



Mohamed Mahgoub Azooz • Parvaiz Ahmad

Legumes under Environmental Stress

Yield, Improvement and Adaptations



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CHAPTER 9

Microbial strategies for the improvement of legume production in hostile environments

Dilfuza Egamberdieva¹, Vyacheslav Shurigin¹, Subramaniam Gopalakrishnan² and Ram Sharma³

¹Department of Microbiology and Biotechnology, Faculty of Biology and Soil Science, National University of Uzbekistan, Tashkent, Uzbekistan

²International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India

³International Center for Agricultural Research in the Dry Areas (ICARDA), Tashkent, Uzbekistan

9.1 Introduction

Various biotic and abiotic factors limit crop productivity, affecting nearly 1 billion people around the world (Munns & Tester, 2008; UNEP 2009). Malnutrition is recognized as the world's most serious health problem, while agricultural development is considered as the most effective sector in reducing hunger and poverty, through improvements in crop productivity (Godfray *et al.*, 2010). Crop losses due to salinity and drought are a major area of concern in coping with increasing food requirements (Egamberdiyeva *et al.*, 2007; Shanker & Venkateswarlu, 2011; Davranova *et al.*, 2013). Salinity alone affects 33% of the world's potential arable land, whereas 950 million ha of salt-affected lands occur in arid and semi-arid regions (UNEP, 2008). Major factors increasing salinity include irrigation of cultivated lands with saline water, poor cultural practices and low precipitation. The ability of plants to take up water are inhibited by salinity stress, causing ion imbalance and, in turn, resulting in a reduction of root and shoot growth (Munns, 2002).

In such situations, the presence of salt-tolerant genetic variants in a particular crop is a prerequisite for its successful cultivation (Mahmood *et al.*, 2000). Numerous studies have been reported on the management and identification of salt-tolerant crops such as cotton, wheat, maize and alfalfa (Chauhan & Singh, 2000; Soltani *et al.*, 2012). In addition, organic farming practices, such as application of farm manure, compost, biofertilizer and recycling of crop residues and/or green manures, also improve degraded soils affected by salinity (Pathma & Sakthivel, 2012; Adesemoye & Egamberdieva, 2013).

Legumes are very important crop plants for human and animal consumption, and the use of legumes in crop rotations can be a useful management strategy to increase the supply of nitrogen to non-legume plants (Lüscher *et al.*, 2011; Nyfeler *et al.*, 2011). The legume-*Rhizobium* symbiosis is known to be the most efficient system for biological nitrogen fixation (BNF) through nodulation in legume roots (Molla *et al.*, 2001). It has been estimated that some 44–66 million tons of N₂ are fixed annually by leguminous plants, providing nearly half of all N used in agriculture worldwide (Alberton *et al.*, 2006).

The response of legumes to environmental stresses such as salinity and drought varies and depends on soil type, salt toxicity and climatic factors (Cordovilla *et al.*, 1995; Serraj *et al.*, 2001; Predeepa & Ravindran, 2010; Jabborova *et al.*, 2013a). Numerous studies have shown that soil salinity inhibits legume growth and development and decreases nodulation and N₂ fixation (reviewed by Zahran, 1999; Mensah & Ihenyen, 2009; Egamberdieva *et al.*, 2013a). Grain legumes are known to be salt-sensitive crops, but differences in salt tolerance exist among genotypes (Dua, 1992; Gandour, 2002). Genotypic variation in leguminous crops for traits affecting nodulation and N₂ fixation has been found (Montealegre *et al.*, 1995). Bliss (1993) observed that the selection and breeding of common bean cultivars tolerant to salinity could improve nodulation and N₂ fixation. Thus, the selection of improved chickpea cultivars with salt-tolerant symbioses is an absolute necessity to enable its cultivation in salt-affected soils. Available reports indicate that legumes show improved

yield and nodulation when co-inoculated with plant-growth-promoting rhizobacteria (PGPR) under hostile environmental conditions compared to inoculation with rhizobia alone (Rokhzadi *et al.*, 2008; Yang *et al.*, 2009; Jabborova *et al.*, 2013b).

This chapter examines recent studies on the impact of salt and drought stresses on legumes and the genotypic variation among legumes for germination, seedling growth and other plant traits under hostile conditions, and the microbial technologies that can improve growth, development and symbiotic performance of legumes. In addition, some possible mechanisms of plant resistance to stress, growth stimulation and improved symbiotic performance by rhizobacteria are described.

9.2 Abiotic stresses affecting legume crop productivity

9.2.1 Plant growth and stress

Previous studies have shown that soil salinity and drought decrease rhizobial colonization, inhibit infection processes and nodule development, and reduce N_2 fixation and nitrogenase activity in legumes (Zahran & Sprent, 1986; Zahran, 1999; Kulkarni *et al.*, 2000; Serraj, 2002; Egamberdieva *et al.*, 2013b). In earlier reports the inhibition by salinity of growth, nodulation and N fixation was observed in chickpea (Singh *et al.*, 2001), common bean (Ferri *et al.*, 2000) and lentil (Golezani & Yengabad, 2012). In subsequent studies, saline soil conditions inhibited germination and seedling growth, nodulation and biomass accumulation in soybean (Essa, 2002; Li *et al.*, 2006). Similar findings were observed for soybean by Hamayun *et al.* (2010), where the plant length, biomass, chlorophyll content, number of pods, 100-seed weight and yield were all significantly reduced by salinity stress. Leaf chlorosis, leaf bleaching and necrosis were also observed as effects of salt stress on soybean (Parker *et al.*, 1987). In lentil, plant growth and nodulation were significantly reduced over the whole growing season under saline soil conditions (Van Hoorn *et al.*, 2001). The decreased nodule formation resulted in reduction of leghaemoglobin content and N_2 fixation activity (Parida & Das, 2005). Limitation of oxygen diffusion in the nodules could be the reason for inhibition of nitrogenase activity and respiration of the nodules (Serraj *et al.*, 1995). Further, salt stress affects protein synthesis, lipid metabolism and photosynthesis, and

reduces the growth of roots and root hairs, thereby decreasing sites for potential rhizobial infection and further nodule development (Katerji *et al.*, 2001; Bouhmouch *et al.*, 2005). The decrease in root growth was related to endogenous levels of phytohormones such as gibberellins, abscisic acid, jasmonic acid and salicylic acid, which declined under NaCl-induced salt stress (Debez *et al.*, 2001).

Several reports have indicated that germination and seedling growth of chickpea are reduced in saline soils, with responses varying according to cultivars (Gandour, 2002; Al-Mutawa, 2003). Krouma (2009) evaluated the growth, nodulation, nitrogen fixation and ionic repartition in two chickpea varieties, and found that the salt-tolerant cultivar was able to protect its photosynthetic and symbiotic apparatus against the toxic Na^+ and Cl^- ions.

We have also observed a significant effect of salinity (salt concentration) on germination, and on shoot and root length of chickpea genotypes (Table 9.1). The 29 genotypes differed significantly for germination and shoot and root length. There was a significant genotype \times salinity interaction on germination and shoot and root length. According to Almansouri *et al.* (2001), seed germination is usually the most critical stage in seedling establishment. According to Sadiki and Rabih (2001), chickpea is a salt-sensitive species, and conditions of 25 mM NaCl resulted in a 71% reduction in growth. We have also observed that most chickpea genotypes were salt sensitive, with germination capacity decreasing with increasing salinity. The present result agrees with the work of Gandour (2002) and Vadez *et al.* (2007), who observed decreases in percentage germination and seedling emergence of chickpea with increases in salinity. Atak *et al.* (2006) and Neamatollahi *et al.* (2009) pointed out that higher salinity may reduce germination due to higher osmotic pressures. The seeds of six chickpea genotypes, namely Jahongir, Uzbekiston-32, Lazzat, Zimistoni, Flip 1-22 and Flip 1-31, showed better germination (40–45%) than other chickpea genotypes. According to Tejavathi *et al.* (1988) the ability of a seed to germinate under salt stress indicates that it has genetic potential for salt tolerance. The 29 genotypes of chickpea differed in their response to different salinity levels (Table 9.2).

The reduction in seed germination rate (at 10 days after sowing), as compared to the respective controls, was less than 25% for Sino, Flip 1-01, Flip 1-04, Flip 1-05, Flip 1-19, Flip 03-27c and Flip 06-155c. Seeds of

Table 9.1 Germination^a of various soybean genotypes 2, 6 and 10 days after sowing in Petri dishes at different concentrations of NaCl.

Time Salinity (dS/m)	2 days			6 days			10 days		
	0	5.0	10.0	0	5.0	10.0	0	5.0	10.0
Variety									
Jahongir	5.3	4.8	2.5	12.0	11.5	4.0	14.5	12.5	8.5
Uzbekistan-32	11.0	6.0	2.8	15.3	13.5	4.3	16.5	14.3	8.0
Lazzat	5.8	5.0	4.5	10.3	6.0	6.3	15.3	10.0	8.3
Zimistoni	10.3	9.0	3.8	12.8	13.3	4.0	18.3	15.0	9.0
Xalima	4.3	6.0	0.1	9.5	9.3	2.5	16.8	15.0	6.5
Miroz	4.0	2.3	0.5	7.5	4.8	2.3	17.3	11.3	6.3
Muqtadir	4.5	5.5	3.3	7.0	7.3	4.5	9.0	8.3	5.3
Xisor-32	8.8	8.0	3.8	9.0	8.0	5.8	9.3	8.8	6.5
Sino	3.5	2.8	1.0	7.0	5.3	2.3	8.0	7.0	3.3
Flip 1-01	7.5	2.8	1.3	10.0	4.8	3.0	13.8	10.5	4.0
Flip 1-04	3.8	2.8	0.8	7.5	4.3	2.3	14.0	11.0	4.8
Flip 1-05	3.0	0.8	0.3	5.5	4.3	1.0	12.3	9.5	2.8
Flip 1-19	7.8	4.8	0.3	12.8	10.5	2.5	19.0	16.3	5.0
Flip 1-21	7.5	5.0	3.8	11.0	8.3	5.5	19.3	15.8	7.5
Flip 1-22	12.3	8.8	2.0	14.8	11.3	3.0	18.8	15.0	8.3
Flip 1-29	9.0	7.8	1.5	12.0	9.0	2.3	17.5	15.3	5.3
Flip 1-31	2.8	2.8	0.8	6.5	5.3	3.8	15.0	11.3	8.5
Flip 1-33	13.8	4.5	1.8	17.0	12.3	4.3	19.3	17.0	6.3
Flip 03-102c	6.5	6.0	3.5	8.3	7.8	4.3	10.3	9.3	5.5
CIEW-45	6.0	7.0	6.0	9.0	8.8	8.8	10.3	9.3	8.8
Flip 05-69 c	5.3	3.5	5.0	7.5	7.0	5.0	9.3	8.8	6.3
Flip 03-74c	5.0	5.0	4.5	7.5	6.5	6.3	9.0	8.0	6.8
Flip 06-102c	4.8	4.5	4.0	6.5	7.5	6.3	9.5	9.0	7.0
Flip 06-66	5.5	6.0	5.0	8.5	7.0	6.3	10.3	9.5	7.8
Flip 05-65	5.0	6.5	5.8	7.0	8.3	6.5	9.8	10.0	7.3
Flip 06-124c	6.3	7.0	6.0	8.8	7.8	6.0	9.8	9.0	7.0
Flip 06-80c	8.0	7.8	6.5	9.0	8.5	7.3	9.5	9.3	8.8
Flip 03-27c	7.5	6.5	1.3	8.0	7.5	2.8	9.8	8.8	3.0
Flip 06-155c	1.5	5.5	1.8	2.8	7.3	4.3	3.0	7.5	2.0
LSD (5%)	1.4	2.0	1.4	1.4	1.8	1.4	0.6	1.8	1.4
Mean	6.4	5.3	2.9	9.3	8.0	4.4	12.9	11.1	6.3
CV (%)	14.0	23.2	29.1	12.1	14.0	19.1	7.7	10.1	13.23

^aNumber of germinated seeds.

CV, coefficient of variation; LSD, least significant difference.

Table 9.2 Mean squares from analysis of variance for various plant traits of 29 genotypes of chickpea at three concentrations of NaCl.

Source	df	Germination	Shoot length	Root length
Replication	2	1.7	1.9	3.1
Concentration	2	1018.4**	1219.3**	841.9**
Genotype	28	66.3**	63.9**	15.4**
Genotype × concentration	56	16.6**	29.8**	19.3**
Error	181	1.0	1.3	1.0

**Significant at $P=0.01$.

Jahongir, Uzbekistan-32, Lazzat, Zimistoni, Flip 1-22, Flip 1-31, CIEW-45 and Flip 06-80c showed better germination (40–45%) at 10 dS/m salt concentration than other chickpea genotypes (Table 9.1). Seed germination was decreased slightly with increasing salinity levels for Xisor-32, CIEW-45, Flip 06-66, Flip 06-124c, Flip 06-80c and Flip 03-27c. In contrast, significant differences between NaCl treatments were observed for seed germination ($P < 0.05$) at 5 and 10 dS/m.

In our work the increase in salt concentration reduced shoot and root length in all genotypes, and it seemed to reduce the availability of the nutrients required for the growth and development of the plants. Growth response of chickpea to NaCl treatment (0, 5 and 10 dS/m) varied with the genotype, as shown by shoot and root lengths. The influence was more pronounced at 10 dS/m salinity level (Table 9.3). Genotypes Flip 1-04, Flip 1-05, Flip 1-22, Flip 06-66, Flip 03-27c, Flip 06-155c and Sino were

Table 9.3 Effect of salinity on shoot and root length of chickpea genotypes (seedlings were grown in a gnotobiotic sand system for 3 weeks).

Genotype	Root length (cm)			Shoot length (cm)		
	Salinity (dS/m)					
	0	5.0	10.0	0	5.0	10.0
Jahongir	16.7	16.2	15.8	11.2	10.4	9.4
Uzbekistan-32	14.3	13.2	13.9	10.5	14.0	9.9
Lazzat	15.1	15.2	12.1	15.8	11.9	10.5
Zimistoni	18.4	14.9	9.3	22.6	13.3	7.6
Xalima	17.0	9.4	10.6	15.4	13.3	10.5
Miroz	18.6	18.5	13.6	14.3	12.5	8.8
Muqtadir	15.6	14.3	11.6	10.4	9.6	7.7
Xisor-32	16.2	13.4	10.5	17.6	12.8	7.8
Sino	19.0	14.9	0.0	19.1	13.3	0.0
Flip 1-01	18.4	15.3	12.4	16.7	12.2	8.8
Flip 1-04	16.4	14.0	11.7	11.7	11.8	10.5
Flip 1-05	19.6	15.9	6.3	11.3	13.3	9.0
Flip 1-19	16.2	16.7	11.6	12.9	13.0	10.0
Flip 1-21	19.8	17.1	10.9	15.5	13.8	10.2
Flip 1-22	7.7	6.1	5.7	12.4	10.6	8.3
Flip 1-29	17.9	16.4	10.0	15.5	13.8	10.2
Flip 1-31	21.2	19.7	14.4	13.9	12.1	10.0
Flip 1-33	13.0	11.6	10.9	14.5	11.5	8.5
Flip 03-102c	16.3	16.1	11.3	13.2	11.4	8.9
CIEW-45	19.6	16.9	10.8	13.5	11.3	8.8
Flip 05-69c	22.5	16.9	11.4	13.1	11.3	9.0
Flip 03-74c	14.2	13.2	9.9	14.5	12.2	10.2
Flip 06-102c	22.0	11.0	10.5	14.5	12.0	9.8
Flip 06-66	12.6	11.5	6.8	14.1	11.8	8.6
Flip 05-65	11.4	9.6	8.1	12.2	9.8	9.2
Flip 06-124c	18.1	15.8	10.4	11.9	11.6	9.3
Flip 06-80c	22.2	15.6	14.2	18.7	13.3	8.7
Flip 03-27c	16.1	14.3	0.0	16.7	13.2	0.0
Flip 06-155c	24.7	5.4	0.0	14.5	11.2	0.0
LSD (5%)	2.4	1.6	1.5	1.8	1.8	1.1
Mean	17.7	14.2	10.0	14.4	12.2	8.0
CV(%)	8.4	6.9	9.3	0	9.0	1.8

CV, coefficient of variation; LSD, least significant difference.

found to be salt-sensitive whereas genotypes Jahongir, Uzbekistan-32, Xalima, Miroz, Xisor 32 and Flip 06-102 were found to be salt-tolerant at 10 dS/m salinity level. There was a significant interaction between salt and shoot and root growth ($P < 0.05$) (Table 9.2).

However, other genotypes remained intermediate in their salt tolerance with respect to seedling shoot and root length. This would suggest the possibility of exploiting genotypic variation in chickpea for tolerance to specific concentrations of salts. The present observations are in line with earlier reports in bean (Kaymakanova, 2009), ground nut (Mensah *et al.*, 2006) and chickpea (Al-Mutawa, 2003), where increased salinity also led to decreased radicle lengths.

Salinity also affects uptake by plants of nutrients such as phosphorus, nitrogen and potassium (Egamberdiyeva & Hoflich 2004). According to Van Hoorn *et al.* (2001) salinity strongly reduced soil nitrogen content, probably through inhibiting nitrogen fixation and soil biological activity responsible for transformation of organic nitrogen. Salehi *et al.* (2008) reported that salt stress reduced plant dry weight, nitrogen content and the number of active nodules in alfalfa cultivars.

9.2.2 Rhizobia-legume symbiosis and stress

It has been reported that the colonization and infection of root hairs by rhizobial cells is sensitive to environmental stresses (Zahran, 1999; Räsänen *et al.*, 2003). Several environmental stresses, including salinity, drought, extreme temperature and nutrient deficiency, are known to decrease survival and proliferation of rhizobia in the rhizosphere, and inhibit the infection process leading to symbiotic association with their plant host (Biswas *et al.*, 2008; Ali *et al.*, 2009). Salt inhibits the absorption of Ca, which reduces the growth of roots, root tips and root hairs, thereby decreasing sites for potential rhizobial infection and further nodule development (Bouhmouch *et al.*, 2005). The number of rhizobial cells was found to be reduced in the root of soybean, common bean and chickpea grown under salt stress conditions (Zahran & Sprent, 1986; Bouhmouch *et al.*, 2005).

We have also observed that increased salt content decreased the ability of *Rhizobium galegae* sv. *officinale* cells to colonize goat's rue roots (Egamberdiyeva *et al.*, 2013b). Similar observations were made about the decrease of root colonization of liquorice (*Glycyrrhiza uralensis*) by *Mesorhizobium* sp. under salt stress, where

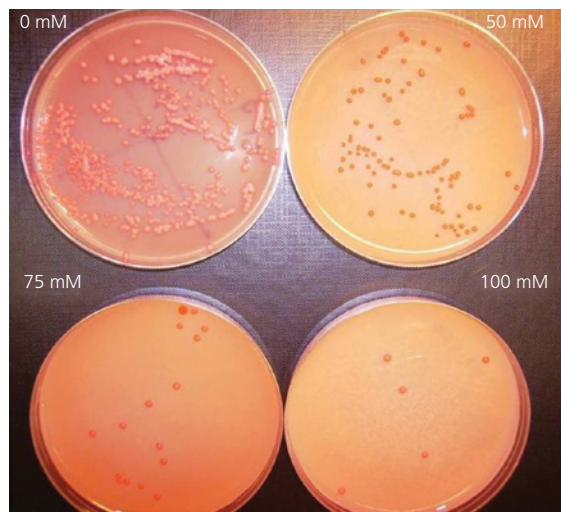


Figure 9.1 The effect of NaCl concentration on the colonization of *Mesorhizobium* sp. in the rhizosphere of *Glycyrrhiza uralensis*.

a salt concentration of 100 mM NaCl totally inhibited bacterial survival (Figure 9.1).

However, the species vary in tolerance to environmental stresses (Sridhar *et al.*, 2005; Wei *et al.*, 2008; Biswas *et al.*, 2008). The rhizobial strains from various grain legumes may tolerate 100–300 mM concentrations of NaCl (Predeepa & Ravindran, 2010). Leguminous plants growing in saline environments require both the rhizobia and the host to be tolerant to salt. The salt tolerance of rhizobia is important for improved symbiotic performance of legumes under stress conditions, where they may enhance the nodulation and nitrogen fixation ability of plants (Shamseldin & Werner, 2005; Ali *et al.*, 2009). Salt-tolerant strains of rhizobia improved the salt tolerance of host plants (Zou *et al.*, 1995; Hashem *et al.*, 1998; Shamseldin & Werner, 2005). Zahran (1999) reported that rhizobia use distinct mechanisms for osmotic adaptation to salt stress. Rüberg *et al.* (2003) reported the accumulation of low-molecular-weight organic solutes (osmolytes) by rhizobia to equilibrate internal and external osmotic concentrations under salt stress.

9.3 Improving legume yield by inoculation with rhizobacteria

The utilization of root-associated bacteria that interact with plants to mitigate the effects of various stresses opens a novel, inexpensive and advanced technology for

combating the problems of salinity (Egamberdiyeva & Hoflich, 2003; Berg *et al.*, 2010; Pliego *et al.*, 2011; Lugtenberg *et al.*, 2013). In our previous work, we reported increased dry matter yield, nodulation, seed yield and protein content of soybean seeds grown under saline soil after inoculation with salt-tolerant strains of *Bradyrhizobium* spp. (Egamberdiyeva *et al.*, 2004). Similar results were observed for chickpea, where shoot, root length and dry matter increased after inoculation of PGPR strain under stressful soil conditions (Mishra *et al.*, 2010).

The tripartite bacterial-mycorrhizal-legume symbiosis also showed improvement in legume growth and development under stressful conditions. For example, Estévez *et al.* (2009) observed that co-inoculation of *Rhizobium tropici* CIAT899 with *Chryseobacterium balustinum* Aur9 improved growth and symbiotic performance of salt-stressed soybeans compared with the single inoculation (CIAT899). Molla *et al.* (2001) also showed that total root length, root number, dry matter, root hair development, number of nodules and shoot dry matter of soybean were significantly increased by *Azospirillum lipoferum* and *Bradyrhizobium japonicum*. Dardanelli *et al.* (2008) also observed improved rhizobia-legume symbioses under saline conditions by co-inoculation of common bean with *Rhizobium* and *Azospirillum* strains.

Dual inoculation of *Rhizobium* with *Azotobacter* showed increased nodulation and plant growth of faba bean under drought conditions (Dashadi *et al.*, 2011). Han and Lee (2005) also observed alleviation of induced stress in plants and improvement in root and shoot growth of soybean by dual inoculation with *Bacillus subtilis* and *Bradyrhizobium japonicum*.

There are also several reports on the improvement of nodulation and plant growth of legumes by phosphate-solubilizing bacteria. For example, Rosas *et al.* (2006) reported on the positive effect of phosphate-solubilizing bacteria *Bradyrhizobium japonicum* and *Pseudomonas putida* on the root and shoot growth of soybean. Similar results were observed by Kumar and Chandra (2008), who showed that phosphate-solubilizing bacteria improved the symbiotic performance of introduced rhizobia in field-grown lentils. In another study, *Pseudomonas* spp. inoculated together with rhizobia significantly increased the number of pods per plant, number of seeds per pod per plant and seed yield per hectare of soybean (Argaw, 2012). Phosphate-solubilizing bacteria (PSB) are known

to help plants to acquire more phosphorus from soil, thus stimulating P uptake by plants and also improving nodulation and nitrogen fixation (Elkoca *et al.*, 2008). El-Azouni (2008) observed significant increases of dry matter, N and P uptake and yield of soybean grown in Egyptian soil inoculated with the phosphate-solubilizing fungi *Aspergillus niger* and *Penicillium italicum*. In another study, the sulphur-oxidizing bacteria *Thiobacillus* sp. stimulated nodule number, plant biomass and yield of groundnut, and increased soil available S (Anandham *et al.*, 2007).

Rabie and Almadini (2005) observed that the dual inoculation of faba bean (*Vicia faba*) with nitrogen-fixing bacteria (NFB) *Azospirillum brasilense* and the arbuscular mycorrhizal (AM) fungus *Glomus clarum* increased salt tolerance of plants and improved growth and development under saline conditions. In their study significant effects of inoculation were reported in the plants for salinity tolerance, mycorrhizal dependency, phosphorus level, phosphatase enzymes, nodule number, nitrogen uptake, protein content and nitrogenase enzymes.

A greenhouse experiment demonstrated that the salt tolerance of goat's rue was clearly improved when the plant was inoculated with its own specific symbiont *Rhizobium galegae* sv. *officinalis* and *Pseudomonas extremorientalis* TSAU20 (Egamberdiyeva *et al.*, 2013). Dual inoculation with *Rhizobium* and plant growth-promoting rhizobacteria (PGPR) *Pseudomonas* could also enhance formation of nodules on soybean grown in salinized potting soil. In recent studies, Aamir *et al.* (2013) demonstrated that combined inoculation of mung bean enhanced nodulation, grain yield (up to 30%) and protein content (up to 48%) compared to an individually inoculated control.

The survival of introduced PGPR strains in the roots of legumes is important, as bacteria may deliver biologically active compounds directly to the rhizosphere of plants. We have investigated the effect of salinity on the colonization of two selected plant-growth-promoting bacteria *P. extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 in the rhizosphere of common bean, using a gnotobiotic sand system, and determined their survival on the plant roots in pot experiments under salinated soil conditions (Egamberdiyeva, 2011). Results showed that bacterial strains were able to colonize the rhizosphere of bean at the highest salinity (12.5 dS/m) and were also able to survive in the root of bean grown in saline soil.

9.4 Biomechanisms regulating growth and development

Although the mechanisms playing in the PGPR stimulation of plant growth are not yet well understood, it is likely that PGPR can promote plant growth through several different mechanisms, which might function in tandem or separately, and depend on the species of plant and bacteria, the environment, and the nature and degree of stress (Ashraf *et al.*, 2004; Adesemoye & Egamberdieva, 2013; Berg *et al.*, 2013). Mechanisms by which bacteria are able to promote plant growth and prevent physiological plant disorders caused by salinity include production of phytohormones like indole acetic acid (IAA), gibberellic acid and cytokinins (Mishra *et al.*, 2010), solubilization of phosphates and micronutrients (Medeot *et al.*, 2010), production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of stress ethylene in the roots (Dey *et al.*, 2004) and symbiotic nitrogen fixation (Ardakani *et al.*, 2009). It has also been reported that PGPR strains can produce exopolysaccharides (EPSs), which may bind Na and decrease the content of Na available for plant uptake. Dardanelli *et al.* (2008) suggested that inoculation with *Azospirillum* spp. combined with rhizobia in common bean induced the synthesis of flavonoids by roots.

9.4.1 Phytohormone production

Phytohormones play an important role in plant physiology, and regulate many aspects of plant development, including the differentiation of vascular tissues, elongation growth, apical dominance, lateral root initiation, and stress responses and adaptation (Sharma *et al.*, 2005; Egamberdieva, 2009; Javid *et al.*, 2011). The exogenous application of auxins to alfalfa (Gruodien & Zvironaite, 1971), groundnut (Srinivasan & Gopal, 1977) and mung bean (Hayat *et al.*, 2008) promoted plant growth and nodulation. Abiotic stresses inhibit phytohormone synthesis in plants, resulting in decreased plant growth and development. According to Figueiredo *et al.* (2008) drought stress causes a change in the balance of plant hormones, like cytokinin, zeatin, IAA and gibberellins in common bean. Phytohormones produced by root-associated bacteria will be taken up by plant cells, and can stimulate plant cell proliferation; this mechanism might be responsible for the enlarged root system and increased number of infection sites prior to nodulation, especially under stressed conditions

(Tanimoto, 2005; Tilak *et al.*, 2006; Jabborova *et al.*, 2013a). Root-associated bacteria synthesize and release phytohormones such as auxins and gibberellins as secondary metabolites because of the rich supplies of substrates exuded from the roots (Egamberdiyeva & Hoflich, 2002; Tsavkelova *et al.*, 2007; Shahab *et al.*, 2009). Nutman (1977) reported that the IAA-producing abilities of *Rhizobium* strains have a positive influence on plant growth and also play an important role in the formation and development of root nodules. In another study Sridevi and Mallaiah (2007) reported IAA production by 26 *Rhizobium* strains isolated from the root nodules of the green manure crop, *Sesbania sesban*.

The comparative effects of auxin (IAA) and IAA-producing bacteria on the amelioration of salt stress on seedling growth of soybean under saline conditions were studied by Jabborova *et al.* (2013b). They observed that seedling growth of soybean was inhibited at 100 mM NaCl concentration and salt stress reduced the length of root by up to 56%. The plant growth regulator IAA did reverse the growth-inhibiting effect of salt stress to a certain extent in both shoot and root, whereas the IAA-producing bacterial strain *Pseudomonas putida* TSAU1 significantly increased seedling root growth by up to 29% in non-salinated conditions and by up to 86% at 100 mM NaCl compared to control plants. This study suggests that the application of low concentrations of auxin (IAA) or IAA-producing bacteria may improve the growth of soybean seedlings, which could enhance the tolerance of plants to soil salinity.

9.4.2 ACC (1-aminocyclopropane-1-carboxylate) deaminase

Ethylene is one of the endogenous hormones that play important roles in plant growth and development. Its production by plants is also considered as one of the stress responses, and is closely associated with various stress factors such as salinity, drought, metal toxicity and nutrient deficiency (Lynch & Brown, 1997; Schmidt, 2001; Li *et al.*, 2009). It has been reported that in leguminous plants, extra ethylene production inhibits nodulation by rhizobia (Hirsch & Fang, 1994). The level of ethylene in stressed plants can be reduced by ACC deaminase enzyme, which can cleave the ethylene precursor ACC to α -ketobutyrate and ammonium (Hontzeas *et al.*, 2005; Glick *et al.*, 2007). It has been reported that many root-colonizing PGPR have the ability to produce ACC deaminase and may enhance the survival of

seedlings under stressed conditions (Glick *et al.*, 1998). We have also observed in our previous work that *Pseudomonas trivialis* 3Re27 was able to utilize ACC as an N source, indicating the presence of ACC deaminase, and thereby increase the salt tolerance of goat's rue under salinated soil conditions (Egamberdieva *et al.*, 2013b). Similar results were observed by Shaharoon *et al.* (2006), who reported that co-inoculation of *Bradyrhizobium* with PGPR isolates possessing ACC deaminase activity enhanced nodulation in mung bean compared with inoculation with *Bradyrhizobium* alone. In another study Ma *et al.* (2003) reported that ACC deaminase-producing *R. leguminosarum* could lower ethylene production in pea roots and improve nodulation. Similar results were observed for lentil (Shahzad *et al.*, 2010; Zahir *et al.*, 2011) and chickpea (Aslam *et al.*, 2010). Arshad *et al.* (2008) studied the effect of ACC deaminase-producing PGPR strains on plant growth of pea under water stress conditions, and found that plant inoculation with PGPR decreased the effects of water stress and improved plant growth and yield. PGPR strains that produce ACC deaminase showed positive effects on plant growth of chickpea (Roopa *et al.*, 2012) and lentil (Zafar-ul-Hye *et al.*, 2013), resulting in increased number of nodules, root and shoot growth, and yield of plant under stressful conditions.

9.4.3 Cell wall degrading enzymes

The complete erosion of the plant cell wall through which the bacterial symbiont penetrates to establish an intracellular endosymbiotic relationship with the host is a key event of the infection process (Robledo *et al.*, 2008). Cellulase enzyme may help rhizobia penetrate more easily into intercellular spaces of root cells, which may result in development of more nodules. Sindhu and Dadarwal (2001) explained the improvement of nodulation and symbiotic performance of rhizobia in legumes through production of hydrolytic enzymes such as cellulases by root-colonizing *Pseudomonas* strains. In their study, co-inoculations of five cellulase-producing *Pseudomonas* strains with *Mesorhizobium* increased the number of nodules and especially nodule biomass in chickpea. Robledo *et al.* (2008) showed that both the cell-bound cellulase enzyme from *R. leguminosarum* bv. *trifolii* and the purified enzyme could erode cell walls at the tip of the root hair of the host, white clover, making a localized hole of sufficient size to allow rhizobial cell penetration. Co-inoculation of the cellulase-producing

strain *P. trivialis* 3Re27 with *R. galegae* HAMBI 540 significantly increased nodulation and nitrogen content of fodder galega, whereas cellulase-negative *P. extremorientalis* TSAU20 showed no significant stimulation (Egamberdieva *et al.*, 2010).

9.5 Conclusions and future prospects

The negative effect of abiotic factors such as salinity on plant growth, development and yield has been described by many studies. Agricultural biotechnology, particularly the use of rhizobia or consortia of plant-beneficial microbes, can be an effective approach for enhancing a plant's tolerance to adverse environmental stresses, increasing legume productivity and the supply of biologically fixed N at low cost under stressed conditions. Several mechanisms of action are used by PGPR to alleviate salt stress, improve symbiotic performance of legumes and stimulate plant growth; however, more detailed studies are needed on the induction of salt stress tolerance at the plant tissue, cell and molecular levels. Recent studies have demonstrated that tripartite bacterial-legume symbioses represent the best microbial strategy for arid and saline regions, and are of great interest as the subject of future research. Revelations about the mechanisms of PGPR action on improving legume symbioses open new doors for improving the efficacy of microbial strategies under harsh environments.

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