Chapter 1
Plant-Growth-Promoting Rhizobacteria: Potential Candidates for Gibberellins Production and Crop Growth Promotion

Sang-Mo Kang, Muhammad Waqas, Abdul Latif Khan and In-Jung Lee

Introduction

Rhizosphere, the layer of soil influenced by plant root (Saharan and Nehra 2011; Antoun and Prévost 2005), is known to play pivotal role in plant growth and development (Hrynkiewicz and Baum 2012). Highest proportion of microbial groups such as bacteria, fungi, nematodes, protozoa, and microarthropods inhabit rhizosphere (Lynch and Whipps 1990; Raaijmakers 2001; Morgan et al. 2005). Members of these microbial groups have beneficial, neutral, or harmful effects on the plant growth (Nihorimbere et al. 2011; Bais et al. 2006). The rhizosphere is diversely populated by bacteria known as rhizobacteria. Rhizospheric bacteria feed on the available soil nutrients and root exudates of plants (Bais et al. 2006; Rovira 1991; Dodd et al. 2010). Currently, the term plant-growth-promoting bacteria (PGPB) is used to encompass all those bacteria that enhance plant growth (Tarkka et al. 2008; Brencic and Winans 2005). However, among PGPB, plant-growth-promoting rhizobacteria (PGPR) are studied more because of their ability to colonize the plant roots (Kamilova et al. 2005; Sturz and Nowak 2000). Due to potential application of the beneficial effects of PGPR, scientists from multiple discipline have been involved to elucidate the underlying mechanisms of plant growth. PGPR influence the plant growth through direct or indirect mechanisms. In direct mechanism, PGPR facilitate the growth promotion by nutrient acquisition and alter the physiological signaling by synthesizing bioactive constituents (Welbaum 2004; Brimecombe et al. 2007), while in indirect mechanism, PGPR enhance plant growth via a set of biocontrol mechanisms. Some PGPR decrease or combat the adverse effects of pathogenic microorganisms, by colonizing plants in

S.-M. Kang · M. Waqas · A. L. Khan · I.-J. Lee (✉)
Agronomy, School of Applied Biosciences, Kyungpook National University, 80 Daehakro, Bukgu, Daegu, Kyungpook 702–701, Republic of Korea
e-mail: ijlee@knu.ac.kr

M. Miransari (ed.), *Use of Microbes for the Alleviation of Soil Stresses, Volume 1*,
DOI: 10.1007/978-1-4614-9466-9_1,

high population during pathogen attack (Nihorimbere et al. 2011). These PGPR are capable of producing antagonistic metabolites such as antibiotics (Compant et al. 2005; Haas and Défago 2005), siderophores (RodrÍgueza and Fragaa 1999), HCN (Ahmad et al. 2008), phenazines (Pierson and Pierson 2010), pyoluteorin (Nowak-Thompson et al. 1999), pyrrolnitrin (Hwang et al. 2002). Furthermore, the PGPR must be able to deliver the chemical constituents in right amount, time, and place to effectively combat the adverse effects of pathogenic attack (Lugtenberg and Kamilova 2009).

In case of direct mechanism, PGPR can stimulate plant growth in the absence of pathogenic attack by secreting plant growth substances. Nitrogen-fixing bacteria such as *Bradyrhizobium* and *Rhizobium* fixes atmospheric N_2 by reducing it into ammonia that can be used by legume plants as a nitrogen source (Franche 2009). Some PGPR help in plant growth by their enhanced potential to solubilize soil phosphate (Bertrand et al. 2000). PGPR have also recently known to produce phytohormones such as auxin, cytokinin, and gibberellins which are synthesized through plant-secreted precursors (Baca and Elmerich 2003). These bacteria-derived phytohormones subsequently facilitate plant growth by promoting cell division under varying environmental conditions. In abiotic stresses, like salinity, drought, and heavy metal, the ethylene production is stimulated in plants, which subsequently inhibits plant growth. Some PGPRs have shown the ability to stimulate the activity of enzymes called 1-aminocyclopropane-1-carboxylate deaminase (ACC) that can hydrolyze ACC into 2-oxobutanoate and ammonia via modulation of plant hormonal level (Glick 2005; Mayak et al. 2004). Glick et al. (1998) previously reported that the continuous exudation of ACC from plant roots under abiotic stress is converted by PGPRs containing ACC deaminase and might be used for their own growth (Siddikee et al. 2010; Nadeem et al. 2010).

Looking at the great potential of PGPR, in this chapter, we focused on gibberellins producing PGPR and its role in abiotic stress particularly drought and salinity stress. Gibberellins (GAs) are ubiquitous plant hormones that elicit various metabolic function required during plant growth like seed germination, stem elongation, sex expression, flowering, formation of fruits, and senescence (Hedden 1997; Hedden and Kamiya 1997). Exogenous applications of GAs (GA_3 and GA_4) have been reported to improve plant growth and biomass while counteracting abiotic stresses in plants (Hedden and Kamiya 1997). The production of such plant growth regulators like auxin, cytokinin, and gibberellins by PGPR can give an additional support to the growth of host plants (Joo et al. 2004, 2005, 2009; Kang et al. 2009, 2010). There are few previous studies (Table 1.1) which elucidated the GA production by PGPR (Joo et al. 2004, 2005, 2009; Kang et al. 2009, 2010; Atzhorn et al. 1988; Bastian et al. 1998; Bottini et al. 1989; Gutierrez-Manero et al. 2001; Janzen et al. 1992; Mansour et al. 1994); here, we further elaborated the role of PGPR in plant growth regulation during abiotic stress.

Table 1.1 PGPR species reported for producing gibberellins

PGPR species	GAs potential	References
Acetobacter diazotropicus	GA_1, GA_3	Bastian et al. (1998)
Azospirillum lipoferum	GA_1, GA_3	Bottini et al. (1989)
Azospirillum brasilense	GA_1, GA_3	Janzen et al. (1992)
Bacillus licheniformis	GA_1, GA_3, GA_4, GA_{20}	Gutierrez-Manero et al. (2001)
Herbaspirillum seropedicae	GA_3	Bastian et al. (1998)
Rhizobium phaseoli	GA_1, GA_4	Atzhorn et al. (1988)
Bacillus pumilus	GA_1, GA_3, GA_4, GA_{20}	Gutierrez-Manero et al. (2001)
Bacillus pumilus CJ-69	GA_1, GA_3, GA_4, GA_5, GA_7, GA_8, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, GA_{44}	Joo et al. (2004)
Bacillus cereus MJ-1	GA_1, GA_3, GA_4, GA_7, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, GA_{34}, GA_{36}, GA_{44}, GA_{53}	Joo et al. (2004)
Bacillus macroides CJ-29	GA_1, GA_3, GA_4, GA_7, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24},GA_{34}, GA_{36}, GA_{44}, GA_{53}	Joo et al. (2004)
Acinetobacter calcoaceticus	GA_1, GA_3, GA_4, GA_9, GA_{12}, GA_{15},GA_{19}, GA_{20}, GA_{24}, GA_{53}	Kang et al. (2009)
Burkholderia cepacia	GA_1, GA_3, GA_4, GA_9, GA_{12}, GA_{15}, GA_{20}, GA_{24}	Joo et al. (2009)
Promicromonospora sp.	GA_1, GA_4, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, GA_{34}, GA_{53}	Kang et al. (2012)

Gibberellin Biosynthesis in PGPR

Phytohormones are organic in nature and effective in very low amount. They are usually synthesized in tissues of plants and are transported to their specific site of action. Upon transport to the targeted tissues, the hormone causes physiological changes in plants such as fruit ripening, lateral root formation, flowering, and bud initiation. Each response is often the result of antagonistic or synergistic action of two or more hormones. Plant physiologists had categorized the hormones into five major groups: auxins, gibberellins, ethylene, cytokinins, and abscisic acid. Recently, two new hormones have also been recognized and known as brassinosteroids and strigolactones. Gibberellin is responsible for active role in seed germination, seedling emergence, stem and leaf growth, floral induction, and flower and fruit growth. Similarly, gibberellin production by PGPR promotes the growth and yield of many crop plants. A small number of PGPR have been identified to produce gibberellins (GA). These PGPR regulate the plant hormone level in three ways either by direct synthesis of GA itself, de-conjugation of glucosyl gibberellins, and change of inactive status of gibberellins into active GA (Lucangeli and Bottini 1997; Piccoli et al. 1997, 1999; Cassán 2001).

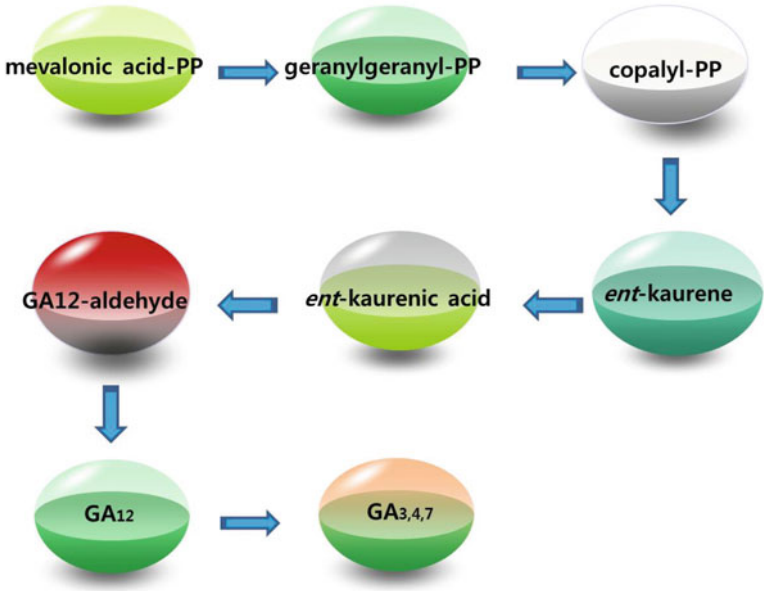

Fig. 1.1 Proposed and comparative GA biosynthesis pathway in bacteria based on the current knowledge from plant and fungi

In bacteria, the elucidation of GA biosynthesis pathway is based upon the knowledge from plants and fungi. Usually, GAs are biosynthesized from geranylgeranyl-PP (Fig. 1.1), which is converted into ent-kaurene via ent-copalyl diphosphate, and ent-kaurene is converted into GA_{12}-aldehyde via ent-kaurene oxidase and ent-kaurenoic acid oxidase. GA_{12}-aldehyde is then oxidized into GA_{12} and metabolized into other GA (Fig. 1.1; Baca and Elmerich 2003; Bomke and Tudzynski 2009). Morrone et al. (2009) have also reported the involvement of *operan* whose enzymatic composition indicates that gibberellin biosynthesis operate a third independent assembled pathway relative to plants and fungi. The reported pathway has superficial similarity to plants instead of fungi. GAs have been identified and isolated from higher plants, fungi, and bacteria. Up until now, 136 GAs from higher plants (128 species), 28 GA from fungi (7 species), and only 4 GA (GA_1, GA_3, GA_4, and GA_{20}) from bacteria (7 species) have been identified (Table 1.1; Hedden and Thomas 2012).

Gibberellin Quantification and Analysis in Microbial Culture

Until now, universal methods to quantify and analyze the gibberellins from microbes does not exist. However, modern analytical techniques such as GC-MS and LC-MS have enabled the plant physiologist to analyze and quantify the minute

quantities of GA in any culture sample. These advance equipments are sufficiently sensitive and selective to measure any phytohormones including GA at low concentrations. For gibberellin quantification and analysis, the microbes are grown initially in specific cultural broth. After a period of time (one week or ten days), the pure cultural filtrate (CF) is separated from growing cells by centrifugation or filtration. Onward several tedious steps are involved to remove interfering substances and bring it to a stage to be analyzed for the presence of GA. The concentrations of GA are very low (ng ml^{-1}) in the cultural broth of bacteria and require very sensitive methods for their detection. The analytical procedure must be able to identify the GA from other components of secondary metabolites. Furthermore, the choice of extraction and purification depends on analyte, type of analysis to be performed, and the equipments available. For GA characterization, extensive purification and standardization with pure substances are needed. The steps followed for GA analysis must eliminate potential impurities from analyte.

Extraction and Purification of Microbial Cultural Filtrate for Gibberellins

For extraction and purification of microbial cultural filtrate, the required strains are grown in nutrient broth (100 ml) for 7 days at 30 °C (shaking incubator at 200 rpm) (Kang et al. 2009, 2010; Lee et al. 1998). The culture and bacterial biomass are separated by centrifugation (2,500× g at 4 °C for 15 min). The culture medium (50 ml) is used to extract and purify GA as described by Kang et al. (2009). GAs have functional groups, highly oxidized and may be relatively labile to extreme pH in aqueous solutions. In alkaline conditions, epimerization is another reason due to which the extraction and purification procedures should be performed within certain range of pH like 2.5–8.5 (Urbanova et al. 2011). All the process of purification and especially the aqueous solution containing GA must be handled at temperature below 40 °C. Therefore, the pH of CF is adjusted to 2.5 using 6N HCl and partitioned with ethyl acetate (EtOAc) to obtain the extract. Before partitioning, deuterated stable GA internal standards (20 ng; [17, 17-$^{2}H_2$] GA_1, GA_3, GA_4, GA_7, GA_{12}, GA_{19}, GA_{24}, GA_{34}, and GA_{53}) are added in the CF. Tritiated GA, i.e., [1, 2-$^{3}H_2$] GA_9 and [1, 2-$^{3}H_2$] GA_{20} are also added (can be obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia). The organic layer is vacuum dried and added with 60 % methanol (MeOH), while the pH is adjusted to 8.0 ± 0.3 using 2N NH_4OH. The bacterial cultures are subjected to chromatographic and mass spectroscopy techniques for identification and quantification of GA (Table 1.2).

Table 1.2 GC-MS analysis of HPLC fractions from ethyl acetate fractions of bacterial culture

Fraction no.	GA	KRI[a]		m/z (%, relative intensity of base peak)[b]				
6–8	GA_8	2,818	Sample	594(100)	448(25)	379(20)	375(15)	238(28)
		2,818	standard	596(100)	450(24)	381(21)	375(11)	240(26)
12–14	GA_1	2,674	Sample	506(100)	448(20)	313(17)	491(13)	377(12)
		2,674	standard	508(100)	450(19)	315(14)	493(11)	379(13)
24,25	GA_{20}	2,485	Sample	418(100)	375(45)	403(14)	359(12)	301(13)
		2,485	standard	420(100)	377(45)	405(13)	361(10)	303(11)
26–28	GA_{44}	2,789	Sample	432(63)	238(41)	417(12)	373(17)	207(100)
		2,789	standard	434(62)	240(39)	419(10)	375(16)	209(100)
29–31	GA_{19}	2,600	Sample	434(100)	374(59)	402(41)	462(10)	375(57)
		2,600	standard	436(100)	376(57)	404(40)	464(9)	377(55)
37,38	GA_{53}	2,450	Sample	448(47)	251(30)	235(30)	389(25)	241(18)
		2,450	standard	450(47)	253(28)	237(28)	391(25)	243(19)
42–44	GA_{12}	2,335	Sample	300(100)	240(31)	328(31)	360(2)	285(19)
		2,335	standard	302(100)	242(32)	330(29)	362(2)	287(20)

[a] *KRI* Kovats retention index

[b] Identified as methyl ester trimethylsilyl ether derivatives by comparison with reference spectra and KRI data (Gaskin and MacMillan 1991)

Chromatography for Purification

The extracts are passed through a Davisil C18 column (90–130 μm; Alltech, Deerfield, IL, USA). The eluent is reduced to near dryness at 40 °C in vacuum. The samples are then dried onto celite and then loaded onto SiO_2 partitioning column (deactivated with 20 % water) to separate the GA as a group from more polar impurities. GAs are eluted with 80 ml of 95:5 (v / v) EtOAc: hexane saturated with formic acid. This solution is dried at 40 °C in vacuum, redissolved in 4 ml of EtOAc, and partitioned three times against 4 ml of 0.1 M phosphate buffer (pH 8.0). Dropwise addition of 2N NaOH is required during the first partitioning to neutralize residual formic acid. One gram of polyvinylpolypyrrolidone (PVPP) is added to the combined aqueous phases, and this mixture is slurried for 1 h. The pH is reduced to 2.5 with 6N HCl. The extract is partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction is dried in vacuum, and the residues are dissolved in 3 ml of 100 % MeOH. This solution is dried in a Savant or a steam of nitrogen. The dried samples are subjected to preparative high-performance liquid chromatography (HPLC) for fractionations. To improve the purification efficiency, a 3.9 × 300 m Bondapak C18 column (Waters Corp., Milford, MA, USA) is used and eluted at 1.0 ml/min with the following gradient: 0–5 min, isocratic 28 % MeOH in 1 % aqueous acetic acid; 5–35 min, linear gradient from 28 % to 86 % MeOH; 35–36 min, 86–100 % MeOH; and 36–40 min, isocratic 100 % MeOH. Forty-eight fractions of 1.0 ml are collected.

GC/MS: SIM for Hormonal Analysis

Qualitative and quantitative analysis is very important for the GA produced by bacterial strains. GA identification requires physicochemical detectors having the ability to distinguish structurally unique compounds from each other. Only nuclear magnetic resonance (NMR) and mass spectrometry (MS) are commonly used techniques to fulfill this condition. MS is more useful than NMR as it is very sensitive to analyse the extremely low concentration of GA. However, NMR is useful for identification of unidentified GA and completes structure elucidation of known GAs. Liquid chromatography has also been remained a choice of qualitative analysis of derivatized GA. Moreover, the lack of efficiency to selectively detect (UV or fluorescence) the carboxylic acid derivatization has limited its use (Urbanova et al. 2011; Crozier and Durley 1983; Reeve and Crozier 1978; Heftmann et al. 1978; Morris 1978). Another great achievement of MS in terms of tandem instruments has improved the identification of GA and made easy the qualitative analysis (Urbanova et al. 2011). Here, we will focus on the qualitative analysis of GA through MS in combination with gas chromatography, and the scheme of whole process is described in Fig. 1.2. In GC-MS, the samples are injected and converted into gas form and then introduced into mass spectrometer ion source serving as a highly versatile GC detector (Urbanova et al. 2011; Hedden 1986).

The fractions are then prepared for gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM) system (6890N Network GC System, and 5973 Network Mass Selective Detector; Agilent Technologies, Palo Alto, CA, USA). Inside GC, derivatization of GA is important for enhancing their volatility to reproduce good peaks. Before analysis with GC-MS, the ethereal diazomethane and BSTFA (N,O-bistrimethyl silyltrifluoroacetamide) or MSTFA (N-methyl-N-trimethyl silyltrifluoroacetamide) are added to GAs to decrease the polarity of the emergent molecule and, more importantly, improve its mass spectral characteristics (step 1, Fig. 1.2). For each GA, 1 µl of sample is injected in GC/MS (step 2, Fig. 1.2); inside GC column, GA are separated (step 3, Fig. 1.2) and introduced

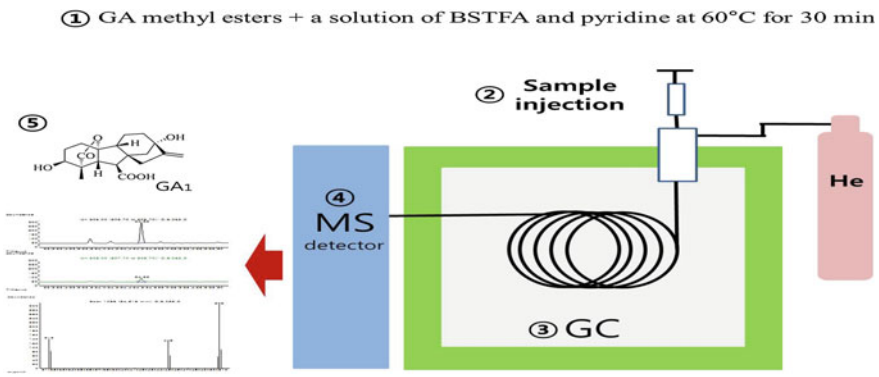

Fig. 1.2 Schematic process of GA identification through GC/MS SIM analysis

into the mass spectrometer (step 4, Fig. 1.2), where they undergo extensive fragmentation (Table 1.2).

The bacterial CF containing GA are calculated from the peak area ratios of sample GA to corresponding internal standards (step 5, Fig. 1.2). The retention time/identity of GA is determined using hydrocarbon standards to calculate the Kovats retention index (KRI) value. The KRI confirms the identity of GA. The GA quantification is based on the peak area ratios of non-deuterated (extracted) GA to deuterated GA (Kovats 1958).

Crop Growth and Abiotic Stress

Crop growth is the accumulative irreversible increase in crop plants. Abiotic and biotic stresses, mostly due to anthropogenic activities, cause losses to the crop yield. This is impossible until we understand inside the plant knowledge that how it interacts with outside environment including beneficial microbes (Mittler 2006) in abiotic stress. In abiotic stresses drought, salinity, and extreme temperature are most common all over the world (Khan et al. 2011). The interaction in such harsh conditions is very complex and may vary from crop to crop and growth stages. The impact is also highly variable on plant growth and biomass production (Tuteja 2007). Drought stress reduces the plant cell water potential and turgor pressure followed by increase in solute concentrations in the cytosol. In response to drought, increase in ABA, compatible osmolytes, and overproduction of reactive oxygen species occur. Overall, the important process for growth and development like acquisition of mineral and cellular metabolism are arrested (Khan et al. 2011; Lisar et al. 2012; Christensen et al. 2007; Munns and Tester 2008).

Salinity has devastated the crop production on more than 45 million hectares of irrigated land around the globe (Munns and Tester 2008; Carrillo et al. 2011). Salinity stress creates osmotic stress, ion toxicity, nutritional disorders, oxidative stress, change in metabolic functions, membrane disintegration, genotoxicity, and negatively influences cell division and expansion (Mittler 2006; Carrillo et al. 2011; Zhu 2007; Hossain et al. 2007, 2008; Türkan and Demiral 2009). The fluctuation in climatic conditions due to global warming has tremendously changed the general pattern of crop plant growth (Mahajan and Tuteja 2006; KohIba 2002; Shah et al. 2011). A high temperature exposure can injure the plant cell and cause cell death in a minute (Schöffl et al. 1999; Wahid et al. 2007). Overall, combination of such stresses cause starvation, growth retardation, abridged ion flux, and production of toxic compounds and reactive oxygen species (Wahid et al. 2007; Howarth 2005; Smertenko et al. 1997; Heidarvand and Amiri 2010; Wang et al. 2003), hence reducing the crop yield.

Different crop plants have devised various strategies to cope abiotic stresses and possess a cascade of signals ranging from primary to secondary responses. In primary response, plant maintains cell ionic and osmotic balance, which is followed by secondary response of activation of hormone, and secondary metabolites

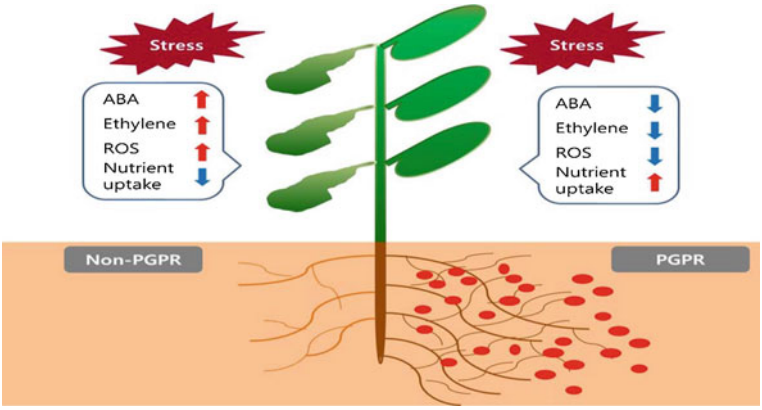

Fig. 1.3 Mechanism involved in PGPR role in crop tolerance against abiotic stress. *The upward arrow* indicates activation of effects, while *the downward* shows reduction

formation occurs. As we know, different abiotic stresses share some common symptoms and mitigation strategies (Hossain et al. 2007; Mahajan and Tuteja 2006; Wang et al. 2003). For example, drought and salinity cause ionic and osmotic stress, and in both cases, plant activates genes related to stress resistance and brings ionic and osmotic homeostasis through salt overly sensitive genes pathway or other related pathways. Drought and low temperature cause the same damage (disintegration of membrane, dehydration, and solute leakage). In perception of both stresses, crop plants either turn on detoxification signaling or activate stress genes which control damage and repair of cell membrane (Fig. 1.3; Lisar et al. 2012; Carrillo et al. 2011; Mahajan and Tuteja 2006; Wahid et al. 2007).

Sustainability of agricultural production is very important to fulfill the growing demands of food for human population. However, there is a need to minimize such abiotic stress in an eco-friendly way (Wang et al. 2003). Use of PGPR as a biocontrol and a biofertiliser seems an ideal strategy to mitigate various extreme environmental conditions like salinity, drought, and temperature stress (Fig. 1.3).

GA-Producing PGPRs and Crop Growth Amelioration

The ability of PGPR to produce phytohormones is one of the most important mechanisms by which many rhizobacteria promote plant growth (Spaepen et al. 2007; Martínez-Viveros et al. 2010). Several fungal and bacterial species are reported for phytohormone production (Tsavkelova et al. 2006). The phytohormone producing ability is widely distributed among bacteria associated with soil and plants. Research on PGPR has established that it can stimulate plant growth through the production of auxins, gibberellins, and cytokinins (Spaepen et al. 2008; Bottini et al. 2004; Timmusk et al. 1999), or by regulating the high levels of endogenous ethylene in the plant (Table 1.3; Glick et al. 1998).

Table 1.3 Reported PGPR species and their role in plant growth and development

PGPR species	Target plants	Observed effects	Reference
Pseudomonas fluorescens	Bean	Higher lignin content	Anderson and Guerra (1985)
Serratia plymuthica	Cucumber	Against disease	Benhamou et al. (2000)
Pseudomonas aeruginosa	Bean	Increased activity of phenlyalanine ammonia lyase	De Meyer et al. (1999)
Pseudomonas corrugata	Cucumber	Induced peroxidase (PO) activity	Chen et al. (2000)
Azospirillum brasilense *Azospirillum lipoferum*	Maize and rice	Gibberellin production	Cassán et al. (2001)
Bacillus subtilis	*Arabidopsis*	Elevated levels of L-malic acid	Thimmaraju et al. (2008)
Bacillus cereus	Tomato	Induced systemic resistance	Bernardo de et al. (2006)
Variovorax paradoxus	Indian mustard	Cadmium tolerant	Belimov et al. (2005)
Acinetobacter calcoaceticus	Cucumber, Chinese cabbage, crown daisy	Gibberellin production—Phosphate solubilization	Kang et al. (2009)
Rhizobium	Rice	Produced auxin (IAA) and gibberellins	Yanni et al. (2001)
Bacillus amyloliquefaciens	Tomato	Nutrient (nitrogen and phosphorus) uptake	Adesemoye et al. (2009)
Azotobacter	Wheat	Antifungal activity produced IAA	Zarrin and Sharon (2010)
Brevibacterium iodinum *Bacillus licheniformis* *Zhihengliuela alba*	Pepper	ACC deaminase producing	Siddikee et al. (2010)
Stenotrophomonas maltophilia	*Arabidopsis*	Production of siderophores and chitinases	Domenech et al. (2007)
Pseudomonas monteilii *Cronobacter dublinensis* *Bacillus* spp.	Sweet basil	Nutrient uptake, antagonist	Rakshapal et al. (2013)
Azospirillum lipoferum	Maize	Accumulation of free amino acids, soluble sugars, proline, and soluble protein contents	Qudsia et al. (2013)

(continued)

Table 1.3 (continued)

PGPR species	Target plants	Observed effects	Reference
Azospirillum sp., *Pseudomonas* sp.	Canola	Antioxidant enzymes and Microelements	Noorieh et al. (2013)
Azospirillum brasilense *Gluconacetobacter diazotrophicus* *Herbaspirillum seropedicae* *Burkholderia ambifaria*	Tomato	Fixing atmospheric nitrogen, protecting the host plant from pathogens	Anna et al. (2013)
Bacillus pumilus *Micrococcus* spp. *Mesorhizobium* sp.	Brassicaceae	Effective metal immobilizing	Wafae et al. (2013)
Pseudomonas aeruginosa	Chickpea	Uptake of nitrogen and phosphorus (P) Production of phytohormone (IAA)	Jay et al. (2013)

Gibberellin-producing PGPR are very little known for their plant growth promotion. In PGPR, the phytohormones are secondary products and are suggested for beneficial effects in plant growth. Several types of PGPR have been identified for their potential to produce gibberellins. These are isolated from rhizosphere and preliminarily selected for plant growth promotion. Plant growth promotion by PGPR species that produce GAs has been previously reported (Bastian et al. 1998; Gutierrez-Manero et al. 2001; Atzhorn et al. 1988). In cultures of wild-type and mutant strains of *Rhizobium phaseoli*, Atzhorn et al. (1988) found GA_1 and GA_4 along with smaller quantity of GA_9- and GA_{20}-like compounds. In another experiment, Bastian et al. (1998) detected phytohormones indole-3-acetic acid and gibberellins GA_1 and GA_3 from chemically defined cultures of *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae*. Both bacteria are associated with Gramineae species in endophytic mode of life and were found to promote plant growth and yield (Table 1.1).

Gutierrez-Manero et al. (2001) isolated the plant-growth-promoting rhizobacteria (PGPR), *Bacillus pumilus* and *Bacillus licheniformis*, from the rhizosphere of alder (*Alnus glutinosa* [L.]). Full-scan gas chromatography–mass spectrometry analyses on extracts of these media showed the presence of GA_1, GA_3, GA_4, and GA_{20} in addition to the isomers 3-epi-GA_1 and iso-GA_3. Bioassay data showed that all the three strains have a strong growth-promoting activity in alder seedlings.

Joo et al. (2004) isolated *Bacillus cereus*, *B. macroides,* and *B. pumilus* and found the production of GA_5, GA_8, GA_{34}, GA_{44}, and GA_{53} for the first time by bacteria. The newly identified PGPR were also evaluated for growth promotion in red pepper which showed that they not only enhanced different plant growth parameters but also increased endogenous gibberellin level (Joo et al. 2004, 2005).

PGPB are also investigated in vegetables. In one experiment, Kang et al. (2012) investigated the symbiotic effect of gibberellin and organic acids producing PGPR (*Acinetobacter calcoaceticus*) on cucumber plant growth. In symbiotic association, the PGPR has significantly ameliorated cucumber plants to higher growth. The PGPR application had higher shoot length, plant biomass, and chlorophyll contents as compared to distilled water and nutrient broth-treated control plants. The bacterial culture-treated plants have also increased the amino acid and crude protein contents as compared to control plants. The improved effects were also observed by the regulation of stress-related abscisic acid which was significantly lower in PGPR-inoculated plants as compared to controls. Contrarily, the endogenous GA quantity was up-regulated, indicating the activation of GA biosynthesis pathway by which it increased the shoot lengths of cucumber plant.

Similar studies were also investigated in tomato plants. *Promicromonospora* sp. SE188 was producing gibberellins and had higher phosphate solubilisation potential. Its inoculation to the tomato plants resulted in higher plant biomass and shoot length as compared to distilled water-treated control plants. The presence of *Promicromonospora* sp. SE188 significantly up-regulated the non-C-13 hydroxylation GA biosynthesis pathway ($GA_{12} \rightarrow GA_{24} \rightarrow GA_9 \rightarrow GA_4 \rightarrow GA_{34}$) in tomato plants as compared to the control plants. Endogenous abscisic acid was significantly down-regulated in the presence of *Promicromonospora* sp. SE188. Contrarily, endogenous salicylic acid was significantly higher in the tomato plant after *Promicromonospora* sp. inoculation as compared to the control.

Karako and Aksoz (2006) isolated the potent *Pseudomonas* sp. from soil of olive waste. The *Pseudomonas* sp. was capable of producing gibberellins. However, no investigation was reported on plant growth promotion. Furthermore, on optimization of nutrient broth, the *Pseudomonas* sp. yielded the highest level of gibberellic acid (285.06 mg/l) upon incubation at 30 °C for 72 h at pH 7 using rotary shaker under dark conditions.

The role of ecological significance must be considered when using PGPR. Barea et al. (1976) isolated fifty phosphate-dissolving bacteria from rhizospheres of various crop plants. Assessing their potential to secrete gibberellins, IAA, and cytokinins, only 29 rhizobacterial strains were active to produce gibberellins.

Another study showed that mutualistic symbiosis of maize and *Pseudomonas* fluorescent enhanced the drought stress tolerance of the host (Ansary et al. 2012). Results showed that drought stress triggered a change in plant phytohormonal balance, including an increase in leaf proline and abscisic acid content, and a decline in auxin, gibberellin, and cytokinin synthesis. In comparison with control, plants inoculated with *P. fluorescens* showed highest level of proline, abscisic acid, auxin, gibberellin, and cytokinin in the leaves. This study indicates that application of PGPR can enhance phytohormone content of maize under water-deficit stress conditions. In addition to maize, *Pseudomonas* strains associated with rapeseed exhibited higher growth and more oil yield in drought stress (Arvin et al. 2012). Results showed that drought stress reduced yield up to 152.5 %, oil content, and yield components. It was also concluded that inoculation treatment had better effects than either no inoculation (control) or co-inoculation.

From the semi-arid ecosystem of south-east Spain, Kohler et al. (2008) isolated PGPR along with arbuscular mycorrhizal fungi and rhizobium bacteria. The symbiotic association was evaluated alone or in combination with each other using *Anthyllis cytisoides* L., a test plant. The parameters evaluated were biomass accumulation and allocation, N and P uptake, N_2-fixation (15N), and specific root length. Many microbial combinations were effective in improving plant development, nutrient uptake, N_2-fixation, or root system quality. It was also concluded that beneficial microbes native to the environment are more effective than the exotic species and instead of selecting a multifunctional microbial inoculum. Appropriate microbial combinations can be recommended for a given biotechnological input related to improvement of plant performance.

To assess the effects and intensity of abiotic stress tolerance of GA-producing PGPR, Kang et al. (2012) applied novel strains, viz., *Promicromonospora* sp. SE188, *Burkholderia cepacia* SE4, and *A. calcoaceticus* SE370 to cucumber plants. The experimental design comprised of eight sets of cucumber (*Cucumis sativus* L) plants with (1) PGPR interactions; (2) non-PGPR interactions; (3) PGPR interactions salt; (4) non-PGPR interactions salt; (5) PGPR interactions drought; and (6) non-PGPR interactions drought. *B. cepacia* SE4, *Promicromonospora* sp. SE188, and *A.calcoaceticus* SE370 were assessed for their potential to resist high salinity (120 mM) and drought (15 % PEG) stress continuously for 7 days. Parameters like plant growth parameters, relative water content, electrolytic leakage, antioxidant activities, and endogenous hormonal regulation were studied. Other functional biochemicals like crude protein contents, amino acids, and nitrogen content were also evaluated. Overall, the effect was very satisfactory, and the application significantly enhanced the growth parameters of the plants. However, *B. cepacia* SE4 was more prominent to extend the abiotic stress tolerance in cucumber plants. Such kind of studies should be extended to other important agronomic crops to save the agriculture loss during harsh climatic conditions.

Future Perspectives

Our current knowledge about PGPR is still very limited, and to understand it better, we have to explore, isolate, and screen the PGPR wealth available with different agricultural crops. More investigations are needed to analyze and assess the role of active PGPR in crop growth under various abiotic environmental circumstances like salinity and drought. Furthermore, the mechanism needs to be explored in phytohormonal regulation (abscisic acid, salicylic acid, jasmonic acid, and gibberellins) during the PGPR interaction with crop host plants under abiotic stress, to further improve strategies for sustainable crop production.

Acknowledgments This work is supported by Korean Ministry of Environment through "The Eco-Innovation Project" and Basic Science Research Program through the National Research Foundation of Korea (NRF) founded by the Ministry of Education, Science and Technology (2012-0008183).

References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbial Res 163(Suppl 2):173–181

Anderson AJ, Guerra D (1985) Responses of bean to root colonization with *Pseudomonas putida* in a hydroponic system. Phytopathology 75:992–995

Anna LB, Alessandra S, Claudia E, Paola C, Maddalena DG (2013) In vitro and in vivo inoculation of four endophytic bacteria on *Lycopersicon esculentum*. New Biotechnol (in press). http://dx.doi.org/10.1016/j.nbt.2013.01.001

Ansary MH, Rahmani HA, Ardakani MR, Paknejad F, Habibi D, Mafakheri S (2012) Effect of Pseudomonas fluorescent on proline and phytohormonal status of maize (Zea mays L.) under water deficit stress. Ann Biol Res 3(2):1054–1062

Antoun H, Prévost D (2005) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 1–38

Arvin P, Vafabakhsh J, Mazaheri D, Noormohamadi G, Azizi M (2012) Study of drought stress and plant growth promoting rhizobacteria (PGPR) on yield, yield components and seed oil content of different cultivars and species of *Brassica* Oilseed Rape. Ann Biol Res 3(9):4444–4451

Atzhorn R, Crozier A, Wheeler CT, Sandberg G (1988) Production of gibberellins and indole-3-acetic acid by *Rhizobium phaseoli* in relation to nodulation of *Phaseolus vulgaris* roots. Planta 175:532–538

Baca BE, Elmerich C (2003) Microbial production of plant hormones. In: Elmerich C, Newton WE (eds) Associative and Endophytic Nitrogen-fixing Bacteria and Cyanobacterial Associations. Kluwer Academic Publishers, The Netherlands

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2007) The role of root exudates in rhizosphere interactions with plants and other organisms. Ann Rev Plant Biol 57:233–266

Barea JM, Navarro E, Montoya E (1976) Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria. J Appl Microbiol 40(2):129–134

Bastian F, Cohen A, Piccoli P, Luna V, Baraldi R, Bottini R (1998) Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically defined media. Plant Growth Regul 24:7–11

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Biol Biochem 37:241–250

Benhamou N, Gagné S, Quéré DL, Dehbi L (2000) Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium *Serratia plymuthica* on the protection against infection by *Pythium ultimum*. Biochem Cell Biol 90(1):45–56

Bernardo de AH, José RVJ, Reginaldo da SR, Harllen SAS, Maria CBP (2006) Induction of systemic resistance in tomato by the autochthonous phylloplane resident *Bacillus cereus*. Pesq Agropec Bras 41(8):1247–1252

Bertrand H, Plassard C, Pinochet X, Touraine B, Normand P, Cleyet-Marel JC (2000) Stimulation of the ionic transport system in Brassica napus by a plant growth-promoting rhizobacterium (*Achromobacter* sp.). Can J Microbiol 46:229–236

Bomke C, Tudzynski B (2009) Diversity, regulation, and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. Phytochemistry 70:1876–1893

Bottini R, Fulchieri M, Pearce D, Pharis RP (1989) Identification of Gibberellins A1, A3, and iso-A3 in cultures of *Azospirillum lipoferum*. Plant Physiol 90:45–47

Bottini R, Cassán F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503

Brencic A, Winans C (2005) Detection of and response to signals involved in host-micobe interactions by plant-associated bacteria. Microbiol Mol Biol R 69:155–194

Brimecombe MJ, De Leij FAAM, Lynch JM (2007) Rhizodeposition and microbial populations. In: Pinton R, Veranini Z, Nannipieri P (eds) The rhizosphere biochemistry and organic substances at the soil-plant interface. Taylor & Francis Group, New York

Carrillo G, Troch PA, Sivapalan M, Wagener T, Harman C, Sawicz K (2011) Catchment classification: hydrological analysis of catchment behavior through process-based modeling along a climate gradient. Hydrol Earth Syst Sci 15:3411–3430

Cassán F, Bottini R, Schneider G, Piccoli P (2001) *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA20 and metabolize the resultant aglycones to GA1 in seedlings of rice dwarf mutants. Plant Physiol 125:2053–2058

Chen C, Bélanger RR, Benhamou N, Paulitz T (2000) Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol Mol Plant Pathol 56:13–23

Christensen JH, Hewitson B et al (2007) Regional climate projections. In: Solomon S, Qin D, Manning M et al (eds) Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, New York

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Crozier A, Durley RC (1983) Modem methods of analysis of gibberellins. In: Crozier A (ed) The biochemistry and physiology of Gibberellins, vol I. Praeger Scientific, New York, pp 485–560

De Meyer G, Capieau K, Audenaert K, Buchala A, Métraux JP, Höfte M (1999) Nanogram amounts of salicylic produced by *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. Mol Plant Microbe Interact 12:450–458

Dodd IC, Zinovkina NY, Safronova VI, Belimov AA (2010) Rhizobacterial mediation of plant hormone status. Ann Appl Biol 157:361–379

Domenech J, Ramos SB, Probanza A, Lucas GJA, Gutierrez MFJ (2007) Elicitation of systemic resistance and growth promotion of *Arabidopsis thaliana* by PGPRs from *Nicotiana glauca*: a study of the putative induction pathway. Plant Soil 290:43–50

Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59

Gaskin P, MacMillan J (1991) GC-MS of Gibberellins and related compounds: methodology and a Library of Spectra. Cantock's Enterprises, Bristol

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68

Gutierrez-Manero FJ, Ramos-Solano B, Probanza A, Mehouachi J, Tadeo FR, Talon M (2001) The plant-growth-promoting rhizobacteria *Bacillus pumilis* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol Plant 111:206–211

Haas D, Défago G (2005) Biological control of soilborne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Hedden P (1986) Gas Chromatography/Mass spectrometry. In: Linskens HF, Jackson HF (eds) Modern methods of plant analysis, New series, vol 3. Springer, Berlin, pp 1–22

Hedden P (1997) The oxidases of gibberellin biosynthesis: their function and mechanism. Physiol Plant 101:709–719

Hedden P, Kamiya Y (1997) Gibberellin biosynthesis: enzymes, genes, and their regulation. Annu Rev Plant Physiol Plant Mol Biol 48:431–460

Hedden P, Thomas SG (2012) Gibberellin biosynthesis and its regulation. Biochem J 444:11–25

Heftmann E, Saunders GA, Haddon WF (1978) Argentation high performance liquid chromatography and mass spectrometry of gibberelline esters. J Chromatogr 156:71–77

Heidarvand L, Amiri RM (2010) What happens in plant molecular responses to cold stress? Acta Physiol Plant 32:419–431

Hossain MM, Sultana F, Kubota M, Koyama H, Hyakumachi M (2007) The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiol 48:1724–1736

Howarth CJ (2005) Genetic improvements of tolerance to high temperature. In: Ashraf M, Harris PJC (eds) Abiotic stresses: plant resistance through breeding and molecular approaches. Howarth Press Inc., New York

Hrynkiewicz K, Baum C (2012) The potential of rhizosphere microorganisms to promote the plant growth in disturbed soils. In: Malik A, Grohmann E (eds) Environmental protection strategies for sustainable development. Springer, Berlin, pp 35–64

Hussain TM, Chandrasekhar T, Hazara M, Sultan Z, Saleh BK, Gopal GR (2008) Recent advances in salt stress biology–a review. Biotechnol Mol Biol Rev 3:8–13

Hwang J, Chilton WS, Benson DM (2002) Pyrrolnitrin production by *Burkholderia cepacia* and biocontrol of Rhizoctonia stem rot of poinsettia. Biol Control 25:56–63

Iba K (2002) Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annu Rev Plant Biol 53:225–245

Janzen R, Rood S, Dormar J, McGill W (1992) *Azospirillum brasilense* produces gibberellins in pure culture and chemically-medium and in co-culture on straw. Soil Biol Biochem 24:1061–1064

Jay PV, Janardan Y, Kavindra NT, Ashok K (2013) Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecol Eng 51:282–286

Joo GJ, Kim YM, Lee IJ, Song KS, Rhee IK (2004) Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus Bacillus macroides* and *Bacillus pumilus*. Biotechnol Lett 26:487–491

Joo GJ, Kim YM, Kim JT, Rhee IK, Kim JH, Lee IJ (2005) Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. J Microbiol 43:510–515

Joo GJ, Kang SM, Hamayun M, Kim SK, Na CI, Shin DH, Lee IJ (2009) *Burkholderia* sp. KCTC 11096BP as a newly isolated gibberellin producing bacterium. J Microbiol 47:167–171

Kamilova F, Validov S, Azarova T, Mulders I, Lugtenberg B (2005) Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environ Microbiol 7:1809–1817

Kang SM, Joo GJ, Hamayun M, Na CI, Shin DH, Kim HY, Hong JK, Lee IJ (2009) Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. Biotechnol Lett 31:277–281

Kang SM, Hamayun M, Joo GJ, Khan AL, Kim YH, Kim SK, Jeong HJ, Lee IJ (2010) Effect of *Burkholderia* sp. KCTC 11096BP on some physiochemical attributes of cucumber. Eur J Soil Biol 46:264–268

Kang SM, Khan AL, Muhammad H, Zabta KS, Kim YH, Joo GJ, Lee IJ (2012a) *Acinetobacter calcoaceticus* ameliorated plant growth and influenced gibberellins and functional biochemical. Pak J Bot 44(1):365–372

Kang SM, Khan AL, Hamayun M, Hussain J, Joo GJ, You YH, Kim JG, Lee IJ (2012b) Gibberellin-producing *Promicromonospora* sp. SE188 improves *Solanum lycopersicum* plant growth and influences endogenous plant hormones. J Microbiol 50(6):902–909

Karako S, Aksoz N (2006) Some optimal cultural parameters for gibberellic acid biosynthesis by *Pseudomonas* sp. Turk J Biol 30:81–85

Khan AL, Hamayun M, Ahmad N, Hussain J, Kang SM, Kim YH, Adnan M, Tang DH, Waqas M, Radhakrishnan R, Park ES, Lee IJ (2011) Salinity stress resistance offered by endophytic fungal interaction between *Penicillium minioluteum* LHL09 and *Glycine max*. L. J Microbiol Biotechnol 21(9):893–902

Kovats E (1958) Gas-chromatographische charakterisierung organischer Verbindungen. Teil 1: retentionsindices aliphatischer halogenide, alkohole aldehyde und ketone. Helv Chim Acta 41:1915–1932

Kohler J, Hernandez JA, Caravaca F, Roldŕn A (2008) Plant-growth-promoting rhizobacteria and abuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. Funct Plant Biol 35:141–151

Lee IJ, Foster K, Morga PW (1998) Photoperiod control of gibberellin levels and flowering in sorghum. Plant Physiol 116:1003–1011

Lisar YS, Motafakkerazad R, Hossain MM, Rahman IMM (2012) Water stress in plants: causes, effects and responses. In: Rahman IMM (ed) Water stress. ISBN:978-953-307-963-9. InTech, Available from: http://www.intechopen.com/books/water-stress/water-stress-inplants-causes-effects-andresponses

Lucangeli C, Bottini R (1997) Effects of *Azospirillum* spp. On endogenous gibberellin content and growth of maize (Zea mays L.) treated with uniconazole. Symbiosis 23:63–72

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10

Mahajan S, Tuteja N (2006) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158

Mansour FA, Aldesuquy HS, Hamedo HA (1994) Studies on plant growth regulators and enzymes production by some bacteria. Qatar UnivSci J 14(2):281–288

Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by Rhizobacteria. J Soil Sci Plant Nutr 10(3):293–319

Mayak S, Tirosh T, Glick B (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19

Morgan JAW, Bending GD, White PJ (2005) Biological costs and benefits to plant–microbe interactions in the rhizosphere. J Exp Bot 56:1729–1739

Morris RO, Zaerr JB (1978) 4-bromophenacyl ester of gibberellins, useful derivatives for high performance liquid chromatography. Anal Lett A 11:73–83

Morrone D, Chambers J, Lowry L, Kim G, Anterola A, Bender K, Peters RJ (2009) Gibberellin biosynthesis in bacteria: separate ent-copalyldiphosphate and ent-kaurene synthases in *Bradyrhizobium japonicum*. FEBS Lett 583:475–480

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nadeem SM, Zahir ZA, Naveed M, Asghar HN, Arshad M (2010) Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Sci Soc Am J 74:533–542

Nihorimbere V, Ongena M, Smargiassi M, Thonart P (2011) Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol Agron Soc Environ 15(2):327–337

Noorieh B, Arzanesh MH, Mahlegha G, Maryam S (2013) The effect of plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress. J Appl Environ Biol Sci 3(1):17–27

Nowak-Thompson B, Chaney N, Wing JS, Gould SJ, Loper JE (1999) Characterization of the pyoluteorin biosynthetic gene cluster of *Pseudomonas fluorescens* Pf-5. J Bacteriol 181:2166–2174

Piccoli P, Lucangeli D, Schneider G, Bottini R (1997) Hydrolysis of [17,17-$_2$H^2]Gibberellin A20-Glucoside and [17,17-$_2$H^2]Gibberellin A20-glucosylester by *Azospirillum lipoferum* cultured in a nitrogen-free biotin-based chemically-defined medium. Plant Growth Regul 23:179–182

Piccoli P, Masciarelli O, Bottini R (1999) Gibberellin production by *Azospirillum lipoferum* cultured in chemically-defined medium as affected by oxygen availability and water status. Symbiosis 27:135–146

Pierson LS, Pierson EA (2010) Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. Appl Microbiol Biotechnol 6(6):1659–1670

Qudsia B, Noshinil Y, Asghari B, Nadia Z, Abida A, Fayazul H (2013) Effect of *Azospirillum* inoculation on maize (*Zea mays L.*) under drought stress. Pak J Bot 45(S1):13–20

Raaijmakers JM (2001) Rhizosphere and rhizosphere competence. In: Maloy OC, Murray TD (eds) Encyclopedia of plant pathology. Wiley, New York, pp 859–860

Rakshapal S, Sumit KS, Rajendra PP, Alok K (2013) Technology for improving essential oil yield of *Ocimum basilicum* L. (sweet basil) by application of bioinoculant colonized seeds under organic field conditions. Ind Crop Prod 45:335–342

Reeve DR, Crozier A (1978) Quantitative analysis of plant hormones. In: Hillman JR (ed) Isolation of plant growth substances. Cambridge University Press, London, pp 17–41

RodrÍgueza H, Fragaa R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech Adv 17(4–5):319–339

Rovira AD (1991) Rhizosphere research, 85 years of progress and frustration. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic, The Netherlands, pp 3–13

Saharan BS, Nehra V (2011) Plant growth promoting Rhizobacteria: a critical review. Life Sci Med Res (LSMR-21)

Schöffl F, Prandl R, Reindl A (1999) Molecular responses to heat stress. In: Shinozaki K, Yamaguchi-Shinozaki K (eds) Molecular responses to cold, drought, heat and salt stress in higher plants. R.G. Landes Co., Austin, pp 81–98

Shah F, Huang J, Cui K, Nie L, Shah T, Chen C, Wang K (2011) Impact of high-temperature stress on rice plant and its traits related to tolerance. J Agri Sci 1–12

Siddikee MA, Chauhan PS, Anandham R, Han GH, Sa T (2010) Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J Microbiol Biotechnol 20:1577–1584

Smertenko A, Draber P, Viklicky V, Opatrny Z (1997) Heat stress affects the organization of microtubules and cell division in *Nicotiana tabacum* cells. Plant, Cell Environ 20:1534–1542

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Spaepen S, Dobbelaere S, Croonenborghs A, Vanderleyden J (2008) Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. Plant Soil 312:15–23

Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Appl Soil Ecol 15:183–190

Tarkka M, Schrey S, Hampp R (2008) Plant associated micro-organisms. In: Nautiyal CS, Dion P (eds) Molecular mechanisms of plant and microbe coexistence. Springer, New York, pp 3–51

Thimmaraju R, Kirk JC, Paul WP, Harsh PB (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–1556

Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. Soil Biol Biochem 31:1847–1852

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006) Microbial producers of plant growth stimulators and their practical use: a review. Appl Biochem Micro 42:117–126

Türkan T, Demiral T (2009) Recent developments in understanding salinity tolerance. Environ Exp Bot 67:2–9

Tuteja N (2007) Abscisic acid and abiotic stress signaling. Plant Signal Behav 2:135–138

Urbanova T, Tarkowska D, Strnad M, Hedden P (2011) Gibberellins–terpenoid plant hormones: biological importance and chemical analysis. Collect Czech Chem Commun 76(12):1669–1686

Wafae A, Christophe S, Jean LM, Ali B (2013) Effect of nickel-resistant rhizosphere bacteria on the uptake of nickel by the hyperaccumulator *Noccaea caerulescens* under controlled conditions. J Soils Sediments 13:501–507

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223

Wang MC, Bohmann D, Jasper H (2003) JNK signaling confers tolerance to oxidative stress and extends lifespan in Drosophila. Dev Cell 5:811–816

Welbaum G, Sturz AV, Dong Z, Nowak J (2004) Fertilizing soil microorganisms to improve productivity of agroecosystems. Crit Rev Plant Sci 23:175–193

Yanni YG, Rizk RY, Abd El-Fattah FK, Squartini A, Corich V, Giacomini A, de Bruijn F, Rademaker J, Maya-Flores J, Ostrom P, Vega-Hernandez M, Hollingsworth RI, Martinez-Molina E, Mateos P, Velazquez E, Wopereis J, Triplett E, Umali-Garcia M, Anarna JA, Rolfe BG, Ladha JK, Hill J, Mujoo R, Ng PK, Dazzo FB (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. trifolii with rice roots. Aust J Plant Physiol 28:845–870

Zarrin K, Sharon LD (2010) Characterization of bacterial endophytes of sweet potato plants. Plant Soil. doi:10.1007/s11104-009-9908-1

Zhu JK (2007) Plant salt stress. Wiley, New York