Impact of soil salinity on the plant-growth – promoting and biological control abilities of root associated bacteria

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ABSTRACT

The effectiveness of plant growth – promoting bacteria is variable under different biotic and abiotic conditions. Abiotic factors may negatively affect the beneficial properties and efficiency of the introduced PGPR inoculants. The aim of this study was to evaluate the effect of plant growth – promoting rhizobacteria on plant growth and on the control of foot and root rot of tomatoes caused by Fusarium solani under different soil salinity conditions. Among the five tested strains, only Pseudomonas chlororaphis TSAU13, and Pseudomonas extremorientalis TSAU20 were able to stimulate plant growth and act as biological controls of foot and root rot disease of tomato. The soil salinity did not negatively affect the beneficial impacts of these strains, as they were able to colonize and survive on the roots of tomato plants under both saline and non-saline soil conditions. The improved plant height and fruit yield of tomato was also observed for plants inoculated with P. extremorientalis TSAU20. Our results indicated that, saline condition is not crucial factor in obtaining good performance with respect to the plant growth stimulating and biocontrol abilities of PGPR strains. The bacterial inoculant also enhanced antioxidant enzymes activities thereby preventing ROS induced oxidative damage in plants, and the proline concentrations in plant tissue that play an important role in plant stress tolerance.

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1. Introduction

The increase in harsh abiotic stresses such as drought, salinity and abrupt changes in temperature are part of the main consequences of climate change. These stresses have led to loss of soil organic matter and other forms of soil degradation that negatively affect agricultural productivity (Ahmad et al., 2015). Another important consequence of climate change and abiotic stresses is the increased infection/infestation of plants by pathogens and pests (Chakraborty, 2013). Intensive research attempts are underway to improve plant growth, and tolerance to various abiotic stresses, and to protect plants from soil borne pathogens using plant growth promoting rhizobacteria (PGPR) which have great potential for sustainable crop production (Lugtenberg and Kamilo, 2009; Berg and Martinez, 2015; Egamberdieva et al., 2015a, 2015b, 2016).

Root associated microbes, including endophytes, closely cooperate with each other and can mediate important physiological processes, especially nutrient acquisition and plant fitness to abiotic stresses (Berg et al., 2013; Abd_Allah et al., 2015). Plants inoculated with PGPR produce more root hairs and take up mineral and microelements more efficiently from the soil. The growth of several plants is enhanced by PGPR treatment, e.g. lentil (Lens culinaris) (Faisal, 2013), pea (Pisum sativum L) (Meena et al., 2015), cucumber (Cucumis sativus), (Egamberdieva et al., 2011), rice (Oryza sativa) (Yadav et al., 2014) and soybean (Glycine max) (Egamberdieva et al., 2015b). PGPR also induces systemic tolerance...
to various abiotic stresses in plants such as salinity, drought and heavy metals through alteration of plant physiology (Wang et al., 2012). The beneficial traits of plant growth promoting bacteria include the ability to synthesize biological active compounds such as plant growth stimulators (Parry et al., 2016; Egamberdieva et al., 2017), osmolytes (Berg et al., 2013), antifungal compounds (Landa et al., 2004), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme (Ali et al., 2014). The strain Pseudomonas was able to suppress soybean root disease caused by fungal pathogens (Susilowati et al., 2010) and showed antagonistic activity against several fungal pathogens, such as Fusarium oxysporum, and Rhizoctonia solani. In another study, P. agglomerans and Bacillus sp. reduced the charcoal root rot of soybean caused by Macrophomina phaseolina under greenhouse conditions (Vasebi et al., 2013). An induced systemic resistance in plants against foliar pathogens by PGPR was also reported (Choudhary et al., 2007a, 2007b). In addition some reports have suggested that some PGPR induces systemic tolerance (IST) in plants through elevated antioxidant responses at the levels of enzyme activity and metabolite accumulation (Hashem et al., 2015, 2016; Jha et al., 2011). The antioxidant defence system plays a major role in plant adaptation to salinity stress that allow the scavenging of reactive oxygen species (ROS) (Ahanger et al., 2014).

The variability in the effectiveness of biologicals is of concern when used under different conditions or cropping systems. Hostile environmental conditions are deleterious for the root associated microbiome and effective functioning of the introduced PGPR inoculants (Landa et al., 2004). In earlier studies Landa et al. (2001) observed an effect of temperature on plant growth and biological control ability of PGPR Pseudomonas fluorescens. In their study Fusarium wilt of chickpea was suppressed by these rhizobacteria at 30 °C, but not at 25 °C at which temperature disease potential was high. Interestingly this suppression was related to the production of extracellular metabolites that inhibit F. oxysporum, and the root colonization and plant growth stimulating abilities of rhizobacteria, which was higher at 30 °C (Landa et al., 2004). In this study, we evaluated the effect of plant growth promoting rhizobacteria on plant growth and on the control foot and root rot disease of tomato caused by Fusarium solani under different conditions of soil salinity.

2. Materials and methods

2.1. Soil, seeds and bacterial strains

The soil used for pot experiments was selected from deep tillage (0–40 cm) irrigated agricultural fields in the Tashkent (non-saline, EC 1.3 dS m⁻¹) and Syr-Darya Provinces (affected by salinity, EC 7.1 dS m⁻¹) of Uzbekistan. Soils with an EC of greater than 4.0 dS m⁻¹ soil were considered saline. The characterisation of the experimental soil used in the current study are described in Table 1. Tomato seeds (Lycopersicon esculentum cv. Bella) were obtained from Enza Zaden, the Netherlands. The bacterial strains Pseudomonas putida TSAU1, P. extremorientalis TSAU6, P. chlororaphis TSAU13, P. extremorientalis TSAU20, and P. aurantiaca TSAU22 were obtained from the culture collection of the Faculty of Biology, National University of Uzbekistan. These strains were previously isolated from the rhizosphere of wheat grown in salinated soil (Egamberdieva and Kucharova, 2009). All Pseudomonas strains were grown on King’s B agar medium (KB; Difco Laboratories, Detroit, MI, USA) at 28 °C. The fungal pathogen Fusarium solani was obtained from the National University of Uzbekistan and was grown on potato dextrose agar plates (PDA; Difco Laboratories, Detroit, MI, USA).

2.2. Fungal isolate

The fungal pathogen Fusarium solani was previously isolated from diseased tomato plants grown in salinated Uzbek soil that showed typical Fusarium foot and root rot symptoms. The procedure for the isolation and identification of pathogen from tomato root was described in previous work by Egamberdieva et al. (2011). Briefly, a small piece of tissue from the diseased plant was plated on potato dextrose agar (PDA) and incubated at 28 °C in the dark for 5 days. A single microconidial culture was prepared from isolate. The pathogenicity test was carried out on tomato under controlled growth chamber conditions. A randomized complete block design experiment with five replicates was carried out to examine the pathogenicity of the isolated phytopathogen (F. solani) to fulfill Koch’s postulates. Healthy tomato seedlings that had developed their second set of true leaves were transplanted into plastic pot (500 ml capacity) containing sand:peat-moss: vermiculite mixture (1:1:1, w/w/w) which infested with the phytopathagen (F. solani) as 2 ml of spore suspension (4 × 10⁷ spore/ml). Control pots (without fungal pathogen) were used as references. The pots were incubated in growth chamber for five weeks at 27 ± 2 °C, then symptoms of root rot disease were evaluated using the following formula.

\[
\text{Disease incidence(%) = } \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100
\]

After 5 weeks, a piece of diseased root from a sick plant was removed and plated on a PDA plate followed by incubation for 5 days to isolate the pathogen. Prior to identification, fungus was grown on sterile filter paper placed on PDA agar. The filter paper containing the fungal hyphae was collected and ground in liquid nitrogen. DNA was isolated from pulverized fungal biomass using the Nucleon Phytopure kit (Amersham Biosciences GmbH, Freiburg, Germany). To identify the fungal isolates, the mtSSU rDNA sequences of two strains were analyzed. The mtSSU rDNA fragments were amplified using MS1 and MS2 primers (Zeng et al., 2003) and sequenced by ServiceXS (Leiden, the Netherlands). The sequences of the fragments were compared with those in GenBank using the BLAST program. The sequences of the two analyzed strains showed 99% similarity with the mtSSU rDNA sequence from Fusarium solani f. sp. glycines isolate 1-potato (GenBank Acc. N. AF125026). Therefore the isolates can be referred to as Fusarium solani on the basis of their mtSSU rDNA fragment sequences.

### Table 1

Soil characterization.

| Soil      | EC dS m⁻¹ | K⁺ | Ca²⁺ | Mg²⁺ | CO₃⁻ | N  | P  | C指的是 | Na⁺ | Cl⁻ |
|-----------|-----------|----|------|------|------|----|----|--------|-----|-----|
| None-saline | 2.3      | 5.92 | 53.4 | 23.7 | 16.1 | 1.06 | 1.30 | 8.69    | 600.2 | 52.0 |
| Saline    | 7.1       | 6.58 | 67.4 | 24.6 | 17.6 | 0.95 | 1.23 | 7.19    | 813.1 | 94.2 |

EC = Electrical Conductivity. K⁺ = exchangeable potassium. Ca²⁺ = exchangeable calcium. Mg²⁺ = exchangeable magnesium. Na⁺ = exchangeable sodium. CO₃⁻ = carbonate and Cl⁻ = chloride. C指的是 = total C. CO₃²⁻ = total organic C. N = total nitrogen. P = total phosphorus.

* Non-saline soil was collected from Tashkent province. Saline soil from Syrdarya province of Uzbekistan.

EC = Electrical Conductivity. K⁺ = exchangeable potassium. Ca²⁺ = exchangeable calcium. Mg²⁺ = exchangeable magnesium. Na⁺ = exchangeable sodium. CO₃⁻ = carbonate and Cl⁻ = chloride. C指的是 = total C. CO₃²⁻ = total organic C. N = total nitrogen. P = total phosphorus.

* Non-saline soil was collected from Tashkent province. Saline soil from Syrdarya province of Uzbekistan.
2.3. Salt tolerance of tomato plants

The tomato seeds were sterilized by immersion in 70% ethanol for 5 min followed by an immersion in 0.1% HgCl₂ for 1 min, after which they were washed several times with sterile water. Germination tests were carried out in Petri dishes (95 mm x 15 mm) containing 1% water agar. Salinity conditions were generated by the addition of NaCl to final concentrations of 75, 100 and 125 mM NaCl. Twenty healthy and surface-sterilized tomato seeds were placed in each Petri dish and were arranged in a randomized complete block design with three replications. Eventually, the Petri dishes were covered with a polyethylene sheet to avoid the loss of the moisture through evaporation and were kept in the plant growth chamber at 28 °C. The seeds were observed daily and the percent of germination was recorded after three days of incubation. Seeds were considered to have germinated when the emergence of radicles were over 0.2 cm long. The lengths of roots and shoots of germinated seedlings were measured and recorded.

2.4. Plant growth promotion

The effect of inoculation with selected bacterial strains on the growth of tomato was studied under non saline and saline soil condition. Bacterial inoculants were prepared and the tomato seeds were sterilized as described in Egamberdieva et al. (2011). Briefly, Pseudomonas strains were grown overnight in KB broth, and cell suspension was centrifuged. The cell pellets were suspended with phosphate-buffered saline (PBS; 20 mM sodium phosphate, 150 mM NaCl, pH 7.4). Tomato seeds were coated with bacteria by dipping the seeds in bacterial suspensions (cell densities of 10⁷–10⁸ cells/ml).

Inoculated seedlings were sown in the plastic pots (7 cm diameter; 10 cm deep) and were set-up in a randomized design with ten replications. The treatments were: (i) un-inoculated seeds, and (ii) seeds inoculated with bacterial strains. The tomato plants were grown under non saline and saline soil for six weeks under open natural conditions with temperatures ranged between 26 °C and 28 °C during the day and between 12 °C and 14 °C at night. At harvest, the shoot lengths were measured and whole plants were dried to constant weight at 100 °C and were weighed.

2.5. Biological control of tomato root rot by bacterial strains

Seeds were germinated and the seedling were sown into a plastic pot (capacity 500 ml) filled with non-saline and saline soil. The treatments were as follows: (i) control, without pathogen and bacteria, (ii) control with pathogen, and (iii) pathogen and bacteria. To prepare the fungal inoculant Erlenmeyer flasks (250 ml capacity) containing Potato-dextrose broth (Difco Laboratories, Detroit, MI, USA) were inoculated with 0.5 cm agar disc cut from 7 days old culture of the pathogenic fungi, F. solani. The flasks were incubated at 28 ± 2 °C under shaking status (110 rpm) for ten days. The spores were removed using sterile glass wool and were adjusted to a concentration of 2 x 10⁵ spores/ml. Randomized complete block designs were used for treatments with four replications. Each treatment contained 24 replicate pots. The plants were grown for five weeks under open natural conditions with temperatures ranging between 26 °C and 28 °C during the day and between 12 °C and 14 °C at night and were watered as needed. At harvest, plants were removed from the soil, and roots were washed and examined for the symptoms of root rot.

2.6. Survival of bacterial strains on plant roots

Antibiotic resistant strains were used to determine the effect of salinity on the survival of inoculated strains. Rifampicin resistant mutants of Pseudomonas putida TSAU1, P. extreomorientalis TSAU6, P. chlororaphis TSAU13, P. extreomorientalis TSAU20, and P. aurantiaca TSAU22 were obtained by plating the parental strain onto KB agar plates containing 200 μg/ml rifampicin. After incubating for 5 days at 28 °C, antibiotic resistant colonies having colony morphology and growth rate similar to the the parental strain were selected and transferred onto KB agar plates containing rifampicin. The plant seeds were sterilized, germinated and coated with rifampicin resistant mutants as described above. Plants were grown in plastic pots filled with potting soil for four weeks under greenhouse condition as described above. The plants were watered when necessary and salinity condition was established by addition of 75 mM NaCl. At harvest, the adhering soil was removed from the plant roots and 1 g of roots was shaken in 9 ml of sterile phosphate-buffered saline (PBS; 20 mM sodium phosphate, 150 mM NaCl, pH 7.4). The number of bacteria in the resulting suspensions was determined as colony forming units (CFU) using the dilution-plate method and KB agar containing 200 μg/ml rifampicin. After incubation for 2–3 days at 28 °C, the number of rifampicin resistant colonies was counted.

2.7. Greenhouse experiments

The plants which showed the best performance with respect to stimulating plant growth and controlling root rot of tomato plants were evaluated for their effect on tomato growth and yield under greenhouse conditions. The greenhouse experiment was carried out in the Tashkent province of Uzbekistan using a calcareous sierozem soil with 2.4% organic matter, N 0.1%, P 1.34%, K 7.1% and soil EC value of 5.6.dSm and pH 7.8. The bacterial inoculants were prepared as described by Egamberdieva et al. (2011). The coated seed with bacterial inoculant and uncoated seeds were sown in pots (one seed per pot), containing a mixture of soil and biohumus (2:1, v/v). Seedlings were initially kept in a small greenhouse under semi-controlled environmental conditions (temperature fluctuating between 18 and 22 °C). When seedlings reached the 2–4 leaf stage, they were transferred to an un-heated greenhouse and transplanted in six rows in each experimental plot (3 m x 2.5 m). Intra-row spacing was 50 cm and rows were 60 cm apart. Four replicate plots per treatment were used (total 12 of treatments), and the experiment was set up in a randomized complete block design. The temperature in the greenhouse ranged between 24 and 27 °C during the day and between 14 and 17 °C at night. At harvest the plant height and total fruit yield were determined. The N, P, and K acquisition of plants grown under field conditions were determined. The shoots were separated from the roots and dried in an oven at 75 °C for 48 h and then were powdered. Total nitrogen (Nₜ) was determined after dry combustion using a CNS elemental analyzer (LECO Corporation, St. Joseph, MI) according to DIN ISO 15178 (2001). The P, K, and Mg contents were analyzed according to DIN 38414-S (1983).

2.8. Antioxidant enzyme activity

The plant extract used to measure enzymes activities was prepared as described by Ahmad et al. (2015). Briefly, fresh leaves (10 g) were crushed in 50 volumes of 100 mM Tris-HCl (pH 7.5) containing 5 mM DTT (Dithiothreitol), 10 mM MgCl₂, 1 mM EDTA (Ethylene diaminetetra acetic acid), 5 mM magnesium acetate, 1.5% PVP-40 (Polyvinylpyrrolidone), 1 mM PMSF (phenylmethane-sulfonyl fluoride) and 1 μg ml⁻¹ aprotinin. The homogenate was filtered using a cheese cloth and centrifuged for 15 min at 10,000 rpm. The enzyme-containing supernatant was collected after centrifugation. For the analysis of APX activity, tissues were separately homogenized with 2 mM AsA. All experiments were performed at 4 °C. Activity of superoxide dismutase (SOD)
Effect of selected plant growth promoting bacteria on shoot length and dry weight of tomato growing in non-saline (EC value 2.3 dS/m) and saline soil (EC value 7.1 dS/m).

(EC 1.15.1.1) was estimated according to Van Rossum et al. (1997) following the photoreduction of nitroblue tetrazolium (NBT). The activity of SOD was expressed as enzyme unit (EU) mg⁻¹ protein. One unit of SOD was defined as the amount of protein causing 50% decrease in the SOD-inhibitable NBT reduction. The method of Nakano and Asada (1981) was followed to determine the ascorbate peroxidase (APX) activity, with the absorbance read at 290 nm. The APX activity was expressed as EU mg⁻¹ protein. Catalase (CAT) (EC 1.11.1.6) activity was determined according to Luck (1974). The activity of CAT was calculated using the extinction co-efficient of 36 × 103 mM⁻¹ cm⁻¹ and expressed as EU mg⁻¹ protein.

2.9. Statistical methods

The statistical analyses were performed using the analysis of variance package included in Microsoft Excel 2007. Mean comparisons were conducted using a least significant difference (LSD) test (P = 0.05), student’s t-test.

3. Results

3.1. Germination of seeds and seedling growth under salt stress

The effects of salinity on seed germination of tomato were evaluated in Petri plates using NaCl concentrations of 75, 100 and 125 mM. Seed germination was decreased slightly with increasing salt concentrations (from 75 to 125 mM NaCl), compared to the control seeds (water only). The observed seed germination was 89 ± 4.6% with distilled water and 76 ± 3.5% at 75 mM NaCl, 61 ± 4.9% at 100 mM NaCl and 47 ± 3.9% at 125 mM NaCl concentration. The highest salinity assayed (125 mM) inhibited the root and shoot length of tomato seedling grown in pots for 10 days. NaCl concentrations of 100 mM reduced the dry weight of tomato by 27% ± 1.0 and 125 mM NaCl by 33% ± 0.6 compared to the untreated control (Table 2).

3.2. Plant growth in pots

The bacterial strains Pseudomonas putida TSAU1, Pseudomonas sp. TSAU5, P. extremorientalis TSAU6, P. chlororaphis TSAU13, P. extremorientalis TSAU20, and P. aurantiaca TSAU22 were selected to assess their plant growth stimulation properties in pot experiments. Three strains Pseudomonas sp. TSAU5, P. chlororaphis TSAU13, and P. extremorientalis TSAU20 significantly (P < 0.05) increased plant length by 13, 26, and 19% compared to the untreated control, respectively (Table 2). The dry weight of tomato was significantly increased only by strain P. extremorientalis TSAU20. Saline soil had a negative effect on the stimulation of tomato plant growth by several strains, but not P. extremorientalis TSAU20. The bacterial strain TSAU20 increased the shoot length and dry weight of tomato plants by 28%, and 27% in a significant way compared with the untreated control (Table 2).

3.3. Biological control of tomato root rot

The assayed strains were also evaluated for their biological control potential against tomato root rot caused by Fusarium solani under non-saline and saline soil conditions. The results showed that in non-saline soil that lacked F. solani, the percentage of diseased plants was 18%, while disease symptoms increased to 36% in saline soil (Table 3). All tested bacterial strains were able to

### Table 2

Effect of selected plant growth promoting bacteria on shoot length and dry weight of tomato growing in non-saline (EC value 2.3 dS/m) and saline soil (EC value 7.1 dS/m).

| Bacterial strains | Non-saline | Saline |
|-------------------|------------|-------|
|                   | Shoot length | Root length | Dry weight | Shoot length | Root length | Dry weight |
| Control           | 8.2         | 5.7     | 0.151      | 7.6         | 5.6         | 0.140      |
| Pseudomonas putida TSAU1 | 7.4         | 4.6     | 0.134      | 7.3         | 5.0         | 0.140      |
| P. extremorientalis TSAU6 | 7.2         | 4.9     | 0.125      | 7.4         | 5.5         | 0.126      |
| P. extremorientalis TSAU20 | 6.9         | 5.0     | 0.128      | 7.2         | 5.2         | 0.140      |
| P. chlororaphis TSAU13 | 7.3         | 5.4     | 0.190      | 7.2         | 5.2         | 0.132      |
| P. extremorientalis TSAU20 | 8.0         | 5.4     | 0.151      | 6.3         | 4.6         | 0.126      |
| P. aurantiaca TSAU22 | 10.8        | 8.0     | 0.156      | 8.0         | 6.5         | 0.127      |
|                   | 9.4         | 7.5     | 0.127      | 8.3         | 6.6         | 0.124      |
|                   | 8.9         | 6.8     | 0.132      | 7.1         | 6.1         | 0.113      |
| P. extremorientalis TSAU20 | 9.7         | 6.8     | 0.171      | 8.8         | 6.9         | 0.163      |
| P. aurantiaca TSAU22 | 9.8         | 7.4     | 0.188      | 9.7         | 7.3         | 0.178      |
|                   | 9.6         | 6.9     | 0.209      | 9.9         | 6.9         | 0.156      |
|                   | 9.1         | 7.0     | 0.169      | 8.3         | 6.9         | 0.154      |
|                   | 8.7         | 7.5     | 0.160      | 7.6         | 6.4         | 0.138      |
|                   | 8.5         | 6.5     | 0.157      | 7.5         | 6.0         | 0.138      |

* Expressed as cm per plant.
* Expressed as gram per plant.
* Significantly different from the control at P < 0.05.

### Table 3

Biological control of tomato root rot by PGPR bacterial strains.

| Treatments                                      | Disease (%) |
|------------------------------------------------|-------------|
|                                                  | Non-saline soil | Saline soil |
| Control                                         | 40.6 ± 8.1    | 71.9 ± 8.1 |
| Pseudomonas putida TSAU1                        | 38.1 ± 9.9    | 48.2 ± 6.1 |
| P. extremorientalis TSAU6                       | 30.2 ± 4.9    | 50.3 ± 9.4 |
| P. chlororaphis TSAU13                          | 23.4 ± 5.4    | 42.2 ± 7.9 |
| P. extremorientalis TSAU20                       | 14.1 ± 7.9    | 25.0 ± 5.1 |
| P. aurantiaca TSAU22                            | 36.3 ± 8.2    | 40.9 ± 8.3 |

Bacteria were coated on pre-germinated tomato seeds, plants were grown under open natural conditions in pots containing non-saline (EC value 2.3 dS/m) and saline soil (EC value 7.1 dS/m) infested with F. solani spores. ±SD: Standard deviation.

* Significantly different from the negative control at P < 0.05.
control tomato root rot caused by *F. solani* compared to the pathogen infected control plants without bacteria (Table 3). Two strains *P. chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 reduced the incidence of disease in plants by 23, and 14% under non saline conditions and by 42, 25% in saline soil compared to the *Fusarium* -infected control plants, respectively.

### 3.4. Survival of bacterial strains in the tomato root

The survival of bacterial strains in the rhizosphere of tomato grown under two different soil conditions was determined using rifampicin resistant mutants of *Pseudomonas putida* TSAU1, *P. extremorientalis* TSAU6, *P. chlororaphis* TSAU13, *P. extremorientalis* TSAU20, and *P. aurantiaca* TSAU22. The results showed that all five bacterial strains were able to colonize and survive on the roots of tomato plants (Table 4). However, their colonization was partly inhibited under salt stress (75 mM NaCl). Among these bacterial strains the rhizosphere colonization by *P. chlororaphis* TSAU13, and *P. extremorientalis* TSAU20 was higher under both soil conditions.

### 3.5. Greenhouse trials

The best selected bacterial strains *P. chlororaphis* TSAU13, and *P. extremorientalis* TSAU20 which showed growth stimulation in non

Table 4
The survival (log10 (CFU/g root) of rifampicin resistant mutants of bacterial inoculants in the rhizosphere of tomato grown under non saline and saline conditions.

| Treatments                     | Survival (log10 (CFU/g root) of rifampicin resistant mutants bacterial inoculants) |
|--------------------------------|---------------------------------------------------------------------------------|
|                                | Non-saline                          | Saline                        |
| *Pseudomonas putida* TSAU1     | 3.45 ± 0.15                         | 2.15 ± 0.18                   |
| *P. extremorientalis* TSAU6    | 3.58 ± 0.11                         | 2.75 ± 0.17                   |
| *P. chlororaphis* TSAU13       | 3.61 ± 0.19                         | 2.83 ± 0.18                   |
| *P. extremorientalis* TSAU20   | 3.65 ± 0.22                         | 2.81 ± 0.28                   |
| *P. aurantiaca* TSAU22         | 3.29 ± 0.21                         | 2.43 ± 0.29                   |

±SD: Standard deviation.

Table 5
Effect of *Pseudomonas* strains (TSAU13 and TSAU20) on tomato growth and fruit yield in greenhouse experiments.

| Treatment | Plant height (cm) | Fruit yield (kg/m²) | Elements uptake (%) |
|-----------|-------------------|---------------------|---------------------|
|           |                   |                     | Nitrogen | Phosphorus | Potassium |
| None      | 125 ± 3.7          | 13.9 ± 0.9           | 2.4 ± 0.2 | 0.42 ± 0.02 | 1.91 ± 0.06 |
| TSAU13    | 145 ± 5.1          | 15.9 ± 1.4           | 2.9 ± 0.3 | 0.49 ± 0.03 | 1.89 ± 0.08 |
| TSAU20    | 157 ± 4.2          | 17.0 ± 0.7           | 3.2 ± 0.1 | 0.52 ± 0.01 | 2.10 ± 0.10 |

Tomato plants were grown in greenhouse for four months, the temperature range was: 22–24 °C at day and 12–14 °C at night.

* Significantly different from the negative control at P < 0.05.

Table 6
Effect of *P. extremorientalis* TSAU20 on hydrogen peroxide (H₂O₂) glutathione and chlorophyll contents in tomato plants.

| Soil       | Treatment | Plant weight g/plant | Hydrogen peroxidea | Prolineb |
|------------|-----------|----------------------|--------------------|----------|
| Non-saline | Control   | 0.78 ± 0.07          | 70.25 ± 1.31       | 0.36 ± 0.03 |
|            | TSAU20    | 0.82 ± 0.02          | 50.43 ± 1.96       | 0.82 ± 0.04 |
| Saline     | Control   | 0.29 ± 0.03          | 210.9 ± 2.07       | 1.46 ± 0.16 |
|            | TSAU20    | 0.38 ± 0.05          | 112.8 ± 1.54       | 3.61 ± 0.06 |

a mM/g fresh weight.

b nM/g fresh weight.

* Significantly different from the negative control at P < 0.05.
Fig. 1. Effects of P. extremorientalis TSAU20 inoculation on antioxidant enzyme activities. A, glutathione reductase (GR); B, ascorbate peroxidase (APX); C, superoxide dismutase (SOD) and D, catalase (CAT). Tomato plants were grown in a greenhouse for six weeks in potting soil under non saline and saline soil condition. Columns represent means for four plants (N = 4) with error bars showing standard deviation.

4. Discussion

The plant beneficial microbes improve plant growth, nutrient acquisition, and stress tolerance to various abiotic stresses such as drought and salinity, and control plant fungal disease (Egamberdieva and Hoflich, 2003; Berg et al., 2013; Hashem et al., 2016; Vardharajula et al., 2011). The alleviation of salt stress in plants by PGPR inoculants has been shown on various crops and vegetables, including licorice (Egamberdieva et al., 2016), and chili pepper (Park et al., 2013). In our study, four PGPR strains stimulated plant growth of tomato, but the activity of some bacterial inoculants was reduced under saline soil condition. It has been reported that the root colonization and plant growth stimulating traits of plant growth promoting bacteria affected by biotic and abiotic factors, such as indigenous microorganisms, temperature, drought, salinity, and soil type (Compant et al., 2010). Salt tolerant and root colonizing bacteria which are physiologically adapted to abiotic stress could survive in such harsh environment and help plant to tolerate salt stress (Ahmad et al., 2013; Cho et al., 2015; Egamberdieva et al., 2015a, 2016). The strain P. extremorientalis is a salt tolerant and potential root colonizing bacteria (Egamberdieva and Kucharova, 2009) that showed the best performance in plant growth stimulation of tomato under non saline and saline soil condition. Yao et al. (2010) reported that Pseudomonas putida strain isolated from alkaline soil could increase salt tolerance of cotton and growth in saline soil. We have also observed plant growth stimulation and biological control of cucumber by salt tolerant P. extremorientalis TSAU20 and P. chlororaphis strains under saline soil condition (Egamberdieva et al., 2011).

Previous studies showed that higher saline conditions increased the susceptibility of plants to plant pathogens (Sanogo, 2004; Triky-Dotan et al., 2005), e.g. severity of Phytophthora root and crown rot in tomato was higher in salt affected soil (Swieckil and MacDonald, 1991). We have also observed a higher incidence of tomato root rot in salinated soil. The inoculation of tomato with PGPR strains reduced tomato root rot caused by F. solani compared to the pathogen infected control plants under both non-saline and saline soil conditions. Rangarajan et al. (2003) also reported that Pseudomonas strains exhibiting antibiosis activity suppressed both bacterial leaf blight and sheath blight diseases in rice under both natural and saline soil conditions.

The survival of introduced bacteria in the plant root is crucial to generate significant effect and is dependent on the physiological adaptation of the introduced cells and, biotic and abiotic factors (Rekha et al., 2007; Cho et al., 2015). In earlier studies the negative effect of salt stress on the colonization of bacteria introduced into the rhizosphere was observed (Sato and Jiang, 1996). It has been found that salt tolerant bacteria that are able to survive in the rhizosphere of plants grown under harsh environmental condition through their persistence and proliferation in semi-arid soils (Paul and Nair, 2008; Egamberdieva et al., 2013). The bacterial strains were salt tolerant (up to 3% NaCl) and showed potential root colonising abilities for wheat (Egamberdieva and Kucharova, 2009) and common bean (Egamberdieva et al., 2011). In our current study the colonization potential of P. chlororaphis TSAU13 and P. extremorientalis TSAU20 strains was not inhibited by salt stress since they were able to colonize tomato root under saline soil conditions. The potential colonization ability of introduced strains is reported as one of the mechanism in the beneficial effects of introduced strains (Lugtenberg et al., 2001) and this ability of strains in our study was not inhibited by salinity. The involvement of auxin synthesized by bacterial inoculants in stimulation of root system of plants was reported previously (Remans et al., 2008; Egamberdieva, 2009). The bacterial strains P. chlororaphis TSAU13 and P. extremorientalis TSAU20 which were able to grow in medium containing up to 4% NaCl, were able to produce indole...
3-acetic acid in medium containing up to 3% NaCl and showed antagonistic activity against plant fungal pathogens (Egamberdieva et al., 2009). The salinity did not have a negative effect on their plant beneficial traits. The best selected strains increased plant growth and fruit yield of tomato under greenhouse condition. A similar observation was reported by Almahrabri et al. (2013), where an increased shoot dry weight, plant height and fruit yield was observed by inoculating tomato plants with P. putida and P. fluorescens. An improved root growth by bacterial inoculants facilitated plants to have better access to soil minerals such as nitrogen (N), phosphorus (P) and potassium (K). Abiotic stress is known to inhibit root systems, thus reducing plant ability to acquire nutrient resources in soil (Hashem et al., 2014). In our study, tomato plants inoculated with TSAU 13 and TSAU20 strains showed better N and P uptake compared to un-inoculated plants.

Salt stress leads to enhanced cell membrane leakage due to an increased peroxidation of membrane lipids resulting in the loss of membrane integrity (Ahmad et al., 2015). It has been demonstrated that root associated microbes may reduce the peroxidation of membrane lipids through an enhancement in free radical scavenging mechanisms, thereby strengthening the ability of membranes to withstand abiotic stress. (Alqarawi et al., 2014; Abd_Allah et al., 2015). The accumulation of compatible osmolytes in plant tissues is increased under abiotic stress by their active role in osmotic adjustment, which helps plants to survive during hostile condition (Ahanger et al., 2014). We have also observed an enhanced concentration of proline in tomato leaves inoculated condition (Ahanger et al., 2014). Arbuscular mycorrhiza with enhanced concentration of proline in tomato leaves inoculated with arbuscular mycorrhizal fungi under salt stress. Saudi J. Biol. Sci. 22, 274–283.

Ahanger, M.A., Hashem, A., Abd_Allah, E.F., Ahmed, P., 2014. Arbuscular mycorrhiza in crop improvement under environmental stress. In: Ahmad, P., Rasool, S. (Eds.), Emerging Technologies and Management of Crop Stress Tolerance, vol. 2. Academic Press, USA, pp. 69–95.

Ahmad, P., Hashem, A., Abd_Allah, E.F., Alqarawi, A.A., John, R., Egamberdieva, D., Guelc, S., 2015. Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (Brassica juncea L.) through antioxidant defense system. Front. Plant Sci. 6, E688. http://dx.doi.org/10.3389/fpls.2015.00688.

Ahmed, M., Zahir, Z.A., Khalid, M., Nazli, F., Arshad, M., 2013. Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mango under salt-affected conditions on farmer’s fields. Plant Physiol. Biochem. 63, 170–176. http://dx.doi.org/10.1016/j.plaphy.2012.11.024.

Ali, S., Charles, T.C., Glick, B.R., 2014. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol. Biochem. 80, 160–167. http://dx.doi.org/10.1016/j.plaphy.2014.04.003.

Almahrabri, O.A., Massoud, S.I., Abdelmoneim, T.S., 2013. Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi J. Biol. Sci. 20, 57–61. http://dx.doi.org/10.1016/j.sjbs.2012.10.004.

Alqarawi, A.A., Abd_Allah, E.F., Hashem, A., 2014. Alleviation of salt-induced adverse impacts via mycorrhizal fungi in Ephedra pygmaea Forsk. J. Plant Interact. 9 (1), 802–810.

Berg, G., Alavi, M., Schmidt, C.S., Zachow, C., Egamberdieva, D., Kamilova, F., Lugtenberg, B., 2013. Biocontrol and osmoprotection for plants under saline conditions. In: de Bruijn, Frans J. (Ed.), Molecular Microbial Ecology of the Rhizosphere. Wiley-Blackwell, USA, http://dx.doi.org/10.1002/9781118297674.ch55.

Berg, G., Martinez, J.L., 2015. Friends or foes: can we make a distinction between beneficial and harmful strains of the Stenotrophomonas maltophilia complex? Front. Microbiol. 6, 241. http://dx.doi.org/10.3389/fmicb.2015.00241.

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. Methods Enzymol. 113, 484–490.

Chakraborty, S., 2013. Migrate or evolve: options for plant pathogens under climate change. Glob. Change Biol. 19 (7), 1985–2000. http://dx.doi.org/10.1111/j.1365-2486.2012.02205.

Choudhary, D.K., Prakash, A., Johri, B.N., 2007a. Induced systemic resistance (ISR) in plants: mechanism of action. Indian J. Microbiol. 47, 289–297. http://dx.doi.org/10.1007/s12087-007-0054-2.

Cho, S.T., Chang, H.H., Egamberdieva, D., Kamilova, F., Lugtenberg, B., Kuo, C.H., 2015. Genome analysis of Pseudomonas fluorescens PCL1751: a rhizobacterium that controls root diseases and alleviates salt stress for its plant host. PLoS ONE, 10.1038/sjolsj.2011.341.

Choudhary, D.K., Prakash, A., Johri, B.N., 2007b. Induced systemic resistance (ISR) in plants: mechanism of action. Indian J. Microbiol. 47, 289–297. http://dx.doi.org/10.1007/s12087-007-0054-2.

Compant, S., Clément, C., Sessitsch, A., 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42, 669–678. http://dx.doi.org/10.1016/j.soilbio.2009.12.026.

Egamberdieva, D., Wirth, S., Jabborova, D., Raisanen, L.A., Berg, G., Liao, H., 2017. Coordination between Bradyrhizobium and root colonizing Pseudomonas alleviates salt stress in soybean (Glycine max L.) through altering root system architecture and improving nodulation. Plant J. 121 (1), 100–107. http://dx.doi.org/10.1111/tpj.1429415.2017.192412.

Egamberdieva, D., Li, Li., Lindström, K., Raisanen, L., 2016. A synergistic interaction between salt tolerant Pseudomonas and Mesorhizobium strains improves growth and symbiotic performance of liquorice (Glycyrrhiza uralensis Fisch.) under salt stress. Appl. Microbiol. Biotechnol. 100 (6), 2829–2841. http://dx.doi.org/10.1007/s00253-015-7147-3.

Egamberdieva, D., Jabborova, D., Hashem, A., 2015a. Pseudomonas induces salinity tolerance in cotton (Gossypium hirsutum) and resistance to Fusarium root rot through the modulation of indole-3-acetic acid. Saudi J. Biol. Sci. 22 (6), 773–779. http://dx.doi.org/10.1016/j.sjbs.2015.04.019.

Egamberdieva, D., Jabborova, D., Berg, G., 2015b. Synergistic interactions between Bradyrhizobium japonicum and the endophyte Stenotrophomonas maltophilia and their effects on growth, nodulation and nutrition of soybean under salt stress. Plant Soil 405, 35. http://dx.doi.org/10.1007/s11104-015-2661-8.

Egamberdieva, D., Berg, G., Lindström, K., Raisanen, L.A., 2013. Alleviation of salt stress by symbiotic Galega officinalis L. (goat’s rue) co-inoculation of rhizobium with root colonising Pseudomonas. Plant Soil 369 (1), 453–465. http://dx.doi.org/10.1007/s11104-013-1586-3.

Egamberdieva, D., Kuchkurov, Z., Davranov, K., Berg, G., Makarova, N., Azarova, T., Chuty, V., Tikhonovich, I., Chebotar, V., Tikhonovich, I., Validov, S., Lugtenberg, B., 2011. Bacteria able to control foot and root rot and to promote growth of cucumber in salinized soils. Biol. Fertil. Soils 47, 197–205. http://dx.doi.org/10.1007/s00374-010-0653-3.

Egamberdieva, D., Kuchkurov, Z., 2009. Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol. Fertil. Soils 45, 561–573. http://dx.doi.org/10.1007/s00374-009-0366-y.
Heidari, M., Golpayegani, A., 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria in rice plants. J. Agric. Biol. Sci. 6, 6–11.

Heidari, M., Mousavinik, S.M., Golpayegani, A., 2011. Plant growth promoting rhizobacteria. Ann. Rev. Phytopathol. 49, 1438–1444. http://dx.doi.org/10.1007/978-1-4614-1719-3_9.

Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. Ann. Rev. Phytopathol. 47, 281–288. http://dx.doi.org/10.1146/annurev.phyto.39.1.461.

Paegle, A., AlNajadi, A., Shaheen, A., Alqarawi, A., Al-Huqail, A.A., Wirth, S., Egamberdieva, D., 2015. Response of chile pepper to Phytophthora capsici in relation to soil salinity. Plant Dis. 88, 205–209. http://dx.doi.org/10.1094/PDIS.2014.8.205.

Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., AlDeebah, A., Egamberdieva, D., 2014. Alleviation of abiotic salt stress in Oryza sativa through induced plant growth promoting rhizobacteria. J. Agri. Biol. Sci. 6, 6–11.

Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., Al-Huqail, A.A., Egamberdieva, D., 2015. Arbuscular mycorrhizal fungi enhance salinity tolerance of Panicum turgidum Forsk by altering photosynthetic and antioxidant pathways. J. Plant Inter. 10, 230–242. http://dx.doi.org/10.1007/s11738-014-05202-5.

Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., Al-Huqail, A.A., Egamberdieva, D., 2014. Alleviation of abiotic salt stress in Oryza sativa through induced rhizobacteria. J. Agri. Biol. Sci. 6, 6–11.

Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., AlDeebah, A., Egamberdieva, D., 2015. Arbuscular mycorrhizal fungi enhance salinity tolerance of Panicum turgidum Forsk by altering photosynthetic and antioxidant pathways. J. Plant Inter. 10, 230–242. http://dx.doi.org/10.1007/s11738-014-05202-5.

Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., Al-Huqail, A.A., Egamberdieva, D., 2014. Alleviation of abiotic salt stress in Oryza sativa through induced plant growth promoting rhizobacteria. J. Agri. Biol. Sci. 6, 6–11.

Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., AlDeebah, A., Egamberdieva, D., 2015. Arbuscular mycorrhizal fungi enhance salinity tolerance of Panicum turgidum Forsk by altering photosynthetic and antioxidant pathways. J. Plant Inter. 10, 230–242. http://dx.doi.org/10.1007/s11738-014-05202-5.