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The root growth of wheat plants, the water conservation and fertility status of sandy soils influenced by plant growth promoting rhizobacteria

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Abstract Plant growth promoting rhizobacteria were isolated and characterized from sandy soils in Pakistan. The role of the rhizobacteria, in association with plant growth regulators, was studied on the roots of wheat grown under water stressed conditions. The plant growth promoting rhizobacteria were characterized on the basis of colony morphology, biochemical traits and identified on the basis of 16S-rRNA gene sequencing which identified the selected isolates *Planomicrobium chinense*, *Bacillus cereus* and *Pseudomonas fluorescens*. Antibacterial and antifungal activities were determined. The fresh cultures (24 h old) of isolates were used to soak the seeds for 2–3 h prior to sowing. The growth regulators salicylic acid and putrescine were applied to the plant as foliar spray at three leaf stage. The plant growth promoting rhizobacteria produced exopolysaccharides that formed soil aggregation around roots of the plants and significantly enhanced water holding capacity of sandy soil. The relative water content (80%) of leaves and root fresh (80%) and dry weight (68%) were higher in plant growth promoting rhizobacteria inoculated plants. The nutrient content of rhizosphere soil of treated plants was also enhanced (Ca 35%, K 34%, Mg 52% and Na 42%) over stressed controls. Integrative use of effective plant growth promoting rhizobacteria in combination with salicylic acid appears to be an effective eco-friendly approach to increase drought tolerance in wheat plants to combat desertification.

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1 Introduction

Plant growth-promoting rhizobacteria stimulate health and productivity of plants by the solubilisation of minerals, promoting root growth and suppressing root diseases. The predominant belong to *Azospirillum, Bacillus, Pseudomonas, and Agrobacterium* (Khan and Bano 2016a; Viveros et al. 2010). Plant roots exude a variety of nutrients, organic compounds, and signals that attract microbial populations (Bais et al. 2006; Drogue et al. 2012; Pothier et al. 2007; Shukla et al. 2011). The resultant bacterial community associated with the plant roots has been termed the rhizo-microbiome (Chaparro et al. 2013). Its composition is different from the microbes found in the adjacent soil (Bulgarelli et al. 2013; Chaparro et al. 2013; Raynaud et al. 2008). Since the root exudates vary in relation to the root development stage and plant genotypes, the rhizo-microbiome also fluctuates (Aira et al. 2010; Berg and Smalla 2009; Bouffaud et al. 2012; Bulgarelli et al. 2013; Chaparro et al. 2013). These rhizobacteria have been shown to increase root growth that leads to a system with a larger surface area and enhanced number of root hairs (Mantelin and Touraine 2004).

Nutrients and water are taken up by plants through the rhizo-sheaths; the soil stuck firmly to the crop roots (McCully and Canny 1988; Watt et al. 1994) that deliver a carbon supply to the microbial community involved in cycling minerals to the growing plants (Nannipieri et al. 2003). Rhizo-sheath formation involves exopolysaccharides (EPS) (Czarnes et al. 2000). These polymers are produced and released by soil micro-

organism associated with roots of the plants (Gilck et al. 1999; Vanhaverbeke et al. 2003). They have roles such as biofilm formation (Bhaskar et al. 2005), protection of the bacteria from dehydration (Pal et al. 1999), and binding of toxic metals (Fusconi and Godinho 2002) which make them of value in bioremediation (Allison 1998). Bacteria like *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Streptococcus mutans* are known for their EPS production (Vimala and Lalithakumari 2003).

The present study set out to isolate and characterize the plant growth promoting rhizobacteria from sandy soils in three areas of Pakistan: Karak, Cholistan and Bhakkar. The role of these bacteria was evaluated on the root growth of wheat under water stressed conditions that prevail in the areas from which the soils were collected.

2 Materials and methods

The soil samples used in this study came from three areas in Pakistan.

Karak is a district in the province of Khyber Pakhtunkhwa (KPK), Pakistan. It is 124 km from Peshawar on the main indus highway between Peshawar and Karachi and located at 33°7′12 N 71°5′41E. The climate is hot during the summers, with temperatures up to 40–45 °C. The main crops of the district include wheat (*Triticum aestivum*), bajra (*Pennisetum glaucum)*, maize *(Zea mays)* and pulses.

The Cholistan desert is a part of the Great Indian Desert with an area of 26,332 Km^2. It lies inside the southeast quadrant of Punjab between 27° 42′ and 29° 45′ North latitude and 69° 52′ and 73° 05′ East longitude. It has extreme summer temperatures (above 50 °C) along with extended droughts. The main crop of the desert are pulses.

Bhakkar is a district in the province of Punjab, Pakistan. It is located in the west of the Punjab province. Bhakkar has a desert climate with 18% Humidity. There is only 213 mm of rainfall during the year. It has extreme summer temperatures (above 45 °C). The main crops are wheat (*Triticum aestivum L.*), gram (*Cicer arietinum*), guwara (*Cyamopsis tetragonoloba*) and bajra (*Pennisetum glaucum)*.

The experimental work was carried out in the field under natural condition in the sandy area at Grot chowk, 20 km away from Khushab, Punjab. It is the driest and hottest district with diverse topography, located at 32° 17 48 N to 72° 21 9 E having arid hills with bushy vegetation in its north (Soon Sakesar Valley). The central part is an irrigated lowland plain and southern part is a hot dry thar desert with sparce vegetation. The temperature ranges from 25 to 48 °C in summer and 19–29 °C in winter with average annual precipitation of 521 mm (Table 1).

Seeds of two wheat varieties (i.e., Drought sensitive (Galaxy-13) and tolerant (Pak-2013) were provided by the National Agricultural Research Centre, Islamabad.

2.1 Collection of soil samples

Bacterial colonies were isolated from the rhizosphere soil samples collected from Karak,

Bhakkar and Cholistan. The soil samples were collected at 6 in. depth from top soil from Karak, Bhakar and Cholistan desert. The pH and electrical conductivity (EC) of soil samples were measured according to the method of McKeague (1978) and McLean (1982) separately.

2.2 Isolation and growth of bacteria from soil

Serial dilution method was followed for bacterial isolation from the three different soil samples. One gram of soil sample was suspended in distilled water (9 ml), stirred for 1 h with magnetic stirrer, the soil suspension thus obtained was centrifuged (3000 rpm) for 10 min. the supernatant was collected for which decimal dilutions were made. 20 ml aliquots from three dilutions i.e., 10^{-1}, 10^{-5}, and 10^{-7} were spread on Luria Bertini (LB) agar plates and nurtured for 2 days. After incubation different colonies were streaked on LB agar plates, 4–5 times until pure colony is obtained in each case.

2.3 Sterilization of seeds

Surface sterilization of seeds was done by shaking in 95% ethanol, followed by shaking in 10% Clorox. Subsequently, autoclaved distilled water (3–4x) was used for washing of seeds.

2.4 Method of inoculation

A fresh culture of bacteria was prepared by inoculating bacteria in LB broth (28 °C). The inoculated LB culture was then incubated in shaker for 2 days, thereafter, centrifuged at 10,000 rpm for 10 min, pellet was obtained after removal of supernatant and was suspended in distilled water. The optical density (at 660 nm) was adjusted to be 1. The inoculated broth was used for seed soaking for 3 h and then the seeds were sown in the field.

2.5 Characterization of isolates

2.5.1 Morphology and enzyme activity

For identification of bacterial strains, fresh cultures obtained after 24 h of their growth on agar plates (Miller 1972) and were studied under microscope. The catalase activity was

Table 1 Experimental work plan

Symbol	Treatments	Symbol	Treatments
T1	Seeds inoculated with P1	T2	Seeds inoculated with P1 + Sprayed with salicylic acid and putrescine
T3	Seeds inoculated with P2 and P3	T4	Seeds inoculated with P2 and P3+ Sprayed with SA and Putrescine
T5	Seeds inoculated with P1, P2 and P3	T6	Seeds inoculated with P1, P2 and P3+ Sprayed with salicylic acid and putrescine (consortium)
T7	Plants Treated with salicylic acid (Foliar spray)	T8	Plants treated with putrescine (foliar spray)
T9	Stress control (seeds grown in the sandy soil)	T10	Irrigated control

P1: *Planomicrobium chinense*, P2: *Bacillus cereus*, P3: *Pseudomonas fluorescens*

assessed using the method of MacFaddin (1980) and the method of Steel (1961) was followed for the detection of oxidase in the bacterial cells.

2.5.2 DNA extraction

The TY broth was inoculated with single colony of fresh bacterial cultures which was grown in shaker (Excella E 24, New Brunswick Scientific USA) overnight. The culture was than centrifuged at 12,000 rpm at 4 °C for 10 min. The pellet of the cells were resusupended and lysed using lysis buffer. The protein and cell debris were discarded by centrifugation after adding 60 ml NaCl (5 M). The supernatant obtained was transferred to another test tube to which chloroform was added and the tube was inverted 50X for proper mixing with the chloroform. The process of centrifugation was repeated and the DNA was precipitated in the 100% ethanol which was air dried. The DNA was dissolved again in autoclaved distilled water, the purity of DNA and its concentration was determined by nanodrop spectrophotometer at 260 nm (Chen and Kuo 1993).

2.5.3 PCR-amplification and 16S rRNA sequence analysis

Amplification of genomic DNA of bacterial isolates was done by the method of Weisburg et al. (1991). The primer used for PCR-amplification has the nucleotide sequence as fd1 (AGAGTTTGATCCTGGCTCAG) and rd1 (AAGGAGGTGATCCAGCC). The reaction mixture contained genomic DNA (50ug), $MgCl_2$ (1.5 mM), buffer (10X), Taq DNA polymerase (1u), dNTP mix (0.2 mM) and 10 moles of each primer. The volume was adjusted to 25ul using autoclaved pure water. The operating conditions were as follows:

The 30 cycles of the reactions were repeated through following reaction sequences: denaturation for 30 s at 94 °C, annealing at 55 °C for 30 s, extension for 2 min at 72 °C and one additional cycle for chain elongation for 10 min at 72 °C. The amplified PCR products were electrophoresed on 1.2% (w/v) agarose gel with DNA ladder (1 kb) as molecular marker. The gel was stained with 0.01gm/ml ethidium bromide and examined under UV trans-illuminator lamp.

2.5.4 Sequencing

The approximately 1400 bp purified PCR products were sequenced by using primers 27 F AgAgTTTgATCM TGG CTC Ag, 1492R TAC ggY TAC CTT gTTACg ACT T, 518 F CCA gCAgCCgCggTA ATA Cg, and 800R TAC CAgggT ATC TAA TCC. Sequencing was accomplished by means of Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA). Sequencing products were resolute on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA) at the Macrogen, Inc., Seoul, Korea.

2.5.5 Phosphorous solubilization index

Pikovskaya's media was transferred into sterilized petri plates and pin point inoculation was made with 24 h old culture of the isolated plant growth promoting rhizobacteria. The inoculated plates were incubated for 7 days (28 °C). SI (solubilization index) was calculated by the formula of Pikovskaya (1948).

$$\mathrm{SI} = \text{Colony diameter(cm)} + \text{halozone diameter(cm)}/\text{Colony diameter(cm)}$$

2.5.6 Antibacterial and antifungal activities of isolated plant growth promoting rhizobacteria

Antibacterial and antifungal activities were determined by Agar well Diffusion method (Navarro et al. 1996) and

agar tube dilution method (Washington and Sutter 1980) respectively. Test tubes containing sterile sabouraud dextrose agar (SDA) were incubated with bacterial and fungal strains (*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Helminthosporium sativum, Fusarium solani*) and the test organisms (plant growth promoting rhizobacteria) while being kept in a slanted position. Growth inhibition was observed after an incubation period of 7 days in comparison to uninoculated controls withoutplant growth promoting rhizobacteria.

2.5.7 Extraction, purification and characterization of exopolysaccharides

Mineral salt medium (optimized) was used for culturing EPS-producing bacteria (Bramchari and Dubey 2006). The plant growth promoting rhizobacteria cultures in this medium were than centrifuged at 15,000 rpm (10 min) after 10 day incubation period. The supernatant was mixed with 2 fold ice cold ethanol (95%) for EPS extraction and the complete precipitated solution was cooled at 4 °C and EPS was obtained from overhead solution (Kumar et al. 2011). The extracted EPS were lyophilized with Labonco lyophilizer at 3000 psi and stored at room temperature (Bramchari and Dubey 2006). Slight amounts of lyophilized EPS were deferred in 2 ml of benzene, water, chloroform, acetone, ethanol, and methanol for determination of its solubility. The mixture was vortexed and stabilized for some time until the pellet development was observed.

2.5.8 Relative water content (RWC)

Relative water content (LRWC) was estimated for fresh leaves according to Weatherly (1950).

$$\mathrm{RWC}(\%) = [(\mathrm{FW}-\mathrm{DW})/(\mathrm{TW}-\mathrm{DW})] \times 100$$

FW: represents sample fresh weight; TW: sample turgid weight; DW: sample dry weight.

2.5.9 Root fresh and dry weight

Fresh weight of roots of five plants was measured with the help of an electronic balance. The roots were than dried in oven at 70 °C till constant weight and their dry weight was measured.

2.5.10 Soil organic matter

Soil organic matter was calculated according to the method described by Mylavarapu (2009).

2.5.11 Data analysis

The data were examined statistically by factorial ANOVA using statistics software (STATISCA version. 8.1). All treatments were tested in an experiment using randomized complete block design (RCBD) with four replicates.

3 Results

3.1 Alignment of 16S rRNA sequence

For the plant growth promoting rhizobacteria isolate P1 from the rhizosphere of wheat (*Triticum aevestivum L.*) grown in the arid region of Karak (7% moisture content), a total length of sequence with 1480 nucleotide bases was obtained. The assessment of the nucleotide sequence with data nucleotide bank exhibited maximum sequence resemblance of 99% with 1478/1480 bases with that of *Planomicrobium chinense* strain (ACC No.: JP007457.1). For the isolate P2 obtained from the arid region of Bhakkar (6% moisture content), the total length of sequence with 1483 nucleotide was attained. The evaluation of the nucleotide sequence with data nucleotide bank indicated sequence similarity of 99% (1482/1483 bases) with *Bacillus cereus* strain (ACC No.: CP003187.1). For the isolate P3 attained from arid region of Cholistan desert (4% moisture content), the total length of sequence with 1474 nucleotide was obtained. The evaluation of the nucleotide sequence with data nucleotide bank showed maximum sequence similarity of 99% (1472/1474 bases) with *Pseudomonas fluorescens* (ACC No.: GU198110.1).

3.2 Phosphorus solubilization index

The three plant growth promoting rhizobacteria were phosphate solubilizer, *Bacillus cereus* was the most effective phosphorus solubilizer with phosphorus solubilisation index of 2.986. The phosphorus solubilization indices for *Planomicrobium chinense* and *Pseudomonas fluorescens* were 2.142 and 1.444, respectively.

3.3 Antibacterial and antifungal activities of plant growth promoting rhizobacteria isolates

Planomicrobium chinense and *Bacillus cereus* inhibited the growth of bacterial strains i.e., *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia and Escherichia coli*. The cultures of *Pseudomonas fluorescens showed* no response against *Pseudomonas aeruginosa* (Table 2).

Maximum inhibition (58%) in mycelial growth of *Helminthosporium sativum* was detected due to *B. cereus* Whereas, *P. chinense* and *P. fluorescens* were ineffective

Table 2 Antibacterial and Antifungal activities of selected PGPR

Antibacterial activities						Antifungal activities (% Inhibition)	
Isolates	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*K.pneumonia*	*E. coli*	*H.sativum*	*F. solani*
P. chinense	+	+	+	+	+	–	–
P. putida	+	–	+	+	+	–	73%
B. cereus	+	+	+	+	+	58%	92%

+ – present, – –absent

against *Helminthosporium sativum. B. cereus* dramatically repressed the growth of *Fusarium solani* (92%) followed by *P. fluorescens* (73%) (Table 2).

3.4 Solubility and chemical composition of exopolysaccharides (EPS)

The lyophilized EPS was solvable in water and unsolvable in benzene, acetone and chloroform (Table 3). The chemical composition of plant growth promoting rhizobacteria induced EPS shown that the sugar and protein contents of EPS were 95% and 98% in *P. chinense*, 93% and 96% in *B. cereus* and 92% and 98% in *P. fluorescens,* respectively. However, highest (92%) uronic acid was found in *B. cereus*.

3.5 Soil moisture content

The moisture content of the rhizosphere soil was increased in all the treatments over un-inoculated control (Fig. 1). The maximum increase (88%) were recorded in T5 > T6 > T7 > T8 for the tolerant variety which was even greater (41%) than the irrigated control and ranked as T6 > T7 > T8 > T5 for the sensitive variety. In T1 (P1 inoculation) and T4 (coinoculation with P2 + P3 along with salicylic acid and putrescine) the soil moisture content of sensitive variety was higher than that of the tolerant variety. T2 and T4 had equal % increase in soil moisture content of tolerant variety. T6, T7 and T8 significantly enhanced the soil moisture content over uninoculated control and irrigated control in the sensitive variety. Thus, demonstrating that addition of salicylic acid and Putrescine had significant effect on the soil moisture content.

3.6 Relative water content

All the inoculated treatments enhanced the relative water content of leaves over uninoculated control (Fig. 2). The relative water content of the tolerant variety was higher than that of the sensitive variety. Whereas, effect of T3 (coinoculation of P1 and P2) was at par for both tolerant and sensitive variety. Both the sensitive and tolerant varieties were more responsive to salicylic acid. Plants of sensitive variety, receiving the foliar spray of salicylic acid enhanced (89%) the relative water content. The effect of T4 (P1 and P2 is association with salicylic acid and putrescine) was higher than the effect of T3 (coinoculation of P1 and P2). Highly significant increase (86%) in tolerant variety was due to T4 followed by T7 = T2. Thus demonstrating that the addition of salicylic acid and putrescine had significant stimulatory effect on relative water content. The salicylic acid itself was also stimulatory to enhance water holding capacity of soil and relative water content of plants but to increase the root biomass they perform better working synergistically with the plant growth promoting rhizobacteria alone or more so in the consortium (e.g., T7 versus T8).

3.7 Nutrient analysis of rhizospheric soil

The total NO_3-N content was increased in all the treatments over uninoculated stressed control (Fig. 3). In T2 (P1 in association with salicylic acid and putrescine), T3 (coinoculation of P2 and P3) and T8 (plants sprayed with putrescine), the total NO_3-N of rhizosphere soil were higher in sensitive variety than that of the tolerant variety. Maximum increase (60% and 27%) in NO_3-N content was due to T3 (coinoculation of P2 and P3) and T3 = T6 as compared to uninoculated stressed

Table 3 Chemical characterization of EPS

Serial No.	PGPR Strains	Sugar content (ug/g)	Protein content (ug/g)	Uronic acid (ug/mg)
01	*Planomicrobium chinense*	6402.3	817.6	1.11
02	*Bacillus cereus*	5005.7	609.1	1.13
O3	*Pseudomonas fluorescence*	4797.9	593.4	1.1

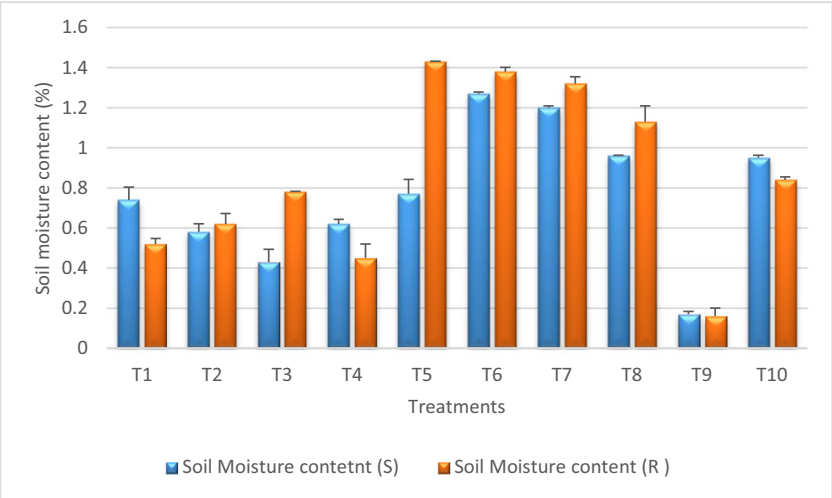

Fig. 1 Moistute content of rhizosphere soil of droght sensitive and tolerant wheat variety

control (T9) and irrigated control (T10) respectively. Plants sprayed with salicylic acid (T7) and putrescine (T8) enhanced (54%) the NO_3-N content as compared to untreated control. While in tolerant variety maximum increase (58% and 25%) was due to T6 (coinoculation of P1, P2 and P3 in association with salicylic acid and putrescine) as compared to uninoculated stressed control and irrigated control respectively. Whereas, total P-content was higher in T1, T2, T4, T5 and T6 but maximum increase (70 and 60%) was in plants sprayed with salicylic acid (T7). The % increase of nutrients was always higher in the tolerant variety.

Plant growth promoting rhizobacteria increased the K, Mg, Ca and decreased Na content in soil (Table 4). The K content was higher than Na Ca and Mg contents (T10). Both salicylic acid and putrescine either used alone or in combination with plant growth promoting rhizobacteria increased (41 and 51%) the Ca and K content over untreated (T9) and irrigated control (T10) in both the sensitive and tolerant varieties. The P1 and P2 inoculations increased the Ca and K contents of plants significantly over control. Significant increase (67%) in Mg and Na content of soil sample was observed due to P1 inoculation (T1) in sensitive variety and combined treatment of P1 and salicylic acid (T2) in tolerant variety as compared to stressed (T9) and irrigated control (T10). The K contents were higher in tolerant variety over that of stressed and irrigated control except T1, T3 and T4 treatments.

3.8 EC and pH

The EC was higher in rhizosphere soil in sensitive variety than that of tolerant variety in T3 (coinoculation of P2 and P3) and T5 (coinoculation of P1, P2 and P3) (Table 4). Electrical

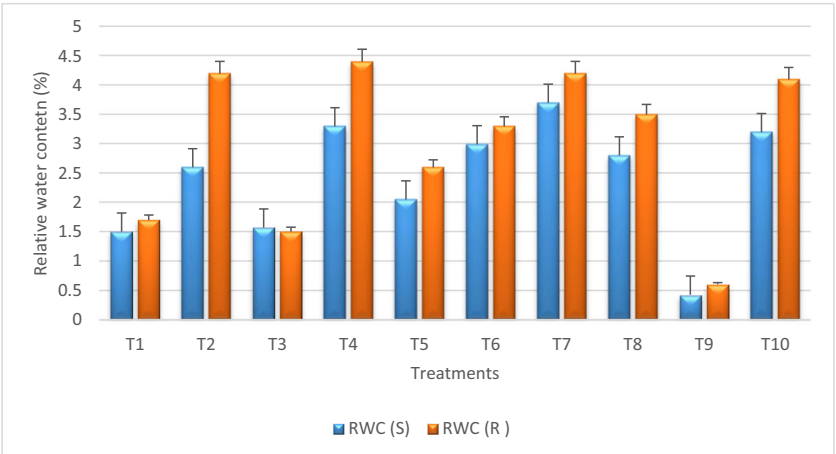

Fig. 2 Relative water content of leaves of sensitive and tolerant wheat variety. **T1-**Seeds inoculated with P1; **T2-** Seeds inoculated with P1 + Sprayed with SA and Putrescine; **T3-** Seeds inoculated with P2 and P3; **T4-** Seeds inoculated with P2 and P3+ Sprayed with SA and Putrescine; **T5-** Seeds inoculated with P1, P2 and P3; **T6-** Seeds inoculated with P1, P2 and P3+ Sprayed with SA and Putrescine; **T7-** Plants sprayed with Salicylic acid; **T8-** Plants sprayed with Putrescine;**T9-**Untreated uninoculated control; **T10-**irrigated control. **(P1:** *Planomicrobium chinense***, P2:** *Bacillus cereus***, P3:** *Pseudomonas fluorescence)*

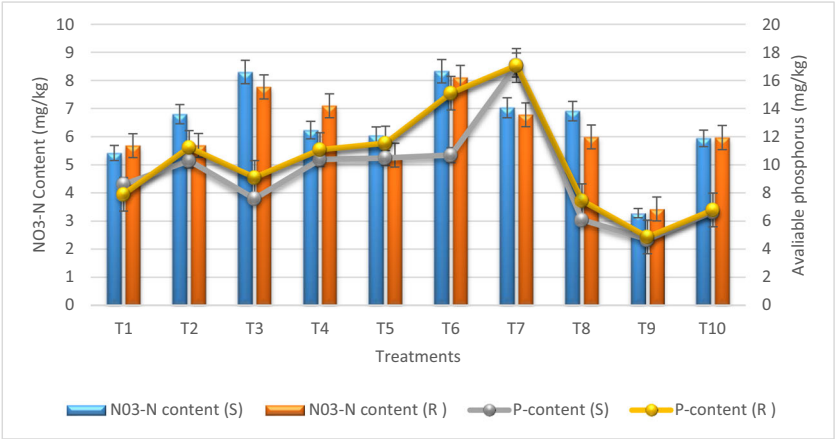

Fig. 3 Total Nitrate-nitrogen and available P-content of wheat rhizospheric soil

conductivity was 30% higher in soil samples treated with T3 in sensitive variety as compared to uninoculated control (T9) Whereas, in tolerant variety maximum increase (25%) was recorded in T1 and T1 = T4 = T6 = T10. Overall, the treatments had shown no significant differences in the pH of soil samples.

3.9 Root fresh and dry weight

The root fresh weight was significantly increased (70%) in all the treatments over un-inoculated stressed control (Fig. 4) but T6 (in both the varieties) and T2 (tolerant variety only) showed significant increases (27% and 37%) over irrigated control. T6 (P1, P2 and P3 in association with salicylic acid and putrescine) had 28% and 22% increases over T5 (coinoculation of P1, P2 and P3) and T4 (P2 + P3 in association with salicylic acid and putrescine). The root dry weight was increased in all the treatments over un-inoculated stressed control though the values were lower than that of irrigated control. In T5 and T6 the dry weights of sensitive variety were (31% and 17%) higher than that of tolerant variety. Higher increases were recorded in treatments which ranked as T4 > T2 > T6 > T8.

3.10 Na/K and Na/Ca ratio

The Na/K and Na/Ca ratios were lower in the irrigated control of the tolerant variety as compared to sensitive variety (T4). Although, salicylic acid and putrescine treatments exhibited higher Na/K and Na/Ca ratio and also increased the plant growth promoting rhizobacteria modulated Na/K and Na/Ca ratio but the tendency of plant growth promoting rhizobacteria were to decrease these ratio over stressed control, response was higher in the tolerant variety. Combined treatment of P2 and P3 was more effective as compared to P2 and P3 in presence of salicylic acid and putrescine. T6 (consortium) had low Na/K ratio than all other treatments. Whereas, the highest Na/Ca ratio was also recorded in T1 (P1 inoculum) of sensitive

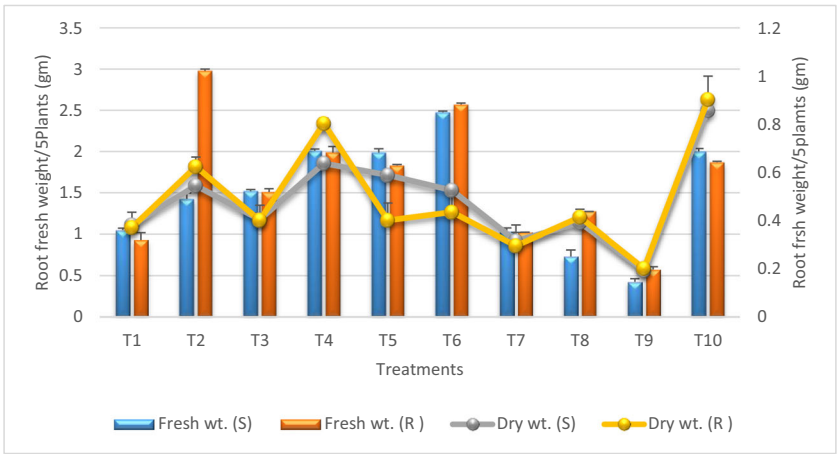

Fig. 4 Root fresh and dry weight/5-Plants of sensitive and tolerant wheat variety

variety. The combined treatment of P1, P2 and P3 (T5) had higher Na/Ca ratio than that of consortium along with salicylic acid and putrescine (T6) (Table 4).

4 Discussion

Plant growth promoting rhizobacteria inhabit plant roots and have substantial effects on plant growth particularly under stress conditions (Khan and Bano 2016b; Glick et al. 2007). These plant growth promoting rhizobacteria stimulate plant growth by a range of mechanisms including production of phytohormones, suppression of pathogenss, phosphate solubilisation, and enhanced nutrient uptake (Ashrafuzzaman et al. 2009; Martinez-Viveros et al. 2010; Zahir et al. 2003).

Phosphorus comprises the major macronutrient after nitrogen; typically present in insoluble form and cannot be used by the plants (Peccia et al. 2013; Pradhan and Sukla 2006). The present investigation revealed that the plant growth promoting rhizobacteria *Bacillus Cereus* and *Planomicrobium chinense* had greater phosphorus solubilisation, antifungal and antibacterial activity and exhibited greater exopolysaccharides production. These factors are thought to enable plants to withstand under water deficit conditions in sandy soils. The production of greater amounts of sugar, protein and uronic acid in their exopolysaccharides may account for the enhancement of the fresh and dry matter production by the roots of wheat seedlings. Similar results were reported previously (Naseem and Bano 2014; Vimala and Lalithakumari 2003). An augmented mass of soil aggregate around roots of the inoculated wheat plants and a highly significant positive correlation ($r = 0.866$, $p < 0.01$) of water unsolvable soil saccharides with rhizo-sheath/root ratio showed the influence of the bacterial exopolysaccharides in combining soil around roots over other soil microbial secretions or plant mucilages (Bezzate et al. 2000; Watt et al. 1993). Robertson and Firestone (1992) recommended that the rise in exopolysaccharides production by *Pseudomonas* during dehydration is required to ensure defence of this strain in sandy soil. An exopolysaccharides matrix adjacent a bacterial colony may slow down the drying process, thereby increasing the time available for metabolic adjustment.

It is noteworthy that salicylic acid augmented the effects of plant growth promoting rhizobacteria particularly in the case of the drought sensitive variety. The relative water content was greater than in the irrigated control in T2 (P1 in association with salicylic acid and putrescine), =T4 (P2 + P3 in association with salicylic acid and putrescine) = T7 (only salicylic acid). Possibly the drought sensitive variety faces the problem of secondary stresses such as osmotic and oxidative stress and plants treated with salicylic acid are better able to resist these.

In the drought sensitive variety salicylic acid alone and in association with the plant growth promoting rhizobacteria (T6) were more effective, demonstrating the positive role of salicylic acid in maintenance of water budget and turgidity of plants and augmenting the plant growth promoting rhizobacteria. This was evident only in T7 (salicylic acid alone) in case of sensitive variety though T4, T2 = T6 were effective and were at par to irrigated control. The relative water content (RWC) and lower electrolyte ion leakage (EL) in plants exposed to drought is indicative of relative tolerance to water stress (Fisher 2000; Pereyra et al. 2006). The evidence suggests that bacterial inoculation of plants increases their relative water content and decreases their % electrolyte leakage as compared with uninoculated plants (Naveed et al.

Table 4 Ca, Mg, Na, K contents and Na/K and Na/Ca ratio of rhizosphere soil of the wheat plants un-inoculated and inoculated with EPS-producing bacteria

Treat.	Ca (S) mg/Kg	Ca (R) mg/Kg	Mg (S) mg/Kg	Mg (R) mg/Kg	Na (S) mg/Kg	Na (R) mg/Kg	K (S) mg/Kg	K (R) mg/Kg	Na^+/K (S)	Na^+/K (R)	Na^+/Ca (S)	Na^+/Ca (R)	EC (S)	EC (R)
T1	1.51	1.60	2.08	0.80	2.13	1.07	13.46	14.13	0.15	0.07	1.42	0.66	100	120
T2	1.79	2.14	1.81	2.67	1.46	1.98	13.58	16.66	0.10	0.11	0.86	0.93	100	100
T3	1.71	1.58	1.88	0.70	1.30	1.02	11.76	12.83	0.11	0.09	0.76	0.65	130	100
T4	1.74	1.61	2.19	0.88	1.27	0.84	15.04	12.24	0.08	0.06	0.76	0.52	100	120
T5	1.75	1.80	1.92	1.11	1.65	1.01	14.30	16.83	0.11	0.06	0.94	0.56	120	110
T6	1.77	2.07	1.44	1.43	1.08	1.42	14.92	18.53	0.07	0.07	0.61	0.69	110	120
T7	1.73	2.28	1.11	1.82	1.46	1.91	14.45	17.78	0.10	0.10	0.84	0.84	100	100
T8	1.37	1.43	0.99	1.22	1.23	1.70	12.96	16.26	0.09	0.10	0.90	1.19	100	110
T9	1.10	1.30	1.00	0.78	0.87	0.96	9.968	12.17	0.08	0.07	0.79	0.74	90	90
T10	1.07	1.18	0.64	0.97	0.94	0.81	6.862	10.70	0.13	0.07	0.89	0.69	110	120

T1-Seeds inoculated with P1; **T2-** Seeds inoculated with P1 + Sprayed with SA and Putrescine; **T3-** Seeds inoculated with P2 and P3; **T4-** Seeds inoculated with P2 and P3+ Sprayed with SA and Putrescine; **T5-** Seeds inoculated with P1, P2 and P3; **T6-** Seeds inoculated with P1, P2 and P3+ Sprayed with SA and Putrescine; **T7-** Plants sprayed with Salicylic acid; **T8-** Plants sprayed with Putrescine;**T9-**Untreated uninoculated control; **T10-**irrigated control

2014; Sandhya et al. 2010). In addition, a mycorrhizal association along with plant growth promoting rhizobacteria can also benefit plants growing under drought conditions particularly in drought sensitive cultivars (Subramanian et al. 1997).

The relative water content (RWC) is correlated with root fresh weight in our study. Data revealed that the salicylic acid and putrescine alone (T7 and T8) or in combination with plant growth promoting rhizobacteria performed better in maintaining relative water content than that of stressed uninoculated control but were not as effective as in treatments T2 > T6 > T4 = T5 which were greater than or at par to irrigated control for both the varieties; indicating that the effects of plant growth promoting rhizobacteria was significantly augmented by salicylic acid and putrescine and that *Planomicrobium chinense,* (T1) appeared an effective plant growth promoting rhizobacteria bioinoculant in the tolerant variety, but not in the drought sensitive variety. The treatment with T1 (P1) alone was not as stimulatory as P1, P2 and P3 in association with salicylic acid and putrescine. Our findings concur with those of Sandhya et al. (2010). They demonstrated that bacterial inoculation of maize seeds with *Pseudomonas spp.* strains improved plant biomass, relative water content, leaf water potential, root adhering soil/root tissue ratio, aggregate stability and mean weight diameter and decreased leaf water loss.

With respect to moisture in the rhizosphere, T6 (P1, P2 and P3 in association with salicylic acid and putrescine) was the best treatment as it helped to retain maximum moisture in the rhizosphere soil and also resulted in the maximum root fresh weight that was even greater than that of irrigated control. The T5 > T6 > T7 > T8 treatments also have soil moisture higher than that of the irrigated control but the root fresh weight was higher than irrigated C (T10) only in T6 (P1, P2 and P3 in association with salicylic acid and putrescine) and T2 (P1 in association with salicylic acid and putrescine) for both the sensitive and tolerant varieties. Grover et al. (2011) reported that plant growth promoting rhizobacteria enhanced retention of soil moisture, and adaptation in agriculture crops exposed to abiotic stresses. Our results are also in line with the findings of Dobbelaere et al. (2001) who assessed the effect of inoculation by *Azospirillum brasilense*on on the growth of spring wheat. They observed that inoculated plants had increased dry weight in both the root system and the upper plant parts.

Plant growth promoting rhizobacteria have been reported to secrete phytohormones in soil that modulate the endogenous levels of phytohormones like IAA and GA (Achard et al. 2009; Patten and Glick 2002). These stimulate cell division and cell elongation and hence increase root biomass, also stimulate nutrient and water uptake as observed in the present investigation (Farwell et al. 2006). An increase in phytohormone (Gibberellic acid, Indole-3-acetic acid and Abscisic acid) production in wheat plants inoculated by *Bacillus cereus* was also reported by Hassan and Bano (2015). *Bacillus cereus* was less effective than *P. chinense* and the consortium was more effective for sensitive variety. This may be attributed to their higher P-solubilizing potential and exopolysaccharides production by *P. chinense* (P1) and *P. fluorescens* (P3).

The results demonstrate that total NO3-N content and phosphorus contents was enhanced in plant growth promoting rhizobacteria inoculated plants. Salicylic acid in particular augmented this particularly with respect to phosphorus content. Salicylic acid occurs in plants in very minute quantities. It has been recognised as a vital signalling factor in plants (Alvarez 2000). Khan et al. (2010) found that foliar sprays of salicylic acid (100 ppm) resulted in higher uptake of nutrients from soil while putrescine sprays had inhibitory effect on phosphorus accumulation but enhanced the total NO3-N content. Furthermore the Na/K and Na/Ca ratio were increased. The observed increase in the ratio of Na/K or Na/Ca in plants inoculated with the plant growth promoting rhizobacteria consortium particularly in the sensitive variety is worth mentioning. Meloni et al. (2001) reported that osmotic adjustment of roots and leaves was predominantly controlled by Na/K and Na/Ca accumulation. Drought sensitivity had been reported in plants as being due to a lower Na/K ratio (Sairam et al. 2002). Increased K concentrations under stress conditions may help to reduce sodium uptake required for maintaining the necessary osmotic balance (Mahajan 2005).

In conclusion, it is inferred from the data obtained that salicylic acid in combination with plant growth promoting rhizobacteria in presence of putrescine promote root growth and improves the relative water content in comparison with control plants grown in the sandy soils. The exopolysaccharides produced by plant growth promoting rhizobacteria were found to enhance root growth and biomass. The integrated use of plant growth promoting rhizobacteria along with salicylic acid appears to be a promising and eco-friendly approach for increasing drought tolerance in wheat plants and also for the rehabilitation of unproductive desert lands.

References

Achard P, Gusti A, Cheminant S, Alioua M, Dhondt S, Coppens F, Genschik P (2009) Gibberellin signalling controls cell proliferation rate in Arabidopsis. Curr Biol 19(14):1188–1193

Aira M, Gómez-Brandón M, Lazcano C, Bååth E, Domínguez J (2010) Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. Soil Biol Biochem 42(12):2276–2281

Allison AG (1998) Exopolyaccharide production in bacterial biofilm. Biofilm J 3(2):1–19

Alvarez ME (2000) Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Pl Mol Biol 44:429–442

Ashrafuzzaman M, Hossen FA, Ismail MR, Hoque A, Islam MZ, Shahidullah SM, Meon S (2009) Efficiency of plant growth-promoting rhizobacteria (Plant growth promoting rhizobacteria) for the enhancement of rice growth. Afr J Biotechnol, 8 (7)

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68(1):1–3

Bezzate S, Aymerich S, Chambert R, Czarnes S, Berge O, Heulin T (2000) Disruption of the *Paenibacillus* polymyxa levansucrase gene impairs its ability to aggregate soil in the wheat rhizosphere. Environ Microbiol 2:333–342

Bhaskar PV, Grossart HP, Bhosle NB, Simon M (2005) Production of macroaggregates from dissolved exopolymeric substances (EPS) of bacterial and diatom origin. FEMS Microbiol Ecol 53(2):255–264

Bouffaud ML, Kyselková M, Gouesnard B, Grundmann G, Muller D, MOËNNE-LOCCOZ YV (2012) Is diversification history of maize influencing selection of soil bacteria by roots? Mol Ecol 21(1):195–206

Bramchari PV, Dubey SK (2006) Isolation and characterization of exopolysaccharides produced by *Vibrio harveyi* strain VB23. Lett Appl Microbiol 43:571–577

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EV, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS ONE 8:e55731. doi:10.1371/journal.pone.0055731

Chen WP, Kuo TT (1993) A simple and rapid method for the preparation of Gram –ve bacterial genomic DNA. Nucleic Acid Res 21(9):2260

Czarnes S, Hallett PD, Bengough AG, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport. Eur J Soil Sci 51:435–443

Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Aguirre JF, Kapulnik Y, Brener S (2001) Responses of agronomically important crops to inoculation with *Azospirillum*. Funct Plant Biol 28(9):871–879

Drogue B, Dore H, Borland S, Wisniewski-Dyé F, Prigent C (2012) Which specificity in cooperation between phytostimulating rhizobacteria and plants? Res Microbiol 163:500–510. doi:10.1016/j.resmic.2012.08.006

Farwell AJ, Vesely S, Nero V, Rodriguez H, Shah S, Dixon DG, Glick BR (2006) The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site. Plant Soil 288(1–2):309–318

Fisher DB (2000) Long distance transport. In: Buchanan BB, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Biology, Rockville, pp 730–784

Fusconi R, Godinho MJL (2002) Screening for exopolysaccharide producing bacteria from sub- tropical polluted groundwater. Braz J Biol 62(2):363–369

Gilck BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperical college press, London, pp 187–189

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACCdeaminase. Crit Rev Plant Sci 26:227–242

Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27(5):1231–1240

Hassan TU, Bano A (2015) Role of carrier-based biofertilizer in reclamation of saline soil and wheat growth. Arch Agron Soil Sci 61(12):1719–1731

Khan N, Bano A (2016a) Role of Plant Growth Promoting Rhizobacteria and Ag-nano particle in the Bioremediation of heavy metals and Maize growth under Municipal wastewater irrigation. Int J Phytoremediation 18(3):211–221. doi:10.1080/15226514.2015.1064352

Khan N, Bano A (2016b) Modulation of Phytoremediation and Plant growth with the treatment of plant growth promoting rhizoacteria, Ag nanoaprticle and untreated municipal wastewater. Int J Phytoremediation 18(12):1258–1269. doi:10.1080/15226514.2016.1203287

Khan N, Syeed S, Masood A, Nazar R, Iqbal N (2010) Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. Int J Plant Biol 1(1):1

Kumar AM, Anandapandian KTK, Parthiban K (2011) Production and characterization of exopolysaccharides (EPS) from biofilm forming marine bacterium. Braz Arch Biol Technol 54:259–265

MacFaddin (1980) Biochemical Tests for Identification of Medical Bacteria, pp 51–4. Williams and Wilkins, Baltimore

Mahajan S (2005) Tuteja N (2005) Cold, salinity and drought stresses: An overview. Arch Biochem Biophys 444:139–158

Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot 55:27–34. doi:10.1093/jxb/erh010

Martinez-Viveros O, Jorquera MA, Crowley DE, Gajardo GMLM, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10(3):293–319

McCully ME, Canny MJ (1988) Pathways and processes of water and nutrient movement in roots. Plant Soil 111:159–170

McKeague JA (1978) Manual on soil sampling and methods of analysis. Can Soc Soil Sci 30:66–68

McLean EO (1982) Soil pH and lime requirement. In Page, A. L., R. H. Miller and D. R. Keeney (eds.) Methods of soil analysis. Part 2 - Chemical and microbiological properties. (2nd Ed.). Agronomy 9:199–223

Meloni DA, Oliva MA, Ruiz HA, Martinez CA (2001) Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. J Plant Nutr 24(3):599–612

Miller JH (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 354–358

Mylavarapu R (2009) UF/IFAS Extension Soil Testing Laboratory (ESTL) Analytical Procedures and Training Manual. Circular 1248, Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida

Nannipieri P, Ascher J, Ceccherini M, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54(4):655–670

Naseem H, Bano A (2014) Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. J Plant Interact 9(1):689–701. doi:10.1080/17429145.2014.902125

Navarro V, Villarreal M, Rojas G, Lozoya X (1996) Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. J Ethnopharm 53(3):143–147

Naveed M, Hussain MB, Zahir ZA, Mitter B, Sessitsch A (2014) Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul 73(2):121–131

Pal S, Manna A, Paul AK (1999) Production of (poly-hydroxy butyric acid) and exopolysacharide by *Azotobacter beijerinckii* WDN - 01. World J Microbiol Biotech 15:15–2

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl Environ Microbiol 68(8):3795–3801

Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman JB (2013) Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends Biotechnol 31(3):134–138

Pereyra MA, Zalazar CA, Barassi CA (2006) Root phospholipids in *Azospirillum* inoculated wheat seedlings exposed to water stress. Plant Physiol Biochem 44:873–879

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17(362):e370

Pothier JF, Wisniewski-Dyé F, Weiss-Gayet M, Moënne-Loccoz Y, Prigent-Combaret C (2007) Promoter-trap identification of wheat seed extract-induced genes in the plant-growth-promoting rhizobacterium *Azospirillum brasilense* Sp245. Microbiology 153: 3608–3622. doi:10.1099/mic.0.2007/009381-0

Pradhan N, Sukla LB (2006) Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr J Biotechnol 5:850–854

Raynaud X, Jaillard B, Leadley PW (2008) Plants may alter competition by modifying nutrient bioavailability in rhizosphere: a modeling approach. Am Nat 171(1):44–58

Robertson EB, Firestone M (1992) Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas sp*. Appl Environ Microbiol 58:1284–1291

Sairam RK, Rao KV, Srivastava GC (2002) Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci 163(5):1037–1046

Sandhya V, Ali SZ, Grover M, Reddy G, Venkateswarlu B (2010) Effect of plant growth promoting *Pseudomonas spp*. on compatible solutes, antioxidant status and plant growth of maize under drought stress. Plant Growth Regul 62(1):21–30

Shukla KP, Sharma S, Singh NK, Singh V, Tiwari K, Singh S (2011) Nature and role of root exudates: efficacy in bioremediation. Afr J Biotechnol 10:9717–9724

Steel KJ (1961) The oxidase reaction as a toxic tool. J Genetics Microbiol 25:297–306

Subramanian KS, Charest C, Dwyer LM, Hamilton RI (1997) Effects of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize. Can J Bot 75(9): 1582–1591

Vanhaverbeke C, Heyraud A, Mazeau K (2003) Conformational analysis of the exo-polysaccharide from *Burkholderia caribensis* strain MWAP71: Impact on the interaction with soils. Biopolymers 69: 480–497

Vimala P, Lalithakumari D (2003) Characterization of exopolysaccharide (EPS) produced by *Leuconostoc sp*. V 41. Asian J Microbiol Biotechnol Environ Sci 5(2):161–165

Viveros OM, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10(3):293–319

Washington JA, Sutter VL (1980) Dilution susceptibility test agar and micro broth dilution procedure. anual of clinical Microbiology, 3rd edn. American society of microbiology, Washington, p 453

Watt M, McCully ME, Jeffree CE (1993) Plant and bacterial mucilages of maize rhizosphere: comparison of their soil binding properties and histochemistry in a model system. Plant Soil 151:151–165

Watt M, McCully ME, Canny MJ (1994) Formation and stabilization of rhizosheaths of *Zea mays L*. Plant Physiol 106:179–186

Weatherly PE (1950) Studies in the water relations of the cotton plant. 1. The field measurement of water deficits in leaves. New Phytol 49: 81–97

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) I6S-ribosomal DNA amplification for phylogenetic study. J Biotechnol 173:16–22

Zahir ZA, Arshad M, Frankenberger WT (2003) Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv Agron 81:97–168