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Coordination between *Bradyrhizobium* and *Pseudomonas alleviates* salt stress in soybean through altering root system architecture

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ABSTRACT

It is a well accepted strategy to improve plant salt tolerance through inoculation with beneficial microorganisms. However, its underlying mechanisms still remain unclear. In the present study, hydroponic experiments were conducted to evaluate the effects of *Bradyrhizobium japonicum* USDA 110 with salt-tolerant *Pseudomonas putida* TSAU1 on growth, protein content, nitrogen, and phosphorus uptake as well as root system architecture of soybean (*Glycine max* L.) under salt stress. The results indicated that the combined inoculation with USDA 110 and TSAU1 significantly improved plant growth, nitrogen and phosphorus contents, and contents of soluble leaf proteins under salt stress compared to the inoculation with the symbiont alone or compared to uninoculated ones. The root architectural traits, like root length, surface area, project area, and root volume; as well as nodulation traits were also significantly increased by co-inoculation with USDA 110 and TSAU1. The plant-growth promoting rhizobacteria (PGPR) *P. putida* strain TSAU1 could improve the symbiotic interaction between the salt-stressed soybean and *B. japonicum* USDA 110. In conclusion, inoculation with *B. japonicum* and salt-tolerant *P. putida* synergistically improved soybean salt tolerance through altering root system architecture facilitating nitrogen and phosphorus acquisition, and nodule formation.

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Rhizobacteria; soybean; salt stress; nutrient uptake; soluble leaf protein

Introduction

Soil salinity is considered as one of the most serious environmental problems in arid and semi-arid regions that cause economic losses in agriculture (Rozema and Flowers 2008). Plant growth can be considerably limited, caused by different mechanisms such as osmotic effects, specific ion-toxicity, and/or nutritional disorders (Mantri et al. 2012; Hashem et al. 2015). Higher salt concentrations inhibit the growth of main and lateral roots by suppressing cell division and elongation (Zolla et al. 2010). It causes a limitation of soil area for the root system to gain access to larger pools of water and nutrients. It has been reported that legumes, especially their symbiotic performance are inhibited by abiotic stress such as drought and salinity (Hashem et al. 2016). The very early symbiotic events, the colonization and infection of root hairs by rhizobial strains, are reported as sensitive to abiotic stresses (Räsänen et al. 2004; Egamberdieva et al. 2013, 2015; Karmakar et al. 2015). Subsequently, the number of rhizobial cells colonizing legume roots, nodulation, and the rate of nitrogen fixation are reduced, which finally result in poor plant growth in salt-affected soils (Latrach et al. 2014). Inhibition of nodule formation in leguminous plants under salt stress has been reported which was due to extra ethylene production by plants (Gresshoff et al. 2009), and changes in auxin levels in roots (Liu et al. 2015).

Plants are colonized by microbes in the phyllosphere, or rhizosphere that closely cooperate with each other through synthesizing biologically active compounds and mediate important physiological processes (Kim et al. 2011; Berg

et al. 2015; Hashem et al. 2016). Such synergies among root associated microbes are known to induce beneficial effects on plants, via modulation of plant metabolites and plant defense systems against various stresses, including drought and salinity (Ali et al. 2014; Ahmad et al. 2015; Cho et al. 2015). It has been well reported that plant growth could be promoted through rhizobacteria colonization under various conditions by modifying the root system, enhancing mobilization, and uptake of several essential elements such as N, P, and K (Parray et al. 2016). A dual inoculation of various leguminous plants with rhizobia and root colonizing beneficial bacteria improved plant growth, nodulation in licorice (Egamberdieva et al. 2015, 2016), alfalfa (Martinez et al. 2015), soybean (Han & Lee 2005), goat's rue (Egamberdieva et al. 2013), and mung bean (Ahmad et al. 2013). There are several reports on the mechanisms of action, by which PGPR can modulate plant physiological properties. Considerable research has been done so far about the mechanisms used by PGPR to stimulate plant growth and for alleviation of salt stress including the production of phytohormones, aminocyclopropane-carboxylate (ACC) deaminase, exopolysaccharides, and osmolytes (Spaepen et al. 2007; Kim et al. 2014). Through producing the phytohormone auxin, root colonizing bacteria stimulate the number of root hairs, and length (Egamberdieva & Kucharova 2009). Consequently, increased root surface area by PGPR, helps to explore soil and absorb more mineral nutrients to sustain plant growth. For example, *Phyllobacterium brassicacearum* STM196 isolated from the roots of field-grown oil seed rape enhanced

shoot and root growth of *Aradiopsis thaliana*, through altering its root architecture and hormonal signaling pathway (Galland et al. 2012). Despite these benefits, studies concerning the elucidation of interactions of root colonizing bacteria and rhizobia in hostile conditions are scarce. This knowledge is essential to understand the interactions among microbial partners in the rhizosphere and their potential effect on plant stress tolerance under hostile environmental conditions.

Soybean (*Glycine max*) is an important grain legume in the world, source of food for man and livestock (Dwevedi & Kayastha 2011), which is also sensitive to salt stress. The salt stress inhibits the shoot and root biomass, nodule formation, number of pods, and yield of soybean (Hamayun et al. 2010). In this study we investigated how a root-colonizing *Pseudomonas* strain and rhizobia mediate the response of soybean to salt stress. The specific objectives were to evaluate (i) whether the co-inoculation of *Bradyrhizobium* symbionts with root colonizing *Pseudomonas* could restore growth and N, P acquisition of soybean under salt stress, and (ii) how synergistic interactions of microbes mediate root system architecture and nodulation in soybean exposed to salt stress.

Materials and methods

Bacterial strains and culture conditions

The root colonizing, salt-tolerant strain *Pseudomonas putida* TSAU1 was obtained from the Faculty of Biology, National University of Uzbekistan. The strain was isolated by an enrichment procedure, which selects for enhanced root colonizers able to produce indole-3-acetic acid (Egamberdieva & Kucharova 2009). *Bradyrhizobium japonicum* strain USDA110 was received from the culture collection of the Root Biology Centre, South China Agricultural University, Guangzhou, China. *P. putida* TSAU1 was grown on King's B agar (KB) (King et al. 1954) and *B. japonicum* USDA110 on tryptone yeast extract agar (TY) (Beringer 1974) at 28°C.

Determination of salt tolerance and PGP activities of bacteria

Salt tolerance of *B. japonicum* USDA 110 was tested in yeast extract mannitol (YEM) broth medium containing 200, 400, and 600 mM NaCl. The growth of bacteria was determined by spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) at 620 nm after 24, 48, and 72 h of incubation at 28°C. The production of indole-3-acetic acid (IAA) was determined according to the method of Bano and Musarrat (2003) with small modification. The strain *B. japonicum* USDA110 was grown in TY broth containing 250 mM NaCl either with or without tryptophan (100 µg/ml) and incubated at 28°C. The phosphate-solubilizing activities of *B. japonicum* USDA110 and *P. putida* TSAU1 were determined according to the method of Sperber (1957). The strains were plated onto agar plates containing 0.5% tricalcium phosphate [Ca₃(PO₄)₂] supplemented with 20 and 350 mM NaCl and incubated at 28°C for five days. The presence of a clear halo around the bacterial colonies indicates the strain is able to solubilize mineral phosphate.

In order to measure ACC deaminase activity of bacterial strains, rhizobial cells were grown in TY medium and *Pseudomonas* in KB broth, and after 24 h the cells were sedimented and washed with phosphate buffer (PBS; 20 mM sodium

phosphate, 150 mM NaCl, pH 7.4). The bacterial cells were grown in 5 mL minimal medium (BM) (Lugtenberg et al. 1999); (i) supplemented with 3.0 mM of either 1-ACC as the sole N-source (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) (to test ACC utilization) or of (NH₄)₂SO₄ (positive control) as the sole N source, and (ii) without added N-source (negative control).

Plant test under hydroponic conditions

The soybean seed cultivar YC03-3 was used for plant growth experiments. The seeds were obtained from the South China Agricultural University, Guangzhou. The seeds were surface sterilized in 10% v/v NaOCl and germinated on paper tissue towels soaked in 0.5 mM CaSO₄ for 7 days in a dark room at 25°C (Liao et al. 2001).

The strain *B. japonicum* USDA110 was grown in TY broth for 48 h and *P. putida* TSAU1 in KB broth for 24 h. The cells were washed in phosphate buffer and supernatant was adjusted to 10⁸ CFU ml⁻¹. For dual inoculation the cell suspensions of two strains were mixed in a ratio 1:1 and vortexed. Sterile seedling were placed into bacterial suspension for 30 min and were then transplanted into hydroponic plastic pots (one plant per pot, five replicates) containing two liters of low-nitrogen containing Hoagland plant nutrient solution (Lynch et al. 1990). Two saline conditions of 50 and 75 mM NaCl used for plant growth and nutrient solutions were changed every three days. The treatments were as follows: (i) uninoculated seedlings, (ii) seedlings inoculated with *B. japonicum* USDA110 alone, and (iii) seedlings co-inoculated together with *B. japonicum* USDA110 and *P. putida* TSAU1. The pots were placed in greenhouse with an average temperature of 29/20°C (day/night), and a relative humidity of 48/83% v/v (day/night). After 40 days soybean plants were harvested, shoots separated from roots, and dried at 75°C for 48 h.

Determination of nitrogen and phosphorus content

The nitrogen and phosphorus contents of root and shoot were determined from dried powdered biomass. The total nitrogen content was determined by Kjeldahl method using a nitrogen analyzer (Kjeldahl 2300; FOSS, Hoganas, Sweden). The phosphorus content was determined using a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) as described by Murphy and Riley (1962).

Determination of soluble leaf proteins

Fresh leaves of each plant (*N* = 5) were frozen in liquid nitrogen, grounded using a cold mortar, and macerated in 1.0 ml of 100 mM Tris buffer (pH 8.0). The extract was then centrifuged at 27,000 g for 10 min at 4°C. The soluble leaf protein content was determined by the method of Bradford (1976).

Root morphological and architectural traits

The plants were carefully removed from hydroponic plastic pots and roots were separated. The fresh root was washed with de-ionized water, and then the root morphology was analyzed by using a scanner system (Expression 4990, Epson, CA) with a blue board as background. Digital images were analyzed using Win RHIZO software (Régent

Instruments, Québec, Canada), and root morphological and architectural parameters, such as total root length, average root diameter, root volume, root surface area, and project area were quantified (Zhao et al. 2004). The number of nodules per plant root was determined using a stereomicroscope.

Statistical methods

Comparisons between treatments were carried out by using univariate and multivariate ANOVA (SPSS 15.0 for Windows) for the data sets, which were usually normally distributed and/or the error variance of dependent variable was equal across groups. Tukey's test was applied after ANOVA to compare means at $P < .05$.

Results

Salt tolerance and PGP activities of the two inoculant strains

The symbiotic strain *B. japonicum* USDA110 was able to grow up to 350 mM NaCl in YEM broth. The IAA synthesis of the strain was 0.8 µg/ml in medium without NaCl addition, and 0.4 µg/ml in 100 mM NaCl but did not produce IAA in nutrient broth containing 250 mM NaCl (Table 1). The presence of a nitrogen source, tryptophan, did not either induce IAA production. *B. japonicum* USDA110 was also not capable of solubilizing mineral phosphorus from tricalcium phosphate used in Sperber's solid medium. Instead, *P. putida* TSAU1 was able to solubilize mineral phosphate on the above-mentioned medium containing up to 200 mM NaCl but not in the presence with 350 mM NaCl. It was previously shown that *P. putida* TSAU1 tolerated up to 800 mM NaCl, and produced auxin hormone IAA in the KB medium containing up to 250 mM NaCl (Egamberdieva & Kucharova 2009).

Effect of bacterial inoculation on soybean growth

The growth of soybeans under hydroponic conditions was strongly impaired by salinity when soybeans were not inoculated at all. Compared with non-stressed plants, the weight of shoots and roots were significantly reduced, on average by 55% in 50 mM NaCl and by 75% in 75 mM NaCl (Figure 1(A,B)). The combination of the symbiotic *B. japonicum* USDA 110 with *P. putida* TSAU1 enhanced root and shoot weight of non-stressed soybeans, the difference between uninoculated and co-inoculated plants being significant (Figure 1(A,B)). Salt-stressed soybeans inoculated with the symbiotic *B. japonicum* USDA 110 alone grew slightly better than uninoculated stressed plants. The shoot weight increased by 20% in 75 mM NaCl (Figure 1(A)), but no effect was observed in 50 mM NaCl. Root weight was increased by 10% in both 50 mM and 75 mM NaCl (Figure 1(A)). The combination of *B. japonicum* USDA 110 and *P. putida* TSAU1 improved the growth of soybeans in 50 mM NaCl more than the inoculation with the symbiont alone. Co-inoculated soybeans had significantly greater (35%) shoot weights, and root weights were stimulated by 20%.

Salt stress significantly inhibited the formation of nodules of soybeans inoculated with *B. japonicum* USDA110 alone. In presence of 50 mM and 75 mM NaCl, plants formed only 45% and 10% of the number of nodules induced under

non-stressed conditions, respectively (Figure 1(D)). The PGPR *P. putida* strain TSAU1 improved the symbiotic interaction between salt-stresses soybean and symbiotic *B. japonicum* USDA110. Co-inoculated soybeans produced two times more nodules in presence of 50 mM NaCl and 75% more nodules in 75 mM NaCl compared to plants inoculated with the symbiont *B. japonicum* USDA 110 alone under similar conditions (Figure 1(D)). Under non-saline conditions, co-inoculated soybeans also produced significantly more (50%) nodules than single-inoculated ones (Figure 1(D)).

Concentration of soluble protein, nitrogen, and phosphorus

Both inoculation treatments applied to non-stressed soybeans increased the content of total nitrogen and phosphorus in plant tissues as well as that of soluble leaf proteins. An increased NaCl level gradually decreased the content of soluble leaf proteins, being 25% lower in 75 mM NaCl than under non-stressed conditions (Figure 1(C)). Similarly, the content of soluble leaf proteins responded positively to both inoculation treatments regardless of whether soybeans were affected by salinity or not (Figure 1(C)). The combination of two bacteria produced even better results since co-inoculated, salt-stressed soybean tissues contained significantly more leaf proteins (on average 75%) than uninoculated salt-stressed ones (Figure 1(C)).

The differences in nutrient contents between uninoculated soybeans and those inoculated together with *B. japonicum* USDA110 and *P. putida* TSAU1 were significant, the nitrogen content being improved by 35%, that of phosphorus by 40% and leaf proteins by 35%, (Figure 2(A,B)). In uninoculated plants, the contents of total nitrogen and phosphorus were similar for plant tissues in 50 mM NaCl as well as for those of non-stressed ones. Nutrient contents were, however, decreased in 75 mM NaCl, nitrogen content by 25% and phosphorus content by 15% (Figure 2(A,B)). Both inoculation treatments, either *B. japonicum* USDA 110 alone or combined with *P. putida* TSAU1, enhanced nutrient contents in salt-stressed soybean tissues (Figure 2(A,B)). Interestingly, in comparison with non-stressed plants the content of total nitrogen was increased with increasing salinity levels among inoculated soybeans. In 50 mM NaCl, both single and co-inoculation improved nitrogen contents on average by 15%. Furthermore, single and co-inoculated plant tissues affected by 75 mM NaCl contained on average 30% more nitrogen than similarly inoculated plants grown in 50 mM NaCl, the differences between the latter treatments were significant (Figure 2(A)). After inoculation with *B. japonicum* USDA110 alone, the phosphorus content was higher in salt-stressed soybeans than in uninoculated ones. The highest phosphorus content was detected from co-inoculated soybean tissues grown in 75 mM NaCl. These plants contained significantly more phosphorus (63%) than uninoculated plant tissues in 75 mM NaCl (Figure 2(B)). The inoculation with *B. japonicum* USDA110 alone increased phosphorus content by 35% and 65% in 50 mM and 75 mM NaCl, respectively.

Root morphological and architectural parameters

The salt stress affected root morphological and architectural parameters of soybean grown in hydroponic conditions.

Table 1. Root morphological traits of soybeans under salt stress as affected by inoculation with *B. japonicum* USDA110 and in combination with *P. putida* TSAU1.

Salinity	Treatment	Total root length, cm	Root surface area, cm ²	Projected area, cm ²	Root diameter, mm	Root volume, cm ³
0 mM NaCl	Control	1425.3 ± 86c	541.1 ± 60b	140.6 ± 28.6b	0.727 ± 0.12a	9.5 ± 1.4b
	USDA110	1850.7 ± 104b	579.6 ± 81ab	180.9 ± 29.5a	0.731 ± 0.05a	12.7 ± 1.6a
	USDA + TSAU1	2180.5 ± 87a	637.6 ± 54a	204.3 ± 26.3a	0.808 ± 0.14a	13.8 ± 2.9a
50 mM NaCl	Control	1136.7 ± 107b	234.1 ± 41a	98.9 ± 2.3a	0.553 ± 0.02b	3.9 ± 0.4a
	USDA110	1291.2 ± 61a	239.60 ± 46a	104.7 ± 6.8a	0.596 ± 0.01b	5.0 ± 1.5a
	USDA + TSAU1	1366.2 ± 53a	247.5 ± 37a	109.7 ± 9.6a	0.769 ± 0.05a	4.8 ± 1.6a
75 mM NaCl	Control	919.2 ± 91b	115.5 ± 49b	50.8 ± 4.4b	0.501 ± 0.03a	2.0 ± 0.3a
	USDA110	980.8 ± 30ab	144.3 ± 40a	61.0 ± 6.9a	0.506 ± 0.05a	2.1 ± 0.8a
	USDA + TSAU1	1024.9 ± 68a	149.3 ± 34a	53.7 ± 2.0b	0.528 ± 0.04a	2.6 ± 0.4a

Note: Soybeans were grown for 40 days under hydroponic conditions in 0, 50, and 75 mM NaCl. Values are the means of four replicates with standard deviation (SD), and different letters indicate significant differences between treatments at $P < .05$ (Tukey's t -test).

The root length, surface area, projected area, diameter, and root volume of control (uninoculated) plants steadily decreased by 33%, 74%, 64%, 31%, and 79% when the concentration of NaCl was increased up to 75 mM, respectively (Table 1). Roots were significantly longer (29%) in control plants (0 mM NaCl) and 16% longer in 50 mM NaCl than uninoculated roots (Table 1). The root volume of inoculated plants with USDA110 and grown in non-saline condition was increased by 33%. Other root parameters such as root surface area, projected area and root diameter were not affected by single inoculation under normal as well as under salt stress conditions. Synergistic effects of co-inoculation with rhizobia and root colonizing *P. putida* TSAU1 were detected, especially under salt stress condition. The total root length, as well as surface area, project area and root volume of co-inoculated plants grown under non stress condition were significantly longer than roots inoculated with the symbiont alone or uninoculated ones (Table 1). A dual inoculation of soybean enhanced the shoot length (48%), projected area (45%), and diameter of root (45%) compared to uninoculated stressed plants and single inoculation with *B. japonicum* USDA 110 alone (Table 1, Figure 3). The combination of *B. japonicum* USDA 110 and *P. putida* TSAU1 slightly improved the root growth of soybeans at 75 mM NaCl

condition, however the differences between uninoculated soybeans and those inoculated together with both strains were not significant (Table 1).

Discussion

In our study, the growth and nodulation of soybean grown in hydroponic conditions was negatively affected by salinity, that is, 50 mM and 75 mM NaCl. Saline conditions are known to inhibit plant growth through decreasing water availability and oxidative damage to cells (Rasool et al. 2013; Ahmad et al. 2015). The growth of root and shoot of soybean inoculated with the symbiotic *B. japonicum* USDA110 alone was significantly decreased at 50 mM NaCl condition. Co-inoculation of soybean with the symbiont *Bradyrhizobium* USDA110 and the salt-tolerant PGPR *P. putida* TSAU1 reduced unfavorable effects of NaCl under hydroponic conditions. Co-inoculated soybeans grown in 50 mM NaCl and 75 mM NaCl had higher shoot biomass than single-inoculated ones under corresponding conditions. Similarly, Estevez et al. (2009) reported that co-inoculation of *Ensifer* (*Sinorhizobium*) *fredii* SMH12 with *Chryseobacterium balustinum* Aur9 improved growth of mildly salt-stressed (25 mM NaCl) soybeans grown in sterile quartz

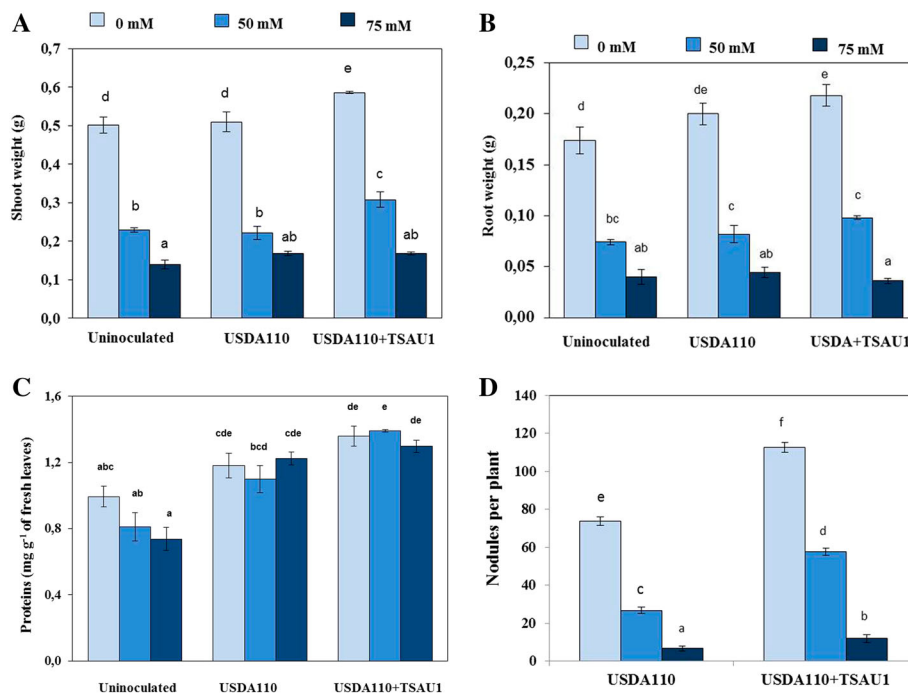


Figure 1. The dry weight of shoots (A), roots (B), leaf protein contents (C) and nodule number (D) of soybean, when plants were inoculated with the combination of *B. japonicum* USDA110 and *P. putida* TSAU1 or with *B. japonicum* USDA110 alone. Plants were grown for 40 days under hydroponic conditions at 0, 50, and 75 mM NaCl nutrient solution. Columns represent means for five plants ($N = 5$) with standard error bars. Columns with different letters indicate significant differences between treatments at $P < .05$ (Tukey's t -test).

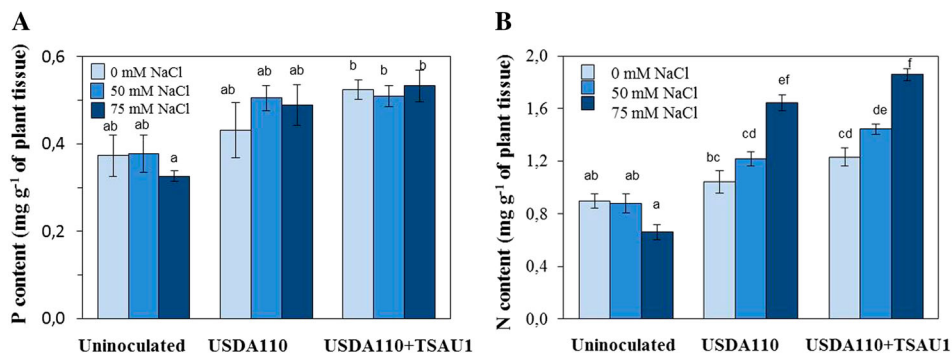


Figure 2. The phosphorus (A), and nitrogen (B) contents of soybean, when plants were inoculated with the combination of *B. japonicum* USDA110 and *P. putida* TSAU1 or with *B. japonicum* USDA 110 alone. Plants were grown for 40 days under hydroponic condition at 0, 50, and 75 mM NaCl nutrient solution. Columns represent means for five plants ($N = 5$) with standard error bars. Columns with different letters indicate significant differences between treatments at $P < .05$ (Tukey's t -test).

sand compared with the single inoculation (SMH12). *B. japonicum* USDA110 combined either with *Bacillus subtilis* or *Serratia proteamaculans*, or with both of them, also alleviated salt stress of soybeans grown in sterilized soil (Han & Lee 2005).

In our study, the nitrogen content of plant tissue was decreased only in uninoculated soybeans in 75 mM NaCl whereas it remained at the same level in 50 mM NaCl and under non-stressed conditions. Transcriptome analysis of *Phaseolus vulgaris* roots (Hiz et al. 2014) indicated that salt stress enhanced or up-regulated genes involved in metabolism of nitrogen-containing compounds. Hiz et al. (2014) gave two explanations for these events, when the growth of salt-stressed plant ceases or is retarded, (i), the consumption of nitrogen decreases and nitrogen starts to accumulate inside plant tissues, or (ii), the salt-stressed plant tries to maintain its ionic and osmotic concentration. It seems, nevertheless, that salt stress does not manifest itself in a decrease of nitrogen contents. For example, high concentrations of soluble and insoluble nitrogen were found in leaves and roots of salt-stressed mung bean (*Phaseolus aureus*) (Imamul-Huq & Larher 1983), or in shoots of faba bean (*Vicia faba*) (Cordovilla et al. 1999).

In earlier reports, Parida and Das (2005) observed a decreased content of phosphorus in plants grown under salt stress. Accordingly in our study, the phosphorus content in uninoculated plants was decreased in 75 mM NaCl. Inoculation of soybeans either with *B. japonicum* alone or combined with *P. putida* TSAU1 enhanced phosphorus contents nearly similarly in all plants, regardless of whether soybeans were grown under salt stress or not. An increase in phosphorus uptake by plants through co-inoculated *Sinorhizobium ciceri* and phosphate-solubilizing *Pseudomonas* has been reported for chickpea (Messele & Pant 2012). In our study, *P. putida* TSAU1 was able to solubilize phosphate under 1% (170 mM) NaCl condition providing more phosphorus to soybean under salt stress.

The content of soluble leaf protein has been used as an indicator of the physiological status of plants, as it reflects availability of nitrogen for growth and development (Andrews et al. 1999). A positive correlation was found between plant growth, salt tolerance, nitrogen content, and protein concentration in tissue, which indicates the protective role of soluble proteins under stress conditions (Singh et al. 1987). In our study, the soluble leaf protein content in

uninoculated soybean tissues decreased with the increase in salinity, indicating that proteins have been degraded. Interestingly, when soybeans were inoculated with bacteria, salt-stressed plant tissues contained more soluble leaf proteins than uninoculated ones under similar conditions. In earlier reports, salt stress was reported to decrease the content of soluble proteins in shoots, roots and/or nodules in salt-sensitive *P. vulgaris* (Ashraf & Bashir 2003), but the content was increased in salt-tolerant *Phaseolus acutifolius* (Yurekli et al. 2004). This fact suggested that a dual inoculation of soybean with *B. japonicum* and *P. putida* improved salt tolerance of plants, thereby maintain a higher concentration of soluble protein in plant tissues.

Roots play a key role in nutrient acquisition by plants and adaptation to abiotic stresses. The root system architecture (Duan et al. 2014) and the nodulation process (Bouhmouch et al. 2005) are particularly sensitive to salt stress. When Tu (1981) used higher NaCl concentrations for soybeans grown in pots filled with silica sand, root hairs showed a weak response to bradyrhizobial inoculation at 140 mM (0.8%) NaCl, and shrinkage of root hairs was evident at 260 mM (1.5%) NaCl. Nodulation was totally inhibited at 210 mM (1.2%) NaCl. High salt concentrations inhibited root elongation, thus reducing plant ability to explore nutrient resources in soil. A combination of *P. putida* and *B. japonicum* affected the root system, causing an increase in root length, surface area, root volume, and also nodule number of soybean under salt stress. An increased root biomass and an alteration of the root architecture as a result of root colonization by bacteria, may have enhanced the capacity of a plant to acquire and utilize more nutrients. A similar observation was reported by Gamalero et al. (2008) who showed enhanced root system and salt tolerance in cucumber by *P. putida* UW4 under 50 mM NaCl condition.

The inhibition of nodules and N fixation in leguminous plants by salinity was observed in several legumes (Dardanelli et al. 2010). Several phenomena might partly explain the reduced nodulation of stressed leguminous plants. One reason could be that salt stress causes an inhibition of colonization of host cells by rhizobium, curling of root hairs, thus inhibit the formation of infection threads (Dardanelli et al. 2010). Thus, Egamberdieva et al. (2013) found, that under salt stress (75 mM NaCl) the root length, formation of nodules and root colonization of *Galega officinalis* by *Rhizobium galegae* sv. *officinalis* was inhibited.

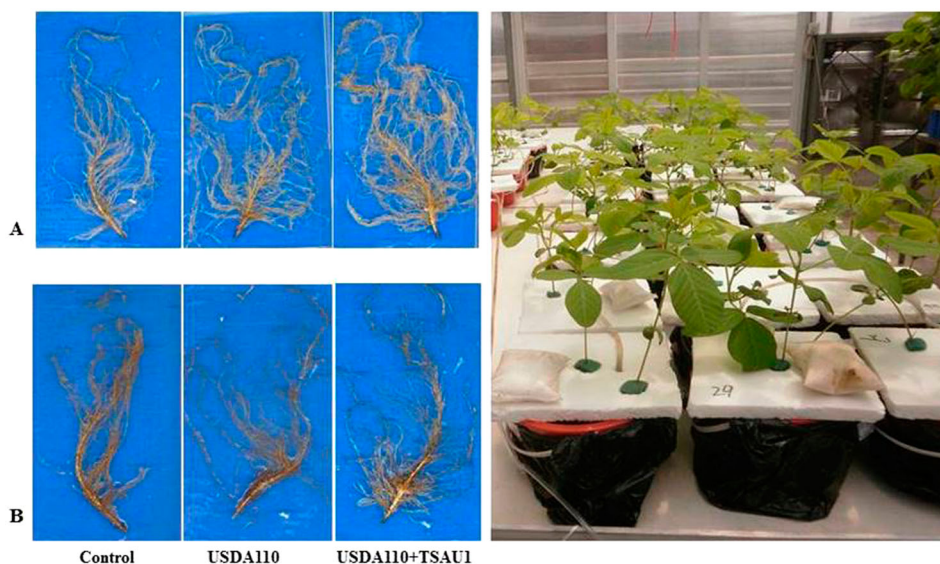


Figure 3. The root morphology of soybean, when plants were inoculated with *B. japonicum* USDA 110 alone or with the combinations of *B. japonicum* USDA 110 and *P. putida* TSAU1. Plants were grown for 40 days under hydroponic conditions in nutrient solution supplemented with 0 mM (A), and 75 mM NaCl (B).

Furthermore, an involvement of microbial phytohormones in root elongation, lateral root initiation (López-Bucio et al. 2007), and nodule formation (Sudadi 2012) was observed. Some authors reported an essential role of auxin levels in host plants in the formation of nodules (Pacios-Bras et al. 2003). The IAA producing strain *P. putida* TSAU1 stimulated the root system, and nodulation of soybean under stress conditions. A modulation of the root system architecture by root associated microbes was related to the production of plant growth-regulating substances, for example, auxin (López-Bucio et al. 2007), and ACC deaminase which reduce ethylene levels in plant tissues (Glick 2014). *P. putida* TSAU1 appears to have several plant growth promoting activities, such as production of IAA and ACC deaminase activity which might explain its ability to mitigate salt stress in soybean (Egamberdieva & Kucharova 2009). Similar observations were reported by Sudadi (2012), that an exogenous application of IAA on soybean slightly enhanced nodule number and root dry weights. We suppose that ACC deaminase suppressed synthesis of ethylene by salt-affected soybeans, while IAA intensified root growth, thereby facilitating absorption of nutrients, increasing availability of water-soluble phosphates, and the formation of nitrogen-fixing nodules.

In conclusion, our results indicate that synergistic interactions between soybean-Bradyrhizobium and root colonizing *Pseudomonas* improved salt tolerance and plant growth of soybean through modulation of the root-system architecture, thus facilitating nitrogen and phosphorus acquisition under salt stress. These results will support developing combinations of inoculants for improving plant growth and symbiotic performance of leguminous under hostile conditions such as salinity.

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Disclosure statement

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