chloroplast membrane and acts as scavenger for the lipid peroxyl radicals. The role of another antioxidant glutathione was explained by Jimenez et al. (1997) that they

**Table 16.3** Role of siderophores in plants against RKN infection

| S. no | Plant species | PGPR | Role of siderophores in RKN resistance | References |
|---|---|---|---|---|
| 1. | *Paspalum distichum* | *Bacillus megaterium* DH14 | Enhanced siderophore production to induced growth promotion and reduced nematode population density | Groover et al. (2020) |
| 2. | *Capsicum annuum* | *Bacillus subtilis* | Improved pathogen resistance | Huang et al. (2017) |
| 3. | *Capsicum annuum* | *Bacillus* spp. | Better growth and yield of the plant and induced siderophores | Yanti and Nasution (2017) |
| 4. | Tomato | *Chryseobacterium* | Effective siderophore production | Radzki et al. (2013) |
| 5. | Sugarcane | *Kluyvera ascorbata* | Siderophore overproduction | Burd et al. (2000) |
| 6. | *Brassica napus* | *Microbacterium* G16 | Increased siderophore production | Sheng et al. (2008) |
| 7. | *Capsicum annuum*, carrot | *Rhizobium* | Higher siderophore synthesis | García-Fraile et al. (2015) |
| 8. | *Ocimum sanctum* | *Achromobacter xylosoxidans* Fd2 | Stimulated growth and release of phytohormones and siderophores | Barnawal et al. (2012) |
| 9. | *Ocimum sanctum* | *Serratia ureilytica* spp. | Effective siderophore production | Barnawal et al. (2012) |
| 10. | *Capparis spinosa* | *Pseudomonas stutzeri* CSP03 | Enhanced siderophore production to induced growth promotion | El-Sayed et al. (2014) |
| 11. | *Capparis spinosa* | *Bacillus subtilis* TTP02 | Higher siderophores, phytohormones, zinc, and phosphate solubilization | El-Sayed et al. (2014) |
| 12. | *Capparis spinosa* | *Pseudomonas putida* PHP03 | Antagonistic potential of microbes toward nematodes by microbe-mediated synthesis of siderophores and phytohormones | El-Sayed et al. (2014) |

actively participate against abiotic and biotic stresses. Glutathione is also known to be actively involved in reducing sulfur and in the administration of the thiol–disulfide content.

PGPRs in soil act as protectors against soilborne plant pathogens that include RKNs (Sharma and Sharma 2015). There are several PGPR species as *Bacillus* sp., *Pseudomonas* sp., *Burkholderia*, *Arthrobacteria*, *Clostridium*, *Rhizobium*, and *Azotobacter* in soil that regulate the RKN impact on plants by the adoption of various mechanisms (Burkett-Cadena et al. 2008; Tian et al. 2007; Gupta et al. 2017a; Sidhu 2018; Prasad et al. 2019). These PGPRs causes inhibition of RKNs by the production of various toxins and metabolites and activates the enzymatic activities and potential of various enzymatic and nonenzymatic antioxidants (Cetintas et al. 2018; Sharma and Sharma 2017). There are many reports in the literature suggesting that the application of PGPRs enhanced the level of these antioxidants in plants under nematode stress. This enhancement is directly proportional to maintaining redox status of plants that is induced by PGPR, and the free radical scavenging is further followed by reduction of poisonous radicals into nontoxic form (Mollavali et al. 2016). Along with this, Gupta et al. (2017a) also suggested that the increase in the level of antioxidants can also be due to the upregulation of genes that are involved in the biosynthesis of these antioxidants. Furthermore, Khanna et al. (2019a) revealed that the supplementation of soil with *Pseudomonas aeruginosa* and *Burkholderia gladioli* enhanced the nonenzymatic antioxidant contents in the form of glutathione, ascorbic acid, and tocopherol in *Lycopersicum esculentum* infected by *Meloidogyne incognita*. Same study was conducted by Khanna et al. (2019b) on 45-day-old plants raised under field conditions and studied the effect of PGPR on nonenzymatic antioxidants, and the active role of PGPR in upliftment of these nonantioxidants in plants under nematode stress is found. Another study by Gupta et al. (2017a) suggested that *Bacillus megaterium, Trichoderma harzianum,* and *Glomus intraradices* individually and in consortia also help in the management of *Meloidogyne incognita*. They also showed that these microbes when used in consortia help in the enhancement of antioxidant potential of total phenols and flavonoids in *Matricaria recutita* L., respectively (Table 16.4).

### 16.6.6 Antinematicidal Compounds or Toxins

PGPR and nematodes within soil affect the functioning of each other in different ways. They both affect the plant activities in the rhizosphere in which they exist. PGPRs help in the growth and development of plant and also protect the plants against nematode stress (Singh et al. 2017). Some PGPR such as bacteria are able to produce several secondary metabolites which are also active against these nematodes. Secondary metabolites help in the protection of plants both directly and indirectly. In direct mechanism, it involves the use of several enzymes, VOCs, gases that affect the nematode community. In indirect mechanism, nematode population is decreased by alteration of the rhizospheric factors in the soil (Marin-Bruzos and Grayston 2019). For instance, some bacterial strains are known to produce cry proteins during the sporulation stage that proves to be highly toxic toward these nematodes (Guo et al. 2008). A well-documented *B. thuringiensis* helps in the protection of plants by the production of cry proteins (endotoxins) against nematode attack (Bravo et al. 2007). These cry proteins cause the degradation of intestine by attaching to the epithelial cells of the nematodes. After the attachment to the epithelial cells of the nematodes, these proteins cause pores and vacuoles formation further leads to the degradation of the nematode (Marroquin et al. 2000). About 54 families of these endotoxins have been identified so far. Among all the identified cry proteins, Cry 5, Cry 6, Cry 55, Cry 12, and Cry 13 are known to have nematicidal activity (Frankenhuyzen 2009). Cry proteins as Cry 21 Fa1 and Cry 21 Ha1 which are produced from *B. thuringiensis* DB27 also show nematicidal activity (Iatsenko et al. 2014).

**Table 16.4** Antioxidant potential of PGPR against RKN infection in plants

| S. no. | Plant species | PGPR | Role of antioxidants against RKN resistance | References |
|---|---|---|---|---|
| 1. | *Pinus massoniana* | *Pseudomonas* and *Stenotrophomonas* | Modulated antioxidant capacities | Fu et al. (2020) |
| 2. | *Solanum lycopersicum* | *Burkholderia gladioli* | Elevated antioxidative capacities with higher ascorbic acid, glutathione, and tocopherol content | Khanna et al. (2019a) |
| 3. | *Lycopersicum esculentum* | *Pseudomonas aeruginosa* | Enhancement in the levels of total antioxidants and nonenzymatic antioxidants (ascorbic acid, glutathione, and tocopherol content) | Khanna et al. (2019b) |
| 4. | *Matricaria recutita* | *Bacillus megaterium, Trichoderma harzianum*, and *Glomus intraradices* | Increased total antioxidants to cope nematode infection in plants | Gupta et al. (2017a) |
| 5. | *Gossypium hirsutum* | *Pseudomonas aeruginosa* | Escalated antioxidant activities | Rahman et al. (2016) |
| 6. | *Pinus* sp. | *Serratia* | Higher antioxidant levels to combat oxidative stress | Vicente et al. (2016) |
| 7. | *Glycine max* | *Bacillus simplex* | Induced expression of AsA to counteract oxidative damage | Xiang et al. (2016) |
| 8. | *Vernonia anthelmintica* | *Pseudomonas aeruginosa* and *Trichoderma harzianum* | Accelerated antioxidant activities | Shafique et al. (2015) |
| 9. | *Ocimum basilicum* | *Bacillus* sp. | Maximized antioxidant capacities for scavenging free radicals | Gupta and Pandey (2015) |
| 10. | *Azotobacter chroococcum* | *Abelmoschus esculentus* L. | Enhanced ascorbic acid content with significant reduction in nematode infection | Safiuddin et al. (2014) |

Many gaseous compounds are also released by PGPR in the rhizosphere. These compounds modulate the metabolism of amino acids culminating into the degradation of nematodes (Mcsorley 2011). Hydrogen cyanide is one such gas helpful in protecting plants against nematode stress. A study reported by Nandi et al. (2015) found that *Pseudomonas chlororaphis* PA23 produces hydrogen cyanide which repelled *C. elegans* proliferation from soils so as to protect them from being attacked by nematodes. Moreover, *Pseudomonas aeruginosa* PA01 also produces hydrogen cyanide to paralyze the nematodes (Gallagher and Manoil 2001). Apart from this, various secondary metabolites produced by different bacterial strains also exhibit the nematicidal activity. To illustrate, Liu et al. (2010) found another mechanism in *B. thuringiensis* to kill nematodes. It comprises thuringiensin, an adenine nucleoside that inhibits RNA polymerases at binding site after competition with ATP molecules and ultimately kills nematodes. In addition, uracil, 9H-Purine, and dihydrouracil produced by *B. cereus* and *B. subtilis* also showed nematicidal activity against *M. exigua* J2 (Oliveira et al. 2014). It was found by Liu et al. (2010) that *B. amyloliquefaciens* FZB42 also produces plantazolicin that also possess nematicidal activity. Above all, Gao et al. (2016) found that *B. cereus* S2 produces sphingosine which attacks on the genital tract of nematode *C. elegans* and disturbs their reproduction and therefore, leading to the destruction of the nematode.

## 16.7 Conclusions and Future Perspectives

Agricultural activities are dependent on the combinatorial multifaceted biological processes within soils that eventually cause modification of agrosystems for better outcome of plant growth and development. Beneficial microbes such as PGPR enhance the ecosystem by their positive actions and also inhibit the penetration of pathogens and pests specifically RKNs. Likewise, PGPR within rhizosphere prevent the root damage from severe pathogens. With evolving studies and use of latest technological advancements, the biological control of RKNs has been prevailing in the present era. PGPR metabolic products now hold a special place in world market as an effective strategy of RKN biocontrol. The PGPR induces the antioxidative defense potential of plants by releasing these metabolites. Moreover, they trigger the enzyme systems, phytohormone levels, and phenolic compounds to combat the adverse effects of nematode infections. Apart from this, the microbe-induced siderophore production has also made a great influence on controlling the nematode infection within soils. Therefore, after taking all these aspects into consideration, we can assume that rhizobacteria could be the most powerful toolbox to curb the RKN infection in agricultural fields. In this sense, they uphold a special position and industries have been ransacking the possible ways to produce them on large scale as an innovative product for world market and extensive research and development. Moreover, their optimization followed by formulation would also help us to develop them as most potent biocontrol agents on both sustainable and economic terms. With the advocates of technology, the agriculture is heading toward sustainability; henceforth, PGPR exploration will unfold many secrets and could be of immense use in present as well as future agricultural practices.

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# Part IV

# Crop Specific Studies with Reference to Antioxidants

# 17

# Disease Management and the Role of Antioxidants in Combating Plant Pathogens upon PGPR Inoculation with Special Reference to Legumes

Bandana Saikia and Prassan Choudhary

## Abstract

Important enzymes like catalase (CAT), peroxidase (POD), phenylalanine ammonia lyase (PAL), etc., have been reported to impart disease resistance in various crops. Also, enzymes like methyl jasmonate and methyl salicylate play a crucial role in imparting induced resistance against the attacking pathogen and production losses. Leguminous plants are known to attract nitrogen via minerals and symbiotic relationship with microbes. Hence, they form a vital part of an agricultural system and play an important role in replenishing soil health. Legumes like chickpea and pigeonpea are prone to various plant pathogens (Collar rot, *Fusarium* wilt, etc.) resulting in huge losses in production to the farmers. Investigations into the role of antioxidants have revealed sufficient evidence of antagonistic effects against such diseases upon PGPR inoculation. This chapter outlines the application of PGPR inoculation with *Serratia*, *Pseudomonas*, *Azotobacter*, *Azospirillium*, and other bacterial agents coupled with the role of antioxidants in curbing major diseases of agriculturally important legumes.

## Keywords

Antioxidants · Legume · Antagonism · Phytopathogen · *Fusarium* wilt

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## 17.1 Introduction

Legume species, known for their high protein content, belong to the Fabaceae family usually characterized by the presence of fruiting body called pod. Some of the important legumes cultivated by man are chickpea, pigeonpea, soybean, common bean, pea, cowpea, lentil, faba bean, and peanut. Crop productions around the world essentially cultivate legumes as they are capable to fix atmospheric nitrogen in soil establishing a unique symbiotic relationship with the soil rhizobia (Graham and Vance 2003). They are cultivated via crop rotations with cereals or vegetables playing a significant role in reducing biotic stresses, enhancing nutrient use efficiency leading to sustainable yield stability. Continuous efforts are undertaken to explore the possible formulations of grain legumes into ready-to-eat food commodities making it an integral part of economically important agricultural produce (Merga and Haji 2019).

Leguminous plants are prone to various bacterial, fungal, and viral diseases which may cause heavy destructions to the crop resulting in losses to the farmers (Aguilera et al. 2011; Desai et al. 2016, 2019). Nene et al. (1996) reported that as many as 172 pathogens can attack chickpea causing huge losses in agricultural produce. *Fusarium* wilt caused by *Fusarium oxysporum* f sp. *ciceri* (FOC) may destroy up to 100% of the crops under severe conditions (Jendoubi et al. 2017). Collar rot of chickpea caused by *Sclerotia rolfsii* may damage up to 30% of the chickpea productions (Sahni and Prasad 2020; Shirsole et al. 2018). Another major disease is dry root rot of chickpea (*Rhizoctonia bataticola*), which has emerged as a menace to the chickpea cultivation in relatively dry areas as the host plant is susceptible to contagion by high temperatures and moisture stress during the flowering to pod filling stage (Sinha et al. 2019). The study also established a correlation between drought stress and pathogen infection. Bacterial blight of cowpea due to *Xanthomonas campestris* pv. *vignicola* may cause up to 92% damage to the yield (Okechukwu and Ekpo 2004). Similarly, bacterial blight in pea owing to the infection of *Pseudomonas syringae* pv. *syringae* reportedly leads to 94% losses in the production and is endemic to dry edible beans (Adhikari et al. 2018; Richardson and Hollaway 2011). Clearly, the susceptibility to diseases presents a great challenge to the farmers and the scientific community alike.

In recent times, globalization along with population boom has presented a huge task to the agricultural community so as to feed the ever-growing population. Steps were taken during the 1930s which promoted the use of chemical fertilizers in an attempt to boost up agricultural produce. Such practices have not only depleted the soil health but also left them prone to various soilborne pathogens. This chapter centers its focus around the disease management strategies deploying plant growth promoting (PGP) bacteria in conjugation with the role of antioxidants. Recent studies have focused on the ability of certain biocontrol agents which not only enhance the growth but also enable the crop to form a first line of defense against various pathogens.

## 17.2 Role of Bacterial spp. as PGPB and Biocontrol Agents

The role of bacterial spp. as a biocontrol agent is a well-established technique to curb deadly plant pathogens. Generally, the use of resistant cultivars is advisable, but the occurrence of new races of pathogen breaks through the plant resistance causing devastation. The advantages of using microbial bio-formulations are immense. It is a bio-friendly approach (green technology) and enhances the systemic resistance in plants. The application of PGPB also raises the nutrient quality and defense-related enzymes which helps to fight against the pathogens. Although an excellent alternative to chemical approaches, the propagation of bio-formulations among the end users is an essentiality. Low performance of bio-formulations on field and against multiple crops can be attributed to a nonuniformity in agricultural practices around the world. In order to establish bio-formulations as trustworthy and global solutions, a number of necessary steps need to be taken. An assessment of the types of bio-formulations available in the market along with future challenges like reclamation of marginal and polluted soils may pave the way ahead for its propagation. Also, this green strategy faces tough competition from synthetic fertilizer industry in terms of crop yield and profit made by the end users. Approximately, 890 synthetic chemicals have been approved globally as pesticides whereas 20,700 marketed products have been estimated to be in circulation (Stenersen 2004).

Although the chemical fertilizers and pesticides enjoy popularity for enhancing productivity as well as action against diseases, they are a threat to environment and soil health. Seepage of chemical fertilizers into the soil not only harms the farmers' fields but travels to nearby places, thus disrupting the ecosystem (Buckler 2018). Karunarathne et al. (2020) recently estimated the death toll due to pesticide poisoning to be around 14 million between 1960 and 2018. Many studies highlight the importance of plant growth promoting (PGP) bacteria instead of chemicals in managing biotic and abiotic stresses that a plant undergoes. PGPR display an important communication with roots of the plant and significantly influence plant augmentation (Majeed et al. 2018). Plant growth is improved by Induced Systemic Resistance (ISR), competitive omission, antibiosis, heavy metal tolerance, and so on. Dogra et al. (2019) used a myriad of microbes including *Pseudomonas citronellis*, *Pseudomonas* sp., *Serratia* sp., *Serratia marcescens*, and *Frateuria aurantia* as PGPR for nutrient enhancement in chickpea. de Andrade Santos et al. (2018) used co-inoculations of *Bradyrhizobium* with *Actinomadura* or *Paenibacillus gramanis* helped in efficient nodulation in cowpea besides imparting salt stress (50 mmol $L^{-1}$ NaCl). Islam et al. (2016) showed two strains of *Proteus mirabilis* (T2Cr and CrP450) along with salicylic acid to improve plant performance under chromium stress. Sarma and Saikia (2014) showed the efficacy of *Pseudomonas aeruginosa* GGRJ21 strain in alleviating drought stress via a strong upregulation of drought-related genes viz. catalase (*CAT1*), dehydration responsive element binding protein (*DREB2A*), and dehydrin (*DHN*) present in mung bean.

Along with PGP traits, the application of these microbes as biocontrol agents against plant pathogens is a key to boost sustainable agriculture. Early reports include that by Fravel et al. (1985) used a alginate-clay matrix bio-formulation

**Table 17.1** Some of the important pathogens affecting the leguminous crops throughout the world

| Pathogen | Disease | Extent of damage (%) | References |
|---|---|---|---|
| *Rhizoctonia bataticola* | Dry root rot | 50–70 | Anjana and Kumar (2008) |
| *Ascochyta rabieii* | *Ascochyta* blight | 70 | Pande et al. (2010) |
| *Fusarium oxysporum* f. sp. *ciceri* | *Fusarium* wilt | 77–94 | Kamdi et al. (2012) |
| *Fusarium udum* | *Fusarium* wilt | 100 | Sharma et al. (2016) |
| *Sclerotium rolfsii* | Collar rot | 70 | Misra (1997) |
| *Sclerotinia sclerotiorum* | Stem root | 10–100 | Singh et al. (1989) |
| *Fusarium solani* | Black rot | 40–50 | Beniwal et al. (1992) |
| *Colletotrichum lindemuthianum* | Anthracnose | 50 | Adebanjo and Bankole (2004) |
| *Leveillula taurica* | Powdery mildew | >80 | Attanayake et al. (2008) |
| *Meloidogyne* spp. | Root knot | 41–100 | Wesemael et al. (2011) |
| *Helicoverpa armigera* | Pod borer | 100 | Pooniya et al. (2015) |
| *Maruca vitrata* | Spotted Pod borer | 84 | Margam et al. (2011) |
| *Melanagromyza obtusa* | Pod fly | 40 | Singh et al. (2013) |

including *Talaromyces flavus*, *Gliocladium virens*, *Trichoderma viride*, and *Pseudomonas cepacia* (Fravel 1985). Sayeed Akhtar and Siddiqui (2008) investigated the antagonistic effects of consortia of *Rhizobium* sp., *Glomus intraradices*, and *Pseudomonas straita* against root-rot disease complex of chickpea caused by *Macrophomina phaseolina* and *Meloidogyne incognita*. Recently, a consortium of *Serratia marcescens*, *Pseudomonas fluorescens*, *Rahnella aquatilis*, and *Bacillus amyloliquefaciens* was used to effectively manage *F. oxysporum* f sp. *ciceris* race 0 and *F. solani* f sp. *pisi* in chickpea rhizosphere (Palmieri et al. 2017). Sharma et al. (2019) characterized five strains of halotolerant *Bacillus* sp. viz. *B. pumilus, B. subtilis, B. licheniformis, B. safensis,* and *B. cereus* as plant growth promoting bacteria showing broad-spectrum antifungal activities and possessing biocontrol traits. Karimi et al. (2012) reported *Bacillus* and *Pseudomonas* spp. as biocontrol agents against *Fusarium* wilt in chickpea (Karimi et al. 2012). Similarly, Figueredo et al. (2017) tested a combination of *Bradyrhizobium* sp. SEMIA6144 with biocontrol agent *Bacillus* sp. CHEP5 against *S. rolfsii* improving its yield by 16.69% in peanut. Table 17.1 highlights some of the major diseases of legumes caused by various pathogens.

A challenging aspect of biocontrol studies is their performance in field conditions. The process of testing biocontrol agents in high densities on field conditions is called as "augmentative biological control" (van Lenteren et al. 2018). Use of fungicide-treated seeds, advanced sowing date, and solarization of soil are usually employed to control *Fusarium* wilt in legumes like chickpea and pigeonpea but with limited success. Gopalakrishnan et al. (2011) reported five actinomycetes species namely *Streptomyces tsusimaensis*, *S. caviscabies*, *S. setonii*, *S. africanus*, and *Streptomyces* spp. which controlled the incidence of *Fusarium* wilt by 4–19% in wilt-sick field of chickpea. Anusha et al. (2019) reported 32% and 31% reduction in *Fusarium* wilt of chickpea by application of *Streptomyces* spp. AC 19 and *Bacillus* spp. BS 20, respectively, in wilt-sick field. The study also reported a significant increase in grain yield by 34% and 28% by *Streptomyces* spp. AC 19 and *Bacillus* spp. BS 20, respectively. Singh et al. (2020a, b, c) reported an interesting observation as the study preferred neem cakes over biocontrol agent (*Trichoderma hazarinum*) as it reduced the galls by 59% caused by *Meloidogyne* spp. in chickpea. The cakes also increased the yield by 168 kg/ha as compared to increase of 51 kg/ha (in case of *Trichoderma hazarinum*) as compared to uninoculated controls. More studies are required to establish the efficacy of biocontrol agents in field conditions so as to commercialize them widely.

## 17.3 Mechanism of Action of Antioxidants in Diseased Plants

Oxidative stress is one of the primary reasons for the development and progression of diseases. Antioxidants play a direct role in plant health and disease prevention by reducing oxidative stress incurred by the plants due to pathogens. These antioxidants work in tandem and follow a complex but strict mechanism of action. Plants continuously produce ROS mainly in chloroplasts via a process known as Mehler reaction (Heldt et al. 2005). Oxidative burst or excess production of ROS is one of the earliest induced responses of plants to stress/pathogen invasion. The production of ROS has two steps: first step begins spontaneously as the pathogen attacks and is weak and transient, while the second step is much more severe and lasting, sustained for several hours (Camejo et al. 2016). For pathogens, ROS also play an important role in their infection processes, and the lack of ROS-producing systems can affect fungal toxicity and their interaction with plants (Kayano et al. 2013). In order to cope with oxidative stress, both plants and pathogens have evolved efficient scavenging systems to modulate ROS homeostasis, which eventually determine the incidence, development, and consequences of diseases in plants.

Plants have gradually developed a well-organized and robust antioxidant system which includes enzymatic (SOD, CAT, POD, APX, GR, DHAR, and MDHAR) as well as nonenzymatic antioxidants (ascorbate, glutathione, tocopherols, thioredoxin, and carotenoids) for rapid scavenging of Reactive Oxygen Species (Sharma et al. 2012). This antioxidant system is efficient in plants which tackles the effect of oxidative stress. It is this antioxidant machinery which effectively controls the ROS formation and their reactivity with biologically important molecules (Puthur 2016). Figure 17.1 depicts the different types of stress a plant has to endure during its life cycle.

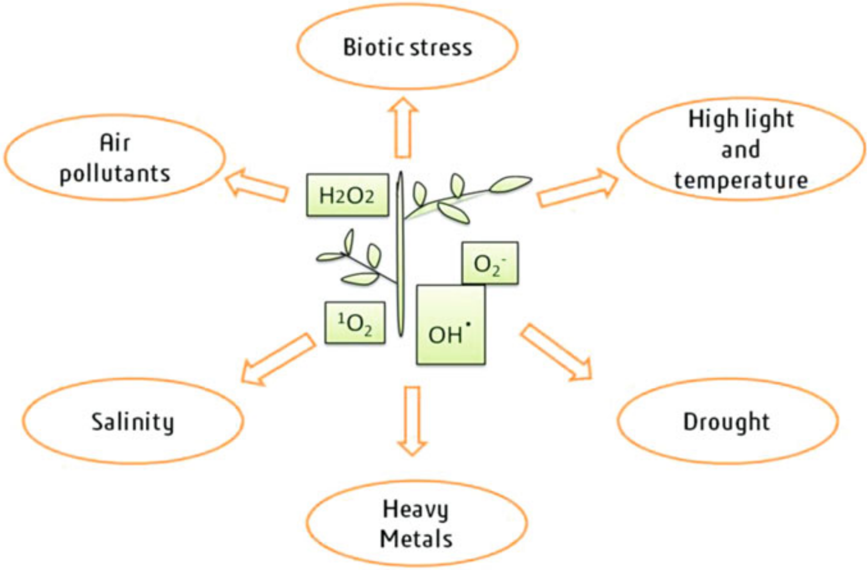

**Fig. 17.1** The figure depicts the various types of stress endured by plants

### 17.3.1 Enzymatic Antioxidants in Plants

Superoxide dismutase (SOD) has three forms in plants viz. copper/zinc, manganese, and iron forms based on their active site biochemistry. It is only in plants that all the three functionally equivalent but structurally different forms of SOD exists. The MnSOD and FeSOD enzymes are structurally quite similar. Studies have even reported that the apoenzymes can function even with either metal (Mn/Fe) present in the active site moiety (Stallings et al. 1984). The Cu/ZnSOD has been reported to be structurally dissimilar. The mechanism of catalysis is essentially involving a protein pocket bordered by positively charged amino acid residues that creates an electrostatic sink for attracting the superoxide anion radicals to the active site. There is an electron transfer by the transition metal present at the active site between two superoxide radicals which then undergoes alternating oxidation/reduction reactions (Bowler et al. 1994).

Similarly, in case of peroxidase, the mechanism is carried out in four distinct steps: (a) binding of peroxide to the heme-Fe(III) to form a very unstable peroxide complex (C 1); (b) oxidation of the iron to generate C 2, a ferryl species with a pi-cation radical in the porphyrin ring; (c) reduction of C 2 by one substrate molecule to produce a substrate radical and another ferryl species, C 3; (d) reduction of C 3 by a second substrate molecule to release a second substrate radical and regenerate the native enzyme. There are many such enzymatic mechanisms built and developed by plants to tackle oxidative stress and fight plant pathogens.

### 17.3.2 Nonenzymatic Antioxidants in Plants

Ascorbic acid, a major redox buffer, uses multiple pathways to combat oxidative stress in plants. Loewus and Kelly (1961) studied the metabolism which converted D-galacturonic acid-1-$^{14}$C to L-ascorbic acid-6-$^{14}$C using detached ripening strawberry fruit by an inversion pathway. Wheeler et al. (1998) explained another pathway for the generation of L-ascorbic acid from L-galactose via the Smirnoff-Wheeler pathway in higher plants. In chloroplast thylakoids, the reaction centers of photosystem I (PSI) and photosystem II (PSII) are the main production site of reactive oxygen species (ROS). Ascorbic acid, under the water-oxidase complex impaired conditions like high light stress serves as an electron donor to PSI and PSII in isolated thylakoids (Gallie 2013). Likewise, plasma membrane is stabilized by glutathione which helps in reducing passive $Na^+$ influx, enhancing plant salt tolerance. Glutathione participates in maintaining cellular redox balance and has a significant role in signaling functions under salt stress in plants (Foyer and Noctor 2005). There are many such examples like thioredoxin, carotenoids, and other antioxidants playing a major role in curbing oxidative stress of diseased plants.

## 17.4 Integrated Approaches with Optimal Use of PGPR Inoculations and Induced Systemic Resistance

PGP Rhizobacteria can help plants to be defiant against biotic stress via direct antagonism or by inducing systemic resistance to pathogens. There are several reports of potential PGP strains which are capable of generating systemic resistance in plants during foreign invasion. During pathogen attack, the presence of host enzymes like SOD, POX, CAT, APX, PR proteins, etc., get altered signifying their role during biotic stress (Vasconcelos and Barreto 2014). Also, there are many ways of applying potential PGP bacteria in order to alter antioxidant enzymes and manage diseases effectively. A recent study by Sahni and Prasad (2020) effectively used PGP strain *Pseudomonas* sp. (PUR 46) integrating it with vermicompost to manage collar rot disease of chickpea (Sahni and Prasad 2020). The study also analyzed levels of SOD, PAL, and POD and their likely part in the induction of resistance integrating vermicomposting and *Pseudomonas* activities in plants showing induced resistance. Egamberdieva et al. (2017) reported *B. subtilis* NUU4 having notable PGP capabilities which improved symbiotic performance of host plant with rhizobia exhibiting antagonistic effects against *Fusaium solani* causing root rot in chickpea. The study further reported a pooled inoculation with *M. ciceri* IC53 and *B. subtilis* NUU4 decreased $H_2O_2$ concentrations with increased proline content compared to the uninoculated plants which clearly indicated an alleviation of adverse effects of salt stress of chickpea.

Seed biopriming, an effective and frequently used technique, allows the bacteria to cling to the seeds and also adaptation of bacteria in existing conditions. Seed biopriming with plant growth-promoting strains are also being used for disease management (Singh et al. 2020a, b). Ghoniem and Belal (2013) tested the in vitro and in vivo application of *Trichoderma longibrachiatum* against *Sclerotia rolfsii* to contain root rot in cowpea (Ghoniem and Belal 2013). Hydro bio priming and drum priming were employed by Tahir et al. (2020) and showed effective disease control with 27.16% and 30.5%, respectively. Sharma et al. (2018) used bio-primed seeds of soybean to increase the crop yield. Recent reports suggest that nearly 700 products were registered, 1400 BCA products were sold, and 175 biopesticide active ingredients are circulating worldwide for their commercialization. In India, the scenario is dim with only 15 biopesticides registration and approval so far under the Insecticides Act 1968 (Mishra et al. 2018).

Issues like storage and marketing of microbial biopesticides significantly confines the extensive use of biocontrol agents in today's agriculture. Time has come to tutor the cultivators and dealers on importance of suitable storage conditions, shelf life, and mode of action of BCAs. The pathogenic variability especially in *Fusarium* spp. causing wilt and other such soilborne phytopathogens in legumes poses a grave challenge targeting to develop potential biocontrol agents. Utmost care must be taken before selecting biocontrol agents for developing its formulations and it is advisable to screen them against multiple pathogens or a large number of races/ isolates/strains of phytopathogens. For field conditions, inoculum potential (aggressiveness and the propagules/unit weight), shelf life, ease of appliance, and purity of the formulation are important. The biological control formulations presently sold in the market have a meager shelf life and lack good quality assurance. Integrating laboratory-based formulations with proper channeling, marketing, and end-user satisfaction are the challenges in the coming future.

## 17.5 Conclusions

Leguminous crops are an integral part of human diet. The use of beneficial bacteria to help plants fight against the diseases is a cost-effective and bio-friendly approach to curb disease incidences. The techniques used like vermi-composting and seed bio-priming with PGP bacteria also adds to soil health and improves the quality of nutrients. Still, there are challenges of propagating such studies and transform them into technologies to be easily used on a large scale. Use of such techniques in the field and by farmers is a challenge yet to be addressed particularly in case of crops other than cereals. If these challenges are met successfully, the path toward sustainable agriculture will be achieved.

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# Rhizobacters as Remedy of Stress Tolerance in Potato

# 18

Sharmistha Banerjee, Shuchi Kaushik, and Rajesh Singh Tomar

## Abstract

Rhizomicrobiome is the microbial community associated with plant roots. Its composition varies from the microbes of nearby surroundings because of the direct effect of bacterial competition for enriched nutrients like sugars, amino acids, flavonols, organic acids, glucosinolates, fatty acids, indole compounds, proteins, and polysaccharides released by plants. As the root soil shows compositional changes, according to stages of plant development and plant genotypes, the rhizomicrobiome also differs accordingly. Rhizosphere is the narrow zone of soil majorly affected by plant roots which grow in its nearby vicinity and is a rich source of microbes and microbial activity, thus better known reservoir of beneficial microbes, particularly bacteria. These free-living soil bacteria both extracellular as well as intracellular endophytes that colonize root and benefit plants by eliciting their growth are termed as PGPR (plant growth-promoting rhizobacteria). Potato (*Solanum tuberosum* L.) is the third largest food crop in the world after rice and wheat on the basis of supply of food quantity. It is a staple food with huge agricultural and climatic regions, poses harvesting area of more than 19 million hectares, production of 374 million tons, and consumption of greater than 239 million tons. Mainly consumed as a fresh food but also used as a raw material for food processing, for example, in French fries and chips industries and also in other industries like ethanol and starch. In addition to low fat and high

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carbohydrate content, potato tuber has balanced nutritional composition with minerals and vitamins that makes this crop an ideal candidate for the human consumption and also in terms of food security globally. It also provides employment to many and act as cash crop. Despite diverse adaptability and distribution to varied climatic and environmental conditions, growth of potato cultivars does not remain uninfluenced by abiotic factors. Water stress (both flood and drought), extreme temperatures (high and low), and ion toxicity (salinity and heavy metal) are the abiotic stresses that potato cultivars encounter in their habitats. Numerous researchers have recognized the importance of PGPR in temperature, drought, and pesticide stress tolerance of crop plants and significant advancements are made in this field but still many gaps are there. Literatures citing the importance of PGPR in tolerance of abiotic stress are limited. Draught, pesticide, and temperature stress tolerance using PGPR is proving to be an emerging area in stress management. Thus, this review is an attempt to explore the indigenous PGPRs present in roots of potato and throw light on the underlying mechanism involved in its stress coping strategy.

**Keywords**

Rhizosphere · Endophytes · Staple · Abiotic · Drought · Toxicity · Temperature · Pesticide

## 18.1 Introduction

The rhizosphere is the thin layer of soil which is usually affected by roots (Dobbelaere et al. 2003). This layer is nutrient enriched in comparison to the bulk soil because of buildup of many different types of plant metabolites like carbohydrates, amino acids, etc., that majorly act as rich source of nutrients and energy for microbes in its vicinity (Gray and Smith 2005). This fact is well proven by the microbial population surrounding the plant roots, particularly 10–100 times, the bacterial concentration is higher in this zone in comparison to the bulk (Weller and Thomashow 1994). Rhizospheric soil is occupied by a wide variety of microbes and bacteria inhabitating in this niche are known as rhizobacters (Schroth and Hancock 1982; Beneduzi et al. 2012).

Rhizobacters respond to the metabolites secreted by roots through chemotaxis, therefore competent bacteria are most likely to modify their metabolism for optimized nutrient collection (Hardoim et al. 2008). Traditionally, rhizobacters are known as soilborne bacteria that inhabits in rhizosphere (Schroth and Hancock 1982), but most of them are able to populate the root surface (exo-root), its surrounding and also enter into the endo-root, i.e., cortex of the root. Nehl et al. (1996), therefore, incorporated the terms both ecto- and endobacteria in rhizobacters. Factually, many of these rhizospheric bacteria are known to be promoters of plant growth and are endophytic in nature (Kloepper and Beauchamp 1992; Sessitsch et al. 2004; Long et al. 2008).

Plant-associated bacteria are categorized into three types: Beneficial, deleterious, and neutral based on how they influence the growth of plants (Dobbelaere et al. 2003). Free-living beneficial soil bacteria are known as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1989). Independent of the mechanism involved in promotion of vegetal growth, PGPR reside in the rhizoplane (surface of the root) or in the root itself (within radicular tissues) or in the rhizosphere (Gray and Smith 2005). It is well proven that only 1–2% of bacteria are known to stimulate the growth of plant in the rhizosphere (Antoun and Kloepper 2001) (Fig. 18.1). Bacteria of various genus have been recognized as PGPR, among them *Pseudomonas* and *Bacillus* spp. are predominant (Podile and Kishore 2006; Beneduzi et al. 2012).

Potato is known to be the world's third largest food crop after wheat and rice on the basis of food supply and quantity (FAO 2019). It is a staple crop with varied agricultural and climatic zones, covers a harvesting zone of more than 19 million hectares, out of which greater than 388 million tons were produced in 2017 and consumption of greater than 239 million tons (FAO 2019). Mainly, it is consumed in fresh form but also can be used as a raw material in various processed foods (e.g., French fries and chips) and in some industries (e.g., Ethanol and Starch industries) (Birch et al. 2012; Watanabe 2015). In addition to high carbohydrate and low fat, potato tuber has balanced nutritional composition with minerals and vitamins that make this crop ideal for balanced human diet and is also vital in context of global food security (Birch et al. 2012; White et al. 2009; Handayani et al. 2019).

Potatoes can be cultivated in both tropical and subtropical regions, examples in highlands of Southeast Asia. Irrespective of the varied distribution and adaptability of potato plant to several climatic and environmental conditions, its growth is not completely uninfluenced by environmental factors. Abiotic constraints like water stress (flood and drought), ion toxicity (heavy metal and salinity), and extreme temperature (low and high) are major challenges that potato cultivators encounter in fields which in turn affect its growth (Bohnert 2007; Handayani et al. 2019). Heat stress tolerance in potato is majorly affected by environment as well as genotype (Mendoza and Estrada 1979; Midmore 1992). Availability of humidity (Mendoza and Estrada 1979), water (Trebejo and Midmore 1990), irradiance (Menzel 1985), nitrogen, and photoperiod are considered as the most crucial environmental parameters that affect heat response in potato (Bensalim et al. 1998).

Presence of higher amount of salts in soluble form in soil present near plant roots is referred to as soil salinity. Such higher concentration of soil poses high osmotic pressure which in turn influences plant growth by limiting the uptake of water and nutrient absorption by roots (Tester and Davenport 2003). Soil salinity is one of the major challenges that influence crop yield, have adverse effects on plant vigor, germination, and productivity (Munns and Tester 2008). Thus, to develop salt-tolerant crop is the aim of many researchers but with a very low success rate (Munns and Tester 2008). Microbes that colonize in the root zone of plants cultivated in saline soil play an important part in coping salt stress because of their ability to tolerate extreme conditions, their interaction with crop plants, and potential deployment techniques (Paul and Lade 2014).

They are engaged in several biotic activities of the ecosystem of soil which make them dynamic for sustainable production of crops and turnover of nutrients (Chandler and Thorpe 1986; Diby et al. 2005a, b). They promote growth of the plants by nutrient mobilization in soil, protect plants from phytopathogens by controlling or inhibiting them, produce several plant growth regulators, bioremediating the polluted soils by sequestering toxic heavy metals and improve structure of the soil and degrading xenobiotic compounds (Braud et al. 2009; Hayat et al. 2010).

It is also predicted that yield of potato crop will reduce gradually by 2055 because of drought and global warming (Holden et al. 2003). In another study by Hijmans in 2003, it was predicted that worldwide potato production will decrease by 18–32% in the projected duration of 2040–2069 due to biotic and abiotic stresses associated with climatic changes (Dahal et al. 2019).

Rhizobacters that are able to produce biofilms and exopolysaccharides (EPS) aid plants to sustain easily in extremities. Further, PGPR also benefits the plants by adopting certain strategies, for example, induction of heat shock proteins and osmoprotectors (Grover et al. 2010). EPS secreted by microorganisms consist of the active component of organic matter of soil (Gouzou et al. 1993). EPS is known to constitute majorly the extracellular matrix of bacteria that shares 40–95% of bacterial weight (Flemming and Wingender 2001). EPS performs many important functions in protecting the plant such as formation of biofilm microbial aggregation, surface attachment, bioremediation and stimulation of plant microbial interaction, etc. (Manca de Nadra et al. 1985). EPS secreting bacteria, i.e., *Azospirillum* responds in a better way against water stress (Bensalim et al. 1998) by improving the texture, structure, and aggregation of soil (Sandhya et al. 2009; Bashan et al. 2004; Verma et al. 2016).

One way to cope up with environmental stress is root growth enhancement and maximizing the uptake of water from soil depth which in turn maintains leaf water relation, a general response toward soil drying (Reid and Renquist 1997). The other way could be to lower ethylene concentration majorly secreted in stress conditions. Thus, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase secreting PGPR produces higher yields and shows better tolerance under drought conditions as it is an immediate precursor of hormone ethylene in plants (Arshad et al. 2008; Zahir et al. 2008; Verma et al. 2016).

## 18.2 Different Types of PGPR

*Pseudomonas*, *Bacillus*, *Enterobacter*, *Serratia, Streptomyces,* and *Klebsiella* are the most common isolates of endophytic bacteria. Endophytes are not only present within the roots, but also in other plant parts, like seeds, stems, unopened flowers, or tubers (Hallmann et al. 1997; Long et al. 2008). Gray and Smith (2005) further distinguished PGPR into two groups: extracellular and intracellular endophytic PGPR (ePGPRs and iPGPRs). iPGPRs have the ability to enter plant cells and can produce specialized structures known as nodules whereas ePGPRs are present in rhizosphere, within the apoplast of the root cortex, or on the rhizoplane but cannot

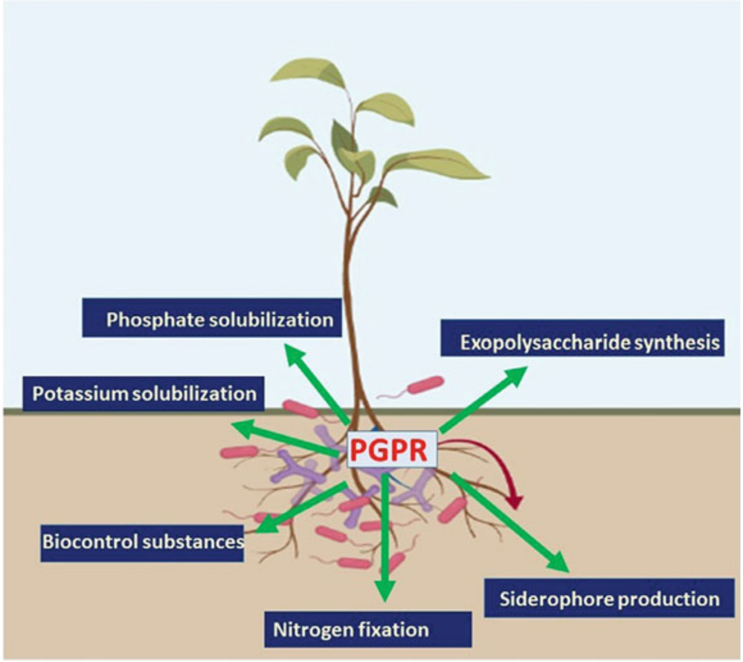

**Fig. 18.1** Role of PGPR in plants. (Adapted from Adedeji et al. 2020)

enter into the cells. According to their location, ePGPRs can be further classified into three categories: one which lives near, but not in contact with the roots; those inhabitating in the surface of roots; and those residing in the spaces between cells of the root cortex (Gray and Smith 2005).

## 18.3 Indigenous PGPR in Potato Plant

Over 60 bacterial genera including Comamonas, Arthrobacter, Curtobacterium, Pantoea, Paenibacillus, Serratia, Enterobacter, Sphingobacterium, Variovorax, Xanthomonas, Agrobacterium, Stenotrophomonas, Pseudomonas, and Bacillus were discovered by culture-dependent technique in rhizospheres of potato (Diallo et al. 2011). Studies of potato endospheres and rhizosphere using culture-independent techniques like sequencing analysis and 16S rRNA gene-based method have also showed the presence of Agrobacterium, Arthrobacter, Bacillus, Curtobacterium, Micrococcus, Sphingobacterium, Streptomyces, and Pseudomonas genus (Garbeva et al. 2001; Smalla et al. 2001; Reiter et al. 2003; Berg et al. 2005; Aloo et al. 2020).

Few researches showed that Pseudomonas sp. and Bacillus sp. are associated with enhancement of phosphorus uptake in potato plants (Hanif et al. 2015), biocontrol activities (Hunziker et al. 2015), Induced Systemic Resistance (ISR) (Ardanov et al. 2011), and production of indole-3-acetic acid (IAA). In a research conducted by

Naqqash et al. (2016), Azospirillum sp., Pseudomonas sp., Rhizobium sp. Pseudomonas sp., and Enterobacter sp. isolated from rhizospheres of potato plants showed plant growth-promoting effects in respect to dry and fresh weight of shoots and roots, total nitrogen levels, and plant height. *Pseudomonas putida* and *Pseudomonas fluorescens* are known to populate the rhizospheres of potato plant specifically (Cirou et al. 2007) and thus could be conferred as growth regulators of potato (Aloo et al. 2020). Bensalim et al. (1998) concluded for first time that the pre-inoculation of potato with *Burkholderia phytofirmans* showed enhancement of length of stem, root, and shoot biomass at elevated temperature (Mishra 2018).

## 18.4 Stress Tolerance Enhancement Through PGPR

Biomass enhancement in plantlets inoculated with rhizobacters was observed and the reason for it may be the induction of root branching and hair formation in roots (Frommel et al. 1991) and thus helps in better uptake of water and nutrient (Bensalim et al. 1998).

Three-way interactions between bacterization, potato genotypes, and temperature showed the vitality of clonal selection of using beneficial microbes for production of potato in the presence of heat stress. Although the clones under study responded in a different way to temperature and bacterization in vitro, particularly in terms of increased temperature and root growth. Bacterial biomass induction may cause changes in concentration of S/R biomass that in turn favored the roots of the plant to survive in heat and water stress which eventually resulted in yield enhancement. Mutant plants that were deficient in Abscisic Acid (ABA) were phenotypically similar to droopy potato plants in appearance and also possessed high stomatal conductance because of accumulation of ABA in a very small amount (Quarrie 1982). This represents the importance of abscisic acid in survival of plants under heat stress conditions (Bensalim et al. 1998).

## 18.5 Mechanism

The mechanisms involved in regulating stress tolerance in plants are combination of both intricate as well as complex, since, plants are nonmotile organisms (Wani et al. 2016). They do not have any other choice rather of giving up or survival by coping up the stress. Improving stress tolerance in crops by traditional breeding is a very long and capital-intensive process, while genetic engineering has its own drawbacks like socio-acceptance and ethical issues. Beneficial microbes are gaining importance in stress management and development of climate change resilient agriculture. Recent researches have devised molecular methods to understand the mechanism of plant–microbe interactions that resulted in induction of the ability to tolerate stress (Backer et al. 2018).

PGPR influence the growth of plant in two different ways: either indirect or direct. Direct stimulation of plant growth by PGPR is either by providing the plant with a chemical such as phytohormones, which is synthesized by bacteria, or helping

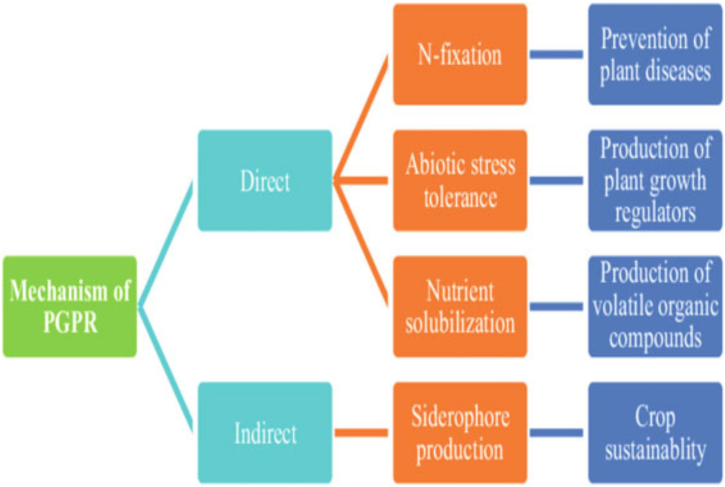

**Fig. 18.2** Mechanism adopted by PGPR in stress alleviation

in the uptake of some nutrients from the environment (Glick 1995). The indirect promotion of plant growth is when PGPR lessen or prevent the negative consequences of one or more pathogenic organisms that infect plants. This may be due to production of antagonistic substances or by induction of resistance against pathogens (Glick 1995). A particular PGPR have the ability to influence plant growth and development by using either one or many of these strategies. PGPR as biocontrol agents can act through many strategies like known production of auxin phytohormone (Patten and Glick 2002), decrease in ethylene concentration (Glick et al. 2007a, b), or nitrogen fixing associated with roots (Döbereiner 1992; Beneduzi et al. 2012) (Fig. 18.2).

## 18.5.1 Direct Mechanics of PGPR

Direct mechanics involved in stress alleviation by PGPR can be summarized under the following heads:

### 18.5.1.1 Biological Nitrogen Fixation

Bacterial strains that pose the ability of nitrogen fixation are categorized into two types. One category includes root/legume-associated symbiotic bacteria that are specific and have the ability to infect the roots and produce nodule, e.g., *Rhizobium strains*. Other category includes bacteria that are known as free-living nitrogen fixers and do not have nodules (Oberson et al. 2013). Examples of such free-living nitrogen fixers include *Azotobacter*, *Burkholderia*, *Azospirillum*, *Paenibacillus,* and *Herbaspirillum* (Goswami et al. 2015; Heulin et al. 2002; Seldin et al. 1984; von der Weid et al. 2002). Though free-living nitrogen fixers cannot enter into the plant cells but then also a very close association is fixed, in which these bacteria can very well live in close proximity to the root such that the atmospheric nitrogen fixed by them is not only beneficial to them alone but also can be uptaken by the plant that in turn provides better absorption of nitrogen (Goswami et al. 2016).

#### 18.5.1.2 Phosphate Solubilization

Next to nitrogen, phosphorous is the most limiting nutrient for plants. Despite huge reserves of phosphorous, it is not present in suitable form which can be readily uptaken by plants. Plants can only absorb mono and dibasic phosphate which are basically phosphate in soluble forms (Jha et al. 2012; Jha and Saraf 2015). Microorganisms mineralize organic phosphorus in soil by solubilizing complex-structured phosphates viz. rock phosphate, tricalcium phosphate, aluminum phosphate, etc., that convert organic phosphorous to inorganic form which ultimately improved the availability of phosphates to plants. These phosphate-solubilizing bacteria use many different mechanisms to solubilize the insoluble forms of the phosphate to soluble forms. The primary mechanism of phosphate solubilization is based on secretion of organic acids by microorganisms due to metabolism of sugars (Goswami et al. 2016).

#### 18.5.1.3 Phytohormone Production

Popular phytohormones including auxins, gibberellins, cytokinins, abscisic acid, and ethylene are commonly synthesized by soil microbes that too specifically the rhizobacters (Arshad and Frankenberger 1998; Patten and Glick 1996). Plants respond to these hormones in the rhizospheric zones that are provided either extrinsically or secreted there by indigenous microorganisms present there. These phytohormones are able to regulate several vital processes like plant cell division and enlargement and also help in extension of both symbiotic and nonsymbiotic association of roots (Glick 2014; Patten and Glick 1996).

#### 18.5.1.4 Indole-3-Acetic Acid (IAA)

IAA is particularly a type of auxin secreted by many rhizobacters. Auxin mainly regulates various phases of plant growth and development like cell division, elongation, tissue differentiation, and also helps in apical dominance. IAA secreted by rhizospheric bacteria majorly targets the root by enhancing its weight, size, branch number, and the surface area that is in direct contact with soil. These changes in turn result in increasing the root potentiality to probe the soil for exchange of nutrients, thus improving plant's nutrition pool and growth capacity (Gutierrez-Manero et al. 2001; Ramos-Solano et al. 2008). IAA also promotes differentiation of adventitious roots from stem because auxin helps in inducing redifferentiation of stem tissue to root tissue. Etesami et al. (2015) investigated that the PGPRs present in rhizoplane, endophytic, and rhizosphere zones can secrete IAA and promote growth of plants (Goswami et al. 2016).

#### 18.5.1.5 Cytokinins

Similarly to IAA, plants also respond to external application of cytokinin. It helps in increased cellular proliferation, root hair formation, root development, inhibition of root elongation, shoot initiation, and many other physiological responses (Amara et al. 2015; Arshad and Frankenberger 1998; Jha and Saraf 2015). Cytokinins are N6-substituted aminopurines which, when applied to plants, affect their development and physiology (Maheshwari et al. 2015; Salisbury and Ross 1992).

#### 18.5.1.6 Gibberellins

Gibberellins are a broad category of phytohormones that consists of 136 different molecules. It is a phytohormone that affects several developmental processes in higher plants which include stem elongation, seed germination, fruit setting, and flowering (Hedden and Phillips 2000).

### 18.5.2 Indirect Mechanics of PGPR

Indirect mechanics involved in PGPR can be summarized under the following subheadings:

#### 18.5.2.1 Siderophore Production

Siderophores are low-molecular weight compounds, usually $<1$ kDa, that contain functional groups which are able to bind iron. The most common functional groups are catechols and hydroximates because the distances in between the two available groups are optimum for binding iron. Concentration of Siderophore in soil is approximately around $10^{-30}$ M. Siderophore-secreting bacteria usually belong to the genus *Pseudomonas*, among them the most-studied organisms are *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* that release two types of siderophores, particularly, pyoverdine and pyochelin (Haas and Défago 2005). Rhizobacters secrete these molecules to enhance their competitiveness, as they have an antibiotic activity which inhibits the growth of other microorganisms and also improve iron nutrition for plants and hence helps in improvement of plant (Glick 1995). Siderophore also restricts the growth of other pathogens by limiting the iron availability, specifically fungi that cannot absorb to absorb iron in iron–siderophore complex form (Shen et al. 2013).

#### 18.5.2.2 Chitinase and Glucanase Production by PGPR

Enzymes capable of degrading cell wall include chitinase, β-1,3-glucanase, protease, and cellulose, and are mainly produced by biocontrol strains of PGPR. They pose direct inhibitory effect on the growth of hyphae of fungal pathogens by degrading their cell wall. Chitinase digest chitin which is an insoluble linear polymer of β-1, 4-*N*-acetyl-glucoseamine that is the major constituent of the cell wall of fungus. β-1,3-glucanase secreted by strains of *Streptomyces* and *Paenibacillus* spp. can easily destroy the cell walls of pathogenic fungus *F. oxysporum* (Compant et al. 2005).

#### 18.5.2.3 Antibiotic Production by PGPR

Use of microbial antagonists against plant pathogens in agricultural crops has been devised as an alternative to chemical pesticides. PGPRs belonging to *Pseudomonas* and *Bacillus* species play a vital role in the suppressing the growth of pathogenic micro-organisms by secreting antibiotics (Goswami et al. 2016).

There are several mechanisms that a plant adapts on exposure to environmental stresses, like salinity, drought, heavy metal toxicity, and nutrient deprivation. It usually includes morphological changes in root. It is a process in which plant hormones play a vital part (Potters et al. 2007). IAA is produced in the plant shoot and is transported down to the root tips (Martin and Elliott 1984). When IAA is present in low concentrations in root, it promotes to enhance cell elongation, resulting in increased root growth, but higher concentration of auxin in the root tips exerts an inhibitory effect on its growth. This inhibition may be due to direct or indirect association of auxin with synthesis of ethylene (Eliasson et al. 1989). Several researches confirmed the association of IAA and the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) (Glick 2003). Furthermore, bacteria colonizing the root zone may also promote such developments in root growth (Bowen and Rovira 1991; Timmusk et al. 2005). As shown in most studies, there are numerous reports where root-associated PGPR exert enhancing effect on plant growth that usually secretes IAA. Plants inoculated with these PGPRs showed positive effect on root growth enhancement and/or enhanced formation of roots and lateral roots which in turn provided higher tolerance level in plants in response to drought conditions. Numerous techniques and mechanisms are known to enhance plant growth by rhizobacters against drought stress and improve its tolerance, but the exact mechanism of enhancement is still unexplored. However, rhizobacter-mediated stress tolerance effects may involve following possible explanations that include: hormone production, for example, gibberellic acid, abscisic acid, auxin, and cytokinin. Enzyme production like 1-aminocyclopropane-1-carboxylate (ACC) deaminase to decrease the concentration of ethylene in the root of growing plants, promoting induced systemic resistance by bacterially derived compounds and formation of bacterial biofilm, i.e., extracellular matrix (Kim et al. 2013; Dimkpa et al. 2009; Timmusk et al. 2011). The extracellular matrix contains a diverse range of macromolecules that are helpful in plant growth and development. Biofilms contains a large number of sugars, oligo- and polysaccharides which play a vital part in bacteria–plant interactions, improvement of water retention capacity, and thus increasing water availability to the root system. Some polysaccharides can retain water by several-fold of their mass (Timmusk and Nevo 2011). It is also observed that even small polysaccharide alginate content in the biofilm can help in maintaining hydrated microenvironment, by increasing the water retention capability (Chang et al. 2007; Verma et al. 2016).

It is also studied that some microorganisms secreting an enzyme ACC-deaminase that is able to hydrolyse ACC decrease the concentration of ethylene as ACC is a precursor of ethylene and can convert to ammonia and α-ketobutyrate; therefore, it cannot have an inhibitory effect on the growth of plant (Shaharoona et al. 2006). Hence, it is quite possible that ACC-deaminase and IAA work in close association to increase root growth and elongation (Shah et al. 1998). However, this mechanism of PGPR is of great agronomic importance under abiotic stress environments, which are supposed to induce increased ethylene production. Patten and Glick in 2002 observed the biosynthesis of siderophore, ACC deaminase, and auxins by some PGPRs that are known to have a positive impact on plant development. Auxin helps in improving root growth, whereas ACC deaminase is potentially involved in decreasing the growth-inhibiting levels of ethylene (Verma et al. 2016).

ST-PGPR uses an array of mechanisms that are directly or indirectly involved in ameliorating salt stress in crops (Egamberdieva et al. 2016; Hashem et al. 2016). Researchers conclude that ST-PGPR is able to produce many phytohormones, like cytokinins, gibberellins, auxins (Dodd et al. 2010), synthesize ACC deaminase (Glick et al. 2007a, b), produce secondary compounds like exopolysaccharides (Upadhyay et al. 2012; Timmusk et al. 2014) and osmolytes (glycine betaines, trhalose, prolines) (Bano and Fatima 2009; Upadhyay and Singh 2015), which aid in regulating the defense system of plants and further activate the antioxidative enzymes of plants in high salinity conditions (Hashem et al. 2016; Egamberdieva et al. 2019).

Phytohormones secreted by ST-PGPR play an important role in modulating physiology of plant under salt-stress conditions (Egamberdieva and Kucharova 2009). The ST-PGPR produces IAA that is required for cell division and elongation in plants in order to cope against salt stress. Some common ST-PGPR producing IAA under salt stress are *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Stenotrophomonas, Pseudomonas*, and *Rahnella* (Egamberdieva et al. 2008, 2018; Piccoli et al. 2011; Abd-Allah et al. 2017). Researches show that under salt stress, yield loss in crops can be reduced with the use of phytohormone producing ST-PGPR. Under salt stress, *P. putida* modulates IAA synthesis in plant tissue and increases the growth parameters of cotton (Yao et al. 2010). It is also observed that inoculation of ST-PGPR resulted in increased uptake of minerals, protected plants from ion toxicity, and enhanced root and shoot growth under saline conditions (Egamberdieva et al. 2017). Apart from auxins, other phytohormones are also known to alleviate the effect of high salinity in plants. For example, production of cytokinins (CK) that are essential in cellular proliferation and differentiation have been reported in salt-tolerant *Bacillus*, *Halomonas*, *Arthrobacter*, *Pseudomonas,* and *Azospirillum* species (García de Salamone et al. 2001; Karadeniz et al. 2006; Naz et al. 2009; TrParray et al. 2016). ABA is also secreted by many strains of ST-PGPR, for example, *Bacillus megaterium*, *Proteus mirabilis*, *Bacillus. licheniformis*, *Achromobacter xylosoxidans,* and *Pseudomonas fluorescens* (Karadeniz et al. 2006; Forchetti et al. 2007; Salomon et al. 2014). Gibberellin secreting bacteria, for example, *Bacillus, Licheniformis*, *Bacillus pumilus,* and *Azospirillium* sp. were also reported by Bottini et al. (2004). There are literatures which have the evidence that ST-PGPR produces more than one type of phytohormones (Egamberdieva et al. 2019).

PGPR showed a significant increase in potato production as the bacteria of *Pseudomonas sp*, *Rhizobium* sp, and *Bacillus* sp in PGPR exert symbiotic association with roots of potato plants (Keerthana et al. 2018). Symbiosis resulted in increased absorption of mineral which ultimately aid in the better growth of the roots and further also increased the plant growth. The symbiosis of PGPR bacteria with the root surface of the potato plants have the ability to initiate hairy root formation (Fahad et al. 2015) and increased branching which in turn changed the regulation of the cortex cells of roots. This interaction resulted in the formation of more hairy roots (Purwantisari et al. 2019). The increased root numbers resulted in increased absorption of water as photosynthetic material in the form of sugar has the ability to reach potato tubers easily (Heidari et al. 2011). In addition, PGPR may play an important role in increasing nitrogen accumulation. Higher nitrogen incorporation led to enhanced production of enzymes and proteins for better physiological activity. The higher nitrogen level also resulted in the formation of chlorophyll, which consequently increased photosynthetic activity and production of sugars (Ibrahim et al. 2010; Purwantisari et al. 2019). Some PGPR, for example, *Azospirillum* (Sp7) potentially synthesized plant hormones that have the ability to replace indole acetic acid (IAA) to stimulate root growth in vegetables (Mohite 2013). In addition, the secretion of gibberellins, cytokines, and auxins by bacteria helped in the production of potato tubers (Fahad et al. 2015; Purwantisari et al. 2019).

## 18.6 Conclusion

PGPRs are plant growth-promoting rhizobacters that are present in the rhizospheric region of soil. They play a major role in alleviating several abiotic stresses which affect the growth, develop, and survival of plants. Potato is known to be the third most major cultivar globally. It plays a vital role in economy as well as in terms of food security. Researchers have predicted that its production will decrease due to stresses like drought, heat, and salinity. So, PGPR in this aspect could be beneficial in contributing to the economy of the country as well as in sufficing the demand of potato. PGPRs regulate these stresses by many different strategies including antibiotic, hormone production. These strategies either reduce the stress or improve the tolerance limit of the plant, thus making it easy to sustain in extreme conditions.

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# Secondary Metabolites, Boon for Plants; Their Role in Defence Mechanism and Antioxidant Activity of *Anthocephalus cadamba*

# 19

Tarubala Sharma, Vishal Khandelwal, Saurabh Gupta, and Shoorvir Singh

## Abstract

Ample information from clinical, biological, epidemiological and chemical investigations supports the antioxidant activity and defence mechanism of certain secondary metabolites, especially phenolics and flavanoids extracted from medicinal plants. Amongst these plants, *Anthocephalus cadamba*, an economically important widely distributed evergreen medicinal tree, secured its position along with therapeutic potential against several health hazards for the betterment of human civilization. Spectroscopic and chromatographic analysis revealed the presence of a wide range of phytochemicals; flavonoids, alkaloids, coumarins, terpenoids, diterpenoids, triterpenes, glycosides, sterols, amides and fatty acids in different parts of plant possessing key role in alteration of various biological activities. This book chapter discusses the antioxidant, free radical scavenging activity of various fractions of *A. cadamba* and defence mechanism of plant against plant pathogens and effects on soil microorganisms under the influence of secondary metabolites especially with phenolics and flavanoids as they alter the mechanism of reactive oxygen species and act upon structures and metabolic pathways of plant predators.

## Keywords

*Anthocephalus cadamba* · Phytochemicals · Antioxidant · Phenolics · Flavanoids

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## 19.1 Introduction

*Anthocephalus cadamba* (kadam tree) also known as Haripriya, God's favourite belonging from family Rubiaceae is a sanctified religious tree, widely distributed in tropical and subtropical regions and have therapeutic potential with their ethnomedical references (Mondal et al. 2020). This traditionally used ornamental *A. cadamba* plant possesses analgesic, antipyretic, anti-inflammatory, antidiarrhoeal, diuretic, laxative, antihepatotoxic, hypolipidemic, antioxidant, antimicrobial, wound healing, anthelmintic, anticancerous, antifilarial antimalarial, sedative, antivenom, gastroprotective, immunomodulatory (Khandelwal et al. 2018) activities along with agroforestry, nanotechnology, toxicological studies, which are newly added applications (Bijalwan et al. 2014). Traditional Indian medicinal systems Charaka Samhita, Sushruta Samhita and Ayurveda advert ethnomedical values of different parts of plant; bark in skin disease, anaemia, uterine complaints, cough, fever, inflammation; leaves in burning sensation, throat infection, inflammation in glands, rheumatism, mouth ulcer, leucorrhoea, increased menstrual flow; fruit in fever, stomatitis, gastric disturbance, lactation in women; root as blood purifier and in urinary tract infection and flowers to give strengthening to body and mind due to the presence of certain secondary metabolites; alkaloids, coumarins, terpenoids, diterpenoids, triterpenes, glycosides, sterols, flavonoids, amides and fatty acids in different parts of plant (Pandey and Negi 2016).

Besides from medicinal uses plant also shows some economical and industrial information as wood is suitable for pulp and paper production along with veneer, plywood, chopsticks, pencils, furniture, matchstick boxes and tea boxes. Ripe fruits of cadamba plants are edible and reported to have significant nutritive value with approximately 75% moisture, 18% carbohydrates, 3% proteins and 2% fat and 40.02 mg/100 gm Fe, 343.7 mg/100 gm Ca, 191.7 mg/100 gm Mg, 2.434 mg/100 gm Zn, 2.362 mg/100 gm Cr and 1.344 mg/100 Cu in edible part of fruit, hence proved it to be an excellent source of minerals in comparison to other consumable fruits (Pandey et al. 2018).

This chapter provides abundance valuable information about antioxidant properties of secondary metabolites present in *A. cadamba* and their role in resistance mechanism against certain plant pathogens.

## 19.2 Photochemistry of *A. cadamba*

Chromatographic and spectrophotometric methods have reported numerous phytoconstituents; alkaloids, coumarins, terpenoids, diterpenoids, triterpenes glycosides, sterols, flavonoids, amides and fatty acids in different part of plant which makes a volumetric impression on its medicinal values. Different parts of plant contain different phytochemicals as bark is rich in tannins, alkaloids, steroids, saponins; leaves contain a wide range of alkaloids, flavonoids, steroids and glycosides; fruits are rich in phenols, flavanoids, anthocyanins, proanthocynidines and minerals as well. Chlorogenic acid is also found in all parts of plant. These phytochemicals reported to show significant activity in In Vitro as well as In Vivo conditions.

Wide array of phytoconstituents along with their medicinal values are listed in Table 19.1.

## 19.3 Antioxidant Activity of Different Parts *of A. cadamba* Plant with Variable Fractions

Antioxidant activity is defined as the limitation of oxidation of proteins, lipids, DNA and other biological molecules that achieved by blocking the propagation stage in oxidative chain reaction. Where primary antioxidant prevents the formation of free radicals, secondary antioxidants indirectly block the formation of free radicals by Fenton's reaction (Shahidi and Zhong 2015). In biological system, free radicals found as the result of natural metabolic processes of oxygen play an important role in cell signalling and caused by exposure towards certain toxicants, radiations, pollutions, household chemicals, sun's rays (UV-A, UV-B and UV-C) and fried and barbequed foods (Synergistic paper) produced as the by-product of cellular redox reaction during the production of ATP (Tungmunnithum et al. 2018). These free radicals lead to the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) like superoxide anion (•O2), hydrogen peroxide ($H_2O_2$) and hydroxyl radical (•OH) which can trap the low density lipoproteins (LDL), damage DNA, oxidize lipids and proteins and alter the structure and functioning of cell signalling molecules resulting in evolution of several health hazards. The biological system of animals itself have some natural enzymes and chemical scavengers like certain endogenous enzymes (superoxide dismutase, glutathione peroxidase and catalase), hormones (estrogen, angiotensin) and dietary antioxidants (α-tocopherol, β-carotene, ascorbic acid, glutathione and uric acid) which can protect cells by free radical damages. Secondary metabolites of plants enhance the activity of these molecules hence plays a key role in protection of animals from such damages (Xu et al. 2017).

Several chemical-based and cellular-based assays are conducted for the assessment of antioxidant capacity of natural/botanical antioxidant products includes Scavenging Free Radicals Assaysl; Trolox equivalent antioxidant capacity (TEAC) with ABTS and DPPH; Reducing the Metal Ions Assays (FRAP and CUPRAC Assays); Folin–Ciocalteu Reagent (FCR) Assay; Oxygen Radical Absorbance Capacity (ORAC) Assay; Total Radical Trapping Antioxidant Potential (TRAP) Assay; Inhibiting the Oxidation of Low-Density Lipoprotein (LDL) Assay; Dichlorofluorecin (DCFH) method and cellular antioxidant activity (CAA) assays (Xu et al. 2017).

Plants secondary metabolites are those organic compounds which are not directly involved in metabolic processes of plant metabolism but have emphatic effect on the same and required for plants' survival against plant pathogens, pests, herbivores and have ample pharmacological importance on human health. These plant-derived chemicals are classified into major three groups: phenolics, terpenes and nitrogen

**Table 19.1** List of phytochemicals present in different parts of *A. cadamba* along with their medicinal values

| Part of plant | Phytochemical | Medicinal value | Reference |
|---|---|---|---|
| Bark | Cadambagenic acid/ phelasingenin, Saponin B, PhelasinA and Phelasin B, Glycosides A, Glycosides B, Cadambine, β- sitosterol, 3β-dihydrocadambine, 3βisodihydrocadambine, 3β-dihydrocadambine −4-oxide, 3’O-caffeoylsweroside | Antioxidant<br>Anti-diabetic<br>Antimicrobial<br>Antiinflammatory<br>Analgesic<br>Antipyretic<br>Sedative<br>Antitumor<br>Antiplasmodia | Sahu et al. (1999, 2000), Chandel et al. (2014) |
| Stem | Narigenin, *β*-sitosterol, prunetin, sakuranetin, apigenin, genkwanin | | Pandey and Negi (2016) |
| Leaves | Chlorogenic acid, β-sitosterol, dihydrocadambine, Kaemferol 3-O-glucoside, Catechin/ Epicatechin, Feruloyquinic acid, Cadambine, Dihydrocadambine, Rutin, Aminocadambine A, Aminocadambine B, Neolamarckines A Neolamarckines B, n-hexadecanoic acid, hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester | Antioxidant<br>Antidiabetic<br>Antimicrobial<br>Antiinflammatory<br>Analgesic<br>Antipyretic<br>Antiplasmodial<br>Anticancer | Liu et al. (2010), Qureshi et al. (2011), Chandel et al. (2012), Zayed et al. (2014) |
| Fruit | Indole alkaloids, saponins, triterpenes and secoiridoids | Antioxidant<br>Anti-diabetic<br>Antimicrobial<br>Antiinflammat ory<br>Antidiarrhoel<br>Anthelmintic<br>Diuretic<br>Antilipidemic | Ganjewala et al. (2013) |
| Flower (essential oil) | Linalool, Geraniol, α-Selinene, 2-Nonanol, Geranylacetate, Linalyl acetate, βPhellandrene, α-Bergamottin, p-Cymol, Terpinolene, Curcumene, Camphene and Myrcene | Economically important | Dubey et al. (2011) |
| Root | Alkaloids, flavonoids, terpenoids and saponins | Antioxidant<br>Anti-diabetic<br>Antimicrobial<br>Anthelmintic<br>Anti-lipidemic | Acharyya et al. (2010, 2011) |

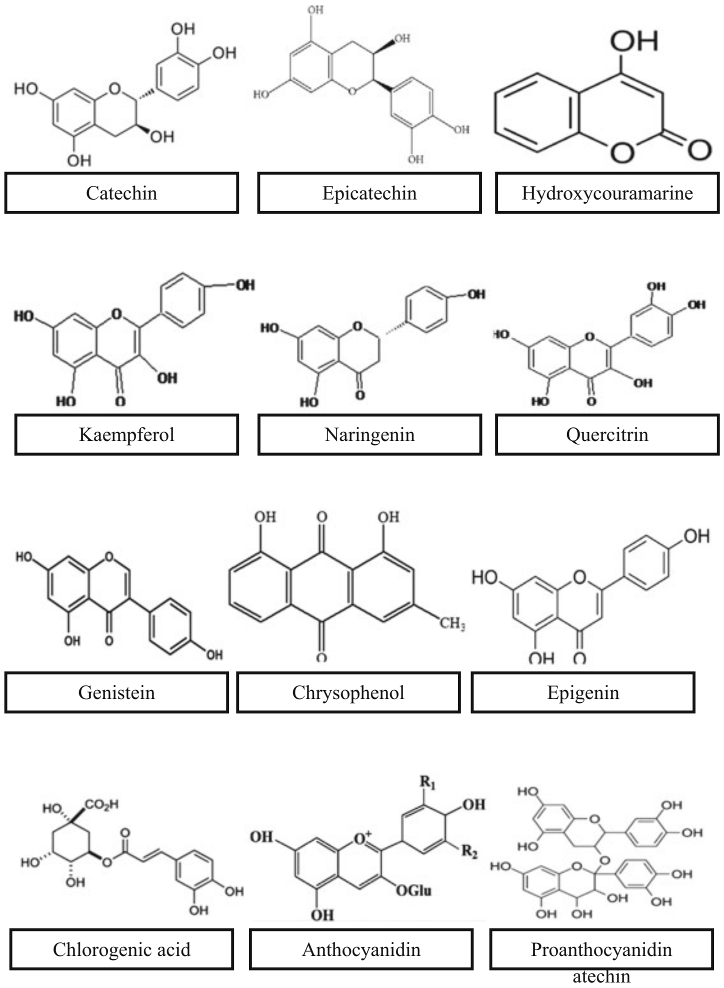
(continued)

**Table 19.1** (continued)

| Part of plant | Phytochemical | Medicinal value | Reference |
|---|---|---|---|
| Seed | Lupeol, β-sitosterol, quinovaic acid | Antimutagenic, antiinflammatory, antileishmanial, antiviral, hypotensive | Gupta et al. (1980), Dwevedi et al. (2015) |

containing compounds in which phenolic compounds (such as phenolic acids, tannins, flavanoids, lignans, stilbenes, coumarins and lignin) are the main group of secondary metabolites having significant antioxidant properties (Zeliha et al. 2018). Phytochemical investigations show ample information about the presence of phenolic components in different parts of *A. cadamba* plant dealing with several health hazards. These antioxidant activity possessing phenolic compounds contains at least one benzene ring attached with one or more hydroxyl groups and range from simple molecule to highly polymerized compound with high molecular weight polymers (Lin et al. 2016). These phenolic compounds and flavanoids defend plant itself, promote its growth under unfavourable conditions and also influence the configuration, substitution, functional group arrangement and number of hydroxyl groups (Yanti et al. 2018) (Fig. 19.1).

**Case Study 1** In a study hydroethanolic extract of *A. cadamba* flowering tops was found to decrease the level of lipid peroxidation product (thiobarbituric acid reactive substances in this case) and increase the activity of peroxidase and catalase (antioxidative enzyme) enzymes resulting in the reduction of oxidative stress in alloxan-induced mice. In hyperglycemic animals and condition of diabetes (Wolff 1993), the level of lipidperoxidation and Reactive Oxygen Species (ROS's) increases due to oxidative stress which damages the nervous tissues by impairment of free radicals in CNS, peripheral nerves, neurons and Schwann cells (Liu et al. 1996). Significant effects of *A. cadamba* extract on oxidative parameter of alloxan-induced diabetic mice are found at the dose of 200 and 400 mg/kg with decreased level of lipid peroxidation products and increase in activity of oxidative enzyme peroxidase and catalase. Phenolic compound of extract increases the activity of antioxidant enzyme of liver, kidney and brain by free-radical-scavenging activity. Extract also suppresses the formation of Nitric Oxide (NO), a well-known toxic reactive free radical of CNS, and inhibits the NO-mediate toxicity in dose-dependent manner. Thus, the results from this study establish the connection of phenolic compounds present in *A. cadamba* flowering top extract in increasing glucose metabolism, serum insulin concentration, protection of pancreas from free radical damages by significant reduction in aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatases (ALP), activities and alteration in the level of thiobarbituric acid reactive substances, peroxidase and catalase.



Catechin

Epicatechin

Hydroxycouramarine

Kaempferol

Naringenin

Quercitrin

Genistein

Chrysophenol

Epigenin

Chlorogenic acid

Anthocyanidin

Proanthocyanidin atechin

**Fig. 19.1** structure of some phenolic component of *Anthocephalus cadamba*

**Case Study 2** In this study conducted by Chandel et al. (2012) different fractions of *A. cadamba* leaves are prepared with ethanol, ethyl acetate, butanol and water. Antioxidant activity is found if these fractions are established with various In Vitro assays. Free radical scavenging activity of these plant extracts is determined by DPPH, ABTS, superoxide scavenging, DNA nicking assay and reducing power assay to confirm the free radical scavenging effect of different fractions of *A. cadamba* leaf extract. Ferric ion (Fe $3^{+}$) reduction is also used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. Although ethanolic extract of *A. cadamba* leaves performs highest antioxidant activity due to the presence of compounds, Chlorogenic acid, Catechin/ epicatechin, Rutin, Feruloyquinic acid, Kaempferol 3-Oglucoside, Glycosides of

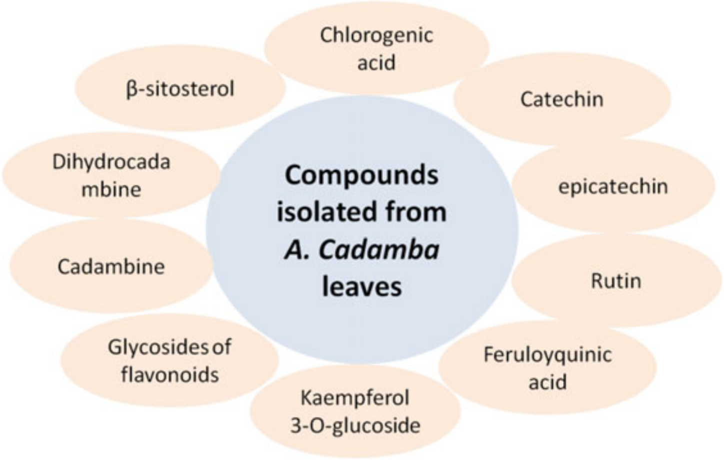


**Fig. 19.2** Identified compounds from the leaves of *A. cadamba*

flavonoids, indole alkaloids and β-sitosterol were identified by ultra-performance liquid chromatography/electrospray ionization quadrupole time of flight mass spectrometry (UPLC-ESI-QTOF). Quantitative estimation of phenolic content also confirms their presence indicating its further use as antioxidant therapeutics. Discussed fractions also show protection of pBR322 plasmid DNA from free radical scavenging (Chandel et al. 2012) (Fig. 19.2).

**Case Study 3** In this study conducted by Kaur and Kumar (2011), methanolic extract of *A. Cadamba* bark was prepared for the evolution of DNA protective activity in DNA protection assay using pBR322 plasmid. Antioxidant activity is determined by DPPH, ABTS and reducing power assays and compared with standard antioxidants like ascorbic acid and butylated hydroxytoluene. Excessive amount of reactive oxygen species (ROS) can break the single and double strands of DNA molecules; oxidize its purines and pyrimidines resulting in structural damage in DNA. Bark extract of *A. cadamba* was found to reduce ABTS radical cations to non-radical forms in a concentration-dependent manner; hence, the plant proved to have potential to protect pBR322 plasmid DNA from the damages caused by hydroxyl radicals generated in Fenton's reaction. Phytochemical analysis determines the total phenolics and flavanoid content in plant extract (Chandel et al. 2014).

**Case Study 4** Increased oxidative stress sometimes results in disorders of lipid metabolism, hyperlipidemia and hypertension. Excess amount of superoxide ions and hydroxyl radicals damages lipid and lipidproteins, especially in the condition of hyperlipidemia caused by oxidative stress modifies the low-density lipoproteins which may also result in atherosclerosis and related cardiovascular diseases (Kumar et al. 2010). Commonly used lipid-lowering drugs for hyperlipidemic disorders does not possess antioxidant property significantly and also have much toxic effects, thus the need of natural antioxidants comes in concern for the treatment of the same. In a prescribed study, root extract of *A. cadamba* plant exhibit antioxidant property by generating $O_2$ and OH free radicals in both enzymic and non-enzymic systems in In Vitro conditions. Chemical investigations of plant suggested the presence of cadambine, 3a and 3b isomers of dihydrocadambine and isodihydrocadambine in leaves and heartwood; cadambagic acid, quinovic acid and b-sitosterol in stem and chlorogenic acid from whole plant (Brown et al. 1974; Brown and Chapple 1976). These active ingredients are supposed to alter the mentioned metabolic processes.

Further, many studies are conducted in reference to antioxidant potential of *A. cadamba* plant. Synergistic effect of *A. cadamba* with 1:1 ratio of seed powder of *Azadirachta indica* and *Syzygium cumini* was followed by DPPH method for the assessment of total flavanoid and phenolic content (Kaur et al. 2016). However, significant antioxidant activity was achieved from these formulations. Khandelwal et al. (2016) also substantiate free radical scavenging activity of hot aqueous extract of *A. cadamba* leaves with DPPH method and found significant percent inhibition of free radicals by plant extract in comparison to ascorbic acid and BHT. Phytochemical investigations suggest the presence of Chlorogenic acid, Catechin/epicatechin, Kaemferol, indole alkaloids, β-sitosterol in cadamba leaves and responsible for its antioxidant activity. Ganjewala et al. (2013) exhibit antioxidant property and phytoconstitution of *A. cadamba* leaves and fruits with fractions of methanol and hexane. Antioxidant property was accessed with DPPH and riboflavin-light NBT assay systems and metal ion chelating and reducing potential determined by ferrous iron–ferrozine complex and potassium ferricyanide methods. All fractions of plant were found to exhibit significant antioxidant activity. Phenolic, flavanoid and proanthocyanine content of leaves and fruit extract are also measured and found to be in the concentration of 48.0 mgGAE/g DW, 103.3 mgQE/g DW and 106.0 mg CE/g DW in leaves and 18.0, 15.4 and 47.6 in fruit extract, respectively.

## 19.4 Resistance Mechanism of *Anthocephalus cadamba* Against Plant Pathogen (*Botryodiplodia theobromae*)

All the plants are provided with complex defence system against the attack of pests, pathogens and certain diseases. *A. cadamba* seedling are one of the preferred plant for nursery as they serve an economical importance along with its medicinal uses and used as shading trees, reforestation, plywood, pulp, paper production. The main problem regarding the nursery of this economical important plant is to combat with pathogen attack and protecting themselves from diseases as every plant is provided with some defence mechanism by synthesizing diversity of secondary metabolites which protect plant from predators and microbes according to their toxic nature and repel microbes and herbivores (Verpoorte and Alfermann 2000). Some secondary metabolites also protect plants from abiotic stress like UV radiations (Schafer and

Wink 2009), thus showing significant importance in the growth and development of plant (Rosenthal 1991). These metabolites act on either physiological activities or act upon certain part of pathogen including enzyme inhibition, DNA alkylation and reproductive system of pathogen (Morrissey and Guerinot 2009). Attack of dieback disease caused by *Botryodiplodia theobromae* is the main problem faced by cultivators during nursery of *A. cadamba* plant. Two types of defence mechanisms shown by *A. cadamba* include structural resistance and biochemical resistance mechanism. Structural resistance of white jaboon seedling can be observed by microscopic analysis of stem using Scanning Electron Microscopy (SEM); on the other hand, biochemical resistance mechanism is analysed by characterizing chemical compounds of *A. cadamba* stem using phytochemistry analysis (Yanti et al. 2018).

Pathogen responsible for dieback disease attack was found to be in the ratio of 10:3 in wounded and non-wounded stem of plant, respectively, while the severity of disease was found to be 62:12 in wounded and non-wounded stem. Yanti et al. (2018) conduct and experiment over jaboon plant in which they consider healthy stem of plant as control and testing plant induced with wounded stem infection method. Several studies conducted on different plants show that plant metabolites play key role in this combat with plant pathogen-borne diseases (Zaynab et al. 2018).

Phytochemical investigation includes alkaloid assays, flavanoid assay, Phenol hydroquinone assay, tannin assays; saponine assays, Steroids and triterpenoids assays and all are found to be positive. Stem of 5-month-old white jabon seedling of plant contained alkaloids, flavonoid, phenyl hydroquinone, tannin, saponin and steroid, while seven-month-old leaves of same plant species have only quinone and steroid compounds (Wali 2014). Variation in the amount of such metabolites depends on the type, age and part of plant. Biochemical investigations reported increase in phenolic content accumulation after pathogen attack. Tannin also acts as an antibacterial substance as it interferes with the cell permeability of pathogen, shrinking its membrane cell, inactivate enzymes, induce protein precipitation and alter the function of genetic material. Saponin, triterpenoid and steroid compounds are also increased after pathogen attack as saponin serves as antimicrobial substance (Astawan and Kasih 2008). However, alkaloid and phenol hydroquinone level decreased at the same time because they serve as enzyme inhibitor and antioxidant along with antimicrobial activity. Alkaloid acts on peptidoglycane of bacterial cell and inhibits the formation of bacterial cell wall viz. leads to death of the cell (Lamothe 2009). Biochemical resistances of white jabon seedling both before and after pathogen attack were found as secondary metabolites such as alkaloids, flavonoids, phenol hydroquinone, tannin, saponin and steroids, and the increase of phenolic compounds, such as flavonoids and tannin, and the increase of saponin, triterpenoids and steroids compounds (Yanti et al. 2018).

## 19.5 Conclusion and Future Prospects

Flavanoids and phenolic compound abundantly distributed in the same species of *Anthocephalus cadamba* plant can serve as interesting choice for development of molecules for drug and medical products. Different amount of secondary metabolites can be achieved by different cultivators; thus, cultivars should be considered for further medical and pharmaceutical research purposes. Geographical area, environmental conditions, nutrient, minerals and microfauna of soil should be analysed to gain potent biological activities of these secondary metabolites. Although many molecular mechanism and signalling pathways of such compounds still need to be understood and hence can be applied further to enhance the knowledge of its economic and industrial importance and for drug development processes.

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# Role of PGPR in Conferring Drought Stress Tolerance in Rice

# 20

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**Abstract**

Rice is one of the widely popular staple foods across the globe. Abiotic stress is known for its devastating effect on crop plants. Drought amid the other abiotic factors has received constant attention as it causes loss in productivity and thus disrupts the efforts toward food security. Drought stress causes havoc in rice productivity as it harms the rice plants at all the developmental stages and the reproductive stages. Concurrently, the effective measures to reduce the loss in yield and development in rice due to drought conditions mostly consist of long-term research efforts. Therefore, it has become an utmost necessity to search for convenient alternatives that are efficient, less time-consuming, cost-effective, and environment friendly. Thus, attention has been paid to Plant growth-promoting rhizobacteria (PGPRs), which stands out as an excellent measure to reduce the stress posed by drought. PGPR could be used for increasing the tolerance mechanisms in drought-affected rice crops and could be established as an excellent choice of the mitigation strategy. We aim to summarize the research efforts of this field in this chapter.

**Keywords**

Drought · Sustainable agriculture · PGPR · Abiotic stress · Stress tolerance · Beneficial microbes

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## 20.1 Introduction

### 20.1.1 Current Agriculture Scenario

Global food security is adversely affected due to loss in crop productivity worldwide. It has been projected that the global population will be increased up to ten billion or more in the next 50 years (Etesami and Maheshwari 2018). In 2015, the Food and Agricultural Organization estimated that there will be around 35% loss of crop productivity globally by 2030 due to environmental disparities. Abiotic factors play a vital role in plant growth and development. The rapid changes in global climatic conditions lead to a drastic reduction in crop productivity. Abiotic stresses like drought, salinity, extreme temperatures, and flood are the major cause of inadequate crop productivity. Being sessile, plants undergo both biotic and abiotic stress conditions throughout their life cycle. Both biotic and abiotic factors play a major role in the growth and development of the plant. Due to human activity, concentration of the greenhouse gases in the atmosphere has increased to the very high extent, resulting in an increase in the earth's temperature. The global warming across the world has caused huge discrepancies in the environmental factors, leading to constant wet or dry condition in some part of the earth, whereas some part of the earth becomes extreme cold or hot. Thus, several adverse conditions like drought, salinity, waterlogging, osmotic stress, oxidative stress, heat, and cold arise. However, around 70% of the global loss in crop productivity occurs due to abiotic stress effects (Lohani et al. 2020). Abiotic stress conditions include heat, cold, drought, salinity, waterlogging, heavy metal toxicity, nutrient deficiency, and oxidative stresses (Choudhury et al. 2017; Jahan et al. 2019). The major abiotic conditions are the drought, salt, and temperature stress, which show a high impact on crop yield across the globe (Krasensky and Jonak 2012). However, the susceptibility to the stress conditions varies depending on the geographical distribution. A lot of physiological and metabolic changes occur in plants on exposure to abiotic stress conditions. Loss in biomass, low photosynthetic rate, alteration in respiration, and transpiration are reported to be the consequences of abiotic stress in the plant (Korkmaz and Dufault 2001). The global crop loss caused by drought stress is recently summed up to 30 billion (Gupta et al. 2020). The water deficit condition causes intracellular damage in plants that affect stomatal opening, respiration, photosynthesis rate, and ATP production, resulting in poor yield and development. Therefore, the crop yield enhancement under drought conditions has become the most difficult challenge to sustain crop productivity.

To survive under unfavorable conditions, plants acclimatize themselves through various abiotic stresses, which requires appropriate signaling. The stress-responsive signaling is mediated through both ABA-dependent and ABA-independent pathway (Fig. 20.1). Scientists have reported that the basic regulation of stress signaling is driven by numerous genes and transcription factors. There are several success stories of identifying genes and TFs involved in stress response (Vashisht and Tuteja 2006; Donoghue et al. 2011; Bihmidine et al. 2013; Nath et al. 2015). A generic signal transduction pathway starts with signal perception, followed by the generation of

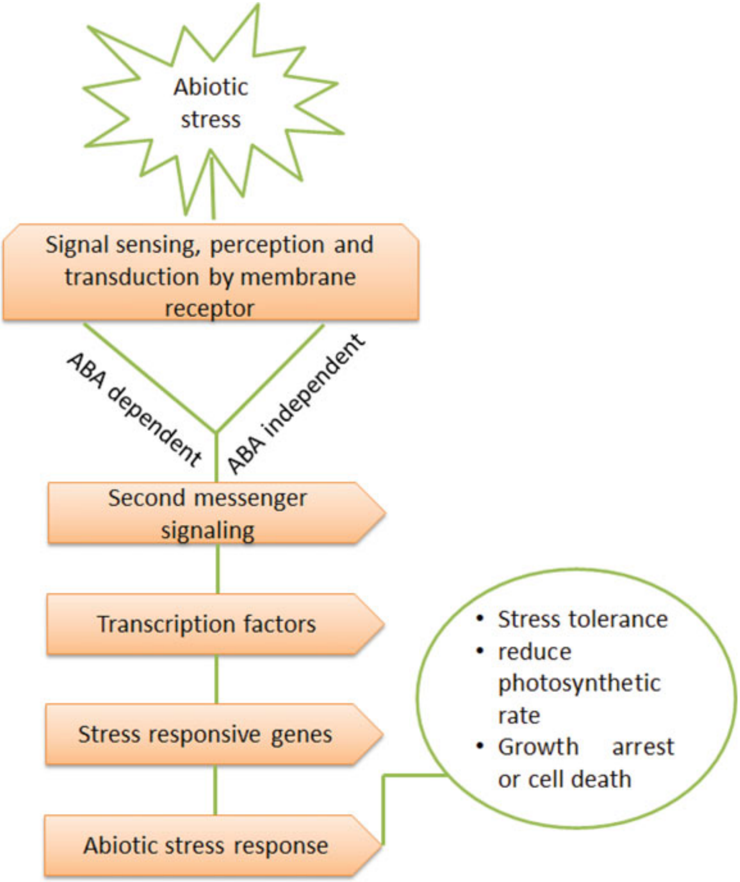

**Fig. 20.1** Schematic presentation of abiotic stress signaling in plants

second messengers (e.g., inositol phosphates and reactive oxygen species [ROS]) (Xiong et al. 2002). A cascade of protein phosphorylation is carried out by second messengers that can modulate intracellular $Ca^{2+}$ levels. This results in the target proteins that are involved in cellular protection or transcription factors controlling specific sets of stress-regulated genes. The products of these genes may participate in the generation of regulatory molecules like the plant hormones, abscisic acid (ABA), ethylene, and salicylic acid (SA). Thus, these regulatory molecules may initiate a second round of signaling that follows the same pattern with the involvement of several other components. The progress in biotechnology and high throughput sequencing have brought immense opportunities to reveal the important genes that are involved in stress response and to unveil the appropriate stress tolerance machinery in plants. But there is a lot more to explore to develop multiple stress-tolerant crops to sustain crop productivity.

Along with the genes and TFs, several microbes are also identified as beneficial organisms to promote the growth and development of the plant. Such microorganisms coordinate to protect the plant from different stress conditions with the regulation of several genes and transcription factors. The soil has a vast range of microbial populations. Depending on the soil's nature like pH and salinity, the microbial community varies. Among these bacteria, some may play a negative or positive role in plant defense mechanisms. Therefore, such microbial communities are categorized as harmful, neutral, and beneficial microbes as per research studies outcome. Plant growth-promoting (PGP) factors are secreted within the plant rhizosphere by several beneficiary bacteria, which are named Plant growth-promoting rhizobacteria (PGPRs) (Bhattacharyya and Jha 2012). PGPR is recently known to be involved in plant protection against both biotic and abiotic stress conditions (Etesami and Maheshwari 2018). To enhance the plant growth by getting rid of the biotic stress like pathogen attack, a large variety of chemical fertilizers and pesticides are widely used in agriculture. However, toxicity caused by synthetic chemical fertilizers and pesticides has become another daunting challenge in developing sustainable crops. No definite scientific solutions yet have arisen to defeat the challenges in developing multistress tolerant crops to sustain food security. Plant growth-promoting bacteria (PGPRs) are considered as one of the most useful microbial communities in the case of crop improvement. The research contribution in PGPR showed its involvement in regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. Therefore, it is also interesting to discover if these PGPRs play any major role in regulating tolerance against abiotic stress in plants. However, the gap between the mechanism of PGPR as biofertilizer and its mode of action in abiotic stress tolerance is still unclear. But, an insightful revelation of PGPR's role in abiotic stress tolerance mechanism in plants would help to translate the research strategy to develop a high yield-oriented multiple stress tolerance crop in the future. In this book chapter, we are going to discuss the role of PGPR in conferring abiotic stress tolerance in plants. Since rice is a staple food all over Asia and India and drought stress is a major critical condition for the plants, we have discussed here the mechanism of PGPR in enhancing drought stress tolerance in rice crops.

## 20.2 Importance of Drought Stress Study

Among all abiotic stress conditions, drought stress has been reported to affect the crop productivity most (Rezayian et al. 2018). As per recent reports, 40% of the global land area is affected by drought (Lohani et al. 2020). Drought stress is mainly caused due to low rainfall, salinity, high and low temperatures, and high intensity of light exposure (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Therefore, drought stress is considered the most common abiotic stress condition that occurs due to various other unfavorable conditions. Optimal water availability is a vital requirement for a plant to grow efficiently. Either water deficit like drought or excess water like flooding or waterlogging has a drastic effect on plant productivity and development. Drought stress is the major abiotic stress that causes an imbalance in intracellular water content. Water deficit conditions occur in almost all areas, especially arid and semiarid regions, and its characteristics may be very different from one region to another. The consequences of drought stress include slow growth rate, impaired photosynthesis, and ion homeostasis, decreased seed germination rate, enhanced production of reactive oxygen species, and eventually, reduced yields (Salehi-Lisar and Bakhshayeshan-Agdam 2016). The negative impact on plant growth quantity and quality depends on the duration and severity of drought stress including the nature and developmental stage of the plant variety (Farooq et al. 2009). Severe water deficit conditions could be lethal to plants as it affects the seed dormancy, flowering fruiting, photosynthetic rate, and all the intracellular metabolic events. Drought stress for short period can enhance the tolerance in plants against drought conditions (Wojtyla et al. 2020). The physiological changes in the plants under drought stress conditions include a higher transpiration rate from leaf surfaces than the water uptake by roots. When the water potential of the soil is lower than the water potential of plant roots, an imbalance in water uptake and water losses occurs in plants (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Severe water deficit condition leads to internal damage of plants rupturing cellular membrane and interrupt enzyme activity. Drought stress also induces a reduction of leaf size, stem elongation, and root proliferation. The impaired water balance inside the plants causes stomatal closure, which affects $CO_2$ assimilation by leaves. Thus, drought stress disturbs nitrogen fixation, respiration, and ATP synthesis and generates a lot of reactive oxygen species. The phytohormones like salicylic acid, auxin, gibberellin, cytokinin, and abscisic acid participate in the regulation of drought stress response. Cell dehydration plants due to drought stress cause osmotic stress. Disruption in osmotic pressure causes a reduction in turgor pressure affecting the chlorophyll content and other essential physiological factors such as the relative water content (RWC), relative electrical conductivity in leaves, stomatal conductance (gs), leaf water potential ($\psi$w), transpiration rates, and malondialdehyde (MDA) content in leaves (Murchie and Lawson 2013; Kaushal and Wani 2016). Abnormal stomatal closure leads to a reduction in photosynthesis and an increase in photorespiration rate. This results in an imbalance of the fixed and utilized carbon in plants as stomatal closure reduces the $CO_2$ availability for photosynthesis. The consequences also include interruption in ATP synthesis and mitochondrial electron transport system. Such hindrance in metabolic activities causes overproduction of reactive oxygen species like superoxide ($O_2-$) and hydrogen peroxide ($H_2O_2$) in different cellular organelles including mitochondria, chloroplasts, and peroxisomes (Noctor et al. 2014; Asada 2020). Therefore, drought stress has been considered as the most important abiotic stress condition affecting the development of major crops as its consequences enhance several other stress conditions like osmotic stress and oxidative stress (Apel and Hirt 2004). Several Low-molecular weight osmolytes, including glycinebetaine, proline, and other amino acids, organic acids, and polyols play a very critical role in sustaining cellular functions under drought stress. Research strategies can be designed to utilize such important osmoprotectants, osmolytes, transcription factors, and phytohormones to develop drought-tolerant crops. The moisture content in soil due to water deficit condition also affects the growth of the microbial communities on the soil. However, many bacterial communities survive with efficient growth in the extreme environments and help to overcome the stress condition. A large variety of microbial populations reside in soil that help in plant growth and development directly or indirectly. Plant growth-promoting rhizobacteria (PGPRs) are one of the most useful bacterial colonies that play an essential role in plant growth and development. A lot of research outcomes showed the involvement of PGPRs in the abiotic stress tolerance mechanisms. The research progress in advanced biotechnological approaches has identified several beneficial bacterial populations, which could be exploited for drought-tolerant crop development.

## 20.3 What Are PGPRs and Their Role in Conferring Stress Tolerance

The soil bacteria that enhance the soil fertility and plant growth are very much essential for the biogeochemical cycle, and such bacteria have been utilized for improvement of crop productivity. Soil bacteria that are beneficial for plant growth are known as PGPRs. Kloepper and Schroth first defined the Plant growth-promoting rhizobacteria (PGPRs) as the soil bacteria that colonize the roots of plants following inoculation onto the seed and that enhance plant growth (Aziz et al. 2012; Kloepper 1993). Kloepper (1993) first proposed the term ‘PGPR’ that was used for a long time, especially for fluorescent *Pseudomonas* involved in the pathogens biological control and enhancing plant growth. In 1981, Kapulnik (1991) extended this term to the rhizobacteria capable of directly promoting plant growth. Nowadays, all bacteria living in the rhizosphere and that are involved in plant growth through one or more mechanisms are referred to as PGPR (Haghighi et al. 2011). PGPR colonizes the rhizosphere of the plants and help the plant in utilizing nutrition, nitrogen, and minerals for efficient growth through crucial plant-microbe interaction. PGPR during colonization multiplies in the seed surrounding region, root exudates, and production of pili occurs to attach to the root surface and to colonize the developing root system (Bloemberg and Lugtenberg 2001; Benizri et al. 2001). Plant Growth Promoting Rhizobacteria (PGPRs) are considered as a group of bacteria that can enhance plant growth and yield via various plant growth-promoting substances and biofertilizers (Singh 2013). In Fig. 20.2, the role of PGPR in plant growth and development conferring abiotic stress tolerance in plants has been shown. PGPRs are classified based on their different functional activities and mode of action. PGPRs are primarily can be categorized into extracellular PGPR and intracellular PGPR. Extracellular PGPR includes bacterial genera such as *Agrobacterium, Arthrobacter, Bacillus*, *Caulobacter, Erwinia*, *Micrococcus*, *Pseudomonas*, and *Serratia*, which may involve in activities in the rhizosphere, rhizoplane, or the spaces of cells of the root cortex (Gray and Smith 2005). The **intracellular PGPR includes** *Allorhizobium, Bradyrhizobium*, *Mesorhizobium,* and *Frankia*, *which may exist* inside the specialized nodular structure of root cells

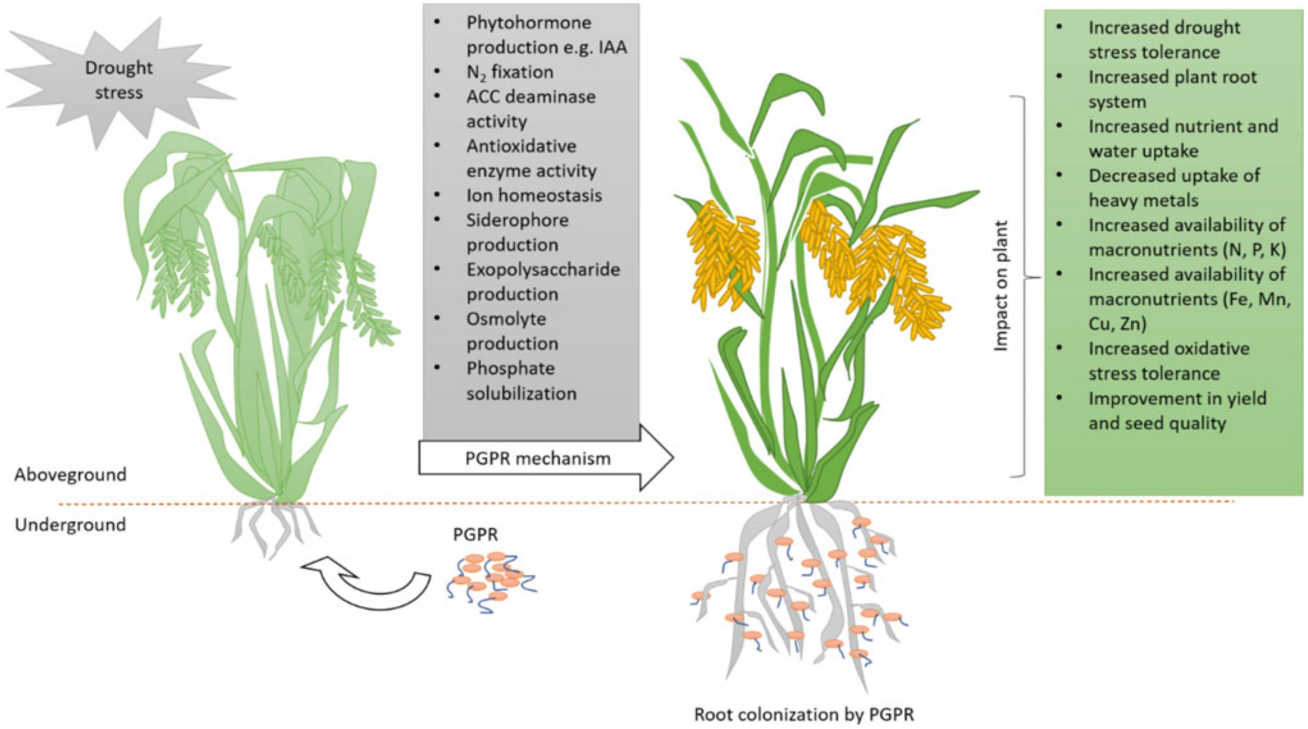

**Fig. 20.2** Role of PGPR in plant growth and development conferring drought stress tolerance in plant

(Verma 2001). PGPR improves the quality and yield of the crops by improving their growth in various ways, which indicates its beneficial qualities to be utilized robustly. Based on different modes of action, PGPRs can be utilized as biofertilizers, biostimulator, rhizomediator, and biopesticides. Looking into the demerits of artificial fertilizers due to their increasing cost and harmful effect on crops, natural beneficial soil microorganisms such as PGPR have gained more popularity as biofertilizer for safe and sustainable agricultural products. PGPR is involved in a wide range of cellular and metabolic machineries like phosphate solubilization, siderophore production, biological nitrogen fixation, rhizosphere engineering, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing (QS) signal interference and inhibition of biofilm formation, phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses, interference with pathogen toxin production, etc. (Bhattacharyya and Jha 2012). Such events help in the plant growth enhancement. Nitrogen fixation in crop plants is a major activity performed by PGPR. In the symbiotic nitrogen-fixation process, bacteria of the Rhizobiaceae are involved. Rhizobiaceae family mainly includes six genera, which are Rhizobium, Sinorhizobium, Mesorhizobium, Allorhizobium, Azorhizobium, and Bradyrhizobium (Okazaki et al. 2004). All of these are collectively referred to as rhizobia, and the differentiated forms of rhizobia in the nodule are called bacteroids. These rhizobia infect the roots of legume plants and produce nodules. In this process, the atmospheric dinitrogen ($N_2$) is converted into ammonia ($NH_3$) by rhizobacteria, which are effectively utilized by host legume plants. The Plant growth-promoting substances produced by PGPR improve plant growth and yield. However, the diversity of PGPR in the rhizosphere along with their colonization ability and mechanism of action has increased the demand of PGPR for various applications in agricultural improvement aspects. The mode of action of PGPR varies depending on the pathways or cellular activities that PGPR is involved with. Several recently known modes of action of PGPR in growth and development of plants include (1) bioremediation of the soils contaminated with heavy metals by sequestering toxic heavy metal species and degrading xenobiotic compounds and improved soil structure (by bacterial exopolysaccharides) (Burd et al. 2000); (2) synthesized enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase, an enzyme involved in decreasing the level of stress-induced ethylene in the root of developing plants (Glick 2005); (3) providing $N_2$ for plant through biological nitrogen fixation; (4) production of siderophores; (5) the generation of phytohormones (e.g., ABA (abscisic acid), GA (gibberellic acid), auxin, i.e., indole-3-acetic acid (IAA), and CK (cytokinins); (6) the control of plant pathogens by different mechanisms like generation of extracellular enzymes hydrolyzing the fungal cell wall, competition for nutrients (niches) within the rhizosphere, induction of systemic resistance (ISR), and the production of antibiotic and siderophores (Compant et al. 2021); (7) solubilization and mineralization of nutrients, particularly mineral phosphate; and (8) improvement of nonbiological stress resistance (Glick 2014; Hayat et al. 2010). There is a lot of research available on PGPR playing role in the growth and development of plant (Glick 2012; Hayat et al. 2010). PGPR enhances growth and development of plant through the synthesis of essential molecules and chemicals, uptake of nutrients from the soil, and lowering or prevention of diseases. PGPR plays a critical role in protecting the plants from phytopathogen or diseases. Rhizobacteria produce certain siderophores, i.e., small metal-binding molecules to protect the soil fertility. Some rhizobacteria also produce certain secondary molecules like hydrogen cyanide (HCN) and/or fungal cell wall degrading enzymes, e.g., chitinase and β-1,3-glucanase, to prevent the phytopathogens, and production of antibiotics also inhibits harmful microbial growth in the plant and in soil (biochemistry and 1986). Synthesis of phytohormones including auxin, cytokinin, gibberellin, ethylene, and abscisic acid by PGPR has a great impact on plant growth and development. Auxin compounds like Indole-3-ethanol or indole-3-acetic acid (IAA) are also secreted by several PGPRs. It has also been reported that some PGPR functions as a sink for 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene in higher plants, by hydrolyzing it into α-ketobutyrate and ammonia. This phenomenon helps to promote root growth by lowering indigenous ethylene levels in the rhizosphere. In addition to that, PGPR also solubilizes mineral phosphates, organic nitrogen, and other nutrients into the rhizosphere region, which enhances the soil structure, organic matter content, and stress resistance in the plants (Hayat et al. 2010).

However, the findings of PGPRs revealing their role in abiotic stress management are a breakthrough. Several recent reports showed that the PGPRs enable agricultural plants to maintain productivity under different stressed conditions in multifarious ways. However, the role of these beneficial bacteria in the management of abiotic stresses has been acquiring importance in recent years. There are numerous free-living, symbiotic, or nonsymbiotic bacteria that are involved in various metabolic activities in plants, which are considered as PGPRs. Apart from nitrogen fixation, phytohormone production, and nutrition uptake, and solubilization, PGPRs are involved with many defensive mechanisms that protect plants from diseases under biotic and abiotic stress conditions. Symbiotic cyanobacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium*, and *Mesorhizobium are the PGPR that helps in nitrogen fixation in plants* (Hayat et al. 2010). Similarly, *Azospirillum*, *Enterobacter*, *Klebsiella*, and *Pseudomonas* species bacteria are the free-living bacteria that have been reported to attach to the root and efficiently colonize root surfaces. It has been reported that PGPRs are involved in salt stress tolerance. Recently, it was shown that rhizobacteria improve the root and shoot growth, chlorophyll content, nutrient uptake, and resistance to diseases. Rhizobacteria confer stress tolerance through biocontrol of phytopathogens in the rhizosphere and by the production of phytohormones and 1-aminocyclopropane-1-carboxylate deaminase, favoring osmolyte accumulation in plants (Okazaki et al. 2004). Higher $K^+/Na^+$ ratio due to plant rhizobacteria interaction confers salt stress tolerance to the plant (Paul and Lade 2014). Many PGPR genera including Pseudomonas, Bacillus, Flavobacterium, Azospirillum, Chryseobacterium, Achromobacter, Sinorhizobium, Bradyrhizobium, Aeromonas, Acetobacter, etc. are reported as bacteria that help in retaining the yield of different crop plants cultivated in salt-affected soils (Hamdia et al. 2004; Dimkpa et al. 2009). PGPRs are recently reported to be involved in drought stress resistance through various physiological and biochemical changes induced by rhizobacteria (Kaushal and Wani 2016). PGPRs induce the production of several metabolic compounds like sorbitol, polyols, mannitol, sucrose, fructan, proline, and ectoine that are associated with plant defense as osmoprotectant against abiotic stresses (Augé 2001). Under different abiotic and abiotic stress conditions, PGPR induces synthesis of small molecules such as carotenoids, ascorbic acid, tocopherols, and anthocyanins that protect plants from oxidative injury and helps in elimination of stress-induced ROS (Piccoli and Bottini 2013). The abiotic stress tolerance in a plant is a network of complex signaling pathways where numerous cell molecules, enzymes, transcription factors, hormones, and metabolites are involved. PGPRs are also involved in the initiation of defense mechanism like phenylpropanoid pathways and lignin biosynthesis by inducing the production of certain plant molecules such phytoalexins, salicylic acid, jasmonic acid, methyl salicylate, and methyl jasmonate that are formed under stress (Naseem et al. 2018). Such molecules act as signaling molecules that trigger a cascade of the stress signaling pathways. Different modes of action of PGPR under drought stress and its effect on plant are mentioned in Table 20.1.

PGPR interacts with the roots through root colonization. PGPR confers different abiotic stress tolerance including drought and oxidative stress tolerance by improving the plant growth via different modes of action.

## 20.4 Growth Stages of Rice Sensitive to Drought

The extent of the harmful effects of drought stress upon crop plants depends on multiple factors like genotype, growth stage, plant species, and also severity and duration of the stressed period. Drought generally hampers plants in almost every growth phase and severely affects the yield by altering multiple physiological and biochemical factors (Gall et al. 2015; Tripathy et al. 2000). The most sensitive growth stages in rice plants are identified as the flowering stages, mid tillering, and panicle initiation (Wopereis et al. 1996). There are three types of drought stresses that commonly affect rice production, viz., early water stress, mild sporadic stress, and late stress affecting the plant stages accordingly (Fukai and Cooper 1995). There are enough reports to state the importance of the roots in devising the avoidance strategy against such stress conditions as roots are involved in the water and nutrient absorption from soil. The targeted assimilation in response to drought stress too gets relocated to roots (Yoshida and Hasegawa 1982; Kim et al. 2020).

## 20.5 Role of PGPR in Drought Tolerance

Drought stress is a major abiotic constraint in the growth and development of plants. Plant growth-promoting rhizobacteria (PGPRs) play a very critical role in the alleviation of drought stress in plants. PGPRs confer drought tolerance by producing exopolysaccharides (EPS), phytohormones, 1-aminocyclopropane-1-carboxylate

**Table 20.1** Role of PGPR in amelioration of drought stress in plants through different mechanisms

| Sl. No. | PGPRs | Crop | Mechanism | Impact on plants | References |
|---|---|---|---|---|---|
| 1 | *Azotobacter chroococcum* strains | Tomato (*Solanum lycopersicum*) | | Increased nutrient availability to drought stressed tomato plants and enhanced water retention | Viscardi et al. (2016) |
| 2 | *Bacillus* spp. strains | Sorghum (*Sorghum bicolor*) | | Enhanced plant growth and biomass with dark greenish leaves due to high chlorophyll content and improved leaf relative water content and soil moisture content | Grover et al. (2014)) |
| 3 | *Azospirillum lipoferum* | Maize | Inducing gibberellins production | Increased gibberellins and ABA levels | Cohen et al. (2009) |
| 4 | *Phyllobacterium brassicacearum* STM 196 | *Arabidopsis thaliana* | Inducing ABA production | Reduced leaf transpiration due to increase level of ABA | Azanesh et al. (2011) |
| 5 | *Bacillus subtilis* IB-21 | Wheat | Inducing cytokinin production | Stimulate rhizodeposition | Kudoyarova et al. (2014) |
| 6 | *Bacillus thuringiensis* AZP2 and *Paenibacillus polymyxa* B | Wheat (Triticumaestivum) | EPS production, high phosphate solubilizing efficiency, and ACC deaminase activity | Improved crop growth and biomass | Timmusk et al. (2014) |
| 7 | Pseudomonas putida strain GAP-P45 | Maize (*Zea mays*) | Exopolysaccharide production | Enhanced plant biomass, relative water content, leaf water potential, and root length | Sandhya et al. (2010) |
| 8 | *Bacillus subtilis* | *Platycladus orientalis* | Inducing cytokinin production | Stomatal conductance | Liu et al. (2013) |
| 9 | *Bacillus* sp. | Potato (*Solanum tuberosum*) | ACC deaminase activity, siderophore production, and phosphate solubilization | Increased photosynthetic efficiency of inoculated plants and expression levels of ROS-scavenging enzymes | (Gururani et al. (2013) |

(continued)

**Table 20.1** (continued)

| Sl. No. | PGPRs | Crop | Mechanism | Impact on plants | References |
|---|---|---|---|---|---|
| 10 | Ochrobactrum pseudogrignonense RJ12, *Pseudomonas* sp. RJ15 and *Bacillus subtilis* RJ46 | Black gram (*Vigna mungo* L.) and garden pea (*Pisum sativum* L.) | Synthesis of siderophore, ACC deaminase activity, indole-3-acetic acid production, and phosphate solubilization | Increased seed germination percentage, root length, shoot length, dry weight of treated plants, and decreased ACC accumulation | Saikia et al. (2018) |
| 11 | Pseudomonas putida strain GAP-P45 | Sunflower (*Helianthus annuus* L.) | Exopolysaccharide production. | Reduced drought stress and increased plant biomass. | Sandhya et al. (2010) |
| 12 | Bulkhorderia cepacia | Pepper (*Capsicum annuum*) | ACC deaminase activity | Increased plant biomass and chlorophyll, a content under drought stress. | Maxton et al. (2018) |
| 13 | *Pseudomonas fluorescens* DR7 | Foxtail millet (*Setaria italica* L.) | Exopolysaccharide (EPS) production and ACC deaminase activity. | Improved seed germination and seedling growth. | Danish et al. (2019) |
| 14 | *Leclercia adecarboxylata* and *A. fabrum* | Wheat | ACC deaminase | Elevated nutrients uptake and high chlorophyll contents | Danish et al. (2019) |
| 15 | *Consortia of P. jessenii, P. synxantha*, and *A. nitroguajacolicus* | *Oryza sativa* | Osmolyte production | Improved plant growth because of proline accumulation | Gusain et al. (2015) |
| 16 | *Azospirillum* spp. *AZ39* and *AZ19* | Maize | Osmolyte production | Increased proline | García et al. (2017) |
| 17 | *Pseudomonas putida*, *Bacillus megaterium* | *Trifolium repens* | IAA production | Increased shoot and root mass | Marulanda et al. (2009) |

(ACC) deaminase, and volatile compounds, inducing accumulation of osmolytes, antioxidants, upregulation or downregulation of stress-responsive genes, and alteration in root morphology (Vurukonda et al. 2016). The useful microorganisms colonize in the (Okazaki et al. 2004) rhizosphere/endorhizosphere of plants and induce physical and chemical changes in the acquisition of drought stress condition. Several secondary metabolite productions are induced such as phenolic, flavonoid, essential oil contents, and soluble sugar, and radical scavenging activity is enhanced due to water deficit condition. The phytohormones including IAA, gibberellins, ethylene, abscisic acid, and cytokinin also take an active part in drought stress tolerance (Ullah et al. 2018). PGPRs induce the production of such phytohormones in response to water deficit conditions. When plants encounter stress condition, the stress-induced signals are sensed by the receptor molecules and then stress signal transduction occurs through a cascade of signaling molecule activation. As a result, the stress response is mediated to escape or tolerate the unfavorable condition. The phytohormones are also responsive to different stress conditions. Different phytohormones act differently on the plant changing its physiological and morphological structure or nature. *Azospirillum* induces IAA production in the plant under water deficit condition and enhances plant's tolerance to drought stress (Dimkpa et al. 2009). IAA actively participates in the growth and development of plants enhancing the root growth, and formation of lateral roots, and root hairs. Similarly, *A. brasilense* Cd also has a great impact on root morphology development by inducing the production of phytohormones especially auxin. This results in efficient water and nutrition uptake conferring drought tolerance in plants. It has been reported that Enterobacter ludwigii and Flavobacterium sp. promote the growth of wheat plants under drought stress (Gontia-Mishra et al. 2016). Recent findings showed that *Enterobacter* sp. and *Bacillus* sp. (12D6) increased branching in wheat (*Triticum aestivum*). *Bacillus* sp. has been observed to be involved in root elongation (Jochum et al. 2019). In maize (*Zea mays*), both *Enterobacter* sp. and *Bacillus* sp. are found to be involved in increased root length, root surface area, and the number of tips. Thus, PGPRs impart drought tolerance in the plant by enhancing the production of stress signaling molecules and osmolytes like ABA and proline. Higher grain yield, higher mineral quality (Mg, K, and Ca), with improved relative and absolute water content, water potential, apoplastic water fraction, and lower volumetric cell wall elasticity were observed after inoculation of *Azospirillum brasilense* Sp245 in wheat (*Triticum aestivum*) under crucial drought stress (Creus et al. 2004). Similarly, *Azospirillum lipoferum* confers drought tolerance through increased production of ABA and gibberellins in maize plants (Cohen et al. 2009). ACC deaminase producing bacteria also help to recover drought stress by increasing seed yield, seed number, and seed nitrogen accumulation and restoring nodulation (Dodd et al. 2005). *A few PGPRs including Pseudomonas fluorescens*, *Enterobacter hormaechei*, and *Pseudomonas migulae* are responsible for promoting seedling germination in foxtail millet under drought condition by producing ACC and EPS (Niu et al. 2018). Under drought conditions, accumulation of reactive oxygen species (ROS) takes place due to cell membrane damage and lipid peroxidation. Several PGPRs reduce ROS production that protects against oxidative stress in plants. It has been reported that *Pseudomonas* sp., *Bacillus lentus*, and *A. brasilense* consortium induce the high activity of glutathione peroxidase and ascorbate peroxidase in *Ocimum basilicum* L. (Heidari and Golpayegani 2012). Similarly, catalase activity in Basil plants (*Ocimum basilicum* L.) was reported to be improved by *Pseudomonas* sp. Several *Pseudomonas* spp. including *P. entomophila*, *P. stutzeri*, *P. putida*, *P. syringae*, and *P. montelli* are involved in the reduction of antioxidant enzymes significantly in maize under water deficit conditions (Sandhya et al. 2010). Similarly, *Bacillus* species are also found to be involved in lowering antioxidant enzymes APX and glutathione peroxidase (GPX) to confer drought tolerance (Vardharajula et al. 2011). The consortium of PGPR containing *P. jessenii* R62, *P. synxantha* R81, and *A. nitroguajacolicus* strainYB3 and YB5 is reported to improve plant growth along with inducing superoxide dismutase, catalase (CAT), peroxidase (PX), and ascorbate peroxidase (APX) and lowering $H_2O_2$ and malondialdehyde (MDA) in Sahbhagi (drought tolerance) and IR-64 (drought-sensitive) rice crop (Gusain et al. 2015). Such research endeavors have enlightened to utilize such beneficial microorganisms to improve crop productivity under critical drought conditions.

## 20.6 Decoding the Mechanism of Action of PGPR on Drought Stress Tolerance in Rice

Drought condition is considered to be one of the devastating environmental stresses for plants as it has always been a challenge for crop yield depending on the intensity the crop is exposed to moderate or intense or short or prolonged drought stress (Lamaoui et al. 2018). The interaction of microbes with plants in normal or stressful conditions displays diverse molecular, biochemical, and physiological roles. This interaction with the root system of plants includes the most complicated biological phenomenon that helps plant activate their adaptive abilities against drought stress through induced defense mechanisms (Armada et al. 2014).

Rice is a crop of tropical and subtropical origin and is usually sensitive to drought conditions (Zu et al. 2017). One of the major factors that affect rice production in different parts of the world is the water deficit that poses serious threat to crop productivity. Microbial communities dominate the plant rhizosphere (the area surrounding the roots) of many crop plants including rice (Lu et al. 2018). In the last few decades, Plant Growth Promoting Rhizobium (PGPR) has been used extensively worldwide for sustainable agricultural practices. To understand the dynamics, diversity, and significance of soil PGPR communities and their active participation in promoting crop production, various investigative research studies are being conducted. The mechanisms by which bacteria can influence plant growth differ among species and strains, and they are directly or indirectly involved. Plant growth-promoting rhizobacteria (PGPRs) promote induced systemic tolerance (IST) and enhanced plant tolerance to abiotic stresses inducing physical and chemical changes in plants (Yang et al. 2009). To elicit IST, PGPR employs a variety of mechanisms that include lowering electrolyte leakage, activation of transcription of stress-responsive genes, changes in root morphology and level of phytohormones, bacterial ACC deaminase activity, and inducing the formation of lateral roots and root hairs. Under varieties of abiotic stress including drought, the plants generate an increased level of reactive oxidative species (ROS) leading to various types of cellular damage such as denaturation of proteins, DNA mutation, and peroxidation of lipids.

Osmotolerant rhizobacterial isolates (Al-19, A82, and M7b) were inoculated in rice seeds in a mixture of two isolates (Al-19 plus M7b, Al-19 plus A82, and A82 plus M7b) and a mixture of 3 isolates under different drought conditions. The plants were grown until they reached the maximum vegetative phase (sterile and nonsterile condition). Under both the conditions, the agronomic traits were found to be improved. All three isolates demonstrated the potential in IAA (Indole acetic acid) production. A82 demonstrated the highest level of IAA synthesis (14.27 μg/mL). Acetylene Reduction Assay analysis showed that M7b had the capability of nitrogen fixation (31.25 ng N/mL medium.h.) (Yuwono et al. 2005).

The application of microbial consortia (*Pseudomonas jessenii* R62, *Arthrobacter nitroguajacolicus* strain YB3 and YB5, and *Pseudomonas synxantha* R81) to Indica rice cultivar (IR-64) grown under drought stress conditions induces the higher activity of antioxidant enzymes such as SOD, peroxidase (POD), catalase (CAT), and APX (Ascorbate peroxidase) along with the increased proline content and lower level of hydrogen peroxide and malondialdehyde (MDA) (Gusain et al. 2015). It was observed in the rice plant inoculated by a consortium of two rhizobacteria Bacillus amyloliquefaciens Bk7 and Brevibacillus laterosporus B4 against cold and drought stress that leaf monodehydroascorbate (MDA) content and electrolyte leakage were decreased and leaf proline and chlorophyll content was increased. Antioxidant enzymes and abiotic stress tolerance genes like OsMYB3R-2, OsDIL, OsDREB1A, and OsCDPK13 genes were significantly upregulated. High production of IAA and siderophores and colonization of the plant roots by both the strains were evident. Also, strain Bk7 exhibited the potential to solubilize inorganic phosphate and form biofilms. (Kakar et al. 2015)

Singh et al. (2020) demonstrated that inoculation with *Trichoderma*, *Pseudomonas*, and their combination minimized the impact of the watering mechanism. The application of the microbes triggered multipronged steps at enzymatic, nonenzymatic, and gene expression levels, which was associated with stress tolerance mechanisms. The PGPR-induced rice plants demonstrated reduced ROS burden by the activation of superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione reductase, and glutathione peroxidase enzymes. Several genes of the important metabolic pathways including superoxide dismutation (*SODs*), phenylpropanoid (*PAL*), $H_2O_2$ peroxidation (*APX*, *PO*), and oxidative defense response (*CAT*) were overexpressed due to PGPR inoculation. Also, they have mentioned in their reports that the expression of *OSPiP* linked to less-water permeability; dehydration-related stress-inducible *DREB* gene, and drought-adaptation gene *DHN* was enhanced in rice inoculated plants.

## 20.7 Crosstalk of Tolerance Mechanism Provided by PGPR During Other Abiotic Stresses

In stress environment like those generated by drought, salinity, waterlogging, heavy metals, and pathogenicity, the endogenous level of ethylene is significantly increased that harms the overall plant growth. Enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase acts as the precursor for the phytohormone ethylene. The PGPR possessing the enzyme secretes it into the rhizosphere, which is further reabsorbed by the roots resulting in the conversion of ethylene. This affects the capacity of the roots to acquire nutrients and water because of the accumulated ethylene that causes poor root growth. ACC deaminase comes into play that degrades the phytohormone ethylene to alpha-ketobutyrate and ammonium. (Jeyanthi and Kanimozhi 2018)

It was observed that under constantly flooded conditions, rice (*Oryza sativa*) seedlings showed increased root elongation when the plant was subjected to PGPR treatment (ACC deaminase producing strain of *Pseudomonas fluorescens* REN1) (Etesami et al. 2014). It was reported by Shakeel et al. (2015) that among the 234 isolates that were obtained from the roots of basmati super rice and basmati-385, two strains were capable of solubilizing potassium (K) and phosphorus (P) in vitro with a solubilization zone of 47–55 mm and 38–46 mm, respectively. The strains promoted Zn translocation toward grains and increased yield of super basmati rice and basmati-385 varieties by 18–47% and 22–49%, respectively. Using the technology of 16S rRNA gene analysis, the above-mentioned two strains were identified as *Bacillus* sp. and *Bacillus cereus* (Shakeel et al. 2015). Nutrient and enhanced water uptake is facilitated by root growth stimulation and effective root area, which is one of the major stress management tools. A strong, healthy, and proliferated root system helps the plant in maintaining optimum growth and development under stress conditions (Adesemoye et al. 2008). Nautiyal et al. (2008) reported that *Bacillus amyloliquefaciens* NBRISN13 (SN13) promotes plant biomass, chlorophyll content enhancement, and increase in proline content under salt stress. The microbe SN13 possesses ACC deaminase activity and auxin-producing properties resulting in overall plant growth and enhanced root surface area. They also observed that SN13 confers tolerance to salinity stress in rice by modulating differential transcription in a set of at least 14 genes including antioxidant enzymes such as CAT.

Another recent report on salinity stress is mentioned where salt-tolerant ACCD-containing plant growth-promoting *Enterobacter* sp. P23 displays plant growth-promoting (PGP) properties that facilitate rice seedling growth. This includes IAA production, phosphate solubilization, HCN production, siderophore production, NH3 production, and ACCD production (Sarkar et al. 2018). Nutrient deficiency also affects plant growth and crop productivity. Rice (Oryza sativa L. var. IR-36) was inoculated by rhizobacteria, viz., *Bacillus amyloliquefaciens* SN13 (SN13) and *Paenibacillus lentimorbus* B-30488 (B-30488) and their consortium. It was observed that parameters such as electrolytic leakage, proline, relative water content, total soluble sugar, and malondialdehyde content were regulated in control plants as compared to treated plants under nutrient-deprived conditions. Different metabolites associated with carbohydrate metabolism to reduce the impact of stress (glucose, fructose, mannose, glucitol, oleic acid, gulonic acid, raffinose, and inositol) were found to be accumulated in treated rice seedlings. (Bisht et al. 2019)

Recent reports by Aw et al. (2019) have demonstrated that As-resistant PGPRs induced grain yield and straw biomass in rice plants. This enhanced growth was due to the IAA, ACC deaminase, and the siderophores produced by PGPR strains and an increase in phosphate available in the soil. Also, PGPR inoculation promoted the activities of POD (peroxidase) and SOD (superoxide dismutase) that acts as scavengers of ROS, leading to reduction of As damage to the plant. A similar report of antioxidative enzyme activities was mentioned by Ghosh et al. (2018)) for heavy metal (As) stress that was facilitated by the inoculation of As-resistant *Bacillus aryabhattai*. Table 20.2 shows the PGPR and its applications on the various abiotic stresses.

## 20.8 Future Perspective

Crops for healthy growth require a number of soil nutrients optimally. These nutrients also help the crop fight the adversities due to changed environmental parameters. Naturally, plants acquire these from the soil generated through the process of decomposition. Gradually, depletion of the nutrients occurs as a result of continuous consumption by the crops from the farmlands, which have made the need for application of chemical fertilizers mandatory. It has also helped to increase the productivity rate to a certain limit, which is the need to meet the ever-increasing global food demand. But in recent years, the negative impacts of the application of chemical fertilizer have created chaos over the world. Over usage of the chemical fertilizers has resulted in infertile lands, hardened soil, emission of greenhouse gases, and consequently air and water pollution. Another concern for the nitrogen-rich fertilizer is contamination of the groundwater. Therefore, application of organic fertilizer is preferred nowadays. Organic supply of nutrients to enrich the crops with essential minerals to sustain adversities is manifold beneficial than the chemical means. PGPR thus has become a breakthrough in the field of agriculture. It is mainly because microbes not only help the crops by releasing the nutrients slowly unlike chemical fertilizers but also enrich the soil leaving it fertile. However, different modes of action of PGPR in plant growth and development enhance the abiotic stress tolerance in plants. Detailed critical study on the mechanism involved in abiotic stress tolerance, mainly drought tolerance, would reveal numerous insightful facts. Advanced biotechnological approaches and genome editing tools like ZFN, TALEN, CRISPR/Cas9, and CRISPR/Cpf could be extensively used to identify the genetic characterization and regulatory role of PGPR in drought tolerance in major crop plants including rice, maize, wheat, and tomato. Therefore, PGPR could be a potent solution for many of the unsolved problems related to agriculture and meeting the global food demand in the near future.

**Table 20.2** List of PGPRs associated with rice plant conferring different abiotic stress

| PGPRs | Cultivar | Abiotic stress | Mechanism and outcome | References |
|---|---|---|---|---|
| *Pseudomonas fluorescens* REN1 | Gohar | Flood condition | Increased root elongation | Etesami et al. (2014) |
| *Bacillus* sp.<br>*Bacillus cereus* | Basmati-385 (Indica)<br>Basmati super rice (Indica) | Nutrient deficiency | Improved growth, yield, and zinc uptake | Shakeel et al. (2015) |
| *Azospirillum lipoferum*<br>*Pseudomonas* sp.<br>*Agrobacterium* sp. | Basmati-385 (Indica) | Nutrient deficiency | Zn translocation, avoid use of costly chemical fertilizer containing Zn | Tariq et al. (2007) |
| *Bacillus amyloliquefaciens* NBRISN13 (SN13) | Narayan (Indica) | Salt stress | Increase in overall plant growth and enhanced root surface area | Nautiyal et al. (2008) |
| *Pseudomonas* strains (PF1 and TDK1) | ADT43<br>IR50 (Indica) | Salt stress | Increase in enzymatic activities (peroxidase, catalase, and nitrate reductase), leading to crop yield | Sen and Chandrasekhar (2015) |
| *Paenibacillus lentimorbus* B-30488 (B-30488), *Bacillus amyloliquefaciens* SN13 (SN13) | IR-36 (Indica) | Nutrient deprivation | Seedling growth enhancement and modulation of carbohydrate metabolism | Bisht et al. (2019) |
| *Bacillus* sp. JBS-28 | Zhendao 14 and Jianyou G2 | Heavy metal (as) accumulation | Increased grain yield and decreased arsenic concentrations | Aw et al. (2019) |
| *Klebsiella* sp. PD3 | (*Oryza sativa* L.) | Phenanthrene (PHE) | Improved growth performance, biomass production, seed germination rate, photosynthetic capacity, antioxidant levels, relative water content and chlorophyll accumulation | Li et al. (2020) |
| *Bacillus pumilus* | KSK-133 | Salinity and high boron | Limited uptake of toxic ions and increased | Khan et al. (2016) |

(continued)

**Table 20.2** (continued)

| PGPRs | Cultivar | Abiotic stress | Mechanism and outcome | References |
|---|---|---|---|---|
| | | | production of antioxidants | |
| *Halobacillus dabanensis* SB-26<br>*Halobacillus* sp. GSP 34 | BRRI dhan 28 | Salinity stress | Improved agronomic traits | Rima et al. (2018) |
| *Enterobacter* sp. | Ratna | Salinity stress | Promote rice seedling growth, decrease in antioxidant enzymes, and stress-induced ethylene | Sarkar et al. (2018) |
| *Bacillus aryabhattai*, *Achromobacter denitrificans*, and *Ochrobactrum intermedium* | Oryza sativa | Salinity stress | Augmenting plant growth and production exhibited a higher amount of atmospheric nitrogen fixation, phosphate solubilization, and indole acetic acid production | Sultana et al. (2020) |

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# Fenugreek-Rhizobium Symbiosis and Flavonoids Under Stress Condition

# 21

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## Abstract

Contaminated soil has adverse effects on legume symbiosis, which lead to disturbance in nitrogen metabolism in plants. Although several studies have been performed on response of biological nitrogen fixation (BNF) under stress condition, less is known about how leguminous plants adjust their BNF process under contaminated soil. Fenugreek (*Trigonella foenum-graecum*) is an annual plant belonging to legume family and majorly grown in Northern region of India for its medicinal property. Northern region of India is mainly affected with dynamic climate that ultimately cause adverse effect on plant cultivation. In addition, due to many functioning industries in this region, nearby agricultural lands are affected with their toxic effluents. These effluents contaminate agricultural soil with heavy metals and salts, where leguminous plants fail to perform rhizobia symbiosis. However, plant releases more number of antioxidants, flavonoids, or other phenolic compounds to cope with such a type of soil stress. In the present chapter, fenugreek plant property and its rhizobial symbiosis are discussed here. Some case studies also mentioned on role of exogenous flavonoids in alleviation of soil stress effects on nodule formation. The present study suggests the use of flavonoid compounds as plant biostimulants for improving BNF process in legume plant under soil stress conditions and also explores new study in this direction.

## Keywords

Fenugreek · Rhizobia · Nitrogen fixation · Stress · Flavonoids

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## 21.1 Background

Fenugreek (*Trigonella foenum-graecum*) is a leguminous annual plant belonging to the Fabaceae family, and consequently, it has the potential to form a symbiotic relationship with nitrogen fixation bacteria (collectively called rhizobia) and can generate a lot of its used nitrogen (Abdelgani et al. 1999). It is a herbaceous plant that grows to around 10–50 cm tall and has bright single flowers with yellow and brown fruits. Its leaves are similar to clover in shape, and pods are thin and crescent-shaped. Fenugreek is a popular traditional spice crop that has been cultivated in the Indian subcontinent for centuries. A few portions of North Africa, Middle East, Mediterranean Europe, China, South-east Asia, Australia, America, Argentina, and Canada are similarly grown in comparison to South Asia. India is the world's biggest fenugreek factory, but with its high domestic consumption, the world' s fenugreek trade does not have a significant share. For the dry and semiarid regions of Asia, Africa, and Latin America, the crop was recommended. The plant was used considerably in many historical cultures across Eurasia for several centuries as a common drilling crop. The Fenugreek plant has traditionally been used for several 100 years in Indian Ayurvedic medicines and ancient Tibetan and China medicines. Cutting-edge experiments have also shown that fenugreek seed and leaves are beneficial for the treatment of multiple disorders including a successful lowering in animal and human studies of blood sugar and blood cholesterol. The crop has the ability to treat diabetic, microbial, and cancer-related diseases as a catholicon. The explanation behind Fenugreek's rich medicinal homes is because of the large range of primary plant products (diosgenin, trigonelline, fenugreekine, galactomannan, and 4-hydroxyisoleucine) (Stewart et al. 2001). In addition, fenugreek seeds comprise a full-size quantity of fiber (phospholipids, glycolipids, oleic acid, linolenic acid, and linoleic acid (Klucas et al. 1983). Fenugreek seeds contain a full size volume of fiber phospholipids, glycolipids, oleic acid, linolenic acid, choline, diet A, B1, B2, and C, nicotinic acid, and a significant variety of useful components. Fenugreek has a common adoption in the manufacturing industry as a chemical crop. The seeds contain a reliable steroid diosgenin source, which complements pharmaceutical companies (Olson and Maier 2000). Fenugreek contains calcium, niacin, and potassium as well as other nutrients. It is one of the phytoestrogens' richest properties and thus an absolutely beneficial seasoning for women who have poor estrogen levels. Fenugreek is also one of the selenium's wealthiest possessions (Wheeler et al. 2001). In addition, the local crop cycles (speed-time rotation) for herbal soil replenishment, for nitrogen fixation, and for feeding the animals as hay or silage can be incorporated without difficulty as a drilled legume and the natural nitrogen fixer (Brito et al. 1994). The crop grows in rainfed circumstances, and as a result, development costs are smaller than various commercial vegetation for semiarid regions.

Despite its incredible nutritional values and medicinal values, little research has been carried out into its genetic improvements and agricultural production growth. The symbiotic relationship of Fenugreek is with rhizobial, which includes active nitrogen-fixing and interaction in the cultivation of agricultural crops. This symbiosis disturbs several biotic and abiotic factors that reduce productivity.

## 21.2 Fenugreek Antioxidants: Metabolites Including Antioxidant Compounds

Primary metabolites are the active compounds found in many medicinal plants (Eidi et al. 2007). In addition, plant extracts can contain a variety of different additives for their antimicrobial activities. Fenugreek seeds are made of high proteins, lysine and L-tryptophans, multilaxant material, and some of its unusual chemicals, along with saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, and trigonelline (Swanston-Flatt et al. 1989). Steroidal saponins (diosgenin, yamogenin, tigogenin, and neotigogenin) are a good idea for inhibiting cholesterol uptake and synthesis and thus its arteriosclerosis capacity.

Fenugreek has also been listed for its robust antioxidant residences, which have resulted in concern for people with coronary heart attacks or most cancers in using the inherent plant-based antioxidants (Sharma et al. 1990). Fenugreek seeds include numerous biological and chemical agents including alkaloids (for example, trigonellin), polyphenols (for instance, kaempferol and quercetin), antioxidants, flavonoids, selenium, carbohydrates (45–60%), proteins (20–30%), lipids (7%), aromatic compounds, saponins, carotenoids, vitamins, and minerals (Bordia et al. 1997). Trigonelline is a large alkaloid ingredient that has a high therapeutic potential, a low toxicity, and a hypoglycemic effect (Sindhu et al. 2012). Selenium helps the network's operations to increase the efficacy of antioxidants and also has beneficial effects upon the intake routinely of a number of cancers, including colon, lung, and prostate (Naidu et al. 2011). It is also an indispensable part of your body's two main antioxidant enzymes, glutathione peroxidase and thioredoxin, which benefit your cardiovascular system and improve your immune system against a broad spectrum of other chronic illnesses (Chatterjee et al. 2009).

According to antioxidative properties, spectrophotometric in vitro investigation exposed the high oxygen radical searching flair of an ethyl acetic acetate (Kaviarasan et al. 2007). Further investigations have likewise demonstrated fenugreek seeds to be a rich wellspring of 80 polyphenols, which has prompted the estimation of numerous compounds by HPLC, in addition to apigenin and a range of kaempferol and quercetin glycosides plus the flavonoids, vitexin, tricin, naringenin, quercetin, and tricin 7-O-β-D-glucopyranoside (Shang et al. 1998; Chatterjee et al. 2009). Likewise, Chatterjee et al. (2009) have recommended a correlation between these polyphenols and fenugreek antioxidant activity. A variety of welfare benefits of fenugreek seeds have been principally endorsed to the prevailing antioxidant activity of high proportion of flavonoids and alkaloids, which depend not just on the ecological states of plant development but also on harvest management. A mixed cropping (i.e., an intercrop) framework with natural manure application can improve fenugreek seed quality while manufacturing an organic product. An intercropped cultivation with legumes can boost the standard of seeds and yield productivity.

## 21.3 Fenugreek-Rhizobia Symbiosis and Flavonoids

The event that occurs initially during the process of nodulation between the two accomplices (specific rhizobia and host legume) is the emission of specific flavonoids by host plant and perceived by microsymbiont (Begum et al. 2001). Flavonoids refer to the auxiliary metabolites in plants delivered along with other aromatic compounds and stimulated rhizobia, which hold the control Nod (nodulation) factor establishment before and during the contamination measures (Zuanazzi et al. 1998). Rice-Evans (2001) demonstrated that flavonoids act as antioxidants and have the ability to shield the cells from oxidative destruction caused by stressful conditions. Plant roots of legume exude flavonoids, which operate as signal molecules that either stimulate or suppress nodulation genes in a number of Rhizobium species (Cooper 2004; Redmond et al. 1986).

These signals trigger the nod gene expression of rhizobia, which is mediated by the nod-D regulatory gene product (Peters et al. 1986). One of the most familiar feedbacks of plants to environmental stress is the synthesis and accumulation of flavonoids (Dixon and Paiva 1995). The phenolic compounds and their derivatives could be associated with the cycle of transformation to harsh or insensitive conditions and act as antioxidants. The fundamental enzyme involved in phenolic synthesis is Phenylalanine ammonia lyase (PAL, EC. 4.3.1.5), which additionally engaged with oxidative stress condition. PAL can deliver ammonium ions from phenylalanine and backbones of t-cinnamate, which lead to increment of various phenolic metabolites. PAL catalyzes a reaction that transforms L-phenylalanine to ammonium particles and trans-cinnamic acid, which is then subsequently modified to enhance distinctive phenolic metabolites (Stewart et al. 2001).

Several products resulted from this pathway, which are strong inducers of rhizobial nodulation genes, are flavones and isoflavones (Redmond et al. 1986). The initial event in nodule formation is the secretion of specific flavonoids, which guide rhizobia to their feasible host legume (Vaishnav et al. 2017). These specific flavonoids could be the basis of transcriptional initiation of the Nod protein that stimulates the rhizobial nodulation genes associated along with the amalgamation of the Nod factor. The entire quantity of isoflavonoids in soybean and yellow lupine was significantly increased by about 15% in cadmium- and 46% in lead-treated plants (Pawlak-Sprada et al. 2011). In light of the previously mentioned data, clearly, there are changes in the flavonoid composition in plants presented to numerous biotic associations, both pathogenic and symbiotic (Kosslak et al. 1987; Muth et al. 2008).

Plants secrete flavonoids that are perceived by compatible bacteria bringing about the stimulation of nodulation genes. These nodulation genes in turn encode enzymes that synthesize a specific lipochitin nodulation signal that consequently activates a large number of early events in the root hair–infection process (Oldroyd et al. 2005). During the infection process, bacteria colonize the plant through the root epidermis and incite the reconstruction of root cortical cell development and the progression of a nodule (Abdel Wahab et al. 1996; Downie 1997; Oldroyd and Downie 2008). Recombinant rhizobia in each one nodule on a root of a legume are beneficial for the expression of foreign genes that facilitate in sequestering heavy metals in contaminated soil (Ferreira de Araújo et al. 2008). Leguminous plants can be employed to expand compost nitrogen and simultaneously eliminate heavy metals from soil (Ike et al. 2007). Investigation on the utilization of Rhizobium inoculants for the establishment of grain legumes demonstrated that it is a less expensive and normally more flourishing agronomic practice for ensuring satisfactory N nourishment of legumes contrasted with the application of N fertilizer.

## 21.4 Effect of Stress on Rhizobia Symbiosis

Rhizobium relationship comprises what is presumably the most popular illustration of plant–microorganism symbiosis (Appelbaum 1990), and it likewise is quite possibly the most complicated relationships: It indulges with a complex signal exchange process (Dénarié et al. 1996), which commences as soon as the seedling starts to grow up (Caetano-Anollés and Gresshoff 1991). A prominent indicator of soil pollution based on the sensitivity of host legumes and rhizobial strains to heavy metals, pesticides, and industrial wastes that has been proposed was the process of nodulation and nitrogen fixation of legumes (Chaudri et al. 1993; Wetzel and Werner 1995; Ibekwe et al. 1997; Abd-Alla et al. 2000, 2012). As a biological framework, nitrogen-fixing symbiosis is a complex process and also sensitive to ecological impacts (Angle et al. 1992). However, due to the inadequate exudation of flavonoids, nodulation process is conceivably affected (Vaishnav et al. 2017). Soils sullied with heavy metal beyond tolerable limits drastically altered the microbial communities network, which is then accompanied by a abstain in nitrogen fixation cycle in various groups of plants. Ahmad et al. (2012) found reduction in the rhizobial population with the enhancement of the metal concentration and recommended that the tenacity of the Rhizobium in contaminated soils was solemnly because of the modification of certain genes, together with those required for symbiosis and, presumably, in nitrogen fixation.

Contaminated soils that contain excessive metal concentrations result in yield losses due to diminishing soil fertility as the microbial activity in soil decreases. The increment in heavy metal concentration, i.e., toxicity, has led to lower down the rhizobial growth and survival chances along with considerable impact on nodulation in legumes (Khan et al. 1998). The inhibitory effects on the growth and activity of both symbionts are accountable for the deleterious effects of heavy metals on nodulation and N2 fixation of Rhizobium-legume symbiosis (Ahmad et al. 2016). The inhibitory effect of Co on rhizobial growth and nod gene expression appeared at the lowest Co concentration (25 mg $\mathrm{kg}^{-1}$) and became toxic with increased Co concentration. To diminish the inhibitory effect of Co on rhizobial growth, combination of hesperetin and apigenin has been added. Chaudri et al. (1993), in a long-term field trial, reported a decline in *R. leguminosarum bv. viciae* and *R. leguminosarum bv. Trifolii* in soils irrigated with sewage sludge containing numerous heavy metals.

In Rhizobium–legume symbiosis, plant is the restrictive factor concerning metal toxicity tolerance. This has been illustrated with Cu, aluminum, Fe, and cadmium (Cd), and this can also sometimes be the case with other stresses as well. Nodules can facilitate plant survival because of the bacteroid counter metal stress by thiol inactivation, further sustaining the fact that symbiosis is mutually beneficial to legume and rhizobia. Reactions to a portion of these metals have been described, e.g., high intercellular carbohydrate and huge cell inclusions increase the resistance of *R. leguminosarum* to Cd, Cu, Ni, and Zn, while the production of thiols has likewise been appeared to counter substantial metal-prompted oxidation (Singh et al. 2001).

## 21.5 Role of Flavonoids in Stress Alleviation

Abd-alla et al. (2014) observed the effect of exogenous flavonoids on alleviation of stress effect on rhizobia symbiosis with fenugreek plant. The authors indicated that high concentrations of Ni cause deleterious effect on nod gene expression in fenugreek. Excessive Ni has been reported to cause harmful effects on the genus Rhizobium and, hence, on nodules formation in a number of leguminous species. The process of signal exchange between rhizobia and the host legume is often attributed by the restraining effects of Ni stress on nodulation and nitrogen fixation. The detrimental effects of Ni stress on the *R. tibeticum*–fenugreek symbiosis can be controlled by the addition of nod gene inducer, i.e., combination of hesperetin and apigenin to *R. tibeticum* inoculums. Thus, this highlights its importance in promoting nodulation, nitrogen fixation, and growth of fenugreek in Ni contaminated soils. According to Santiago et al. (2002), the plant tissues exposed to heavy-metal stresses are usually stimulated by PAL. Fenugreek plants inoculated with induced or uninduced *R. tibeticum* under Co stress along with increased PAL activity confer the tolerance capacity of the plant to protect against oxidative damage. In a recent study, the activity and levels of PAL mRNA were found to be increased on the treatment of soybean (Glycine max) and yellow lupine (Lupinus luteus) with Cd (as $Cd^{2-}$) or Pb (as $Pb^{2-}$) by Pawlak-Sprada et al. (2011). As per the studies conducted by Bolaños-Vasquez and Werner (1997), among the six flavonoids identified in exudates of bean plants, the concentration of daidzein and naringenin after inoculation with several bacteria strains was found to be increased.

The amount of various flavonoids and isoflavonoids in different legume plant species is enhanced due to an infection or elicitation (Kosslak et al. 1987; Gagnon and Ibrahim 1997; Treutter 2005; Subramanian et al. 2007; Wasson et al. 2009). Colonization of lupine roots by symbiotic bacteria stimulated genistein and its derivatives, while during a defense reaction, prenylated genistein and 20-hydroxygenistein derivatives (wighteone, luteone) were synthesized (Bednarek et al. 2003; Muth et al. 2008). 20-hydroxygenistein glucoside and 20-hydroxygenistein-7-*O*-glucoside malonylate are the major compounds induced in the root of yellow lupine treated with $Cd^{2-}$ or $Pb^{2-}$(Pawlak-Sprada et al. 2011).

Fenugreek is a common green leafy vegetable grown in areas of Jajmau, Kanpur (India), indulging treated tannery wastewater for irrigation (Sinha et al. 2007). It has also been observed that soils contaminated with a high level of metals especially the toxic metal Cr have an adverse effect on the physicochemical properties of the soil. And fenugreek plants have exhibited healthy growth even in such stressful conditions. However, the tolerance of the plant grown at tannery waste contaminated sites is still not completely studied. In this relation, a pot experiment was framed and fenugreek plants were grown on soil with different tannery sludge (TS) percentages to know the involvement of various antioxidant substances (carotenoids, ascorbic acid, cysteine, thiol, and free proline contents) induced in response to metal stress.

Nodulation of some legume species increases due to the administration of exogenous nod gene inducers. As in certain alfalfa cultivars, the addition of luteolin resulted in increased nodulation (Kapulnik et al. 1987). Similarly, the addition of quercetin resulted in increased bean nodulation by *R. etli* or *R. tropici* (Hungria and Phillips 1993); the grain yield and nodulation of soybean (Glycine max) increased on pretreatment of *Bradyrhizobium japonicum* with genistein (Zhang and Smith 1996); similarly, nodulation and plant dry-matter accumulation of pea and lentil plants were stimulated by treatment of *R. leguminosarum* with hesperetin and naringenin (Begum et al. 2001). According to Abd-Alla (2011), nodulation of common bean cv. Adzuki increased significantly due to the application of isoflavonoids to the nutrient solution.

## 21.6 Conclusion

The above-reported studies strongly suggest the association of nod gene inducers like flavonoid compounds during inoculant formulation technology with agriculturally acceptable inoculant carriers, which can be directly administered to seeds or furrows during planting in soils. The remediation processes provide a wide range of applications in contaminated environments. This may uplift agriculture by enhancing legume yield in normal soil and soil contaminated with heavy metals by the development of novel inoculant formulation. The inclusion of flavonoid compounds confers the vast potential of revamping rhizobial inoculant formulation.

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# Datura Stramonium: An Overview of Its Antioxidant System for Plant Benefits

# 22

Saurabh Gupta, Kundan Kumar Chaubey, Vishal Khandelwal, Tarubala Sharma, and Shoor Vir Singh

**Abstract**

The interaction of plant with beneficial and/or pathogenic microorganisms can have the potential to trigger active defense reactions in plant. Plants have an innate ability to biosynthesize an ample range of antioxidants (nonenzymatic) capable of attenuating reactive oxygen species (ROS) induced oxidative damage. The defense response of the weed, *Datura stramonium* (belonging to the family Solanaceae), a poisonous vespertine flowering plant, has been evaluated for its vast pharmacological properties having 64 tropane alkaloid compounds with antioxidant potential. Scientifically, *D. stramonium* reported to possess antibacterial, antifungal, anti-inflammatory, insecticidal (organophosphate protective effects), and herbicidal activity. Scientifically, the frequent recreational exploitation of *D. stramonium* has resulted in toxic syndromes. This chapter presents an overview concerning its classical literature and pharmacological aspects and can also be envisaged as a novel source of natural antioxidants and antimicrobial compounds. In future perspectives, potential alteration in superoxide dismutases, guaiacol peroxidases, and catalases, lignin production, and bacterial inoculation will be approached to activate some defense mechanisms and a potentially increase the plant resistance to pathogen attack via increase in enzymatic activity of antioxidants.

**Keywords**

*Datura stramonium* · Pharmacology · Antioxidant activity · Antibacterial activity · Antifungal activity · secondary metabolites

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**Fig. 22.1** Morphological characteristics of *Datura stramonium*

**Taxonomic Classification**

Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Solanales, Family: Solanaceae, Genus: *Datura*, and Species: *Datura stramonium* (Gaire and Subedi 2013).

**Common Names**

Arabic: tatura, nafer, thagher, el-shajara el-muskera, banj tatura, and tatura shaeka; Afrikaans: gewone stinkblaar; English: false castor oil, purple thorn apple, moonflower, and jimsonweed; Chinese: man tuo luo; French: datura stramoine, pomme epineuse, and herbe des taupes; German: Stechapfel; Portuguese: Estramonio and figueira-do-diabo; Italian: Commune, indormia, and stramonio; Spanish: tapa, toloache, datura manzana, and estramonio (U.S. National Plant Germplasm System n.d.).

**Distribution**

*D. stramonium* originates in the United States of America but is now found around the world including Asia, Europe, Africa, and South and North United States (Bayih 2014).

**Regional and Other Names**

Hindi: Sadah-*Datura* and Safed *Datura;* Sanskrit: Umatta-virkshaha; English: Thorn apple; Gujarat: Dhatoria; Bengali: Dhattura; Tamil: Umatai; Marathi: Kanaka (Fig. 22.1).

## 22.1 Introduction

Plants have long been a source of exogenous dietary antioxidants, and two thirds of the world's plant species have medicinal importance along with excellent antioxidant potential (Krishnaiah et al. 2011). Due to rising acknowledgment of natural product, the medicinal plant's demand is increasing in both developing and developed countries. Herbal medicine is an essential part of both modern and traditional systems of our medicines (Kirtikar and Basu 1994). *Datura stramonium* from the family Solanaceae is a widespread yearly plant and grows to 1.5 m high. It is one of the generally acknowledged folk tale medicinal herb with dark green, alternate, simple, broadly ovate, glabrous, and shallowly lobed leaves. The fruit is a 5 cm long 4-valved capsule, which is covered with short spines. Flowers are trumpet-shaped, white, solitary, and large with terminal or in the branch bifurcations. They are androgynous and are pollinated by insects. Datura prefers soil that is moist and rich or even very alkaline, but it barely persists under shade. From prehistoric culture, it was conventionally used for spiritual idealistic purpose all over the world. It was also used for witchcraft purposes in medieval Europe. *Datura* and *Cannabis* were famous to smoke by lord Shiva. People still offer the apple with small thorn during special days and festivals to Shiva icons. Stripped barks are smeared on the surface to treat burns, swellings, and ulcers. Leave extract is consumed orally for the cure of sinus infections and asthma. All parts of the plant have anti-inflammatory property along with CNS stimulation, alopecia, treatment of skin, dental infections, and respiratory decongestion. It causes serious poisoning due to its hallucinogenic property. Severe anticholinergic reaction can occur due to consumption of any part of the plant. It may cause toxicity and sometimes cause diagnostic complications. Death may befall from failure of heart after consuming about 120 seeds, as seeds have the maximum amount and has a fast beginning of action. Thus, for the cure of the muscarinic indications of toxicity due to organophosphate and a number of anticholinergic effects, it can be used as an alternative to atropine. The extensive distribution, the possibility for occurrence in foodstuffs, and the strong toxicity are accountable for the various instances in humans (Das et al. 2012). Approximately ten species of *Datura* have been found, out of these, *Datura stramonium* and *Datura innoxia* are most significant medicinal plants. All over the world, *Datura* has long ago been recognized as a plant hallucinogen and as a medicinal plant. Ancient use of *Datura* in ceremonial rituals and for medicinal purposes could be observed in native Indian subcontinent (Parashuram 2011). Phytochemical screening of *Datura stramonium* extract revealed the presence of Tannins, steroids, cardiac glycosides, anthraquinones, saponins, triterpenes, flavonoids, and alkaloids (Shagal et al. 2012). The healing actions are often due to the presence of single or more constituents like saponins, cardiac glycosides, tannins, and alkaloids. The major alkaloids hyoscyamine and scopolamine and several minor alkaloids like tigloidin, aposcopolamine, apoatropin, hyoscyamine N-oxide, and 7-hydroxyhyoscyamine have been identified in *Datura* species (Das et al. 2012). Scopolamine and Atropine are central nervous system depressants and are competitor antagonists of muscarinic cholinergic receptors. All plant parts are toxic, but ripe seeds contain the highest amount of alkaloids (Oseni et al. 2011; Shagal et al. 2012), and amino acids (alanine, glutamate, tyrosine, and phenylalanine) were also isolated from the seeds (Lewis et al. 1970). Numerous cases have been reported of chance poisoning by *D. stramonium* due to the accidental consumption of these plants (Devi et al. 2011). Along with the cytotoxic activity of crude extracts from *Datura stramonium*, it has exposed a number of potential benefits to the future traditional medicine for the management of both livestock and human diseases.

## 22.2 Pharmacological Activity

### 22.2.1 Antioxidant Potential

**Chemical Constituents** *D. stramonium* contains a variety of alkaloids (0.2–0.6%) including atropine, hyoscamine, and scopolamine (Soni et al. 2012). Sixty-four tropane alkaloids have been detected from *D. stramonium* including important alkaloids hyoscyamine, scopolamine (hyoscine), atropine, and protein albumin. Atropine is produced from hyoscyamine by racemization. The alkaloids scopoline, aponor scopolamine, 3-(hydroxyacetoxy)-tropane, 3-hydroxy-6-(2-methylbutyryloxy)tropane, 3-tigloyloxy-6-propionyloxytropane, 3a-tigloyloxy-6-hydroxytropane, 3,7-dihydroxy-6-tigloyloxytropane, 3-phenylacetoxy-6,7-epoxytropane, 7-hydroxyhyoscyamine, 3a,6a-ditigloyloxytropane, and 3-phenylacetoxy-6-hydroxytropane are reported for the first time in *D. stramonium*. Ditiglyol esters of 3,6,7-trihydroxytropane and 3,6-dihydroxytropane have been isolated from the roots of *D. stramonium* in addition to hyoscine, hyoscyamine, tropine, and pseudotropine. It also contains 6-hydroxyhyoscymine, campesterol with anolide, metelodine, acetyl derivatives of caffeic, p-coumaric, skimmianine, ferulic acid, stigmasterol, daturataturins A and B, steroidal glycosides, chrysins, flavonoids, quercetin, and their esters. With astramonolide and coumarins, umbelliferone and scopolin are also present in the *D. stramonium* (Soni et al. 2012; Singh and Singh 2013).

Aqueous and methanolic extracts of different parts of *Datura stramonium* were evaluated for antioxidant potential using DPPH, super oxide, and Nitric oxide radical scavenging tests and exhibited a wide range of antioxidant potential. Methanolic extracts of flowers showed highest antioxidant potential at a concentration of 100 μg/mL that inhibited 99.0, 96.0, 86, and 56.45% of DPPH radical, Super oxide radical, Nitric oxide radical, and Linoleic acid peroxidation, respectively (Fatima et al. 2014). Methanolic extract of flowers also showed maximum reducing potential. Results opined that flowers of *D. stramonium* showed maximum reducing potential and are promising source of natural antioxidants.

Study by Kumar et al. (2008) has reported the antioxidant in *Datura stramonium* in terms of alkaloid contents varying from 24.6 and 63.3 mg per gram in the extracts and flavonoid contents range from 23.15 and 63.3 mg/gram in the methanolic extract of the *Datura stramonium*. List of above reported alkaloids and flavonoids in this plant showed free radical scavenging and inhibition of hydrolytic oxidative enzymes (Singh and Singh 2013).

Another study by Sharma and her group reported maximum DPPH, superoxide, and ABTS scavenging activity of methanolic *D. stramonium* extract was 92.3% at 1000 μg m/L, 73.1% at 128 μg mL-1, and 84.5 at 128 μg m/L. The maximum nitric oxide radical scavenging activity of the above extract was found to be 63.1 at 1000 µg m/L. The IC50 of the methanolic extract of *Datura stramonium* and standard was found to be 39.48 and 42.0 µg mL-1, respectively (Sharma et al. 2014).

### 22.2.2 Antibacterial Activity

Methanol extracts of *Datura innoxia* and *Datura stramonium* displayed action toward Gram(+) ve bacteria in a dosag-dependent behavior. There was no or very little antibacterial activity reported against *Pseudomonas aeruginosa* and *E. coli* (Takhi and Ouinten 2011). The combined crude alcoholic extract of *Withania somnifera*, *D. stramonium*, *and Terminalia arjuna* was subjected for antibacterial activity against *E. coli, Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus*, and *Klebsiella species* in comparison to standard Ciprofloxacin drug (Sharma and Sharma 2010). The antibacterial results of chloroform, benzene, and ethanol extracts of *Datura stramonium* leaves were studied against *Micrococcus luteus*, *Streptomyces*, *Xanthomonas campestris*, Enterobacter, *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia* and significant zone of inhibition was recorded except *Neisseria* gonorrhoeae (Sharma et al. 2013; Ali Esmail 2017). Other studies also reported broad-spectrum vibriocidal effect of *D. stramonium* (Sharma et al. 2009).

### 22.2.3 Antifungal Activity

The methanolic extract of root and the flower of *D. stramonium* observed a competent antifungal activity against *Rhizoctonia solani* fungus (Iranbakhsh et al. 2010). Acetone extracts of seeds exhibited moderate to good antifungal properties against phytopathogenic fungi (*Aspergillus niger, Aspergillus parasiticus, Penicillium janthinellum, Phytophthora nicotianae, Penicillium expansum, Pythium ultimum, Fusarium oxysporum, Trichoderma harzianum,* and *Colletotrichum gloeosporioides*) with minimum inhibitory concentrations (Mdee et al. 2009; Sharma et al. 2013). Antifungal activity against *Fusarium mangiferae was* examined. Apotion was concocted from *Datura stramonium, Azadirachta indica* (neem), *Calotropis gigantea,* and cow manure (T1). It was followed by alcohol water (70/30 v/v) extracts of *Datura stramonium, A. indica* T2, and *Calotropis gigantea*. The studies verified that when the extract is spread at breaking stage of bud and then again at setting stage of fruit, it shows ecofriendly and a sustainable manner to control floral deformity in mango (Usha et al. 2009).

## 22.2.4 Other Synergistic Activities

### 22.2.4.1 Activity Against Toxicity by Pesticide and Organophosphate

Extract of *Datura stramonium* was effective against toxicity by cypermethrin pesticide (Theodore et al. 2004). Patient's treatment suffering from an organophosphate exposure can exhaust a clinic's whole atropine stock. This increases a need for more atropine stocks or for the alternative antidote development as there is the possibility of having many severe exposures after an attack by terrorist using harmful OP nerve agents. Jimson weed has atropine and other anticholinergic combinations in them, and it is very common and can be easily obtained. They are recreationally used for their crucial anticholinergic effects, and an extract can easily be made by boiling the grinded seeds. The seed extract may be used for OP poisoning treatment as it shows rapid onset of effects. Survival after severe dichlorvos exposure can increase significantly after pretreating patient with *D. stramonium* extracts (Theodore et al. 2004).

### 22.2.4.2 Activity as a Pest and Insect Repellent

Synergistic bioefficacy of *D. stramonium* plant extracts has been reported against *Zabrotes subfasciatus* (bean bruchids: Coleoptera), major storage pests of common bean (*Phaseolus vulgaris* L.) in Ethiopia (Bayih 2014). The alcoholic extracts attained from seed and leaf in *Datura* were examined for repellent properties toward adult two-spotted spider mites in laboratory conditions. Seed and leaf extracts were applied in 145.76 g/L and 167.25 concentrations. It caused 25% and 98% death among adult spider mites after 48 h. These results propose that to control the two-spotted spider mite, *Datura stramonium* extracts can be used (Kurnal and Yalcin 2009). Fruit extract was applied to the scalp to treat dandruff and falling hair. However, the growing plant protected the neighboring plants from insects, which showed an insect repellant activity (Das et al. 2012). The ethanolic extracts of *D. stramonium* leaves were investigated for larvicidal and mosquito repellent properties against *Culex quinquefasciatus, Anopheles stephensi*, and *Aedes aegypti*. However, significant LD50 standards were found against larvicidal activity. Ethanolic extract of *D. stramonium* leaves provided Mosquito repellency (protection time) against above insects at 1.0 percent concentration (Swathi et al. 2012).

### 22.2.4.3 Biopesticide with AntiFungal Activity

Ethanolic leaf extracts (as biopesticides) obtained from *D. stramonium* showed antifungal activities against the *Fusarium oxysporum* (fungal pathogen) of wilt of *Cajanus cajan L.* (pigeon pea). Higher concentration of ethanolic extracts in both in vivo and in vitro showed complete inhibition in linear growth and sporulation in test fungi (Khandare and Salve 2011).

## 22.2.5 Anticholinergic Activity

Alkaloids present in *Datura* are organic esters, which are used as anticholinergic agents clinically. It contains a variety of alkaloids including atropine and scopolamine that can cause anticholinergic poisoning if taken in large doses (Diker et al. 2007). However, the tropane alkaloids are the essential anticholinergic alkaloids isolated from *D. stramonium* and found after second week of seed germination (Robbers et al. 1996).

## 22.3 Conclusion

This brief overview gives extensive knowledge about the uses and further exploitation of biologically active secondary metabolites of *Datura stramonium* and their pharmacological effects against various phytopathogenic agents. Different parts of *D. stramonium* have been reported to contain several alkaloids, flavonoids, steroids, saponins, tannins, phenols, and glycoside proteins that can be approached as rich antioxidant potential to activate some defense mechanisms in plants against pathogens. So far, in vitro and in vivo pharmacological studies have been performed, and some unique alkaloids and flavonoids reported in this plant showed significantly higher free radical scavenging and inhibition of hydrolytic oxidative enzymes as compared to its other species. Therefore, there should be further exploration and quantification of the phytoconstituents present in the plant along with their pharmacological profile.

**Conflict of Interest** No potential Conflict of Interest to declare.

**Authors Contribution** All authors played an active role in drafting this article to achieve important intellectual content and thus agreed of the version to be published.

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# Part V

# Role of Antioxidants in Microbe Mediated Plant Growth Promotion

# PGPR-Mediated Regulation of Antioxidants: Prospects for Abiotic Stress Management in Plants

# 23

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## Abstract

Production and accumulation of reactive oxygen species (ROS) within subcellular components are some of the important responses of plants under abiotic stress condition. ROS accumulation in cells is usually exacerbated, causing oxidative damage to cellular components and rise in electrolyte leakage that ultimately leads to cellular membrane damage and cell death. Hence, it is necessary for the cell to maintain balance between production and elimination of ROS, which is done by the production of antioxidant enzymes and by activating ROS-scavenging mechanisms. PGPRs have been found to enhance the growth of crops under extreme environments by preventing or reducing oxidative damage to plants through the activation of antioxidant defense system. Many reported that under abiotic stress, microorganisms regulate enzymatic antioxidants such as catalase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase as well as nonenzymatic antioxidants such as ascorbate, glutathione, α-tocopherol, flavonoids, and phenolic compounds that protect the plants from oxidative damages. This chapter intends to elucidate the recent advances in understanding the effect of microorganisms on antioxidant defense systems and their action on plants under various abiotic stresses such as drought, salinity, temperature, and heavy metal stress.

## Keywords

Abiotic stress · PGPR · ROS · Antioxidants

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## 23.1 Introduction

Land plants experience various kinds of stresses at one or the other stage during their lifetime which limits them from performing up to their maximum potential. These abiotic stresses such as drought, salinity, low and high temperature, heavy metals, flooding, and UV radiation have significant role in reducing the crop yields drastically and pose a serious threat to food security (Wani et al. 2016; Nguyen et al. 2018; He et al. 2018). Plant response to these abiotic stresses is complex and dynamic and is mainly dependent on the plant parts or tissue affected by the stress (Cramer et al. 2011). The complexity of the symptoms due to stress is determined by the duration and magnitude of the stress (Pandey et al. 2017). Plants activate variety of stress responses to tackle the ill effects such as membrane injury, reactive oxygen species damage, protein denaturation, and dehydration that are induced by a multitude of abiotic stress in general, which are costly to the plant and hence affect their growth (He et al. 2018). The stress response comprises membrane lipid desaturation; accumulation of antioxidants; osmolytes and compatible solutes; triggering of molecular chaperones; production of phytohormones such as abscisic acid, ethylene, salicylic acid, and jasmonic acid (Wani et al. 2016); activation of transcription factors; and the expression of stress-specific genes (He et al. 2018; Atkinson et al. 2013).

A common aspect of most of the adverse environmental conditions is the increased production of reactive oxygen species (ROS) within several subcellular compartments of the plant cell (Van Breusegem et al. 2001). ROS can also occur as a by-product of regular cellular metabolism such as photosynthesis. Under abnormal environmental conditions, reactive oxygen species (ROS) accumulation in cells is usually exacerbated, causing oxidative damage to cellular components that ultimately leads to cell death (Rejeb et al. 2014). An increase in the ROS ($O_2^{\cdot -}$ and $^{\cdot}OH$) accumulation in plants causes a rise in electrolyte leakage and malondialdehyde content (final product of lipid peroxidation) and is responsible for cellular membrane damage (Sharma et al. 2012). In stressful conditions, therefore, it is necessary for the cell to maintain balance between production and elimination of ROS. Despite causing cellular damage, ROS also acts as important signalling molecules that mediates variety of plant responses. To protect against oxidative stress, plant cells produce antioxidant enzymes and activate ROS-scavenging mechanisms (Huang et al. 2019). ROS-scavenging system comprises enzymatic and nonenzymatic antioxidants (Fahad et al. 2017). The enzymatic antioxidants include superoxide dismutase, catalase and ascorbate peroxidase, glutathione peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, and glutathione S-transferase, while ascorbic acid, cysteine, tocopherol, glutathione, and carotenoids serve as nonenzymatic antioxidants.

These built-in metabolic capabilities of the plants to cope up with the fluctuating environmental condition are thought to have been acquired over the period of evolution (Pereira 2016). Based on this, continuous efforts have been taken to improve the plant tolerance against abiotic stresses by various strategies that include physiological approaches, conventional and molecular breeding programs, and genetic engineering (Jha et al. 2014; Choudhary et al. 2018). However, recent attention has turned toward microbe-mediated mitigation of abiotic stresses by the application of beneficial microorganisms which are termed as plant growth-promoting rhizobacteria (PGPRs). Enhanced activity of different antioxidant enzymes and greater accumulation of antioxidants have been reported in response to drought stress in PGPR-inoculated plants, thereby mitigating the adverse effects of drought stress (Han and Lee 2005).

## 23.2 Role of PGPR in Abiotic Stress Alleviation

Microbes, being essential living component of the soil, naturally become associated with the crop production as soon as the seed comes in contact with the soil to begin its life cycle. The association of the microbes with the plants may be at the surface or inside the roots, stem, or leaves interacting symbiotically, associative or free living (Meena et al. 2017). These plan-microbe interactions have been found to improve plant growth by assisting in acquiring nutrients, defending against diseases, and also providing tolerance toward the abiotic stresses (Turner et al. 2013), and hence, such microbes are referred to as PGPRs. Plants send signals in the form of root exudates which chemoattract the rhizobacteria, and these are mobilized toward the roots where they form beneficial interactions (Dimkpa et al. 2009). The cues differ under different stresses that also have an impact on the kind of rhizobacteria colonizing the roots (Lareen et al. 2016). Several stress-tolerant PGPR isolates from various stress conditions have shown to protect plants from abiotic stress by a number of explicit mechanisms. The general mechanisms they employ include phytohormone biosynthesis, nitrogen fixation, potassium and phosphorous solubilization, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production, and exopolysaccharide (EPS) production, inducing metabolic and molecular reprogramming and regulation of stress-related genes and transcription factors (Enebe and Babalola 2018; Kumar et al. 2019; Vurukonda et al. 2016). However, the exact underlying mechanisms are yet to be elucidated.

In plants, phytohormones play crucial roles in response to various abiotic stresses by cross talks among them resulting in synergistic and antagonistic interactions (Peleg and Blumwald 2011). Auxin is involved in modifying plant root architecture allowing them for better adaptation to stressful environments (Kazan 2013). Cohen et al. (2009) have provided evidence that PGPR-produced phytohormones play a vital role in inducing abiotic stress tolerance by regulating phytohormones. The halotolerant PGPR strains *Arthrobacter protophormiae* SA3 and *Dietzia natronolimnaea* STR1 and the water-deficit stress-tolerant strain *Bacillus subtilis* LDR2 have pronounced role in wheat crop by providing tolerance against salt stress and drought stress, respectively, where all the strains exhibited increased synthesis of IAA under the stress conditions (Barnawal et al. 2017). The co-inoculation of ACC deaminase producing PGPR strains *Aneurinibacillus aneurinilyticus* ACC02 and *Paenibacillus* sp. ACC06 in French bean has shown to reduce 60% of ethylene in plants, elevated due to salinity stress, and hence provide tolerance (Gupta and Pandey 2019). Similarly, inoculation with ACC deaminase producing rhizobacteria helped wheat germination under Cd stress (Govindasamy et al. 2015). The enzyme ACC deaminase, present in some PGPR hydrolyzes 1-aminocyclopropane-1-carboxylic acid (ACC) which is an immediate precursor of the plant hormone ethylene whose synthesis at higher levels is detrimental to the plant growth (Gamalero and Glick 2015; Singh et al. 2015). The production of exopolysaccharide is a significant character of the PGPR which participate in protecting the plants from drought and salinity stress (Vurukonda et al. 2016; Egamberdieva et al. 2019). EPS production was greatly enhanced under high osmotic stress (40% PEG 6000) in the osmotolerant PGPR strains *Bacillus* sp. MR D17 and *Bacillus cereus* NA D7 which mitigated deleterious effects of drought stress in mustard (Bandeppa et al. 2018). EPS has its action in soil aggregation that helps to hold moisture and in trapping $Na^+$ ions, making it unavailable for plant absorption (Alami et al. 2000; El-Ghany et al. 2020). Eleven salt-tolerant PGPR strains producing EPS were shown to provide tolerance in wheat crop against salinity stress (Upadhyay et al. 2011).

The generation of reactive oxygen species (ROS) is associated with several abiotic stresses mainly drought, salinity, high temperature, and heavy metal stress which causes damage to the plant tissues (You and Chan 2015; Choudhury et al. 2013). Induced systemic tolerance has been the effective ancillary mechanism operated by PGPRs in reducing the ill effects of abiotic stress in plants (Yang et al. 2009). *Bacillus licheniformis* K11-treated pepper plants under drought stress showed elevated expression of *Cadhn*, *VA*, *sHSP*, *and CaPR-10* genes which are stress-specific (Lim and Kim 2013). The inoculation of PGPR strains *Arthrobacter protophormiae* (SA3), *Dietzia natronolimnaea* (STR1), and *B. subtilis* (LDR2) upregulated the expression of the genes *TaCTR1* (encodes for regulatory component of ethylene signalling pathway) and *TaDREB2* (encodes transcription factor involved in abiotic stress tolerance) in wheat plants under drought and salinity stress (Barnawal et al. 2017). Upregulation of endogenous ABA levels was observed in pepper plants inoculated with the GA-producing PGPR strain *Serratia nematodiphila* PEJ1011 which helped in immediate adaptation to the cold stress (Kang et al. 2015). The application of PGPR has a promising role in reducing these ROS produced under various abiotic stresses by activating ROS-scavenging systems in the crop plants which is the key discussion in this chapter (Table 23.1).

## 23.3 PGPR and Antioxidant Defense Systems of Plants

Abiotic stress usually induces oxidative damage through the accumulation of reactive oxygen species (ROS) (Anjum et al. 2012). Microorganisms have been found to enhance the growth of diverse crops grown under a range of stresses by preventing or reducing oxidative damage to plants through secretion of antioxidants. PGPR-mediated physical or chemical changes in plants may also lead to increased plant tolerance against abiotic stresses, and this is termed as induced systemic tolerance (IST) (Yang et al. 2009). Antioxidants (ROS scavengers) include enzymes such as

**Table 23.1** Upregulation of antioxidant defense system due to inoculation with plant growth-promoting microorganisms under abiotic stress condition

| Plant species | PGPR | Effect | Type of stress | References |
|---|---|---|---|---|
| Wheat (*Triticum aestivum*) | *Bacillus subtilis* Rhizo SF 48 | Increased proline, SOD, and APX activity and a decrease in MDA and $H_2O_2$ contents | Drought stress | Gowtham et al. (2020) |
| Okra (*Abelmoschus esculentus* (L.) Moench) | *Pseudomonas fluorescens* (PF) | Enhanced phenolics, ascorbate (AsA), and glutathione (GSH) and ROS-scavenging enzyme like SOD, CAT, APX, and GPX | Drought stress | Pravisya et al. (2019) |
| Mustard (*Brassica juncea* L.) | *Bacillus cereus* strain NA D7 and *Bacillus* sp. strain MR D17 | Increased in the CAT, APX, GR, SOD, and ascorbic acid in DS mustard genotype. In case of DT genotype, only CAT and ascorbic acid activity increased | Drought stress | Bandeppa et al. (2019) |
| Chickpea (*Cicer arietinum* L.) | *P. putida* and *B. amyloliquefaciens* | Enhanced accumulation of SOD and APX, and reduced the negative effect of ROS on cell damage | Drought stress | Kumar et al. (2016) |
| Green gram (*Vigna radiate*) | *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPB | Increased the activity of catalase | Drought stress | Saravanakumar et al. (2011) |
| Potato (*Solanum tuberosum* L.) | *Bacillus* strains (SR-2-1, SR-2-1/1) | Enhanced auxin production and that ultimately regulated antioxidant enzyme production and uptake of $Na^+$, $K^+$, and $Ca^{2+}$ | Salt stress | Tahir et al. (2019) |
| Chickpea (*Cicer arietinum* L.) | *Rhizobium ciceri*, A-08, EB-80, and isolate-30 | Increased antioxidant activity (APX, SOD, CAT) along with proline and MDA | Salt stress | Yilmaz and Kulaz (2019) |
| Sunflower (*Helianthus annuus*) | *Pseudomonas otitidis* Rhizo SF 7 and *Acinetobacter calcoaceticus* Rhizo SF 9 | Enhanced the enzymatic (APX and SOD) and nonenzymatic (proline) antioxidants, and decreased the MDA content | Salt stress | Singh et al. (2019) |
| Chickpea (*Cicer arietinum* L.) | *Bacillus subtilis* (BERA 71) | Enhanced plant growth via modulation in the antioxidant system, thereby eliminating salt-induced oxidative damage in plants | Salt stress | Abd-Allah et al. (2018) |
| Canola (*Brassica napus*) | *Enterobacter cloacae* HSNJ4 | Alleviated SOD, POD, and CAT activity, ROS-scavenging capacity increased, and reduced MDA content | Salt stress | Li et al. (2017) |

(continued)

**Table 23.1** (continued)

| Plant species | PGPR | Effect | Type of stress | References |
|---|---|---|---|---|
| Maize (*Zea mays* L.) | *Bacillus aquimaris* DY-3 | Improved antioxidant enzymes (superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase) and the non-antioxidant systems | Salt stress | Li and Jiang (2017) |
| *Arabidopsis thaliana* | *Bacillus megaterium* | Enhanced monodehydroascorbate reductase content together with ATP synthase | Salt stress | Erice et al. (2017) |
| Wheat (*Triticum aestivum*) | *Dietziana tronolimnaea* | Increased expression of various antioxidant enzyme genes (GR, GPX, CAT, SOD, and APX) | Salt stress | Bharti et al. (2016) |
| Wheat (*Triticum aestivum*) | *Bacillus safensis* and *Ochrobactrum pseudogrignonense* | Improved antioxidant response | Heat stress | Sarkar et al. (2018) |
| Tomato (*Solanum lycopersicum*) | *Pseudomonas frederickbergensis* OS211, *Flavobacterium glaciei* OB146, *Pseudomonas vancouverensis* OB155, and *P. frederickbergensis* OS261 | Activation of proline synthesis and antioxidant enzymes SOD, APX, and GSH | Chilling stress | Subramanian et al. (2016) |
| Mustard (*Brassica juncea*) | *Bacillus thuringiensis* KVS25 | Regulating the NO levels (signalling molecule), accumulated ROS, and ascorbate-glutathione cycle | AgNP-induced metal toxicity | Vishwakarma et al. (2020) |
| Wheat (*Triticum aestivum*) | *Pseudomonas aeruginosa* | Improved antioxidant enzymatic activity such as SOD, POD, and CAT as well as ascorbic acid (nonenzymatic antioxidant) | Zn toxicity | Islam et al. (2014) |

catalase, superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Vardharajula et al. 2011; Yadav et al. 2014), as well as nonenzymatic molecules such as ascorbate, cysteine, glutathione, α-tocopherol, carotenoids, flavonoids, and anthocyanins (Anjum et al. 2011) that prevent tissues from oxidative damages by quenching and detoxifying ROS (Gill and Tuteja 2010). The induction and regulation of the antioxidative system are necessary to obtain substantial tolerance against oxidative stress, and detoxification of ROS might be a strategy for tolerance against various abiotic stresses (Hasanuzzaman et al. 2012) (Table 23.2).

### 23.3.1 Enzymatic Antioxidant Defense System

Improved antioxidative enzyme activity is often correlated with enhanced tolerance of plants to various abiotic stresses. Recent studies have indicated the role of PGPR in modulating antioxidant enzyme level in various crops in response to abiotic stresses, thereby reducing oxidative damage elicited by stress in plants (Sandhya et al. 2010; Saravanakumar et al. 2011; Vurukonda et al. 2016). In agreement with this, rice plants inoculated with PGPR strains showed high antioxidant enzyme activities which were attributed to improving plant protection against oxidative damage caused by drought (Gusain et al. 2015). Moreover, in certain cases, PGPR-treated plants tend to reduce antioxidant enzyme activities, indicating that the treated plants experienced less stress (Tiwari et al. 2016; Misra and Chauhan 2020).

#### 23.3.1.1 Superoxide Dismutase (SOD)

Superoxide dismutases (SOD) are metalloenzymes which constitute an important element of the plant defense system against free radicals. It catalyzes the conversion of superoxide radical ($O_2^{\cdot -}$) to hydrogen peroxide ($H_2O_2$). PGPRs are known to mitigate the adverse effect of stresses by modulating plants' antioxidant enzyme level, especially SOD enzyme activity. For instance, potato plants inoculated with rhizobacterial strains *Bacillus pumilus* str. DH-11 and *Bacillus firmus* str. 40 induced plant tolerance to drought, salinity, and heavy metal by modulating antioxidative enzyme activity in plants (Gururani et al. 2013). The study showed that the specific activity of SOD enzyme was enhanced significantly (1.7–2.4 times) in inoculated plants as compared to control under different stress conditions which was also positively correlated with the mRNA expression levels of SOD. Increased level of MDA in leaves of mustard exposed to drought stress has been observed in a study by Bandeppa et al. (2019). However, inoculation with PGPR strains *Bacillus cereus* NA D7 and *Bacillus* sp. MR D17 showed significant enhancement in the activity of SOD enzyme and reduction of MDA levels in the stressed plant, thus protecting the plant against oxidative damage. Chiappero et al. (2019) suggested that PGPRs (*Pseudomonas fluorescens* WCS417r and *Bacillus amyloliquefaciens* GB03) alleviate oxidative stress in peppermint plants subjected to drought by improving plant growth and activities of antioxidant enzymes such as peroxidase and superoxide dismutase

**Table 23.2** PGPR-mediated downregulation of plant's antioxidant defense system under abiotic stress condition

| Plant species | PGPR | Effect | Type of stress | Reference |
|---|---|---|---|---|
| Chickpea (*Cicer arietinum* L.) | *Planomicrobium chinense*, *Bacillus cereus*, and *Pseudomonas fluorescens* | Decreased CAT, POD, and SOD activities in the leaves | Drought stress | Khan et al. (2017) |
| Maize (*Zea mays*) | *Burkholderia* sp. strain LD-11 | Reduced the activity of SOD | Drought stress | Fan et al. (2015) |
| Maize (*Zea mays*) | *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2), and *Alcaligenes faecalis* (AF3) | Decreased activity of APX, CAT, and GPX enzymes has also been reported in plants inoculated with EPS-producing bacteria | Drought stress | Naseem and Bano (2014) |
| Soybean (*Glycine max* L.) | *Pseudomonas putida* H-2-3 | Decreased SOD, flavonoids, and radical scavenging activity | Drought stress | Kang et al. (2014b) |
| Maize (*Zea mays*) | *Bacillus* sp. | Reduced APX and GPX activity | Drought stress | Vardharajula et al. (2011) |
| Rice (*Oryza sativa*) | *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* | Reduced lipid peroxidation and superoxide dismutase activity | Salt stress | Jha and Subramanian (2014) |
| Cucumber (*Cucumis sativus*) | *Burkholderia cepacia* SE4, *Promicromonospora* sp. SE188, and *Acinetobacter calcoaceticus* SE370 | Reduced activity of catalase, peroxidase, polyphenol oxidase, and total polyphenol | Salt stress | Kang et al. (2014a) |
| Wheat (*Triticum aestivum*) | *Bacillus subtilis* SU47 and *Arthrobacter* sp. | Reduced antioxidant enzyme activity | Salt stress | Upadhyay et al. (2012) |
| Rice (*Oryza sativa*) | *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* | Reduced SOD and APX activities, signifying a reduced $O_2^-$ scavenging | Salt stress | Jha et al. (2011) |
| Wheat (*Triticum aestivum*) | *Pseudomonas putida* | Reduced membrane injury and antioxidative enzymes such as APX, SOD, and CAT | High temperature | Ali et al. (2011) |
| Wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) | *Bacillus megaterium* M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245, and *Raoultella terrigena* | Reduced ROS content as well as enzymes SOD, POD, and CAT | Freezing injury | Turan et al. (2013) |

and consequently improved cell membrane integrity. Similarly, elevated SOD enzyme activity (in the 7–69% range) was reported in paddy plants when inoculated with rhizobacteria *B. pumilus* and *Pseudomonas pseudoalcaligenes* under salinity stress condition, which alleviated the oxidative damage induced by salt stress (Jha and Subramanian 2013).

### 23.3.1.2 Ascorbate Peroxidase (APX)

Excess of $H_2O_2$ in the cellular system can be reduced into $H_2O$ by ascorbate peroxidase (APX), glutathione peroxidase (GPX), and glutathione reductase (GR), in which APX plays a key role in detoxifying $H_2O_2$ (Caverzan et al. 2012). On exposure to abiotic stress conditions, $H_2O_2$ generation is aggravated in plants. However, inoculation with rhizobacterial strains *B. pumilus* str. DH-11 and 6 *B. firmus* str. 40 led to significant improvement in APX and GR enzyme activity and reduction in $H_2O_2$ level (Gururani et al. 2013). Moreover, genes encoding antioxidative enzymes (APX and GR) were found to increase in plants treated with rhizobacteria. Plant tolerance to abiotic stress was correlated with enhanced transcript level of genes for antioxidative enzymes, indicating that rhizobacteria elicited the stress-related defense pathways (Gururani et al. 2013; Bharti et al. 2016). Similarly, increased APX enzyme activity and increased expression of genes for ROS-scavenging enzymes such as APX and GR were observed in rhizobacteria (*Bacillus megaterium* and *Enterobacter* sp.)-treated okra under salt stress (Habib et al. 2016). This was attributed to enhanced protective mechanisms induced by rhizobacteria in plants against oxidative stress. Contrastingly, Han and Lee (2005) observed decreased activity of APX and GR enzymes under salt stress condition in lettuce plants inoculated with rhizobacteria (Serratia proteamaculans and Rhizobium leguminosarum), indicating that the treated plants encountered less stress.

### 23.3.1.3 Glutathione Peroxidase (GPX)

GPX is another important ROS-scavenging enzyme that catalyzes the reduction of $H_2O_2$ to $H_2O$. Increased GPX level was observed in the plants subjected to abiotic stress conditions. PGPR (*Pseudomonas* sp.)-inoculated basil plants showed significantly higher GPX content in comparison to uninoculated plants under drought stress and thus imparted stress tolerance in inoculated stressed plants (Heidari et al. 2011). Increased enzyme activity was correlated to the upregulation of genes encoding GPX in *Dietzia natronolimnaea* STR1-treated wheat plants under salt stress (Bharti et al. 2016).

### 23.3.1.4 Catalase (CAT)

Inoculation of lettuce (*Lactuca sativa* L.) with PGPR *Pseudomonas mendocina* augmented an antioxidant CAT under severe drought conditions, suggesting that they can be used as inoculants to alleviate the oxidative damage elicited by drought (Kohler et al. 2008). Maize plants inoculated with five drought-tolerant plant growth-promoting *Pseudomonas* spp. strains, namely, *P. entomophila*, *P. stutzeri*, *P. putida*, *P. syringae*, and *P. monteilii*, were subjected to drought stress, and inoculated plants showed significantly lower activity of antioxidant enzymes as compared to uninoculated plants (Sandhya et al. 2010), indicating a lowering of stress in the plants. Decreased activity of CAT and GPX enzymes was reported in maize plants inoculated with EPS-producing bacteria conferring stress tolerance to plants (Naseem and Bano 2014). Okra plants inoculated with PGP were adapted to saline conditions by eliminating ROS through APX, CAT, and SOD activities (Gururani et al. 2013).

### 23.3.1.5 Guaiacol Peroxidase (GPX)

Guaiacol peroxidase antioxidant systems play an important role in protecting plants from oxidative stress (Han and Lee 2005; Paczkowska et al. 2007) and involve a variety of antioxidant enzymes, including superoxide dismutase (SOD), dehydroascorbate reductase (DHAR), glutathione reductase (GR), APX, CAT, and GPX (Caverzan et al. 2012; Debez et al. 2001). In plant systems, enzymes and redox metabolites act in synergy to detoxify ROS. For example, both APX and GPX catalyze the conversion of $H_2O_2$ to water, and CAT converts $H_2O_2$ to oxygen and water. Hahm et al. (2017) reported that activities of antioxidant enzymes (APX, CAT, and GPX) in the leaf extracts of PGPR-inoculated pepper plants were significantly greater than those observed for the uninoculated control plants, regardless of growing conditions (i.e., normal or saline). Gururani et al. (2013) also reported that the activities of ROS-scavenging enzymes, such as APX, CAT, DHAR, GR, and SOD, were enhanced in PGPR-inoculated potato plants exposed to various stressors (salt, drought, and heavy metals). The tolerance of pepper seedlings to salt stress was also correlated with the increased levels of ROS-scavenging enzymes APX, CAT, and GPX.

### 23.3.1.6 Glutathione-S-Transferase (GST)

Glutathione-S-transferases are key enzymes playing important role in herbicide detoxification reactions and peroxide breakdown. GSTs safeguard the plants against chemical-induced toxicity and provide tolerance by catalyzing the S-conjugation between thiol group of GSH and electrophilic moiety in the hydrophobic and toxic substrate (Deavall et al. 2012). After conjugation, the conjugate is either sequestered into the vacuoles or exported from the cells by putative membrane ATP-dependent pump system (Kumar and Trivedi 2018). In rice plants, inoculation with *P. fluorescens* led to the accumulation of 23 rice proteins including GST (Kandasamy et al. 2009). Another important beneficial bacterium, endophytic *Pseudomonas putida*, considerably increased the drought tolerance of chickpea. This beneficial effect was supposedly due to the increased expression of genes involved in biotic stress response (*PR1*) and ROS scavenging, including *GST* (Tiwari et al. 2016). Colonization of black pepper by *P. putida* led to the induction of several host genes that encoded defense-related proteins and GST (Agisha et al. 2017). These transcriptional changes including the induction of *GSTs* may have significantly increased plant's tolerance against stress.

#### 23.3.1.7 Monodehydroascorbate Reductase (MDHAR) and Dehydroascorbate Reductase (DHAR)

*Trichoderma velutinum* considerably suppressed the infection caused by *Rhizoctonia solani* in common bean and markedly induced the expression of several defense genes including *DHAR* and *GSTs* (Mayo et al. 2016). Application of *T. harzianum* increased the growth of melon considerably and activated several GSH-related enzymes in melon leaves (Bernal-Vicente et al. 2015). The endophytic root-colonizing fungus *Piriformospora indica* can promote plant growth significantly and enhance the tolerance of host plants against abiotic and biotic stresses. These beneficial effects were attributed to the elevated antioxidative capacity of *P. indica*-inoculated plants due to the activation of GSH-dependent antioxidative pathways (Waller et al. 2005; Harrach et al. 2013). Thus, the significant upregulation of a tau-class *GST* (*BcGSTU*) was observed in *P. indica*-treated Chinese cabbage roots (Lee et al. 2011; Kao et al. 2016). Furthermore, the accumulation of two GST proteins was explored by a proteomic study in *A. thaliana* roots inoculated with *P. indica* (Peškan-Berghöfer et al. 2004). The MDAR gene coding for monodehydroascorbate reductase was significantly upregulated, and accordingly, the pool of reduced ascorbic acid was found to be increased in *Trichoderma*-treated plants (Mayo et al. 2016).

### 23.3.2 PGPR and Nonenzymatic Antioxidative Defense System of Plants

Plants are also well equipped with a plethora of nonenzymatic antioxidants. Among these, ascorbate, glutathione (GSH), and tocopherols are active molecules and not only act as redox buffers but also affect the expression of genes related to abiotic stress (Foyer and Noctor 2005; Szarka et al. 2012).

#### 23.3.2.1 Ascorbic Acid

Ascorbic acid is the most widely studied nonenzymatic antioxidant in plants. These low-molecular-weight molecules mainly protect metabolic process from toxic oxygen derivatives formed as a result of various types of stresses. It scavenges free radicals by acting as a reductant (Ahmad et al. 2010). Ascorbic acid is also involved in the regeneration of another antioxidant, α-tocopherol. Above all, it is an important cofactor for many enzymes involved in photosynthesis (Gallie 2012). The complete biosynthetic pathway of L-ascorbic acid has not been elucidated, though the ultimate precursor is found to be D-glucose (Foyer and Noctor 2005). Some of the pathways well studied for ascorbic acid synthesis are through L-galactose, galacturonic acid, and myo-inositol, and genes such as *VTC2* and *VTC5* (GDP-L-galactose phosphorylase genes studied from *A. thaliana*) are involved in the synthesis (Szarka et al. 2012). There are some studies proving the antioxidant activity of ascorbic acid. For example, Faroq et al. (2013) carried out seed priming with ascorbic acid in wheat to study its role in drought resistance. The study revealed that osmopriming of wheat with ascorbic acid significantly increased drought resistance. This may be due to higher accumulation of proline with a simultaneous antioxidant action of ascorbic acid and phenolics. This provided better membrane stability as well as tissue water maintenance. Another study conducted by Kandoliya and Vakharia (2015) showed that *P. fluorescens*, a common PGPR, has the ability to elicit antioxidant system including ascorbic acid. This improved the plant's resistance against wilt disease. In a study conducted on oxidative stress tolerance developed in wheat under Zn stress, Islam et al. (2014) found that *Pseudomonas aeruginosa* enhanced the production of ascorbic acid to combat adverse effects of Zn stress.

#### 23.3.2.2 Glutathione

Glutathione (GSH) is a nonprotein thiol that can act as both electron accepter and donor in many biological reactions and directly scavenges free radicals (Xiang et al. 2001; Foyer and Noctor 2005). Accelerated production of ROS due to various forms of abiotic stress was mainly combated by ascorbate-glutathione (AsA-GSH) cycle by eliminating hazardous peroxides (Latowski et al. 2010). High ratio of GSH (reduced form)/GSSG (oxidized form) is required to be maintained in the cell during stress condition. This is accomplished by glutathione reductase (GR), which converts GSSG back to GSH (Trivedi et al. 2013). Studies have stated that the supply of GSH exogenously can reduce ROS production, induced by copper stress (Mosofa et al. 2014). There are several studies proving the influence of PGPR in glutathione production. For example, Li et al. (2020) found that *Kocuria rhizophila* enhanced the production of GSH as well as GSSG in maize plants under salt tolerance. Pawar et al. (2016) studied the effect of two PGPR strains belonging to genus *Pantoea* and *Enterococcus* on mung bean plants grown under salt stress. They observed a 10–30% increase in glutathione in the PGPR-inoculated plants.

#### 23.3.2.3 α-Tocopherols

In general, vitamin E constitutes a liposoluble redox buffer system, mainly present in cell membrane, and provides defense against lipid peroxidation (Foyer and Noctor 2005; Jaleel et al. 2009). The hydroquinone ring in tocopherol is produced through shikimate pathway, and further, biosynthesis is catalyzed by enzymes tocopherol cyclase (VTE1), homogentisate phytyltransferase (HPT1/VTE2), and γ-tocopherol methyltransferase (γ-TMT, VTE4) (Szarka et al. 2012). Antioxidant activity of tocopherols and tocotrienols is associated with its ability to donate phenolic hydrogen to the free radicals. So, these molecules scavenge peroxy radicals, before it can attack lipids, and donate hydrogen to form tocopheroxyl or tocotrienoxyl radicals. Moreover, vitamin E plays a major role in quenching singlet oxygen, mainly by resonance energy transfer (one molecule of α-tocopherol can deactivate up to 120 $^1O_2$ molecules) (Munne-Bosch and Alegre 2002; Szarka et al. 2012). Some of the studies found that foliar spray of α-tocopherol improved the antioxidant system in mung bean (grown under water-deficit stress) and onion (under salt stress) (Sadiq et al. 2016; Semida et al. 2016). Some of the bacteria proven to be PGPR such as *Microbacterium imperiale*, *Kocuria erythromyxa*, and *Terribacillus saccharophilus* were reported to enhance production of α-tocopherol in *Vitis vinifera* L. Malbec (Salomon et al. 2016).

#### 23.3.2.4 Phenolic Compounds and Alkaloids

Phenolic antioxidants are secondary metabolites that have aromatic rings with one or more hydroxyl groups, generated basically by acetate and shikimate pathways. So, phenolics carry out antioxidative functions by chain breaking (stabilization and delocalization of unpaired electron) (Ahmad et al. 2010; Kasote et al. 2015). Phenolics also reduce the membrane fluidity and thereby reduce the free radical diffusion (Schroeter et al. 2002). Chiappero et al. (2019) reported that PGPR species such as *P. fluorescens* and *Bacillus amyloliquefaciens* could induce 30–40% more phenolic accumulation in *Mentha piperita* under severe water stress.

Alkaloid production was also increased, as a result of abiotic stress (Karadge and Gaikwad 2003). Ghorbanpour et al. (2013) reported that tropane alkaloid production increased with the application of *P. fluorescens* under water-deficit stress conditions. Terpenoid indole alkaloids such as vincristine and vinblastine are produced in *Catharanthus roseus*. Studies state that these alkaloids are produced in higher concentrations when grown under chromium stress, as defense mechanism (Rai et al. 2014). *Catharanthus roseus* also showed higher alkaloid accumulation as a result of oxidative stress caused by drought (Jaleel et al. 2007).

## 23.4 Role of PGPR in the Regulation of Antioxidants Under Various Abiotic Stress Conditions

It is imperative to regulate ROS levels through the coordination of ROS production and ROS-scavenging systems to manage oxidative damage and simultaneously modulate signalling events. Microbial inoculants that alleviate plant stress offer a possible cost-effective, environmentally friendly (Dodd and Perez-Alfocea 2012; Shrivastava and Kumar 2015) strategy. They regulate antioxidant production and signalling, thus reducing the impact of abiotic stress-induced oxidative stress on plants.

### 23.4.1 Drought Stress

In drought-stressed soils, plants are highly dependent on the microbial activity to tolerate this environmental stress (Medina and Azcon 2010). Equipped with antioxidant defense systems constituting both enzymatic and nonenzymatic components, plants act in concert to diminish the oxidative trauma outcropping during water-deficit stress via scavenging ROS (Miller et al. 2010; Etesami and Maheshwari 2018). Okra (*Abelmoschus esculentus* L. Moench) plants primed with *P. fluorescens* (PF) enhanced the activity of nonenzymatic antioxidants such as phenolics, ascorbate (AsA), and glutathione (GSH) and ROS-scavenging enzyme like SOD, CAT, APX, and GPX (Pravisya et al. 2019). Bandeppa et al. (2019) inoculated two mustard (*Brassica juncea* L.) cultivars with *B. cereus* strain NA D7 and *Bacillus* sp. strain MR D17 and showed increase in the CAT, APX, GR, SOD, and ascorbic acid in DS mustard genotype. In case of DT genotype, only CAT and ascorbic acid activity were increased in response to inoculation. Timmusk et al. (2014) and Sarma and Saikia (2014) reported that the inoculation of *B. thuringiensis* and *P. aeruginosa* could also increase activity of GR, CAT, and superoxide in wheat and activity of SOD, POD, and CAT in mung bean under drought stress, respectively. Chandra et al. (2018) inoculated wheat (*Triticum aestivum*) with *P. fluorescens* strain DPB15 and *Pseudomonas palleroniana* strain DPB16 and a significant increase in SOD, CAT, GPX, and APX activities was observed. Ordookhani et al. (2010) studied the inoculation of PGPR (*P. putida*, *Azotobacter chroococcum*, and *Azospirillum lipoferum*) and AMF (*Glomus intaradics*, *Glomus mosseae*, and *Glomus etunicatum*) and reported that these individually promoted the tomato growth; however, maximum antioxidant activities were observed in plants co-inoculated with both PGPR and AMF.

Vardharajula et al. (2011) studied the effect of *Bacillus* sp. inoculation in maize plants. Inoculated plants developed protection against drought stress by reducing the activity of the antioxidant enzymes APX and GPX. There was enhanced growth of wheat in terms of root and shoot biomass, height, and foliar nutrient content. In another study, Khan et al. (2017) reported that the combined application of PGPR (*Planomicrobium chinense*, *B. cereus*, and *P. fluorescens*) led to significant decrease in CAT, POD, and SOD activities in the leaves of chickpea grown under drought stress condition.

Gowtham et al. (2020) studied the drought tolerance of wheat crop by inoculating ACCD-positive *B. subtilis* Rhizo SF 48 and observed an increase in proline, SOD, and APX activity and a decrease in MDA and $H_2O_2$ contents. Inoculation of ACC deaminase-positive *P. putida* and *B. amyloliquefaciens* treatment to chickpea plants resulted in the enhanced accumulation of SOD and APX upon imposition of drought stress, thereby assisting the plants in reducing the negative effect of ROS on cell damage (Kumar et al. 2016). Kang et al. (2014b) studied the efficiency of GA-producing *P. putida* H-2-3 on the growth of soybean under drought stress conditions. They observed that the inoculation protected the plants by modulating antioxidant levels via decreasing SOD, flavonoids, and radical scavenging activity.

Govindasamy et al. (2020) studied the impact of multi-trait PGP rhizobacterial endophytes in alleviating drought stress in a senescent genotype of sorghum (*Sorghum bicolor* (L.) Moench). The endophytes provided better cellular osmotic adjustment in leaves ($\geq$1-fold increase in proline accumulation over control). Favorable physiological responses like relative water content (RWC) and cell membrane stability index (MSI) in the inoculated plants during drought stress were observed. Upregulation of drought-responsive genes like *sbP5CS2* and *sbP5CS1* was observed in these endophyte-treated plants as compared to untreated control.

Priming of mung bean (*Vigna radiata* (L.)) crop with *P. aeruginosa* GGRJ21 strain led to stronger upregulation of drought stress-responsive genes, *DREB2A*, *CAT1*, and *DHN*, and also led to the accumulation of antioxidant enzymes (CAT, POX, SOD) (Sarma and Saikia 2014). Kasim et al. (2013) reported a significant increase in the activity of enzymes involved in the plant ascorbate-glutathione redox cycle in wheat crop. There was also upregulation of stress-related genes *APX1*, *SAMS1*, and *HSP17.8*, when the crop was co-inoculated with *B. amyloliquefaciens*

5113 and *Azospirillum brasilense* NO40. Wang et al. (2012) observed that the inoculation of microbial consortium containing PGPR *B. cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21 in cucumber plants enhanced the SOD activity and mitigated the drought-triggered downregulation of the genes *cAPX*, *rbcL*, and *rbcS* encoding cytosolic ascorbate peroxidase.

### 23.4.2 Salinity Stress

Salinity impairs plant growth by causing osmotic imbalance and ion toxicity. Plant health in salinized soils can be improved with the use of microbial inoculants (Lugtenberg et al. 2013). The PGPR-induced antioxidative enzymes are believed to be contributing to the salt stress tolerance in plants also, by eliminating hydrogen peroxide from salt-stressed roots (Gururani et al. 2013). Li et al. (2020) conducted a study in which maize (*Zea mays* L.) plant inoculated with *Kocuria rhizophila* Y1 showed higher plant growth performance, biomass production, seed germination rate, antioxidant levels, and relative water content, and the plant also showed higher transcript levels of genes encoding antioxidants (*ZmGR1* and *ZmAPX1*) and increased ROS-scavenging capacity. Similar study conducted by El-Esawi et al. (2018) in soybean inoculated with *B. firmus* SW5 showed that the expression of antioxidant enzyme-encoding genes (*APX*, *CAT*, *POD*, *Fe-SOD*), and genes conferring tolerance to salinity alleviated the adverse effect of ROS and increased the protein biosynthesis. Inoculation of bacterial consortium of *Bacillus* strains (SR-2-1, SR-2-1/1) with potato (*Solanum tuberosum* L.) enhanced auxin production, and that ultimately regulated antioxidant enzyme production and uptake of $Na^+$, $K^+$, and $Ca^{2+}$ and resulted into a higher tuber yield in salt affected soils (Tahir et al. 2019). Yilmaz and Kulaz (2019) studied the effect of bacterial (*Rhizobium ciceri*, A-08, EB-80, and isolate-30) inoculation in chickpea (*Cicer arietinum* L.) under salt stress. There was increased antioxidant activity (APX, SOD, CAT) along with proline and MDA. Singh et al. (2019) reported that sunflower seed treated with ACCD-producing PGPR isolates (*Pseudomonas otitidis* Rhizo SF 7 and *Acinetobacter calcoaceticus* Rhizo SF 9) enhanced the enzymatic (APX and SOD) and nonenzymatic (proline) antioxidants upon stress induction and decreased MDA content.

Fukami et al. (2018) reported that the co-inoculation with *Azospirillum brasilense* strains and *Rhizobium tropici* strain in maize affected antioxidant enzymes (APX, CAT, and SOD). Inoculation led to upregulation of APX1, CAT1, SOD2, and SOD4 in leaves and APX2 in roots during salinity stress. The reports put forth by Li and Jiang (2017) signified that the efficiency of inoculation with *Bacillus aquimaris* DY-3 in maize (*Zea mays* L.) plants improved the plant response against salt damage through the integration of the antioxidant enzymes (SOD, CAT, POX, APX) and the non-antioxidant systems. Erice et al. (2017) studied the proteomic basis of the plant tolerance to saline soil by inoculating *Bacillus megaterium* to *Arabidopsis* plants and showed that enhanced monodehydroascorbate reductase (MDHAR) content together with ATP synthase helped to overcome the salt stress. *Enterobacter cloacae* HSNJ4 alleviated SOD, POD, and CAT activity in canola seedlings caused by ROS, produced owing to salt stress (Li et al. 2017). ROS-scavenging capacity under salt stress increased, thereby reducing the MDA content. Moreover, the increased expression of various antioxidant enzyme genes such as GR, GPX, CAT, SOD, and APX was observed in wheat inoculated with *D. natronolimnaea* that resulted in increased tolerance to salinity stress (Bharti et al. 2016).

Pinedo et al. (2015) studied the salt tolerance mechanism in *Arabidopsis* plants by inoculating with *Burkholderia phytofirmans* PsJN. Inoculation augmented the accretion of proline and transcription of genes associated with abscisic acid signalling, ROS scavenging, and detoxification. Jha et al. (2014) have reported that *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* inoculation in salt-sensitive rice GJ-17 during salt stress reduced lipid peroxidation and superoxide dismutase activity. Cucumber (*Cucumis sativus*) plants inoculated with PGPR (*Burkholderia cepacia* SE4, *Promicromonospora* sp. SE188, and *Acinetobacter calcoaceticus* SE370) led to reduced activity of catalase, peroxidase, polyphenol oxidase, and total polyphenol (Kang et al. 2014a, b). Wheat plants inoculated with PGPR (*Bacillus subtilis* SU47 and *Arthrobacter* sp.) reduced antioxidant enzyme activity under saline conditions (Upadhyay et al. 2012). Inoculation of rice (*Oryza sativa*) with *P. pseudoalcaligenes* and *B. pumilus* in salt stress condition led to reduction in SOD and APX activities, signifying a reduced $O_2^-$ scavenging and dismutating capacity in the rice cultivar (Jha et al. 2011).

### 23.4.3 Temperature Stress

Temperature is the main factor that decides the distribution of organisms geographically. Each organism has their own narrow range of thermal optima above or below which imposes stress (Nievola et al. 2017). Stress induced by temperature is a significant environmental factor that has an influence on growth and development of plants. Both high and low temperature affect the plants from subcellular level to the whole plant level (Waraich et al. 2012). Production of elevated levels of reactive oxygen species (ROS) is induced in both high- and low-temperature stress to which plants respond by activating the ROS-scavenging system. Several PGPR strains have shown to provide tolerance to temperature-induced stress by regulating the antioxidant system in plants. The inoculation of thermotolerant PGPR strain *P. putida* reduced membrane injury and the activity of several antioxidant enzymes such as APX, SOD, and CAT which indicated lower production of reactive oxygen species that positively correlated with the improved heat stress tolerance in wheat plants (Ali et al. 2011). The treatment of wheat seeds with *B. amyloliquefaciens* UCMB5113 or *Azospirillum brasilense* NO40 reduced the expression of *APX1* and the enzymes involved in ascorbate-glutathione redox cycle and hence provided tolerance to short-term heat stress in the seedlings (Abd El-Daim et al. 2014).

The application of PGPR (*B. megaterium* M3, *B. subtilis* OSU142, *A. brasilense* Sp245, and *Raoultella terrigena*) along with boron in wheat and barley reduced the ROS content as well as antioxidant enzyme activity such as SOD, POD, and CAT under freezing injury, with improved plant growth (Turan et al. 2013). However, some of the reports show contrasting results where the application of PGPR increased antioxidant activity in the plants while providing tolerance to the temperature stress. Priming of wheat seeds with PGPR strains *Bacillus safensis* and *Ochrobactrum pseudogrignonense* showed improved antioxidative response and thereby ameliorated the heat stress which was induced in one-month-old seedlings (Sarkar et al. 2018). In alleviating chilling stress in tomato by the psychrotolerant bacterial strains *Pseudomonas frederickbergensis* OS211, *Flavobacterium glaciei* OB146, *Pseudomonas vancouverensis* OB155, and *P. frederickbergensis* OS261, antioxidant enzymes (SOD, APX, and GSH) were activated along with synthesis of proline in the leaves when exposed to chilling temperature of 15 °C (Subramanian et al. 2016).

### 23.4.4 Light Stress

Light is also one of the stress factors that has an impact on growth and development of the plant. Excess light tends to decrease the rate of photosynthetic electron transport which is mainly due to the damage caused to PSII by ROS, induced by strong light which also inhibits the repair of photodamaged PSII (Szymańska et al. 2017). However, no reports have been found related to the mitigation of ROS produced by light stress in plants by PGPR.

### 23.4.5 Heavy Metal Stress

Buildup of heavy metals such as Cu, Mn, Fe, Ni, Co, Cd, Zn, Hg, and As has been observed in agricultural soils due to various anthropogenic activities such as fertilizer application, disposal of industrial waste, smelting, and sewage disposal. Some of these elements are toxic to the plants affecting their growth, physiology, and development and also affecting the health of animals and humans once it enters the food chain. These heavy metals inactivate or denature the proteins and important enzymes and also interfere with the substitution reactions of necessary metal ions from biomolecules (Ghori et al. 2019; Hossain et al. 2012). Most importantly, heavy metals induce the production of reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\cdot}$), superoxide radical ($O_2^{\cdot -}$), and hydrogen peroxide ($H_2O_2$) that cause lipid peroxidation of cellular membranes. PGPRs that have adapted to heavy metals through a variety of resistance systems mediated by chromosome, transposons, and plasmids are known to protect the plants from toxicity of the heavy metals by various mechanisms such as production of siderophore, organic acid, biosurfactant, phytohormone, and exopolysaccharide and inducing metal-resistant genes.

Inducing plant production of antioxidant enzyme is one of the important mechanisms in reducing the ill effects caused by the heavy metal-induced ROS (Etesami 2018). Metal-resistant, glutathione-producing plant growth-promoting bacteria *Enterobacter ludwigii* SAK5 and *Exiguobacterium indicum* SA22 alleviated Cd and Ni toxicity in rice plants by upregulation of metal stress-responsive genes such as *OsGST*, *OsMTP1*, and *OsPCS1* and by regulation of antioxidant system (Jan et al. 2019). Glutathione is a nonenzymatic antioxidant and can reduce heavy metal stress by removing peroxidase, modulating the cell cycle, and eliminating reactive species (Hossain et al. 2012; Jozefczak et al. 2012). PGPR *Bacillus thuringiensis* KVS25 along with silicon or alone could reduce the AgNP-induced metal toxicity in *Brassica juncea*. Regulation of NO levels (signalling molecule), ROS accumulation, and active ascorbate-glutathione cycle was reported to be the reasons for the alleviation of metal toxicity and improved plant growth (Vishwakarma et al. 2020).

Zn-tolerant *P. aeruginosa* improved uptake of P and N along with an increase in leaf chlorophyll content, total soluble protein, and plant biomass under Zn stress (100 mg/kg of soil) (Islam et al. 2014). The improvement was attributed to the improved antioxidant enzymatic activity such as SOD, POD, and CAT as well as ascorbic acid (nonenzymatic antioxidant) and total phenolics compared to uninoculated Zn-treated plants. Elevated expression of genes involved in ROS scavenging and higher proline content in the potato tubers was induced by the treatment of two PGPR strains *Bacillus pumilus* DH-11 and *Bacillus firmus* 40 providing tolerance to various abiotic stress including drought, salinity, and heavy metal stress (Gururani et al. 2013). The antioxidant enzymes induced by PGPRs contribute to the plant tolerance against heavy metal toxicity stress by eliminating $H_2O_2$ from the roots subjected to heavy metal stress (Sharma et al. 2003; Dimkpa et al. 2009). Contrasting results were reported by Mesa-Marín et al. (2018) where decreased antioxidant enzyme activity and root respiration were observed in the roots of the plants inoculated with PGPR. The exact mechanism of PGPR regulating the antioxidant system is a mystery which needs a much-sophisticated study to uncover.

### 23.4.6 Flooding Stress (Hypoxia)

Flooding is one of the major abiotic stress factors that have a ravaging effect on crop growth and ultimately reduce the yield as most agricultural crops are incapable of handling such stress (Setter and Waters 2003; Normile 2008). Flooding or waterlogging is the condition where the soil is completely saturated with water. The waterlogged soil has very high water table, affecting the normal biological activities (Jackson and Colmer 2005). Flooding is prevalent in the rain-fed ecosystems, especially where poor drainage exists. Waterlogging condition impedes the process of gas exchange in soil and, thereby, reduces oxygen availability leading to a condition called hypoxia. Hypoxic conditions tend to decrease root development and elevate the redox potential between waterlogged soil and plants, which leads to the production of reactive oxygen species (ROS). The soil microbiome is also affected by hypoxia. Plants try to cope up with the flooded conditions by various mechanisms such as altering the plant metabolism, ROS signalling, antioxidative defense, expression of heat-shock transcripts, and accumulation of osmolytes (Tewari and Mishra 2018).

Few studies have reported promotion of plant growth by PGPRs under flooding stress (Liddycoat et al. 2009; Etesami et al. 2014; Ali and Kim 2018; Vargas et al.

2019) mainly by reducing the waterlogging-induced ethylene by ACC deaminase production (Ali and Kim 2018; Etesami et al. 2014). However, studies related to the mitigation of ROS by regulating antioxidant system by PGPR under flooding stress have not been reported.

## 23.5 Conclusion and Future Prospects

Abiotic stresses adversely affect crop growth and productivity worldwide. However, plants employ mechanisms to protect and defend themselves actively against these stresses. For this, plants interact with microorganisms present in the rhizosphere. Among the microorganisms, the most important for agriculture are plant growth-promoting rhizobacteria (PGPR), which increase plant growth and tolerance under different abiotic stresses. PGPR priming modifies certain biochemical and physiological processes to induce systemic tolerance (IST) within the plant system to protect the plants from abiotic stresses. PGPRs help the plants to overcome the stress either by enhancing/reducing antioxidant accumulation in plants or by scavenging ROS generated by plants with bacterial antioxidative enzymes. Inherent antioxidative mechanisms and signalling protect the plants from oxidative stress with PGPR application. However, there is much more to understand about the underlying mechanisms that are repressed/downregulated under stress or triggered/upregulated by inoculation of bacteria or fungi. Moreover, the explanation of the underlying mechanism in the alleviation of abiotic stress by PGPR needs to be better elucidated, and a lot more needs to be unveiled about PGPR-mediated stress tolerance in plants. Nevertheless, current progress in the area conveys that future research has great potential to give new insights for sustainable food production. The future research needs to be in developing efficient microbial formulation for boosting plant performance under stress that substantially reduces the use of chemical fertilizers and pesticides.

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# Prospects of PGPR-Mediated Antioxidants and S and P Metabolism in Plants Under Drought Stress

# 24

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## Abstract

With the ongoing climatic fluctuations, abiotic stressors are the limiting factors which negatively regulate the growth and yield of crop plants. Among them, drought is a major challenge to the field crops, as it leads to the unavailability of nutrients and minerals to the plant system. Agrochemicals, warmer temperatures, high $CO_2$ levels, and shortage in available water resources typically change the frequency and duration of drought stress. Plant growth-promoting rhizobacteria (PGPRs) are well known to make chemical physical and biological interactions in the rhizosphere region, as these microorganisms feed on the root exudates and further help the plants in growth and development by changing the nutrient dynamics and susceptibility to drought stress. Nutrient like phosphorous (P) is present in organic and inorganic forms but is least available to the plants because they mostly form reactive metal complexes in the soil. However, the soil inoculated with PGPRs significantly improves the solubilization of these phosphates into available form. On the other hand, sulfur (S) is a key limiting element in every ecosystem, and it is required for the synthesis of certain plant hormones. It is also an important constituent of few amino acids. Immobilization of these sulfur compounds to available forms is also triggered by these microorganisms. Finally, the PGPRs act against drought stress by regulating phytohormone status; accumulation of compounds like sugars, amino acids, polyamines, and antioxidants; and stress-related gene expressions. Rhizobacteria

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not only induce local responses like mineral mobilization, root elongation, and root architecture but also mediate hormonal regulation for the growth and physiological responses in plants under drought stress. This chapter provides a brief overview on plant-rhizobacteria interaction and PGPR-mediated hormonal and nutrient regulation in plants under drought stress.

**Keywords**

PGPR · Nutrients · Stress · Sustainable agriculture · Plant growth

## 24.1 Introduction

World's population explosion is one of the emerging concerns to the global food security. Food crops are facing multiple challenges from land degradation to biotic and abiotic stresses resulting into low productivity. Urbanization and industrialization are also posing threat to the world's arable land available for agriculture. Environmental challenges such as temperature, salinity, light UV, and drought stress are putting pressure on the growth and yield and show mechanical injuries to the food crops (Vickers et al. 2009). Also, the indiscriminate use of other products such as fertilizers and pesticides to maximize yield and their synthesis leads to emission of greenhouse gases. Thus, climate change is putting extreme pressure on the global crop health management. In 2014, the Intergovernmental Panel on Climate Change published a report which showed its concern regarding greenhouse gases emission and its impacts on the Earth and ocean environment. These gases are most likely to be carbon dioxide ($CO_2$), nitric oxide ($N_2O$), and methane ($CH_4$) with 300 ppm net emission in recent past. These factors influence the weather conditions from temperature fluctuations, to water crisis, to inadequate nutrient supply to the crop plants. Thus, all these factors trigger the level of drought stress (IPOC, Climate change 2014; Eisenhauer et al. 2012). Thus, drought is the major abiotic stress which impedes low crop production by influencing photosynthesis, alters the stomatal physiology, and further increases the photorespiration (Lamaoui et al. 2018). In plants, drought also lowers the nutrient availability, increases the soil salinity, triggers high vapor pressure, and changes osmotic pressure in plant cells which compromises the growth and productivity (Boyer 1982; Wilkinson and Davies 2010).

However, to accomplish the desire of improvement in yield and quality, modern agricultural practices involve the interactions between plants and their beneficial microbe partners. These associations confer the plant's growth and development processes and further show resistance against abiotic stressors (Compant et al. 2010). Plants select those microorganisms which are competent in the rhizosphere with advantageous effects on growth and yield of host plants. The most competent PGPRs belong to the genera such as *Azospirillum*, *Bacillus*, *Arthrobacter*, *Enterobacter*, *Pseudomonas*, *Serratia*, *Rhizobium*, S*treptomy*ces and *Acinetobacter* (Etesami and Beattie 2017).

To develop these plant-microbe associations, plants release a series of organic compounds in the rhizosphere region such as sugars (fructose, xylulose, glucose, sucrose, maltose, ribose), nucleotides, amino acids, fatty acids, vitamins, putrescine, and organic acids (lactic, malic, citric, oxalic, succinic, and pyruvic acids) which account for at least 6–21% of total fixed carbon in plants (Etesami and Beattie 2017; Etesami 2018; Etesami and Maheshwari 2018). These compounds are used as nutrients or signaling molecules between microorganisms and the host plants (Lugtenberg 2015). PGPR inoculation in field crops improves the root length and root architecture. Naseem and Bano (2014) reported that *Alcaligenes faecalis* treatment to maize seeds increases its roots by 10% compared to non-treated seeds under drought. *Bacillus* spp. on the other hand improve shoot length under drought stress in maize crop (Vardharajula et al. 2011). Other crops such as tomato and sunflower associated with PGPRs in the rhizosphere improve their overall growth parameters under drought conditions (Castillo et al. 2013; Calvo-Polanco et al. 2016).

During these associations, microbes also secrete signaling molecules like phytohormones such as indole-3-acetic acid (IAA), jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and ethylene. Phytohormones are the potential growth regulators in plants; they participate in plant's secondary metabolism and significantly play an important role to counter stress responses. Kaushal and Wani (2016) revealed that *Azospirillum lipoferum* inoculation to *Zea mays* plants triggers the synthesis of ABA and gibberellins under drought stress (Cohen et al. 2009). In plants, brassinosteroids under drought stress enhance the water uptake capacity and minimize the ion leakage to maintain the membrane integrity (Bhargava and Sawant 2013). Kang et al. (2014) found that soybean plants treated with rhizobacterium *Pseudomonas* change the GA levels to enhance the growth under drought. Ethylene is known to get triggered under drought which further arrests or limits the root and shoot growth. Certain PGPRs are known to release 1-aminocyclopropane-1-carboxylase (ACC) deaminase which cleaves the ethylene precursor ACC into α-ketobutyrate and ammonia and further inhibits the ethylene activities (Glick et al. 2007; Barnawal et al. 2017a, b).

PGPR inoculations also improve nutrient diffusion in plants such as sulfur, phosphate, nitrate, and magnesium under drought conditions (Selvakumar et al. 2012). Drought alters the bulk flow of nutrients and their availability and transport by changing plant-water turgor pressures. Phosphorous (P) is generally required in traces and is mostly available as organic and inorganic forms in the soil environment. Globally, it is deficient in soil with 5.7 billion ha of land lacking soluble phosphate ($H_2PO_4^-$) which is required for crop production efficiency (Zeng et al. 2016). The most studied and significantly important phosphate-solubilizing rhizobacteria (PSRB) associated with the rhizosphere are *Bacillus*, *Pseudomonas*, *Enterobacter*, *Rhizobium*, *Serratia*, and *Agrobacterium* (Zaidi et al. 2017; Alori et al. 2017). Timmusk et al. (2014) found that *Bacillus* strains KB122 and KB133 improve the root biomass, chlorophyll content, and relative water content in sorghum as these strains has the ability to solubilize phosphate and IAA.

PGPRs also contribute to plant health under drought conditions by enhancing the production of compounds like siderophores, volatile organic compounds (VOCs), exopolysaccharides (EPS), ACC, and antioxidative enzymes. Environmental stress triggers the production of ROS molecules which leads to oxidative damage in plants. These molecules interfere in the biosynthetic and signal transduction pathways of auxin but accelerate the ethylene synthesis (Ghosh et al. 2019). PGPR inoculation lowers the oxidative stress in plants. Reports showed that *Pseudomonas* inoculation to *Ocimum basilicum* L. improves the levels of enzyme catalase which alleviates the oxidative stresses under drought. *Bacillus lentus* can improve the levels of glutathione peroxidase and ascorbate peroxidase in *Ocimum basilicum* L. (Heidari et al. 2011). Mostly, the VOCs produced under drought are likely to be 11-decyldocosane, dodecane, tetradecane, methyl, and benzene. *Pseudomonas putida* (GAP-P45) inoculation to *Arabidopsis thaliana* under drought stress enhances the production of EPS and alleviates the drought stress (Sen et al. 2018).

Plants and PGPRs associations follow certain molecular and regulatory processes in the rhizosphere. Under drought stress, these associations trigger the synthesis of functional proteins as well as regulatory proteins. These complex networking between plants and microbes are evolutionary processes which have evolved accordingly to cope up with the environmental stressors.

## 24.2 Drought Stress: A Major Challenge to the Crop Plants

Optimal quantity of water is highly essential for the plants to survive as the inadequate quantity impedes the overall growth and alters the metabolic activities in plants and hence their yield (Ojuederie et al. 2019). An array of defense mechanisms including suppressed lateral root formation; maintaining membrane stability; carbon dioxide assimilation rate due to reduced photosynthesis; phytohormonal regulation (abscisic acid, cytokinin, gibberellic acid, auxin, and ethylene); and generation of reactive oxygen species ($O^{2-}$, $H_2O_2$, and $^{\cdot}OH$ radicals), antioxidative enzymes (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase), and osmolytes (proline, sucrose, soluble carbohydrates, glycinebetaine, and other solutes) along with induced stress proteins played a pivotal role in maintaining the holistic health of plant and its survival under limited moisture conditions (Farooq et al. 2009; Anjum et al. 2011; Khan et al. 2018). The following table summarizes the effect of water scarcity on the morphological parameters of some important crop plants (Table 24.1).

## 24.3 Mechanisms of PGPR-Mediated Drought Stress Tolerance in Plants

In response to drought stress, plants are equipped with numerous traits and abilities that confer drought tolerance and adaptation within them. For instance, root architecture and morphology are the major aspects that play a pragmatic role in the resistance process (Huang et al. 2014). The root system comprises topological characters, formation of primary and secondary roots, branching system, lateral

**Table 24.1** Summary of the effects of drought on the growth and development of some common plant species

| S. no. | Plant species | Effects | References |
|---|---|---|---|
| 1. | Maize (*Zea mays*) | Leaf rolling, wilting, reduced survival of seedlings, premature flowering and longer anthesis-silk interval, reduced photosynthetic rate due to reduced chlorophyll content, lower leaf area index and number, loss of normal root architecture, overproduction of ROS ($O^{2-}$, $H_2O_2$, $OH^-$) and enhanced malondialdehyde contents, reduced photosynthetic components and nutrient uptake and crop yield | Kakumanu et al. (2012); Aslam et al. (2015); Mao et al. (2015); Chen et al. (2016a, b); Daryanto et al. (2016); Kumar et al. (2016a, b); Bista et al. (2018); Zhang et al. (2018); Hussain et al. (2019); Song et al. (2019); Sah et al. (2020) |
| 2. | Soybean (*Glycine* spp.) | Decreased plant height, root length, and dry biomass accumulation; reduction in shoot biomass and leaf area index; increase in soluble sugar and sucrose in leaves but decreased starch content and chlorophyll content; upregulation of expression levels of sucrose transporter genes (*GmSUC2*, *GmSWEET6*, and *GmSWEET15*) in leaves and roots; decrease in nodule number; impaired nitrogenase activity | Arrese-Igor et al. (2011); Thu et al. (2014); Marquez-Garcia et al. (2015); Basal and Szabo (2020); Du et al. (2020) |
| 3. | Cotton (*Gossypium* spp.) | Severely restricts height, dry weight of leaf and stem, leaf area index, node number, fiber quality, canopy, root development, net photosynthetic rate, transpiration rate, stomata conductance, carboxylation efficiency, and leaf water potential; produces lower and smaller balls; induces expression of stress-related transcription factors and genes such as ROS-scavenging, ABA, and MAPK signaling genes and accumulation of free amino acids, soluble proteins, and carbohydrates; proline content, SOD, and POD activity also enhanced while CAT activity and gibberellic acid content of the roots decreased | Kumar et al. (2001); Showler and Moran (2003); Loka et al. (2011); Wang et al. (2016); Ullah et al. (2017); Khan et al. (2018a, b); Niu et al. (2018a, b); Chen et al. (2019) |

(continued)

**Table 24.1** (continued)

| S. no. | Plant species | Effects | References |
|---|---|---|---|
| 3. | Rice (*Oryza sativa*) | Influences plant development and yield components; reduces plant height, plant biomass, tiller number, leaf area, chlorophyll content, grain size and shape and weight and stomata closure which reduces the leaf $CO_2/O_2$ ratio and inhibited photosynthesis | Sabetfar et al. (2013); Korres et al. (2017); Singh et al. (2018); Zhang et al. (2018); Mukamuhirwa et al. (2019); Yang et al. (2019) |
| 4. | Barley (*Hordeum vulgare*) | Shortened plant height and reduced plant fresh biomass accumulation, number of grains per plant, grain filling duration, tiller number and grain weight per plant, root biomass, and nutrient uptake | Samarah (2005); Alghabari and Ihsan (2018); Bista et al. (2018) |
| 5. | Chickpea (*Cicer arietinum*) | Decreased chlorophyll a and b and total chlorophyll contents both at vegetative and flowering stages, increased proline accumulation in both stages, and reduced crop yield due to low number of pods and enhanced activities of MDA, CAT, GPX, and SOD | Mafakheri et al. (2010); Mohammadi et al. (2011) |
| 6. | Faba bean (*Vicia faba*) | Elevated levels of proline, soluble sugars, and protein contents in the leaves but significantly reduced relative water content, plant height, fresh weight, dry weight, leaf area, relative water content, grain yield, total chlorophyll content, and stomatal conductance | Ammar et al. (2015); Siddiqui et al. (2015); Abid et al. (2017); Belachew et al. (2019) |
| 7. | Wheat (*Triticum aestivum*) | Decreased net photosynthetic rate, stomatal conductance under severe and moderate water stress due to low $CO_2$ availability, total soluble sugars, and proline levels enhanced. Declined plant height, biomass, leaf water potential, osmotic potential, turgor osmotic potential and relative water content, and yield and yield components | Daryanto et al. (2016); Abid et al. (2018); Guo et al. (2018); Zhang et al. (2018) |
| 8. | *Sorghum bicolor* | Reduced biomass, plant height, leaf water and chlorophyll contents, grain yield, sugar and protein and starch contents, and stomatal conductance, while the | Beheshti (2010); Castro-Nava and Ortiz-Cereceres (2012); Khaton et al. (2016); Qadir et al. (2019); Amoah and Antwi-Berko (2020) |

(continued)

**Table 24.1** (continued)

| S. no. | Plant species | Effects | References |
|---|---|---|---|
| | | proline, MDA, electrolyte leakage, $H_2O_2$ and antioxidant enzyme activity and amounts of remobilized dry matter, remobilization efficiency, and remobilization percentage increased significantly | |
| 9. | Mustard (*Brassica* spp.) | Plant height, primary branches per plant, secondary branches per plant, seed yield of mustard, seed fatty acid composition, oil content, protein content, relative water content, osmotic potential and potassium contents, total dry matter, leaf area index, relative growth rate and crop growth rate are greatly reduced; significant reduction in growth and photosynthetic parameters, activity of ribulose-1,5-bisphosphate carboxylase, nitrate reductase, ATP sulfurylase which accounted for decreased nitrogen and sulfur assimilation, and ascorbate content; but pronounced increase in proline metabolism, MDA, $H_2O_2$, GSH, and GSSG | Chauhan et al. (2007); Aslam et al. (2009); Moaveni et al. (2010); Alam et al. (2013); Nazar et al. (2015) |
| 10. | *Phaseolus vulgaris* | Significant reduction in leaf area, dry matter production, number of pods per plant, number of seeds per plant, grain yield, harvest index, seed weight, and enriched carbon isotope abundance ($\delta^{13}C$) in the seed, photosynthetic rate, stomatal conductance, and transpiration along with significant decrease in the concentration of mineral nutrients and amino acids in leaves while higher levels of accumulation of leaf N and proline in the leaves | Ghanbari et al. (2013); Mathobo et al. (2017); Smith et al. (2019) |

root formation, etc. However, the characteristic role of roots and their morphological aspects are based on the soil physicochemical properties (Tuberosa 2012; Barnawal et al. 2019). PGPRs residing within the rhizosphere promote plant growth and also alter and nourish roots so as to provide growth-promoting traits (Gouda et al. 2017).

Moreover, PGPR-mediated changes across roots adhere the plant and induce the total surface area of roots, subsequently causing enhanced water and mineral uptake. As a result, these positive changes ultimately promote total plant growth and metabolism of plant (Timmusk et al. 2014).

Nevertheless, due to abundant benefits of PGPR toward plants in promoting plant growth and yield during drought stress, they are considered as highly valuable. Cohabiting within the rhizosphere, they assist nourishment via phytohormone production, enzyme synthesis, and release of different metabolites and root exudates (Olanrewaju et al. 2017). Moreover, they stimulate nutrient uptake and assist plant growth by serving as carbon source and essential nutrient for plant metabolism (Olanrewaju et al. 2017). In this way, they establish positive colonization among plants and allow them to survive under harsh conditions. Apart from this, their interactions form a microbial niche within the rhizosphere and make the soil fertile for cultivation purposes (Nicolitch et al. 2016). PGPRs assist in Fe sequestration, phosphorous solubilization, exopolysaccharide synthesis and ACC production to protect plants from drought stresses. Therefore, they are very effective due to their multifaceted role for agricultural purposes under unfavorable conditions. The mechanistic action of PGPR for drought stress mitigation has been depicted in Fig. 24.1. Generally, the growth-promoting potential of PGPR is a consequence of traits possessed by them toward plants subjected to drought severity. A study depicted that *Bacillus megaterium* and *B. subtilis* induced mineral uptake, relative water content, stomatal attributes, and overall plant yield under drought-stressed plants (Sahin et al. 2015). Moreover, an investigation carried in *Zea mays* revealed that *Alcaligenes faecalis* promoted seed germination rate, plant biomass, root and shoot length, and water uptake in plants under drought conditions (Nadeem et al. 2013). They speculated that PGPR treatment modulated the root system and enhanced the water transport in microbe-inoculated plants under drought stress. Likewise, *Burkholderia phytofirmans* considerably enhanced morphological parameters in terms of plant biomass during water scarcity (Naveed et al. 2014). It has been attributed that plants inoculated with PGPR regulate plant growth rates and subsequently enhances overall crop productivities. Similar to this, inoculation of *Bacillus* spp. promoted plant growth and biomass of drought-stressed corn plants in contrast to control plants (Vardharajula et al. 2011). Additionally, under drought conditions, PGPR induced biomass of wheat, depicting its direct role in improvement of overall performance of plants under adverse environmental conditions (Timmusk et al. 2014). Another study conducted by Lim and Kim (2013) in pepper plants determined that when application of *B. licheniformis* was done in drought-stressed plants, it induced shoot length, root length, plant biomass, water uptake, and other metabolic activities of plants. The role of PGPR for plant improvement under drought stress has been studied in many other plant species such as *Helianthus annuus*, *Sorghum bicolor*, *Solanum lycopersicum*, *Triticum aestivum*, *Vigna radiata*, *Cicer arietinum* (Castillo et al. 2013; Grover et al. 2014; Kasim et al. 2013; Naveed et al. 2014; Sarma and Saikia 2014).

In addition, PGPR inoculants modulate the physiological characteristics of plants through inducing water balance, photosynthetic pigment content, photosynthetic

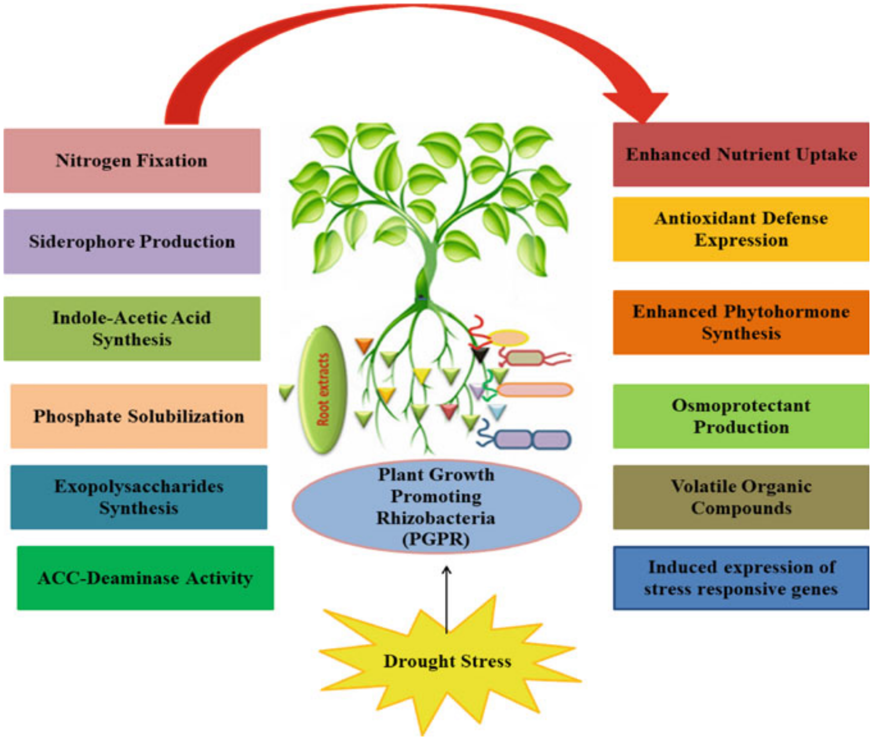

**Fig. 24.1** Mechanism of PGPR-mediated drought stress tolerance in plants

efficiencies, and quantum efficiency while reducing the oxidative stress markers such as MDA and superoxide radicals. It has been studied that PGPR consortium altogether works more efficiently, like in case of cucumber plants where three PGPR species (*B. cereus*, *B. subtilis*, and *Serratia*) declined the MDA and electrical conductivity of plants under drought stress (Wang et al. 2012a, b). Moreover, they also investigated that total chlorophyll, Chl a and Chl b, content was significantly enhanced in the same plants (Wang et al. 2012a, b). Similar trend was observed in the studies conducted by Han et al. (2014) who reported that *B. subtilis* enhanced the pigment levels of stressed plants with progressive decline in the content of MDA in white clover plants. It has been implicated that the potential of PGPR to lower the lipid peroxidation in stressed plants and protect the cell membrane from damage under drought severity is associated with the ability of the strains to produce the protective compounds that combat this stress and enhance the adaptive and defensive power of plants to survive under harsh situations. Also, in case of *Pinus halepensis*, the supplementation of *Pseudomonas fluorescens* triggered quantum efficiency and electron transport chain in plants under drought stress (Rincon et al. 2008). Also, inoculation of *Arabidopsis thaliana* plants with *Azospirillum brasilense* elevated relative water content and improved gaseous exchange parameters in drought-affected plants (Cohen et al. 2015). Furthermore, *Pseudomonas* sp. also enhanced the overall transpiration rate and photosynthetic rate of grapevine plants under drought conditions (Rolli et al. 2017). Therefore, it is quite evident that PGPR treatment leads to improve of plant metabolism during drought conditions, and their potential roles have been explained in the mentioned sections below (Fig. 24.1; Table 24.2).

### 24.3.1 Role of PGPRs in Modification of Phytohormonal Activity in Plants

Hormones are the key regulators in the plant's growth and developmental processes. Also, they play an important role in augmenting plant's growth under drought stress (Fahraji et al. 2014). An array of plant hormones such as IAA, GA, JA, ABA, CK, and ET are the endogenous regulators in plant system (Egamberdieva et al. 2017). Similarly, PGPRs also have the capability to produce or synthesize phytohormones which have the potential to stimulate growth-like activities in plant cells. These hormones alleviate the level of stress during harsh environment, either they promote the growth and developmental activities or they regulate the processes like dormancy and abscission (Liu et al. 2012; Ahmed and Hasnain 2014). PGPR-associated phytohormones also contribute to enhance the plant's respiration and metabolic processes. Plants inoculated with these PGPRs show a significant increase in yield of crops under drought stress (Chanway and Holl 1994; Barnawal et al. 2017a, b).

#### 24.3.1.1 Indole-3-Acetic Acid (IAA)

IAA is one of the most efficient molecules with diverse physiological roles in plants. In plants, auxin is a key modulator which involves cell division, cell differentiation, cell elongation, tropism, senescence, flowering, and apical dominance (Teale et al. 2006; Ahemad and Kibret 2014). Endogenous IAA levels in plants control the various growth-related pathways associated with auxin, but at the same time, PGPRs also release IAA to the auxin pool (Glick 2012; Parray et al. 2016). Most of the PGPRs known to synthesize auxin are *Acinetobacter*, *Bacillus thuringiensis*, *Pseudomonas chlororaphis*, *Azospirillum*, *Pseudomonas*, etc. (Rolli et al. 2015). Bacteria synthesize auxin in the rhizosphere region with respect to the available root exudates. IAA-producing bacteria mostly follow the intermediate indole-pyruvic acid pathway. In indole-pyruvic acid pathway, microorganisms use ipdC gene which codes for indole-pyruvate decarboxylase to convert indole-3-pyruvic acid (IPyA) to indole-3-acetaldehyde (Ahmed and Hasnain 2014). Active IAA acquisition helps in plant-rhizobacteria associations which trigger the defense mechanism against pathogen and environmental stress (Parray et al. 2016; Spaepen and Vanderleyden 2011).

Plants inoculated with IAA-producing bacteria are found to have enlarged roots with multiple root hairs and typically accelerate lateral root formation (Dimkpa et al. 2009). Also, the plants show resistance against drought stress by improving the potential for nutrient and water uptake (Egamberdieva and Kucharova 2009; Vacheron et al. 2013). Report says that tomato plants (*Solanum lycopercum*)

**Table 24.2** PGPRs and their influence under drought stress

| S. no. | PGPRs | Plant species | Effects | References |
|---|---|---|---|---|
| 1 | *Pseudomonas putida* strainGAP-P45 | *Helianthus annuus*, *Zea mays* | Increase biomass, root length, relative water content, and water potential in leaves | Sandhya et al. (2010) |
| 2 | *Bacillus* sp. | *Solanum tuberosum* | Enhance the photosyntheticefficiency as well as expression levels of ROS-scavenging enzymes | Gururani et al. (2013) |
| 3 | Bacillus spp. strains KB122, KB129, KB133, and KB14 | *Sorghum bicolor* | Enhanced chlorophyll content, leaf relative water content, and soil moisture content | Grover et al. (2014) |
| 4 | *Pseudomonas fluorescens*, *Enterobacter hormaechei*, and *Pseudomonas migulae* | *Setaria italica* | Stimulated seed germination, seedling growth, soil moisture, and root-adhering soil/root tissue ratio | Niu et al. (2018a, b) |
| 5 | *Acinetobacter calcoaceticus* | *Sambucus williamsii* | Increases the photosynthetic efficiency of plants | Liu et al. (2019a, b) |
| 6 | *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Achromobacter xylosoxidans*, and *Leclercia adecarboxylata* | *Zea mays* | Stimulates photosynthetic rate, stomatal conductance, chlorophyll a, total chlorophyll, and carotenoids contents | Danish et al. (2020) |
| 7 | *Azospirillum brasilense* | *Solanum lycopersicum* | Increases the root hair growth | Molina-Favero et al. (2008) |
| 8 | *Burkholderia phytofirmans* strain PsJN | *Triiticum aestivum* | Reduced oxidative stress, and increased mineral components of wheat | Naveed et al. (2014) |
| 9 | *Bacillus thuringiensis* AZP2 and *Paenibacillus polymyxa* B | *Triiticum aestivum* | Stimulate crop growth and biomass | Timmusk et al. (2014) |
| 10 | *Proteus penneri*, *Pseudomonas aeruginosa*, and *Alcaligenes faecalis* | *Zea mays* | Ameliorate plant biomass, leaf area, and other growth parameter | Naseem and Bano (2014) |
| 11 | *Azotobacter chroococcum* | *Solanum lycopersicum* | Increases nutrient availability and water retention | Viscardi et al. (2016) |
| 12 | *Burkholderia cepacia* | *Capsicum annuum* | Increased plant biomass andchlorophyll a content underdrought stress | Maxton et al. (2018) |
| 13 | *Bacillus megaterium*, *Bacillus subtilis* | *Lactuca sativa* | Enhance plant growth, yield nutrient element content, leaf relative water content, and stomatal conductance | Sahin et al. (2015) |

(continued)

**Table 24.2** (continued)

| S. no. | PGPRs | Plant species | Effects | References |
|---|---|---|---|---|
| 14 | *Bacillus* and *Enterobacter* spp. | *Mucuna pruriens* | Enhanced plant biomass whereas there is a reduction in ACC concentration in leaves and roots | Saleem et al. (2018) |
| 15 | *Ochrobactrum pseudogrignonense*, *Pseudomonas*, and *Bacillus subtilis* | *Pisum sativum*, *Vigna mungo* | Increased seed germination percentage, root length, shoot length, and dry weight, and reduced ACC accumulation | Saikia et al. (2018) |
| 16 | *Variovorax paradoxus* RAA3, *Ochrobactrum anthropi* DPC9, and various other *Pseudomonas* spp. | *Triticum aestivum* | Improved plant growth and foliar nutrient concentration, and also enhanced antioxidant properties | Chandra et al. (2019) |
| 17 | *Pseudomonas aeruginosa* and *Bacillus amyloliquefaciens* | *Capsicum annum* | Enhance biomass production, chlorophyll content, and nutrient uptake | Gupta et al. (2019) |
| 18 | *Bacillus thuringiensis* | *Lavandula dentata* | Increases K and proline, whereas decreases glutathione reductase (GR) and ascorbate peroxidase(APX) | Armada et al. (2014) |
| 19 | *Agrobacterium fabrum*, *Bacillus amyloliquefaciens* | *Triticum aestivum* | Increases grain yield and biomass | Zafar-ul-Hye et al. (2019) |
| 20 | *Leclercia adecarboxylata* and *A. fabrum* | *Triticum aestivum* | Increase nutrient uptake and high chlorophyll contents | Danish et al. (2019) |
| 21 | *Pseudomonas aeruginosa* PM389, *Pseudomonas aeruginosa* ZNP1, *Bacillus endophyticus* J13, *and Bacillus tequilensis* j12 | *Arabidopsis thaliana* | Increases phytohormones (IAA, cytokinin, gibberellins) and EPS secretion | Ghosh et al. (2019) |
| 22 | *Pseudomonas putida* GAP-P45 | *Arabidopsis thaliana* | Increase polyamine biosynthetic genes | Sen et al. (2018) |
| 23 | *Streptomyces pactum* | *Triticum aestivum* | ABA accumulation upregulation of drought-resistant-related genes. Prevent oxidative damage | Li et al. (2019) |
| 24 | *Azospirillum* spp. AZ39 and AZ19 | *Zea mays* | Increase proline content | García et al. (2017) |

inoculated with *A. brasilense* are known to produce nitric oxide that shows regulatory cross talks with IAA signaling pathway and further helps in adventitious root growth formation (Molina-Favero et al. 2008). Tea clones TV1, TV19, and TV20 inoculated with *Enterobacter lignolyticus* strain TG1 produce a sufficient amount of IAA which leads to the increase in root lengths and biomass with respect to non-treated plants or control (Dutta et al. 2015). Table 24.3 shows the ameliorative roles of PGPR-associated (auxin) IAA on different food crops under drought stress.

#### 24.3.1.2 Abscisic Acid (ABA)

ABA, a sesquiterpene, is one of the signaling hormones that work under stress conditions (Zhang et al. 2006). In plants, endogenous ABA modulates various physiological processes under biotic and abiotic challenges. However, it also controls the processes like fruit ripening, seed development, and other growth and developmental processes in plants. In plants, ABA also improves the water uptake capacity under drought stress (Zhang et al. 2009). ABA is not restricted in plants but also gets synthesized in other organisms like algae, bryophytes, fungi, and bacteria (Takezawa et al. 2011).

The PGPRs associated with ABA synthesis belong to *Bacillus*, *Azospirillum*, and *Pseudomonas* (Salomon et al. 2014). Plants show certain physiological responses under drought such as decrease in photosynthesis and pigment content and show disparity in antioxidant production (Hu et al. 2006). Under drought stress, ABA secretions get activated in roots, and further, it reaches to leaves and assists stomatal closure under water-deficient conditions. Cohen et al. (2009) reported that *Azospirillum lipoferum* produces ABA in maize crops under drought conditions. Also, dehydrated cells trigger the signaling for ABA synthesis under water-deficient environment (Kaushal and Wani 2016).

Studies reveal that *Arabidopsis thaliana* under drought stress when inoculated with *Phyllobacterium brassicacearum* strain STM 196 (PGPRs) improves the ABA levels in the rhizosphere which further moves into the plant tissues and lowers the transpiration rates (Bresson et al. 2013). Liu et al. (2013) found that *Bacillus subtilis* inoculation to *Platycladus orientalis* under drought stress synthesizes PGPR-associated ABA which assists the plants to improve the ABA levels in shoots and increases stomatal conductance.

#### 24.3.1.3 Salicylic Acid (SA)

Phytohormone production is one of the mechanisms of PGPR for maintaining the proper plant growth and development under normal as well as in stress condition. Majority of PGPR strains produce salicylic acid (SA) and indole-3-acetic acid (IAA) in large amount as compared to other phytohormones (Jochum et al. 2019). There are various investigatory reports showing positive stimulatory effects of salicylic acid in combination with PGPR. Ali et al. (2014a, b) reported that antagonistic rhizobacteria in combination with salicylic acid treatments induced systemic resistance in *Triticum aestivum.* Additionally, PGPRs like *Azospirillum*, *Pseudomonas*, and *Bacillus* species in combination with salicylic acid (1 mM) cause stimulation in plant height, fresh weight, dry weight, chlorophyll b, total chlorophyll, and yield of essential oils

**Table 24.3** Role of PGPR to regulate the IAA production and their effects in plants

| S. no. | Stress | Rhizobacteria | Phytohormone fluctuated | Plant species | Effects | References |
|---|---|---|---|---|---|---|
| 1. | Drought | *Bacillus thuringiensis* | IAA | *Triticum aestivum* | Lateral root growth and density and length of roots gets improved. Also improves root hair density | Timmusk et al. (2014) |
| 2. | Drought | *Azospirillum* | IAA | *Triticum aestivum* | Root growth and lateral root development | Arzanesh et al. (2011) |
| 3. | Drought | *B. thuringiensis* | IAA | *Lavandula dentate* | Improve nutrient uptake physiology and metabolism of plant | Armada et al. (2016) |
| 4. | Drought | *P. putida* | IAA | *Trifolium repens* L. | Improves root and shoot biomass. Also improves water content | Marulanda et al. (2009) |
| 5. | Drought | *B. megaterium* | IAA | *Trifolium repens* L. | Improves root and shoot biomass. Also improves water content | Marulanda et al. (2009) |
| 6. | Drought | *Phyllobacterium brassicacearum* strain STM 196 | IAA | *Arabidopsis thaliana* | Increases lateral roots | Bresson et al. (2014) |
| 7. | Drought | *Bacillus* spp. strain KB122 | IAA | *Sorghum bicolor* | Increase chlorophyll content, relative water content, and soil moisture | Grover et al. (2014) |
| 8. | Drought | *Azotobacter chroococcum* strains 67B and 76A | IAA | *Lycopersicum* | Improves nutrient and water retention capacity | Viscardi et al. (2016) |
| 9. | Drought | *Bacillus megaterium* TV6D | IAA | *Lactuca sativa* | Increases plant growth, stomatal conductance, and plant yield | Sahin et al. (2015) |
| 10. | Drought | *Bacillus* spp.<br>*Enterobacter* spp. | IAA | *Mucuna pruriens* L. | Enhances plant biomass | Saleem et al. (2018) |
| 11. | Drought | *Bacillus subtilis* TV12H | IAA | *Lactuca sativa* | Leaf relative water content improves | Sahin et al. (2015) |
| 12. | Drought | *Ochrobactrum pseudogrignonense* RJ12 | IAA | *Vigna mungo* L. | Improves seed germination, root length, shoot length, and dry weight | Saikia et al. (2018) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 13. | Drought | *Bacillus subtilis* RJ46 | IAA | *Vigna mungo* L. | Improves seed germination, root length, shoot length, and dry weight | Saikia et al. (2018) |
| 14. | Drought | *Azospirillum lipoferum* | IAA | *Zea mays* | Stomata closure with low transpiration | Etesami and Maheshwari (2018) |
| 15. | Drought | *Bacillus licheniformis* K11 | IAA | *Piper nigrum* | Growth of plants | Lim and Kim (2013) |
| 16. | Drought | *Rhizobium phaseoli* (MR-2) | IAA | *Triticum aestivum* | Improves growth, biomass, and drought tolerance index | Hussain et al. (2014) |

in *Hyssopus officinalis* (Sharifi 2017). *Likewise*, foliar treatment of salicylic acid at three leaf stages along with PGPR treatments of *Planomicrobium chinense* strain P1 and *Bacillus cereus* strain P2 enhances the significant accumulation of Cd, Pd, and Ni in the rhizosphere and increases shoot length, root length, dry biomass, and chlorophyll and carotenoid content in *Helianthus annuus* (Khan et al. 2018a, b). Similar report showing SA foliar spray at the rate 200 mg/l with combined treatment of *Azospirillum brasilense* SARS 1001 and *Azotobacter chroococcum* SARS 302 increases the chlorophyll content, relative water content, stomatal conductance, and soil microbial population and also showed inhibitory impacts on proline content, thus improving yield-related traits, productivity, and nutrient uptake (N, P, K) under water-deficit compared to the control treatment in *Triticum aestivum* L. cv. Sakha 95 (Hafez et al. 2019). Khan et al. (2019) also reported that in *Bacillus subtilis*, *Bacillus thuringiensis*, and *Bacillus megaterium*, salicylic acid (SA) in combination with putrescine (Put) spray at the rate of 150 mg/l enhances chlorophyll, protein, and sugar contents and also improves drought conditions in *Cicer arietinum* (chickpea).

#### 24.3.1.4 Jasmonic Acid (JA)

PGPRs are used to improve the performance of plants by regulating the production of phytohormones (Buchanan et al. 2000). Jasmonic acid is an important phytohormone that is increased by PGPR in plants (Zebelo et al. 2016). JA and *Bacillus subtilis* strain have the ability to activate transcription of JA-responsive genes, which are involved in prevention of pathogenic infection (Veselova et al. 2015). Some of the rhizobacteria-treated plants show significantly increased synthesis of jasmonic acid as shown in Table 24.4.

#### 24.3.1.5 Ethylene (ET)

Microbe/plant interaction regulates the expression of phytohormones (Ambreetha et al. 2018). Ethylene concentration in the rhizosphere is moderately regulated by PGPR (Glick 2014). Ethylene generally acts as the growth inhibition hormone but with rhizobacteria helps plant growth by lowering ethylene concentration (Glick 2005). Some of the role of PGPR in regulation of ethylene is shown in Table 24.5.

### 24.3.2 Exopolysaccharide Production

Exopolysaccharides (EPSs) are the microbial polysaccharides which are produced in microbial cell and are then secreted out. In the environment, these EPSs are very important because they play a critical role in adhesion of bacteria to any other solid surface, interaction between cells, and protection of cell (Nicolaus et al. 2010). These EPSs produced by the microorganisms are very helpful in the protection in various environmental conditions because they serve a part of the protective mechanism or reserve material (Rehm 2010). The composition of EPSs varies from species to species, and these EPSs are composed of mostly carbohydrates and some noncarbohydrates as acetate, pyruvate, phosphate, and succinate (Staudt et al. 2012). According to the reports of Kumar et al. (2007), most of the bacteria produce

**Table 24.4** Role of PGPR to regulate the jasmonic acid production and their effects in plants

| S. no. | Rhizobacteria | Phytohormone fluctuated (name) | Plant species | Effects | References |
|---|---|---|---|---|---|
| 1. | *Bacillus* spp. | Jasmonic acid | *Gossypium* | – Higher transcription level of jasmonic acid-related genes<br>– Increase plant defense against insect | Zebelo et al. (2016) |
| 2. | *Bacillus amyloliquefaciens* | Jasmonic acid | *Arabidopsis thaliana* | – Systemic resistance via JA-dependent pathway with the suppression of miR846 expression by *B. amyloliquefacien*s FZB42 | Xie et al. (2018) |
| 3. | *Pseudomonas chlororaphis* O6 | Jasmonic acid | *Arabidopsis thaliana* | – Jasmonic acid marker genes VSP1 and PDF1.2 were up-regulated in colonized plants | Cho et al. (2013) |
| 4. | *Burkholderia phytofirmans* PsJN | Jasmonic acid | *Arabidopsis thaliana* | – Increasing the expression of defense-related genes | Poupin et al. (2013) |
| 5. | *Pseudomonas chlororaphis* O6 | Jasmonic acid | *Arabidopsis thaliana* | – Increasing the expression of defense-related genes | Cho et al. (2008) |
| 6. | *Bacillus subtilis* | Jasmonic acid | *Cucumis melo* | – Activation of induced systemic resistance which induce the synthesis of JA | García-Gutiérrez et al. (2013) |
| 7. | *Endophytic diazotrophic* | Jasmonic acid | *Prosopis strombulifera* | – Synthesize of JA in plant roots | Piccoli et al. (2011) |
| 8. | *Pseudomonas putida* LSW17S | Jasmonic acid | *Arabidopsis thaliana* | – Induced priming in plant | Ahn et al. (2007) |
| 9. | *Achromobacter xylosoxidans* and *Bacillus pumilus* | Jasmonic acid | *Helianthus annuus* L. | – Enhance the jasmonic acid content in shoot | Castillo et al. (2013) |
| 10. | *Achromobacter xylosoxidans*, *Alcaligenes*, *Bacillus pumilus* | Jasmonic acid | *Helianthus annuus* L. | – Increase the production of JA | Forchetti et al. (2007) |

**Table 24.5** The role of PGPR in regulation of ethylene with effects on plants

| S. no. | Rhizobacteria | Phytohormone fluctuated (name) | Plant species | Effects | References |
|---|---|---|---|---|---|
| | | | | – | et al. (2013) |
| 1. | *Azospirillum brasilense* and *Herbaspirillum seropedicae* | Ethylene | *Zea may* | – Decline in the ethylene content due to the production of ACC deaminase by PGPR | Cohen et al. (2009) |
| 2. | *Bacillus licheniformis* | Ethylene | *Piper nigrum* | – Decline in the ethylene content | Lim and Kim (2013) |
| 3. | *Bacillus subtilis* | Ethylene | *Trigonella* spp. | – ACC content reduced | Barnawal et al. (2013) |
| 4. | *Bacillus subtilis* | Ethylene | *Oryza sativa* L. | – ACC declined | Barnawal et al. (2017a, b) |
| 5. | *Achromobacter xylosoxidans*, *Pseudomonas oryzihabitans*, *Variovorax paradoxus* | Ethylene | *Solanum tuberosum* | – Decline rhizosphere ACC concentrations | Belimov et al. (2015) |
| 6. | *Pseudomonas fluorescens* | Ethylene | *Arabidopsis thaliana* | – Downregulation of ethylene-related genes | Wang et al. (2005) |
| 7. | *Pseudomonas Chlororaphis* | Ethylene | *Arabidopsis thaliana* | -Upregulation of ethylene response gene (HEL) | Cho et al. (2013) |
| 8. | *Bacillus* spp. and *Enterobacter* spp. | Ethylene | *Mucuna pruriens* | – Reduce ethylene production | Saleem et al. (2018) |
| 9. | *Achromobacter piechaudii* | Ethylene | *Solanum lycopersicum* L. and *Piper nigrum* seedlings | – Decline in ethylene concentration under drought stress<br>– Increased fresh and dry weight | Mayak et al. (2004) |

| | | | | | |
|---|---|---|---|---|---|
| 10. | *Bacillus subtilis*, *Bacillus subtilis*, and *Bacillus safensis* | Ethylene | *Zea mays* | – Reduction of ethylene | Misra and Chauhan (2020) |
| 11. | *Streptomyces* spp. | Ethylene | *Oryza sativa* L. | – Declining the ethylene content | Jaemsaeng et al. (2018) |
| 12. | *Arthrobacter protophormiae* | Ethylene | *Pisum sativum* | – ACC content and ethylene production declined | Barnawal et al. (2014) |
| 13.` | *Variovorax paradoxus* 5C-2 | Ethylene | *Arabidopsis thaliana* | – Decline in ethylene production | Chen et al. (2013) |
| 14. | *Methylobacterium fujisawaense* | Ethylene | *Brassica campestris* | – Reduce ethylene production | Madhaiyan et al. (2006) |
| 15. | *Agrobacterium tumefaciens* | Ethylene | *Cucumis melo* | – Inhibited ethylene production | Nonaka et al. (2008) |
| 16. | *Enterobacter* sp. | Ethylene | *Oryza sativa* L. | – Decline in ethylene production | Sarkar et al. (2018) |
| 17. | *Bacillus subtilis* | Ethylene | *Solanum lycopersicum* L. | – Reduced ethylene production | Tahir et al. (2017) |

either homopolysaccharides or heteropolysaccharides. These homopolysaccharides need specific substrate, whereas in case of the heteropolysaccharides, the residues are produced intracellularly (Nwodo et al. 2012). Protection of cells from desiccation, effect of antibiotics, predation, antibodies, and antimicrobial substances under different biotic and abiotic stress is caused by the EPSs (Mata et al. 2006; Kumar and Moody 2009; Kumar et al. 2007; Donot et al. 2012; Ordax et al. 2010). The reports of Donot et al. (2012) suggest that EPSs help the microbial diversity because they provide a substrate for the growth of microbes.

There is extensive progress made in the field of elucidating the genetic and biosynthetic mechanism that is involved in the exopolysaccharide synthesis. EPS production occurs both extracellularly and intracellularly (Li and Wang 2012). Expression of enzymes and availability of sugar precursors are various metabolic and physiological parameters that play important role in the regulation of biosynthesis (Delbarre-Ladrat et al. 2014). Bacterial EPSs are produced intracellularly and after their generation are exported to the extracellular environment. In case of homopolysaccharides, these are synthesized outside the cells with the help of enzymes that help in the conversion of the substrate into the polymer (Li and Wang 2012).

The mechanisms involved in the production of EPSs include Wzx/Wzy-dependent pathway, the synthase-dependent pathway, and the ATP-binding cassette (ABC) transporter-dependent pathway. Another mechanism involved is the extracellular synthesis with the help of a singe sucrose protein. In case of intracellular production of EPSs, the precursor molecules inside the cell are transformed with the help of enzymes to produce sugar, whereas in case of the extracellular generation of EPSs, cleavage of disaccharides or trisaccharides by the addition of monosaccharides takes place (Schmid and Sieber 2015). Activated sugars are linked by GTFs in a specific sequence to a lipid carrier until there occurs the formation of repeating units (either regular or irregular) from nucleotide precursors. Intermediates from the carbon metabolism that are generated intracellularly are the sources of precursors involved in the intracellular production of EPSs. These precursors include sugar nucleotides as nucleoside diphosphate sugars, nucleoside diphosphate sugar derivatives, and nucleoside diphosphate sugar acids (Rehm 2010).

GTFs also help in catalyzing the biosynthesis of heteropolysaccharides. In its biosynthesis, there are many intracellular steps, and the last step involving the polymerization of repeating units occurs extracellularly, whereas in case of homopolysaccharides, their biosynthesis occurs extracellularly with the help of GTFs (Badel et al. 2011).

### 24.3.3 Role of Volatile Organic Compounds (VOCs)

Microbial VOCs are lipophilic signaling compounds with low molecular weight below 300 g/mol or 300 Da, very low boiling point, high vapor pressure (0.01 kPa at 20 °C), and high evaporative nature. Such kinds of high evaporative nature and vapor pressure help these VOCs to diffuse easily through various kinds of mixtures of solids, liquids, and gases (Vaishnav et al. 2017) over a short and long distance in any kind of medium, i.e., air, soil, and water (Kanchiswamy et al. 2015a, b). However, VOC production is species-specific. These VOCs are mainly involved in signaling by acting as a stimulus which activates the series of signal responsible for regulating physiological processes and growth-promoting factors inside the plant system that in turn leads to further activation of signaling among organisms, with its surroundings, and cell to cell signaling which finally contributes to plant health (Bailly and Weisskopf 2012; Kai et al. 2016).

Volatile organic compounds (VOCs) are eco-friendly and alternative sources for sustainable development. Soil microbes especially plant growth-promoting rhizobacteria (PGPR) produce gaseous organic compounds known as volatile organic compounds (VOCs). Inside the soil, these rhizobacteria strains (VOCs) cause alteration in root architecture. Further, these microbial volatile organic compounds (mVOCs) are responsible for promoting plant health, inducing systemic resistance/tolerance (ISR/IST) in plants (Panpatte et al. 2017), and sustaining soil health. Not only PGPRs are capable of producing VOCs inside the soils, but also plants are capable of producing and releasing wide ranges of volatile organic compounds mainly from different plant parts like root, shoot fruits, leaves, and flowers (Dudareva et al. 2006). These volatile compounds are part of 1% of secondary metabolites of plant system which were able to cross freely inside the membranes of cell and are also able to release inside the soil or atmosphere. However, inside the plant system, VOCs are generally emitted from leaves in low levels, but under stress condition, there was rise in the levels of VOCs (Opriş et al. 2013). Further, these microbial volatile organic compounds (mVOCs) structurally contain plethora of bioactive chemical groups (Lemfack et al. 2018; Kanchiswamy et al. 2015a, b) responsible for inducing growth in various plants species, such as alkanes, alkenes, alcohols, ester, ketones, benzenoids, pyrazines, sulfides, furans, and terpenes (as shown in Table 24.6).

### 24.3.4 Siderophore Production

Siderophores are small, low-molecular-chelating, peptidic compounds generally produced by bacteria, fungi, cyanobacteria, and plants (phytosiderophores). These chemicals mediate the uptake of essential iron (III) from the environment, convert them into soluble complexes, and transport them to the cells, thereby improving iron nutrition (Shen et al. 2013; Raines et al. 2015; Řezanka et al. 2019). Among the multiple uses of these chelating compounds including culturing of unculturable microfauna (Lewis et al. 2010; D'Onofrio et al. 2010; Kim et al. 2011), natural siderophores help in agriculture by increasing the crop output and by suppressing the growth of phytopathogens that damage the major crops by limiting the availability of iron and even reducing heavy metal uptake (Braud et al. 2009; Shen et al. 2013; O'Brien et al. 2014; Řezanka et al. 2019). Thus, siderophore-producing PGPRs are efficiently used for promoting plant health.

**Table 24.6** Bioactive microbial volatile organic compounds as growth inducers in various plants species

| S. no. | Bioactive microbial volatile organic compound (VOCs) | PGPR | Microbial VOC doses range | Plant responses | References |
|---|---|---|---|---|---|
| 1. | 2,3-Butanediol | *Bacillus amyloliquefaciens* | 1 and 100μg | Ameliorate drought stress | Ryu et al. (2003) |
| 2. | 2R,3R-Butanediol and 3-hydroxy-2-butanone (acetoin) | *Bacillus amyloliquefaciens* IN937a and *Bacillus subtilis* GB03 | 20μl | Enhanced growth in *Arabidopsis* plants by modulating expression of genes involved in cell wall structure | Ryu et al. (2004) |
| 3. | 2,3-Butanediol | *Pseudomonas chlororaphis* strain O6 | VOCs produced itself by *Pseudomonas chlororaphis* strain O6 | Prevents water loss by closing the stomata | Cho et al. (2008) |
| 4. | 2-Pentylfuran | *Bacillus megaterium* strain XTBG34 | 0.5μg/μl | Increase in fresh weight | Zou et al. (2010) |
| 5. | Dimethylhexadecylamine | *Arthrobacter agilis* | 8–32μM | Increase fresh weight, stem length, root length, and root density of *Medicago sativa* seedlings | Velázquez-Becerra et al. (2011) |
| 6. | β-Caryophyllene | *Bacterial consortium*, *Serratia* sp. strain DM1, and *Achromobacter* sp. strain | 25–100μM | Enhance root/shoot length, fresh weight, and chlorophyll of *Lactuca sativa* seedlings | Minerdi et al. (2011) |
| 7. | Dimethyl disulfide (DMDS) | *Bacillus ambifaria* | 2μl | Alter the growth and root architecture of *Arabidopsis thaliana* | Huang et al. (2012) |
| 8. | Dimethyl disulfide | *Bacillus* sp. | 50μM | Act as nutritional sulfur source to the tobacco (*Nicotiana attenuata*) seedlings | Meldau et al. (2013) |
| 9. | Dimethyl disulfide and acetophenone | *Burkholderia ambifaria* strains | 1 ng/μl and 1μg/μl | Increase biomass in *Arabidopsis thaliana* | Groenhagen et al. (2013) |
| 10. | 3-Hydroxy-2-butanone | *Bacillus vallismortis* EXTN-1 | 1 and 10 ppm | Increase fresh weight of tobacco | Ann et al. (2013) |

| | | | | | |
|---|---|---|---|---|---|
| 11. | Indole | *Proteus vulgaris* JBLS202 | Up to 10µg/µl | Induce growth in *Arabidopsis thaliana* | Bhattacharyya et al. (2015) |
| 12. | Indole | *Bacillus subtilis*, *Bacillus thuringiensis*, and *Bacillus megaterium* | 50 ng/h | Stimulate root development and regulating auxinsignaling that protects plants from herbivorous insects | Erb et al. (2015) |
| 13. | 13-Tetradecadien-1-ol, 2-methyl-η-1-tridecene, and 2-butanone | *Pseudomonas fluorescens* strain SS101 | 5 and 50 ng | Increase fresh weight of tobacco | Park et al. (2015) |

Under water-deficit conditions also, PGPRs have been found to be involved in siderophore production (Kim et al. 2012). In tea rhizosphere, siderophore-producing *Bacillus megaterium* de Bary TRS-4 helps in the plant growth promotion and reduction of disease intensity (Chakraborty et al. 2006). Similarly, endophytic bacteria *Escherichia coli* (S6 isolate; 2.4 mg/ml) sequestered and described from *Saccharum* spp. and *Pseudomonas* (R6; 3.0 mg/ml) from *Lolium perenne* supplied maximum siderophores, thereby promoting growth of the plants (Gangwar et al. 2017). Siderophores produced by fungi *Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma harzianum* increased length of shoot and root in chickpeas (*Cicer arietinum*) (Yadav et al. 2011). Even siderophore activity of bacterial consortium (*Pseudomonas putida* NBRIRA and *Bacillus amyloliquefaciens* NBRISN13) ameliorated drought stress in chickpea (Kumar et al. 2016a, b). In maize also, *Azotobacter* strains (Az63, Az69, and Az70) were found to be most effective in enhancing plant growth under different drought stress conditions (Shirinbayan et al. 2019).

### 24.3.5 Role of ACC Deaminase-Producing Rhizobacteria

Plant rhizobacteria play a significant role by activating or producing ACC deaminase (Nascimento et al. 2014), which promotes plant growth under stress condition (Glick 2012). ACC deaminase also plays important role in changing the root and shoot morphology (Molina-Favero et al. 2008) and acts as a biocontrol agent against the pathogen (Hao et al. 2011), inducing nodulation in leguminous plants and leaf and delaying flower senescence (Ali et al. 2012). Various rhizobacteria exhibiting ACC deaminase activities have been reported in Table 24.7.

## 24.4 Role of PGPRs in Activating Antioxidative Defense System Under Drought Stress

Plant growth-promoting rhizobacteria form an inexpensive and an indispensable means that hold a promise to promote the overall growth and development in plants. Commonly called as biofertilizers, these are employed either as a single strain or as bacterial consortium for obtaining effective results (Carmen et al. 2016; Alori et al. 2017). PGPRs also help in reducing the detrimental effects of ROS production in plants during stress. This is achieved by activating the antioxidative defense system utilizing both enzymatic and nonenzymatic elements (Ojuederie et al. 2019). The enzymatic forms help in maintaining cellular homeostasis by combatting the oxidative stress and include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) (Chojak-Koźniewska et al. 2017; Choudhury et al. 2017; Ojuederie et al. 2019). On the other hand, plants also produce osmolytes such as proline, glutamate,

**Table 24.7** Role of ACC deaminase-producing rhizobacteria in plants

| S. no. | Rhizobacteria producing ACC deaminase | Role of ACC deaminase | Plant species | References |
|---|---|---|---|---|
| 1. | *Aneurinibacillus aneurinilyticus and Paenibacillus* sp. | – Alleviate the negative effects of salinity, and promote the plant growth by increasing the shoot and root length and biomass. | *Phaseolus vulgaris* | Pandey and Gupta (2019) |
| 2. | *Variovorax paradoxus*, *Pseudomonas* spp., *Achromobacter* spp., *and Ochrobactrum anthropi* | – Enhance the plant growth and foliar nutrient | *Triticum aestivum* L. | Chandra et al. (2019) |
| 3. | *Agrobacterium fabrum or Bacillus amyloliquefaciens* | – Improve growth and yield | *Triticum aestivum* L. | Hartmann et al. (2006) |
| 4. | *Bacillus* sp. | – Increased the photosynthetic ROS-scavenging enzymes<br>– Enhanced plant growth and biomass | *Solanum tuberosum* | Gururani et al. (2013) |
| 5. | *Burkholderia phytofirmans* strain PsJN | – Oxidative stress declined and increased minerals | *Triticum aestivum* L. | Naveed et al. (2014) |
| 6. | *Bacillus thuringiensis* AZP2 and *Paenibacillus polymyxa* B | – Increase biomass, growth and the photosynthesis | *Triticum aestivum* | Timmusk et al. (2014) |
| 7. | *Azotobacter chroococcum* strains 67B and 76A | – Water availability increased under drought stress and improved nutrients | *Solanum lycopersicum* | Viscardi et al. (2016) |
| 8. | *Burkholderia cepacia* | – Chlorophyll improved<br>– Improve plant growth | *Capsicum annuum* | Maxton et al. (2018) |
| 9. | *Pseudomonas fluorescens* DR7 | Seedling improve | *Setaria italica* L. | Niu et al. (2018a, b) |
| 10. | *Bacillus megaterium* TV 6D<br>*Bacillus subtilis* TV 12H | – Improve plant growth | *Lactuca sativa* | Sahin et al. (2015) |
| 11. | *Bacillus subtilis* | – Shoot and root length increases | *Pisum sativum* L. | Saikia et al. (2018) |
| 12. | *Pseudomonas aeruginosa* | – Increase chlorophyll content and biomass | *Capsicum annum* L. | Gupta et al. (2019) |
| 13. | *Pseudomonas fluorescens* REN1 | – Increase root length and tolerant to flooded environment | *Oryza sativa* | Etesami (2018) |

(continued)

**Table 24.7** (continued)

| S. no. | Rhizobacteria producing ACC deaminase | Role of ACC deaminase | Plant species | References |
|---|---|---|---|---|
| 14. | *Bacillus amyloliquefaciens* | – Improved tolerance to salt and enhanced chlorophyll content | *Zea mays* | Chen et al. (2016a, b) |
| 15. | *Consortia of Bacillus* isolate 23-B *and Pseudomonas* 6-P *with Mesorhizobium ciceri* | – Enhance proline concentration<br>– Improve seed germination and plant growth | *Cicer arietinum* | Sharma et al. (2013) |
| 16. | *Enterobacter aerogenes* (LJL-5), *Pseudomonas aeruginosa* (LJL-13) | – Enhance the shoot length and yield of plant | *Alfalfa* | Liu et al. (2019a, b) |
| 17. | *Azotobacter chroococcum* CAZ3 | – Enhance production | *Zea mays* L. | Rizvi and Khan (2018) |
| 18. | *Pseudomonas aeruginosa* | – Enhance seed germination | *Brassica juncea* | Aka and Babalola (2016) |
| 19. | *Pseudomonas fluorescens and Pseudomonas putida* | – Increase shoot root length | *Brassica napus* | Grobelak et al. (2015) |
| 20. | *P. fluorescens* 002 | – Increase biomass production | *Zea mays* | Zerrouk et al. (2016) |
| 21. | *Pseudomonas fluorescens* | – Increase chlorophyll content and root shoot length | *Cucumis sativus* | Nadeem et al. (2016) |
| 22. | *Pseudomonas simiae* | – Increase lateral roots<br>– Stomata closure | *Vigna radiata* | Kumari et al. (2016) |
| 23. | *Pseudomonas aeruginosa* strain 2CpS1 | – Promote plant growth by increasing height and biomass | *Triticum aestivum* | Meena et al. (2015) |
| 24. | *Pseudomonas* strain | – Increase photosynthetic rate and chlorophyll content | *Vigna radiata* | Ahmad et al. (2013) |
| 25. | *P. fluorescens* YsS6<br>*Pseudomonas migulae* | – Promote flowering and bud formation | *Solanum lycopersicum* | Ali et al. (2014a, b) |
| 26. | *Agrobacterium fabrum, Bacillus, and Bacillus amyloliquefaciens* | – Improve plant growth and yield | *Triticum aestivum* | Zafar-ul-Hye et al. (2019) |
| 27. | *Leclercia decarboxylata and A. fabrum* | – Nutrient content is improved in plant | *Triticum aestivum* | Danish et al. (2019) |
| 28. | *Bacillus* spp. | – Enhance plant growth | *Zea mays* | Misra and Chauhan (2020) |
| 29. | *Bacillus subtilis* | | | |

(continued)

**Table 24.7** (continued)

| S. no. | Rhizobacteria producing ACC deaminase | Role of ACC deaminase | Plant species | References |
|---|---|---|---|---|
| | | – Protect plant from oxidative damage<br>– Promote plant growth | *Solanum lycopersicum* L. | Gowtham et al. (2020) |
| 30. | *Enterobacter oryzendophyticus*, *Pseudomonas putida*, and *Pseudomonas lini* | – Protect the plant from downy mildew and promote growth | *Papaver somniferum* L. | Barnawal et al. (2017a, b) |

glycine betaine, carnitine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose, and oligosaccharides that mitigate oxidative stress (Ali et al. 2017) (Table 24.8).

## 24.5 PGPRs and Nutrient Mobilization Under Drought Stress

As plants are immobile, they face various abiotic stresses such as waterlogging, extreme temperature, drought, salinity, and heavy metal stress. These stresses are responsible for affecting different physiological and metabolic activities in plants. Drought is one of the important abiotic factors affecting crop growth and reduces the yield of crop globally. This is due to alterations caused in the photosynthetic efficiency and other physiological activities of the cells. Due to drought, there occurs closing of the stomata resulting in the reduction in the amount of $CO_2$ responsible for photosynthesis and photorespiration increases. As a result, photosynthetic metabolism is affected resulting in the production of ROS (Pinheiro and Chaves 2011; Chaves et al. 2009). Due to stomatal closing, the production of superoxide and hydrogen peroxide increases, and these increases accelerate the water cycle (Asada et al. 2006). In drought, seed germination is either delayed or stopped because for the seed germination adequate moisture is required (Cairns et al. 2013). Drought is also responsible for affecting plant growth, cell elongation, and cell division because of cell dehydration resulting in the osmotic stress (Kaushal and Wani 2016). Plants adjust themselves in drought condition in order to survive in several ways following different mechanisms as plants complete their life cycle before the start of drought or avoid drought by withstanding high water status or by sustaining their metabolic activities (Li et al. 2009; Aroca et al. 2009).

Besides various mechanisms adopted by plants, the use of PGPRs is a cost-effective method of improving crop production and thereby relieving stress in plants (Alori et al. 2017). The PGPRs help in iron sequestration, synthesis of exopolysaccharides and beneficial enzymes, and mobilization of phosphorus in soil (Glick 2014). In addition to these benefits, the microbes in the rhizosphere enhance nutrient availability and uptake and thus cause plant growth.

Among various mechanisms, phosphate solubilization by the phosphate-solubilizing bacteria is also one of the method adopted by PGPRs to help plants in

**Table 24.8** Effects of PGPR on plant species (singly/consortium) and antioxidative defense system of plants under drought stress

| S. no. | Plant species | PGPR strain/ consortium | Effects on antioxidative defense system | References |
|---|---|---|---|---|
| 1. | *Arabidopsis thaliana* | *Azospirillum brasilense* Sp245 strain | Increase in proline and lipid peroxidation | Cohen et al. (2015) |
| 2. | *Cucumis sativa* | *Bacillus cereus* strain AR156B, *B. subtilis* strain SM21, and *Serratia* sp. strain XY21 | Reduction in leaf monodehydroascorbate content and increased proline and SOD activity in leaves | Wang et al. (2012a, b) |
| 3. | *Hyoscyamus niger* | *Pseudomonas putida* strain (PP) and *Pseudomonas fluorescens* strain (PF) | Stimulated activities of SOD and POD while CAT decreased, and increased proline accumulation, and improved alkaloid content | Ghorbanpour et al. (2013) |
| 4. | *Solanum tuberosum* | *Bacillus pumilus* strain DH-11 and *Bacillus firmus* strain 40 | Increased proline content and level of APX, SOD, and CAT | Gururani et al. (2013) |
| 5. | *Sorghum bicolor* | *Bacillus* spp. strains KB122, KB129, KB133, and KB142 | Increased sugar, chlorophyll, and proline content | Grover et al. (2014) |
| 6. | *Vigna radiata* | *Pseudomonas fluorescens* strain Pf1; *Bacillus subtilis* EPB5, EPB22, and EPB 31 | Increased content of proline, CAT, and POD | Saravanakumar et al. (2011) |
| | | *Pseudomonas aeruginosa* | Increased activity of SOD, POX, and CAT | Sarma and Saikia (2014) |
| 7. | *Zea mays* | *Pseudomonas entomophila* strain BV-P13; *P. stutzeri* strain GRFHAP-P14; *P. putida* strain GAP-P45; *P. syringae* strain GRFHYTP52; *P. monteilii* strain WAPP53 | Increased antioxidant enzymes APX, CAT, and GPX activities and increased levels of proline, sugars, and free amino acids | Sandhya et al. (2010) |
| | | *Bacillus amyloliquefaciens* strain HYD-B17; *B. licheniformis* strain HYTAPB18; *B. thuringiensis* strain HYDGRFB19; | Increase in antioxidant enzymes and enhanced production of proline, amino acids and soluble sugars along with improved protein content in leaves | Vardharajula et al. (2011) |

(continued)

**Table 24.8** (continued)

| S. no. | Plant species | PGPR strain/ consortium | Effects on antioxidative defense system | References |
|---|---|---|---|---|
| | | *Paenibacillus favisporus* strain BKB30; *B. subtilis* strain RMPB44 | | |
| | | EPS-producing *Pseudomonas* sp. | Significantly reduced activities of SOD, POD, CAT, and proline | Naseem and Bano (2014 |
| | | *Ochrobactrum* sp. NBRISH6 | Significant lower expression of genes involved in antioxidant enzyme activities (SOD, CAT, APX, GPX, and PPO), proline, and soluble sugar content | Mishra et al. (2020) |
| 8. | *Oryza sativa* | *Pseudomonas fluorescens* strain P2; *P. jessenii*R62; *P. synxantha* R81; *Bacillus cereus BSB* 38(14B); *Arthrobacter nitroguajacolicus* strain YB3 and strain YB5 | Higher proline content and enhanced activities of SOD, CAT, POD, and APX but lower $H_2O_2$ and MDA in leaves | Gusain et al. (2015) |
| | | *Bacillus amyloliquefaciens* Bk7 and *Brevibacillus laterosporus* B4 | Significant decrease in leaf MDA content and electrolyte leakage, increased leaf proline and chlorophyll contents, and increased activities of SOD and CAT by threefold and 3.6-fold, respectively | Kakar et al. (2016) |
| | | *Pseudomonas fluorescens* (*Pf*) (OKC; JN128891); *Trichoderma asperellum* (*Th*) (T42; JN128894) | Overexpression of genes of key metabolic pathways of phenylpropanoid (PAL), SOD, $H_2O_2$ peroxidation (APX, PO), and CAT resulting in enhanced activities of POD, APX, GPX, and GR | Singh et al. (2020) |
| 9. | *Lavandula dentate* | *Bacillus thuringiensis* | Decreased GR and APX activities | Armada et al. (2014) |

(continued)

**Table 24.8** (continued)

| S. no. | Plant species | PGPR strain/ consortium | Effects on antioxidative defense system | References |
|---|---|---|---|---|
| 10. | *Ocimum basilicum* | *Pseudomonades* sp., *Bacillus lentus*, and *A. brasilense* | Increased CAT, APX, and GPX activities | Heidari and Golpayegani (2012) |
| 11. | *Triticum aestivum* | *Bacillus thuringiensis* | Increased activity of GR, CAT, SOD, and alginate | Timmusk et al. (2014) |
| 12. | *Mentha piperita* | *Pseudomonas fluorescens* WCS417r and *Bacillus amyloliquefaciens* GB03 | Higher activities of POD and SOD, reduced proline level, and higher accumulation of total phenolic content | Chiappero et al. (2019) |
| *13.* | *Glycine max* | *P. putida* H-2-3 | Declined SOD, flavonoids, and radical scavenging activity | Kang et al. (2014) |
| 14. | *Cicer arietinum* | *Pseudomonas putida* NBRIRA and *Bacillus amyloliquefaciens* NBRISN13 | Modulated activities of SOD, CAT, lipid peroxidase (LPX), and PAL | Kumar et al. (2016a, b) |
| | | *P. putida* MTCC5279 (RA) | Significant decrease in MDA accumulation, SOD, and CAT activities | Tiwari et al. (2016) |
| 15. | *Fragaria ananassa* Duch. | *Paenibacillus polymyxa* RC05; *P. polymyxa* RC35; *Pseudomonas fluorescens* RC77; *P. fluorescens* RC86; *P. putida* RC06; *P. putida* 29/2; *Rhodococcus erythropolis* RC9 | Increased total phenolic content, trolox equivalent antioxidant capacity, and GR, GST, CAT, POD, SOD, and APX activities but decreased MDA and $H_2O_2$ contents | Erdogan et al. (2016) |
| 16. | *Trifolium arvense* | *Pseudomonas azotoformans* ASS1 | Enhanced chlorophyll, CAT, POD, SOD, and proline contents in leaves but lower concentrations of malondialdehyde | Ma et al. (2017) |

combating the drought stress. Phosphorus is an important nutrient required by plants for their optimum growth. Phosphorus present in soil is mostly in the form of insoluble, organic, and inorganic compounds, and the soluble forms are present only in low concentration and therefore cause limited plant growth (Zaidi et al. 2009). Phosphorus-solubilizing bacteria convert immobile phosphorus in the form of organic phosphorus which is easily used by plants. In some bacterial strains, there is coexistence of both phosphorus solubilization and mobilization (Tao and Zhi-biao 2008).

### 24.5.1 Phosphate Solubilization

Among different PGPRs, the most effective phosphorus-solubilizing bacteria belong to the genera *Rhizobium*, *Bacillus*, and *Pseudomonas*. Some of the examples of major phosphorus-solubilizing bacteria and their host plants are *Azotobacter chroococcum* that helps in solubilizing phosphorus in wheat (Kumar and Narula 1999), *Bacillus megaterium* in tea (Chakraborty et al. 2015), *Enterobacter agglomerans* in tomato (Kim et al. 1998), *Pseudomonas chlororaphis*, and *Pseudomonas putida* in soybeans (Cattelan et al. 1999).

There are mainly two mechanisms which are followed by the bacteria for the phosphorus solubilization. These bacteria release low-molecular-weight organic acids such as ketogluconic and gluconic acids and thereby affect the mobility of phosphorus through the ionic interactions (Tao and Zhi-biao 2008). In another mechanism, the solubilization of phosphorus occurs with the help of phosphatases. These phosphatases help in unbinding the phosphates from the organic matter, thereby solubilizing and increasing the mobilization of the phosphorus (Goldstein 1995). The schematic representation of solubilization of phosphorus in soil with the help of phosphorus-solubilizing bacteria is given in Fig. 24.2.

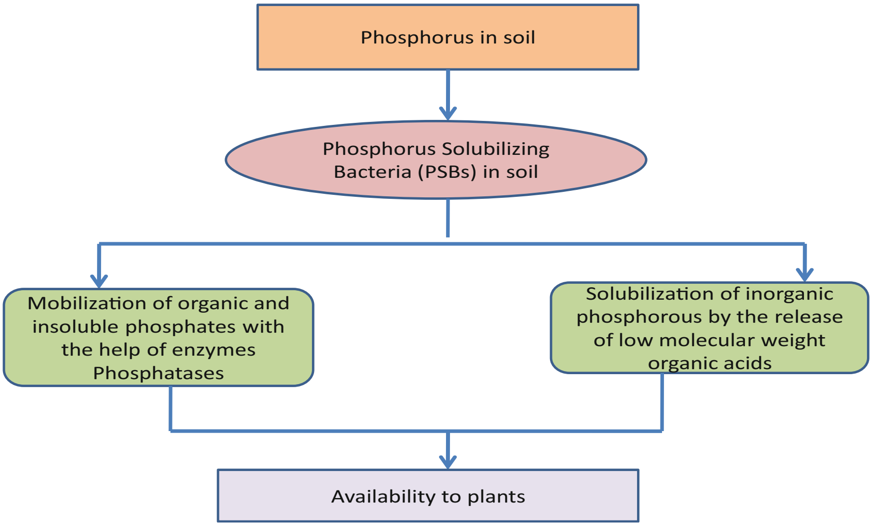

**Fig. 24.2** Mobilization and solubilization of phosphorus in soil

Enzyme phosphatases help in the mineralization of the insoluble and organic phosphorus where the organic acids are used for the solubilization of inorganic phosphorus (Glick 2012). It has been reported by Zaidi et al. (2009) that when phosphorus-solubilizing bacteria are applied, these bacteria also help in the availability of other elements and biological nitrogen fixation.

### 24.5.2 Sulfur Mobilization Under Drought Stress

Sulfur, one of the essential plant nutrients, is required for the growth and development of plants. S is mainly absorbed through the roots in the form of $SO_4^{2-}$ ions. Sulfur plays an important role in various physiological and biochemical activities of plants, viz., formation of chlorophyll; synthesis of oil, proteins, and important constituent of three S-containing amino acids like cysteine, cystine, and methionine; and improving crop yield and quality. Drought stress reduces the growth of several crops like maize, barley, wheat, and rice by decreasing nutrient diffusion and bulk flow of various water-soluble nutrients such as magnesium, calcium, nitrate, silicon, and sulfate (Selvakumar et al. 2012). Generally, nutrients are carried to the roots by water; drought stress is known to impact on the availability and transport of nutrients (Vandana et al. 2020). But microorganisms help to mobilize organically and inorganically bound sulfur which the plant can then readily utilize. In one of the study reported by Fox et al. (2014), it was observed that rhizobacteria-amended biochar enhances mobilization of S and P resulting in improved plant growth of *Lolium perenne*.

Microbial inoculants improve the soil health by mobilizing the micronutrients of soil like S, Mn, Mg, Fe, Mo, B, Zn, and Cu and making them available to the plants. In one of the study conducted at University of Agricultural Sciences, Bangalore (Karnataka), on red sandy clay loam soil with soybean crop, it was observed that with the application of 100% NPKS + Zn, B, Mo + rhizobium + PSB, available phosphorus and sulfur were low (36.4 and 13.5 kg/ha, respectively) as compared to control which might be due to uptake of residual sulfur and phosphorus present in the soil by the crop (Kumar et al. 2017). There are two critical processes in sulfur cycling in soils: immobilization of inorganic sulfur and mobilization of organically bound sulfur. Both of these are thought to be microbially mediated. Microbes act as catalyst in transformation of organic sulfate pool consisting of ester sulfur and carbon-bounded sulfur into inorganic sulfate in soil. This inorganic sulfate is later absorbed/utilized by plants (Kertesz and Mirleau 2004).

## 24.6 Molecular Mechanism and Gene Expression Associated with PGPRs and Plants Under Drought Stress

Drought tolerance is a highly intricate process and associated with the regulation of multiple gene expressions induced during drought stress (Lang and Buu 2008). Segregation mapping and quantitative trait loci (QTL) analysis provide molecular basis of drought tolerance. These analyses have been carried out in many crops like wheat, maize, rice, cotton, and barley, and 51 QTL for ten traits on ten different chromosomes under water-deficit condition have been identified. Out of these, 22 QTL were responsible for various root characteristics associated with drought stress tolerance (Guo et al. 2013). Drought stress induces the expression of two major sets of genes, viz., functional gene and regulating gene (Chinnusamy et al. 2004). Functional genes expressed in various products under drought stress include chaperons, late embryogenesis abundant (LEA) proteins, antifreeze proteins, mRNA-binding proteins, osmoprotectants, water channel proteins, detoxifying enzymes, key enzymes for osmolyte biosynthesis, free radical scavengers, and various protease gene products which directly protect the cell against the stress (Bray 2002).

The products of the regulating genes regulate the expression of others gene in response to drought stress such as transcription factors, protein phosphatases, kinases (mitogen-activated kinases (MAPKs)), calcium-dependent protein kinases (CDPKs) and SOS kinases and enzymes involved in phospholipid metabolism, and other signaling molecules such as calmodulin-binding protein (Xiong et al. 2002). In transgenic plants, transcription factors regulate the expression of various stress-inducible genes which in turn enhanced the abiotic stress tolerance by encoding key enzymes regulating biosynthesis of compatible solutes such as amino acids (e.g., proline), quaternary and other amines (e.g., glycinebetaine and polyamines), and a variety of sugars and sugar alcohols (e.g., mannitol, trehalose, galactinol, and raffinose) (Bartels 2005). LEA protein- and heat shock protein-coding genes also conferred drought tolerance in transgenic plants by alleviating the cellular damage. It has been observed that galactinol synthase (GolS) (a key enzyme involved in raffinose family oligosaccharide biosynthesis)-encoding gene, induced by drought, cold, and ABA, is involved in drought stress tolerance in transgenic *Arabidopsis* (Umezawa et al. 2004) (Fig. 24.1).

In semiarid and arid areas, drought stress is ameliorated through PGPR inoculation of plants. PGPRs colonize in the rhizosphere of plants and promote plant growth through direct or indirect mechanisms (Ali et al. 2017). PGPRs isolated from stressed area also help in providing resistance to host plants against various environmental stresses by modulating in root morphology (Sandhya et al. 2010; Belimov et al. 2009). *Bacillus* sp. helps to overcome the drought stress in maize plants by accumulating compatible solute such as amino acid, quaternary amines, and sugar that prevent degeneration process (Vardharajula et al. 2011). *Bacillus subtilis*-inoculated water-stressed maize plants showed improvement in plant biomass, relative water content, leaf water potential, and root-adhering soil/root tissue ratio. Another microbe named *Phyllobacterium brassicacearum* STM196 strain, isolated from the rhizosphere of oilseed rape, enhanced water use efficiency, along with biomass under water-deficit conditions (Bresson et al. 2014). Wang et al. (2012a, b) reported that *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21 conferred induced systemic tolerance to drought stress in cucumber plants (Table 24.9).

**Table 24.9** Responses of drought-stressed plants to PGPRs

| Plant species | PGPR | Response | Reference |
|---|---|---|---|
| Maize | *Bacillus* sp. | Induce accumulation of compatible solute such as amino acid, quaternary amines, and sugar that prevent degeneration process. Also, enhance plant biomass, relative water content, leaf water potential, and root-adhering soil/root tissue ratio | Vardharajula et al. (2011) |
| Oilseed rape | *Phyllobacterium brassicacearum* STM196 | Improve water use efficiency and plant biomass | Bresson et al. (2014) |
| Cucumber | *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21 | Induced systemic tolerance to drought stress | Wang et al. (2012a, b) |

To improve the stress tolerance in plants, plant biotechnologists are focusing on molecular level through genetic engineering on genes encoding stress-protective proteins, ion transport proteins, compatible solutes, antioxidants, heat shock, late embryogenesis-abundant proteins, and transcription factors for gene regulation. Various microbial genes have been frequently used to engineer traits involved in stress tolerance like transgenic tobacco plants carrying bacterial gene coding for mannitol-1-phosphate dehydrogenase (mt1D). This leads to increase in level of mannitol in their cytoplasm and provides tolerance against salt stress and physiological drought. Transgenic plants with imt1 gene encoding for myo-inositol-o-methyltransferase enzyme involved in synthesis of inositol were more drought and salt tolerant as compared to the wild-type plants. The structural gene TPS1 of *Saccharomyces cerevisiae* that encodes for trehalose was fundamentally expressed in transgenic potato plants. The TPS1 transgenic potato plants have been seen to have essentially improved drought resistance as compared to wild type. Genetically engineered rice plants with *E. coli* trehalose biosynthetic genes (otsA and otsB) also showed enhanced abiotic stress tolerance (Vandana et al. 2020).

Molecular studies on alleviation of drought stress by PGPR gene expression are a powerful tool to understand the complex responses of a plant to their environment. The entire set of transcripts that are expressed within a cell or organism at a particular developmental stage or under different environmental conditions constitute the transcriptome. There are various technologies available for assaying the transcriptome like hybridization-based microarrays and RNA sequencing (Wang et al. 2009; Vurukonda et al. 2016). At the transcriptional level, inoculation of PGPR *Paenibacillus polymyxa* B2 enhanced the drought tolerance of *Arabidopsis thaliana*. RNA display confirmed that an mRNA transcription of a drought response gene, EARLY RESPONSE TO DEHYDRATION 15 (ERD15), was augmented in inoculated plants compared to uninoculated plants (Timmusk and Wagner 1999).

**Table 24.10** Induction of stress-responsive genes in drought-stressed plants by PGPR

| Plant species | PGPR | Drought-responsive genes | References |
|---|---|---|---|
| *Arabidopsis thaliana* | *Paenibacillus polymyxa* B2 | Induction of EARLY RESPONSE TO DEHYDRATION 15 (ERD15) genes | Timmusk and Wagner (1999) |
| Pepper | *B. licheniformis* K11 | Increased expression of stress protein genes like Cadhn, VA, sHSP, and CaPR-10 | Lim and Kim (2013) |
| Wheat | *Bacillus amyloliquefaciens* 5113 and *Azospirillum brasilense* NO40 | Upregulation of stress-related genes APX1, SAMS1, and HSP17.8 | Kasim et al. (2013) |
| *Arabidopsis thaliana* | *Pseudomonas chlororaphis* O6 | Transcripts of the jasmonic acid marker genes, VSP1 and PDF1.2; salicylic acid-regulated gene, PR-1; and ethylene response gene, HEL, were upregulated | Cho et al. (2013) |
| Sugarcane | *Gluconacetobacter diazotrophicus* PAL5 | ABA-dependent signaling genes activated | Vargas et al. (2014) |

Using 2-D polyacrylamide gel electrophoresis (2D-PAGE) and differential display polymerase chain reaction (DD-PCR), it was observed that drought-stressed pepper plants inoculated with *B. licheniformis* K11 expressed six different stress proteins. Among the stress proteins, specific genes of Cadhn, VA, sHSP, and CaPR-10 showed more than a 1.5-fold increase in treated plants compared to control (Lim and Kim 2013). Priming of wheat with *Bacillus amyloliquefaciens* 5113 and *A. brasilense* NO40 also confers resistance against drought stress. The expression of stress-related genes like APX1, SAMS1, and HSP 17.8 and activities of antioxidative enzymes were upregulated to overcome the deleterious effects of drought stress in wheat plants (Kasim et al. 2013). PGPR inoculation also downregulates the expression of stress-signaling genes. In the *P. chlororaphis* O6-colonized *A. thaliana*, transcripts of the jasmonic acid marker genes, VSP1 and PDF1.2; salicylic acid-regulated gene, PR-1; and the ethylene response gene, HEL, were upregulated as compared to control plants (Cho et al. 2013).The association between the diazotroph, *Gluconacetobacter diazotrophicus* PAL5, and sugarcane cv. SP70–1143 under drought stress activates the ABA-dependent signaling genes conferring drought resistance in sugar cane cv. SP70-1143 (Vargas et al. 2014) (Table 24.10) (Fig. 24.3).

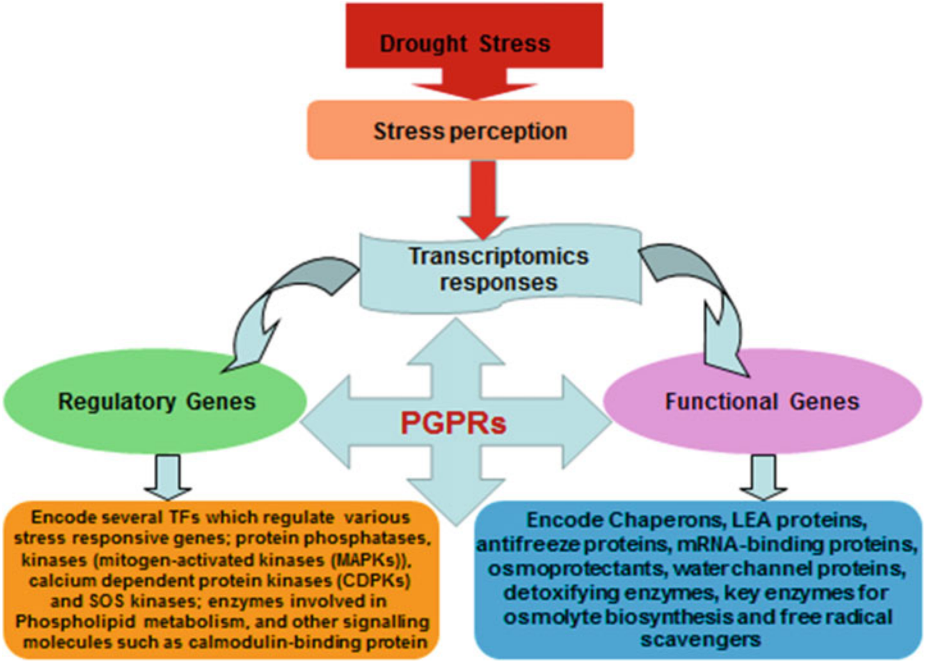

**Fig. 24.3** PGPR-associated gene expression in plants under drought stress

## 24.7 Conclusion

Plants undergo beneficial associations with the soil microbes to counter harsh environmental conditions. This interplay between both the partners is an example of evolutionary coexistence. Under stress conditions, PGPRs help the plants by altering their physiological, biochemical, and molecular processes. Also, they change the endogenous hormonal status in plants by influencing various biosynthetic pathways. Thus, there is a need for exploitation of these PGPRs to meet the maximum yield and to improve the health of food crops by improving nutrient mobilization under unfavorable environmental conditions. Also, the molecular pathways associated with these plant-microbe interaction to counter drought and other stresses need more research to have better understandings of their mechanisms of action.

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# Prominence of Antioxidant Potential of Plants and Its Induction by Interaction with Microorganisms

# 25

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## Abstract

Plant-microbe interaction is of vital importance for improving plant growth and soil structure and controlling plant disease. It is not shocking that such cooperative relationships, where plants provide food for rhizospheric microbes and in exchange microbes help in plant growth and stress resolution, are essentially the basis of sustainable agriculture. In addition, certain plant-microbe interaction contributes to a variety of defence-responsive behaviours including regulation of level of antioxidant in plant by modifying the enzymes, control of quorum-sensing process and stimulation of phenylpropanoid pathway to overcome the pathogen threat. The natural antioxidants in food and medicinal plants are widely spread. These natural antioxidants particularly flavonoids, phenolic acid and carotenoids have a broad range of biological effect.

## Keywords

Plants · Microorganisms · Antioxidant · Potential · Interaction · Biological effect

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## 25.1 Introduction

A large number of destructive organisms such as pathogens and insects are threatening plant species which limit plant growth. To obtain higher yields, a sustainable approach to plant growth promotion and the control of plant diseases is being introduced. Generally, plant species communicate with diverse range of desirable and undesirable microorganisms that provide a base for linking the other members living above and below the ground (Pineda et al. 2010). The rhizospheric interaction may actually influence plant growth by expending either favourable, relatively neutral or unfavourable impact. The interaction of plant-microbes is driven by signal-transduction pathways that enable plants to evaluate the immune response amid physiological stress. The advantageous microbiota is believed to increase intrinsic immunity level of plant towards pathogen attack by triggering a variety of defensive responses which include improvement of the plant's antioxidant level by altering defence-related enzymes, regulation of quorum-sensing activities and stimulation of phenylpropanoid process that leads to production of phenolics and accumulation of lignin.

## 25.2 Type of Antioxidants

*Antioxidants* are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called "free-radical scavengers." The sources of *antioxidants* can be natural or artificial. In animals, antioxidants are compounds capable of counteracting the adverse effects that arise in animal tissues through the physiological phase of oxidation. In case of plants, they produce reactive oxygen species (ROS) as a by-product of aerobic metabolism which can damage the cells. Antioxidants are capable of deactivating those free radicals and prevent them to attack further. These can be nutrients or enzymes that assist in a chemical reaction (Kumar et al. 2017). It is possible to classify antioxidants by solubility and occurrence. According to solubility, antioxidants can be water-soluble or fat-soluble. And on the basis of occurrence, they are divided into natural antioxidants and synthetic antioxidants.

### 25.2.1 Type I: Antioxidants Based on Solubility

*Water-soluble antioxidant*, also called as hydrophilic antioxidants, includes ascorbic acid, glutathione and lipoic acid (Kumar et al. 2017). It scavenges free radicals present inside the cells. Ascorbic acid, also known as vitamin C, is important for the maintenance of the blood vessels, skin and bones (Gupta and Sharma 2006). Glutathione is a tripeptide structure produced by the liver and prevents early signs of ageing. Lipoic acid can be obtained from spinach, carrots, red meat, etc. which improves the metabolism.

*Fat-soluble antioxidant* plays a key role in sustaining a balanced body. It includes carotenes, lycopene, vitamin A and ubiquinol. These can be synthesised by the cells or taken through diet as well. Fat-soluble antioxidants protect cell membrane from damage and from lipid peroxidation. Vitamin A and beta-carotene improve the vision and gastrointestinal health and even foster the immune system. Beta-carotene is known to be the best quencher of singlet oxygen (Huang et al. 2005). Ubiquinol is a type of coenzyme Q10 and synthesised within the body.

### 25.2.2 Type II: Antioxidants Based on Occurrence

*Natural antioxidants* are present in food, green vegetables and medicinal plants. There are broad variety of biological effects of these natural antioxidants especially polyphenols and carotenoids including anti-ageing, anticancer and anti-inflammatory. Phenolic acid constitutes the derivatives of cinnamic acid and benzoic acid (Xu et al. 2017).

Some of the food and medicinal plants that possess antioxidant activity are *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Aloe vera*, *Ocimum sanctum*, *Mangifera indica*, *Solanum nigrum*, *Daucus carota*, *Solanum tuberosum*, *Asparagus racemosus*, *Zingiber* officinale, etc. (Kumar et al. 2012). The various natural antioxidants with their source are mentioned in the table below.

*Synthetic antioxidants* include butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) that was originally created to protect petroleum from oxidative gumming. They have similar structure and antioxidant property.

Also, there are some enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) present inside the body that act as antioxidants. Superoxide dismutase breaks the structure into hydrogen peroxide which in turn is converted into water and oxygen by catalase. And glutathione is a detoxifying agent (Huang et al. 2005).

## 25.3 Plant and Microbe Interactions

Plant interacts with microbe in different forms and in many ways. Both can favour each other, and also, there can be negative impact of their interactions. Definitely, plant fringe benefits to the associated microbe by direct or indirect means. Plant produces few compounds such as amino acids, polysaccharides, sugars, plant growth regulators and secondary metabolites to fascinate the microbe and provide them sheltered habitat; the microbe in turn emanates some compounds which favour the growth of plant and make the plant resistant to various stresses (Schirawski and Perlin 2018). In aquatic ecosystem, microbe and plant interactions depend upon mutual advantage where the plant provides organic carbon and oxygen to the microbe and the microbe gives essential minerals and metabolites for the growth of the plant (Srivastava et al. 2017). Most commonly, microbes and plants share two different types of symbiotic relationship. Either it is endophytic meaning microbes are living within the plant without causing harm or it can be ectophytic in which microbes remain outside the plant. Endophytic bacteria can help plant directly or indirectly as they produce plant growth regulators, perform nitrogen fixation and also metabolise the complex sugar into simpler ones (Taghavi et al. 2009). Endophytes include nitrogen-fixing diazotrophs (Nielsen et al. 2001) and other nutrient assimilators such as arbuscular mycorrhizal fungi (AMF) (Šraj-Kržič et al. 2006). The examples of ectophytes are ammonia-oxidising bacteria (Wei et al. 2011) and methanotrophic bacteria (Sorrell et al. 2002). Mycorrhizal type of symbiosis is commonly found in land plants. In aquatic ecosystem, the interrelatedness of plant and microbe depends upon the accessibility of various nutrient ions for their growth. Excess of nutrients can cause eutrophication of water body which form the cyanobacterial bloom and might be responsible for toxin production (Giaramida et al. 2013). Hence, the aquatic plants absorb the unnecessary nutrient from the water and supress the algal growth. Some of the free-floating macrophytes which can remove the excessive nutrient ions are *Ipomea aquatica*, *Eichhornia crassipes*, etc. (Srivastava et al. 2017).

Mainly, microbe colonises the rhizosphere part of plant, and *Streptomyces* species is one such example which can grow filamentously into the roots and upper parts of the plant. It would protect the plant from harmful bacteria by releasing antibiotics. It also acts as a biocontrol agent and has plant growth-stimulating ability (Vurukonda et al. 2018). These growth-promoting bacteria can aid both in phytoremediation and in the sustainable production of biomass (Montalbán et al. 2017). Rhizobacteria, mycorrhiza and Firmicutes make plant survival possible under metal stress by involving in some mechanisms like volatilisation, metal complexation, efflux and enzymatic detoxification (Glick 2010). Heavy metals like Pb, As and Mn can be removed from the polluted soil by using aggregation of microbes (Zhang et al. 2015). The soil bacteria *Bacillus amyloliquefaciens* GB03 can remarkably revamp the resistance to drought stress of ryegrass (Su et al. 2017). Some other mechanisms like nutrient mobilisation and production of exopolysaccharides help the plant's survival in adverse environment (Vardharajula et al. 2011). Therefore, there is nothing wrong in saying that the microbes could be used to alter or improve the plant growth ability and make them more tolerant to various stresses.

But the negative effect of this interactions is also there, as microbes can be pathogenic to plants. Fungi may be a huge warning to plant well-being, and they are highly host-specific, and host switching may lead to any fungal disease (Borah et al. 2018). Pathogens also use chemical signals in the early stage of identification of host and infection. Some antifungal or antimicrobial compounds like phytoanticipins, phytoalexins and some secondary metabolites are effective against fungus and other pathogenic microbes (Morrissey and Osbourn 1999). Major crop destruction is caused by mainly root-knot nematodes and cyst nematodes (Bird 2004).

Plants face different biotic and abiotic stresses which affect its productivity, survivability and the food quality as well. So, one of the best alternatives to these stresses is to use the microbial community which can enhance the plant growth in the unfavourable conditions. The microbes stimulate the plant growth by controlling the phytohormones, better nutrition, and siderophore production and improve the antioxidant system. Moreover, the microbial inoculants are more safer than the chemical fertilisers and cause nearly zero harm to the environment and human health (Berg 2009).

### 25.3.1 Soybean (*Glycine max* (L.) Merr) Plant and Microbe Interaction

Soybean is indeed a significant crop in the world as more of an essential food source for protein and cooking oil. Soybean yields better yield with the help of biological nitrogen fixation than by using nitrogen fertilisers. Many well-known plant growth-promoting rhizobacteria such as rhizobium, arbuscular mycorrhizal fungi and *Azospirillum* species are more frequently been residing in the soybean rhizosphere. They significantly improve plant growth and efficiency by producing IAA and other phytohormones, release siderophore against pathogenic microbes and also induce systemic resistance (Shameer and Prasad 2018). Soybean is nodulated by both slow- and fast-growing rhizobia. The accumulation of rhizobium in the rhizosphere of soybean even in non-nodulating variety reinforces the concept that the recruiting of rhizospheres could be an effective approach in the further development of rhizoplanes which encourages symbiotic relationship amongst bacteria and host species (Bulgarelli et al. 2013). Root exudates of soybean including antioxidants are being researched in the scope of association with rhizobacteria promoting plant growth and destruction of harmful toxins like polycyclic aromatic hydrocarbons (PAHs) (Guo et al. 2017). Treatment with soybean phenolic compounds to PAH-contaminated soil led to a considerable rise in the oxidation of PAHs by soil microorganisms (Guo et al. 2017). Pre-incubation of the *Bradyrhizobium japonicum* with genistein, a type of signal molecule, seems to be very effective for soybean and *B. japonicum* symbiosis under salt stress (Miransari and Smith 2009).

The study of the bacterial rhizosphere populations of hairy roots suppressed in isoflavone synthase made it clear that isoflavones inflict slight yet substantial impact on microbial populations, specifically for Comamonadaceae and Xanthomonadaceae (White et al. 2017). Soybean crops cultivated in three different soils often have higher microbiota in the rhizosphere than those in the roots, and that is close to the findings in soybean and alfalfa (Xiao et al. 2017). Soybean rhizobial populations exhibit clear biogeographic trends that are influenced by regional climatic and ecological influences like availability of iron in soil and its pH (Vinuesa et al. 2008). Therefore, it indicates that rhizosphere microbiota plays a crucial role in the regulation of rhizobia-soybean symbiosis and also in the adaptation of plants to stress environment (Fig. 25.1).

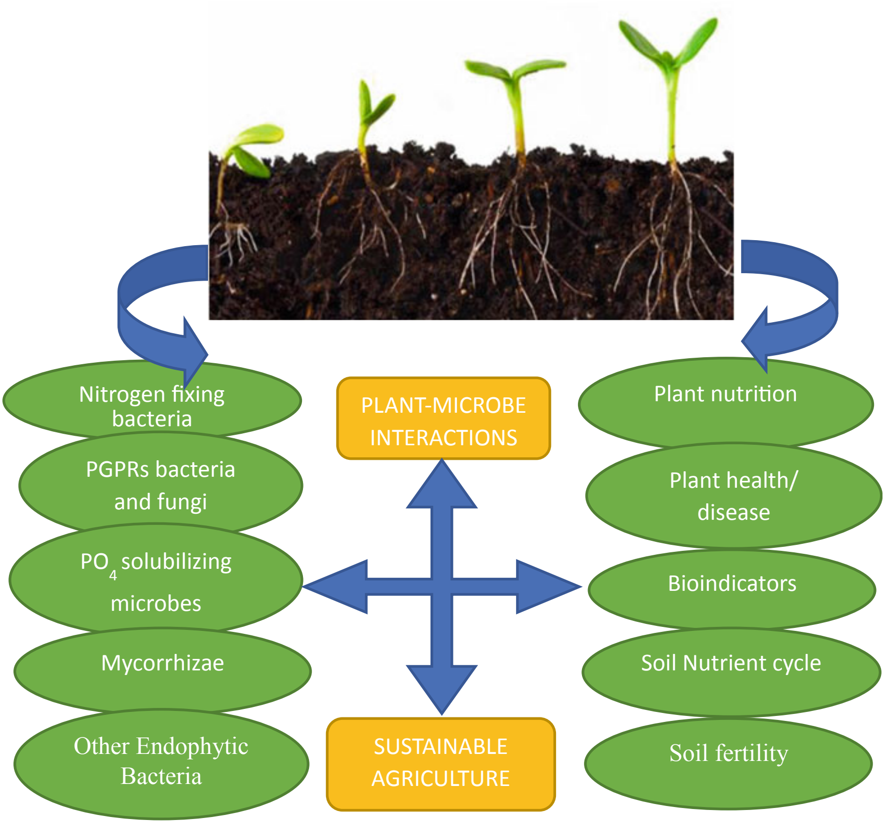

**Fig. 25.1** Plant-microbe interactions in the rhizosphere

## 25.4 How Antioxidants Play an Important Role in Microbe and Plant Interaction

Favourable interaction of plant and microbe involves endophytes, mycorrhizal fungi and plant growth-promoting rhizobacteria that upgrade the growth of plant by increasing the nutritional level and make plant resistant to biotic as well as abiotic stresses (Harrison 2005; Berendsen et al. 2012). It is assumed that the microbes stimulate the plant's immune system to invasive pathogens by inducing a variety of defence responses including regulation of quorum-sensing activities, enrichment in level of antioxidants and initiation of phenylpropanoid pathway which leads to production of phenolic and lignin deposition (Mishra et al. 2015). Pathogen infection in plants results in the production of reactive oxygen species which lead to destruction of cell. To control this killing effect of ROS, the plant produces antioxidant enzymes like superoxide dismutase and catalase that can scavenge the free radicals.

Under unfavourable conditions, the plant raises the activity of antioxidant enzymes (Table 25.1).

The major defence pathway followed by plants is the phenylpropanoid pathway that catalyses trans-cinnamic acid from L-phenylalanine (Dixon and Paiva 1995) which can further synthesise phenolics with strong antimicrobial properties (Nicholson and Hammerschmidt 1992). Soil microflora in rhizosphere provides favourable environment to plants to elevate the antioxidant status. There are reported many microbes that enhance the antioxidant enzymes in plant and contribute in providing resistance against pathogens. Rhizobacterial strains and *Serratia marcescens* were found to increase the activity of phenylalanine ammonia lyase (PAL), lipoxygenase and polyphenol oxidase (PPO) in betelvine and tomato after the attack by *Phytophthora nicotianae* and *Pseudomonas syringae*, respectively (Diallo et al. 2011). Similar observation is recorded in another study by Jain et al. (2012); the microbial consortium elevates the level of PAL, PPO and SOD and increases the tolerance in pea plant against *Sclerotinia sclerotiorum*. Singhai et al. (2011) reported that the *Pseudomonas* species increase the tolerance against potato scab disease caused by *Streptomyces scabies* by inducing the level of PAL.

### 25.4.1 Role of Flavonoids

Flavonoids are synthesised not only within the plant but also a part of root exudate. The synthesis and release of flavonoids can be seen under biotic and abiotic stresses. Due to the deficiency of nutrients such as phosphorus (Juszczuk et al. 2004) and nitrogen (Coronado et al. 1995) in the soil, the production of flavonoids is induced by microbes. Flavonoids, including genistein, quercetin and kaempferol, can make iron available by metal reduction from Fe(III) to Fe(II) (Cesco et al. 2010).

Root exudate flavonoids are able to regulate the nod genes in rhizobia. The very first and foremost flavonoid to act as inducer of nod genes was luteolin, isolated from *Medicago sativa*, and 7,4′-dihydroxyflavone (DHF) from *Trifolium repens* (white clover) (Peters et al. 1986). It is also very fascinating to note that some flavonoids act as nod genes repressor as well. For instance, the isoflavonoids medicarpin and coumestrol were found to regulate the nod factors negatively in *Sinorhizobium meliloti* (Zuanazzi et al. 1998).

Quorum-sensing signals are synthesised by most bacteria, and the so far best studied signals belong to the class of acyl homoserine lactones (AHLs), which are used by many Gram-negative bacteria. This activates the expression of hundreds of bacterial genes, many of which are important in plant-microbe interactions, including genes responsible for biofilm formation; nitrogen fixation; synthesis of degradative enzymes, exopolysaccharides and toxins; and motility and conjugation (González and Marketon 2003). A number of land plants have been shown to synthesise quorum-sensing mimics, which can both inhibit and stimulate AHL-dependent genes in various reporter strains (Gao et al. 2003). The first mimic signal identified from plants was lumichrome, a riboflavin derivative (Rajamani et al. 2008). A flavonoid identified from the medicinal tree *Combretum*

**Table 25.1** List of antioxidants from different plant sources

| S. no. | Antioxidants | Subclasses | Examples | Source | References |
|---|---|---|---|---|---|
| 1. | Phenolic acid | Cinnamic acid | p-Coumaric<br>Caffeic<br>Ferulic | Blueberry, kiwi, cherry, plum, apple, cereal grains | Manach et al. (2004) |
| | | Benzoic acid | Gallic acid<br>Hydroxybenzoic acid | Red fruits, black radish | Manach et al. (2004) |
| 2. | Flavonoids | Flavanols | Quercetin | Onion | Erlund (2004) |
| | | Flavanones | Hesperidin<br>Narirutin<br>Naringin | Citrus fruits | Erlund (2004) |
| | | Catechins | Aglycones | Tea, red wine | Erlund (2004) |
| | | Flavones | Apigenin<br>Luteolin | Red pepper, celery | Erlund (2004) |
| | | Anthocyanin | Cyanidin<br>Delphinidin<br>Pelargonidin | Plums, eggplant, berries | Erlund (2004) |
| | | Isoflavonoids | Genistein<br>Daidzein | Soybeans, soya products | Liggins et al. (2000) |
| 3. | Carotenoids | Beta-carotene | | Mango, pumpkin, carrot, nuts, oil palm | Mezzomo and Ferreira (2016) |
| | | Lycopene | | Tomato | Borguini and da Silva Torres (2009) |
| | | Lutein | | Leafy vegetables such as broccoli, spinach, peas, lettuce | Abdel-Aal et al. (2013) |
| | | Zeaxanthin | | Red marine algae | Abdel-Aal et al. (2013) |
| 4. | Lipoic acid | | | Spinach, potato, carrot, red meat | Gupta and Sharma (2006) |
| 5. | Ascorbic acid | | | Citrus fruits | Dinis et al. (1994) |
| 6. | Vitamin A | | | Sweet potato, egg yolk, milk, carrot | Dinis et al. (1994) |
| 7. | Vitamin E | | | Almonds, soy bean, nuts | Dinis et al. (1994) |

*albiflorum*, catechin, which also occurs in many other plant species, also showed activity as a quorum-sensing mimic, although at rather high concentrations of between 0.125 and 4 mM (Vandeputte et al. 2010).

Flavonoids are expected to participate during fungal invasion and arbuscule formation within the root. The fungal infection in roots leads to the initiation of flavonoid pathway, and the accumulation starts before the infection, and it might vary with the stages of infection and to distinct symbionts (Harrison 1993). The fungal invasion activates the defence mechanism inside the roots that incorporate the production of flavonoid phytoalexin.

Antioxidants carry the property of antimicrobial toxins and hinder the root pathogens. Any attack induces the de novo synthesis of phytoalexin that inhibits bacteria and fungi. These molecules may also be retained in inactive state to serve as broad-spectrum phytoanticipins to provide rapid protection against later attacks (Lattanzio et al. 2015). Quercetin also has antimicrobial property. It inhibits the ATPase activity by binding to the GyrB subunit of DNA gyrase at concentration more than 80μM and also hampers the growing fungus *Neurospora crassa* (Parvez et al. 2004).

Flavonoids are to be considered as allelochemicals in rhizosphere. *Striga*, the parasitic weed, was one of the major threats to the crops of Africa and needs to be checked. Isoflavonoids from root exudate were found to mimic the forage legume *Desmodium uncinatum* and restrict the growth of weed (Hooper et al. 2010).

### 25.4.2 Role of Phenolic Acids

Plants release some of the phenolic compounds into the rhizosphere. Microbes present in the soil recognise the antioxidants and use them for their different types of interactions with the plants.

Coumarins accumulate due to the infection caused by various pathogens like virus, fungi and bacteria. The level of coumarin accumulation is directly proportional to the level of disease resistance. For instance, the tree *Platanus occidentalis* is resistant against the fungal pathogen *Ceratocystis fimbriata* associated with the multiplication of coumarin scopoletin at the site of infection (Modafar et al. 1995).

### 25.4.3 Role of Vitamins

Vitamins have a significant role in the biology of green plants and algae, but sometimes, it is difficult to synthesise certain vitamins, so it is beneficial for plants to be associated with the microorganisms that can generate vitamins for them. Many species of algae can be cultured only when the media are supplemented with the vitamins; e.g. thiamine, biotin and cobalamin are needed for the growth of auxotroph freshwater algae (Smith et al. 2007). The plant hormones along with the vitamins enhance the benefits of PGPB on legume plants. For example, vitamins produced by *Azotobacter* species elucidate the positive effect of bacteria on the plant and their relationship with other microbes in the rhizosphere (Revillas et al. 2000). The pesticides can be harmful to the production of vitamins by PGPB. One of the pesticides named profenofos used on the cells of *Azospirillum brasilense* leads to crucial declining in the production of vitamins like thiamine, niacin and pantothenic acid; intracellular level of ATP; and nitrogen fixation, but on the other hand, the diazinon showed no negative effect on *A. brasilense* (Gómez et al. 1999). There is a positive relation between the vitamins released by the rhizosphere and the capacity to solubilise the dicalcium phosphate by PGPB (Baya et al. 1981). Ascorbic acid produced in root nodules helps in various activities, but the senescence of nodules decreased the level of ascorbate and likewise reduced its antioxidant property (Matamoros et al. 2006).

### 25.4.4 Role of Carotenoids

Carotenoids are involved in photoprotection and transmit colouration to plants, animals and bacteria. One study showed that the loss of carotenoids in cells of *Pantoea* sp. YR343 leads to oxidative damage, deformity in plant root colonisation, production of indole-3-acetic acid and biofilm formation (Bible et al. 2016). Carotenoids are also significant in the management of oxidative stress linked to herbivorous and pathogen attack in plants. After the attack of pathogen, there will be the systemic declining of carotenoids, catalase and non-protein thiols in plants (Bi and Felton 1995).

## 25.5 Conclusion

Positive interaction of plants with microbes empowers the plant to grow well and enhances their fitness without any chemical treatment. Microbes tend to stimulate the defence responses in plants against pathogen by regulating quorum-sensing activities and increasing the level of antioxidants which can scavenge the free radicals produced. Antioxidants such as phenolic acid carry the property of antimicrobial toxins. The amount of flavonoid production increases under nutrient deficiency in plants. Flavonoids regulate the activity of nod genes and show significant effect in fungal infection. Likewise, vitamins also improve the growth and enhance the function of plant growth-promoting bacteria. Therefore, we can conclude that antioxidants obtained from food and medicinal plants have been increasingly researched for their dietary functions and health benefits. Antioxidants improve the relation between plant and microbe and enhance the soil fertility. But there is still a need to learn more about the plant-microbe interaction.

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# Biomolecular Painstaking Utilization and Assimilation of Phosphorus Under Indigent Stage in Agricultural Crops

# 26

Shamim Ahmed, Aritra Roy Choudhury, Swapan Kumar Roy, Jeongyun Choi, R. Z. Sayyed, and Tongmin Sa

## Abstract

Phosphorus (P) is one of the most critical plant nutrients for all living organisms. Plants are known to be photosynthetic eukaryotes, and P plays significant role on photophosphorylation, respiration, and energy storage and transfer. It also regulates protein synthesis, cell division, and development of new tissue. Plants can take up the primary forms of phosphorus as $H_2PO_4^-$ and $HPO_4^{2-}$ (orthophosphates). In plants, this P concentration ranges is only 0.1–0.5% due to the challenges in the management of phosphorus. Although the content of total organic (P) and inorganic (Pi) form in the earth's crust is high, the availability of orthophosphates is low and heterogeneous in almost all natural and agricultural ecosystems. In this review, we emphasize to gather the existing knowledge about P and propose how to increase the availability of orthophosphates by assimilation. Apart from this, several research findings including next-generation DNA and RNA sequencing coupled with other omic platforms have advanced

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considerably on the molecular basis of plant nutrition and how plants respond to nutritional stress. Moreover, we shed light on the factors involved on phosphorus uptake by plant, as well as critical responses under P starvation and metabolic activation to mitigate P deficiency.

**Keywords**

Orthophosphates · P starvation · Omic platforms · Next-generation sequencing · Metabolic activation

## 26.1 Background

Phosphorus (P) is an essential element for all living organisms including plants and classified as a macronutrient. It is the second highest essential nutrient element followed by nitrogen, but only 0.2% of P concentration is available in plant organic materials (Shi and Schulin 2018). P plays a significant role in the natural development and proper maturity of plants, photosynthesis, respiration, energy storage and transfer for cell division and enlargement, and several other processes. But usually, this has not been found in pure elemental form except only available as phosphates in many minerals. P for commercial uses mainly comes from mining and heating calcium phosphate (Roessler 1990). Though active P is least available in nature, it is essential for metabolic processes and a regulatory factor for protein synthesis of plants (Abel et al. 2002). P is associated with the complex structure of nucleic acid and energy transformations as a component of ATP, structurally consisting of adenosine, an organic compound, and three phosphate molecules (Westheimer 1987). Phosphorus availability is controlled by three primary factors: soil pH, amount of organic matter, and proper placement of fertilizer. Acidic soil should be limed to bring soil pH to ideal levels (6–7). Orthophosphate ($HPO_4^{-2}$ or $H_2PO_4^-$) is the available ionic form to be taken up by the plant from the soil solution. Soil pH is one of the indicators to determine the availability of this two ionic form, as more $HPO_4^{-2}$ is taken up at higher soil pH (Kaur et al. 2017). Although the content of total organic (P) and inorganic (Pi) form in the earth's crust is high, the availability of orthophosphates ($H_2PO_4^-$ and $HPO_4^{-2}$) is low and heterogeneous in almost all natural and agricultural ecosystems (López-Arredondo et al. 2013). Pi availability strongly depends on several factors, such as soil pH, cation presence, and its rapid conversion by soil microorganisms into organic forms that are not directly available for plant uptake (Alatorre-Cobos et al. 2009). The adverse effect of these factors is noticeable in the high amounts of Pi fertilizers used each year. Of particular importance, approximately 20–30% of the P fertilizers applied is effectively exploited by cultivated plants (Fageria et al. 2017). Given the need to increase food production to keep pace with the growing population, the global demand for N and P fertilizers is steadily increasing, reaching an estimated 120 Mt. of elemental N and 47 Mt. of $P_2O_4$ by 2018 (Heuer et al. 2017). Consequently, ~40–70% N, ~80–90% P, and ~50–70% K of the total applied conventional fertilizers are lost to the environment due to different soil dynamics (Fageria 2014). Therefore, P represents one of the significant constraints to increase crop productivity in arable lands. Immobilization and mineralization of soil P are similar to nitrogen. If added organic materials have a C/P ratio of $>300$, there will be net immobilization, and if $<200$, there will be net mineralization of phosphorus (Wang et al. 2017). When plants are exposed to P deprivation, they alter their metabolism to scavenge and conserve internal Pi. A model crop, white lupin is highly efficient for phosphorus acquisition strategy. Also, plant vacuoles play a fundamental role in storage and remobilization of various nutrients, including phosphorus. The storage of P in vacuoles varies by organism and tissue. Though the case of P and its importance are not a recent issue, in this review, we try to highlight recent findings which discovered how plants with limited P availability can cope up and assimilate under P starvation. It might be helpful for the excessive use of Pi and improve management practices through the use of that molecular technique to recover P starvation of plant before agricultural point of view for crop production.

## 26.2 Soil Properties on P Availability

Phosphorus (P) adsorption onto soil minerals decreases its bioavailability as compared to the free, orthophosphate P form in solution (Chase et al. 2018). Soil properties and changing climate intrinsically affect crop growth and productivity. P availability often limits on its application and regulation processes during fertilization.

### 26.2.1 Organic Matter

The mineralization rate of P from organic matter (OM) decomposition mostly depends on plenty of environmental factors such as rainfall and temperature, moisture, aerobic and anaerobic condition, and soil salinity in the form of electrical conductivity (EC) (Saha et al. 2017). P releases slowly from OM decomposition in cool dry climates and more quickly in warm, humid climates. Similarly, in an anaerobic condition, P releases much slowly on wet saturated soil whereas faster on well-aerated (higher oxygen levels) soil (Broschat 2005). An organic compound in soils increases P availabilities by the formation of organophosphate complexes. Plants more easily assimilate this complex compounds, anion replacement of $H_2PO_4^-$ on adsorption sites and Fe/Al oxide protective cover, and reduce P adsorption, increasing the quality of organic P mineralized to inorganic P. In soil solution, plants absorb extremely low concentration of soluble P (often less than 1 lb./ac) due to soil pH. The range of soil pH highly moderates the availability of orthophosphate. P availability in most soils is at a maximum near pH 6.5. At low pH values, P fixation is mostly from reaction with Al/Fe oxides and precipitation as $AlPO_4$ and Fe $PO_4$ (Läuchli and Grattan 2017; Nathan 2017).

### 26.2.2 Soil Physiochemical Properties

The main factors that affect phosphate fixation in the soil are nature and amount of soil components, over-liming, soil pH, other ions, organic matter, and temperature. More than 90% of total P is present as insoluble and fixed forms including primary phosphate minerals, humus P, and insoluble phosphate of Ca, Fe, Al, and P fixed by hydrous oxides and silicate minerals (Mengel and Kirkby 2012). There are three pools in soil phosphorus amendment: non-labile P, labile P, and solution P (Costa et al. 2016). Phosphorus is often found as sinks or available in fraction in the soil. In an experiment, labile and non-labile fractions of P and its transformations in soil under *Eucalyptus* plantations in Brazil show statistical amendments of available P. The availability of P between that variable pools is different in range. For example, labile P, moderately labile P, and the occluded P varied accordingly between 6% and 16%, 28% and 40%, and 6% and 19%. The P associated with Ca (HCl-Pi) varied between 0.4% and 3%, and the residual P varied between 35% and 47% (Costa et al. 2016). Soil P cycle was hypothetically composed of four P pools (latent variables): the organic pool, occluded pool, inorganic pool, and available pool. Usually, the availability of P is high in inorganic and occluded pool. Organic pool acts predominantly on available and inorganic pools, which is a sink of P that demonstrates the high level of the recalcitrance of this pool.

Carbon is often the factor limiting microbial biomass in soil. The C/P ratio of the organic amendment is commonly used as an indicator of fertilizer quality and therefore plant P uptake from organic amendments (OA). If C/P is high, more C is added to soil to reach the same level of P addition than for OA with a low C/P (Ren 2017). Therefore, if large amounts of C are added with OA, there is a rapid increase in microbial biomass and microbial demand for P (Malik et al. 2013). Soil microorganisms immobilize P when C/P ratios of OA are higher than 20, reducing the amount of P available for plant uptake (Mackay et al. 2017; Malik et al. 2013). When temperatures are right for good plant growth, they affect P availability very little. High temperatures encourage organic matter decomposition. But when temperatures are too high or too low, they can restrict P uptake by the plant.

One of the most crucial factors of P availability is tied up of phosphorus (phosphates) by soil minerals. After immediate fertilizer application, a large percentage of P is available for root uptake by the plants. In soil solution in the surface of soil, minerals are found to be bind tightly of this P. Roots may still use this P. In the long run, most of the bound P are significantly reduced with its plant availability and become a part of the structure of the mineral (Fig. 26.1).

## 26.3 Phosphorus Use Efficiency (PUE)

On a global scale, it is estimated that about 50% of agricultural soils are deficient in P (Lynch 2011), either because of insufficient P replacement into agricultural systems or because of P-fixing soil properties rendering P unavailable to plants. Besides increased acquisition of soil P, efficient utilization of acquired P is also considered an

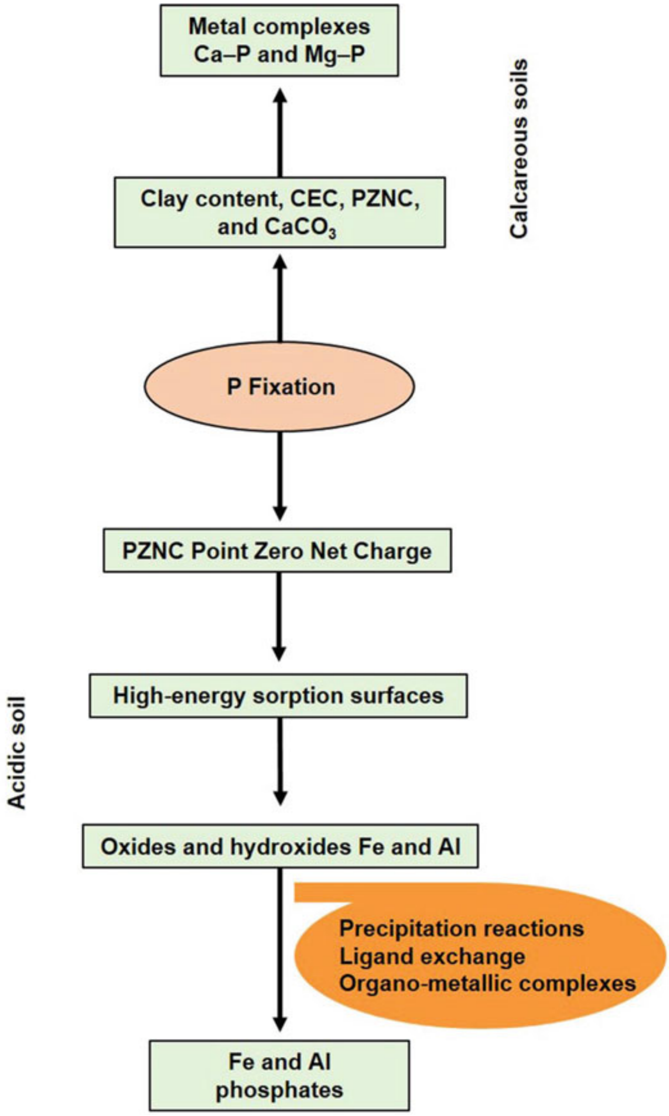

**Fig. 26.1** Phosphorus (P) fixation in acidic and calcareous soil

important adaptation for plant growth on low-P soils. Phosphorus utilization efficiency refers to the ability of a plant species/genotype to produce higher dry matter per unit of P absorbed (Balemi and Negisho 2012). For low-input conditions, crops are needed with very efficient, high-affinity Pi uptake capacity and, at the same time, high internal PUE (Rose and Wissuwa 2012). For instance, in rice (*Oryza sativa*), the PUE is only about 25% (Dodermann and Fairhurst 2000), providing considerable scope for improvement. P-fixing soils are generally soils with low or high pH that

**Table 26.1** Some common terms used to assess phosphorus use efficiency (PUE)

| Term | Description | References |
|---|---|---|
| Agronomic PUE | Yield increase per unit P applied | Hammond et al. (2009) |
| P use efficiency (I) | Grain yield per nutrient supplied | Manske et al. (2001) |
| P use efficiency (II) | Shoot biomass per unit P uptake | Wissuwa et al. (1998) |
| P uptake efficiency (I) | Total aboveground nutrient per unit P applied | Osborne and Rengel (2002) |
| P uptake efficiency (II) | Total P accumulated per unit root weight or length | Liao et al. (2008) |
| P acquisition efficiency | Total P in the plant per unit P applied | Osborne and Rengel (2002) |
| P utilization efficiency | Grain yield per unit P uptake | Manske et al. (2002) |
| Shoot P utilization efficiency (I) | Shoot biomass per unit P uptake | Su et al. (2006) |
| Shoot P utilization efficiency (II) | Shoot biomass per unit P uptake (shoots and roots minus seed P reserve) | Osborne and Rengel (2002) |
| Biomass utilization efficiency | Biomass yield per unit P uptake | Su et al. (2009) |
| P harvest index | Grain P concentration per total P uptake | Batten (1992) |
| P efficiency ratio (I) | Grain yield per unit P uptake | Jones et al. (1989) |
| P efficiency ratio (II) | Shoot growth at low P relative to shoot growth at high P | Ozturk et al. (2005) |
| Relative grain yield | Grain yield at low P relative to grain yield at high P | (Graham (1984) |
| Root efficiency ratio | P uptake in tops per unit root dry weight | Jones et al. (1992) |

cause complexation of P by aluminum (Al) or iron (Fe) and, in alkaline soils, by calcium (Haefele et al. 2014). Plants have developed a suite of adaptive mechanisms to respond to Pi deficiency and the exudation of organic acids (to solubilize P complexes) or phosphatases and phytases (to access organic P) (Hammond et al. 2004; Lopez-Arredondo et al. 2014). In accordance with this, the importance of arbuscular mycorrhizal (AM) symbiosis for Pi uptake and as an extension of the root surface area has been studied in great detail (Gutjahr and Parniske 2013; Lambers et al. 2013) (Table 26.1).

## 26.4 Phosphorus in Physiology

As mentioned before, phosphorus (P) is an essential building block of cell components involved in regulation of many biochemical and physiological processes; it is also a component of biomembranes in phospholipids and structural element of nucleic acids (Müller et al. 2015). Roots of plants acquire P as the inorganic phosphate ion (Pi) through Pi transporters (Yang et al. 2017). In such kind of environments, plants thrive themselves by using morphological, physiological, molecular, and biochemical adaptations process. Multiple genes that encode for phosphate transporters are present and confirmed by molecular studies that have been expressed differentially. Some are firmly upregulated when phosphate supply is inadequate, and under such conditions, high-affinity phosphate membrane transporters are either activated or depressed (Kaur et al. 2017). Other phosphate uptake systems are constitutive which means they are present a priori and are not affected by changes in phosphate concentration in the nutrient medium. The pH in the apoplast controls the $H_2PO_4^-$ the uptake rate. At the same $H_2PO_4^-$ concentration in the apoplast (2.50 mmol/m$^3$), phosphate uptake at pH 4 was three times greater than at pH 6 (Mengel and Kirkby 2012).

Phosphate is readily mobile in plants and can be translocated in upward or downward directions. Phosphate taken up by roots is translocated via the xylem mainly to fast-growing young laminae where it is required for leaf expansion and growth. In a study of P transport and assimilation in intact castor bean plants, Jeschke et al. (1996) observed that young leaves are supplied not only by phosphate taken up by the roots but also by phosphate from somewhat older leaves. This is particularly so when phosphate uptake by the roots is insufficient to meet the demand of the young leaves. The re-translocated phosphate from hydrolysis of organic P is supplied via the phloem. Phosphorus remobilized from mature leaves is also transported via the phloem but is directed to the roots. Inorganic P is present in phloem sap in substantial concentrations and makes up most of the total P (Hall and Baker 1972; Jeschke et al. 1996). P deficiency often results in increased shoot sugar levels (Ganie et al. 2015). The phloem loading, therefore, enhances the amount of sucrose which leads to translocation from shoots to roots by the accumulation of sugars in shoots. When the sucrose from shoots are delivered to the roots, the plant roots act as a P starvation by the signal of morphological, biochemical, and gene expression changes (Müller et al. 2015).

## 26.5 Functions of Plant Cluster Root Under P-Limiting Condition

White lupin, the cluster root (CR)-forming plant is, therefore, a role model for studying morphological and biochemical adaptations under P deficiency. White lupins form proteinoid in densely or clustered lateral rootlets, which often get exposed immediately after P-limiting condition. As a result of access to a vast volume of soil, this kind of CR formation increases the surface of the root system. Compare to non-CRs, this formation of roots can acquire ten times more P for attribution to their morphology (Heuer et al. 2017). The rhizospheric soil of lupin plant contains surprising amounts of organic acids mainly malate and citrate as well as protons by root exudation. The soil solution in the rhizosphere is becoming lower in soil pH. Around that root-forming area, Pi liberates by reaction with the complexes of Al, $Fe^{3+}$, or Ca ions or ligand exchange. Moreover, for access to the soil organic P pool by hydrolyzing P monoesters, these CRs secrete an acid phosphatase (Gilbert et al. 1999; Tadano and Sakai 1991; Wasaki et al. 2003). White lupin is a crop plant with a highly efficient phosphorus acquisition strategy. P-deficient lupin plants had much lower shoot P concentrations, but this did not affect their shoot biomass negatively (Müller et al. 2015). This may arise new understanding about the physiology of plant P acquisition and provide a complete idea to elucidate the strategy of defining targets for breeding other P-efficient crops (Fig. 26.2).

## 26.6 Role of Vacuoles in Phosphorus Storage and Remobilization

Various nutrients including P are stored and remobilized in vacuoles which play a fundamental role for that (Yang et al. 2017). P stored in vacuoles vary in form for every organism and tissue. Algae and yeast store polyphosphates (polyPs), whereas plants store Pi and inositol phosphates (InsPs) in vegetative tissues and seeds, respectively. The importance of vacuolar P is in buffering the cytoplasmic Pi concentration to maintain cellular homeostasis when the external P supply fluctuates and presents additional roles for vacuolar polyP and InsP besides being a P reserve.

In yeast, polyP is synthesized and transported into vacuoles via the vacuolar transporter chaperone (VTC) complex. Pi is released from the hydrolysis of polyP by the polyphosphatase Ppn1 and subsequently exported out of the vacuole by the Pho91 Pi transporter. Inositol phosphates (InsPs) or inositol pyrophosphates (PP-InsPs) may regulate the activity of VTC complexes by their interaction with the SYG1/PHO81/XPR1 (SPX) domain (Yang et al. 2017) (Fig. 26.3a).

In plant vacuoles, AtPHT5 mediates Pi influx in *Arabidopsis*, whereas OsSPX-MFS3 mediates Pi efflux in rice. In seeds, storage of *myo*-inositolhexakisphosphates (InsP6) inside vacuoles is mediated by multidrug resistance-associated protein (MRP)-type ABC transporters. During seed germination, InsP6 is hydrolyzed by purple acid phosphatases (PAPs) into Pi and inositol, which are released into the cytosol by Pi transporters and inositol transporters (presumably INT1), respectively. An additional unidentified Pi export system is expected and indicated (Yang et al. 2017) (Fig. 26.3b).

Potential pathways for recycling P are from RNA or phospholipids via autophagy and multivesicular body (MVB)-vacuolar fusion. Inside the vacuoles, RNA is degraded by RNase and then phosphatases (AP, yeast alkaline phosphatase, or plant acid phosphatase) to release Pi and nucleosides, which are exported by Pi transporters and equilibrative nucleoside transporter 1 (ENT1), respectively. Pi is also liberated from phospholipid catabolism via a series of enzymatic reactions: 3′-NMP, 3′-nucleotide monophosphate; GPD, glycerophosphodiester; and GDPD, glycerophosphodiester phosphodiesterase (Yang et al. 2017) (Fig. 26.3c).

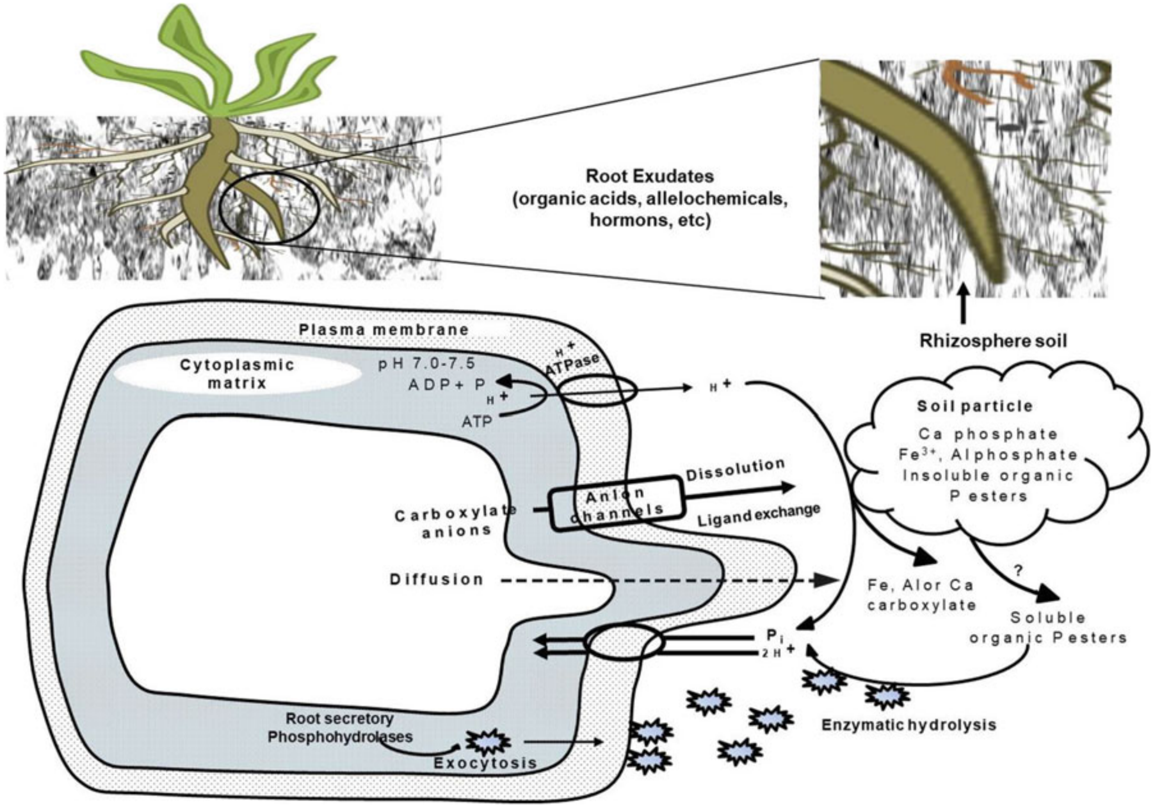

**Fig. 26.2** The ability of the cluster roots of white lupin to excrete organic acid is associated with several physiological mechanisms

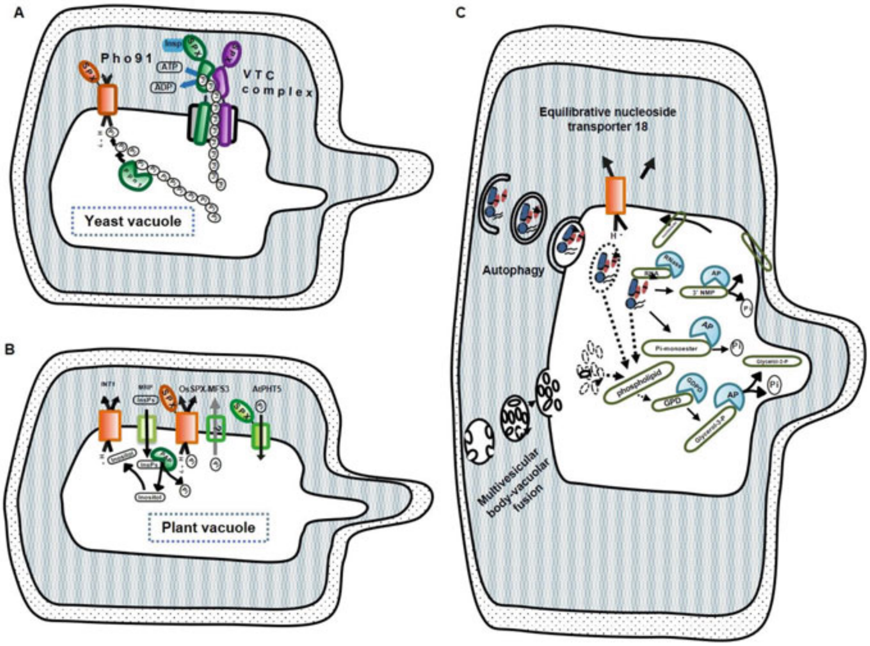

**Fig. 26.3** Storage and remobilization of phosphorus from vacuole both in yeast (**a**) and in plant (**b**) with its potential pathways for recycling P from phospholipids via autography and multivesicular body vacuolar fusion (**c**)

## 26.7 Phosphorus Signaling in Plants: Biochemical and Molecular Approach

Phosphoesters are essential for protein phosphorylation, whereas dephosphorylation of Pi is also a key regulator of signal transduction (Müller et al. 2015). Evolving an efficient P-signaling system, plants are compelled to develop a series of interconnected responses to conserve and remobilize internal Pi, due to critical nature of Pi nutrition. Pi acquisition from the external environment also increases (Kaur et al. 2017). Forward and reverse genetics approaches combined with analyses of whole transcriptomes, obtained by high-throughput sequencing technologies, allow the identification of master controllers of the different signaling pathways that modulate plant responses to Pi deficiency. Genes involved in Pi uptake, translocation, and recycling have been characterized, and their possible roles in enhancing phosphate use efficiency (PUE) have been evaluated (López-Arredondo et al. 2013).

Root system architecture and morphological adaptations of roots have been altered in low external Pi availability that results in enhancement of root hair formation with increase soil exploration and Pi acquisition. Although implicated, the role of auxin and ethylene in Pi-mediated root responses is presently unclear. Pi limitation induces a set of Pi-starvation-inducible (*PSI*) genes, whose products promote intracellular Pi recycling and extracellular Pi mobilization and acquisition. Induction of at least some *PSI* genes is regulated by the transcription factor PHR1 and is influenced by the whole-plant Pi status, possibly mediated by cytokinin. Pi and phosphite repress morphological and molecular Pi-starvation responses (Abel et al. 2002).

It has been observed that large fluctuations of Pi in soils and the intracellular concentrations of Pi in plants are highly regulated to maintain homeostasis. For this, plants have developed a series of interconnected responses to conserve and remobilize internal Pi and increase Pi acquisition from the external environment (Abel et al. 2002; Yang et al. 2017). The level of phosphate within the plant is also nonuniform as it depends on its physiological demands. To elicit localized signaling cascades, the signaling molecules need to move intracellularly or intercellularly, but the molecules acting as long-distance signals can travel through the vascular system, via either xylem or phloem, to distant target sites (Liu et al. 2009). Regardless of sufficient Pi status in shoots under low-Pi medium, physical contact of the primary root tip was essential to attenuate primary root growth.

Unusual change in primary root growth under low Pi indicates that the root tip, including the meristem region and root cap, may represent a site to sense local Pi (Svistoonoff et al. 2007). P5-type ATPase-encoded *phosphate deficiency response 2* (*PDR2*) disruption exhibits an inflated short-root phenotype under Pi deficiency owing to meristem exhaustion (Ticconi et al. 2004, 2009). There is an observation established between the pattern of root development and root meristem activity that it depends on P availability. Pi likely functions as a local signal after application of phosphite (Phi), an analog of Pi. On the other hand, low phosphorus insensitive (*lpi*), low phosphate root (*lpr*), and phosphate starvation insensitive (*psi*) mutants display long primary roots under Pi deficiency. The gene responsible for *psi* mutant phenotype is the same allele as *LPR1*/*LPR2* (Wang et al. 2010). *PDR2 and LPR1*/*LPR2* are genetically interacted with each other, which is epistatic to *PDR2* (Ticconi et al. 2009). That means, once changes in external Pi are sensed, *PDR2* may function together with *LPR1*/*LPR2* to regulate the meristem activity in endoplasmic reticulum (ER)-resident pathway. PDR2 may act upstream to control the output of *LPR1*/*LPR2* negatively. These observations established an association between pattern of root development and root meristem activity in response to Pi availability (Kaur et al. 2017).

Pi uptake from the rhizosphere is a function carried out by proton ($H^+$)/Pi symporters, directly called Pi transporters. Four gene families of Pi transporters (PHT1, PHT2, PHT3, and PHT4) have been identified in *Arabidopsis*, and their respective orthologs have been found in other plant species (López-Arredondo et al. 2013). Recently, a role in tolerance to the low-Pi availability of *AtPHT1;9* was observed in *Arabidopsis AtPHT1;9*-overexpressing lines, in which 20–30% more shoot fresh weight was produced than in wild-type plants under Pi-limiting conditions (Remy et al. 2012). In rice, overexpression of *OsPT1* results not only in a twofold higher Pi content than in wild-type plants but also in a higher number of tillers per plant, independent of the Pi-fertilization regimen (Seo et al. 2008). In general, overexpression of *PHT* genes moderately increases Pi content and biomass accumulation, whereas the constitutive expression of regulatory elements, such as transcription factors (TFs), microRNAs, signaling intermediates, or some TF activity modifiers, apparently increases Pi accumulation in plant tissues.

Interestingly, in null mutants of the *AtSIZ1* (a positive regulator of phosphate starvation response 1 (PHR1) activity by simulation) gene, a root phenotype similar to that described for *PHR2*-overexpressing plants was associated with changes in auxin accumulation (Miura et al. 2011). Although SIZ1 and PHR1 are components of the same signaling pathway that regulate many responses to Pi deficiency, these molecular controllers might play opposite roles in root development (Miura et al. 2011). Among the efforts to improve the PUE, three attempts are considered promising. These attempts are considered when it is implemented in essential crops, and the field trials are successful. However, the attempts are the use of phythases to metabolize phytate, the use of citrate synthases to overproduce citrate, and the use of phosphite dehydrogenase to use phosphite (Phi) instead of Pi as fertilizers. These enzymes are collectively called phytases and are classified into four groups according to their catalytic properties: (1) histidine acid phosphatases (HAPs), (2) purple acid phosphatases (PAPs), (3) Cys phosphatases, and (4) β-propeller phytases (BPPs) (Brinch-Pedersen et al. 2002; Ma et al. 2009) (Figs. 26.4 and 26.5).

## 26.8 Metabolic Alterations During Phosphorus Deprivation

The main carbon source at the early stages of the plant is usually from shoot-derived carbohydrates which are required for their growth and development. This can also act as a signaling molecule for promoting or inhibiting the growth of plants (Hammond and White 2008). The abiotic stress response, especially in white lupin (*Lupinus albus* L.), can be investigated through this, which makes carbohydrates more important. This plant has a high adaptive ability under the P-deficient soil. An experiment was conducted by Müller et al. (2015) to elaborate the hypothesis of the metabolic alteration during phosphorus deprivation on early and later growth stages of white lupin plants. Early response to P deficiency in white lupins was to adapt with carbohydrate partitioning between shoot and root. P deficiency (−P) compared with P-sufficient (+P) plants was calculated to determine the relative concentration of metabolites by a response ratio of −P/+P. The sugars sucrose, fructose, and glucose were significantly reduced in shoots at an early stage of P deficiency. However, in both cluster and non-cluster roots, the levels of sucrose decreased by half, but fructose and glucose levels were not affected after the same period. The levels of sucrose, glucose, or fructose neither on shoot nor on roots exhibited a significant change in later stage. Maltose (degraded form of starch) was increased at early shoot development but reduced in a later stage, but in case of the cluster and non-cluster roots, it remains unchanged at both time points. The phosphorylated metabolites were strongly reduced in P-deficient shoots and roots. This reduction was intensified with the duration of phosphorus deficiency. Moreover, Glycerol-3-P

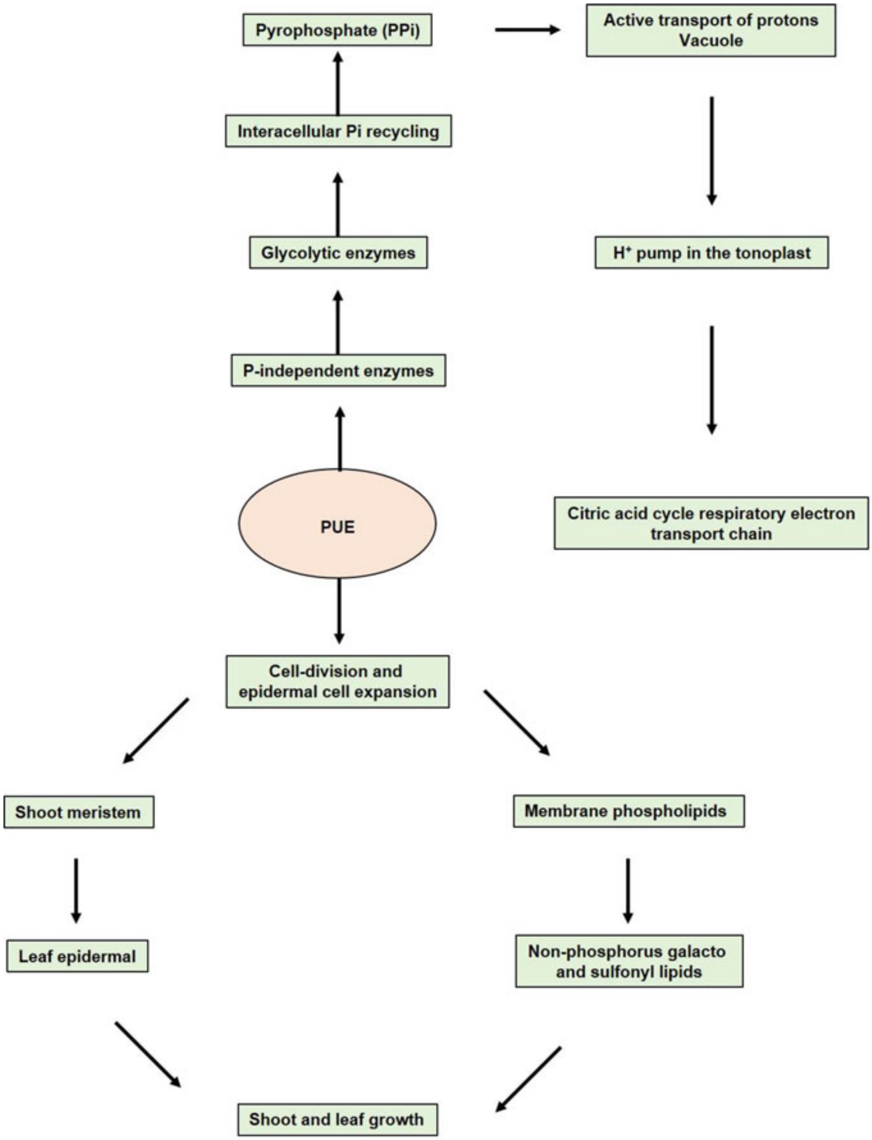

**Fig. 26.4** Phosphorus use efficiency (PUE) and related cellular physiological and metabolic changes toward the phenotype of plants

has a crucial role in plant cells, being a component of the glycerophospholipids of biomembranes. There are many organic acids of the tricarboxylic acid (TCA) cycle that exhibited elevated levels. Cluster root (CR)-forming plants were found with the highest increase of citrate followed by *cis*-aconitate (Tables 26.2, 26.3, and 26.4).

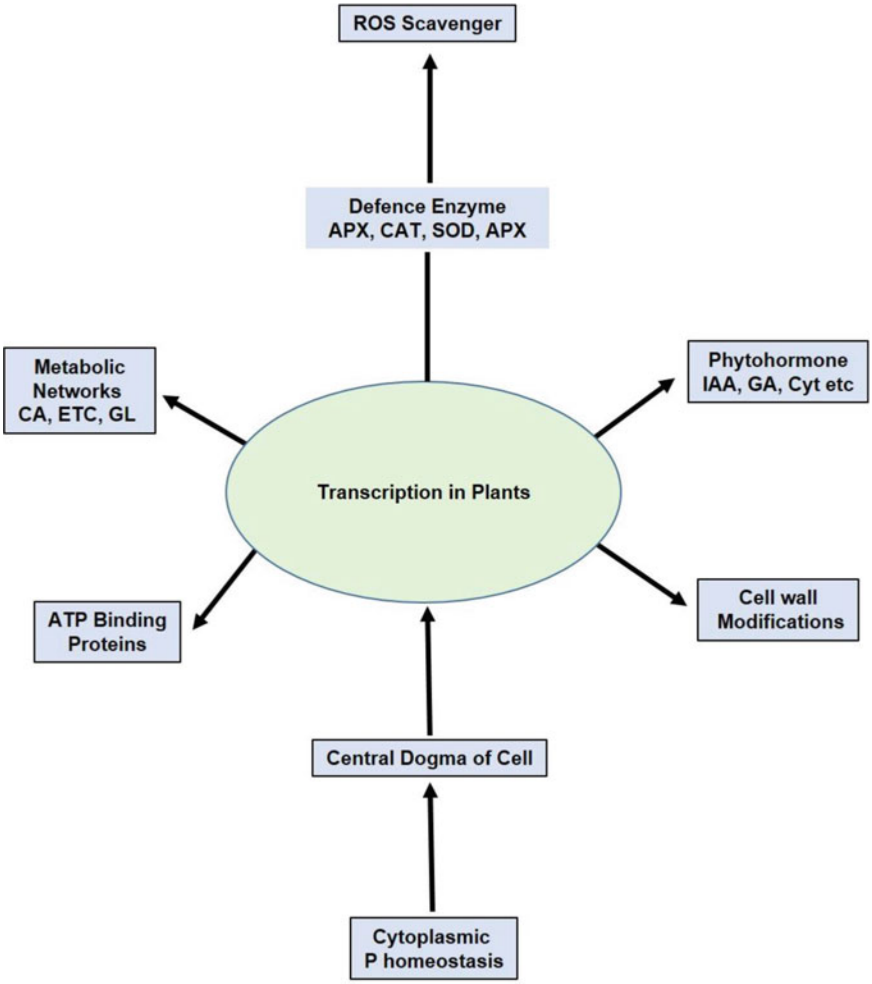

**Fig. 26.5** Cytoplasmic phosphorus homeostasis and related cellular physiological and metabolic functions

CRs of white lupin plants accumulate large amounts of citrate and exude them into the rhizosphere for mobilization of unavailable P. CRs are getting this citrate from their TCA cycle and accumulate them in the rhizosphere. On the other hand, while citrate and *cis*-aconitate accumulated in CRs were high, the levels of isocitrate were reduced. When tissue is becoming P-deficient, about half of the measured amino acids were increased. Strong reactions in both shoots and roots were found in the later stage of P deficiency, namely, in tryptophan, followed by asparagine and leucine. Fumarate in both shoots and CRs was significantly increased. Succinate exhibited elevated levels in all P-deficient tissues compared with control plants. It was also observed that at a later stage of P deficiency in white lupin plant, some of

**Table 26.2** List of reducing metabolites on both early and later stages of P deficiency on shoots and roots of plant (a case study on white lupin (*Lupinus albus* L.) by Müller et al. (2015)

| Non-cluster roots in early stage of development[a] | Non-cluster roots in later stage of development[a] | Cluster roots in early stage of development[a] | Cluster roots in later stage of development[a] | Shoots in early stage of development[a] | Shoots in later stage of development[a] |
|---|---|---|---|---|---|
| 1. Glucose-6-P | 1. Glucose-6-P | 1. Glucose-6-P | 1. Glucose-6-P | 1. Glucose-6-P | 1. Glucose-6-P |
| 2. Fructose-6-P | 2. Fructose-6-P | 2. Fructose-6-P | 2. Fructose-6-P | 2. Fructose-6-P | 2. Fructose-6-P |
| 3. Glycerol-3-P | 3. Glycerol-3-P | 3. Glycerol-3-P | 3. Glycerol-3-P | 3. Myo-inositol-P | 3. Glycerol-3-P |
| 4. S-Methylcysteine | 4. Myo-inositol-P | 4. Sucrose | 4. Myo-inositol-P | 4. Fructose | 4. Myo-inositol-P |
| 5. Sucrose | 5. Mannitol | 5. Myo-inositol-P | 5. Mannitol | 5. Glycerol-3-P | 5. α-Ketoglutarate |
| 6. Myo-inositol-P | 6. Isocitrate | 6. Mannitol | 5. Isocitrate | 6. Sucrose | 6. γ-Aminobutyrate |
| 7. Mannitol | 7. Trehalose | 7. Isocitrate | 6. Pinitol | 7. Glucose | 7. Glutamate |
| 8. Gluconate | 8. γ-Aminobutyrate | 8. Melibiose | 7. Gluconate | 8. Pinitol | 8. Uracil |
| 9. Glucono-1-5-lactone | 9. Pinitol | 9. Gluconate | 8. Shikimate | 9. Xylose | 9. Pyruvate |
| 10. Naringenin | 10. Myo-inositol | 10. Naringenin | 9. Glycerate | 10. Myo-inositol | 10. Mannitol |
| 11. 2-Aminoadipate | 11. Gluconate | 11. Pinitol | 10. γ-Aminobutyrate | 11. Gluconate | 11. Trehalose |
| 12. Melibiose | 12. Glycerate | 12. Shikimate | 11. Myo-inositol | 12. Mannitol | 12. Myo-inositol |
| 13. 2-Hydroxyglutarate | 13. Maltose | 13. Threonine | 12. Uracil | 13. Glycerate | 13. Pinitol |
| 14. Isocitrate | 14. *Cis*-aconitate | 14. Proline | | 14. Threonine | 14. Cis-Aconitate |
| 15. Isoleucine | 15. Glutamate | 15. Maltose | | 15. 2-Hydroxyglutarate | 15. 2-Aminoadipate |
| 16. Maltose | 16. α-Ketoglutarate | 16. Glycine | | 16. 2-Aminoadipate | 16. Glucono-1-5-lactone |
| 17. Myo-inositol | | | | 17. Tryptophan | 17. Gluconate |
| 18. Tyrosine | | | | 18. S-Methylcysteine | 18. Glucose |
| 19. Phenylalanine | | | | 19. Phenylalanine | 19. Glucuronate |
| 20. Threonine | | | | 20. Isocitrate | 20. Fructose |
| 21. Lysine | | | | 21. Isoleucine | |

(continued)

**Table 26.2** (continued)

| Non-cluster roots in early stage of development[a] | Non-cluster roots in later stage of development[a] | Cluster roots in early stage of development[a] | Cluster roots in later stage of development[a] | Shoots in early stage of development[a] | Shoots in later stage of development[a] |
|---|---|---|---|---|---|
| 22. Ornithin | | | | 22. Glycine | |
| 23. Citrullin | | | | 23. Naringenin | |
| 24. Arginine | | | | 24. Leucine | |

[a] P deficiency (−P) compared with P-sufficient (+P) plants was calculated to determine the relative concentration of metabolites by a response ratio of −P/+P

**Table 26.3** List of increasing metabolites on both early and later stages of P deficiency on shoots and roots of plant (a case study on white lupin (*Lupinus albus* L.) by Müller et al. (2015)

| Non-cluster roots in early stage of development[a] | Non-cluster roots in later stage of development[a] | Cluster roots in early stage of development[a] | Cluster roots in later stage of development[a] | Shoots in early stage of development[a] | Shoots in later stage of development[a] |
|---|---|---|---|---|---|
| 1. Leucine | 1. Tryptophan | 1. Leucine | 1. Tryptophan | 1. Adenosine | 1. Tryptophan |
| 2. Adenosine | 2. Asparagine | 2. Adenosine | 2. Citrate | 2. Maltose | 2. Asparagine |
| 3. Succinate | 3. Leucine | 3. Citrate | 3. S-Methylcysteine | 3. 4-Hydroxyproline | 3. Adenosine |
| 4. 4-Hydroxyproline | 4. Succinate | 4. Asparagine | 4. Asparagine | 4. Fumarate | 4. Succinate |
| 5. Asparagine | 5. Citrate | 5. Adenine | 5. Leucine | 5. γ-Aminobutyrate | 5. Phenylalanine |
| 6. Uracil | 6. Proline | 6. Cis-Aconitate | 6. Adenine | 6. Maleate | 6. Isoleucine |
| 7. Maleate | 7. Ornithin | 7. Succinate | 7. Cis-Aconitate | 7. *Cis*-aconitate | 7. Naringenin |
| 8. Fructose | 8. Citrullin | 8. Ribose | 8. Adenosine | 8. Glucono-1-5-lactone | 8. Leucine |
| 9. Adenine | 9. Arginine | 9. Tryptophan | 9. Naringenin | 9. Succinate | 9. Sucrose |
| 10. Shikimate | 10. Adenine | 10. Pyruvate | 10. Ornithin | 10. Shikimate | 10. Ribose |
| 11. Ribose | 11. Adenosine | 11. Uracil | 11. Citrullin | 11. Asparagine | 11. Melibiose |
| 12. Glycerate | 12. 4-Hydroxyproline | 12. Fumarate | 12. Arginine | 12. Pyruvate | 12. Fumarate |
| 13. Citrate | 13. Glucuronate | 13. α-Ketoglutarate | 13. Tyrosine | 13. Uracil | 13. Shikimate |
| 14. Fumarate | 14. Fumarate | 14. Glucuronate | 14. 2-Hydroxyglutarate | 14. Citrate | 14. Lysine |
| 15. Glucuronate | 15. Tyrosine | 15. Phenylalanine | 15. Proline | 15. Arginine | 15. Rhamnose |
| 16. Pinitol | 16. 2-Hydroxyglutarate | 16. Maleate | 16. 2-Aminoadipate | 16. Citrullin | 16. Maleate |
| 17. Rhamnose | 17. Sucrose | 17. Tyrosine | 17. Glucuronate | 17. Ornithin | 17. Ornithin |
| 18. Xylose | 18. Fructose | 18. γ-Aminobutyrate | 18. Ribose | 18. Melibiose | 18. Citrullin |
| 19. Tryptophan | 19. Ribose | 19. S-Methylcysteine | 19. Pyruvate | 19. Lysine | 19. Arginine |
| 20. Glycine | 20. Naringenin | 20. Glutamate | 20. Lysine | 20. Rhamnose | 20. Threonine |

(continued)

**Table 26.3** (continued)

| Non-cluster roots in early stage of development[a] | Non-cluster roots in later stage of development[a] | Cluster roots in early stage of development[a] | Cluster roots in later stage of development[a] | Shoots in early stage of development[a] | Shoots in later stage of development[a] |
|---|---|---|---|---|---|
| 21. *Cis*-Aconitate | 21. Isoleucine | 21. Rhamnose | 21. Maleate | 21. Glutamate | 21. S-Methylcysteine |
| | 22. Rhamnose | 22. Xylose | 22. Fumarate | | 22. Glycerate |
| | 23. Xylose | 23. Fructose | 23. Isoleucine | | 23. Adenine |
| | 24. Pyruvate | 24. Glucose | 24. Succinate | | 24. Citrate |
| | 25. Threonine | 25. Myo-inositol | 25. Glycine | | |
| | 26. Maleate | 26. Glycerate | 26. Threonine | | |
| | 27. Glucose | 27. Lysine | 27. Phenylalanine | | |
| | 28. Glycine | 28. Isoleucine | 28. Rhamnose | | |
| | 29. S-Methylcysteine | 29. Ornithin | 29. α-Ketoglutarate | | |
| | 30. Phenylalanine | 30. Citrullin | 30. Maltose | | |
| | 31. 2-Aminoadipate | 31. Arginine | 31. Xylose | | |
| | | | 32. Fructose | | |
| | | | 33. Glucose | | |
| | | | 34. Melibiose | | |
| | | | 35. Trehalose | | |
| | | | 36. Glutamate | | |
| | | | 37. Glucono-1-5-lactone | | |

[a] P deficiency (−P) compared with P-sufficient (+P) plants was calculated to determine the relative concentration of metabolites by a response ratio of −P/+P

**Table 26.4** List of unchanged metabolites on both early and later stages of P deficiency on shoots and roots of plant (a case study on white lupin (*Lupinus albus* L.) by Müller et al. (2015)

| Non-cluster roots in early stage of development[a] | Non-cluster roots in later stage of development[a] | Cluster roots in early stage of development[a] | Cluster roots in later stage of development[a] | Shoots in early stage of development[a] | Shoots in later stage of development[a] |
|---|---|---|---|---|---|
| 1. Trehalose | 1. Melibiose | 1. 2-Hydroxyglutarate | Sucrose | 1. Glucuronate | 1. Maltose |
| 2. Pyruvate | 2. Shikimate | 2. 2-Aminoadipate | | 2. Adenine | 2. Xylose |
| 3. γ-Aminobutyrate | 3. Uracil | 3. Glucono-1-5-lactone | | 3. Ribose | 3. Glycine |
| 4. Glutamate | 4. Glucono-1-5-lactone | | | 4. α-Ketoglutarate | 4. 2-Hydroxyglutarate |
| 5. Proline | | | | 5. Proline | 5. Isocitrate |
| 6. α-Ketoglutarate | | | | | 6. Proline |

[a] P deficiency (−P) compared with P-sufficient (+P) plants was calculated to determine the relative concentration of metabolites by a response ratio of −P/+P

the amino acid like tryptophan, tyrosine, and shikimate and a precursor of the aromatic amino acid phenylalanine were found to be decreased by half on CRs. P-deficient plants increased the levels of nucleosides and nucleotides as degradation residues by scavenging Pi from degradation of RNA and ATP. Adenosine, a constituent of adenosine phosphates (such as ATP, NADPH/NADH, and RNA) was clearly increased in all P-deficient tissues. The highest increase of adenosine was found in the early development stage of shoots. Furthermore, adenine and ribose, the constituents of adenosine, exhibited elevated levels. Usually, in plant organ, CRs were the only organs that elevated the levels of all three metabolites adenosine, adenine, and ribose at both early and later stage of development. The RNA-specific metabolite uracil did not accumulate at all in CRs.

## 26.9 Conclusion

The requirement of crop varieties with high nutrient use efficiency has been discussed historically. Increasing food production and improving food quality have always been needed worldwide. However, the increasing fertilizer consumption rates focused on human and animal food production, and the growing population has alerted research groups to develop urgently more efficient agricultural schemes to use natural resources more rationally. In the past, the limited knowledge on plant metabolism affected the development of these improved varieties, but the tools are now available to genetically modify many plant species. Systems biology has accelerated the discovery of regulatory elements in several pathways with the potential to improve plant performance in the field. As discussed above, there are numerous attempts to improve nutrient use in plants through the manipulation of enzymes and proteins directly involved in uptake and assimilation of phosphorus, but only a few have produced results sufficiently promising for their commercial application. Because of the storage feature of vacuoles, an understanding of reallocation and recycling of vacuolar P has potential applications in agriculture. Modulation of these systems may help in improving crop nutritional value and assist in crop adaptation to a low-P environment. The limited availability of P in young lupin plants decreases sugar concentrations in the shoot. It suggests an adaption of carbohydrate partitioning between shoot and root as an early response to the limited available P. The biosynthesis of organic acids is increased in CRs, with significant accumulation. Interactions between plant roots play an important role in determining the performance of individuals in natural communities as well as crop productivity in agroecological systems. It is dependent on morphological (root architecture) and physiological plasticity of roots (e.g., exudation of organic and inorganic compounds, nutrient uptake) in response to the soil environment.

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# Plant Antioxidant System Regulates Communication Under Abiotic Stress for Enhanced Plant Productivity

## 27

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**Abstract**

Agricultural crops are exposed to various abiotic and biotic stresses which cause considerable losses in production system. The changing climatic condition and degradation of the environment have led to more severe effects of abiotic factors on agricultural crops. It has been estimated that until 2050, around 7.6 million km$^2$ of the world's area would be vulnerable to abiotic stresses. These abiotic challenges are known to affect the plants by increasing the generation and accumulation of reactive oxygen species (ROS) to which plants respond by an antioxidant defense system. Many new developments have showed that ROS not only is harmful but also has major function as signaling molecule in plants. The functions of ROS in communication have made deciphering their role in the same as very eminent. In this chapter, we will discuss the plant antioxidant system in regulating the ROS concentration and their deployment to increase plant's tolerance to abiotic stresses.

## 27.1 Introduction

All the living organisms living on this earth have a set of relationships with the other living organisms and the nonliving things. These relationships are always in a balance which is maintained by numerous natural processes. Eventually, all the

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individual organism influences the environment it thrives in and also gets influenced by the environment (Xie et al. 2019). Due to the drastic changes in climate, the environmental conditions are now becoming harsher, and prevalence of various environment-related challenges like salinity, extreme temperature, drought, flood, metal toxicity, and air pollutants is increasing (Pereira 2016; Raza et al. 2019). All the organisms including plants get affected by these abiotic challenges. For animals, it is easier to ward off these stresses as they have the ability to move. Plants however are immobile and hence have developed various strategies to combat these abiotic stresses and survive. Most of these abiotic challenges cause oxidative stress in plants either directly or indirectly through generation and accumulation of reactive oxygen species (ROS) (Hossain et al. 2015; Farnese et al. 2016; Foley et al. 2016). In the state of oxidative stress, plants exhibit growth retardation symptoms like abscission, root gravitropism, polar cell growth, senescence, and lignin biosynthesis (Mugnai et al. 2014; Shi et al. 2014a, b; Mangano et al. 2016; Goldental-Cohen et al. 2017; Muñoz and Munné-Bosch 2018). Oxygen is one of the most important elements for the growth and development of plants but is equally nefarious in its reduced forms which are altogether known as ROS.

ROS are natural by-products of aerobic processes that are carried out in different cell organelles like mitochondria, peroxisomes, chloroplasts, and plasma membrane (Apel and Hirt 2004). They are categorized into two major groups, namely, free radicals and non-radicals. The free radicals consist of hydroxyl radical ($OH^{\cdot}$), superoxide radical ($O_2^{\cdot -}$), hydroperoxyl radical ($HO_2^{\cdot}$), and alkoxy radical ($RO^{\cdot}$), while the non-radicals consist of singlet oxygen ($^1O_2$) and hydrogen peroxide ($H_2O_2$) (Hasanuzzaman et al. 2020). When the level of ROS is higher than the defense mechanisms of plants, they are highly reactive toward different biochemical, physiological, and cellular functions. They cause disruption of cell membrane through lipid peroxidation, carbohydrate deoxidation, protein denaturation, and destruction of nucleic acids (Martínez et al. 2017; Van Ruyskensvelde et al. 2018). Oxidative stress is the outcome of this uncontrolled damage to the cellular compartments (Dat et al. 2000; Mittler 2002). Plants have thus developed mechanisms for cellular detoxification which maintains the homeostasis (Apel and Hirt 2004; Kushwaha et al. 2019). These defense mechanisms for scavenging of ROS and maintaining of homeostasis are composed of several enzymatic and nonenzymatic antioxidants. Antioxidants are small group of structurally diverse compounds generated by aerobic organism to counteract imbalances in oxidative stress. For proper growth and development of plant, existence of ideal balance of ROS is very necessary, allowing proper functioning of biological redox process (Mittler 2017).

ROS are not always villainous in nature, since at a relatively lower or moderate concentrations they act as major molecules in cell signaling pathways of plants which control the antioxidative system. They bring an upregulated action of this antioxidative system and thus ensure tolerance against oxidative disturbances (Dat et al. 2000; Vranová et al. 2002; Singh et al. 2019). The ROS generated due to environmental stresses are recognized by sensors that transmit the signal to the nucleus of cell through redox reactions and mitogen-activated protein kinase

(MAPK) pathway. As a result, stress-responsive transcription factors (TFx) are activated which binds to their respective cis-acting elements (CREs), thereby causing a transcriptional reprogramming leading to modifications of existing proteins and/or translation of new proteins (Foyer and Noctor 2016). Many other studies have also shown that ROS has an essential role in different other natural processes related to cell differentiation and proliferation (Mittler 2017). In addition to ROS, many other reactive element derivatives such as that of nitrogen, carbon, and sulfur also play a significant role in imparting abiotic stress tolerance in plants through a cross talk (Yamasaki et al. 2019).

## 27.2 Plant Antioxidants

The antioxidative system of plants is composed of various enzymatic and nonenzymatic antioxidants that function together in coherence with ROS generation pathway for maintenance of ROS homeostasis. As stated earlier, the antioxidants are composed of enzymatic and nonenzymatic counterparts as $OH^{\cdot}$ and $^1O_2$ can only be detoxified by the low-molecular-weight nonenzymatic antioxidants. On one hand, the underlying mechanisms of ROS production in all the stressed plants are universal, while on the other, antioxidant defense mechanisms involved in ROS scavenging vary according to the plant species (Uzilday et al. 2015). A brief description about both the antioxidant counterparts is described below.

## 27.3 Enzymatic Antioxidants

### 27.3.1 Superoxide Dismutases (SODs)

It is an enzymatic antioxidant molecule that operates as a physiological barrier for plants while combating abiotic and biotic challenges. SOD is a member of class metalloproteins and belongs to protein enzymes family. It is classified as EC 1.15.1.1, SOD. It can catalyze superoxide ($O_2^-$) into oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$) in aerobic organism and in some non-aerobic organism (Wang et al. 2009). It is well known that within a cell, SOD acts as preeminent defense against ROS. They are generated at any location inside the cell. Since $O_2^-$ is formed at any part of the cell where ETC is present, $O_2^-$ activation occurs at various compartments of organelles. However, chloroplast, peroxisomes, and mitochondria are major ROS generators (Fridovich 1986). The cellular membrane is impermeable to the superoxide molecules since it is composed of phospholipids (Takahashi and Asada 1983). So, presence of SODs in all the compartment organelles is important for removal of $O_2^-$ radicals. Plants have multiple SODs unlike other organisms, which play different physiological roles and help them to combat several environment-imposed abiotic stresses like salinity, cold, and drought (Bowler et al. 1994; Van Camp et al. 1994; Gill and Tuteja 2010). Based on metal cofactors present, SODs are of three

**Table 27.1** Types of plant SODs

| Sl. no. | Metal cofactor | Occurrence | Sensitivity | Structure |
|---|---|---|---|---|
| 1. | Iron-SOD (Fe-SOD) | Plastids, mitochondria, cytosol, and peroxisomes | $H_2O_2$ | Homodimeric and tetrameric |
| 2. | Manganese-SOD (Mn-SOD) | Peroxisomes and mitochondria | $CHCl_3$/ $CH_3CH_2OH$ | Homodimeric and homotetrameric |
| 3. | Copper/zinc-SOD (cu/Zn-SOD) | Plastids, peroxisomes, mitochondria, and cytosol | $H_2O_2$ and KCN | Homodimeric and homotetrameric |

types, viz., Fe-SOD, Mn-SOD, and Cu/Zn-SOD whose structure and sensitivity are described in Table 27.1.

Fe-SODs probably constitute the earliest group of SODs. They are found in both prokaryotes and eukaryotes. The Fe-SOD is resistant to KCN inhibition, but they are sensitive to $H_2O_2$. They are present in organelles like mitochondria, chloroplast, cytosol, and peroxisomes. Fe-SODs are further categorized into two well-defined groups of which one is homodimeric. The homodimeric ones contain two identical 20 kDa proteins with Fe in the core. They have been isolated from the *E. coli*, purple sulfur bacteria, and some plant species like *Ginkgo biloba* (Yost and Fridovich 1973; Kanematsu and Asada 1978; Salin and Bridges 1980). The Mn-SOD is found in cell wall also in addition to mitochondria and peroxisomes, while the Cu/Zn-SOD exists only in cytosol and plastids.

### 27.3.2 Catalases (CATs)

They are iron-containing, principal $H_2O_2$-scavenging enzyme antioxidant, which has systematic nomination as EC 1.11.1.6. CATs are mainly present in peroxisomes. Since they covert $H_2O_2$ to $O_2$ and $H_2O$, they prevent aggregation of harmful hydrogen peroxide in the cells (Garg and Manchanda 2009). They have the capacity to reduce $H_2O_2$ directly and hence are needed for detoxification of ROS during abiotic stresses. Plant-produced CATs can be grouped into three classes, namely, class I CAT, class II CAT, and class III CAT. Class I are the most prominent ones which function during removal of $H_2O_2$ that are formed during photosynthesis. Class II are produced mostly in vascular tissues of plants. There function is unknown in plants although they may have some role in lignification of tissues. They are mainly studied for disease development and resistance. Class III are found most abundantly in seeds and young plants. Their major function is to remove $H_2O_2$ that are produced in glyoxalate cycle during degradation of fatty acids (Willekens et al. 1994).

### 27.3.3 Glutathione Peroxidases (GPXs)

They constitute multiple isozymes having systemic nomination as EC 1.11.1.9. They act as catalyst during reduction of hydrogen peroxide and its derivatives into alcohol which are toxic to cell (Dixon et al. 1998). Additionally, they also detoxify lipid peroxidation products that are formed after the action of ROS. Plant GPXs are of three types, namely, glutathione transferases (GST, EC 2.5.1.18), selenium-dependent GPX (EC 1.11.1.19), and non-selenium-dependent phospholipid hydroperoxide GPX (PHGPX). The PHGPX shows response when plant suffers from salinity (Gueta-Dahan et al. 1997).

### 27.3.4 Ascorbate Peroxidases (APXs)

They have systemic nomination as EC 1.1.11.1. APXs play major role in plant antioxidant system which reduce $H_2O_2$ to water (Kangasjärvi et al. 2008). They have an extortionate affinity for $H_2O_2$ in comparison to PODs and CATs. Additionally, they also play an imperative role in ROS signaling (Davletova et al. 2005).

### 27.3.5 Glutathione Reductases (GRs)

They have systemic nomination as EC 1.6.4.2. They are primarily present in stroma of chloroplast although they can also be additionally present in peroxisomes, mitochondria, and cytosols.

## 27.4 Nonenzymatic Antioxidants

### 27.4.1 Ascorbic Acid

It is also known commonly as vitamin C. It is found in almost all cell organelles of plants. Ascorbic acid in plants is mainly present in reduced form, i.e., ascorbate, and its concentration in cytosol and mitochondria ranges from 20 mM and 20–300 mM, respectively. It protects the metabolic processes of plants from $H_2O_2$ as well as from free radicals. It is also responsible for influencing the actions of various enzymes, thereby reducing the disturbances caused by oxidative process (Pourcel et al. 2007).

### 27.4.2 Glutathione

It is present in almost all the cell compartments like ER, mitochondria, chloroplast, and cytosol in its reduced form (Millar et al. 2003). However, its amount is predominately higher in chloroplasts as compared to other organelles.

### 27.4.3 α-Tocopherols

They are found in all plant parts (Srivalli et al. 2003). They are predominantly present in chloroplast and mainly responsible for protecting plant cell from damage caused due to photooxidation.

### 27.4.4 Carotenoids

Carotenoids are found in all plants. They can protect the plant cell from peroxidative damage when present in high concentrations.

## 27.5 Mechanism of Antioxidant-Based Stress Responses

The stress imposed by abiotic factors on plants results in production of free radicals or ROS in plant cells. They are deleterious to organic macromolecules like nucleic acids, proteins, and lipids present in plant cells. This in turn interferes with crop growth and production (Aly et al. 2012). Cells communicate with each other via producing various biological stimuli called cell signals. These cell signals enable the plants to transmit the outside information inside the plant cells, leading them to communicate. This mechanism imparts capability to plants to sense the ROS and appropriately induce the cellular responses. Redox-sensitive proteins are the major players of this process as they can be subjected to reversible reactions in plant cells in accordance to its redox state (Shao et al. 2005). These redox-sensitive proteins also execute the service through numerous downstreaming signaling molecules (Li and Jin 2007).

### 27.5.1 Calcium Signaling During Abiotic Stresses

Calcium is one of the major essential nutrients for plants which has a major part to play as secondary messenger to relay the message during abiotic challenges (Mahajan et al. 2008). The varying cytosolic concentration of $Ca^{+2}$ (calcium ion) is well studied and proven for the transduction of abiotic stimuli like salinity, temperature stress, and osmotic stress (Sanders et al. 2002). The stimulus-specific change in the concentration of calcium ion in the cytosol is known as the calcium signature. The abiotic stress-imposed calcium signature is dependent on the type, rate of development, exposure, and type of cell affected by the stress (Plieth et al. 1999; Knight et al. 1997; Kiegle et al. 2000). Depending upon the subcellular location, magnitude, and duration of production of calcium ion, the cross talk signaling may occur (Ahmad et al. 2010). Any alteration in the cytosolic calcium signature is perceived by a set of specific proteins which are called as the calcium sensors. In abiotic stress signaling, calcium-dependent protein kinases (CDPKs) and

$SOS_3$ sensors are responsible for coupling of calcium signals to phosphorylation cascades (Ahmad et al. 2010).

### 27.5.2 Role of ABA in Signaling

Abscisic acid (ABA) is one of the hormones that is produced by plants and is known to play an indispensable role during abiotic challenges and thus is also referred to as stress hormone. With the advent of abiotic stresses, the genes responsible for biosynthesis of ABA get switched on which leads to its increased production. The precursor of ABA synthesis is β-carotene. The gene responsible for ABA biosynthesis is *ZEP* gene; this gene is present in every plant part (Xiong et al. 2002).

## 27.6 Antioxidant System Under Abiotic Stresses

For the mitigation of adverse impacts imposed by ROS, crop plants activate their antioxidant defense system. However, based on the genotypes, plant species, duration, and type of abiotic stress obtruded, the antioxidant defense ability of crop plant may vary. A brief description about the functioning of antioxidant system under different abiotic challenges is described below.

### 27.6.1 Drought

There are numerous researches that have proven that the plants activate their antioxidant system as adjusting mechanism to combat drought (Hasanuzzaman et al. 2018). It has been shown that when two canola varieties were subjected to drought stress, they had higher level of phenols, CAT, and POD in comparison to the control ones (Akram et al. 2018). Similarly, mung bean seedlings subjected to drought also had increased levels of GST, GR, APX, and GPX in comparison to the control suggesting the role of antioxidant in imparting drought tolerance to the plants (Nahar et al. 2017a, b). In another similar study pertaining to two varieties of sorghum, the $H_2O_2$ level was observed to be increased from 28.9% to 54.9% in the respective cultivars. In response to the drought stress, the SOD and APX levels increased to 1.1–1.6% and 0.9–1.7% in the respective cultivars (Guo et al. 2018). The tomato plants also respond to drought and increased generation of $H_2O_2$ and $O_2^{\cdot -}$ through alleviated production of APX, CAT, and POD by 77%, 66%, and 110%, respectively.

### 27.6.2 Salinity

There are numerous findings pointing toward the essential role of crop antioxidant machinery for amelioration of salt stress (Table 27.2). The tolerance of barley and

**Table 27.2** Antioxidant defense in plants under salt stress

| Sl. no. | Salinity level | Antioxidant defense | Crop | References |
|---|---|---|---|---|
| 1. | 100 mM | Melatonin increased APXs, PODs, SODs, and CATs by 16%, 15%, 29%, and 23%, respectively | Cucumber (*Cucumis sativus*) | Zhang et al. (2020a, b) |
| 2. | 250 mM | Melatonin increased the accumulation of Fe/Zn-SOD as well as PODs and APXs | Alfalfa (*Medicago sativa*) | Cen et al. (2020) |
| 3. | 100 mM | SODs, CATs, and GRs doubled and APXs tripled when treated with nitrogen | Wheat (*Triticum aestivum*) | Ahanger et al. (2019) |
| 4. | 100 mM | SODs, APXs, GRs, and CATs enhanced by 91%, 144%, 49%, and 33%, respectively | Black mustard (*Brassica juncea*) | Jahan et al. (2020) |

wheat against high salinity is due to the actions of enzymatic antioxidants (Zeeshan et al. 2020). The augmentation of silicon in mung bean increases its tolerance to salinity as it enhances the activity of antioxidants which ultimately keeps check on ROS, EL, and MDA levels (Ahmad et al. 2019). The application of salicylic acid to the wheat plants under salt stress increases its tolerance against the same by doubling CATs, SODs, and APXs while reducing the $H_2O_2$ three folds (Alsahli et al. 2019). Similarly, a combined application of jasmonic acid and humic acid in sorghum enhances the tolerance of sorghum to salt stress by increasing the levels of APXs (Ali et al. 2020).

### 27.6.3 Flood

By initiating antioxidant defense machinery, several crop species have the ability to survive under water-logged conditions (Table 27.3).

### 27.6.4 Temperature

To cope up with the high as well as low temperature, plants activate their antioxidant defense system in similar way like other abiotic stress factors. Kumar et al. (2013) observed that in sensitive chickpea cultivars (ICC14183 and ICC5912), activity of APX and GR decreased, thereby increasing the amount of $H_2O_2$ by twofold under high temperature. A similar report was communicated in germinating rice seeds that at higher temperatures, the levels of SODs and CATs were reduced (Liu et al. 2019). There was also a report of elevated POXs and CATs in wheat crop grown at 30 °C. At lower temperature of 8 °C in cucumber plants for 8 days, there was an increased generation of $H_2O_2$ and $O_2^{\cdot-}$ to which the plant responded by alleviating its Cu/Zn-SOD and Fe-SOD by three- and twofold, respectively (Zhao et al. 2016). An elevated level of $H_2O_2$ and $O_2^{\cdot-}$ was also observed in rice plants when exposed to

**Table 27.3** Antioxidant defense in plants under water logging

| Sl. no. | Flood (days) | Antioxidant defense | Crop | References |
|---|---|---|---|---|
| 1. | 12 | Increased SODs and CATs activities | Sorghum (*Sorghum bicolor*) | Zhang et al. (2019) |
| 2. | 21 | In both genotypes sensitive (TF 57) and tolerant (TF 58), the activities of PODs, SODs, and CATs increased | Barley (*Hordeum vulgare*) | Luan et al. (2018) |
| 3. | 15 | PODs, CATs, and SODs activities enhanced by 57%, 33%, and 7%, respectively, compared to control | Tomato (*Solanum lycopersicum*) | Rasheed et al. (2018) |
| 4. | 2, 4, 6, and 8 | MDHAR and APXs activities increased by 55% and 61%, respectively | Sesame (*Sesamum indicum*) | Anee et al. (2019) |

12 °C along with increased level of CATs and SODs (Han et al. 2018). A significant increase in the level of CATs was observed in watermelons exposed to low temperature (Shi et al. 2014a, b).

### 27.6.5 Heavy Metals

The antioxidant activity is positively correlated to the metal toxicity tolerance as it chelates out metal as well as detoxifies ROS (Gratão et al. 2019; Mahmud et al. 2019). GSH plays a major role in reducing the metal toxicity in assistance by GST, as GSH conjugates with metals (Kumar et al. 2019). Moreover, GSH also is the precursor of phytochelatins that has the capability of binding with the metal ions and facilitating their transport. The description of antioxidants against heavy metal stress in some of the plants is proved in Table 27.4.

## 27.7 Transgenic Approach for Manipulation of Plant Antioxidant System for Alleviated Tolerance Against Abiotic Stresses

There are several strategies for strengthening the plant antioxidant system in order to combat the negative impacts of abiotic stresses. Many transgenic technologies have been deployed for manipulating the same in plants. The alleviated transgenic antioxidant systems have also been proven to impart tolerance in plants to the abiotic challenges. These transgenic manipulations mainly upregulate the level of both enzymatic and nonenzymatic antioxidants by overexpressing the gene (s) responsible for their production (Rajeevkumar et al. 2015). It was shown that the negative impacts of drought were reduced by the upregulated APXs and Cu-Zn-SOD expressions in transgenic tobacco plants as compared to their non-transgenics (Faize et al. 2016). Similarly, an upregulated expression of Cu-Zn-SOD and APXs in

**Table 27.4** Antioxidant defense in plants under metal toxicity

| Sl. no. | Metal toxicity | Crop | Cultivar | Antioxidant defense | References |
|---|---|---|---|---|---|
| 1. | 0.5–1.0 mM $CdCl_2$ exposure for 2 days | Mustard (*B. napus*) | BINA Sharisaha-3 | Enhanced level of APXs and GRs | Hasanuzzaman et al. (2017) |
| 2. | 0.5 mM $AlCl_3$ exposure for 2 and 3 days | Mung bean (*Vigna radiata*) | BARI Mung-2 | Enhanced level of SODs, GRs, APXs, GPXs, and GSTs | Nahar et al. (2017a, b) |
| 3. | 0.25 and 0.5 mM $NiSO_4{\cdot}7H_2O$ exposure for 2 days | Rice (*Oryza sativa*) | BRRI dhan54 | Enhanced level of APXs, DHAR, MDHAR, and GRs by 114%, 31%, 116%, and 104%, respectively, at 0.5 mM | Hasanuzzaman et al. (2019) |
| 4. | 100 μM $NiCl_2$ exposure for 2 days | Pea (*Pisum sativum*) | – | Fivefold increase in GSH accumulation, sixfold increase in both CATs and APXs activity, 16-fold increase in SODs activity, and threefold increase in GRs activity | El-Amier et al. (2019) |
| | 100 μM of $CdCl_2$ exposure for 3 days | | | Threefold increase in GSH accumulation, tenfold increase in SODs activity, eightfold increase in APXs and CATs activity, fourfold increase in GRs activity | |

chloroplasts of transgenic sweet potato aided in recovering of plants from the impacts of drought. This in turn enhanced the resistance of transgenic sweet potato to drought stress (Lu et al. 2010). In transgenic potato plants, upregulated expression of *GLOase* multiplied the level of ascorbic acid by 141% in comparison to the non-transgenics during the conditions of salt, mannitol, and methyl viologen stresses (Hemavathi et al. 2010). A brief outline about the transgenic plants with enhanced activity of antioxidant defense system has been presented in Table 27.5.

## 27.8 Conclusion and Future Prospects

Abiotic stresses impose major restriction in the production and productivity of crop plants all over the world. Thus, it is very much understood that there is a need to find the ways which would embellish the crop tolerance to the abiotic stresses, by understanding the physiological, molecular, cellular, and biochemical mechanisms

**Table 27.5** Transgenic plants and their enhanced antioxidant defense system

| Sl. no. | Abiotic stress | Genes | Source plants | Examined plants | Transgenic activities | References |
|---|---|---|---|---|---|---|
| 1. | Salinity | *PaSOD* and *RaAPX* | *Potentilla atrosanguinea* (cinquefoil) and *Rheum australe* (red-veined pie plant) | *S. tuberosum* (potato) | Two- to sixfold increase in SODs and 5- to 11-fold increase in APXs | Shafi et al. (2017) |
| 2. | Salinity | *GmMyB84* | *Glycine max* (soybean) | *G. max* (soybean) | CATs, SODs, and PODs significantly enhanced | Zhang et al. (2020a, b) |
| 3. | Salinity | *VvWRKY30* | *Vitis vinifera* (grape) | *Arabidopsis thaliana* | CATs, SODs, and PODs significantly enhanced | Zhu et al. (2019) |
| 4. | Osmotic stress | *SoCYP85A1* | *Spinacia oleracea* (spinach) | *Nicotiana tabacum* (tobacco) | Improved activities of PODs and SODs by 1.3–1.5 and 1.36–1.39, respectively | Duan et al. (2017) |
| 5. | Drought | *MdATG18a* | *Malus domestica* (apple) | *M. domestica* (apple) | CATs and PODs increased by 1.57 and 2.05 | Sun et al. (2018) |
| 6. | Drought | *ZmSO* | *Zea mays* (maize) | *N. tabacum* (tobacco) | Increased activity of GSH by 64% and 88% in overexpressed lines | Xia et al. (2018) |
| 7. | Metal toxicity | *OsSultr1;1* | *O. sativa* (rice) | *A. thaliana* | Enzyme activity enhanced | Kumar et al. (2019) |
| 8. | Water logging | *BnERF2.4* | *B. napus* | *A. thaliana* | SODs, PODs, and CATs activity enhanced | Lv et al. (2016) |
| 9. | Water logging | *DaAPX* | *Dioscorea alata* (water yam or winged yam) | *A. thaliana* | APXs activity enhanced | Chen et al. (2019) |
| 10. | High temperature (48 °C) | *MdATG18a* | *M. domestica* (apple) | *M. domestica* | Increased SODs, PODs, CATs, ascorbic acid, and GSH levels | Huo et al. (2020) |
| 11. | Low temperature (4 °C) | *AtDREB1A* | *S. lycopersicum* (tomato) | *A. thaliana* | SODs increased by 29.49%<br>CAT increased by 21.34% | Karkute et al. (2019) |

of stress response in plants. By applying the knowledge of plant physiology and crop husbandry judiciously, it is possible to mitigate the losses caused by the same. The major problem caused by these stresses is ROS accumulation which causes damages to the plants. ROS scavenging is a vital part of plant's defense mechanism. Initially, ROS was recognized for its damaging by-products produced during aerobic mechanism. But now, it is an established fact that they also act as signaling molecules in plants. Moreover, to understand the harmful impacts of abiotic stresses, it is imperative to decipher the underlying mechanisms of tolerance and/or resistance in plants. Since the detoxification system in plants for the accumulated ROS is very complex and additionally controlled at multiple levels, it is quite challenging to decipher the exact functioning of each component. This also leaves us with a drawback that changing one component might not alter the whole antioxidative defense system (Lee et al. 2009). Therefore, more emphasis should be given on outlining the roles of each components that are involved in the antioxidant-based defense pathway for achieving tolerance to multiple abiotic stresses in a single crop plant.

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# Nematophagous Fungi in Antioxidant-Mediated Defense Against Plant Parasitic Nematodes

# 28

S. Saranya, Basavaraj Teli, Jyotika Purohit, R. K. Singh, and Anirudha Chattopadhyay

## Abstract

Nematophagous fungi play vital role in plant growth promotion and antioxidant defense response against many plant parasitic nematodes as well as plant pathogens. Huge bio-diversity and wider adaptability to various soil habitats, make them prominent predacious fungi that can be utilized in integrated pest management practices for sustainable crop production. Oxidative stress generated in the plants due to various biotic and abiotic factors leads to the imbalance in the host cellular mechanisms resulting in the cell death. Application of nematophagous fungi is one of the option to mitigate the oxidative stress. External root application of these nematophagous fungi not only parasitised on the plant parasitic nematodes with the help of trapping structure, but also induced the production of the various antioxidants to maintain the free radical ion synthesis directly and indirectly in the host plants. Thus, nematophagous fungi act as the best alternative for the management of plant parasitic nematodes and other pathogens to mitigate the oxidative stress and to maintain the plant health through antioxidant production.

## Keywords

Nematophagous fungi · Antioxidants · Oxidative stress · Biological control · Plant growth promotion

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## 28.1 Introduction

Plants are known to suffer with lots of biotic and abiotic stresses. Biotic stress includes all living organisms which cause damage to plants, viz., insects and nematodes. Among biotic stresses, plant parasitic nematodes play a vital role in limiting crop productivity. It was estimated that plant parasitic nematodes cause an annual crop loss of 10–15% that amounts to about $78 billion, and it directly impacts sustainable food production (Lima et al. 2018).

One of the control measures to eliminate the activity of nematodes in plant is the usage of nematophagous fungi. Nematophagous fungi are a group of carnivorous fungi which specially trap, kill, and digest nematodes (Braga and de Araújo 2014; Degenkolb and Vilcinskas 2016; de Freitas Soares et al. 2018). They are found in almost every natural soil and also in several other substrates. Use of antioxidants for managing the nematodes is very effective as most of the nematode infections resulted in generation of free radicals or ROS. Research on epidemiology strongly propounded that plants containing or supplied with antioxidants have a capacity to check out the disorders caused by free radicals and ROS. Recent data revealed that nematophagous fungi are not only biocontrol agents of plant parasitic nematodes but also potential producers of a variety of antioxidants, viz., catalase (CA), peroxidase (POX), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD), and polyphenol oxidase (PPO) which help the plants to mitigate the oxidative stress created by nematodes during the infection process (Singh et al. 2013; Singh et al. 2019a, 2019b; Mostafanezhad et al. 2014).

Oxidative stress is a kind of physio-chemical stress which occurs as a result of imbalance in the activity of free radicals and antioxidants in any biological system that leads to the tissue damage, and induces the accumulation of reactive oxygen species (ROS) in the cells and tissues (Sies 1997; Mittler 2002; Miller et al. 2010; Hussain et al. 2019). These free radicals and reactive oxygen species in turn react with other biomolecules and attain stability, thereby activating oxidative stress in plants (Asada 1987). ROS is produced frequently in cytosol, mitochondria, plastids, peroxisomes, and apoplasts in plants (Asada 1999). Excess generation of free radicals and reactive oxygen species results in damaging the DNA, RNA, protein, and lipids which ultimately leads to cell death and causes mutations with uncontrolled cell division.

Oxidative stress is found to be the root cause of occurrence and development of many diseases. The amount of lipid peroxidized in the biological system can be used as an indicator to detect the amount of reactive oxygen species (ROS) that induce systemic damage in the organisms (Popova and Popov 2002). Resistant plants have innate ability to synthesize these antioxidant enzymes. But susceptible plants fail to produce such antioxidant enzymes against biotic and abiotic stresses. Under these circumstances, nematophagous fungi can be used for supplying antioxidant enzymes against oxidative damages caused by biotic and abiotic factors. Thus, nematophagous fungi play a vital role not only in controlling plant parasitic nematodes but also in inducing structural and biochemical defense in plants (Singh et al. 2013).

In this chapter, we will discuss the various antioxidants produced by nematophagous fungi (NPF) and their role in promoting structural and biochemical defense in plants during various biotic and abiotic stresses.

## 28.2 What Are Antioxidants and Different Antioxidants in Plants?

According to Halliwell and Gutteridge (1995), antioxidants are the compound which ward off, retard, or eliminate oxidative damage. In other words, the substances which check or reduce the impairment caused to cells by free radicals produced during biotic and abiotic stress, are known as antioxidants. These antioxidants at lower concentration hold back, reduce, or retard the production of free radicals in plants during stress and ultimately hinder the oxidation process. Apart from this, antioxidants also act as the reducing agents by removing ROS intermediates and terminating many reactions, thereby inhibiting other oxidative stresses (Khlebnikov et al. 2007; Mehta and Gowder 2015).

Antioxidants are natural free radical scavengers (Halliwell 2001; Castelli et al. 2019). Though reactive oxygen species (ROS) are harmful, they serve as the important antioxidant defense signaling molecules, i.e., these ROS help to protect the cells against the damage caused by them by activating antioxidant defense mechanism in plants (Ahmad et al. 2010). During oxidative stress created by biotic and abiotic agents, a protective action is created in the cells by the antioxidant system.

The plant cells contain a variety of antioxidant mechanisms which serve to protect the plant cells and tissues against the damages caused by free radicals. These mechanisms include (1) chelating the trace elements to suppress the generation of free radicals or inhibiting the enzyme production and (2) scavenging the free radicals and ROS by stimulating the endogenous antioxidant defenses. Based on these mechanisms, antioxidants are characterized as primary antioxidants, secondary antioxidants, and tertiary antioxidants (Halliwell and Gutteridge 1995; Mehta and Gowder 2015).

### 28.2.1 Primary Antioxidants

Primary antioxidants are those antioxidants which provide first line of defense to plants during pathogenic infection (Ighodaro and Akinloye 2018), i.e., they check out or limit the generation of free radicals or reactive oxygen species in plant cells. These primary antioxidants include catalase (CA), superoxide dismutase (SOD), polyphenol oxidase (POX), phenylalanine ammonia lyase (PAL), and glutathione peroxidase (POX).

### 28.2.2 Secondary Antioxidants

These antioxidants function as scavengers of free radicals and impede the chain initiation or break the propagation of chain. They donate free electrons to free radicals and neutralize them, and these antioxidants in turn are converted into free radicals, but are less injurious to the biological system. Further, other antioxidants in this group neutralize these new radicals. Thus, this group is also referred to as scavenging antioxidants. This group includes ascorbic acid, glutathione, and α-tocopherols.

### 28.2.3 Tertiary Antioxidants

They serve as third line of defense (Ighodaro and Akinloye 2018) by repairing the impairment caused by free radicals to proteins, DNA, RNA, and lipids. This group includes polymerase, peptidase, protease, nuclease, and glycosylase.

### 28.2.4 Naturally Occurring Antioxidants

Plants have inherent potential to generate a variety of enzymatic and nonenzymatic antioxidants (Jaleel et al. 2008; Shi and Zhu 2008; Sharma and Dietz 2009; Ashraf 2009; Ahmad et al. 2010) which have the capacity to reduce reactive oxygen species-prompted oxidative stress. Once, the naturally occurring antioxidants in the cell are disturbed and get deficient, and oxidative stress appears; thereby making the plant cells more prone to attack by external agents like diseases and nematodes. Although plants can synthesize antioxidants naturally for the better management of free radicals, which cause oxidative stress in plants; they need external supply of antioxidant to improve its defense system.

### 28.2.5 Types of Antioxidants

Antioxidants are of two types, viz., enzymatic and nonenzymatic (Fig. 28.1). The enzymatic antioxidants disintegrate and detach the free radicals, whereas the nonenzymatic antioxidants intersperse the chain reactions of free radicals. Antioxidants may be either endogenous or exogenous (Mehta and Gowder 2015). Endogenous antioxidants are a group of antioxidants which are present in the plants naturally, and it provides defense against both abiotic and biotic stresses. Whenever an imbalance comes about between naturally occurring antioxidant and free radicals produced as a result of infection by pathogens or insects or nematodes, oxidative stress is generated in the plants. So the plants need some supplementary antioxidants. Those antioxidants which are supplied externally to the plants in addition to the antioxidants, are known as exogenous antioxidants. These exogenous antioxidants not only protect the plants from oxidative stress but also trigger the level of

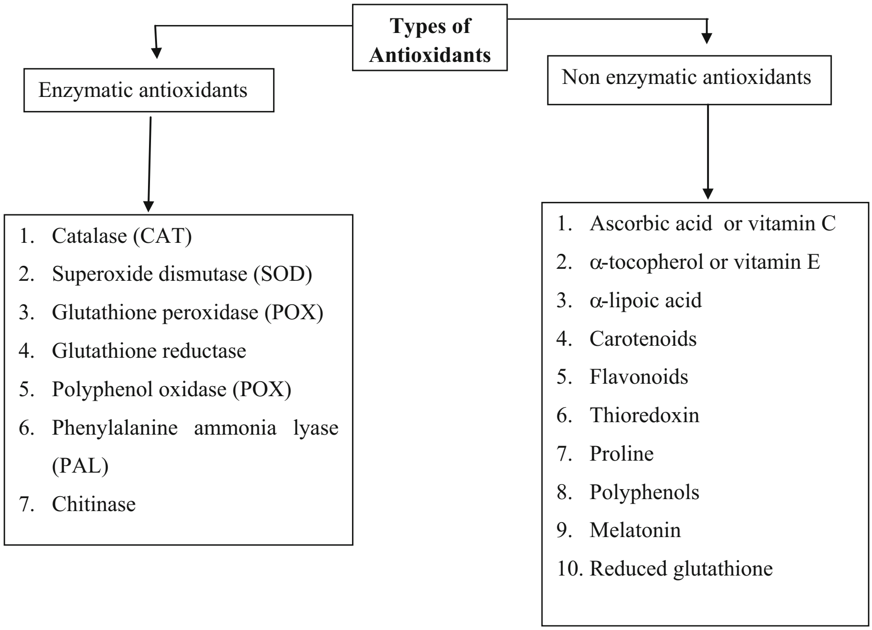

**Fig. 28.1** Types of antioxidants produced within plant system

endogenous antioxidants, thereby increasing the immunity of plants against stress condition.

## 28.3 How Antioxidants Are Released by Nematophagous Fungi with the Plant Interaction?

The plant infestation by parasitic nematodes leads to the initiation of oxidative stress in plants, which stimulate the generation of free radicals like hydrogen peroxide, superoxide ions, and hydroxyl ions and trigger the activity of ROS in plants. As a result, the defense system in plants gets broken. In addition to these free radicals, NADPH oxidases were also known to be resulted from nematode infestation.

Due to excess synthesis of free radicals and reactive oxygen species, plants start losing their natural immunity, and the DNA, protein, and lipids get damaged and require supplementary antioxidants for their growth and survival. Although various chemicals are available to supply antioxidants to plants, various bio-agents can be the novel alternative considering their cost-effectiveness, bio-efficacy, and environmental safety. One such option is the exploitation of nematophagous fungi as the bioagents and plant growth promoter. Nematophagous fungi are used not only to control nematodes, but also supply antioxidant to plants efficiently; thereby reducing the free radical production. Thus, nematophagous fungi prevent the further multiplication of nematodes and improve the defense mechanism in plants.

Nematophagous fungi (NPF) represent diverse predatory fungi species, which kill the plant parasitic nematodes through various mechanism (Chattopadhyay and Singh 2015) and put an end to the infection, colonization, and multiplication of parasitic nematodes within plants (Barron 1977; Hsueh et al. 2013). For so many years, these nematophagous fungi are known to act only as biocontrol agent for the plant parasitic nematodes. For better understanding, their biodiversity with seasonal/periodic distribution, ecological specificity, and biological activities against different plant parasitic nematodes are deciphered (Singh et al. 2014; Chattopadhyay and Singh 2015). Nowadays, they are known to synthesize a variety of antioxidants, which are not only prevent free radical formation in plants, but also help to stimulate the endogenous antioxidants present in the plants. Apart from predacious fungi, some edible mushrooms, i.e., *Pleurotus ostreatus*, is showing inhibitory action against *Meloidogyne graminicola* amended with organic substrates (Singh et al. 2019a, 2019b). NPF like *Paecilomyces lilacinus*, *Arthrobotrys oligospora*, *Pochonia chlamydosporia*, *Catenaria anguillulae*, *Haptoglossa dickii*, *Nematoctonus leiosporus*, *Stylopage grandis*, and *Pleurotus* sp. are known to produce a variety of enzymatic antioxidants (Table 28.1) and increase the host defense mechanism against nematode infection. They either directly target the plant parasitic nematodes with their different trapping structure (Chattopadhyay and Singh 2015) or else influence the host plant to regulate the growth promotion activity (Singh et al. 2014) to beat the various stresses developed against the host. NPF play a role in targeting plant pathogens occurring in the same host (Singh et al. 2012).

The inoculation of these nematophagous fungi in nematode-infested soil helps to reduce the nematode population and induce the activity of various enzymatic antioxidants, viz., superoxide dismutase, catalase, polyphenol oxidase, phenylalanine ammonia lyase, and peroxidase, to strengthen the histological and cellular defense at the time of nematode infestation (Singh et al. 2013; Mostafanezhad et al. 2014; Kamali et al. 2015).

In addition to the direct parasitization on the nematodes, the nematophagous fungi are known to colonize the host plant rhizosphere and become endophytes (Larriba et al. 2015). It may be either ectorhizosphere colonization or endorhizosphere colonization (Lopez-Llorca et al. 2006). Ectorhizosphere colonization, in a sense, is the colonization of external root surface of host plant (Persmark and Jansson 1997; Bordallo et al. 2002), whereas endorhizosphere colonization means colonizing the internal tissues of roots like the epidermis and cortex, but not vascular tissues (Lopez-Llorca et al. 2002; Bordallo et al. 2002). During nematode infection, the free radicals released by the host plant due to oxidative stress are recognized by these nematophagous fungi present in the rhizosphere region of host plants which in turn release antioxidants to neutralize the toxic effects of free radicals and help the plants to mitigate the stress created. Thus, a tritrophic interaction is seen between nematophagous fungi, plants, and plant parasitic nematodes. Figure 28.2 shows the direct and indirect interaction of nematophagous fungi to the plant parasitic nematodes in enhancing the antioxidants productions in the plants. In direct interaction, the nematophagous fungi will trap the nematodes infecting the plants and regulate the modulating stress by producing enhanced antioxidants in order to mitigate the ROS molecules. Similarly, in indirect interaction, the nematophagous

**Table 28.1** Antioxidant enzyme production by nematophagous fungi against plant parasitic nematodes

| S. no. | Nematophagous fungi | Trapping structure | Against nematode | Host plant | Antioxidant enzyme released | Function/role/results | References |
|---|---|---|---|---|---|---|---|
| 1. | *Arthrobotrys oligospora* | Adhesive nets | *Meloidogyne hapla* and *Heterodera schachtii* | Sugar beet | – | Less production of antioxidants | Andersson (2013) |
| | | | *Meloidogyne* sp. | Tomato | Peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, and some other phenolic compounds | Induction of biochemical and structural defense and reduced nematode population | Mostafanezhad et al. (2014) |
| 2. | *Monacrosporium haptotylum* | Adhesive knobs | *Meloidogyne hapla* and *Heterodera schachtii* | Sugar beet | Thioredoxins, CAT, and SOD | Less production of antioxidants | Andersson (2013) |
| 3. | *Arthrobotrys dactyloides* | Constricting rings | *M. hapla* and *H. schachtii* | Sugar beet | – | | |
| 4. | *Monacrosporium cionopagum* | Adhesive branches | *M. hapla* and *H. schachtii* | Sugar beet | – | | |
| 5. | *Drechslerella dactyloides* NDAd-05 | Constricting rings | *Meloidogyne incognita* | Tomato | PAL, SOD, peroxidase, chitinase, β-1,3-glucanase | Induction of structural defense and lower nematode infection | Singh et al. (2019a, 2019b, 2020) |
| 6. | *Dactylaria brochopaga* NDDb-15 | Constricting rings | *M. incognita* | Tomato | PAL, SOD, peroxidase, chitinase, β-1,3-glucanase | Induction of structural defense and lower nematode infection | |
| 7. | *Trichoderma harzianum* | – | *M. javanica* | Tomato | PAL | Reduction of wilt disease complex | Kamali et al. (2015) |

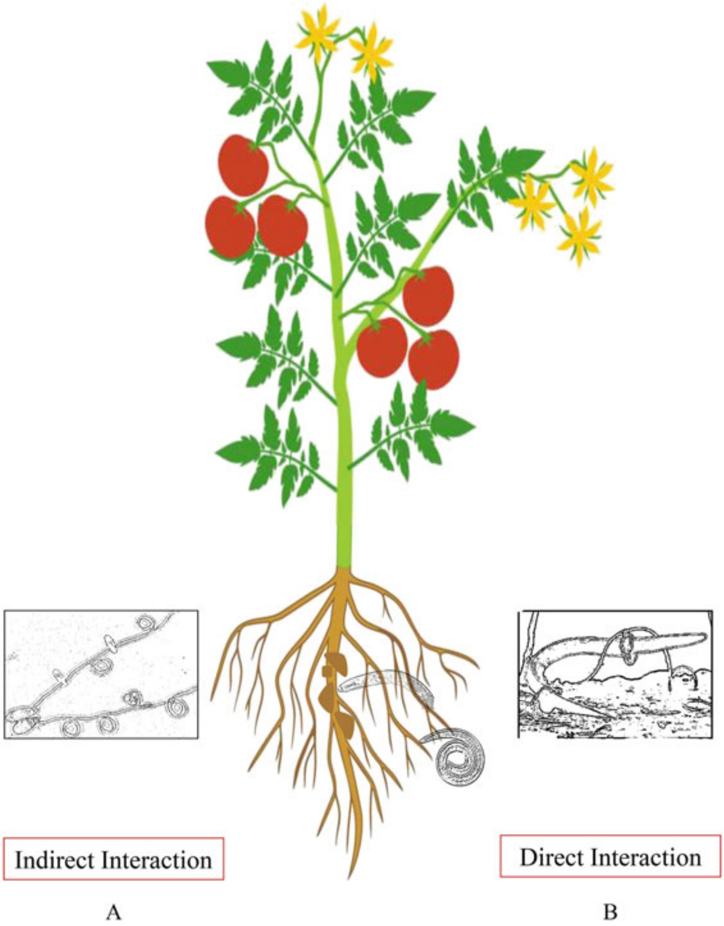

**Fig. 28.2** Direct and indirect interaction of nematophagous fungi in enhanced antioxidants production

fungi will colonize the roots portion and help in producing the antioxidants whenever necessary.

## 28.4 Mode of Action of NPF-Based Antioxidants

### 28.4.1 Superoxide Dismutase (SOD)

Among all the antioxidants, SOD is the first to detoxify the free radicals (Qin et al. 2017). The conversion of singlet oxygen radical or superoxide radical to hydrogen peroxide and molecular oxygen is catalyzed by SOD, thus making the free radicals like superoxide to potentially less hazardous form (Ighodaro and Akinloye 2018).

SOD uses various metal ions as cofactors, viz., iron, manganese, zinc, and copper (Fridovich 1995; Dringen et al. 2005; Gill and Tuteja 2010; Karuppanapandian et al. 2011). Based on metal ions used, SOD is classified as follows:

| Type of SOD | Occurrence |
|---|---|
| Fe-SOD | Prokaryotes and chloroplast of some plants |
| Mn-SOD | Prokaryotes and eukaryotes (in mitochondria) |
| Zn/Cu-SOD | Mainly in eukaryotes |

## 28.4.2 Catalase (CA)

Catalase is a commonly occurring antioxidant enzyme that exists almost in all living organisms which use oxygen. It catalyzes and reduces hydrogen peroxide into water and oxygen in cells affected with oxidative stress with the help of iron or manganese as cofactors (Droge 2002; Chelikani et al. 2004). This was the first discovered and characterized antioxidant enzyme:

$$H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O.$$

## 28.4.3 Glutathione Peroxidase (POX)

The conversion of hydrogen peroxide into water and lipid peroxides to their alcoholic states is catalyzed by this intracellular antioxidant enzyme with the help of selenium as a cofactor (Góth et al. 2004; Ighodaro and Akinloye 2018). Thus, it is also known as selenocysteine peroxidase:

$$\mathrm{ROOH} + 2\mathrm{GSH} \rightarrow H_2O + \mathrm{ROH}.$$

This enzyme helps in hindering lipid peroxidation and limits the production of free radicals and reactive oxygen species and eventually protects the plant cells from oxidative stress (Gill and Tuteja 2010; Ighodaro and Akinloye 2018).

Peroxidase contains a pathogenesis-related protein (PR-9) which catalyzes phenolic radical formation, thereby decreasing $H_2O_2$. It also increases the defense responses in plant by synthesizing lignin and cross-linking the cell wall proteins, so as to reinforce cell wall by polymerization of hydroxyl cinnamyl alcohols through hydrogen peroxide-dependent pathway and ultimately reduces the $H_2O_2$ levels.

### 28.4.4 Polyphenol Oxidase (PPO)

Polyphenol oxidases are a group of antioxidants which are involved in converting phenolic substrates to o-quinines with the help of copper as cofactors (Lalezar et al. 2016). These quinines in turn interact with oxygen and act as a highly reactive molecule, and produce nonenzymatic secondary reactions to form melanin which leads to enzymatic browning of tissues. The synthesis and function of PPO are still unclear.

### 28.4.5 Phenylalanine Ammonia Lyase (PAL)

This enzyme acts as a catalyst during deamination of phenyl alanine, an amino acid in phenylpropanoid pathway, and leads to the production of cinnamic acid. PAL is known to produce various polyphenolic compounds (MacDonald and D'Cunha 2007; Lalezar et al. 2016).

## 28.5 Can Antioxidants Produced by NPF Help the Plants for Structural and Biochemical Defense Against Plant Parasitic Nematodes?

The first and foremost step in defense mechanism is recognition of pathogen or parasite. Once the nematode attacks the plants, the plants inoculated with nematophagous fungi can recognize the nematode-originated molecules called ascarosides, which acts as nematode-associated molecular patterns (NAMPs) (Butcher et al. 2007; Srinivasan et al. 2008, 2012; Hsueh et al. 2013). These NAMPs act as the key molecules to trigger the innate immunity during plant-nematode interaction (Butcher et al. 2008; Pungaliya et al. 2009; Choe et al. 2012). Upon recognition or detection of these NAMPs with the help of plant pattern recognition receptors (PRRs), the down-stream defense signaling pathways are activated to safeguard themselves from nematode infection via., hypersensitive response (HR) and sudden cell death by generating reactive oxygen species (ROS). This immunity triggered in plants may be either pattern-triggered immunity (PTI) or effector-triggered immunity (ETI). PTI and ETI are produced as a result of jasmonic acid signaling which is responsible for induced systemic resistance. These molecular patterns are recognized by the plants treated with nematophagous fungi, and systemic resistance is promoted in plants rapidly during nematode infection. This process is collectively called defense priming (Conrath et al. 2001; Conrath et al. 2015; Martinez-Medina et al. 2016; dos Santos et al. 2017).

Plants have inherent immunity to promote local and systemic defense systems against various biotic and abiotic stress-initiating agents (Ramirez-Prado et al. 2018). Most of the resistant plants counteract against the biotic and abiotic stresses by initiating the innate defense mechanism like excess production of ROS or by certain hypersensitive reaction (HR) like programmed cell death (PCD). Since obligate parasites like nematodes and certain fungi and bacteria require living host cells for their growth and development, the hypersensitive response like sudden death of cell helps the plant to resist their multiplication to healthy cells. This is known as systemic acquired resistance (SAR) and is mediated by salicylic acid-based compounds. But susceptible plants fail to activate innate defense system during nematode infection. In that case, if nematophagous fungi are inoculated, they interact with the free radicals produced in the plants, and the various antioxidants present in the nematophagous fungi neutralize the free radicals and stimulate the innate defense mechanism of plants, thereby promoting the structural and biochemical defense in the plants. Many reports are there, which delineate that the amount of these defense-related biomolecules like peroxidase, catalase, phenyl ammonia lyase, polyphenol oxidase, and superoxide dismutase gets increased by the application of nematophagous fungi during oxidative stress created in plants by nematode infection (Chen et al. 2000; Wu et al. 2009; Singh et al. 2013; Mostafanezhad et al. 2014).

The antioxidants produced by nematophagous fungi, viz., CAT, POX, PPO, SOD, and PAL, are known to promote the biochemical defense in plants during stress condition by neutralizing the toxic free radicals like superoxide, nitrous oxide, and hydroxyl radicals and help in accumulation of phenolic compounds. Higher accumulation of phenolic compounds in the plants helps in improving the biochemical defense system in the plants suffering from stress caused by nematodes during interaction with host plants. Apart from this, structural defense is also promoted by the nematophagous fungi in which it helps in deposition of callose, pectin, and lignin in host plant. Accumulation of these substances in the roots of the plants helps to mitigate the stress induced by nematodes during infection. Mostafanezhad et al. (2014) reported that lignification of roots was observed at 45 days after inoculation of nematophagous fungi, and this is due to lignin production or transportation of monolignols. This lignification starts by activating the phenylpropanoid pathways.

Among the various antioxidants, viz., peroxidase, catalase and superoxide dismutase are found to be involved in terminating the activities of reactive oxygen species and toxic effect of free radicals, while other antioxidants like polyphenol oxidase, and phenylalanine ammonia lyase are found to be involved in strengthening the cell wall by lignification process. Thus, these antioxidants play a vital role in creating structural defense in plants during oxidative stress. Further, nematophagous fungi help in accumulation of pectic substances in plant roots that strengthen the cell wall and also reduce the intercellular gaps, thereby reducing the nematode infection. Thus, nematophagous fungi can promote structural and biochemical defense in plants against nematode infection by promoting induced systemic resistance in plants.

## 28.6 Role of NPF-Produced Antioxidants Against Various Biotic and Abiotic Stresses

The antioxidants produced by nematophagous fungi, viz., PAL, CAT, SOD, POX, and PPO, are known to help the plants in mitigating various biotic and abiotic stresses. When the nematophagous fungi are inoculated in the plant suffered under various stress, they produce certain enzymes which react with the free radicals produced as a result of oxidative stress and neutralize the toxic effects created by them. Furthermore, the nematophagous fungi also help to promote the innate defense-related biomolecules which are present in the plants. The enzymes that are found in leaves of host plant are responsible for creating defense in plants against biotic stress.

Phenylalanine ammonia lyase (PAL) is considered to be the primary enzyme responsible for creating induced systemic resistance (ISR)-like reaction in plants through phenyl propanoid pathway during biotic and abiotic stress created in plants. Also, PAL is the key enzyme involved in the generation of certain polyphenolic compounds, viz., lignins, phenylpropanoids, and flavonoids (Yang et al. 2009; Singh et al. 2009, 2012, 2013; Gao et al. 2010). During biotic and abiotic stress conditions, there will be an elevation in the level of toxic hydrogen peroxide and superoxide radicals in the plants. The enzymes catalase and superoxide dismutase produced by nematophagous fungi help in detoxifying these free radicals like hydrogen peroxide and superoxide, respectively, and also disintegrate the chain reactive oxygen species.

Peroxidase (POX) is considered to be one of the important antioxidant enzymes, because it catalyzes many essential biological processes which promote plant defense mechanism during oxidative damage that occurs during stress condition (Passardi et al. 2005; Gupta 2010; Lalezar et al. 2016). For example, polymerization of suberin (Arrieta-Baez and Stark 2006), cross-linkage of extensin (a structural protein) (Jackson et al. 2001), and lignin synthesis (Almagro et al. 2009) are catalyzed by peroxidase enzyme. Polyphenol oxidase (PPO) catalyzes the biosynthesis of quinines from polyphenols. Quinine is an antimicrobial compound which generates resistance in plants against certain pathogenic microbes. PPO is also involved in cell wall thickening by producing lignin, which helps the plants to resist the damage caused by oxidative stress (Constabel and Barbehenn 2008; Tran et al. 2012; Lalezar et al. 2016).

## 28.7 Conclusion

In this article, the various effects of free radicals, antioxidants produced by nematophagous fungi within plant system are addressed; their mode of action and their role in promoting various defense mechanisms against biotic and abiotic stress are also discussed. ROS act as signaling molecules which help in growth and development of plants, generation of hypersensitive response, systemic acquired resistance, production of stress hormone, and programmed cell death in plants as a result of pathogen infection. The enzymatic antioxidants play a vital role in eliminating free radicals, encouraging the host defense mechanism, and promoting the growth of plants, thereby checking out the activity of nematodes. They also improve the induced systemic resistance (ISR) in plants. Thus, use of nematophagous fungi-derived antioxidants is not only safe, but it is a safe and eco-friendly measure to combat the impairment caused by oxidative stress caused by plant parasitic nematodes. Awareness among farmers about the multiple benefits of nematophagous fungi as biocontroller and plant defense promoter should be created. Large-scale commercial production of various nematophagous fungi should be promoted.

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# Biopriming and Nanopriming: Green Revolution Wings to Increase Plant Yield, Growth, and Development Under Stress Condition and Forward Dimensions

# 29

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## Abstract

In biopriming, beneficial microorganisms or biological control factors are employed in roots or seeds that improve plant growth or disease control through various mechanisms, including the production of plant hormones, antibiotics, or enzymes. *Trichoderma* spp. produce regulatory growth factors that increase germination speed and dry weight of stems and roots under drought stress conditions. Formation of the colony in plant roots by *Trichoderma* spp. results in increased root growth and expansion, more crop production, increased resistance to abiotic stress, and availability of nutrients. *Trichoderma* fungus increases the solubility and availability of phosphorus and microelements to the plant. This increase improves the growth, vigor, and disease resistance in plants resistant to pathogens and drought. Various studies have reported that biopriming with PGPR in combination (combining different bacteria and fungi) under conditions of salinity and water deficit can increase the relative water content and growth of seedlings and even pigments and protein content. Generally, biopriming with

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PGPR improves water status in stressful seedlings, and physiological and biochemical parameters have been improved as the result of the severe effects of water stress. Biopriming occurs naturally with soil microorganisms, and it is a safe and nonpolluting action as well as a controlling agent for environmental diseases; in the meantime, it is an effective preplanting treatment. This chapter will try to introduce PGPR, priming and their types, the role of biopriming in germination, chlorophyll rate, function, function components, increased plant growth and development under stress, and production of substances such as ACC deaminase, siderophore, phytohormones, and antioxidants, and bio-stabilization of nitrogen and dissolution of insoluble inorganic and organic phosphates should be evaluated.

**Keywords**

Abiotic stress · ACC deaminase · PGPR · Phosphate solubilization · Siderophore

## Abbreviations

| | |
|---|---|
| ACC | 1-Aminocyclopropane-1-carboxylic |
| BNF | Biological nitrogen fixation |
| CAT | Catalase |
| CFU/ml | Colony-forming units/ml |
| GPX | Glutathione peroxidase |
| HCN | Hydrogen cyanide |
| HPLC | High-performance liquid chromatography |
| IAA | Indoleacetic acid |
| ISR | Induced systemic resistance |
| NA | Nutrient agar |
| NUE | Nitrogen use efficiency |
| PDA | Potato dextrose agar |
| PGPR | Plant growth-promoting rhizobacteria |
| ROS | Reactive oxygen species |
| SAR | Systemic acquired resistance |
| SMP | Solid matrix priming |
| SOD | Superoxide dismutase |

## 29.1 Introduction

Soil pollution and environmental hazards caused by reckless and excessive use of chemical fertilizers on agricultural products have been a major issue in today's agriculture. Besides, the risk to human health has led to a strict regulatory framework for the use of synthetic chemicals in agriculture. Biofertilizers have been proposed as the most practical environmentally friendly solution to these problems. Since the introduction of the first case, they have gained a great deal of consumer acceptance. Bio-stimulants are substances that contain living microorganisms that enhance plant growth and maintain plant growth and soil health by increasing the supply or availability of primary nutrients to the host plant (Ali-Soufi et al. 2016, 2019).

Seed biopriming through plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) is one of the effective methods of increasing the growth rate and the efficiency of nutrient uptake by the plant. It reduces dependence on chemical fertilizers, and it is an attractive option for farmers with low physical resources and income. The most prominent agents that are widely used in biopriming include *Trichoderma* spp., *Pseudomonas* spp., *Glomus* spp., *Bacillus* spp., *Agrobacterium* spp., and *Gliocladium* spp. (Ali-Soufi et al. 2017a, c, 2019).

In this chapter, we discuss the potential of biopriming to improve crop growth and nutrient efficiency and present an assessment of existing organisms with crop species and key constraints.

## 29.2 Seed Priming

Seed priming is a physiological method that improves seed function for acceleration and harmonious germination (Amozadeh and Fazeli-Nasab 2012; Mohammadi and Amiri 2010). During this treatment, a controlled amount of water is absorbed by the seed to start metabolic activities (Lechowska et al. 2019; Sayyed et al. 2019). In priming, the water uptake level in the seeds is controlled to improve germination, seedling establishment, and function (Bradford 1986; Feghhenabi et al. 2020; Naik et al. 2018). Seed priming appears to increase germination by reducing damage to proteins, RNA, and DNA (Nguyen et al. 2017; Zope et al. 2019b; Abbas Dokht and Edalat Psheh 2002; Ambehabati et al. 2020). The acceleration of germination in primed seeds is due to increased levels of alpha-amylase, ATP, RNA, and DNA synthesis and improved function of mitochondria (Afzal et al. 2008; Harris et al. 2008).

Poor seedling establishment due to drought is one of the most important problems in semiarid regions. So, increasing germination speed is very important in improving plant establishment (Fazeli-Nasab et al. 2016; Kaur et al. 2003; Saxena and Sayyed 2018). This can be achieved through seed that improves seed germination and initial establishment of the plant (Farooq et al. 2006; Mousavi and Omidi 2019), utilization of environmental inputs, early maturity, increasing the quantity and quality of the product (Harris et al. 2008), and improving food quality of seeds (Fazeli-Nasab and Sayyed 2019; Mousavi and Omidi 2019). Seed priming with biological treatments can be a way to control the speed of seed degradation process during storage and improve its effects after storage (Bakhit et al. 2017). Seed priming can improve physiological reactions in seeds under environmental stress conditions and thus significantly increase stress resistance. In primed seeds that are exposed to stress in their substrate, the degradation of macromolecules, nucleic acids, and oxidative reactions leading to the production of toxic and harmful substances such as free radicals is far less than seeds are untreated.

## 29.3 Types of Priming

A large number of seed priming methods have been used in agriculture in the past until now (Shumaila and Ullah 2020). These techniques include hydro-priming (aqueous pre-treatment), halo priming (absorption of water in nonaqueous saline solution), osmo-priming (water absorption in various osmotic solutions), matrix priming (seed treatment with wet solid matrix), and biopriming (pre-treatment in biological solutions and priming with plant growth hormones) (Aluko et al. 2020; Dhage and Anishettar 2020; Rasouli et al. 2020).

### 29.3.1 Matrix Priming

One of the priming methods is the use of solid matrices with low matrix potential, which is one of the matrix improvement methods and is called SMP (Pandya et al. 2018). The usable solids must have several special properties, such as low matrix potential, very low solubility in water, high water-holding capacity, high contact surface, non-toxicity, and ease of adhesion to the seed. Specific vermiculite, silt, and microcellular compounds (mainly soil particles containing silica, hydrated lime, and water) are commonly used which have high matrix potential and low osmotic potential. Seeds primed with solids will allow enough water to be absorbed to complete the pre-germination stages, but the amount of water absorbed will not be sufficient for root emergence (Ashraf and Foolad 2005; Bhamare et al. 2018). Perlite and vermiculite have been used as effective matrices (Patel et al. 2018; Vasei Kashani et al. 2015)

### 29.3.2 Hydro-Priming

Aqueous pre-treatment is a method in which the seeds are first soaked in water and then dried so that germination processes begin, but the root does not come out of the seed (Das et al. 2020; El Enshasy et al. 2020). This technique is the simplest and cheapest way to treat seeds, and it is very suitable for preserving the environment, but in some cases, it causes the seeds to not absorb water evenly (Bradford 1986; Das et al. 2020; Rowse et al. 2001; Zope et al. 2019a). Hydro-priming improves important germination indices such as germination percentage, root length, stem length and normal seedling percentage, in increasing plant tolerance to environmental stresses and better establishment and greater uniformity (Farahani et al. 2011; Tatari et al. 2020; Afzal et al. 2002; Singh et al. 2019).

### 29.3.3 Halo Priming

Halo priming is soaking the seeds in different concentrations of mineral salts (such as NaCl, $CaCl_2$, $ZnSO_4$) that improve germination and monotonous emergence of seedlings under adverse environmental conditions (Afzal et al. 2008; Das et al. 2020; Kour et al. 2019).

### 29.3.4 Osmo-priming

Osmo-priming refers to placing dry seeds in solutions with osmotic potentials, such as polyethylene glycol, sorbitol, or mannitol, followed by drying the seeds before planting. The lower the osmotic potential of the treatment solution, the less water can be absorbed by the seed (Anwar et al. 2020; Black and Bewley 2000; Bradford 1995; Saberali and Shirmohamadi-Aliakbarkhani 2020; Michel and Kaufmann 1973; Yadav et al. 2019).

### 29.3.5 Biopriming

Biopriming (priming of seeds with biofertilizers) uses beneficial microorganisms or biocontrol factors in the roots or seeds that provide improved plant growth or disease control through various mechanisms including the production of plant hormones, antibiotics, or enzymes (Bennett and Whipps 2008b; Syamsuwida and Sudrajat 2020; Gharib et al. 2008; Syamsuwida and Sudrajat 2020; Bennett and Whipps 2008a, b). Seed bio-enhancement by using fungi and bacteria that increase plant growth is one of the most effective methods of seed priming, and it is gradually being replaced by chemical treatments (Bashan et al. 2004; Rana et al. 2020).

#### 29.3.5.1 Biopriming Steps Based on Bacterial Microstructure

In step one, to prepare the NA culture medium, 5.6 g of NA and 1.25 g of agar were mixed in 250 ml of distilled water in an Erlenmeyer flask. To disinfect the culture medium, Erlenmeyer was placed in an autoclave at 121 °C for 2 h. The disinfected culture medium was poured into 90 mm flakes to cool.

In step two, bacterial isolates were cultured with a laboratory tube (loop) from the samples taken (Koohkan et al. 2020; Piri et al. 2020) on NA culture medium in a zigzag pattern and placed in an incubator at 28 °C for 24–48 h for bacterial growth. Then, by pouring the suspension into the cuvette, in the spectrophotometer, the absorption rate was calibrated on the number 0.5 in a wavelength of 600 nm. When it gives us the number 0.5 at a wavelength of 600 nm, it means that it is the same density suitable for seed inoculation (inoculum density: $10^8$ CFU/ml). Seeds were subjected to bacterial inoculation treatments for 2 h. To attach the suspension to the seeds, 0.1% Arabic gum was used, but no gum is needed in mucilage seeds.

#### 29.3.5.2 Biopriming Steps Based on Fungi Microstructure

In step one, 5.25 g of PDA and 0.62 g of agar in 125 ml of distilled water were mixed in an Erlenmeyer flask to prepare the culture medium. To disinfect the culture medium, Erlenmeyer was placed in an autoclave at 121 °C for 2 h. Pour the disinfected culture medium into 90 mm petri dishes to cool.

Using a loop, the colonies of fungal strains were cultured on PDA medium and placed in an incubator (Parsian Teb model) at 27 °C. After growing the fungi and abundant sporulation for 10 days, the spores were poured into the Erlenmeyer sterol with the culture medium. In the next step, the fungal colonies along with the culture medium were removed in a square shape and placed in 20 ml of distilled water. It should be noted that a shaker can be used to separate the fungal colonies from the culture medium.

To study the concentration of the fungal suspension inoculum using a hemocytometer slide (blood cell count), the spores were counted under a microscope (suspension of $10^7$ spores per milliliter suitable for seed inoculation). After achieving the appropriate density, the fungicides of the seeds were placed in this suspension for 2 h. After inoculation, the culture was performed in a petri dish on top of the paper.

Many physiological mechanisms occur during primings, such as the repair of damage to seed cells, the progression of metabolic events that occur in the second phase of water uptake leading to root-out, and events such as division, better endosperm, and storage materials that allow the fetus to grow more and increase its efficiency (He et al. 2010; Jie 2002). To increase the growth components as well as control the soil diseases, in this method, biological factors such as fungi and bacteria are used instead of chemical treatment. Beneficial released bacteria (rhizosphere) are often called plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 2004; Pascale et al. 2020). These bacteria, either directly (nitrogen fixation, production of plant growth regulators, production of ACC deaminase enzyme, increase the absorption of various elements for the plant, production of vitamins and other plant growth stimulants) or indirectly (production of antibiotics or antibiotics, depletion of iron rhizosphere. Competition with harmful species for root transmission, production of enzymes that break down the cell wall of plant pathogenic fungi, systemic resistance in plants and also by increasing plant resistance to abiotic stresses) can increase plant growth.

Growth-stimulating bacteria also release high levels of vitamin B, nicotinic acid, heterodoxies, and other compounds into the soil, which leads to increased plant growth (Ramakrishnan and Selvakumar 2012). On the other hand, accelerating the germination process is a prerequisite for establishing good vegetation and efficient use of resources and increasing function (Farooq et al. 2019; Harris 1996). In biopriming, the seed is primed with a bacterial biocontrol substance, followed by incubation of the seed under heat and moisture conditions. Biopriming occurs naturally with soil microorganisms, and it is a safe, pollution-free operation as well as a controlling factor in environmental diseases while also being an effective prognostic treatment (Entesari et al. 2013a).

#### 29.3.5.3 The Necessity of Seed Biopriming

Soil quality depends not only on its physical and chemical properties but also on its very close biological properties. Microbial communities play an important role in the production of agricultural ecosystems. A significant number of bacterial and fungal species of soil have functional relationships with plants and have beneficial effects on their growth. Today, it is believed that the interrelationships between plant roots and soil microorganisms have been affected by human interventions through agricultural and industrial activities. Since in a soil profile along with the plant, the root environment is the center of gravity of the energy in the soil, any change in soil fertility management, whether or not fertilization imbalance, or the use of organic matter, etc., can have a major impact on soil-plant coexistence and subsequently affect agricultural production (Entesari et al. 2013a).

Currently, to maintain and increase soil fertility in sustainable agriculture, biofertilizers have been proposed as a suitable alternative to chemical fertilizers (Wu et al. 2005). Biofertilizers contain preservatives and dense populations of one or more beneficial soil microorganisms or their metabolic products that are used solely to provide the nutrients needed by plants in an agricultural ecosystem. The use of beneficial organisms under the title of biofertilizers is considered as the most natural and desirable solution to keep the vital system of the soil alive and active (Ali-Soufi et al. 2017b; Darzi et al. 2011).

A group of these bacterial species that can help plants belong to the genera *Pseudomonas flavescens*, *Azotobacter* spp., *Spirillum* spp., and *Bacillus* spp., and the phosphate-dissolving bacteria include a group of tiny organisms that are capable of converting phosphorus insoluble in soil into an available plant-soluble form. The most important genera of this family are *Pseudomonas* spp. and *Bacillus* spp.

It is reported (Hoseini-Moghaddam et al. 2018) that drought stress poses negative effects on all germination indices of fennel. The occurrence of drought stress in the germination stage is associated with a decrease in water potential in the seedbed, which results in a decrease in germination rate and percentage and seedling growth. Inoculation of fennel seeds by *Trichoderma harzianum* and *Pseudomonas fluorescens* bacteria has been able to reduce the negative effects of drought stress by improving germination and seedling growth indices. The best results were obtained in the use of CHA0 bacteria and T39 fungus under optimal conditions and stress, and the use of these treatments revealed a suitable solution to counteract the destructive effects of stress. Based on the obtained results, it has been recommended that inoculation of fennel seeds before planting with T39 strain of *Trichoderma harzianum* and CHA0 strain of *Pseudomonas fluorescens* is a suitable solution to increase germination percentage, seed vigor, and drought resistance.

## 29.4 Plant Growth-Promoting Rhizobacteria (PGPR)

The types of rhizobacteria that have a positive effect on plant growth and function are called plant growth-promoting rhizobacteria (PGPR). The term was first coined by Kloepper and Schroth (1978), and until later years, it was used only for a variety of rhizosphere bacteria that indirectly provide plant health and growth by controlling plant pathogens (Beneduzi et al. 2012; Fazeli-Nasab and Sayyed 2019; Bhattacharyya and Jha 2012; Fazeli-Nasab and Sayyed 2019; Kenawy et al. 2019; Mayak et al. 2004; Meena et al. 2016; Bloemberg and Lugtenberg 2001).

Among plant growth-promoting bacteria, the genus *Pseudomonas* due to its wide distribution in soil, ability to colonize the rhizosphere of many plants, and production of a wide range of plant-stimulating traits such as auxin (Patten and Glick 2002a, b), ACC deaminase enzyme (Penrose and Glick 2003), siderophore (Meyer 2000), salicylic acid (Maurhofer et al. 1998), chitinase (Ajit et al. 2006), hydrogen cyanide (Schippers et al. 1990), and phosphate solvent (Rashid et al. 2004) has a direct and indirect effect on plant growth. One of the direct mechanisms affecting the growth of bacteria is the production of phytohormones such as auxin, cytokinin, and gibberellin and inhibition of ethylene production (Chinnusamy et al. 2004; Fazeli-Nasab et al. 2021). Other mechanisms by which growth-promoting bacteria (PGPR) improve plant growth under stress conditions include improved water uptake and nutrients by plant, growth of plant root, prevention of airborne falls, increasing viscosity, and biological stabilization in molecular nitrogen (Khan et al. 2019; Renaut et al. 2004).

### 29.4.1 *Pseudomonas flavescens* Bacteria

Among the plants that stimulate plant growth, *Pseudomonas* bacteria are of particular importance due to their widespread distribution in the soil, ability to colonize the rhizosphere of many plants, and production of a wide variety of metabolites. These gram-negative bacteria belong to the division Proteobacteria, classis Proteobacteria-*Υ*, ordo Pseudomonales, familia Pseudomonaeae, and genus Pseudomonas (Couillerot et al. 2009).

Among the bacteria, *Pseudomonas flavescens* are among the plant growth-promoting bacteria that are present in most agricultural soils and have different growth-promoting properties. These bacteria increase soluble and absorbable phosphorus, especially through the dissolution of insoluble organic and inorganic phosphates (Abo-Elyousr and El-Hendawy 2008; Alipour and Sobhanipour 2012).

*Pseudomonas* bacteria have a wide range of plant growth-promoting traits such as auxin production (Linu et al. 2019; Pandey and Gupta 2019; Patten and Glick 2002b), production of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, phosphate solubilization (Rashid et al. 2004), and production of siderophore, salicylic acid, chitinase, and hydrogen cyanide that directly and indirectly increases plant growth (Haas and Défago 2005; Harman 2006; Mehnaz and Lazarovits 2006). *Pseudomonas flavescens* increase the function and dry weight of various plants by producing domestic acid and phosphate-soluble acids.

The production of cytokinin in the root by bacteria causes the increase of the lateral roots and in leaf the production of chloroplasts with expanded gravity and chlorophyll, and also, the production of photosynthetic enzymes is done faster (Sakakibara 2006). Inoculation of seeds with *Pseudomonas flavescens* increases plant dry weight, iron uptake, and maize chlorophyll (Alipour and Sobhanipour 2012; Mishra et al. 2011; Zabihi et al. 2011).

## 29.5 Mechanism of Effectiveness of Growth-Promoting Bacteria on the Plant

### 29.5.1 Increase Plant Growth

The effects of PGPR bacteria on plant growth are both direct and indirect effects (Jadhav et al. 2017; Zahir et al. 2004). In the direct state, PGPR types stimulate and increase plant growth by using nitrogen biological stabilization mechanisms, increasing uptake and solubility or dissolving nutrients, producing plant growth hormones, producing various vitamins, producing iron-chelating factors, and dissolving phosphate (Fazeli-Nasab and Sayyed 2019; Lucy et al. 2004).

Indirectly, using various antagonistic mechanisms neutralizes or modulates the harmful effects of plant pathogens, thereby increasing plant growth. Competition for material uptake and occupation of suitable sites for pathogen activity and production of antibiotics and hydrogen cyanide (HCN) are the most important mechanisms used in this method (Glick 1995; Rostami and Azhdarpoor 2019; Saeidi and Fazeli-Nasab 2019; Vessey 2003; Van Loon 2007).

Direct mechanisms of plant growth promotion include P solubilization, production of phytohormone, and increased N2 fixation (Ali-Soufi et al. 2019; Glick 1995; Vessey 2003; Mirshekari et al. 2009; Zahir et al. 2004; Gutiérrez Mañero et al. 2003; Bharathi et al. 2004; Pan et al. 1999; Egamberdieva and Kucharova 2009; Ahmadi et al. 2004; Bacilio et al. 2003; Broek et al. 1999; Hubbell et al. 1981).

### 29.5.2 Siderophore Production Capacity

Siderophores are low-molecular-weight organic compounds and chemical ligands with a strong affinity for binding to iron III. Some strains of PGPR bacteria can secrete siderophore in iron deficiency conditions. In addition to providing the iron needed by the plant, this mechanism also has an indirect effect on plant growth. This effect is achieved by reducing the growth of plant pathogens and reducing the access of pathogens to iron (Guerinot 1991). Siderophores are produced by different groups of soil microbes. *Pseudomonas* is one of the most important producers of siderophore (Nelson 2004). The special importance of siderophores among the types of microbial metabolites released in the rhizosphere is related, on the one hand, to the key role of iron in vital metabolic processes in plants and, on the other hand, to the specific properties of the element iron in the soil. The role of potent strains in the production of siderophore in the control of plant pathogens has also been proven (Xiao et al. 2020).

### 29.5.3 Production of ACC Deaminase

The enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase breaks down ACC and catalyzes it to ammonium and alpha-bactobutyric acid (Fazeli-Nasab and Sayyed 2019). Since ACC is an excellent precursor to ethylene production in plants, by removing this substance, the amount of ethylene in plants is reduced, and consequently, its negative effects are reduced. The enzyme has so far only been detected in microorganisms, and the *Rhizobium* strains have been able to successfully overcome the negative effects of ethylene on rooting and elongation of roots in legumes and nonlegumes.

### 29.5.4 Production of Phytohormones

Some PGPR strains increase plant growth by interfering with known phytohormone concentrations (Fazeli-Nasab and Sayyed 2019). These phytohormones affect the root growth pattern of plants and cause the production of larger roots, with more branching and more effective surface (Vessey 2003). In some cases, even in insufficient levels of nitrogen fertilizers, inoculation of plants with PGPR bacteria has increased plant growth, in which case there are certainly other mechanisms, including the production of growth regulators such as indoleacetic acid. PGPRs have been shown to increase plant growth (Frankenberger Jr and Arshad 2020).

### 29.5.5 Biological Nitrogen Fixation

Nitrogen is a key nutrient for crop production. Arable soils lose significant amounts of nitrogen annually due to leaching, which drastically reduces the amount of total nitrogen available for crop growth (Peoples et al. 1995). Also, in conventional agricultural systems, the length of the fallow period is shortened or eliminated, and the crop operations are applied intensively, which destroys crop soils, and as a result, in most agricultural soils, crop yields are limited by a lack of adequate nitrogen supply. Under such conditions, the utilization of stabilized atmospheric nitrogen as a symbiosis by non-native and nonsymbiotic plants in non-dark plants through the biological nitrogen fixation (BNF) process is not an appropriate option. As an appropriate option, it provides the nitrogen input required by arable soils and helps to replace soil nitrogen reserves. Various estimates have determined the participation rate of the nitrogen biodegradation process in soil nitrogen supply of 44–200 kg/ha/year and an average of 140 kg of pure nitrogen per hectare per year (Mahmud et al. 2020; Söderlund and Rosswal 1982).

### 29.5.6 Solubilization of Mineral and Organic Phosphates

Several reports show the ability of different bacterial strains to dissolve the mineral compounds of insoluble phosphates (Khan et al. 2009). Phosphate-solubilizing bacteria reduce the soil pH in the rhizosphere by mechanisms such as the secretion of organic acids such as gluconic acid, oxalic acid, and citric acid, thereby increasing the solubility of insoluble phosphorus (Illmer and Schinner 1995). Among organic acids, gluconic acid seems to be the most abundant factor in the dissolution of mineral phosphates (Dalai 1977). Mineralization of most organic compounds of phosphorus is done by phosphatase enzymes, also called phosphohydrolase (Rodríguez and Fraga 1999). Soil bacteria of different genera of *Rhizobium*, *Pseudomonas*, and *Bacillus* can produce significant amounts of phosphatase enzymes (Kirchner et al. 1993).

### 29.5.7 The Role of Growth-Promoting Bacteria in Drought Stress Conditions

Identification of drought tolerance mechanisms in plants is one of the most important strategies to reduce the harmful effects of stress in agriculture. By identifying these mechanisms, as well as how environmental stresses affect plant growth, it is possible to achieve newer methods to deal with these nonliving stresses. One solution is to use rhizospheric bacteria to increase plant growth, of which *Pseudomonas* bacteria are a minor part. These bacteria, including biological sources, directly and indirectly improve plant growth (Azad et al. 2017; Fazeli-Nasab and Sayyed 2019). Growth-promoting rhizosphere bacteria can develop plant root systems, increase nutrient uptake, improve soil structure and water-holding capacity, reduce sodium uptake, and increase the expression of genes responsible for resistance to salinity and drought that cause the plant to tolerate environmental stresses (Patten and Glick 2002a, b). One of the characteristics of growth-promoting bacteria is the improvement of water and nutrient uptake by plants and the effect on improving germination under environmental stress. For example, reducing the effects of ethylene on stress on plant growth can be achieved through the use of bacteria with the ability to produce the enzyme ACC deaminase, which can be even more important in dry soil conditions (Fazeli-Nasab and Sayyed 2019). Growth-promoting bacteria have been reported to improve wheat resistance under stress by producing hormones such as auxin (Yazdani-Biuki et al. 2011).

### 29.5.8 The Role of Growth-Promoting Bacteria in Germination

Among plant growth-promoting bacteria, *Pseudomonas* bacteria have special importance due to their widespread distribution in soil, ability to colonize the rhizosphere of many plants, and production of a diverse range of metabolites. These bacteria have a wide range of plant growth stimulants such as production of auxin, salicylic acid, chitinase, and hydrogen cyanide, which directly or indirectly increase plant growth (Haas and Défago 2005). Growth-promoting bacteria have been reported to increase the germination index and the longitudinal index of seedling vigor in rice (Mia et al. 2012). It should be noted that the growth-promoting bacteria of the plant increase the germination speed in the fennel plant compared to the control (Khoshvaghti et al. 2013). Application of *Pseudomonas fluorescent* and *Putida* growth promoting bacteria in *Althaea officinalis* and *Sinapis arvensis* increased the germination percentage of these plants compared to the control (Golpayegani et al. 2010). The effect of growth-promoting bacteria on sorghum seeds has been reported (Prathibha and Siddalingeshwara 2013), where the growth-promoting bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* have increased the percentage of germination in sorghum seeds. The effects of seed inoculation with *Rhodococcus* sp., *Corynebacterium* sp., *Mycobacterium* sp., *Bacillus* sp., *Azotobacter* index, *Pseudomonas aeruginosa*, and *Pseudomonas* were studied. The growth and yield of evergreen flowers have showed (Mehrban and Fazeli-Nasab 2017; Sheikhi 2014) that growth-promoting bacteria have improved germination characteristics such as germination percentage and speed, germination rate coefficient, and seedling vigor index in this plant.

Examining the effects of rhizobacterial inoculation of plant growth stimulants from *Acetobacter*, *Pseudomonas*, and *Azospirillum* on some germination and seedling characteristics of *Asclepias procera* has showed (Bahmani et al. 2014) that biopriming with plant growth-promoting bacteria increases plant germination speed. *Pseudomonas fluorescens* growth-promoting bacteria of $CHA_0$, $PF_1$, $PF_2$, $PF_{75}$, $PF_{52}$ strains increased germination indices and flaxseed seedlings (*Linum usitatissimum* L.) under different storage conditions (Bakhit 2015). Inoculation of flax seeds with growth-promoting bacteria has increased the germination rate by 32% compared to non-inoculated seeds (Wu et al. 2014).

Growth-promoting bacteria increase germination percentage, germination speed, shoot and root length, and *Plantago ovata* seed vigor index compared to the control treatment (Hamzi et al. 2012). The effect of growth-promoting bacteria from *Azotobacter* sp., *Azospirillum* sp., and *Pseudomonas putida* on germination and early growth of artichokes has been reported (Jahanian et al. 2012). Inoculation of summer barley with growth-promoting bacteria has increased the dry weight of stems and roots in it (Suarez et al. 2015). The effect of growth-promoting bacteria on germination and growth of safflower seedlings at different temperatures and increasing root length in the inoculation of safflower seeds with different strains of *Pseudomonas fluorescens*, *Acetobacter*, and *Azospirillum* have been reported (Hamzi et al. 2012). By examining the effect of seed biopriming on the germination characteristics of Shirazi balangu (*Lallemantia royleana* L.), it has been reported (Hassanzadeh Delavi et al. 2015) that inoculation of Shirazi balangu seeds with *Pseudomonas fluorescens* bacteria increased the stem length of balangu, and the increase in shoot length in *Trichoderma* seed inoculation did not differ significantly from untreated treatment.

The effect of biopriming with PGPR on germination percentage in saline conditions is different from different bacterial strains and different plant cultivars. Biopriming applications with bacterial strains significantly increased seed germination percentage under saline conditions. These results indicate that biopriming with PGPR in saline conditions can be useful to obtain a higher seed germination percentage in radish (Kaymak et al. 2009). Sunflower seedlings (*Helianthus annuus* L. cv. PAC36) inoculated with *Azotobacter chroococcum* and *Bacillus polymyxa* separately and in combination were examined, and it was found that the relative water content and seedling growth in combined inoculation conditions compared to other conditions (control without inoculation or even inoculation with single bacteria) have increased. Water stress did not significantly reduce RWC, growth, and dry mass of inoculated seedlings. Inoculated seedlings also grew more even under water stress and produced more pigments and protein content than under water stress. In general, PGPR has improved the water status of stressed plants, and physiological and biochemical parameters have also improved as a result of the severe effects of water stress (Singh et al. 2015).

*Pseudomonas fluorescens* has increased growth parameters in drought stress conditions and has significantly improved growth control due to drought and by increasing wet and dry weight. Native PGPRs can be used as an appropriate tool in enhancing biomass performance and alkaloid content in medicinal plants, as it is an environmentally consistent approach (Jaleel et al. 2007).

## 29.6 Plant Growth-Promoting Fungi

### 29.6.1 *Trichoderma harzianum* Fungus

This fungus belongs to the phylum Ascomycota, subclass Pyrenomycetes, order Hypocreales, and family Hypocreaceae, and its genus form is called *Hypocrea* (Elkelish et al. 2020). *Trichoderma* species usually grow rapidly in different culture media. The activity of these fungi is antagonistic. Antagonists from two pathways (systemic acquired resistance (SAR) and induced systemic resistance (ISR)) can induce resistance in plants, and these two pathways are affected by salicylic acid, ethylene, and jasmonate. Antioxidant enzymes are signals to activate these pathways (Segarra et al. 2007). Antioxidant metabolites such as vitamin E, ascorbic acid, and other compounds, especially in seeds and leaves, play an important role (Bailly 2004; Fazeli-Nasab and Fahmide 2020). Antioxidant enzymes including catalase, peroxidase, superoxide dismutase, and other enzymes eliminate and inactivate active oxygen species (Bailly 2004). The enzyme catalase directly breaks down hydrogen peroxide ($H_2O_2$) (Jiang and Huang 2001; Keikhaie et al. 2018). The enzyme ascorbate peroxidase in the glutathione-ascorbate cycle uses ascorbate as an electron donor to break down hydrogen peroxide and superoxide radicals during the Haber-Weiss reaction.

*Trichoderma* fungi also increase the solubility of phosphorus and microelements and increase the availability of these elements to the plant. Increasing nutrient uptake as a result of *Trichoderma* fungi activity can increase plant growth and vigor and make the plant resistant to pathogens. Other mechanisms of *Trichoderma* fungi include biological control of soil diseases by enzyme secretion (Sagar et al. 2020;

Singh et al. 2007), antibiotic production (Saxena et al. 2020) and penetration into the body of pathogenic fungi (Luh Suriani et al. 2020), elimination of poisoning and increased sugar transfer and amino acid in plant roots, induction resistance to environmental stresses, increased nutrient uptake by increasing nutrient solubility, secretion of growth hormones, and production of xylanase and cellulase enzymes that can directly stimulate ethylene production in plants in response to the presence of pathogens (Gravel et al. 2007).

In the last two decades, the approach to sustainable agriculture and the production of a healthy crop inspired by nature have accelerated. Meanwhile, the use of fungi and improving the absorption of nutrients and activating the living environment of the soil and stimulating food cycles have been considered by researchers in agricultural sciences and natural resources (Raval et al. 2020; Sharma et al. 2003). Growth-inducing soil bacteria along with fungi can increase biomass and mineral uptake even under stressful conditions (Adesemoye et al. 2009). These fungi increase the resistance to nonliving stresses during the growing season by improving root growth, plant water-holding capacity, and nutrient uptake (Mastouri et al. 2010; Yazdani et al. 2012). Studies show that *Trichoderma* fungi, through special mechanisms such as the secretion of the enzymes xylanase and cellulase, which can directly stimulate the production of ethylene in the plant, stimulate the transmission of the disease in the presence of the pathogen and stimulate the immune response in the presence of the pathogen and amino acids in plant roots to cause induced resistance to stress and biological control of soil diseases (Harman 2006; Khan et al. 2020).

*Trichoderma* has shown to increase the growth of a wide range of crops such as tobacco (*Nicotiana* sp.), tomato (*Lycopersicum esculentum*), pepper (*Piper nigrum*), eggplant (*Solanum melongena*), cloves, and chrysanthemums (Chang et al. 1986; Deepa et al. 2020).

### 29.6.2 The Role of *Trichoderma* in Germination and Plant Growth

*Trichoderma* is one of the most resistant microorganisms to toxins and natural and synthetic chemicals, and it can break down some of them such as hydrocarbons, chlorophenol compounds, and polysaccharides. Some *Trichoderma* strains are opportunistic invaders that grow rapidly and produce many spores. They are also strong producers of antibiotics and enzymes. The combination of these characteristics has led to their being found in agricultural soils and meadows, forests, swamps, deserts, and in climatic zones such as the tundra, Antarctica, the equator, as well as lakes and dead plant remains (Monte 2001; Sharma et al. 2020). Researchers have shown that *Trichoderma* species increase plant growth under certain mechanisms. Among the mechanisms mentioned are biological control of soil diseases (Kalam et al. 2020), production of antibiotics (Basu et al. 2021), penetration into the body of pathogenic fungi (Ilyas et al. 2020), detoxification, increased transfer of sugar and amino acids in plant roots, and induction of resistance to environmental stresses. The increase in nutrient uptake was noted by increasing the solubility of the element, secretion of growth hormone and quasi-hormones, and production of cellulose enzyme, which can directly stimulate the production of ethylene in the plant in response to the presence of pathogens (Fazeli-Nasab et al. 2018; Gravel et al. 2007). In soybeans inoculated with *Trichoderma* fungi, the results have been shown that *Trichoderma* fungi increase the rate of cumulative emergence of seedlings compared to the control (without inoculation) (Yazdani et al. 2012).

In inoculation of *Trichoderma* fungi on plant seeds, it has been reported (Mehrban and Fazeli-Nasab 2017; Vinale et al. 2004) that T22 from *T. harzianum* and P1 from *T. atroviride* avoided increasing the frequency of growth of lettuce, tomato, and pepper. The results showed that the yield increased by up to 30% compared to plants that were not treated with *Trichoderma*. Isolation of *Trichoderma harzianum* in low concentration caused the highest germination percentage in chickpea seeds compared to the control (Ghorbani et al. 2011). Impregnation of seeds with *Trichoderma* increases the activity of antioxidants and reduces the number of reactive oxygen species, and this is the property of induction of resistance that occurs by this antagonist (Mastouri et al. 2010). Increased germination and growth of tomato seedlings due to the inoculation of *Trichoderma* under nonliving stresses such as drought have been reported (Mastouri et al. 2010). Strains T36, T39, T13, T29, T40, and T43 of *Trichoderma harzianum* increased the germination indices and seedlings of flaxseed in different storage conditions (Bakhit 2015).

*Trichoderma* can help the plant overcome inanimate stresses such as drought, salinity, cold, and heat. Tomato seeds treated with *T. harzianum* showed a high rate of germination at an osmotic potential of 0.3 ms and were more resistant in dry conditions (Harman and Shoresh 2007; Wani et al. 2016). Using the proteomics method, the effect of T22 on corn was investigated, and it was found that 205 proteins are produced in the presence of *Trichoderma*, the most common of which are proteins produced in the carbohydrate cycle, in particular those involved in the production of glycolytic and tricarboxylic acids that are effective in combating drought stress (Shoresh and Harman 2008). Cocoa seedlings reported during the study (Bae et al. 2009; Basu et al. 2021) that were treated with *Trichoderma* produced proteins called tonoplasts, which are found in plants as channels. In the plant, these proteins act as membrane channels that selectively transport water out. The expression of such proteins and the formation of such membranes induce a dry reaction.

Increasing the level of indoleacetic acid by *Trichoderma* fungi increases and accelerates the growth of plants (Martínez-Medina et al. 2014). *Trichoderma* fungi produce growth regulatory factors that increase the germination rate and dry weight of radish stems and roots. Also, seed treatment with *Trichoderma* may improve the condition and long-term quality of the plant. *Trichoderma* growth fungi have increased the length of seedlings in cucumbers and bitter squash compared to control of drought stress (Lo and Lin 2002). Similar results have been reported by increasing the length of roots and stems (Dubey et al. 2007) as well as increasing yield (Rojo et al. 2007) by *Trichoderma*. The effect of *Trichoderma* fungi on germination and seedling indices of pepper has been investigated, and it has been found that these fungi increase the germination percentage and longitudinal index of seedling vigor in this plant under drought stress (Asaduzzaman et al. 2010).

*Trichoderma* fungi and growth-promoting bacteria have been effective in improving the germination and growth of rice seedlings (*Oryza sativa* L.) under the stress of the copper element. The effect of *Trichoderma* fungi on germination and seedling indices of pepper seeds was investigated, and it was found that *Trichoderma* fungal strains did not increase germination indices including germination percentage and rate and seedling indices compared to prim treatment (Bahmanesh et al. 2012). The effect of *Trichoderma* fungi on germination and seedling indices of pepper seeds was investigated, and it was found that *Trichoderma* fungal strains increased germination indices such as germination percentage and rate and seedling indices compared to no-priming treatment (Rahman et al. 2012). Seedling emergence rate from the soil, plant height, leaf area, and plant dry weight were significantly increased by *Trichoderma* inoculation compared to the control (Sharma et al. 2013). Besides, growth was reported by the treatment of *Trichoderma* fungus in other plants such as peas (Zheng and Shetty 2000), tomatoes (Gravel et al. 2007), wheat (Cavalcante et al. 2008), and onions (Altintas and Bal 2008).

## 29.7 The Effect of Seed Biopriming on Plant Growth and Development Under Drought Stress Conditions

Germination percentage in canola cultivars inoculated with different isolates of *Pseudomonas* has been higher than the control (Jalili et al. 2009). Similar results have been reported in plants such as barley (Şahin et al. 2004), corn (Pal 1998), and sugarcane (Sundara et al. 2002) in increasing percentage and germination speed. In biopriming, bacteria adhere to the seed surface and synthesize indoleacetic acid (IAA) in response to tryptophan or other amino acids secreted from the seed. Indoleacetic acid may be absorbed by seeds and added to its endogenous source, stimulating plant cell growth and elongation. Also, a large amount of ACC (ethylene precursor) released from the seed, which is produced by the stimulation of indoleacetic acid, is hydrolyzed by the bacterium ACC enzyme (Jalili et al. 2009). Growth-promoting bacteria have increased germination percentage, root and shoot length, and longitudinal index of artichoke seedling vigor (Jahanian et al. 2012). Besides, biofertilizers such as growth-promoting bacteria have increased the percentage and speed of germination and the weight of coriander (Shirkhodaei et al. 2014).

*Trichoderma* spp. have been reported (Patel et al. 2016) to produce growth regulatory factors that increase germination speed and dry weight of stems and roots under drought stress conditions. Colonial formation in plant roots by *Trichoderma* increases root growth and expansion, increases crop production, increases resistance to non-life-threatening stresses, and makes food available (Harman 2006; Sayyed et al. 2015). *Trichoderma* fungus increases the solubility of phosphorus and microelements and increases the availability of these elements to plants. Increasing the absorption of nutrients as a result of the activity of *Trichoderma* fungi can also increase the growth and vigor of the plant and make the plant resistant to pathogens and drought (Singh et al. 2007).

## 29.8 Effect of Seed Biopriming on Root Growth

Several bacteria, including *Azospirillum*, cause changes in the physiology and morphology of the host plant root. Among the physiological changes of the root, we can name the increase of proton flow in the wheat root, which increases the absorption of minerals by the plant root, because the increase of proton flow in the root acts similar to acidification of the rhizosphere, which is the main mechanism for dissolving minerals. The main changes in the root morphology of plants inoculated with *Azospirillum* sp. can be an increase in cell division in the root, an increase in the number of capillaries, an increase in the number of lateral roots (subbranch), a decrease in the distance between the tip of the root and the area of capillaries, and an increase in the number of branching of capillaries (Baldani et al. 1983; Sharma et al. 2016).

## 29.9 The Effect of Seed Biopriming on Yield and Yield Components

Seed biopriming has the potential to increase plant growth, yield, and nutritional value of the final product. This treatment also increases the uniform germination of seeds, which leads to rapid uniformity and high yield of the crop, thus improving the quality and yield of the harvest (Mahmood et al. 2016). Numerous mechanisms have been reported to determine the role of biopriming to increase the nutritional value of plant products, namely, phosphate dissolution, increasing $N_2$ stabilization, increasing the production of plant growth-promoting compounds such as phytohormones, antibiotic production, and organic decomposition (Sinha et al. 2010).

*Rhizosphere* microbes play a very important role in increasing the uptake of the three essential substances N, P, and K (Sarma et al. 2015). It has been well known that N, P, and K are the main components of enzymes, hormones, amino acids, and genetic materials in plants that are involved in various physiological processes of plants (Chevalier and Rossignol 2011; Krouk et al. 2010). Nutrient uptake of N, P, K, Na, Ca and organic matter in seeds, foliage and pericarp of chickpeas bio-primed with *Pseudomonas fluorescens* OKC and *Trichoderma asperellum* T42. In addition to increasing the quality, quantity of nutrients of seeds, foliage, pericarp, phenolic and protein content, carbohydrate content, flavonoid content, are also increased. But pea vigor has decreased during seed biopriming (Yadav et al. 2017). There has also been an increase in the number of branches, the number of grains per plant, the number of grains per graft, the weight of a thousand grains, the percentage of oil, and the grain yield in safflower primed with *Pseudomonas* (Sharifi 2012).

*Trichoderma asperellum* T42 has increased the effectiveness of nitrogen consumption in tobacco (Singh et al. 2018). Also, *Pseudomonas fluorescens* OKC, *Trichoderma asperellum* T42, and *Rhizobium* sp. RH4 have played an important role in increasing seed germination and seedling growth in chickpea (Yadav et al. 2013). It has also been reported that some specific microbes produce vitamins and other nutrients, such as *Azotobacter vinelandii* ATCC 12837 and *A. chroococcum* H23 strain; some members of the B vitamin group, namely, niacin, pantothenic acid, thiamine, riboflavin, and biotin, are produced in chemical media after 72 h of growth (Revillas et al. 2000). Therefore, it can be claimed that proper seed biopriming can improve plant nutritional value and harvest.

The positive effect of inoculation with *Azospirillum* sp. on plant height, leaf size, root length and volume, and dry matter content in different types of cereals has been proven (Wani 1990). The results of various studies have shown that the application of strains of *P. fluorescens* and *P. putida* has increased root and shoot length in canola and tomato and also yield in rice, wheat, and sugar beet (Rodrı́guez and Fraga 1999; Shaikh et al. 2018). *Azospirillum* inoculation of plants has also caused significant changes in various growth parameters in cereals (Sonawane et al. 2018). Inoculation of potato seeds with biofertilizers before planting has been able to produce potato tubers with a larger diameter and average weight (Mirshekari and Alipour 2013).

## 29.10 The Effect of Biopriming on Chlorophyll Content Under Drought Stress Conditions

Plant growth-promoting rhizobacteria (PGPR) increase chlorophyll content in plants (Reshma et al. 2018). This increase appears to be due to the greater uptake of elements such as iron and magnesium by antioxidant enzymes such as catalase and peroxidase. The role of these enzymes in chlorophyll synthesis is an important factor (Kavino et al. 2010). With increasing chlorophyll rate and photosynthesis, finally, the rate of assimilation and carbohydrate in rice increases and the accumulation of dry matter produced. Inoculation of basil seed (*Ocimum basilicum*) with *Pseudomonas* increases leaf chlorophyll content (Heidari and Golpayegani 2012). The higher the chlorophyll content, the higher the photosynthesis rate of the plants. However, higher concentrations of chlorophyll can also be attributed to increased phosphorus uptake. This is because the release and exit of triose phosphates from the chloroplast are regulated by phosphorus. The net uptake of inorganic phosphate into chloroplasts regulates the release of organic matter synthesized by photosynthesis from chloroplasts. High leaf chlorophyll content in bacterial inoculation treatments can be a reason for increasing photosynthesis and yield. Inoculation of wheat seeds with *Pseudomonas* bacteria increased the significance of chlorophyll content (Rasouli Sadaghiani et al. 2009). Also, in a study on the effect of growth-promoting microorganisms in corn, there was a significant increase in chlorophyll and carotenoid content (Vinay et al. 2016).

## 29.11 Biopriming and Antioxidant Enzymes in Drought Stress Conditions

Rhizosphere microorganisms, especially beneficial bacteria and fungi, can improve plant function against environmental stress conditions and thus increase yield directly and indirectly. Drought stress, like other environmental stresses, causes the accumulation of reactive oxygen species (ROS) in cells. ROSs include several molecules such as superoxide ($O_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (OH), and other reactive molecules in the cell are produced in cell organs such as mitochondria and chloroplasts due to electron cycle imbalances and are highly reactive with cellular macromolecules such as proteins. They have fats and DNA, so they cause damage to various parts of the cell membrane (Zhu 2000). Plants use a range of antioxidant enzymes to counteract drought stress and avoid photosynthetic degradation. Oxygen-free radicals are metabolized by several antioxidant enzymes such as glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (Zhu 2000). In the study of antioxidant enzyme activity on inoculated wheat seeds with growth-promoting bacteria, an increase in grain yield and catalase and glutathione peroxide enzymes was observed; this increase has resulted in a reduction in damage from active oxygen species (chamaani et al. 2012).

## 29.12 The Combined Effect of Nanopriming and Biopriming on Growth Components

The effect of nanopriming and biopriming on growth components of *Onobrychis sativa* Lam. in laboratory conditions was investigated, and the results showed that maximum amount of stem length (6.76 cm) and altitude coefficient (1.5) in the treatment of potassium nano-silicate (1000 mg/l), the highest percentage of germination percentage (8.5%) and germination rate (46) under the treatment of nano-titanium (1000 mg/l), and the highest amount of seedling fresh weight (3.37 g), seed vigor index (0.45) and seedling tissue water content (91.6) were observed in 2% effective microorganism treatment (Moameri et al. 2018).

The effect of biopriming on germination components, physiological traits, antioxidant enzymes, and control of bean *Rhizoctonia* disease (*Phaseolus vulgaris* L.) was investigated, and it was concluded that seeds treated with zinc sulfate and combined with *Trichoderma* weighed more than the rest of the team. Dried seedlings had higher nitrogen content. The highest iron content (265.6 mg/kg) was obtained from seeds primed with zinc sulfate and treated with UTPF5 bacteria. Seeds treated with salicylic acid and treated with UTPF5 bacterium had higher ascorbate peroxidase (APX; 17.4 U/mg proteins activity) than other experimental treatments (Entesari et al. 2013b).

Effect of nitrogen and seed biopriming with growth-stimulating bacteria on yield, speed, and period of filling of sunflower seeds (*Helianthus annuus* L.) has been studied, and it has been determined that with increasing levels of nitrogen fertilizer and the use of growth-promoting bacteria, grain yield, plant height, head diameter, number of grains per head, oil yield percentage, and protein yield percentage have increased. The response of grain yield to seed biopriming was not the same as that of growth-promoting bacteria at nitrogen fertilizer levels. The highest yield was 160 kg of nitrogen per hectare and seed biopriming with nitrogen. Maximum grain weight, velocity, and effective grain filling period were estimated at high levels of nitrogen and application of growth-promoting bacteria; therefore, to increase grain yield in the climatic conditions of Ardabil (Iran), sunflower seed biopriming with *Azotobacter* sp. is suggested (Seyed Sharifi and Nazarli 2013). The effect of PGPRs on plant growth and development has been studied separately, and it has been found that the relative content of water and seedling growth under hybrid inoculation conditions has increased compared to other conditions (control without inoculation and even inoculation with a single microorganism) (Singh et al. 2015).

## 29.13 Conclusion and Future Views

With the rapid development of genomic technology, significant efforts are made to decipher the nonbiological stress responses of plants; many challenges still lie in discovering the complexity of stress message pathways. As a result, seed priming with PGPR has the potential to improve resistance to biological and abiotic stresses of plants, which provides new opportunities to maintain sustainable crop production to feed a growing population in the face of changing environmental conditions. On the other hand, in seed priming with PGPR, the combined application of biostimulant bacteria and fungi with each other and even with nitrogen fertilizer will be a sustainable solution to improve the ability to harvest the seed and create a stable yield in the field of sustainable production. However, it is hoped that the use of efficient microbes will reduce the dependence on chemical fertilizers for potential plant yields. Success in identifying the role of PGPR in stress tolerance at the molecular level will help demonstrate the positive effects of seed priming with PGPR and their alternatives in improving stress tolerance in a wide range of crops. However, more research is needed to discover the mechanism of PGPR, especially with stress response genes.

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