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Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescens* on growth and pigment composition of radish plants (*Raphanus sativus*) under NaCl stress

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Abstract

Soil salinity is one of the most severe factors limiting growth and physiological response in *Raphanus sativus*. In this study, the possible role of plant growth promoting bacteria (PGPB) in alleviating soil salinity stress during plant growth under greenhouse conditions was investigated. Increasing salinity in the soil decreased plant growth, photosynthetic pigments content, phytohormones contents (indole-3-acetic acid, IAA and gibberellic acid, GA_3) and mineral uptake compared to soil without salinity. Seeds inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens* caused significantly increase in fresh and dry masses of roots and leaves, photosynthetic pigments, proline, total free amino acids and crude protein contents compared to noninoculated ones under salinity. The bacteria also increased phytohormones contents (IAA and GA_3) and the contents of N, P, K^+, Ca^{2+}, and Mg^{2+} but decreased ABA contents and Na^+ and Cl^- content which may contribute in part to activation of processes involved in the alleviation of the effect of salt.

Additional key words: mineral uptake; photosynthetic pigment; phytohormones; plant growth promoting bacteria; radish growth; salt stress.

Introduction

Soil salinity is an enormous production problem for vegetable crops. Salt stress affects all the major processes such as growth, yield, photosynthesis, protein synthesis and lipid metabolism (Parida and Das 2005). Salt induces osmotic stress by limiting absorption of water from soil and ionic stress resulting from high concentrations of potentially toxic salt ions within plant cells (Kohler *et al.* 2009). Saline stress also affects many physiological activities related to the accumulation of ions (Lee *et al.* 2008). Salinity was shown to increase the uptake of Na^+ or decrease the uptake of Ca^{2+} and K^+ in leaves which lead to nutritional imbalances (Neel *et al.* 2002).

Salt in the soil water inhibiting plant growth involves two aspects: one is the osmotic or water-deficit effect of salinity when the presence of salt in the soil solution reduces the ability of the plant to take up water and this leads to slower growth; the other is the salt-specific or ion-excess effect of salinity when excessive amounts of salt entering the transpiration stream will eventually injure cells in the transpiring leaves and this may further reduce growth (Munns *et al.* 2006). Meanwhile, photosynthesis is the most important process affecting plant growth under saline conditions (Sudhir and Murthy 2004).

There are numerous reports on photosynthetic characteristics under salt stress (Qiu *et al*. 2003, Koyro 2006, Wei *et al*. 2006). Generally, photosynthesis is inhibited by salt stress (Qiu *et al*. 2003, Koyro 2006). Salt stress also affects photosynthetic components (Qiu *et al*. 2003) and chloroplast ultrastructure (Fidalgo *et al*. 2004).

During recent years, a new biocontrol approach has been developed to protect plants from salt stress in soil by treating crop seeds and seedlings with PGPB (Yue *et al.* 2007). PGPB are a group of bacteria that actively colonize plant roots and increase plant growth and yield (Yang *et al.* 2009). PGPB can either directly or indirectly improve the extent or quality of plant growth (Glick 1995). PGPB can directly facilitate the proliferation of plants by fixing atmospheric nitrogen, producing siderophores (Fe-III chelating agent) which can solubilize and sequester iron and provide it to plants, phytohormone

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Abbreviations: ABA – abscisic acid; ACC – 1-aminocyclopropane-1-carboxylate; Car – carotenoid; CAS – chrom azurol S; Chl – chlorophyll; DM – dry mass; FM – fresh mass; GA_3 – gibberellic acid; GC – gas chromatography; HCN – hydrogen cyanide; IAA – indole-3-acetic acid; LSD – least significant difference; PGPB – plant growth promoting bacteria; PVK – Pikovskaya; RT– retention time; SD – standard deviation.

production like indole-3-acetic acid, cytokinin and gibberelin, which can enhance various stages of plant growth, solubilizing minerals such as phosphorus and synthesizing enzymes that can modulate plant growth and development (Mayak *et al.* 2004b). Indirect stimulation of plant growth includes a variety of mechanisms by which the bacteria prevent phytopathogens from inhibiting plant growth and development by any one or more of several different mechanisms including the production of antibiotics, lytic enzyme and hydrogen cyanide (Glick and Bashan 1997). In addition, PGPB can protect plants from the deleterious effects of some environmental stresses including flooding (Grichko and Glick 2001), drought (Mayak *et al.* 2004a), salt (Mayak *et al.* 2004b), and phytopathogens (Harman and Bjorkman 1998).

PGPB increase water-use efficiency, fresh and dry masses of plants (Mayak *et al.* 2004b) and render the plants more tolerant to salt stress by improving antioxidant status and physiological response (*e.g* proline used as osmoregulant) of plants (Han and Lee 2005). PGPB also produce several other growth promoting substances including IAA, GA_3 and ABA (Perrig *et al.* 2007).

Numerous PGPB of the genera *Pseudomonas, Bacillus, Arthrobacter, Azospirillum, Klebsiella*, *Enterobacter, Azotobacter, Herbaspirillum, Burkholderia, Rhizobium*, *Gluconacetobacter, Alcaligenes,* and *Serratia* have been isolated from the rhizosphere of various crops and noted for their synergistic effects on plant growth (Rabie and Almadini 2005).

The aim of the present study is to determine the effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on plant growth and some physiological properties of radish plants grown under salt stress.

Materials and methods

Preliminary study: In this study, different bacterial strains were used in the preliminary study such as *Bacillus subtilis, Bacillus licheniformis, Pseudomonas fluorescens,* and *Rhizobium sp.* We found that *B. subtilis* and *P. fluorescens* increased the growth parameters in radish plants and alleviated the adverse effect of NaCl. We also did the test on the ability of these four strains in the grown in different NaCl concentrations. We found that *Bacillus subtilis* and *Pseudomonas fluorescens* have the ability to grow in different NaCl concentrations (1–10%).

Bacterial strains *B. subtilis* and *P. fluorescens* were obtained from microbial culture collection at Faculty of Agriculture, Ain Shams University, Egypt. Each strain was maintained on its respective agar media; *Pseudomonas* on King's B medium (King *et al.* 1954) and *Bacillus* on a nutrient agar.

Characterization of strains for PGP traits

Production of indole-3-acetic acid: Indole-3-acetic acid (IAA) production was detected as described by Brick *et al.* (1991).

ACC-deaminase activity assay was quantified by monitoring the amount of α-ketobutyrate that was produced by the deamination of ACC as described by Honma and Shimomura (1978).

Production of ammonia: Bacterial strains were tested for the production of ammonia in peptone water as described by Cappuccino and Sherman (1992).

Production of HCN: Bacterial strains were screened for the production of hydrogen cyanide by adapting the method of Lorck (1948).

Phosphate-solubilization test was conducted qualitatively by streaked the bacterial culture on the surface of Pikovskaya (PVK) agar medium according to Nautiyal (1999).

Siderophore production was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chrom azurol S (CAS). Orange halos around the colonies on blue were indicative for siderophore production

Enzymatic activities: Bacterial strains were tested for the enzymatic activities such as catalase as described by Gagnon *et al.* (1959). Protease, amylase, and cellulose activities were measured by the clearing zone techniques as described by Barrow and Feltham (1993), Ammar *et al.* (1991), and Cattelan *et al.* (1999).

Greenhouse experiment: Seeds of *Raphanus sativus* cv. Longioinnatus (white radish) were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. For seed treatments, seeds were soaked in the bacterial suspension (*B. subtilis* and *P. fluorescens*) of 10^7 CFU ml^{-1} amended with sucrose (0.2%) to facilitate the adherence of the bacteria to the seeds and in distilled water (which served as the control) for 2 h at 27°C. Then, the seeds were air-dried before use. Radish seeds were sown during December 2010/2011 into pots (25 cm in diameter) containing equal amounts of homogeneous soil. The soil characteristics were as follows: sandy loam in texture, sand 80%; silt 15.5%; clay 4.5%; pH, 7.8; EC 0.4 dS m^{-1} and organic matter 0.45%. Each treatment contained three pots. The experiment was divided into three groups; the first group was irrigated with water and served as control. The second group was irrigated with 75 mM NaCl. The third group was irrigated with 150 mM

NaCl. This experiment was conducted under environmental conditions (day length 12–14 h, temperature 20–22°C and humidity 70%). Plants were grown in the dark and in the light under circadian illumination 12 h dark/12 h light (200 μmol m^{-2} s^{-1} PAR). After 45 days from sowing the plants were collected to determine fresh and dry masses of roots and leaves. Photosynthetic pigments were determined according to Vernon and Seely (1966). Proline content was determined in roots and leaves as described by Bates *et al.* (1973). Total free amino acids content was estimated according to Moore and Stein (1954). Crude protein was determined using micro Kjeldahl method. The total protein was calculated multiplying the evaluated nitrogen by 6.25 (Indrayan *et al.* 2005). The method of plant hormones extraction was essentially similar to that adopted by Shindy and Smith (1975). The frozen samples were ground in cold 80% methanol, followed by triple extraction with fresh methanol for 2 h at 0°C. To estimate the amounts of acidic hormones, the plant hormone fractions and standards were methylated according to Vogel (1975), ready for gas chromatography (GC) analysis. The retention time (RT) and the area of peaks of authentic samples were used for the identification and characterization of peaks of samples under investigation. Na^+, Ca^{2+} and Mg^{2+} were determined in the acid digested by atomic absorption spectrometry according to A.O.A.C. (2005). N, P and K^+ were determined according to A.O.A.C. (1995). For chloride determination, Cl^- was determined by the silver ion-titration method according to Bozcuk (1970).

Statistical analysis: The data were statistically analyzed using *F*-test and LSD at 5% and 1% levels of probability according to *SAS*-Programme (1982).

Results and discussion

Field salinization is a growing problem worldwide. It was estimated that the gradual increase in salt content in irrigated soils has been considered as one of the main threats against crop production (Bacilio *et al.* 2004). The use of elevated doses of fertilizers, as well as pesticides, may have negative and unpredictable effects on the environment and contribute to the contamination of soil, water and natural areas. Such impacts pose a serious threat to human and animal health. An interesting option for decreasing the use of chemical fertilizers could be the exploitation of PGPB. These bacteria may provide a natural and harmless means to improve the growth and yield of crops especially under environmental stresses (Zahir *et al.* 2004).

In this study, two strains of PGPB (*B. subtilis* and *P. fluorescens*) were examined in the greenhouse experiments for their ability to ameliorate the inhibitory effect of salt on radish plants. The results illustrated in Table 1 showed multiple plant growth promoting traits of these bacterial strains. *B. subtilis* and *P. fluorescens* were also siderophore producers (Table 1). Siderophores are low-molecular-mass compounds that are produced and utilized by bacteria as iron (Fe) chelating agents. Siderophore producing PGPB can prevent the proliferation of pathogenic microorganisms by sequestering Fe^{3+} in the area around the root (Siddiqui 2006).

B. subtilis and *P. fluorescens* produced HCN which is a volatile, secondary metabolite that suppresses the development of microorganisms.Various studies attribute a disease-protective effect to HCN production (Siddiqui *et al.* 2006). Being produced commonly by rhizosphere, HCN is a gas known to negatively affect root metabolism and root growth and is a potential and environmentally compatible mechanism for biological control of pathogen. HCN is also known to inhibit the electron transport, disrupting the energy supply to the cells, ultimately leading to death of the pathogen.

Moreover, *B. subtilis* produced catalase, protease, cellulase, and amylase enzymes. On the other hand, *P. fluorescens* produced catalase and protease but not cellulase and amylase. Production of these enzymes was reported as a mechanism by which the bacteria prevent phytopathogens from inhibiting plant growth. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical, and chemical stresses. These results are in accordance with Joseph *et al.* (2007) who reported that all the bacterial strains (*Bacillus, Pseudomonas*, *Rhizobium*, and *Azotobacter*) were able to produce catalase. *B. subtilis* and *P. fluorescens* produced ammonia (Table 1). Production of ammonia is reported as another important trait of PGPB that may indirectly influence the plant growth (Joseph *et al.* 2007).

Both fresh and dry masses of roots and leaves of radish plants significantly decreased with increasing salt concentrations. Co-inoculation with *B. subtilis* and *P. fluorescens* caused highly significant increase in the fresh and dry masses of roots and leaves in both unstressed and salt-stressed conditions (Fig. 1). These results are in accordance with Yildirim *et al.* (2008) who found that

Table 1. Biochemical characteristics of bacterial strains. + means that these compounds were produced by bacterial strains.

Bacterial characterization	*B. subtilis*	*P. fluorescens*
IAA production	+	+
ACC-deaminase activity	+	+
Ammonia production	+	+
HCN production	+	+
P-solubilization	+	+
Siderophore production	+	+
Catalase	+	+
Cellulase	+	-
Protease	+	+
Amylase	+	–

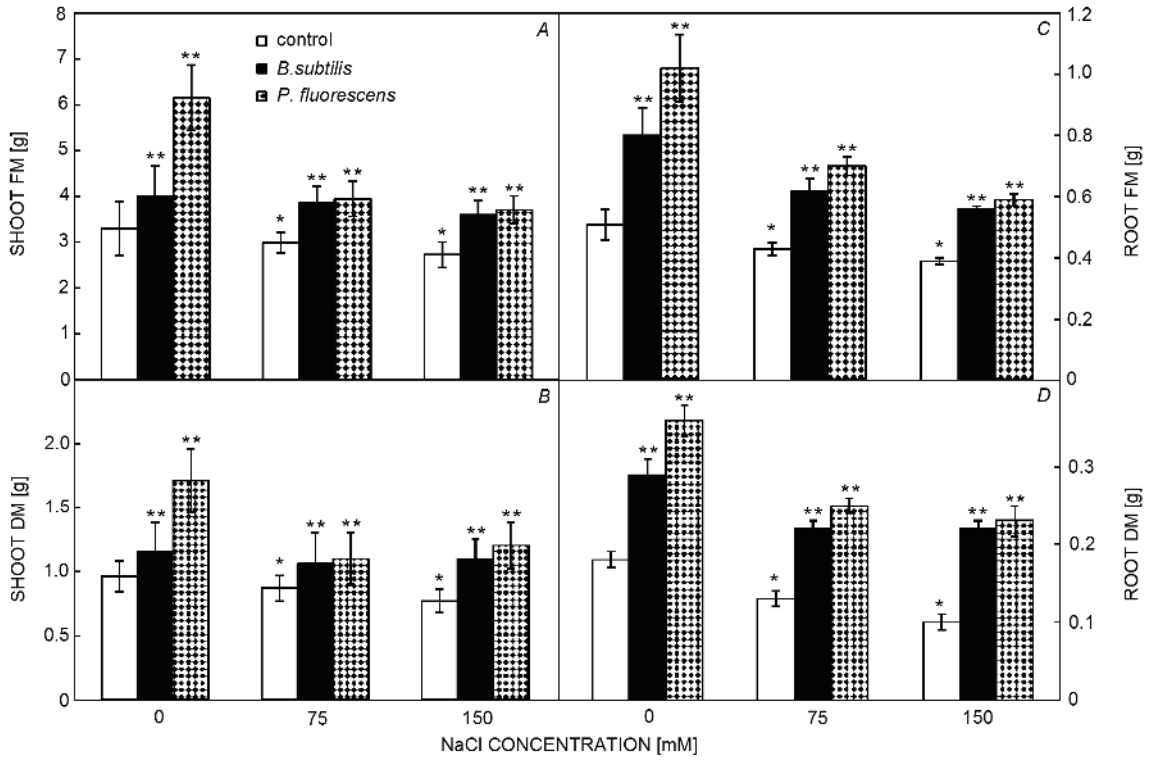

Fig. 1. Effect of *B. subtilis* and *P. fluorescens* on root fresh mass (*A*) and dry mass (*B*), shoot fresh mass (*C*) and dry mass (*D*) of radish plants at different salinity levels. Means ± SD (n = 10) of measurements on each ten plants. Means followed by * and ** are highly significant decrease and increase different at $P \leq 0.01$, respectively, according to least significant difference (LSD) test.

fresh and dry shoot and root masses of radish plants significantly decrease by salt treatment. While bacterial treatments under salinity conditions have positive effects on fresh and dry shoot and root masses. Also, Bano and Fatima (2009) showed that salt stress markedly decreased shoot and root dry masses of of *Zea mays* plants. Co-inoculation with both *Rhizobium* and *Pseudomonas* increased the dry mass of shoots and roots in both unstressed and salt-stressed conditions. In addition, Stajković *et al.* (2011) found that co-inoculation with *Rhizobium*, *Pseudomonas* sp. and *Bacillus* sp. improved shoot dry mass in bean plants compared to inoculation with *Rhizobium* alone.

The decline in growth observed in many plants subjected to salt stress is often associated with a decrease in their photosynthetic activity (Hajlaoui *et al.* 2006). The decrease in photosynthesis induced by salt stress can be associated with the partial stomatal closure and/or the nonstomatal factors (das Neves *et al.* 2008).

B. subtilis and *P. fluorescens* cause stimulation in plant growth under salt stress probably due to the production of ACC deaminase by the two strains (Table 1). This enzyme facilitates plant growth as a consequence of the fact that it sequesters and cleaves plant-produced ACC (an immediate precursor of ethylene biosynthesis in higher plants) into α-ketobutyrate and ammonia. The bacteria utilize the ammonia evolved from ACC as a source of N and thereby lowering the level of ethylene in the plant. In turn, decreased ethylene levels allow the plant to be more resistant to a wide variety of environmental stresses. These results are in the same line with Saravanakumar and Samiyappan (2007) who previously reported that *P. fluorescens* containing ACC deaminase activity enhanced the saline resistance in groundnut plants and increased yield as compared with that inoculated with *Pseudomonas* strains lacking ACC deaminase activity.

Chl *a*, Chl *b*, Chl *a*/*b* ratio and carotenoids contents in leaves of radish plant were significantly decreased with increasing NaCl concentrations, while co-inoculation with PGPB alone or in combination with NaCl significantly increased Chl *a*, Chl *b*, Chl *a/b* ratio, carotenoids and total photosynthetic pigment contents in leaves of radish plants (Fig. 2). These results are in accordance with Yildirim *et al.* (2008). *Pseudomonas* and *Bacillus* caused accumulation in Chl content. These results may be due to the effect of *Pseudomonas* and *Bacillus* in biofertilizer, or due to increase the ACC-deaminase enzymes in PGPR treated plants which slow down Chl degradation or probably due to the increase of the photosynthetic rate or the role of N nutrition in producing growth-promoting substances resulting in more efficient absorption of nutrients, which main components of photosynthetic pigments and consequently the Chl content was increased.

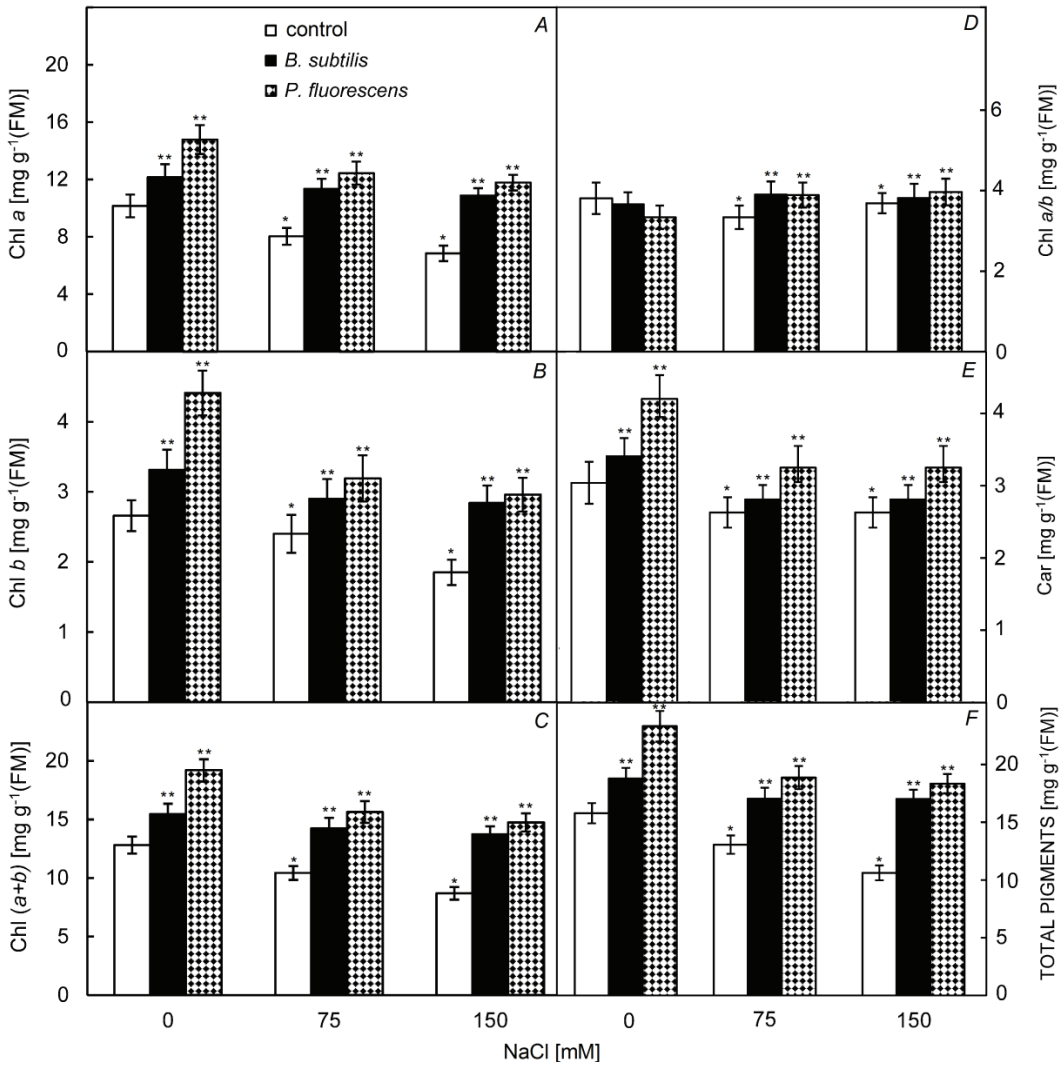

Fig. 2. Effect of *B. subtilis* and *P. fluorescens* on Chl *a* (*A*), Chl *b* (*B*), Chl (*a*+*b*) (*C*), Chl *a*/*b* (*D*), Car (*E*) and total pigments (*F*) in leaves of radish plants at different salinity levels. Means ± SD (n = 3) of measurements on each three plants. Means followed by * and ** are highly significantly decrease and increase different at $P \leq 0.01$, respectively, according to least significant difference (LSD) test. Chl – chlorophyll; Car – carotenoids.

The severe reduction in photosynthetic pigments in radish leaves in response to salinity treatment might be ascribed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and/or due to damage of the chloroplast thylakoid. The observed severe reduction in Mg^{2+} ions in salt-treated plants which are essential for Chl biosynthesis reinforced the view that salinity decreased Chl biosynthesis.

The accumulation of proline and total free amino acids contents in roots and leaves of the co-inoculation treatment particularly under salt stress may contribute to cellular adaptation to salt stress (Table 2). These results are in accordance with Mahajan and Tuteja (2005) who found that proline accumulation can contribute to the adjustment at the cellular level, may act as an enzyme protectant and stabilize the structure of macromolecules. Proline also acts as a major reservoir of energy and nitrogen for utilization upon exposure to salinity. In addition, Bano and Fatima (2009) found that salt stress significantly increased proline content of *Zea mays* leaves as compared to that of control. Co-inoculation of *Rhizobium* and *Pseudomonas* significantly increased the leaf proline content compared to salinized plants. Similarly, Jaleel *et al.* (2008) found that NaCl-stressed (80 mM) *Dioscorea rotundata* plants showed increased free amino acid content as compared to unsalinized plants.

Proline content increased in radish plants inoculated with *Bacillus* and *Pseudomonas* under NaCl stress, which may be due to upregulation of proline biosynthesis pathway to keep proline in high levels, which helps in maintaining cell water status, protects membranes and proteins from stress or increased Ca^{2+} accumulation also affects the osmoregulation capacity by increasing the content of proline, leading to a higher water potential

Table 2. Effect of *B. subtilis* and *P. fluorescens* on proline, total free amino acid, crude protein and phytohormones contents of radish plants at different salinity levels. Means ± SD (n = 3) of measurements on each three plants. Means followed by * and ** are highly significant decrease and increase different at $P \leq 0.01$, according to least significant difference (LSD) test.

Treatment	Proline [µg g^{-1}(FM)]		Total free amino acid [µg g^{-1}(FM)]		Crude protein [g (100 g)$^{-1}$(DM)]		IAA [mg (100 g)$^{-1}$(FM)]	GA_3 [mg (100 g)$^{-1}$(FM)]	ABA [mg (100 g)$^{-1}$(FM)]
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Leaves	Leaves	Leaves
Control	12.7 ± 0.94	15.5 ± 1.06	171.7 ± 3.54	250.0 ± 4.33	12.07 ± 0.83	14.48 ± 0.71	10.11 ± 0.43	15.68 ± 0.65	0.806 ± 0.06
B. subtilis	14.1** ± 0.99	17.9** ± 1.15	202.1** ± 4.65	322.5** ± 4.85	18.31** ± 0.91	16.88** ± 0.78	18.36** ± 0.69	24.93** ± 0.99	0.698* ± 0.04
P. fluorescens	16.8** ± 1.02	21.7** ± 1.22	267.4** ± 4.77	463.7** ± 5.50	20.69** ± 1.02	24.57** ± 1.14	19.34** ± 0.78	63.99** ± 2.11	0.448* ± 0.01
75 mM NaCl	13.5** ± 0.86	22.7** ± 1.26	263.8** ± 3.25	272.4** ± 3.81	10.86* ± 0.58	12.07* ± 0.58	8.10* ± 0.33	9.87* ± 0.44	0.926** ± 0.07
75 mM NaCl + *B. subtilis*	14.9** ± 0.93	24.8** ± 1.34	307.5** ± 4.94	341.3** ± 4.11	14.48** ± 0.67	15.69** ± 0.74	21.43** ± 0.82	22.21** ± 0.89	0.633* ± 0.05
75 mM NaCl + *P. fluorescens*	17.2** ± 1.10	27.4** ± 1.46	323.9** ± 4.89	379.0** ± 4.62	15.69** ± 0.70	18.31** ± 0.87	22.48** ± 0.84	43.67** ± 1.35	0.575* ± 0.05
150 mM NaCl	14.7** ± 0.96	29.8** ± 1.54	305.2** ± 4.77	329.7** ± 4.00	8.23* ± 0.36	10.86* ± 0.43	7.40* ± 0.23	6.47* ± 0.33	1.215** ± 0.12
150 mM NaCl + *B. subtilis*	16.4** ± 1.05	33.2** ± 1.50	337.5** ± 5.54	386.2** ± 4.69	18.32** ± 0.98	15.73** ± 0.79	17.73** ± 0.63	21.85** ± 0.73	0.784* ± 0.09
150 mM NaCl + *P. fluorescens*	21.5** ± 1.13	34.1** ± 1.58	380.4** ± 5.66	428.3** ± 5.74	19.53** ± 1.00	16.44** ± 0.82	18.30** ± 0.74	34.19** ± 1.22	0.656* ± 0.08
LSD at 5%	0.53	1.31	13.32	14.01	0.77	0.80	1.12	3.64	0.047
1%	0.77	1.88	19.16	20.14	1.10	1.15	1.61	5.23	0.068

Table 3. Effect of *B. subtilis* and *P. fluorescens* on mineral content of radish plants at different salinity levels. Means ± SD (n = 3) of measurements on each three plants. Means followed by * and ** are highly significant decrease and increase different at $P \leq 0.01$, according to least significant difference (LSD) test.

Treatment	K^+ [g (100 g)$^{-1}$(DM)]		P [g (100 g)$^{-1}$(DM)]		N [g (100 g)$^{-1}$(DM)]		Na^+ [g (100 g)$^{-1}$(DM)]		Cl^- [g (100 g)$^{-1}$(DM)]		Ca^{2+} [g (100 g)$^{-1}$(DM)]		Mg^{2+} [g (100 g)$^{-1}$(DM)]	
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
Control	1.868 ± 0.36	2.385 ± 0.30	0.418 ± 0.03	0.434 ± 0.02	1.931 ± 0.26	2.317 ± 0.35	0.183 ± 0.01	0.171 ± 0.01	0.51 ± 0.02	3.71 ± 0.16	0.500 ± 0.06	0.894 ± 0.07	0.43 ± 0.02	0.38 ± 0.01
B. subtilis	2.213** ± 0.41	2.586** ± 0.36	0.585** ± 0.04	0.498** ± 0.02	2.929** ± 0.33	2.700** ± 0.39	0.160* ± 0.01	0.126* ± 0.01	0.16* ± 0.01	2.50* ± 0.13	0.547** ± 0.05	0.928** ± 0.08	0.48** ± 0.02	0.46** ± 0.02
P. fluorescens	2.242** ± 0.39	2.700** ± 0.39	0.629** ± 0.06	0.528** ± 0.03	3.310** ± 0.40	3.931** ± 0.42	0.137* ± 0.01	0.080* ± 0.01	0.12* ± 0.01	1.93* ± 0.09	0.593** ± 0.06	0.987** ± 0.08	0.49** ± 0.02	0.58** ± 0.02
75 mM NaCl	1.797* ± 0.25	2.098* ± 0.25	0.247* ± 0.01	0.393* ± 0.01	1.738* ± 0.15	1.931* ± 0.26	0.205** ± 0.01	0.262** ± 0.02	0.80** ± 0.03	4.77** ± 0.25	0.431* ± 0.04	0.802* ± 0.07	0.37* ± 0.01	0.29* ± 0.01
75 mM NaCl + *B. subtilis*	2.026** ± 0.28	2.500** ± 0.31	0.496** ± 0.03	0.524** ± 0.02	2.317** ± 0.21	2.510** ± 0.29	0.171* ± 0.01	0.148* ± 0.01	0.33* ± 0.01	2.31* ± 0.20	0.523** ± 0.05	0.971** ± 0.08	0.46** ± 0.02	0.43** ± 0.02
75 mM NaCl + *P. fluorescens*	2.039** ± 0.30	2.672** ± 0.33	0.506** ± 0.04	0.461** ± 0.03	2.510** ± 0.26	2.930** ± 0.30	0.160* ± 0.01	0.126* ± 0.01	0.37* ± 0.01	2.19* ± 0.21	0.563** ± 0.05	0.994** ± 0.08	0.49** ± 0.03	0.47** ± 0.01
150 mM NaCl	1.810* ± 0.26	1.868* ± 0.27	0.131* ± 0.01	0.365* ± 0.03	1.317* ± 0.12	1.738* ± 0.22	0.251** ± 0.02	0.319** ± 0.01	1.79** ± 0.16	6.19** ± 0.30	0.431* ± 0.03	0.662* ± 0.05	0.30* ± 0.01	0.25* ± 0.01
150 mM NaCl + *B. subtilis*	1.984** ± 0.29	2.555** ± 0.34	0.474** ± 0.02	0.479** ± 0.04	2.931** ± 0.34	2.517** ± 0.27	0.171* ± 0.01	0.117* ± 0.01	0.35* ± 0.02	3.23* ± 0.18	0.570** ± 0.05	1.009** ± 0.08	0.53** ± 0.04	0.45** ± 0.01
150 mM NaCl + *P. fluorescens*	1.997** ± 0.28	2.644** ± 0.35	0.491** ± 0.02	0.498** ± 0.04	3.124** ± 0.39	2.631** ± 0.27	0.165* ± 0.01	0.105* ± 0.01	0.31* ± 0.01	3.04* ± 0.20	0.620** ± 0.06	1.025** ± 0.08	0.58** ± 0.04	0.48** ± 0.01
LSD at 5%	0.03	0.06	0.03	0.01	0.12	0.13	0.008	0.015	0.103	0.273	0.012	0.023	0.016	0.020
1%	0.04	0.08	0.05	0.02	0.18	0.18	0.011	0.022	0.149	0.392	0.017	0.034	0.02	0.029

gradient and thereby improving the water uptake and growth under stress. Also, proline plays a major role in osmoadaptation through increase in osmotic pressure that shifts the dominant osmolyte from glutamate to proline.

Salinity stress decreased crude protein content in roots and leaves of radish plants (Table 2). One characteristic of saline stress is the removal of potassium ions by plant roots, which causes physiological imbalance because potassium is necessary for protein synthesis. Potassium loss causes diminished plant growth and development (Chen *et al*. 2007). If the stress is prolonged, it could affect protein synthesis and eventually cause it to decline. A decrease in the protein level in salt-stressed plants may be attributed to a decrease in protein synthesis; the decreased availability of amino acid and the denaturation of enzyme involved in amino acid and protein synthesis.

Co-inoculation with PGPB increased crude protein content in roots and leaves of radish plants under salt stress. These results are in accordance with Tawfik (2008) who found that crude protein content in cowpea plants was higher in rhizobacterin salinized plants than nonsalinized plants.

One of the most commonly reported direct plant growth promotion mechanism by bacteria is the production of plant growth substances (phytohormones) such as auxins (Table 2). IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin (Table 2). IAA may function as important signal molecule in the regulation of plant development, influencing many cellular plant processes, such as the cell elongation (Tsavkelova *et al.* 2006). They promote seed germination, root elongation which improve uptake of essential nutrients thus increasing plant growth (Zimmer *et al.* 1995).

B. subtilis and *P. fluorescens* were considered as the best studied IAA producers and could improve the growth of plants and alleviate the effect of growth inhibitors by declining ABA content of plants (Patten and Glick 2002). Increased production of IAA and inhibited production of ABA in inoculated radish plants may be a good mean of protection against salt stress and promotion of radish growth in the fields.

Gibberellins regulate all aspects of the life history of plants, from seed germination to vegetative growth and flowering. Many microorganisms that interact with plants can synthesize hormones similar to those produced by the plant as growth regulator, such as auxins, gibberellins and cytokinines. ABA increased in leaves of radish plants under salinity stress. This increase in ABA contents hinder radish growth and development. It is due to the fact that ABA synthesized in plants upon salinity stress, triggers closure of stomata thereby reducing water loss. Mechanically, the closure is facilitated by a reduction of the internal pressure (turgor) in the guard cells which is achieved by a concerted efflux of potassium ions and anions, sucrose removal and malate conversion into osmotically inactive starch.

ABA is a genetic stress hormone that has multiple functions, including induction of genes involved in osmotic stress protection. Salt-induced ABA mediated the inhibition of leaf expansion and limited the accumulation of Na^+ and Cl^- in leaves of radish plants. ABA could also provoke carbohydrate accumulation by putatively blocking sucrose export from mature leaves, contributing to osmotic adjustment during the early phase of salinity. On the other hand, a high ABA level was less maintained in PGPB-treated plants providing the development of antistress reactions. Maintaining high level of ABA in growth regulators treated plants under salt stress promotes protective reactions, which decreased injurious effects on growth and accelerate growth resumption. Finally, ABA induces late-embryogensis abundant proteins, osmoprotectants and osmolyte biosynthesis genes.

Salinity stress caused highly significant increase in the Na^+ and Cl^- content and decrease in the contents of K^+, Mg^{2+}, and Ca^{2+} in the roots and leaves of radish plants. Seed inoculation of radish with *B. subtilis* and *P. fluorescens* significantly increased concentration of P, N, K^+, Mg^{2+}, and Ca^{2+} in the roots and leaves of radish plants but decreased Na^+ and Cl^- (Table 3).

It is probable that salt stress causes nutrient deficiency due to competition between Na and other nutrients such as K^+, Mg^{2+}, and Ca^{2+}. Other possibility is that reduction in uptake of mineral nutrients under saline conditions may occur due to Na-induced blockage or reduced activity of the transporters, resulting in ionic imbalance of K^+, Ca^{2+}, and Mg^{2+} as compared to Na^+. After treating with *B. subtilis* and *P. fluorescens*, reduction the Na^+ uptake of plants and/or the increased K^+ uptakes compared to control treatment under salt stress The PGPB strains can produce bacterial exopolysaccharides which bind cations including Na^+ (Geddie and Sutherland 1993) and decrease the content of Na^+ available for plant uptake, thus helping alleviate salt stress in plants. These results are in accordance with Yildirim *et al.* (2008) who found that bacteria-inoculated radish plants under salt stress have lower Na^+ and Cl^- contents and higher NPK contents compared with noninoculated plants.

Phosphate solubilizing and N_2 fixing bacteria can improve the N and P nutrition of plants and stimulate the plant growth (Puente *et al.* 2004). The highest P accumulation exhibited by the co-inoculation treatment may be important in mitigating salt stress by overcoming the scarce P availability in saline soils. In this respect, Han and Lee (2005) observed that N, P, and K^+ concentrations increased in soybean plants under salinity stress due to inoculation with PGPB. Also, Stajkovic *et al.* (2011) who found that significant positive effect of co-inoculation with *Pseudomonas* sp. and *Rhizobium phaseli* on N and P contents of common bean plants compared to inoculation with *Rhizobium* alone. Bacterial inoculations in radish plants could alleviate deleterious effects of salt stress. This phenomenon can be explained by a couple of

mechanisms: bacterial inoculation can restrict Na^+ and Cl^- uptake and enhance the uptake of other nutrients such as N, P, K^+, and Ca^{2+} positively. This may be due to the reason that PGPB can produce bacterial exopolysaccharides which bind cations, especially Na^+, in roots, thus preventing their transfer to leaves and helping alleviate salt stress in plants (Ashraf *et al.* 2004).

Conclusion: The present study demonstrates that salinity stress caused reduction in some morphological and biochemical parameters in radish plants but caused accumulation in proline, total free amino acids and ABA content. In addition, PGPB can protect plants from the deleterious effects of salinity stress by the production of hormones (IAA, GA, and ABA), osmoregulation (proline), increase mineral and nitrogen and production of siderophore. PGPB could offer an economical and simple treatment to salt-sensitive radish plants, helping to solve the production problems caused by high salinity. *P. fluorescens* treatment could showed better effect to alleviate the salt stress in radish plants than *B. subtilis*.

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