Soil Environ. 37(2): 178-189, 2018
DOI:10.25252/SE/18/61522
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Wheat (*Triticum aestivum* L.) growth promotion by halo-tolerant PGPR-consortium

Lubna Rajput[1,2], Asma Imran[1*], Fathia Mubeen[1] and Fauzia Y. Hafeez[1,3]
[1]National Institute for Biotechnology and Genetic Engineering (NIBGE), Jhang Road, Faisalabad, Pakistan
[2]Plant Physiology Section, Agriculture Research Institute, Tandojam, Pakistan
[3]Department of Biosciences, COMSATS Institute of Information Technology, Chak Shahzad Campus, Islamabad, Pakistan

Abstract

Salinity is among the major environmental factors that significantly affects the global crop production. Inoculation with plant growth promoting rhizobacteria (PGPR) increases crop productivity because PGPR convert the un-available forms of nutrients to plant-available form. With the aim to develop saline-soil specific biofertilizer, bacteria were isolated from rhizosphere of wheat grown in saline soil (EC 7.63 dS m^{-1}). Of total 21, eight bacteria showed halophilic (up to 65g L^{-1} NaCl), and four showed alkaliphilic (up to pH 9.5) trait; 12 isolates produced indole-3-acetic acid (411.5-9.33 mg L^{-1}), 15 bacterial isolates solubilized inorganic tri-calcium phosphate (17.5-6.7 mg L^{-1}), 14 isolates exhibited ACC-deaminase activity, and only one isolate solubilized the insoluble ZnO. A consortium of three potential PGPR strains (SAL-12, SAL-17, SAL-21; having multiple PGP traits) was tested for two years in laboratory and field experiments for wheat productivity with half dose of chemical fertilizer (NPK) under induced and natural salinity. The comparison of results with a non-halophilic wheat inoculum (BioPower containing Azospirillum and Pseudomonas spp.) indicated that both halo-tolerant and non-halo tolerant PGPR-consortia with reduced fertilizer dose have potential to increase the growth and yield of wheat in saline conditions. The relative increase in yield induced by halo-tolerant consortia was however, significantly better as compared to non-halo-tolerant PGPR inoculum that may be attributed to salt tolerance potential and stable PGP activities of PGPR indigenous to stressed environment. The study suggests using eco-friendly, cost-effective PGPR-biofertilization (inoculation) technology for wheat productivity in saline environments with reduced application of chemical fertilizers.

Keywords: Salinity, PGPR, *Aeromonas*, wheat, IAA, ACC-deaminase, seed inoculation

Introduction

The presence of excessive salts (especially sodium) in soils induces ionic, osmotic and oxidative stress in plants. Salinity inhibits seed germination (Singh *et al.*, 2008; Goswami *et al.*, 2014), respiration, cytosolic protein production (legheamoglobin), shoot dry weight, shoot-N contents, and photosynthetic activity of the plant (Borucki and Sujkowska, 2008; Abd-Alla, 2014).

Indigenous micro flora of salt affected soils contains mechanisms to overcome and combat with the salt (Mayak, 2004; Orhan, 2016). They contain the enzyme ACC deaminase which hydrolyzes the ethylene precursor ACC to ammonia and α-ketobutyrate (Glick *et al.*,1998; Arshad *et al.* (2007) and results in reduced endogenous ethylene ultimately stimulating the root development (Hirsch and Fang, 1994) and plant growth (Nakbanpote *et al*., 2014) Bacteria from salt-stressed environment may contain plant beneficial traits *e.g*., nitrogen fixation (Nakbanpote *et al.*, 2014), production of plant hormones (Bilal *et al.*, 1990; Malik *et al*., 1991), and solubilization of phosphate compounds (Srinivasan *et al.*, 2012; Soni *et al.*, 2013). Inoculation with these plant growths promoting rhizobacteria (PGPR) promotes growth and yield of different crops in a range of environmental and ecological conditions (Zhang *et al.*, 2012) even under salt stress (Rajput *et al.*, 2013; Singh and Jha, 2016).

Being in arid to semi-arid climatic zones, Pakistani soils are prone to salinity. Salinized area is increasing at the rate of 10% annually due to various reasons and multiple factors (Rengasamy, 2006). It has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011). Rapidly growing population, urbanization, continuous reduction in the agricultural land, constant decrease in soil fertility urgently need to devise feasible strategies for converting saline lands into economically viable entities. Salt tolerant crop varieties are practical solution for saline agriculture, but it requires a lot of time, input and investment for variety development (Dwivedi *et al.*, 2010). On the other hand, induction of salt-tolerance by biofertilization of crops with salt-tolerant bacteria is an integrated and cost-effective solution to the salinity (Egamberdieva *et al*., 2013). Rhizosphere microbiome has direct impact on overall plant growth, yield and sustenance so this microbiome can be manipulated for introduction of salt tolerance in crops (Yadav *et al*., 2017).

*Email: asmaaslam2001@yahoo.com

Keeping in view the increasing problem of salinity, our aim was to formulate a halophilic microbial inoculum for sustaining the wheat yield under salinity as wheat is the staple crop of Pakistan and moderately sensitive to salinity. We have identified salt tolerant PGPR that confer salt tolerance in wheat on one hand and promote its growth on the other hand with lesser input of chemical fertilizer. The results indicate the growth promoting potential of a "dual-purpose PGPR-consortium" based on halo tolerant *Aeromonas* species which may be introduced as saline biofertilizer as an integrated solution for crop (wheat)-farming in the emerging saline lands of the country with less reliance on chemical fertilizers.

Materials and Methods

Bacterial isolation, growth and salt tolerance

Wheat plants growing at Biosaline Research Station-II (BSRS-II) Pakka Anna (31°24/ N and 73º05/E) were carefully uprooted and rhizosphere soil tightly adhering to the root was removed. Bacteria were isolated from soil by serial dilution plating as described by Somasegaran and Hoben (1994). Twenty-one colonies were tested for salt tolerance by growing in LB-broth supplemented with 100-600 mM NaCl concentration at 28±2ºC for 4 days. Growth was confirmed by taking the culture OD at 660. Extreme salt tolerance was checked onto halophilic agar medium (containing 65g L^{-1} salt) plates as described by Akhtar *et al.* (2008).

Physiological & Biochemical Characterization

Colony morphology, gum production, cell morphology, motility, acid/alkali production, reaction for amino-peptidase, cytochrome oxidase and catalase enzymes as well as intrinsic antibiotic resistance pattern were checked as described in detail earlier (Imran *et al.,* 2015). Extreme tolerance to alkali was tested on solid alkaliphilic medium as described by Akhtar *et al.* (2008). Gram's staining was done following the Vincent (1970).

Bioassays for plant growth promoting traits

Nitrogenase activity of bacteria was checked through acetylene reduction assay (Hardy *et al*., 1973) in semi-solid NFM medium using a Gas Chromatograph (Thermoquest, Trace GC, Model K, Rodon Milan, Italy) equipped with a hydrogen flame ionization detector (FID). Activity was measured and expressed in ηmol of ethylene formed per hour as described by Park *et al.* (2005)

For IAA production, bacteria were grown in LB-broth (both with and without salt) supplemented with 100 mg L^{-1} tryptophan. The IAA was detected by colorimetric method (Gordon and Weber, 1951) and quantified by ethyl acetate extraction method (Tien *et al.,* 1979) using HPLC (Perkin Elmer, USA).

P-solubilization was checked by spot inoculation onto Pikoviskaya's agar while quantification was carried out in Pikoviskaya's broth by Phospho-molybdate blue color method (Murphy and Riley, 1962) using spectrophotometer (Camspec M350). Zinc solubilization was tested by spot inoculation on LGI medium (Cavalcante and Döbereiner, 1988) supplemented with 0.1% zinc oxide.

The ACC-Deaminase activity was checked in Dworkin–Foster (DF) minimal-salt medium (Dworkin and Foster, 1958) containing 0.5M ACC as sole nitrogen source at different concentrations of salt (100-600 mM).

Identification of potential halo-tolerant PGPR

Genomic DNA was extracted from bacterial strains SAL-12, SAL-17 and SAL-21 using standard procedure (Maniatis *et al.,* 1982) and used to amplify the 16S rRNA gene with primers P1 / P6 using conditions as described earlier (Tan *et al.,* 1997). The amplified products were got sequenced commercially from Macrogen (Korea). The gene sequence was analyzed using sequence scanner software package; compared with others in the GenBank database using the NCBI BLAST (Altschul *et al.,* 1990). The sequences were submitted to the database and the accession numbers were obtained.

Plant inoculation assays

Formulation of PGPR consortium and experimental set-up

As the PGPR obtained from BSRS-II contain variable potential for salt tolerance and a range of different plant growth promoting properties, hence, a consortium was formulated containing potential strains (SAL-12, SAL-16, SAL-17) after confirming compatibility by standard well-cut method (Chin *et al*., 2001). The bacterial strains were grown in LB broth up to an OD of 0.45, cells were harvested by centrifugation, mixed (1:1:1 ratio) in 0.85% saline to get a consortium of halo-tolerant bacteria (PGPR-consortium) for plant inoculation.

Two pot experiments were designed in completely randomized design (CRD) with a moderately salt tolerant wheat variety TJ-83. Surface sterilized seeds were germinated on water agar (1%) in dark at 20±2°C and transplanted to pots after 3 days of germination.

Pot experiment 1

To evaluate the effect of induced salinity on the early seedling and vegetative growth, the experiment was conducted in sterile salinized sand as described by Rajput *et*

al. (2013). One mL of inoculum was applied at the base of seedling after 2 days of transplantation. Inoculated plants vegetated in non-salinized sand served as positive while non-inoculated plant vegetated in salinized sand served as negative controls. The plants were maintained in growth room, watered with Hoagland (Hoagland and Arnon, 1950) solution as per requirement and harvested 45 days after germination. Four replicates were maintained per treatment. Data regarding morphological traits were recorded and compared to the positive and negative controls.

Root colonization was analyzed by fluorescent staining with acridine orange at 7th day post inoculation. The plants were carefully up-rooted; roots were cut in to smaller sections, placed on glass slide and stained for 10 minutes with dye (0.1 mg mL^{-1} distilled water). The stained roots were washed and observed under confocal laser scanning microscope (Fluo view, FV 1000, Olympus) using argon ion laser line at 502-525 nm. The images were captured and processed using FluoView software (Olympus).

Pot experiment 2

This experiment was set up to evaluate the P-solubilization potential of bacteria *in vitro*. The set up was like pot experiment 1. Sterilized sand (450 g) was thoroughly mixed (5-times) with 3g tri-calcium phosphate (dissolved in 10 mL water). The sand was dried and mixed aseptically and filled in pots. Three days old germinated seedlings were transplanted to pots. Plants were maintained in growth room, watered with Hoagland solution and harvested 45 days after germination. Plants without bacterial-inoculation and without TCP (phosphorus) were used as negative control. Plants were maintained in growth room, watered with Hoagland (Hoagland and Arnon, 1950) solution as per requirement and harvested 45 days after germination. Four replicates were maintained per treatment. Data regarding morphological traits were recorded and compared to the positive and negative controls.

Field experiment

On-site field evaluation of PGPR consortium was conducted for two consecutive years at BSRS-II. The experiments were laid out in randomized complete bock design (RCBD) in 25m^2 plots with three replications each. The evaluated treatments were: Full NPK (Full fertilizer control), Half NPK (Half fertilizer control), *BioPower* + ½ NPK (non-halophilic PGPR inoculum control congaing *Azospirillum* and *Pseudomonas* spp.), PGPR-consortium + ½ NPK (halo tolerant PGPR consortium). Fertilizer recommendation for variety TJ-83 is 3 bags DAP (in three split doses), 2 bags SSP (before sowing) and seed rate is 50 kg ha^{-1} in normal soil (Kazi *et al*., 2016). Because this was degraded saline soil with poor fertility, so fertilizer DAP and potassium (Engro Chemicals, Pakistan) were added during the field preparation as per following rate i.e., phosphorus 90 kg ha^{-1} and potassium 60 kg ha^{-1}. In full N control 170 kg ha^{-1} Urea was added while in ½ N plots 85 kg ha^{-1} was added. Seed was coated with bacterial inoculum using filter mud as carrier material and sown @ 75 kg seeds / ha. The crop was irrigated with brackish water three times during whole season. Data on morphological and yield traits were recorded at maturity (130 days after planting). Five plants were taken from 1 m^2 of each replicate plot to estimate the data and means were calculated.

Statistical analysis

In vitro quantification studies on salt tolerance, P-solubilization, nitrogenase activity and IAA production were carried out with three independent replicates and means were calculated. The data was averaged over 4 replicates (from pots) and three replicates (field) and subjected to analysis of variance (ANOVA) using MSTAT-C. Significance was measured at LSD 0.05. Regression and correlation was performed using SPSS (Version 17).

Results and Discussion

Ever increasing saline ecosystems have drastically reduced plant growth and crop yield (Ahmad *et al.,* 2010; Ashraf *et al.,* 2012). The development of halophytes (through breeding or genetic engineering) remains mostly unsuccessful due to the genetic and physiological complexity of the salt tolerance trait (Araus *et al.,* 2008; Dwivedi *et al.,* 2010). The PGPR mediated plant's salinity tolerance is a well-known phenomenon (Glick, 2005; Lugtenberg and Kamilova, 2009; Pliego *et al.,* 2011; Egamberdieva *et al.,* 2013) and offers economically practicable approach for combating salinity at large scale.

The present study was conducted with the aim to screen the bacterial population present in the rhizosphere of wheat so that a "dual-purpose PGPR inoculum" may be developed to be used in salt effected soils of Pakistan for growing wheat.

Isolation and characterization of halo-tolerant bacteria

Twenty-one bacterial isolates were obtained from wheat rhizosphere with variable potential for salt and pH tolerance. The bacterial isolates showed variable no. of cells (cfu) in salinized medium (Figure 1A). Morpho-physiological characteristics (Table S1) showed variable characters regarding gram's reaction, acid production, catalase and oxidase reaction, motility and cell/colony morphologies. This heterogeneity of bacterial population from a single host may be attributed to the soil conditions (especially salt) which plays a vital role in shaping the structure and activities of rhizo-microbiome (Ofek *et al.,* 2006) as it influences the quantity and/or quality of root exudates (Nelson and Mele, 2007).

Supplementary Table 1: Phenotypic characteristics of bacterial isolates from wheat rhizosphere soil investigated in this study

Isolate	Catalase	Oxidase	Production of		Solubilization of		Resistant to antibiotic				Cell Shape	Colony colure on LB agar	Gram's reaction	Reaction on BTB medium		Motility	Growth on medium	
			IAA*	ACC - deaminase	P**	Zn**	Strep.	Gent.	Neo.	Amp.				Acidic	Alkali		Halo-philic	Alkali-philic
SAL-1	+	+	186.6	-	-	-	-	-	-	-	Very short rods	White	-	+	-	+	-	-
SAL-2	+	-	151.7	-	+	-	+	-	-	+	Very short rods	Yellow	+	+	-	+	-	-
SAL-3	-	+	-	+	6.7	-	-	-	-	-	Very short rods	Yellow	+	-	+	+	-	-
SAL-4	-	-	-	-	8.4	-	-	-	-	-	Thin rods	Off-white	-	+	-	+	+	-
SAL-5	+	+	-	+	10.3	-	+	-	-	-	Small rods	Off-white	-	+	-	+	-	-
SAL-6	+	+	-	+	13.9	-	+	-	-	-	Short rods	Off-white	-	+	-	+	-	-
SAL-7	+	+	197.2	+	-	-	-		-	-	Very short rods	Off-white	-	-	+	-	-	-
SAL-8	-	-	-	+	10.1	-	-		-	-	Rods	Off-white	+	+	-	-	-	-
SAL-9	+	-	-	-	8.42	-	-		-	-	Short rods	Off-white	-	+	-	-	-	-
SAL-10	-	-	186.6	+	10.2	-	+	-	-	-	Short rods	Off-white	+	-	+	-	-	-
SAL-11	+	+	-	+	10.7	-	-	-	-	-	Long rods	Off-white	-	-	+	+	+	+
SAL-12	+	+	348.3	+	13.1	+	-	-	-	-	Short rod	Off-white	-	-	+	+	+	+
SAL-13	+	+	-	+	11.6	-	+	-	-	-	Small rods	Off-white	-	-	+	+	+	-
SAL-14	-	-	248.3	+	11.7	-	-	+	+	-	Small rods	Off-white	-	-	+	+	-	-
SAL-16	+	+	295.1	+	17.5	-	-	-	-	-	Long rods	Off-white	-	-	+	+	+	+
SAL-17	+	+	411.5	+	12.7	-	+	+	+	+	Short rod	Yellow	-	-	+	+	+	-
SAL-18	+	+	167.46	-	-	-	-	-	-	-	Long rods	White	-	-	+	-	+	-
SAL-19	-	-	-	-	-	-	+	+	+	-	Very short Rods	White	+	+	-	+	-	-
SAL-20	-	-		-	-	-	-	-	-	-	Short Rods	White	-	+	-	+	-	-
SAL-21	+	+	118.5	+	11.5	-	+	+	+	-	Very short rods	White	-	+	-	+	+	+
SAL-22	+	+	9.33	+	-	-	-	-	-	-	Rods	Off-white	-	+	-	+	-	-

*Indole acetic acid (IAA), Phosphate-solubilization and Zn Solubilization checked at 100 mM NaCl concentration;
Strep=Streptomycin 10µg/mL, Gent=Gentamycin 10µg/mL, Neo=Neomycin 30µg/mL, Amp=Ampicillin 10µg/mL
+= The character/reaction is present; - = The character/reaction is absent; BTB-medium=LB supplemented with Bromothyml blue

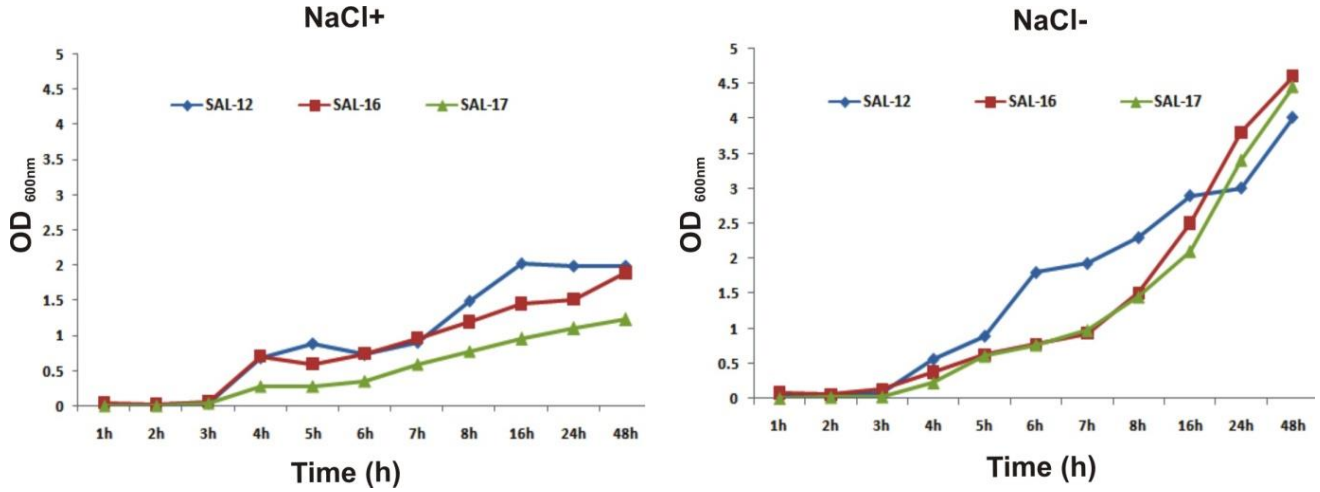

Figure 1(A): Growth (optical density) of bacteria in LB-medium supplemented with NaCl (600 mM)

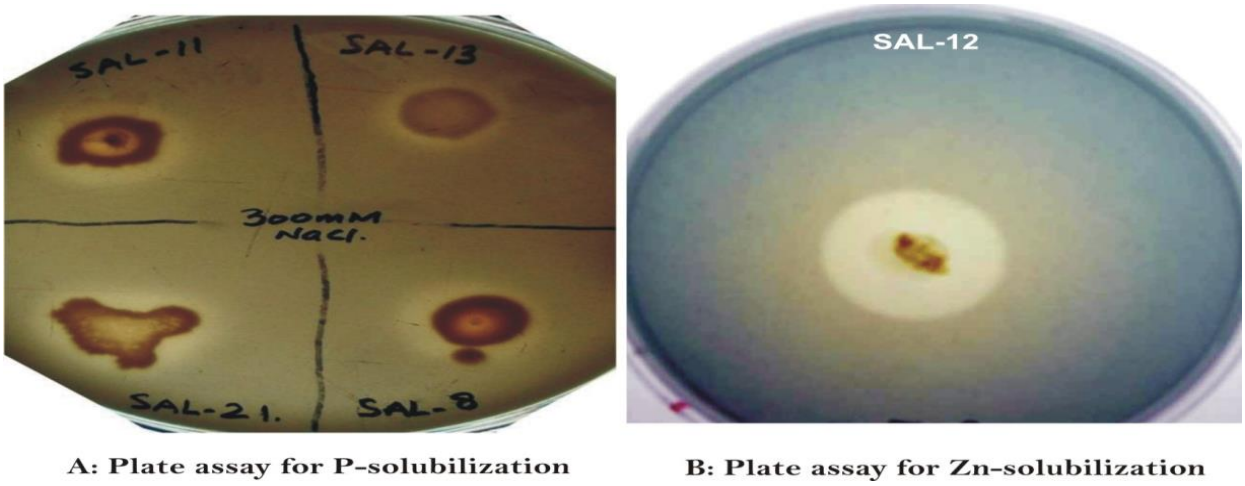

Figure 1(B): Plate assay showing solubilization of inorganic phosphate on Pikoviskaya 's agar plate containing 300 mM NaCl (A) and Zn-solubilization on LGI medium containing 100mN NaCl by different bacteria

Bioassays and identification of potential halo-tolerant PGPR

The bacteria containing multiple traits or a consortium of bacteria having a blend of different plant growth promoting (PGP) traits is more beneficial as it can compensate multiple growth requirements of plants. Of 21 tested isolates, 12 bacterial isolates showed IAA production (Table S1) of which SAL-17 showed IAA production up to 400 mM salt while 13 isolates showed ACC-deaminase activity of which six isolates showed ACC-deaminase activity up to 300 mM NaCl. Production of IAA and ACC-deaminase was found to be a widespread phenomenon among the bacterial isolates screened from BSRS-II environment both traits are important mediators for stress tolerance and stimulate root growth in a coordinated fashion (Li, 2000). Indole acetic acid promotes root growth by increasing number and length of adventitious root that lead to enhanced nutrient uptake (Majeed *et al.*, 2015; Naqqash *et al.*, 2016) especially in saline soil (Orhan, 2016). The ACC-deaminase significantly reduces the level of plant hormone 'ethylene' (Glick, 2005) that consequently improves the stress tolerance of crop plants (Mayak *et al.*, 2004; Bianco and Defez, 2009, 2012). The IAA production and ACC-deaminase activity of isolates up to 400mM of NaCl shows the stability of these traits in BSRS-II isolates. Auxin production of *Azospirillum brasilense* spp. is reported up to 200 mM (Nabti *et al.*, 2007) but our strains showed higher tolerance and IAA production up to 300 mM which makes them candidate of choice for biofertilizer production for salt-affected soils.

Phosphate solubilizing and nitrogen fixing microorganisms provide bio-available phosphate and nitrogen

for plant growth and are vital traits for biofertilizer production. Of 21 tested isolates, 14 isolates showed P-solubilization of which eight showed P-solubilization up to 300 mM NaCl (Figure 1B); SAL-12 solubilized insoluble zinc at 0 mM salt. Many BSRS-II isolates showed P-solubilization ability up to 300 mM NaCl. The difference in relative increase (in root length, shoot length, root area) by bacterial inoculation with and without TCP shows the mobilization/utilization of TCP by inoculated consortium. *Aeromonas* spp. from rhizosphere of chickpea, mustard and wheat with P-solubilization ability have been reported by Kundu *et al.* (2009). We could not obtain any diazotroph from BSRSII environment due to higher levels of salts present in the soil as high salinity and pH drastically affect the nitrogenase activity of bacteria (Dicker and Smith, 1981; Moradi, 2011).

Partial 16S *rRNA* gene sequences of SAL-12 (1279 bp) showed 98% homology to the un-cultured *Aeromonas* sp. clone SS36E121 (GU356308), SAL-16 (859 bp) showed 97% homology to *A. salmonicida* sp., SAL-17 (1414 bp) showed 99% sequence homology to the *A. salmonicida* strain AF-1 (KU359246) and SAL-21 (1411bp) showed 99% homologies to uncultured bacterium clone 740 (FR853630) and *A. salmonicida* strain AF-1 (KU359246). These sequences were submitted to Gen Bank under

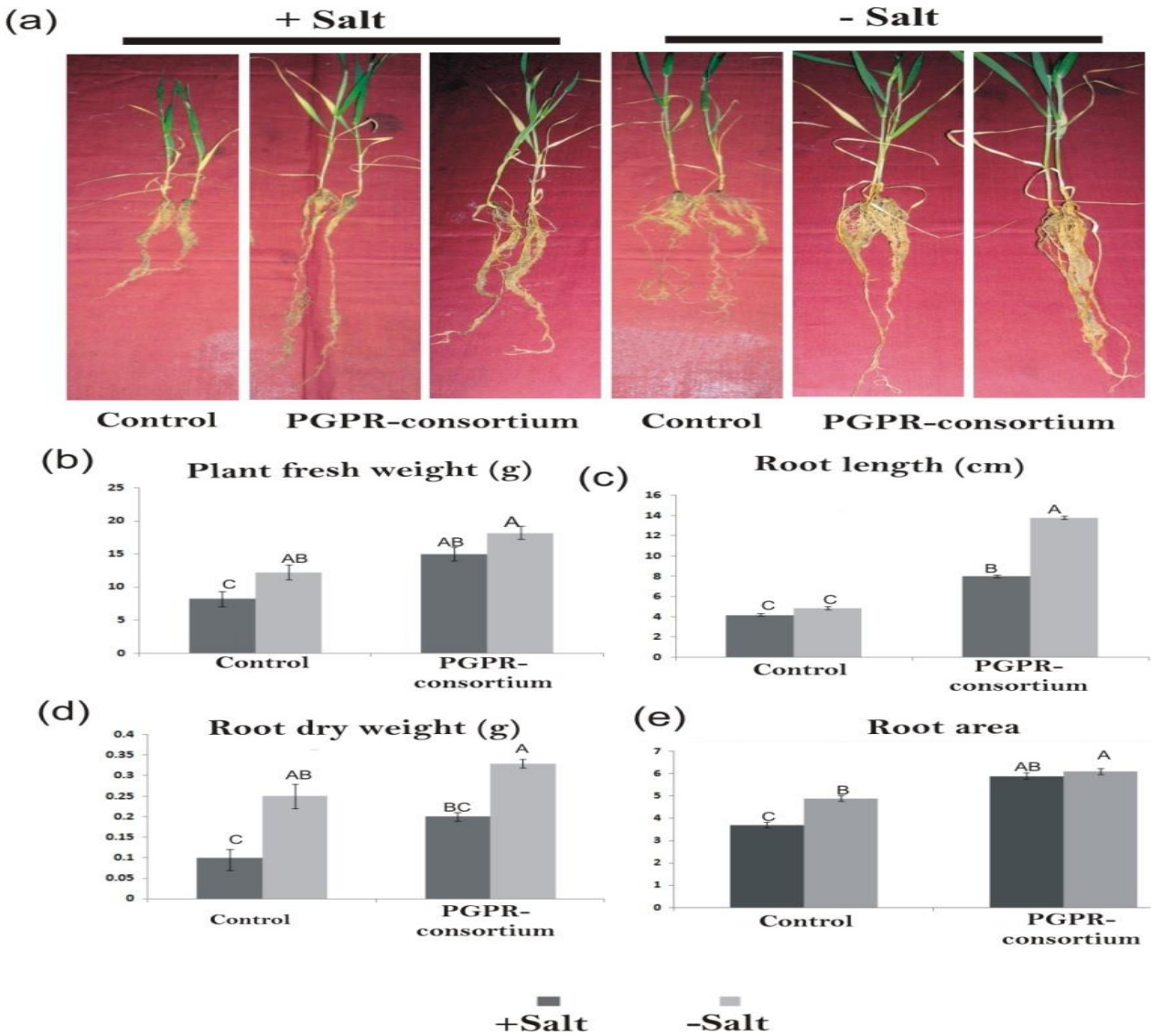

Figure 2: Representation of shoot and root growth (a) and response of plant fresh weight (b), root length (c), root dry weight (d) and root area (e) under the influence of PGPR inoculation and NaCl salinity treatments

following accessions: HG763856 (SAL-12), HE573182 (SAL-16), HG763857 (SAL-17), HG763858 (SAL-21). Although regarded as pathogen, the genus *Aeromonas* occupies a wide variety of ecological niches and is a frequent soil inhabitant including rice rhizosphere in saline (Organo *et al.*, 2014) and non-saline soils (Mehnaz *et al.*, 2001). Being ubiquitous in nature, opportunistic pathogens are present in rhizosphere, but the plant root colonization potential of pathogenic strains is very poor as compared to beneficial PGPR (Egamberdieva and Kucharova, 2009). Successful root colonization of aermomonad- consortium on wheat shows the adaptability and selective advantage of inoculated bacteria in saline and non-saline environment.

Plant inoculation assays

Inoculation response and root colonization under induced salinity and P-mobilization

Since bacteria were isolated from saline soil, and few of them even exhibited halophilic character (upto 65 g L^{-1}) therefore, their potential for growth response on wheat under induced salinity was evaluated. The PGPR-consortium significantly promoted the growth of wheat under normal as well as induced salinity. The root and shoot growth of inoculated plants was better (Figure 2a) with an increase of 48% in plant fresh weight (Figure 2b), 181% in root length (Figure 2c), and 32% in root dry weight (Figure 2d) in non-saline conditions. Under salinity, the relative increase was 22% in plant fresh weight and 63% in root length which was significantly higher than the non-inoculated control.

Moreover, the root area of inoculated plants increased significantly as compared to non-inoculated plants (Figure 2e) both with and without salinity. This response may be attributed to bacterial IAA and ACC-deaminase traits present in the inoculated bacteria. Under salinity stress, release of additional (bacterial) auxin could help to maintain root growth, increase water use efficiency under stress (Zahir *et al.*, 2007) and leaf growth (Albacete *et al.*, 2008; Egamberdieva, 2009). A direct correlation has been reported in chickpea seedlings between *in vitro* bacterial ACC-deaminase activity and root growth together with increased number of lateral roots, root length, and root dry weight (Shahzad *et al.*, 2010) showing that ACC-deaminase not only ameliorates salt stress but stimulate shoot/root growth.

Inoculated bacteria were present on the root surface attached to the root hair and in the close vicinity of the root epidermal cells (Figure 3b, c) both in saline and non-saline conditions. The development of root hair was clearly seen on the root surface.

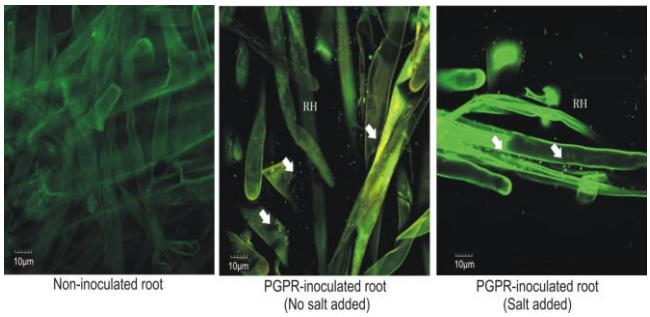

Figure 3: Confocal micrographs of sand-grown wheat roots from non-inoculated control plant (A) compared to plant roots inoculated with PGPR-consortium without salt supplementation (B) and plant roots inoculated with PGPR-consortium with salt supplementation(C) white arrows indicate the presence of inoculated bacteria. The roots were stained with acridine orange and observed under confocal microscope.

RH= root hair, Bar represent 10µm.

P-solubilization experiment showed a significant response of bacterial inoculum as well as the ability of bacterial inoculum to mobilize/utilize the inorganic P (TCP) in the rhizosphere. Generally, PGPR-inoculated plants were healthier and taller (Figure 4a) and inoculation significantly increased root length (82 and 71%), shoot length (17 and 12.6%) and root area (42 and 31%) of wheat with and without TCP, respectively (Figure 4b, c).

Inoculation response under natural salinity in field

The halo-tolerant PGPR-consortium showed a significant potential to promote wheat growth at seedling, vegetative and maturity stage under normal conditions, induced salinity and natural salinity (BSRS-II) with reduced fertilizer (1/2 NPK). The root and shoot growth of inoculated plants was better and roots were highly proliferated, although seed germination and early seedling growth are considered as most salt-sensitive plant growth stages (Rahman *et al.*, 2000; Jamil *et al.*, 2006) causing a significant decrease in shoot and root length in wheat (Egamberdieva, 2009). Halo-tolerant PGPR-consortium showed a stable and significant growth promoting response ($p < 0.05$; Figure 5) for both years. Response was evaluated based upon relative increase (RI) in inoculated plants compared to un-inoculated plants. *BioPower*-inoculated plants showed a relative increase of 54/33% in shoot/root length and 29/36% in shoot/root biomass whereas halo-tolerant PGPR inoculated plants showed relative increase of 60/36% in shoot/root length and 44/64% for shoot/root

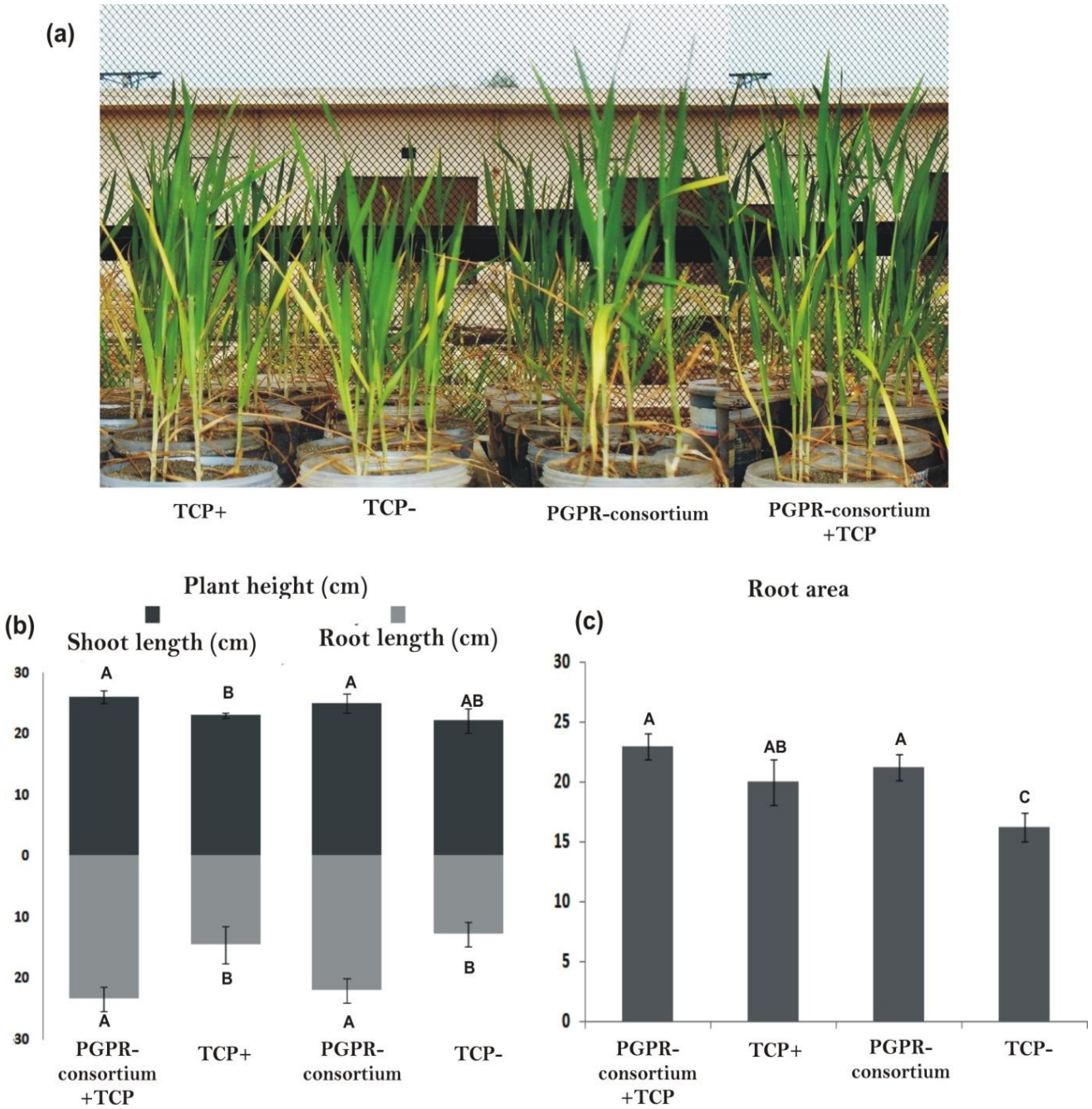

Figure 4: Representation of plant growth (a) and response of shoot length, root length (b), and root area (c) under the influence of PGPR inoculation and TCP-treatments

Different upper-case letters indicate significant differences between means of treatments. Means were compared at α = 0.05 (LSD). The standard error of the means is represented as bars.

biomass. This shows the effectiveness of halo-tolerant PGPR over non-halo-tolerant PGPR (*BioPower*). Similarly, increase in grain weight (8.8 & 5.7%) and yield (73 & 50%) was more in halo-tolerant PGPR in two years than increase in *BioPower* inoculated plants which further validated the effectiveness of this inoculum for saline soil. A positive correlation was observed between plant height & biomass (r= 0.964**), plant height & grain weight (r=0.84**),

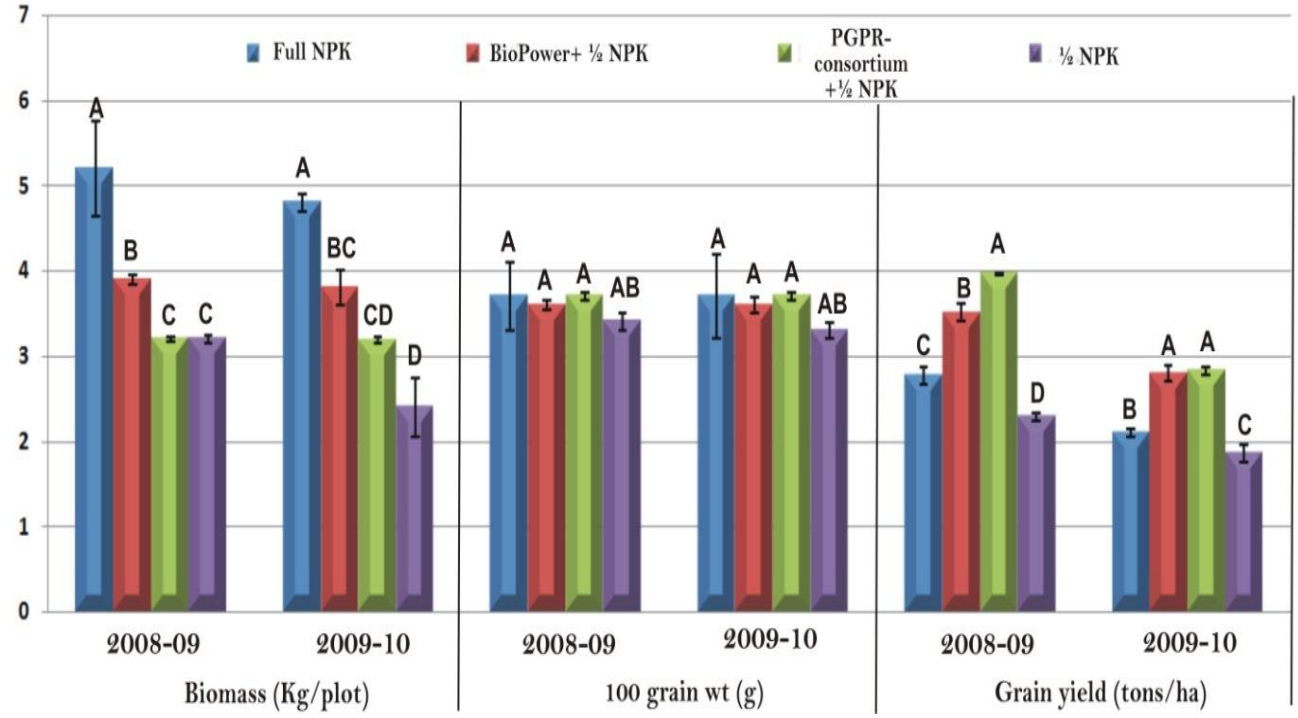

Figure 5: Effect of halo tolerant PGPR consortium on grain yield, grain weight and biomass of wheat variety TJ-83 at Biosaline Research Station II under natural saline condition in year 2008-2009 and 2009-2010

The values are means of three biological replicates. Comparison between treatments was carried out by one-way analysis of variance (ANOVA). Values followed by different letters (a, b, c) are significantly different from each other at 5% level of significance.

biomass & grain weight (r=0.924**) for the year 2009 and shoot length to grain weight (r=0.741) and biomass to grain yield (r=0.868) for the year 2010. A linear regression model effectively modeled the response of grain weight to plant biomass after bacterial inoculation treatment (R^2=*0.853*) explaining more than 85% of the variance in the data. The positive impact of halo-tolerant PGPR was significantly higher as compared to *BioPower* suggesting that the salt-tolerance trait has been co-evolved with PGP traits in saline environment and salinity does not interfere and/or alter the beneficial functioning of these microbes; the capability which was lacking in the *BioPower*. Although growth and yield increase by PGPR inoculation has been reported in wheat under normal (Majeed *et al.,* 2015; Egamberdieva, 2009; Hussain and Hasnain, 2011; Khalid *et al*., 2004) and saline conditions (Rajput *et al.,* 2013; Orhan, 2016) but an *Aeromonas*-based halo-tolerant inoculum with multiple plant growth promoting traits has not been reported.

The Bio-saline Research Station II since last three decades has served as demonstration site for Biosaline Agriculture Technology for stakeholders both at national and international levels. The selection and subsequent use of halo-tolerant PGPR with multiple beneficial activities will serve as an important tool for the facilitation of sustainable agriculture in this environment due to economic feasibility and ecological safety aspects of this technology. The PGPR-based interventions may make the ecosystem rehabilitation efforts economically feasible on a self-sustained basis. Future studies may be directed to elucidate the role of other related factors e.g., drought in determining the host-microbe interactions, adaptability and activities of PGPR in this saline stressed environment.

Conclusions

The findings of present study provide sustainable solution for rehabilitation of saline ecosystems through the cultivation of plants by inoculating them with salt-tolerant PGPR inoculum. The study has shown that PGPR with multiple plant beneficial traits can not only withstand salinity but facilitate plant growth at high salt concentration. The consortium of halo tolerant *Aeromonas* spp. strains having IAA production, ACC-deaminase activity and P-/Zn-solubilizing abilities, outcompeted in terms of plant growth, biomass production and grain yield of wheat with ½ NPK fertilizer. Based on these results, this halo tolerant PGPR-consortium may be recommended as saline biofertilizer for the facilitation of wheat growth in saline environments with lesser input of chemical fertilizer. Furthermore, the inoculum may be safely used as

biofertilizer in non-saline soils for wheat farming with reduced application of chemical fertilizer.

Acknowledgements

The authors are thankful to the management and field staff of Biosaline Research Station-II Pakka Anna and NIBGE for facilitating field experiments.

References

Abd-Alla, M.H., A.W.E. El-enany, M. Khalil and B.S.R Bashandy. 2014. Alleviating the inhibitory effect of salinity stress on nod gene expression in *Rhizobium tibeticum* – fenugreek (*Trigonella foenum graecum*) symbiosis by isoflavonoids treatment. *Journal of Plant Interactions* 9(1): 275-284.

Ahmad, M.S.A., M. Ashraf and Q. Ali. 2010. Soil salinity as a selection pressure is a key determinant for the evolution of salt tolerance in Blue Panicgrass (*Panicum antidotale* Retz.). *Flora* 205: 37-45.

Akhtar, N., M.A. Ghauri, A. Iqbal, M.A. Anwar and K. Akhtar. 2008. Biodiversity and phylogenetic analysis of culturable bacteria indigenous to Khewra salt mine of Pakistan and their industrial importance. *Brazilian Journal of Microbiology* 39: 143-150.

Albacete, A., M.E. Ghanem, C. Martinez-Andujar, M. Acosta, J. Sanchez-Bravo, V. Martinez, S. Lutts, I.C. Dodd and F. Perez-Alfocea. 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. *Journal of Experimental Botany* 59: 4119-4131.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410.

Araus, J.L., G.A. Slafer, C. Royo and M.D. Serret. 2008. Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Science* 27(6): 377-412.

Arshad, M., M. Saleem and S. Hussain. 2007. Perspectives of bacterial ACC deaminase in phytoremediation. *Trends in Biotechnol*ogy 25: 356-362.

Ashraf, M.Y., A.R. Awan and K. Mahmood. 2012. Rehabilitation of saline ecosystems through cultivation of salt tolerant plants. Pakistan Journal of Botany 44: 69-75.

Bianco, C and R. Defez. 2009. *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. *Journal of Experimental Botany* 60: 3097-3107.

Bianco, C. and R. Defez. 2012. Soil bacteria support and protect plants against abiotic stresses. In: A. Shanker, B. Venkateswarlu. Abiotic Stress in Plants—Mechanisms and Adaptations (eds). doi:10.5772/23310

Bilal, R., G. Rasul, J.A. Qureshi and K. A. Malik. 1990. Characterization of *Azospirillum* and related diazotrophs associated with roots of plants growing in saline soils. *World Journal of Microbiology and Biotechnology* 6: 46-52.

Borucki, W. and M. Sujkowska. 2008. The effects of sodium chloride-salinity upon growth, nodulation, and root nodule structure of pea (*Pisum sativum* L.) plants. *Acta Physiologiae Plantaraum* 30: 293-301.

Cavalcante, V.A. and J. Döbereiner. 1988. A new acid-tolerant nitrogen fixing bacterium associated with sugarcane. *Plant and Soil* 108: 23-31.

Chin, H.S, J.S, Shim, J.M. Kim, R. Yang and S.S. Yoon 2001. Detection and antibacterial activity of a bacteriocin produced by *Lactobacillus plantarum*. *Food Science Biotechnology* 10(5): 461-467.

Dicker, H.J. and D.W. Smith. 1981. Effects of salinity on acetylene reduction (nitrogen fixation) and respiration in a marine *Azotobacter*. *Applied and Environmental Microbiology* 42(4): 740-744.

Dwivedi, S., H. Upadhyaya, P. Subudhi, C. Gehring, V. Bajic and R. Ortiz. 2010. Enhancing abiotic stress tolerance in cereals through breeding and transgenic interventions. In: Plant Breeding Review Janick (ed) 33, Wiley, Hoboken.

Dworkin, M. and J. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *Journal of Bacteriology* 75: 592.

Egamberdieva, D. 2009. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiologiae Plantarum* 31: 861-864.

Egamberdieva, D., G. Berg, K. Lindström and L.A. Räsänen. 2013. Alleviation of salt stress of symbiotic *Galega officinalis* L. (Goat's Rue) by co-inoculation of *Rhizobium* with root colonizing *Pseudomonas*. *Plant and Soil* 369: 453-465.

Egamberdieva, D. and Z. Kucharova. 2009. Selection for root colonizing bacteria stimulating wheat growth in saline soils. *Biology and Fertility of Soils* 45: 563-571.

Glick, B.R. 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC-deaminase. *FEMS Microbiology Letters* 251: 1-7.

Glick, B.R, D.M. Penrose and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of Theoretical Biology* 190: 63-8.

Gordon, S.A. and R.P. Weber. 1951. Colorimetric estimation of indole acetic acid. *Plant Physiology* 26: 192-195.

Goswami, D., P. Dhandhukia, P. Patel and J.N. Thakker. 2014. Screening of PGPR from saline desert of

Kutch: Growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. *Microbiological Research* 169: 66-75.

Hardy, R.W.F., R.C. Burns and R. D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry* 5: 47-81.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Circular., 347. University of California Agriculture Experiments Station, Berkley.

Hirsch, A.M. and Y. Fang. 1994. Plant hormones and nodulation: What's the connection? *Plant Molecular Biology* 26: 5-9.

Hussain, A. and S. Hasnain. 2011. Phytostimulation and biofertilization in wheat by cyanobacteria. *Journal of Indian Microbiology and Biotechnology* 38: 85-92.

Imran, A., M.S. Mirza, T.M. Shah, K. A. Malik and F.Y. Hafeez. 2015. Differential response of kabuli and desi chickpea genotypes towards inoculation with PGPR in different soils. *Frontiers in Microbiology* 6: 859.

Jamil, M, D.B. Lee, K.Y. Jung, M. Ashraf, S.C. Lee and E.S. Rhal. 2006. Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. *Journal of Central European Agriculture* 7: 273-282.

Jamil, A., S. Riaz, M. Ashraf and M. R. Foolad. 2011. Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Science* 30 (5): 435-458.

Jha, B., I. Gontia and A. Hartmann. 2011. The roots of the halophyte *Salicornia brachiata* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. *Plant and Soil* 356:265-277.

Kazi, K.A, I. Bari, M. Ayaz, and K. Noureen. 2016. Crop Development in Lower Sindh Tandojam. Report of National Agromet Centre, Pakistan Meteorological Department (2015-16). Islamabad, Pakistan.

Khalid, A., M. Arshad and Z. A. Zahir. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology* 96: 473-480.

Kundu, B.S., K. Nehra, R. Yadav and M. Tomar. 2009. Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. *Indian Journal of Microbiology* 49: 120-127.

Li, J., D.H. Ovakim, T.C. Charles and B.R. Glick. 2000. An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. *Current Microbiology* 41: 101-105.

Lugtenberg, B. and F. Kamilova. 2009. Plant-growth-promoting rhizobacteria. *Annual Reviews in Microbiology* 63: 541-556.

Majeed, A., M.K. Abbasi, S. Hameed, A. Imran and N. Rahim. 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology* 6: 198.

Malik, K.A., R. Bilal, G. Rasul, K. Mahmood and M.I. Sajjad. 1991. N_2-fixation in plants growing in saline sodic soils and its relative quantification based on ^{15}N natural abundance. *Plant and Soil* 137: 67-74.

Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, USA, p: 545.

Mayak, S., T. Tirosh and B.R. Glick. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry* 42(6):565-572.

Mehnaz, S., M.S. Mirza, J. Haurat, R. Bally, P. Normand, A. Bano and K.A. Malik. 2001. Isolation and 16S *rRNA* sequence analysis of the beneficial bacteria from the rhizosphere of rice. *Canadian Journal of Microbiology* 47: 110-117.

Murphy, J. and J.P. Riley. 1962. Modification solution method for determination of phosphate in natural water. *Analytica Chemica Acta* 27: 31-36.

Nabti, E., M. Sahnoune, S. Adjrad, A.V. Dommelen, M. Ghoul, M. Schmid, A. Hartmann. 2007. A halophilic and osmotolerant *Azospirillum brasilense* strain from Algerian soil restores wheat growth under saline conditions. *Engineering in Life Sciences* 7(4): 354-360.

Nakbanpote, W., N. Panitlurtumpai, A. Sangdee, N. Sakulpone, P. Sirisom and A. Pimthong. 2014. Salt-tolerant and plant growth-promoting bacteria isolated from Zn/Cd contaminated soil: identification and effect on rice under saline conditions. *Journal of Plant Interactions* 9(1): 379-387.

Naqqash, T., S. Hameed, A. Imran, M.K. Hanif, A.M. Khan and J.D.V. Elsas. 2016. Differential growth stimulation response of potato towards inoculation with taxonomically diverse plant growth promoting rhizobacteria. *Frontiers in Plant Science* 7:144.

Nelson, D.R. and P.M. Mele. 2007. Subtle changes in the rhizosphere microbial community structure in response to increased boron and sodium chloride concentrations. *Soil Biology and Biochemistry* 39: 340-351.

Ofek, M., S. Ruppel and Y. Waisel. 2006. Effects of salinity on rhizosphere bacterial communities associated with different root types of Vicia *faba* L. In: M. Ozturk, Y. Waisel, A. Khan and G. Gork. Biosaline agriculture and salinity tolerance in plants (eds). Birkhauser, Basel, pp 1-21.

Organo, N.D., E.S. Paterno, E.F. Delfin, G.B. Gregorio, N.B. Lantican and J.F. Simbhana. 2014. Application of PCR-DGGE in examination of the microbial

community associated with the rhizosphere of rice as affected by salinity. In: Recent Advances in Biofertilizers and Biofungicides (PGPR) for Sustainable Agriculture. M.S. Reddy, R.I. Ilao, S. Patricio, W.D. Dar, W.D. Batchelor, R. Sayyed, H. Sudini, K.V.K. Kumar, A. Armanda and S. Gopalkrishnan. Eds: Cambridge Scholars Publishing.

Orhan, F. 2016. Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). *Brazilian Journal of Microbiology* 47(3): 621-627.

Park, M., C. Kim, J. Yang, H. Lee, W. Shi and, S. Kim. 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiology Research Journal* 160:127–133. doi:10.1016/j.micres.2004.10.003

Pliego, C., F. Kamilova and B. Lugtenberg. 2011. Plant Growth-Promoting Bacteria: Fundamentals and Exploitation. In: D.K. Maheshwari, Bacteria in Agrobiology: Crop Ecosystems (Eds). Springer, Germany, pp 295-343.

Rahman, M.S., T. Matsumuro, H. Miyake and Y. Takeoka. 2000. Salinity-induced ultra-structural alternations in leaf cells of rice (*Oryza sativa* L.). *Plant Production Science* 3: 422-429.

Rajput, L., A. Imran, F. Mubeen and F.Y. Hafeez. 2013. Salt-tolerant PGPR strain *Planococcus rifietoensis* promotes the growth and yield of wheat (*Triticum aestivum*) cultivated in saline soil. *Pakistan Journal of Botany* 45: 1955-1962.

Rengasamy, P. 2006. World salinization with emphasis on Australia. *Journal of Experimental Botany* 57: 1017-1023.

Shahzad, S.M., A. Khalid, M. Arshad and Kalil-ur-Rehman. 2010. Screening rhizobacteria containing ACC-deaminase for growth promotion of chickpea seedlings under axenic conditions. *Soil and Environment* 29(1): 38-46.

Singh, A.L., K. Hariprassana and R.M. Solanki. 2008. Screening and selection of ground-nut genotypes for tolerance of soil salinity. *Australian Journal of Crop Science* 1: 69-77.

Singh, R.P. and P.N. Jha. 2016. Alleviation of salinity-induced damage on wheat plant by an ACC deaminase-producing halophilic bacterium *Serratia* sp. SL-12 isolated from a Salt Lake. *Symbiosis* 69:101-111.

Somasegaran, P. and H.J. Hoben. 1994. Handbook for Rhizobia Methods in Legume-*Rhizobium* Technology. Springer, Heidelberg, New York.

Soni, A., S. Rokad and P. Sharma. 2013. Screening of efficient halotolerant phosphate solubilizing bacteria and their effect on seed germination under saline conditions. *Journal of Scientific Innovative Research* 2(5): 932- 937.

Srinivasan, R., M.S. Yandigeri, S. Kashyap and A.R. Alagawadi. 2012. Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. *Saudi Journal of Biological Science* 19: 427-434.

Tan, Z.Y., X.D. Xu, E.T. Wang, J.L. Gao, E. Martinez-Romero and W.X. Chen. 1997. Phylogenetic and genetic relationships of *Mesorhizobium tianshanense* and related rhizobia. *International Journal of Systematic and Bacteriology* 47(3): 874-879.

Tien, T.M., M.H. Gaskins and D.H. Hubbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* 37: 1016-1024.

Vincent, J.M. 1970. A manual for the practical study of the root nodule bacteria. IBP Hand book No.15.Blackwell Scientific Publications: Oxford England.

Yadav, A.N, P. Verma, D. Kaour, K.L. Rana, V. Kumar, B. Singh, V.S. Chauahan, T.C.K. Sugitha, A.K. Saxena and H.S. Dhaliwal. 2017. Plant microbiomes and its beneficial multifunctional plant growth promoting attributes. *International Journal of Environmental Science and Natural Resources* 3(1): 555601. DOI: 10.19080/IJESNR.2017.03.555601

Zahir, Z.A., A. Munir, H.N. Asghar, B. Shahroona and M. Arshad. 2007. Effectiveness of *rhizobacterium* containing ACC-deaminase for growth promotion of pea (*Pisum Sativum*) under drought conditions. *Journal of Microbiology and Biotechnology* 18: 958-963.

Zhang, J., J. Liu, L. Meng, Z. Ma, X. Tang, Y. Cao and L. Sun. 2012. Isolation and characterization of plant growth-promoting rhizobacteria from wheat roots by wheat germ agglutinin labeled with fluorescein isothiocyanate. *Microbiology Journal* 50(2): 191-198.