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IMPACT OF PLANT GROWTH PROMOTING *BACILLUS SUBTILIS* ON GROWTH AND PHYSIOLOGICAL PARAMETERS OF *BASSIA INDICA* (INDIAN BASSIA) GROWN UDDER SALT STRESS

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Abstract

In this study, the role of a salt-tolerant plant growth-promoting bacterium (PGPR), *Bacillus subtilis*, in the alleviation of salinity stress during the growth of Indian bassia (*Bassia indica* [Wight] A.J. Scott), was studied under ccontrolled growth chamber conditions following seed inoculation. Physiological parameters such as neutral and phospholipids, fatty acid composition as well as photosynthetic pigments, were investigated. Salinity inhibited shoot and root length by 16 and 42%, dry weight by 37 and 23% respectively and negatively affected physiological parameters. Inoculation of unstressed and salt-stressed Indian bassia with *B. subtilis* significantly improved root and shoot growth, total lipid content, the phospholipid fraction, photosynthetic pigments (chlorophyll a and b and carotenoid contents) and also increased oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids in plant leaves compared to uninoculated plants. The salt-tolerant PGPR, *B. subtilis* could act synergistically to promote the growth and fitness of Indian bassia plants under salt stress by providing an additional supply of an auxin (IAA) and induce salt stress resistance by reducing stress ethylene levels.

Key words: *Bassia indica*, Salt stress, *Bacillus subtilis*, Photosynthetic pigments, Phospholipids, Auxin.

Introduction

The salinization of land has become a major environmental issue and has been recognized as the most important economic, social and environmental problem in many regions of the world, including Saudi Arabia (Qadir *et al*., 2007). Previous studies have shown that salinity and drought stress led to a significant decline in plant growth, nutrient uptake and yield of agriculturally and medicinally important plants (Alqarawi *et al*., 2014 a,b; Abd_Allah *et al*., 2015; Egamberdieva *et al*., 2015). Soil salinization has become a great challenge for the rehabilitation of range lands and it affects plant productivity (Kushiev *et al*., 2005). Planting perennial and annual halophytes such as *Bassia indica* [Indian bassia or kochia] (Shelef *et al*., 2012) and *Portulaca oleracea* [purslane] (Ben Asher *et al*., 2012) in salt-affected soils may help to restore abandoned land for sustainable crop production (Kushiev *et al*., 2005; Toderich *et al*., 2008). Indian bassia is a richly branched herb that belongs to the *Chenopodiaceae* family and is mainly found in the Nile River valley (Zahran, 1986). It has rich nutritive value, can be used as fodder for livestock and is considered to be an alternative income source for farmers in salt-affected arid regions (Zahran *et al*., 1992). Indian bassia has the potential to accumulate salts and is therefore considered for salt phytoremediation in constructed wetlands (Shelef *et al*., 2013). It is a desirable goal to combine three agronomically important traits: fast growth, higher biomass, and salt tolerance. The improvement of plant-microbe interactions under salt-stressed soil conditions has been shown to stimulate plant growth, nutrient uptake and alleviate the negative effects of salinity stress (Egamberdieva, 2011; 2012). Strains of plant growth-promoting bacteria (PGPR) may also positively affect the physiological parameters of plants by increasing photosynthetic pigments, total free amino acids, proteins and nitrogen, phosphorous and potassium (NPK) concentrations compared to uninoculated control plants under saline conditions (Berg *et al*., 2013).

The ameliorative effects of PGPR on plant growth under saline conditions have been shown for various plant species, including durum wheat [*Triticum durum*] (Upadhyay *et al*., 2011); brahmi [*Bacopa monnieri*] (Bharti *et al*., 2013); milk thistle [*Silybum marianum*] (Egamberdieva *et al*., 2013a), goat's rue [*Galega officinalis* L.] (Egamberdieva *et al*., 2013b) and basil [*Ociumum basilicm*] (Heidari *et al*., 2011). PGPR may use several mechanisms to promote plant growth and increased plant stress tolerance such as the synthesis of phytohormones like indole-3-acetic acid (IAA), gibberellic acid, or cytokinins (Spaepen *et al*., 2009), production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Dey *et al*., 2004), and production of exopolysaccharides (EPS) (Upadhyay *et al*., 2011).

In the present work, we investigated whether a salt-tolerant PGPR strain of *Bacillus subtilis* could improve tolerance to salt, plant growth and physiological parameters, including neutral and phospholipids, fatty acid composition as well as photosynthetic pigments, of *Bassia indica* plants grown in saline soil. In addition, those traits that might be responsible for the beneficial mechanisms of action by this PGPR were evaluated.

Material and Methods

Plants and bacteria: The mature seeds of *Bassia indica* (Wight; = Kochia indica) A. J. Scott, (Indian bassia) were collected from naturally grown plants in salt marsh vegetation in Madinet El Sadat city, Al-Bu□ayrah Governorate (Egypt). Madinet El Sadat city is west of the Rosetta branch of the Nile, which is located between Wadi Al-Na□rūn and the western edge of the Nile Delta. The city is 58 miles (94 km) northwest of Cairo, in the desert, and covers an area of 19 square miles (48 square km). The overall average annual rainfall in Madinet El Sadat is about 18 mm. The average minimum monthly air temperature is 19.5°C in January and the maximum of 34.4°C is in August, while the soil temperature ranges from 20 to 26.3°C.

The *B. subtilis* (Egyptian isolate) was previously isolated from the rhizosphere of tomato (*Solanum lycopersicum* L.) grown in Sharkia Governorate, Egypt and identified to the species level according to Buchanon *et al*. (1974). The strain has been used as biocontrol agent in previous study (Hashem *et al*., 2013).

Seed germination: Seeds of Indian bassia were sorted to eliminate broken or small seeds. They were surface-sterilized for 5 min with 1% sodium hypochlorite solution followed by 70% ethanol for 3 min, and rinsed five times with sterile distilled water. Seeds were germinated in 85 mm × 15 mm tight fitting plastic Petri dishes with 5 mL of 50 mM NaCl solution according to Shelef *et al*. (2010). Petri dishes were covered with a polyethylene sheet to avoid the loss of moisture through evaporation and placed in a plant growth chamber at 28°C for 10 days exposed to a photosynthetic photon flux density (PPFD) of 1500 µmol m^{-2} s^{-1} (12-h photoperiod). Seeds were observed daily until 10 days after germination, i.e., when the radicle had emerged >0.5 cm.

Plant growth: A pot experiment was carried out using sandy soil collected from Derab Agricultural Research Station, Riyadh, Saudi Arabia. Five replicate pots were used per treatment (N=5). The soil had the following properties (%): sand (73.9); moisture content, 4.27; organic carbon, 0.15; total nitrogen, 0.008; (EC) = 7.12 dS m^{-1}; and pH 7.8. Soil was autoclaved for 3 h at 121°C, cooled then divided among plastic pots.

The formulation of *B. subtilis* strain was carried out as described in our previous work (Hashem *et al*., 2013). The germinated seeds were coated with bacteria by dipping the seedlings in bacterial suspensions at concentrations of 10^7 CFU/ml. Inoculated seedlings were sown in plastic pots containing 2 kg of soil. One seed was sown per plastic pot at a depth of approximately 1.0 cm. Plants were grown for two months in climate-controlled chambers at 28°C with a PPFD of 1500 µmol m^{-2} s^{-1} (18-h photoperiod). Plants were irrigated every five days with 200 mM NaCl solution. Control plants were irrigated with normal tap water. The treatments were: (i) uninoculated plants irrigated with tap water (non-saline); (ii) plants inoculated with *B. subtilis* and irrigated with non-saline water; (iii) uninoculated plants irrigated with saline water; (iii) plants inoculated with *B. subtilis* and irrigated with saline water. After two months, plants were harvested, separated into roots and shoots, rinsed in water, dried at 105°C and subsequently weighed. Fresh shoots were used to estimate photosynthetic pigments and lipid fractions.

Lipids analysis: Total lipids were estimated according to Marsh & Weinstein (1966). Neutral and phospholipids were estimated according to the methods of Amenta (1964) and Rouser *et al*. (1970), respectively. Fatty acid methyl esters were prepared by methanolysis in H_2SO_4-MeOH (Kates, 1972).

Fatty acids: Esters were analyzed by gas liquid chromatography (GLC). The peaks of fatty acid methyl esters were separated, quantified and identified by comparing their retention times with those of an authentic methyl ester standard (Sigma Co., St. Louis, USA). Phospholipids were separated by two-dimensional chromatography with $CHCl_3$-MeOH - 28% (w/v) NH_4OH (13:5:1, v/v/v) for the first dimension and CHC_3- Me_2CO - MeOH-HOAc - H_2O (6:8:2:2:1, v/v/v/v/v) for the second dimension (Rouser *et al*., 1970). Fatty acid methyl esters were prepared by methanolysis in H_2SO_4-MeOH (Kates, 1972) and methyl esters were analyzed by GLC on a Perkin-Elmer Model 910 (Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector (Johnson & Stocks 1971).

Photosynthetic pigments: Photosynthetic pigments were extracted and estimated according to Fadeel (1962). Lipid content was extracted from fresh leaf samples (1.0 g) according to Fölch *et al*. (1957) using chloroform, methanol (2:1, v/v) and 0.05% butylated hydroxytoluene (BHT) was added to all solvents to prevent lipid peroxidation (Cachorro *et al*., 1993).

ACC deaminase: In order to measure growth using ACC as the sole N-source, bacterial isolates were incubated in BM minimal medium (Lugtenberg *et al*., 1999) supplemented with 1.5% NaCl and 3.0 mM of either ACC (Sigma Chemical Co., St. Louis, Missouri, USA) (to test ACC utilization) or of $(NH_4)_2SO_4$ (positive control) as the sole N source. The negative control did not have an added N-source.

Auxin production: The production of IAA was determined according to the method of Bano & Musarrat (2003). The strain was grown in LB agar medium (Luria Bertani) with and without tryptophan (500 µl/mL) and incubated at 28°C. After three days of cultivation, 2 ml aliquots of bacterial cultures were centrifuged at 13,000 ×g for 10 min. One ml of supernatant was transferred to a fresh tube to which 100 µL of 10 mM orthophosphoric acid and 2 mL of reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) were added. After

25 min, the absorbance of the developed pink color was read at 530 nm. The IAA concentration in culture was calculated by using a calibration curve of pure IAA (Sigma Aldrich) as the standard.

Statistical analysis: Data were tested for statistical significance using the analysis of variance package included in Microsoft Excel 2007. Comparisons were done using a Student's *t*-test. Mean comparisons were conducted using a least significant difference (LSD) test (P=0.05).

Results

Plant growth promotion: In this study, we investigated how salt stress affected the growth, photosynthetic pigments, lipid fractions and fatty acids composition of Indian bassia and whether inoculation with a salt-tolerant PGPR strain of *B. subtilis* could restore the growth and physiological parameters of salt-stressed plants. Our results indicate that after plants were grown for two months in 0 or 200 mM NaCl, the root and shoot weight as well as root length responded positively to inoculation treatment (Fig. 1 a,b). Compared with uninoculated non-stressed plants, the length of roots and shoots of stressed plants was reduced by 42-16% while the dry weight of roots and shoots was reduced by 23-37%. The inoculation of plants with *B. subtilis* enhanced the root and shoot length and dry weight of unstressed plant. The roots of plants inoculated with *B. subtilis* were significantly longer (64%) than uninoculated roots. Inoculation also increased root and shoot weight by 33 and 43%, respectively (Fig. 1b). Salt-stressed plants inoculated with *B. subtilis* grew better than salt-stressed uninoculated plants showing a 10% increase in root weight and 20% increase in shoot dry weight (Fig. 1b). Inoculated plants also had significantly longer (38%) roots than uninoculated stressed plants (Fig 1a).

Lipid fractions: Salinity (200 mM NaCl) decreased the total lipid content in plant tissues (leaves) as well as the neutral lipid content. When uninoculated plants were grown in saline soil, total lipid content decreased by 34% compared with non-stressed plants (Fig. 2). Interestingly, inoculation with *B. subtilis* enhanced total lipid content both under saline and non-saline conditions (Fig. 2). Inoculated salt-stressed plants contained significantly more total lipids (61%) than uninoculated salt-stressed plants (Fig. 2). When the lipid content of uninoculated unstressed plants was adjusted to 100%, inoculated plants grown in non-saline soil contained 16% more lipids than uninoculated plants.

Data regarding neutral lipids in plants grown under non-saline and saline soil is shown in figure 3. The concentration of neutral lipids in uninoculated control plants, namely triacylglycerol (TG) and sterol (S), decreased by 43% and 48%, respectively in saline soil (Fig. 3). In contrast, the content of diacylglycerol (DG), sterol ester (SE), and non-esterified fatty acids (NEFA) increased by 18%, 45% and 95%, respectively, compared with that of the control plants. The neutral lipids (TG and S) content responded to inoculation treatments in a similar way as total lipid content, increasing the TG and S content in salt-stressed plant tissues (leaves). In plants grown in saline soil, the difference in TG and S contents between co-inoculated and uninoculated plants was significant (Fig. 3). In plants inoculated with *B. subtilis*, 36% and 64% more TG and S lipids were observed, respectively, compared to uninoculated stressed plants (=100%). The neutral lipids such as DG, SE, and NEFA content did not respond to bacterial inoculation treatments under non-saline and saline soil conditions (Fig. 3).

Data regarding phospholipid fractions (PA-phosphatidic acid; PC- phosphatidyl choline; PE-phosphatidyl ethanol; PG- phosphatidyl glycerol; PI-phosphatidyl inositol; PS- phosphatidyl serine) in plant leaves is shown in figure 4. The concentrations of PC, PE, PG, PI, and PS decreased by 50%, 32%, 45.5%, 50% and 40%, respectively in leaves of plants grown in saline soil. Their concentration increased slightly after inoculation with *B. subtilis* under non-saline soil. Under salinity stress, there was a 69%, 25%, 39%, 52% and 31% increase in PC, PE, PG, PI, and PS concentration, respectively more than the control with PGPR inoculation.

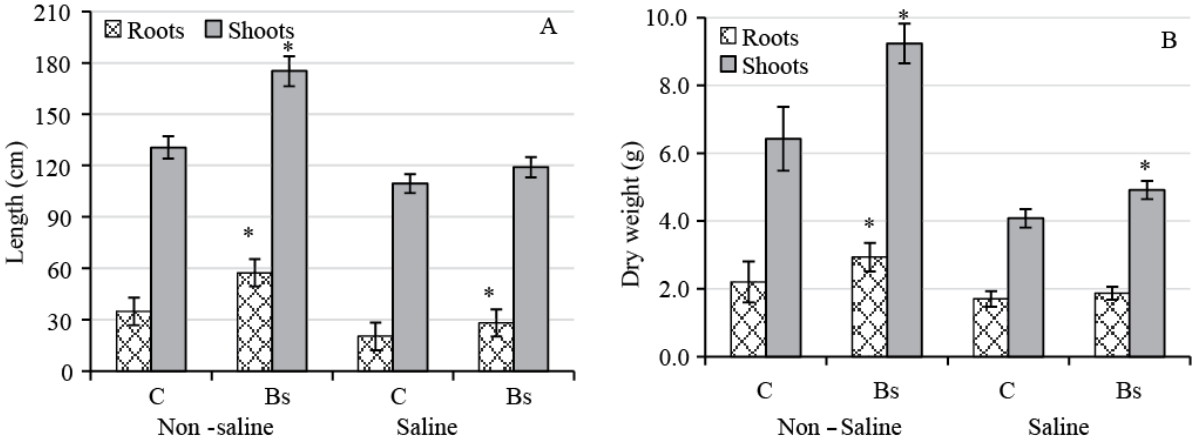

Fig. 1A-B. The effect of *B. subtilis* on root, shoot length (A) and dry weight (B) of *B. indica*. Treatments: C- uninoculated control plants, Bs- plants inoculated with *B. subtilis*. Columns represent means for five plants (N=5) with standard error bars.
*Columns marked with an asterisk differed significantly from uninoculated plants at $p<0.05$.

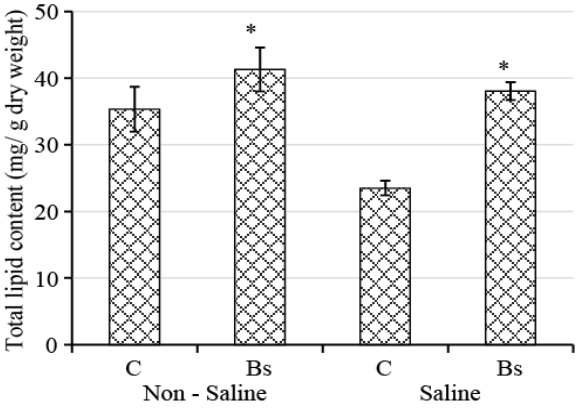

Fig. 2. Total lipid content in leaves of *B. indica* when seedlings were inoculated with *B. subtilis* (C- uninoculated plants, Bs – plants inoculated with *B. subtilis*). Columns represent means for four plants (N=4) with standard error bars.
*Columns marked with an asterisk differed significantly from uninoculated plants at p<0.05.

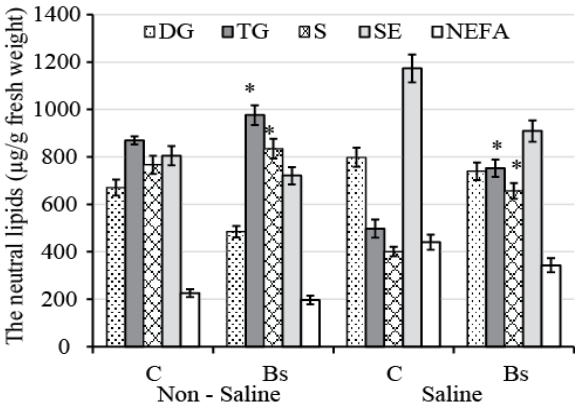

Fig. 3. The neutral lipid contents (µg/g fresh weight, DG- diacylglycerol; TG- triacylglycerol; S- sterol; SE- sterol ester; NEFA - non-esterified fatty acids) in leaves of *B. indica* when seedlings were inoculated with *B. subtilis* (C- uninoculated plants, Bs – plants inoculated with *B. subtilis*). Columns represent means for four plants (N=4) with standard error bars.
*Columns marked with an asterisk differed significantly from uninoculated plants at p<0.05.

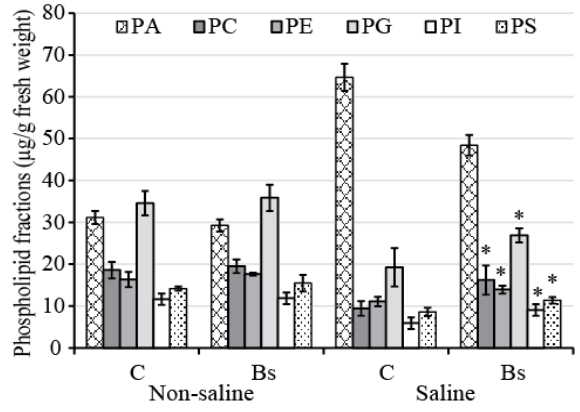

Fig. 4. Phospholipid fractions (PA- phosphatidic acid; PC- phosphatidyl choline; PE- phosphatidyl ethanol; PG- phosphatidyl glycerol; PI- phosphatidyl inositol; PS- phosphatidyl serine) in leaves of Indian bassia (*B. indica*) when seedlings were inoculated with *B. subtilis* (C- uninoculated plants, Bs – plants inoculated with *B. subtilis*). Columns represent means for four plants (N=4) with standard error bars.
*Columns marked with an asterisk differed significantly from uninoculated plants at p<0.05.

Fatty acids composition: The GLC analysis of methylated lipids of Indian bassia irrigated with non-saline water revealed the presence of saturated palmitic (C16:0), margaric (C17), stearic (C18:0), oleic (C18:1), linoleic (C18:2), alpha linolenic acid (C18:3), arachidic (C20), and arachidonic (C20:4) fatty acids accounting for 2.65%, 0.55%, 14.68%, 10.02%, 9.04%, 17.09%, 17.78% and 28.19%, respectively with a total saturation of 35.66% (Table 1). Our results also showed the appearance of C8, C10, C12 and C14 fatty acids in leaves of plants irrigated with saline water accompanied with a significant increase of saturated fatty acids such as C16, stearic (C18) and arachidic (C20) compared to control plants. On the other hand, the percentage of mono-unsaturated [oleic acid (C18:1)] and poly-unsaturated [linoleic (C18:2) and linolenic (C18:3)] fatty acids in Indian bassia decreased significantly with salt stress (Table 1). In general, inoculation did not affect the fatty acid composition (%) of Indian bassia, whereas only oleic acid (C18:1) increased slightly. In comparison to plants irrigated with non-saline water, salt-stressed plants inoculated with *B. subtilis* showed an increase in oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids.

Photosynthetic pigments: Results of the pot study showed that (overall assessment) salinity significantly inhibited growth and decreased the photosynthetic pigments (chlorophyll *a* and *b* and carotenoid contents) of plants. Control plants contained highest concentration of total pigments (21.6 mg/g leaf) while salinity-exposed plants contained lowest total pigment concentrations (6.8 mg/g leaf) (Table 2). When uninoculated plants grown in saline soil were compared with non-stressed plants, total pigment content decreased by 60% (Table 2). The maximum concentration of total pigments was observed in plants inoculated with *B. subtilis* under non-saline (a maximum of 34%) and saline soil (a maximum of 88%) conditions. Data regarding the effect of inoculation on chlorophyll *a* content in plants revealed that the *B. subtilis* strain caused a maximum increase in chlorophyll *a* content in leaves of plants grown in non-saline soil (a maximum of 35% increase over the respective control). At saline stress, this *B. subtilis* strain increased chlorophyll *a* content by as much as 43% more than the control plant. This strain also increased chlorophyll *b* content and carotenoid content relative to control plants grown in non-saline and saline soil, respectively.

In vitro screening for traits involved in plant growth promotion: In order to obtain possible clues about the mechanism(s) underlying the alleviation of salt stress by bacteria, traits possibly involved in plant growth stimulation were tested. The salt-tolerant PGPR strain of *B. subtilis* produced IAA (5.0 µg/mL) in medium containing 3% NaCl. The presence of tryptophan strongly stimulated IAA production in the strain (12.2 µg/mL). The strain could utilize ACC as an N source indicating the presence of ACC deaminase, which plays a role in reducing ethylene levels in plants.

Table 1. The effect of seed treatment with the *B. subtilis* on fatty acids composition* (%) of Indian bassia (*B. indica*) grown under non-saline and saline soil conditions.

Treatments	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	$C_{20:4}$
Non-saline												
Control	0.0	0.0	0.0	0.0	2.65	0.55	14.68	10.02	9.04	17.09	17.78	28.19
B. subtilis	0.0	0.0	0.0	0.0	1.52	0.44	12.63	10.50	15.00	21.47	17.11	21.34
Saline												
Control	2.38	5.15	1.85	1.40	4.31	1.34	19.04	5.94	6.82	14.36	21.95	15.80
B. subtilis	1.16	2.29	0.66	0.60	2.94	0.67	16.46	8.81	9.26	18.54	19.42	19.19
$LSD_{0.05}$	0.29	0.45	0.19	0.28	0.36	0.36	1.49	1.30	0.86	1.42	1.56	1.64

* Caprylic, (C8); capric (C10); lauric (C12); myristic (C14); palmitic (C16); argaric (C17); stearic (C18); oleic (C18:1); linoleic (C18:2); alpha linolenic acid (C18:3); arachidic (C20); arachidonic (C20:4)

Table 2. The effect of seed treatment with *B. subtilis* on photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids and total pigments) in leaves of Indian bassia (*B. indica*) plants grown under non-saline and saline soil conditions.

Treatments	Photosynthetic pigments (mg/g fresh weight)			
	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Total pigments
Non-saline				
Control	11.52	9.18	1.06	21.68
B. subtilis	15.61*	12.02*	1.47	29.10*
Saline				
Control	4.55	1.71	0.52	6.79
B. subtilis	6.53*	5.66*	0.61	12.81*

* Significantly different from uninoculated plants at $p<0.05$

Discussion

B. indica is a drought- and salt-tolerant plant which is well-adapted to grow in saline arid soils and used in the rehabilitation of abandoned fields (Shelef *et al*., 2013). Shelef *et al*. (2009) observed that Indian bassia was able to growth in hydroponic solution at 80 mM NaCl. Although *B. indica* tolerates saline soils, to our knowledge, very little is known about how salt stress affects plant growth and physiological parameters including photosynthetic pigments, lipids and fatty acid compositions in leaves. In our study we observed that soil salinity affected root and shoot growth as well as physiological parameters. A root-associated, salt-tolerant PGPR *B. subtilis* strain was able to alleviate salt stress and improve plant growth and physiological properties of Indian bassia. Yaqoob *et al*. (2013) studied the microbial composition associated with *B. indica* and found that more than half of the microbial strains isolated from the rhizosphere and rhizoplane were able to solubilize inorganic phosphate and produce auxin, which indicates the ability of these bacteria to stimulate plant growth under hostile environmental conditions. In many studies, PGPR could improve the growth of medicinally important plants and alleviate the effect of growth inhibitors by producing phytohormones such as gibberellins, auxin, and cytokinins (Berg *et al*., 2013; Teixeira da Silva & Egamberdieva, 2013; Egamberdieva & Jabborova, 2015; Egamberdieva *et al*., 2015), which are subsequently taken up by plants for growth and development. Moreover, bacterial IAA increases root surface area and length, and thereby provides plants with greater access to soil nutrients.

Lipids play vital roles in the tolerance to several physiological stressors in plants such as drought and salinity (Singh *et al*., 2002). We observed that salinity inhibited neutral lipids and phospholipids in the leaves of Indian bassia. Similar results were reported by Alqarawi *et al*. (2014a) where the lipid content in *Ephedra alata* decreased at higher salt concentrations. Wu *et al*. (1998) also showed a decrease in phospholipid content in the root plasma membrane of salt marsh grass (*Spartina patens*) following NaCl stress. According to Kerkeb *et al*. (2001), the content of PC and PE decrease after exposure to salinity, but the PC/PE ratios are not affected by salinity. Bacterial inoculation increased PC, PE, PG, PI, and PS concentration compared to control plants under saline soil conditions. The fatty acid composition of Indian bassia leaves was also affected by bacterial inoculation. *B. subtilis* increased the percentage of oleic acid ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids. Similar results have been reported by Nosheen *et al*. (2013) in which significantly higher oleic acid ($C_{18:1}$) and linolenic acid ($C_{18:3}$) content was observed after *Azospirillum* treatment.

Our results showed that salinity decreased chlorophyll and total carotenoid contents in the leaves of Indian bassia compared to control plants grown in non-saline soil. Similar results were observed in other studies in which photosynthetic pigments were significantly reduced under NaCl stress (Alqarawi *et al*., 2014a; Hashem *et al*., 2014; Abd_Allah *et al*., 2015). For example, in the leaves of tomato (Hashem *et al*., 2015), and *Bruguiera parviflora* (Parida *et al*., 2004) the contents of chlorophyll *a*, chlorophyll *b*, and carotenoids decreased by NaCl stress. According to Zörb *et al*. (2009), the reduction in pigment content is attributed to the destructive effect of salt stress on chloroplasts in maize. PGPR strain *B. subtilis* increased chlorophyll *a*, *b* and carotenoid contents in leaves of Indian bassia plants grown under both non-saline and saline soil conditions. Mohamed and Gomaa (2012) studied the ability of PGPB *B. subtilis* to ameliorate the inhibitory effect of salt on radish (*Raphanus sativus*) in greenhouse experiments. They found that seeds inoculated with *B. subtilis* significantly increased the fresh and dry weight of roots and leaves, photosynthetic pigments, proline, total free amino acids, crude protein and N, P, K^+, Ca^{2+}, and Mg^{2+} uptake compared to uninoculated control plants under saline conditions. There are other studies in which PGPR strains stimulated the production of biologically active compounds of medicinal plants (Egamberdieva & Teixeira

da Silva 2015; Egamberdieva *et al*., 2015). For example, Bharti *et al*. (2013) observed that salt-tolerant *Bacillus pumilus* stimulated plant growth and bacoside-A content of brahmi (*Bacopa monnieri*). Golpayegani & Tilebeni (2011) observed similar results in which inoculation of basil (*Ocimum basilicum*) with *Bacillus lentus* alleviated the effects of salinity (6 dS m^{-1}) on growth, photosynthesis, mineral content and antioxidant enzymes such as ascorbate peroxidase and glutathione reductase. PGPR strains are also known to stimulate the level of phytohormones in plant tissues. For example, Arkhipova *et al.* (2007) observed increased cytokinin concentrations in lettuce (*Lactuca sativa* L.) treated with a cytokinin-producing PGPR strain *Bacillus* sp. (IB-22). *Azospirillum* strains increased the levels of gibberelic acid in the roots after inoculation of maize seedlings (Fulchieri *et al*., 1993). Increased production of IAA in inoculated plants by PGPR may be a good means of protection against salt stress while promoting plant growth in harsh soil conditions (Egamberdiyeva, 2005). PGPR that release the enzyme ACC deaminase may decrease ethylene levels and enhance salt tolerance of plants, stimulating shoot and root growth under saline soil conditions (Hontzeas *et al*., 2004; Glick *et al*., 2007). Our strain *B. subtilis* was capable to utilize ACC as an N source, indicating the presence of ACC deaminase and increased salt tolerance of Indian bassia, consequently stimulating shoot and root growth in saline soil.

Conclusion

In conclusion, this study demonstrated a clear and positive effect when salt-stressed Indian bassia plants were inoculated with *B. subtilis*. This PGPR strain can assist plants by providing an additional supply of an auxin (IAA) and induce salt stress resistance by reducing stress ethylene levels through the production of ACC deaminase, which might improve root growth and nutrient uptake.

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