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Improved Salinity Tolerance of *Arachis hypogaea* (L.) by the Interaction of Halotolerant Plant-Growth-Promoting Rhizobacteria

Pushp Sheel Shukla · Pradeep K. Agarwal · Bhavanath Jha

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Abstract Salinity adversely affects plant growth and development. Halotolerant plant-growth-promoting rhizobacteria (PGPR) alleviate salt stress and help plants to maintain better growth. In the present study, six PGPR strains were analyzed for their involvement in salt-stress tolerance in *Arachis hypogaea*. Different growth parameters, electrolyte leakage, water content, biochemical properties, and ion content were analyzed in the PGPR-inoculated plants under 100 mM NaCl. Three bacterial strains, namely, *Brachybacterium saurashtrense* (JG-06), *Brevibacterium casei* (JG-08), and *Haererohalobacter* (JG-11), showed the best growth of *A. hypogaea* seedlings under salt stress. Plant length, shoot length, root length, shoot dry weight, root dry weight, and total biomass were significantly higher in inoculated plants compared to uninoculated plants. The PGPR-inoculated plants were quite healthy and hydrated, whereas the uninoculated plant leaves were desiccated in the presence of 100 mM NaCl. The percentage water content (PWC) in the shoots and roots was also significantly higher in inoculated plants compared to uninoculated plants. Proline content and soluble sugars were significantly low, whereas amino acids were higher than in uninoculated plants. The MDA content was higher in uninoculated plants than in inoculated plants at 100 mM NaCl. The inoculated plants also had a higher K^+/Na^+ ratio and higher Ca^{2+}, phosphorus, and nitrogen content. The auxin concentration was higher in both shoot and root explants in the inoculated plants. Therefore, it could be predicted that all these parameters cumulatively improve plant growth under saline conditions in the presence of PGPR. This study shows that PGPR play an important role in inducing salinity tolerance in plants and can be used to grow salt-sensitive crops in saline areas.

Keywords PGPR · Salt stress · *Arachis hypogaea* · Plant growth · Osmolytes · Ion contents

P. S. Shukla · P. K. Agarwal (✉) · B. Jha (✉)
Discipline of Marine Biotechnology and Ecology, CSIR-Central Salt and Marine Chemicals Research Institute, G.B. Road, Bhavnagar 364021, Gujarat, India
e-mail: pagarwal@csmcri.org

B. Jha
e-mail: bjha@csmcri.org

P. S. Shukla
e-mail: pushps@csmcri.org

Introduction

Plants face various biotic and abiotic stresses in adverse environmental conditions. Among these, salinity is a major abiotic factor that suppresses agricultural productivity (Boyer 1982). Approximately 7% of the world's land (Szabolcs 1994) and 20% of the irrigated agricultural land is affected by salinity (Chinnusamy and others 2005; Al-Maskri and others 2010). Abiotic stress indeed is a complex process, which informs cells to adapt themselves at the molecular, biochemical, and physiological levels. Plants follow a number of salt-tolerant mechanisms such as synthesis of osmolytes and polyamines, reducing reactive oxygen species, the antioxidant defense mechanism, ion transport, and compartmentalization. Ion transporters play an important role in reducing the Na^+ load in the cytosol either by excluding Na^+ by plasma membrane *SOS1* (Zhu 2002) or by compartmentalization of Na^+ in the vacuole by *NHX1* (Gaxiola and others 1999). Several "regulatory elements" and "single function genes" related to abiotic stress have been isolated and are being introduced into

plants for stress tolerance by genetic engineering (Ashraf and Akram 2009; Agarwal and Jha 2010).

Plant-growth-promoting rhizobacteria (PGPR) are a group of microorganisms that colonize the root of plants and enhance plant growth either directly or indirectly (Lugtenberg and Kamilova 2009). Directly, PGPR promote plant growth by a higher acquisition of nutrients, increased availability of phytohormones, and a greater uptake of phosphorus and nitrogen (Richardson and others 2009). Indirectly, PGPR affect the status of plants by eliciting an Induced Systemic Resistance (ISR) or Systemic Acquired Resistance (SAR), not allowing the soil-borne phytopathogens to inhibit plant growth (Bloemberg and Lugtenberg 2001). PGPR have improved seed germination, plant weight, harvest yield, and disease resistance (Kloepper and others 1980, 1991, 1999). Zhang and others (2008a) reported that PGPR regulate photosynthesis by decreasing glucose-sensing and abscisic acid level in *Arabidopsis* plants.

Yang and others (2010) coined the term "Induced Systemic Tolerance" for PGPR-elicited tolerance in plants against abiotic stress. Various reports have been published that elucidate the effect of PGPR in relieving abiotic stress in different crop plants (Hamdia and others 2004; Mayak and others 2004; Saravankumar and Samiyappan 2007; Ali and others 2009; Kohler and others 2009; Zahir and others 2009; Sandhya and others 2010). PGPR promote plant growth by changing the selectivity of Na^+, K^+, and Ca^{2+} and maintain a higher K^+/Na^+ ratio in inoculated plants under salt stress (Hamdia and others 2004). PGPR-inoculated plants show a reduction in membrane potential, enhanced lateral root development due to higher nitric acid and IAA production, accelerated osmotic adjustment, and production of ACC deaminase, which further reduces the ethylene-induced stress in plants (Dimkpa and others 2009). Only a few reports have been published on the molecular mechanism of growth promotion by PGPR inoculation under abiotic stresses. Zhang and others (2008b) reported tissue-specific regulation of *HKT1* by the soil bacterium *Bacillus subtilis* GB 03 in *Arabidopsis thaliana*.

Arachis hypogaea (L.) is an important agricultural crop grown in the semiarid region of peninsular India (Kumar and others 2004), and widely grown in the Gujarat State of India. *Arachis hypogaea* is sensitive to salinity (Leidi and others 1992). In our laboratory we had isolated several PGPR from the roots of the *Salicornia brachiata*, a commonly grown halophyte along the Gujarat coast (Gontia 2010). These bacteria revealed growth-promoting traits like indole-3-acetic acid (IAA) production, siderophore production, and ACC deaminase activity (Jha and others 2011). Therefore, in the present study we studied the interaction of these PGPR with *Arachis hypogaea* in control as well as in the presence of NaCl.

Materials and Methods

Microorganisms

Six halotolerant plant-growth-promoting rhizobacteria (PGPR) strains, namely, *Agrobacterium tumefaciens* strain JG-02, *Zhinguelliuella* strain JG-03, *Brachybacterium saurashtrense* strain JG-06, *Vibrio* strain JG-07, *Brevibacterium casei* strain JG-08, and *Haererohalobacter* strain JG-11, used in the present study were isolated earlier in our laboratory from the *Salicornia brachiata*, a succulent halophyte grown in the coastal area of Gujarat, India (Gontia 2010). These bacteria were screened for their salt tolerance capacity at different salt concentrations of 1–20% NaCl in nutrient broth media and 2–4% in Nfb media (Jha and others 2011). The 16S rRNA gene sequences of these strains have been submitted to the NCBI by the accession numbers DQ-458962 (JG-02), EU-937748 (JG-03), EU-937750 (JG-06), EU-937751 (JG-07), EU-937752 (JG-08), and EU-937754 (JG-11). Among these PGPR strains, JG-06 was proposed as a novel species of genus *Brachybacterium* and hence named as *Brachybacterium saurashtrense* (Gontia and others 2011).

Inoculation of PGPR and Salt-Stress Treatment

Arachis hypogaea cv. GG 20 seeds were collected from Gujarat Seed Corporation, Sihor, Gujarat, India. Seeds were surface sterilized with 0.1% $HgCl_2$ and subsequently rinsed four times with sterilized double-distilled water. Seeds of uniform size were placed on sterilized cotton in a tissue culture jar with ½ MS (Murashige and Skoog 1962) major and minor components for germination. Seven-day-old germinated seedlings were placed on a thermocol disk (22-cm diameter) and the disk floated in a plastic pot containing 1,000 ml ½ MS major and minor components in two sets each with 10 plants. Before growing the seedlings in hydroponic culture, initial seed weight, plumule length, and root length were recorded. PGPR were grown in ½ DYGS media at 30°C on 180 rpm for 24 h. A total of 500 µl of primary culture was inoculated in 50 ml of ½ DYGS medium and grown at 30°C on 180 rpm to 0.6 OD_{600}. Cells were collected at 5,000 rpm for 10 min and resuspended in ½ MS medium to 10^8 CFU ml^{-1} at 0.6 OD_{600}. *A. hypogaea* plants were inoculated by 50 ml of the PGPR culture in two sets. After 4 days of acclimatization with PGPR inoculation, 100 mM NaCl was added to one set and the other was kept without salt. Both inoculated and uninoculated treatments were replicated three times. The plants were grown in a culture room at 25 ± 2°C and 16-h/8-h light/dark cycle (350 µmol m^{-2} s^{-1} light intensity). Medium in each pot was changed after 7 days. Two pots

without PGPR inoculums served as uninoculated control for salt-treated and nontreated plants.

Plant Analysis

After 21 days of treatment, the growth parameters of plants were observed. Fresh weight (FW) and total length of each plant were measured. Each plant was divided into root and shoot, excluding the endosperm region, and the FW, length, and dry weight (DW) were recorded after drying in an oven at 70°C for 72 h. Percentage increase in biomass was calculated using the formula:

$$\%\ \text{increase in biomass} = (\text{plant FW} - \text{seedling weight}) / \text{plant FW} \times 100$$

Electrolyte Leakage and Percentage Change in Water Content

Electrolyte leakage was measured according to Lutts and others (1996). One young leaf from the first primary branch toward the distal end of the same size was collected from three plants for each treatment and washed thoroughly with deionized water to remove surface-adhered electrolytes. The samples were placed in closed vials containing 10 ml of deionized water and incubated at 25°C on a rotary shaker for 24 h, and the electrical conductivity of the solution (L_t) was determined using Seven Easy conductivity (Metler Toledo AG 8603, Switzerland). Samples were then autoclaved at 120°C for 20 min and the final electrical conductivity (L_0) was obtained after cooling at 25°C. The electrolyte leakage was defined as follows:

$$\text{Electrolyte leakage}\ (\%) = (L_t/L_0) \times 100$$

Percentage change in water content was calculated as [(FW – DW)/FW] × 100 (Zhang and Blumwald 2001). Each experiment was replicated three times and mean values are given in Table 3.

Lipid Peroxidation

The lipid peroxidation was estimated by determining the malondialdehyde (MDA) concentration produced by the thiobarbituric acid (TBA) reaction according to Hodges and others (1999). Leaf material (0.5 g) was homogenized in 15 ml of 80% alcohol. In one set, 1 ml of extract was mixed with 1 ml of 0.5% (w/v) TBA in 20% (w/v) TCA. In another set, TBA was excluded. The mixture was incubated at 90°C for 30 min and then cooled at room temperature. Samples were centrifuged at 4,000 rpm for 3 min and the absorbance of the supernatant was read at 400, 532, and 600 nm. The concentration of MDA was calculated with the following formulas:

$$A = [(\text{Abs}_{532+\text{TBA}} - \text{Abs}_{600+\text{TBA}}) - (\text{Abs}_{532-\text{TBA}} - \text{Abs}_{600-\text{TBA}})]$$

$$B = [(\text{Abs}_{440+\text{TBA}} - \text{Abs}_{600-\text{TBA}})\,0.0571]$$

$$\text{MDA equivalents}\ (\text{nmol g}^{-1}) = (A - B/15,700)10^6$$

Auxin Analysis

Auxin analysis was carried out according to Andreae and Ysselstein (1959) using the alcoholic extract of shoot and root tissue. One milliliter of alcoholic extract was mixed with 2 ml of Salkowski reagent and incubated at room temperature in the dark for 20 min followed by the spectrophotometric analysis at 535 nm (Andreae and Ysselstein 1956).

Biochemical Analysis

Free proline content in the leaves was determined by using ninhydrin (Bates and others 1973) with minor modifications. Plant tissue (100 mg) was homogenized in 1.2 ml of 3% aqueous sulfosalicylic acid and centrifuged at 13,000 rpm for 10 min. After centrifugation, 500 µl of supernatant was made up to 1 ml with distilled water and reacted with 1 ml of 2% ninhydrin. The mixture was incubated at 90°C for 1 h. The samples were cooled in an ice bath and 2 ml of toluene was added and vortexed for 2 min. The upper phase was aliquoted to read the absorbance at 520 nm in a T80 + UV–Vis spectrophotometer (PG Instrument Ltd., UK). The proline content was calculated by comparing the absorbance with a standard curve drawn from known concentrations of L-proline (Sigma Aldrich, USA) and expressed as µg/mg FW. Total soluble sugars were analyzed by treating 0.1 ml of the alcoholic extract with 3 ml of freshly prepared anthrone reagent [150 mg anthrone in 100 ml of 72% (v/v) H_2SO_4] and placed in a boiling water bath for 10 min according to Irigoyen and others (1992). After cooling, the absorbance at 620 nm was determined using the T80 + UV–Vis spectrophotometer. The calibration curve was made using glucose (Sigma Aldrich, USA) in the range 20–400 µg/ml. Total amino acid content was determined as described by Sandhya and others (2010). One milliliter of the plant extract was treated with 1 ml of 0.2 M citrate buffer (pH 5), 1 ml of 80% ethanol, and 1 ml of ninhydrin (1%) followed by incubation at 95°C for 15 min. The samples were cooled and absorbance was read at 570 nm (Chen and others 2007) using the T80 + UV–Vis spectrophotometer. Total protein content was determined by using 0.5 g homogenized plant tissue in 2 ml of extraction buffer [50 mM Tris–HCl (pH 8.3), 1 mM EDTA, 3 mM DTT, 0.08% ascorbic acid, 1 mM PMSF]. The protein was quantified by the Bradford method (Bradford 1976). For analysis of ion content, 0.2 g of plant tissue was digested with 4 ml of perchloric acid and

nitric acid solution (3:1). The solution was dried on a hot plate and further made to 25 ml with deionized water and filtered through a 0.2-μm filter. Ion content was measured by an inductively coupled plasma optical emission spectrometer (Optima 2000DV, PerkinElmer, USA). The N content was determined by the semi-micro Kjeldahl method [AOAC 1995; method No. Ba 4b-87(90)] after the plant tissue was oxidized and decomposed by sulfuric acid with digestion mixture (K_2SO_4:$CuSO_4$ = 5:1).

Statistical Analysis

Each experiment was repeated three times and the data of ten plants were recorded. The two-factor ANOVA with replicates was carried out using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The critical difference (C.D.) values were calculated at the $p = 0.05$ level to find out the significant differences between the means of different bacterial treatments at both control and 100 mM NaCl stress. The significantly different mean values are indicated by different letters.

Results

Effect of PGPR on Plant Growth

The salt sensitivity assay showed that *Arachis hypogaea* cv. GG 20 seedling growth was affected at 50 mM NaCl and further retarded severely at 100 mM NaCl; therefore, we have selected 100 mM NaCl concentration for all the experiments. The present study showed that *Arachis hypogaea* cv. GG 20 can tolerate NaCl stress in hydroponic culture more efficiently when inoculated with halotolerant PGPR as compared with controls (Fig. 1). All the PGPR showed better plant growth in the hydroponic culture without NaCl. When NaCl was added, the plant length, shoot length, and root length were affected severely in the case of uninoculated plants, whereas growth was less reduced in inoculated plants. In the presence of JG-02, JG-06, and JG-08, the growth was significantly better compared to that with other strains (Tables 1, 8). Plants inoculated with JG-03, JG-07, and JG-11 did not show significantly greater growth, but it was greater in comparison to control plants (Table 1).

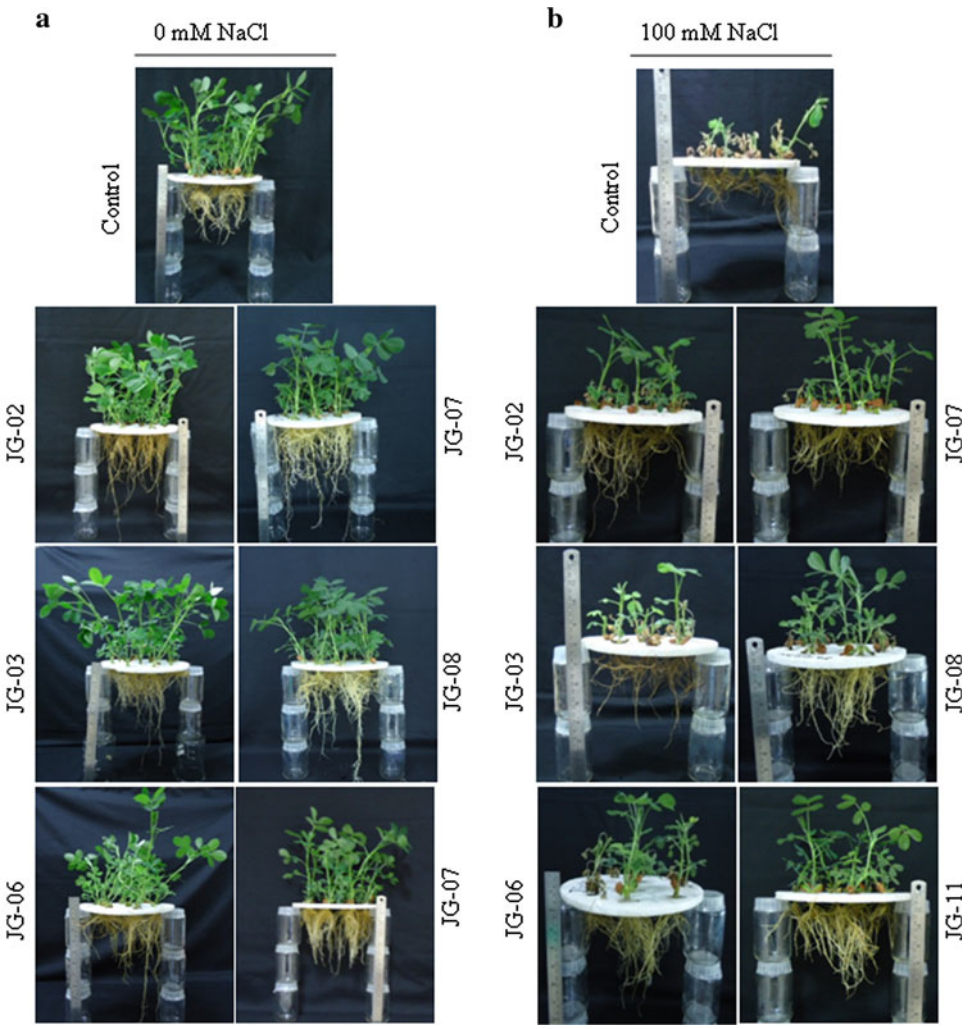

Fig. 1 Twenty-one-day-old *Arachis hypogaea* seedlings grown in ½ MS liquid medium in the presence of different PGPR strains **a** without NaCl and **b** with 100 mM NaCl

Table 1 Effect of various plant-growth-promoting rhizobacteria on plant length, shoot length, and root length under control and NaCl-stress conditions

PGPR strain	Plant length (cm)		Shoot length (cm)		Root length (cm)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Control	39.175 ± 3.376^{a}	16.080 ± 6.973^{b}	22.708 ± 2.88^{a}	05.900 ± 2.44^{b}	15.508 ± 1.606^{a}	06.133 ± 2.588^{b}
JG-02	45.125 ± 5.099^{a}	26.292 ± 8.376^{bc}	26.192 ± 2.77^{a}	12.758 ± 4.82^{c}	18.508 ± 5.705^{a}	13.375 ± 3.856^{a}
JG-03	41.908 ± 4.223^{a}	23.492 ± 4.178^{bc}	24.925 ± 2.652^{a}	11.558 ± 3.90^{bc}	16.942 ± 3.453^{a}	11.750 ± 2.984^{ba}
JG-06	43.275 ± 7.756^{a}	27.875 ± 4.658^{ac}	26.408 ± 3.107^{a}	12.842 ± 2.28^{c}	19.617 ± 6.928^{a}	16.200 ± 3.842^{a}
JG-07	48.583 ± 8.639^{a}	24.125 ± 5.540^{b}	20.292 ± 3.702^{a}	10.725 ± 3.83^{bc}	17.833 ± 7.218^{a}	13.108 ± 3.031^{ba}
JG-08	47.142 ± 8.10^{a}	27.483 ± 5.399^{c}	26.892 ± 3.638^{a}	12.65 ± 3.781^{c}	19.750 ± 6.824^{a}	13.983 ± 2.381^{a}
JG-11	42.708 ± 5.38^{a}	25.700 ± 4.531^{bc}	23.192 ± 4.126^{a}	12.54 ± 2.777^{c}	17.583 ± 5.050^{a}	12.783 ± 3.103^{ba}

Data are given as mean ± SD of three replicates; means followed by *same letters* are not significant at 5% level

Table 2 Effect of various plant-growth-promoting rhizobacteria on percentage increase in total plant biomass, shoot dry weight, and root dry weight under control and NaCl-stress conditions

PGPR strain	% increase in total plant biomass		Shoot dry weight (g)		Root dry weight (g)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Control	72.30 ± 3.55^{ac}	36.56 ± 6.02^{b}	0.453 ± 0.079^{a}	0.098 ± 0.043^{b}	0.0926 ± 0.0198^{a}	0.05260 ± 0.0266^{b}
JG-02	76.60 ± 3.40^{a}	44.76 ± 9.00^{b}	0.595 ± 0.075^{a}	0.269 ± 0.161^{c}	0.1003 ± 0.0148^{a}	0.09042 ± 0.0239^{ab}
JG-03	77.26 ± 4.55^{a}	46.90 ± 8.18^{bd}	0.544 ± 0.197^{a}	0.189 ± 0.184^{bc}	0.1059 ± 0.0408^{a}	0.07900 ± 0.0257^{ab}
JG-06	76.35 ± 3.03^{a}	61.23 ± 9.77^{cd}	0.617 ± 0.071^{a}	0.278 ± 0.088^{c}	0.1150 ± 0.0425^{a}	0.10967 ± 0.0328^{ac}
JG-07	73.64 ± 3.98^{ac}	50.77 ± 7.84^{d}	0.443 ± 0.116^{a}	0.171 ± 0.119^{bc}	0.1471 ± 0.0248^{a}	0.09250 ± 0.0220^{abc}
JG-08	75.98 ± 3.73^{a}	51.64 ± 11.25^{d}	0.487 ± 0.099^{a}	0.274 ± 0.092^{c}	0.1037 ± 0.0337^{a}	0.10750 ± 0.0281^{abc}
JG-11	75.47 ± 4.18^{a}	56.91 ± 10.20^{d}	0.5183 ± 0.135^{a}	0.268 ± 0.109^{c}	0.1053 ± 0.0283^{a}	0.11892 ± 0.0480^{ac}

Data are given as mean ± SD of three replicates; mean followed by *same letters* are not significant at 5% level

Plant biomass was increased at 0 mM NaCl in the presence of all the PGPR; however, at 100 mM NaCl (Table 2), plants inoculated with JG-06, JG-07, JG-08, and JG-11 showed significantly higher biomass. At 0 mM NaCl, the shoot dry weight of inoculated plants was not significantly increased compared to uninoculated plants. However, an increase of 7.5–36% in shoot dry weight was observed in the presence of all PGPR except JG-07. At 100 mM NaCl, JG-02, JG-06, JG-08, and JG-11 yielded significantly increased shoot dry weights. Similar to the shoot dry weight, none of the bacteria showed significantly higher root weight at 0 mM NaCl; however, an increase of 8.31–58.85% was observed in the presence of all the PGPR. In the presence of 100 mM NaCl, JG-06 and JG-11 showed significantly higher root weights (Table 2).

Plants generally show increased electrolyte leakage when exposed to salt stress. In the present study we examined electrolyte leakage under salt stress in inoculated and uninoculated plants (Table 3). Electrolyte leakage was less at 0 mM NaCl and at 100 mM NaCl in the presence of PGPR. JG-03 and JG-06 yielded a significant reduction in electrolyte leakage followed by JG-11, whereas reduction in electrolyte leakage was not significant in plants inoculated with JG-02, JG-07, and JG-08. MDA content was significantly reduced in plants inoculated with JG-02, JG-06, JG-07, and JG-11 at both 0 and 100 mM NaCl compared with uninoculated plants (Table 3). All strains showed no significant increase in percentage water content (PWC) in shoot and root tissue at 0 mM NaCl. At 100 mM NaCl, inoculated plants showed higher PWC in shoot tissue than uninoculated plants. JG-02 showed a maximum increase in water content in shoots. In the case of root tissue under 100 mM NaCl, the increase in PWC was the highest in plants inoculated with JG-06 and JG-11 followed by JG-02, JG-03, JG-07, and JG-08 (Table 3).

Effect of PGPR Inoculation on Biochemical Parameters of Plants

Salinity significantly enhanced Na^+ concentrations and decreased the Ca^{2+}, P and K^+ concentrations in *A. hypogaea* shoots. Ca^{2+} content was significantly higher at 0 and 100 mM NaCl in JG-06-, JG-07- and JG-08-inoculated plants. Higher Na^+ and less K^+ content was observed in

Table 3 Effect of various plant-growth-promoting rhizobacteria on electrolyte leakage of foliage, percentage change in water content of shoots and roots, and MDA equivalents in foliage under control and NaCl-stress conditions

PGPR strain	Electrolyte leakage (%)		Percentage water content (shoot)		Percentage water content (root)		MDA equivalents (nmol g^{-1} FW)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Control	32.40 ± 09.29^{a}	55.02 ± 05.15^{d}	87.430 ± 1.374^{a}	57.739 ± 12.645^{b}	90.739 ± 1.946^{a}	87.091 ± 4.411^{b}	20.619 ± 2.18^{a}	58.758 ± 0.35^{d}
JG-02	30.62 ± 11.79^{ab}	52.67 ± 09.52^{de}	87.930 ± 1.726^{a}	85.242 ± 04.092^{ac}	92.038 ± 0.850^{a}	90.036 ± 0.975^{a}	09.440 ± 0.489^{b}	42.507 ± 3.69^{e}
JG-03	25.72 ± 05.47^{c}	48.70 ± 04.13^{ef}	87.421 ± 1.428^{a}	75.601 ± 08.707^{ac}	91.300 ± 1.622^{a}	90.251 ± 1.417^{a}	20.472 ± 3.79^{a}	26.414 ± 4.29^{f}
JG-06	27.26 ± 04.46^{bc}	46.71 ± 02.76^{f}	88.101 ± 1.822^{a}	81.214 ± 05.284^{ac}	91.023 ± 1.845^{a}	91.118 ± 1.102^{a}	09.462 ± 0.43^{b}	34.784 ± 0.51^{g}
JG-07	28.57 ± 03.34^{ac}	52.87 ± 08.82^{de}	88.414 ± 1.300^{a}	78.673 ± 07.213^{ac}	92.688 ± 1.175^{a}	90.655 ± 1.405^{a}	08.069 ± 0.04^{b}	42.602 ± 1.76^{e}
JG-08	29.44 ± 09.24^{ac}	50.64 ± 09.03^{de}	87.976 ± 1.269^{a}	84.706 ± 06.686^{ac}	92.548 ± 1.119^{a}	90.758 ± 1.560^{a}	19.038 ± 1.74^{a}	43.012 ± 0.62^{e}
JG-11	29.04 ± 01.04^{ac}	45.17 ± 02.97^{f}	87.612 ± 1.638^{a}	74.456 ± 10.826^{ac}	91.664 ± 0.810^{a}	91.402 ± 1.825^{a}	16.371 ± 9.12^{d}	27.397 ± 0.16^{f}

Data are given as mean ± SD of three replicates; mean followed by *same letters* are not significant at 5% level

uninoculated plants at 100 mM NaCl, whereas plants inoculated by JG-06 and JG-08 showed maximum reduced Na^+ concentration. The K^+ concentration and K^+/Na^+ ratio were higher at both 0 and 100 mM NaCl in the presence of all the PGPR (Table 4). Under salt stress, the PGPR-treated plants had almost double the K^+/Na^+ ratio compared to uninoculated plants. Phosphorus content was significantly higher in JG-06- and JG-08-inoculated plants at 0 mM NaCl. However, at 100 mM NaCl, all the strains increased phosphorus content in inoculated plants from 1.33 to 2.79 times (Table 5) Nitrogen content was not changed significantly in inoculated plants without NaCl, whereas in the presence of 100 mM NaCl, plants inoculated with JG-02, JG-06, and JG-08 showed significantly higher nitrogen content compared to uninoculated plants (Table 5).

The response of *A. hypogaea* to salt stress in the presence of PGPR was studied by determining the biochemical status of the plants in terms of proline content, total sugars, total amino acids, total protein, and auxin content. The proline content in the shoots of *A. hypogaea* was significantly reduced in stressed condition when inoculated with all the PGPR. There was a significant decline in total soluble saccharides in the shoots of *A. hypogaea* in the presence of all the strains at both 0 and 100 mM NaCl except JG-11, which showed a significant decline in total soluble saccharides only under salt stress (Table 6). Inoculation by all strains improved amino acid concentration in the shoots of *A. hypogaea* under nonstressed as well as stressed conditions. Plants inoculated with JG-06 showed a maximum increase in amino acid content at 100 mM NaCl (Table 6). Total protein content was significantly higher in plants inoculated with JG-02, JG-06, and JG-08 at both 0 and 100 mM NaCl (Table 6). All PGPR inoculations showed enhanced auxin content in both shoots and roots at 100 mM NaCl compared to uninoculated plants (Table 7).

Discussion

Salinity adversely affects the growth and yield of several crop plants. However, interaction of PGPR with several crops in saline conditions reduces the extent of poor growth and thus helps plants survive in adverse conditions. Our results suggest that PGPR promote better growth of plants under salt treatment. Plants inoculated with JG-06, JG-08, and JG-11 showed good growth and higher salt tolerance than plants inoculated with other strains. The shoot and root lengths were significantly decreased in uninoculated plants under salt stress, whereas in the presence of PGPR their lengths increased significantly. The greater root length of plants is probably due to the availability of higher auxin concentrations. PGPR are known to release IAA and the

Table 4 Effect of various plant-growth-promoting rhizobacteria on Ca^{2+}, Na^{+}, K^{+} ion concentrations and K^{+}/Na^{+} ratios in shoot tissue under control and NaCl-stress conditions

PGPR strain	Ca^{2+} (mg g^{-1} DW)		K^{+} (mg g^{-1} DW)		Na^{+} (mg g^{-1} DW)		K^{+}/Na^{+}	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Control	11.66 ± 1.58^{a}	07.115 ± 0.191^{d}	29.66 ± 1.55^{a}	9.351 ± 1.38^{d}	4.33 ± 0.601^{a}	30.25 ± 0.502^{b}	$06.89 \pm .601^{a}$	0.308 ± 0.041^{d}
JG-02	11.95 ± 2.76^{a}	07.034 ± 0.859^{d}	$33.28 \pm 0.78b^{c}$	15.58 ± 3.52^{e}	4.16 ± 1.159^{a}	29.21 ± 2.091^{be}	08.35 ± 2.52^{b}	0.531 ± 0.083^{de}
JG-03	13.28 ± 0.46^{b}	07.378 ± 2.152^{d}	31.94 ± 5.74^{c}	15.07 ± 0.115^{e}	3.69 ± 0.162^{a}	23.57 ± 0.716^{c}	08.64 ± 1.179^{b}	0.64 ± 0.024^{d}
JG-06	14.42 ± 0.76^{c}	10.960 ± 1.131^{c}	34.32 ± 2.52^{bc}	18.67 ± 0.77^{f}	3.36 ± 0.552^{a}	23.29 ± 5.150^{c}	10.29 ± 0.94^{c}	0.825 ± 0.215^{e}
JG-07	13.18 ± 0.49^{b}	08.50 ± 0.622^{f}	33.57 ± 0.27^{b}	15.532 ± 1.92^{e}	4.14 ± 0.068^{a}	25.19 ± 3.660^{d}	08.11 ± 0.199^{b}	0.62 ± 0.166^{de}
JG-08	14.97 ± 1.36^{c}	09.57 ± 1.421^{g}	29.75 ± 3.60^{a}	16.59 ± 1.37^{e}	3.66 ± 0.417^{a}	22.45 ± 3.465^{c}	08.13 ± 0.119^{b}	0.753 ± 0.177^{e}
JG-11	11.87 ± 0.93^{a}	07.28 ± 1.158^{d}	31.54 ± 2.61^{b}	16.04 ± 0.71^{e}	3.69 ± 0.705^{a}	28.39 ± 2.430^{e}	08.64 ± 0.945^{b}	0.566 ± 0.023^{de}

Data are given as mean ± SD of three replicates; mean followed by *same letters* are not significant at 5% level

Table 5 Effect of various plant-growth-promoting rhizobacteria on concentration of phosphorus and nitrogen in shoot tissue under control and NaCl-stress conditions

PGPR strain	Phosphorus (mg g^{-1} DW)		Nitrogen (mg g^{-1} DW)	
	0 mM	100 mM	0 mM	100 mM
Control	13.120 ± 0.325^{a}	2.89 ± 0.175^{d}	70.026 ± 1.30^{a}	59.076 ± 5.86^{c}
JG-02	13.498 ± 0.667^{a}	4.71 ± 0.028^{f}	69.120 ± 6.95^{a}	62.380 ± 9.98^{d}
JG-03	12.945 ± 0.219^{a}	3.86 ± 0.289^{e}	64.266 ± 1.97^{b}	61.834 ± 1.36^{dc}
JG-06	13.690 ± 1.382^{b}	8.05 ± 0.077^{g}	70.061 ± 1.22^{a}	63.938 ± 3.90^{d}
JG-07	13.175 ± 0.071^{a}	4.09 ± 0.622^{e}	60.674 ± 3.59^{b}	60.622 ± 9.86^{dc}
JG-08	14.540 ± 2.276^{c}	7.62 ± 0.608^{g}	68.403 ± 3.77^{a}	62.544 ± 0.392^{d}
JG-11	13.074 ± 0.072^{a}	5.20 ± 0.071^{f}	69.109 ± 1.59^{a}	61.639 ± 0.096^{dc}

Data are given as mean ± SD of three replicates; mean followed by *same letters* are not significant at 5%

Table 6 Effect of various plant-growth-promoting rhizobacteria on proline content, total soluble sugar, total amino acid, and total protein contents in shoot tissue under control and NaCl-stress conditions

Treatment	Proline content (μg g^{-1} FW)		Total soluble sugar (μg g^{-1} FW)		Total amino acid (μM)		Total protein content (mg g^{-1} FW)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Control	0.1195 ± 0.036^{a}	2.722 ± 0.225^{d}	4.5990 ± 0.0834^{a}	10.366 ± 0.8626^{e}	357.74 ± 29.97^{a}	551.14 ± 10.98^{d}	07.997 ± 0.614^{a}	07.583 ± 1.957^{a}
JG-02	0.2933 ± 0.145^{b}	0.259 ± 0.036^{b}	1.8675 ± 0.0148^{b}	8.5460 ± 0.0141^{f}	462.57 ± 11.61^{b}	708.53 ± 05.29^{e}	14.501 ± 03.14^{b}	26.367 ± 1.206^{g}
JG-03	0.0174 ± 0.008^{c}	2.567 ± 0.329^{e}	3.7535 ± 2.546^{c}	5.8170 ± 0.2291^{g}	397.13 ± 1.57^{c}	732.94 ± 35.02^{e}	07.970 ± 0.295^{a}	10.233 ± 0.028^{d}
JG-06	0.0501 ± 0.014^{ac}	0.526 ± 0.041^{f}	2.8715 ± 0.0388^{d}	8.6945 ± 0.7912^{f}	449.26 ± 35.19^{b}	887.80 ± 32.35^{f}	09.196 ± 01.24^{c}	12.240 ± 1.492^{dh}
JG-07	0.1868 ± 0.034^{a}	0.086 ± 0..017^{ac}	3.4535 ± 0.5692^{c}	9.0245 ± 0.7643^{h}	452.81 ± 14.19^{b}	880.36 ± 49.05^{f}	08.255 ± 0.925^{a}	17.119 ± 1.124^{f}
JG-08	0.2284 ± 0.149^{b}	0.515 ± 0.159^{f}	3.7495 ± 0.2736^{c}	5.4815 ± 0.0445^{g}	468.42 ± 26.26^{b}	701.21 ± 39.90^{e}	11.107 ± 01.15^{d}	11.299 ± 0.999^{d}
JG-11	0.2875 ± 0.055^{b}	0.833 ± 0.310 ^{g}	4.2740 ± 0.1004^{a}	9.4735 ± 0.5748^{h}	448.26 ± 12.32^{b}	808.69 ± 24.31^{f}	06.143 ± 01.79^{e}	15.766 ± 3.602^{b}

Data are given as mean ± SD of three replicates; mean followed by *same letters* are not significant at 5% level

interaction of IAA with plants had been reported to result in higher root length (Patten and Glick 2002; Rajkumar and others 2005; Chakraborty and others 2006). Most of the PGPR-inoculated plants with or without NaCl showed higher auxin content in both shoots and roots (Table 7). PGPR-inoculated plants showed a higher percentage increase in biomass under saline conditions than did uninoculated plants (Table 2). Sodium chloride reduces the mean shoot and root dry weights in control plants, but in the presence of PGPR the shoot and root dry weights were higher in inoculated plants than in controls under salt stress. Similar results were reported for salt tolerance in various plants induced by PGPR (Hamdia and others 2004; Mayak and others 2004; Saravankumar and Samiyappan 2007; Zahir and others 2009). Electrolyte leakage is used to measure the membrane permeability of the cell. Salt stress leads to damage to the plant cell membrane and hence increases its permeability. As a result, the electrolytes present within the membrane leak and accumulate in the surrounding tissues (Wu 2009). In the present study, PGPR-inoculated plants under salt stress showed significantly low electrolyte leakage from shoot tissue, suggesting that PGPR protect the integrity of the plant cell membrane from the detrimental effect of salt (Table 3). One reason for this may be a change in the saturation pattern of membrane phospholipids upon PGPR inoculation, which in turn causes a reduction in membrane potential (Dimkpa and others 2009). Similarly, Bano and Fatima (2009) have reported low electrolyte leakage in *Zea mays* by the inoculation of *Rhizobium* and *Pseudomonas*. *Pseudomonas* inoculation also showed low ion leakage in maize plants under drought stress (Sandhya and others 2010). Malondialdehyde (MDA) is produced as a result of decomposition of polyunsaturated fatty acids of biomembranes and shows greater accumulation under salt stress (Gossett and others 1994). Therefore, MDA is a reflection of the extent of stress induced as well as peroxidative damage caused by reactive oxygen species (Jain and others 2001). In the present study we showed PGPR-mediated reduction in MDA content in inoculated plants compared to uninoculated plants. Thus, in addition to reduced electrolyte leakage, PGPR also prevent plants from oxidative damage caused by salt stress.

Salt stress in plants reduces the osmotic potential of the growth medium and affects water availability, causing physiological drought in plants. In the control plants, the percentage increase in the water content in roots and shoots was significantly low, whereas inoculated plants showed a higher percentage of water content (Table 3). PGPR help the plant by removing the physiological drought under salt stress and increase the water content in the cell. Kohler and others (2009) have observed higher hydration by the interaction of the PGPR *Pseudomonas mendocina* Palleroni

Table 7 Effect of various plant-growth-promoting rhizobacteria on auxin content in shoot and root tissue under control and NaCl stress

PGPR strain	Shoot auxin content (μmol g^{-1} FW)		Root auxin content (μmol g^{-1} FW)	
	0 mM	100 mM	0 mM	100 mM
Control	4.74 ± 0.54^{a}	3.96 ± 0.12^{c}	1.35 ± 0.04^{a}	2.67 ± 0.09^{a}
JG-02	12.78 ± 2.03^{b}	12.91 ± 2.25^{b}	3.63 ± 0.09^{abc}	5.36 ± 0.83^{bc}
JG-03	3.34 ± 1.31^{c}	10.72 ± 0.10^{g}	1.57 ± 0.13^{a}	6.07 ± 0.27^{c}
JG-06	6.17 ± 0.60^{d}	26.37 ± 0.26^{h}	2.84 ± 0.09^{ab}	22.26 ± 5.94^{d}
JG-07	6.35 ± 0.01^{d}	4.62 ± 0.36^{a}	4.085 ± 0.07^{bc}	4.28 ± 0.24^{bc}
JG-08	2.65 ± 0.51^{e}	5.85 ± 0.012^{d}	3.92 ± 0.05^{bc}	2.70 ± 0.32^{ab}
JG-11	7.71 ± 0.82^{f}	8.39 ± 0.15^{f}	4.10 ± 0.09^{bc}	5.65 ± 0.29^{c}

Data are given as mean ± SD of three replicates; mean followed by *same letters* are not significant at 5% level

Table 8 Performance of plants on MS liquid medium with or without 100 mM NaCl and in the presence of different bacterial inoculums

S. no.	Bacterial isolates	Plant length	Shoot length	Root length	% increase in total plant biomass	Shoot dry weight	Root dry weight
1	JG02	↑	↑	↑		↑	
2	JG03					↑	
3	JG06	↑	↑	↑	↑	↑	↑
4	JG07				↑	↑	
5	JG08	↑	↑	↑	↑	↑	
6	JG11		↑		↑	↑	↑

↑, ↓ denote significant increase or decrease at 100 mM NaCl compared to nonbacterial incubation; a blank entry shows that data were not statistically significant at 5% level

in inoculated plants compared to control plants. The higher hydration in inoculated plants leads to better water use efficiency and thus improves photosynthesis and higher biomass (Mayak and others 2004).

Salinity causes an imbalance in the ratio of ion homeostasis in the plant system. Plants try to maintain low salt composition in the cytosol by extrusion through the plasma membrane using the *SOS* pathway or by scavenging in the vacuole through *NHX1* antiporters. Salinity impedes the ratio of Ca^{2+} and K^{+} in the cell. However, an increase in K^{+} concentration can alleviate the deleterious effect of salinity on growth and yield (Giri and others 2007). In the present study we observed a low Na^{+} content and a higher K^{+} content in the presence of PGPR under salinity. Therefore, a higher K^{+}/Na^{+} ratio in plants under salt stress due to the restricted Na^{+} uptake and enhanced K^{+} uptake was observed. Similarly, several PGPR are reported to reduce the salt toxicity in various other plants by lowering the Na^{+} concentration and increasing the K^{+} concentration (Hamdia and others 2004; Nadeem and others 2006; Yildirim and others 2006; Bano and Fatima 2009; Kohler and others 2009).

It has been shown that Ca^{2+} plays a major role as an early signaling molecule at the onset of salinity. In this study, three PGPR significantly increased Ca^{2+} content compared to control plants, and it is evident from the overall performance chart (Table 8) that plants inoculated with these PGPR showed better salt tolerance also. Salinity is responsible for the depletion of available phosphorus from the soil (Richardson and others 2009). Phosphate-solubilizing PGPR have a tendency to solubilize precipitated forms of phosphorus and therefore play an important role in providing phosphorus to the plant system (Kucey and others 1989; Gyaneshwar and others 2002). In this study, PGPR yielded increased phosphorus and nitrogen concentrations in inoculated plants at 100 mM NaCl compared to uninoculated plants. This shows that PGPR strains could improve plant growth by helping to increase nutrient uptake. A similar result was reported by Kohler and others (2009), Bano and Fatima (2009), Nadeem and others (2006), and Han and Lee (2005).

Proline and total soluble sugars are very important biochemical indicators of salinity tolerance in plants (Ashraf and Harris 2004). When plants face salt stress, proline accumulates in the cytosol and helps substantially in cytoplasmic osmotic adjustment (Leigh and others 1981). Proline also helps the plant cell by stabilizing subcellular structures such as membranes and proteins, scavenging free radicals, and buffering cellular redox potential under salt stress (Ashraf and Foolad 2007). In this study, PGPR-treated plants showed low proline content under 100 mM NaCl stress compared to uninoculated plants, but

Table 9 Significant performance of plants on MS liquid medium with or without 100 mM NaCl and in the presence of different bacterial inoculums

S. no.	Bacterial isolates	Electrolyte leakage (%)	PWC (shoot)	PWC (root)	Proline content	MDA content	Total soluble sugar	Total amino acid	Protein content	Auxin content		Na^+	K^+	K^+/Na^+	Ca^{++}	P	N
										Shoot	Root						
1	JG02		←	←	◢↓	▼↓	▼↓	◢↑	◢↑	◢↑	↑		◢↑	◢		↑	←
2	JG03	▼↓	←	←	▼↓	→	▼↓	◢↑	←	▼↑	←	→	◢↑	◢	◢	←	▶
3	JG06	▼↓	←	←	→	▼↓	▼↓	◢↑	◢↑	◢↑	←	→	◢↑	◢↑	◢↑	◢↑	←
4	JG07		←	←	→	▼↓	▼↓	◢↑	←	◢↑	◢↑	→	◢↑	◢	◢↑	◢↑	▶
5	JG08		←	←	◢↓	→	▼↓	◢↑	◢↑	▼↑	◢	→	←	◢↑	◢↑	←	←
6	JG11	→	←	←	◢↓	▼↓	→	◢↑	▼↓	◢↑	◢↑	→	◢↑	◢	←	←	

◢, ▼ denote significant increase or decrease at 0 mM NaCl compared to nonbacterial incubation; ↑, ↓ denote significant increase or decrease at 100 mM NaCl compared to nonbacterial incubation; a blank entry shows that data were not statistically significant at 5% level

the level was higher than the basal level of proline in uninoculated plants under no salt stress. This suggests that PGPR-treated plants do not face much salt stress, therefore, the proline accumulation is less in the presence of PGPR. Soluble sugars constitute about 50% of the total osmotic potential in plant cells during salt stress (Cram 1976). Similar to proline, the soluble sugars in inoculated plants are lower than in uninoculated plants. Different amino acids and nitrogen-containing compounds are accumulated in higher plants under salinity stress (Gilbert and others 1998; Mansour 2000; Ashraf and Harris 2004). Total free amino acids are reported to be high in the foliar parts of different salt-tolerant plants such as sunflower, safflower, *Eruca sativa*, *Medicago sativa*, and *Lens culinaris* (Fougere and others 1991; Hurkman and others 1991; Ashraf 1994; Ashraf and Fatima 1995; Ashraf and Tufail 1995). In this study, PGPR-inoculated plants showed enhanced total amino acid accumulation with or without salt stress compared to uninoculated plants. Higher amino acid contents were observed under drought stress in the presence of plant-growth-promoting *Pseudomonas* sp. (Sandhya and others 2010). The protein content was also higher in inoculated plants growing with or without NaCl. Few of these proteins may have direct correlation with salinity tolerance. Similarly, many proteins directly or indirectly related to plant growth promotion were differentially found expressed in rice plants by the interaction of *Pseudomonas fluorescens* (Kandasamy and others 2009).

In conclusion, these data suggest that the PGPR tested in the present study can play a pivotal role in conferring salt tolerance in plants. From the performance charts (Tables 8, 9), it is evident that all PGPR promote plant growth under salt stress. Among them, *Brachybacterium saurashtrense* strain JG-06, *Brevibacterium casei* strain JG-08, and *Haererohalobacter* strain JG-11 are the best strains in terms of growth promotion under salt stress. *Brachybacterium saurashtrense* showed better growth in *Salicornia brachiata* (Amaranthaceae) also under saline conditions (Jha and others 2011). However, this has to be tested with many other crops to confirm the ability of this PGPR to confer salt tolerance under field conditions. Therefore, it is plausible to mention that PGPR are an effective approach for improving the growth of salt-sensitive plants and this strategy could be applied for sustainable agriculture.

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