Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 3312-3319
Received: 01-05-2018
Accepted: 05-06-2018

Yogendra Singh Gusain
Department of Biological Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

CM Mehta
School of Agriculture, Lovely professional University, Jalandhar, Phagwara, Punjab, India

IP Sharma
Patanjali Research Institute, Haridwar, Uttarakhand, India

AK Sharma
Department of Biological Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Correspondence
Yogendra Singh Gusain
Department of Biological Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Bacterial inoculations ameliorate saline alkali soil stress in contrasting genotype of rice (*Oryza sativa* L.)

Yogendra Singh Gusain, CM Mehta, IP Sharma and AK Sharma

Abstract
The effects of the inoculation of plant growth promoting rhizobacterial (PGPR) strains *Pseudomonas fluorescence strain* P2, *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81, *Pseudomonas koreensis* strainYB1 and *Arthrobacter nitroguajacolicus* strain YB3 on CSR-36 (Salinity tolerance) and IR-64 (Salinity sensitive) genotypes of rice were studied under three level of saline alkali soil stress. The Level I have 9.18 pH and 2.05 ds/m Electrical conductivity (Ec), the Level II have 9.38 pH and 2.47 ds/m Ec, while the Level III have 9.63pH and 3.05 ds/m Ec. PGPRs, *Pseudomonas jessenii*, R62, *Pseudomonas synxantha*, R81 were used as a consortium. Most of the inoculated plants had remarkably higher plant height, fresh weight, chlorophyll, carotenoid content, lowered electrolyte leakage (EL) and Malondialdehyde (MDA) content as compare to uninoculated plants in all the level of stress. The PGPRs efficiently reduced the proline and superoxide dismutase (SOD) activity in both the genotype of rice plants as compare to their respective control. Majority of the inoculated plants had remarkably higher, Phosphorus, potassium ion uptake and lowered sodium ion uptake in shoot as compare to non inoculated plants. In this respect the selected PGPRs helped to reduce the saline alkali soil stress in plants. In overall stress level irrespective of treatments salt sensitive IR-64 showed higher level of EL, MDA content, Phosphorus uptake, sodium uptake and SOD activity as compare to salt tolerant CSR-36.

Keywords: PGPR, Rice, saline alkali stress, proline, malondialdehyde, superoxide dismutase

Introduction
Salinization and alkalization are dynamic soil degradation processes. Saline soil has high concentration of neutral salts (NaCl, Na_2SO_4) which cause osmotic stress and ion-induced injury in plants, while alkaline stress caused by high concentrations of alkaline salts ($NaHCO_3$ and Na_2CO_3) and has additional high pH effect on the plants [1]. Soil salinization and alkalization frequently occur together around 950 million hectare land worldwide [2]. In alkali stress environment root of the plants surrounded by the soil of high pH, which can directly endanger the root growth, membrane stability, cross-membrane potential and interfere in the function of root cells [3]. Alkaline environment can induce the inhibition of growth and photosynthesis, alteration of ion accumulation, antioxidative metabolism and devastation of the structure of root cells, ultimately can leads to cell death [4, 5]. In respect of ionic balance under salt stress condition, the high ratio of K^+/Na^+ can be considered as an important indicator for evaluating salt tolerance of plants [3]. Salinity can cause oxidative stress by enhanced production of reactive oxygen species (ROS) in plants [6], which can act as toxic molecules to cell [7]. In order to protect plants against ROS cells produce antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), and various other nonenzymatic antioxidants [8]. In addition to antioxidant stressed plants can accrue various molecules such as proline in cytosole which can act as osmoregulant and thereby can protect macromolecules and enzyme activity. In this respect such molecules are normally using as indicator of oxidative stress in plants [9]. Comparatively little attention has been given, especially from the aspect of reactive oxygen species (ROS) level and antioxidative enzymes level in the plants to both salt and alkaline mixed soil stress, as such conditions frequently co-occur and both ROS level and antioxidative enzymes level regulate growth of plants under stress conditions [1].

Rice is essential for feeding the world's population, and has immense importance to food security for world population. It is estimated that the global need of rice production will increase from 586 million metric tons (mmt) in 2001 to about 756 mmt by 2030 [10]. This demand can be met from the sustainable use of available land and various conventional and biotechnological approaches. In this track, in addition to produce the high salt tolerant variety of crops to reduce the unfavorable effect of salinity on plants, recently the use of biological

methods to alleviate the consequences of soil stress including salinity on plants gaining attention [11-13]. Among the biological methods, PGPR are most studied soil microorganism for the growth promotion [14, 15] as well as induction of salt tolerant ability in plants by inducing the antioxidants [16-18] and physiological response (e.g proline as osmoregulant) of plants against stress conditions [19]

The present study aimed to investigate the effect of PGPRs on growth promotion, phosphorus uptake, Na^+, K^+ uptake and SOD activity in salt tolerant CSR-36 [20] and salt susceptible IR-64 [21] varieties of rice under different range of saline alkali soil stress condition as well as to investigate the differential effect of saline alkali soil stress in both the genotype of rice.

Materials and Methods

Collection and of physiochemical properties of alkali soil

For present experiment, saline alkali soil was collected from the upper 0-15 cm soil layer from Faizabad district of Uttar Pradesh, India. For the experiment we have made the three different level of saline alkali soil stress by mixing the saline alkali soil with the non alkali soil in 1:1(stress Level III), 1:2 (stress Level II) and 1:3 (Stress Level I) ratio. The soils were analyzed for the physico-chemical properties (Table 1 and Table 2). The soil pH was measured by Beckman Glass electrode pH meter; electrical conductivity (Ec) by EUTECH digital electrical conductivity meter, percent organic matter was calculated according to Jackson [22], available mineralizable nitrogen (kg ha^{-1}) by Alkaline $KMnO_4$ method [23], Available P_2O_5 (kg ha^{-1}) by Olsen's method [24], available potassium (kg ha^{-1}) and Available sodium (kg ha^{-1}) were measured according to [25].

Selection of PGPRs

For the present study plant growth promoting bacterial strains *Pseudomonas jessenii* (R62), *Pseudomonas synxantha* (R81) [26-28], *Pseudomonas koreensis* strain YB1, *Arthrobacter nitroguajacolicus* strain YB3 [29] and *Pseudomonas fluorescence* strain P2, were kindly provided by Rhizosphere biology lab of department of Biological Sciences of G. B. Pant university of Agriculture and Technology Pantnagar. In this study R62 and R81 were used as consortium (R62+R81). Before used as inoculants all the selected bacterial strains were checked for their growth on high pH (pH 10) nutrient agar and found positive by visualized the colony on plates after 2 days on incubation at 28± 2 ^{0}C. For the preparation of culture inoculants, all the strains grow separately in Nutrient Broth medium (Himedia, India) in flasks incubated at 28^{0}C at 120 rpm until the late exponential phase. The final culture cfu was maintained at 10^7 to 10^8 cfu ml^{-1} level.

Table 1: Physiochemical properties of alkaline soil (collected from Faizabad, UP, India) and non alkaline soil (collected from agriculture field of Pantnagar University)

Soil sample	pH	EC (dS/m)	O C (%)	Nitrogen (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	Sodium (Kg/ha)
Saline Alkali soil	10.02	6.20	0.328	175.616	15.004	096.32	354.37
Non alkali soil	08.36	0.85	1.150	186.07	34.910	145.6	016.57

Table 2: Physiochemical properties of the soil of all the alkaline soil stress level.

Saline alkali soil stress	pH	EC (ds/m)	Nitrogen (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	Sodium (Kg/ha)
Level III	9.63	3.050	177.05	21.03	110.21	269.51
Level II	9.38	2.475	179.43	26.23	119.35	207.23
Level I	9.18	2.050	183.53	31.79	133.59	162.31

Rice varieties

Seeds of two genotype of rice, salinity tolerant CSR-36 and salt sensitive IR-64 was kindly provided by the IRRI, Pusa New Delhi, India.

Saline alkali soil stress level and experimental detail

Selected PGPRs were evaluated for their effect on the growth promotion, level of ionic balance, physiological and biochemical status of two genotype of rice under three different saline alkali soil stress conditions. The Level I of alkali soil stress has 9.18 pH and 2.05 ds/m Ec, the Level II have 9.38 pH and 2.47 ds/m Ec, while the Level III have 9.63pH and 3.05 ds/m Ec. The soils of all the levels were sterilized by autoclaving on three consecutive days at 121ºC for 60 min each. After sterilization, 500g soil of each level was filled separately into the pots. The pots were irrigated with sterilized water and left for a day for equilibration. The experiment was performed in greenhouse condition (where temperature: 27± 2ºC, photo period: 16/8h day/night cycle, light intensity: 400$Em^{-2}s^{-1}$, (400-700 nm), and relative humidity: 60% respectively). Rice seeds were surface disinfected by immersion in 70% ethanol and 3% (v/v) sodium hypochlorite for 1 min and 5 min. Seeds were washed thoroughly many times with sterile distilled water then germinated on sterilized Petri dish. Equally germinated seeds of both varieties were taken for sowing. There were three replicate of each treatment, including control, for each variety of rice at each level of saline alkali soil stress (a total of nine replicate of each treatment for each variety at all the level of saline alkali stress). At the time of sowing seeds in pots the bacterial inocula were given to 1ml / pot. Two seedlings per pot were maintained. Tap water was supplied at regular interval during whole experiment. All the replicate was arranged according to complete randomized design. For measuring growth parameter and biochemical status plants were harvested after 55 days of sowing. After harvesting, fresh weight were taken immediately and sample were placed in -80°C for further investigation of physiological and biochemical activity.

Estimation of Chlorophyll and Carotenoid content

Total chlorophyll and carotenoid concentrations were measured using a UV-Visible spectrophotometer (RAY LEIGH, UV-2601) at wavelengths 663 nm and 645 nm followed the method [30].

Measurement of electrolyte leakage

Electrolyte leakage (EL) was estimated according to Dionisio-Sese and Tobita [31]. Fresh leaf samples (0.1 g) were washed with triple DW and cut into small pieces (~1cm segments)

and suspended in test tubes containing 10 ml of de-ionized water and covered with plastic cap. Tubes were incubated in a water bath at 32°C for 2 h. After incubation, electrical conductivity (EC1) of the bathing solution was recorded. These samples were then autoclaved at 121°C for 20 min to completely kill the tissues and release the electrolytes. Samples were then cooled to 25 °C, and final electrical conductivity (EC2) was measured. The percent leakage of electrolytes was calculated using the formula (EC1/EC2) X 100.

Estimation of malondialdehyde (MDA)

For estimation of malondialdehyde (MDA) content leaf material (0.15 g) was homogenized in 2 ml of 0.1% trichloroacetic acid (TCA). Homogenate was centrifuged at 10,000xg for 10 min at 4^0C. MDA content was determined according to procedure of Heath and Packer [32]. The concentration of MDA was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Estimation of proline content

Free proline from leaf sample (0.15 g) was estimated according to Bates *et al*. [33]

Enzyme assay

For SOD enzyme extraction, 0.2 g of leaf samples was homogenized with a pestle in an ice-cold mortar in 5ml cold buffer containing: 50 mm potassium phosphate buffer (p-H 7.0), 1 mm ethylene diamine tetra acetic acid (EDTA) and 1% (w/v) polyvinylpyrolidone (PVP). Whole extraction procedure was carried out at 4 °C. The SOD activity was estimated according to Zhang and Kirkham [34]. Protein was estimated by Bradford [35] method.

Chemical analysis

Chemical analyses were carried out on dry weight basis. Plant samples (0.2g) were digested with the mixture of HNO_3 and $HClO_4$ in the ratio of 9:4 and the extract was made to a definite volume. Total phosphorous was determined by vanadomolybdate phosphoric acid yellow colour method by taking the absorbance in spectrophotometer at 730 nm [36]. Potassium (K) and sodium (Na) assayed from the diacid digested mixture by using a flame spectrophotometer.

Statistical analysis

The data presented here are mean values ± SD. The data of each level of alkali soil stress has three replicate (n=3) for each treatments of individual variety. The data were subjected to factorial analysis of variance (ANOVA), with varieties, stress level and treatments used for analysis and the differences between the means were compared using least significant differences at $P<0.05$. Different letters denote significant differences among treatments (including control) in two varieties.

Results and Discussion

Plant height, root length, shoot fresh weight and root fresh weight increased with the decrease of alkali soil stress from level III to level I. Most of the inoculated plants showed the increased growth in both the varieties of rice as compared to control however the significance levels of various inoculums varied with stress level and varieties used. When both the genotype of rice compared with each other in respect of their control condition salinity tolerance CSR-36 showed elevated effect on shoot and root fresh weight as compared to salinity susceptible IR-64 (Table 3). Here results indicated that inoculation with the selected bacterium could decrease the injurious effects of alkali soil. Generally, salinity and alkalinity can reduce shoot and root dry weight of plants and thus causing lower plant yields [1]. Various authors have also been reported decrease growth and yield of plant under salinity stress [37, 38] and Bacterial inoculation under saline stress [39, 40] and alkalinity stress [12] improves plant growth**.**

Total Chlorophyll and Caretenoid content

The experimental findings (Table 4) reveal that total chlorophyll and carotenoids contents decreased with increase of saline alkali stress. Most of the treated plant showed significantly higher effect on both the parameters under all the level of saline alkali soil stress as compare to their respective control (Table 4). In overall stress level irrespective of treatments total chlorophyll and carotenoids content was slightly high (6.25% and 1.01% respectively) in IR-64 as compare to CSR-36. Chlorophyll concentration in leaf is an indicator of salt tolerance and responds to increasing salinity [41]. Decreased chlorophyll and carotenoids content in plants under saline stress was also reported by Sairam *et al*. [42]. Similarly, Tapias *et al*. [43] observed the higher chlorophyll content in inoculated plants under saline stress and Hamdia *et al* [44], observed the greater activity of total pigments in *Azospirillum brasilense*, inoculated maize plants when grown in the different concentration of salinity.

Table 3: Growth promoting effect of bacterial inoculants on two cultivars of rice under different level of saline alkali soil stress. Mean followed by same letter are not significantly different ($P<0.05$) for a particular trait in two cultivars at all the level of stress.

Rice cultivars	Inoculants	Shoot length (cm)			Root length (cm)			Shoot Fresh wt (gm)			Root fresh wt (gm)		
		Stress Level III	Stress Level II	Stress Level I	Stress Level III	Stress Level II	Stress Level I	Stress Level III	Stress Level II	Stress Level I	Stress Level III	Stress Level II	Stress Level I
CSR-36	control	55.40 ab	57.70 ab	60.80 b	14.60 ab	16.37 ab	17.30 ab	4.83 b	5.18 bc	6.03 cd	5.02 bc	5.32 bc	5.43 c
	YB1	60.00 b	62.93 b	63.97 b	15.27 ab	18.67 b	19.77 b	5.84 cd	6.33 d	6.73 de	6.04 cd	6.18 cd	6.26 cd
	YB3	60.43 b	61.67 b	65.77 b	16.97 ab	19.23 b	20.00 b	4.88 bc	5.59 c	8.36 f	5.55 cd	6.07 cd	9.02 f
	P2	58.77 ab	60.70 b	62.97 b	17.60 ab	18.83 b	19.43 b	5.30 bc	6.49 d	8.66 f	6.31 cd	6.35 d	8.02 ef
	R62+R81	61.67 b	62.17 b	63.33 b	15.23 ab	17.97 ab	19.07 b	5.30 bc	6.22 cd	8.31 f	4.72 bc	6.31 cd	8.60 f
IR-64	control	51.10 a	52.23 ab	59.23 ab	13.93 a	14.43 ab	17.53 ab	4.08 a	5.18 bc	5.88 cd	4.01 ab	4.12 ab	5.71 cd
	YB1	57.60 ab	59.13 ab	61.57 b	15.60 ab	17.20 ab	19.00 b	4.12 ab	5.47 bc	7.71 ef	3.45 a	4.45 b	7.50 e
	YB3	54.90 ab	57.33 ab	59.93 b	16.13 ab	18.10 ab	18.37 b	4.76 ab	5.20 bc	6.82 de	4.62 bc	4.29 ab	6.92 de
	P2	56.63 ab	60.70 b	62.33 b	14.80 ab	16.47 ab	18.27 ab	4.97 bc	6.33 d	7.37 e	4.48 b	5.72 cd	6.93 de
	R62+R81	60.33 b	61.80 b	63.23 b	17.63 ab	18.87 b	18.90 b	5.13 bc	6.21 cd	7.51 e	5.10 bc	5.22 bc	7.12 de

Table 4: Effect of bacterial inoculants on total chlorophyll, carotenoids, electrolyte leakage and MDA content of two cultivars of rice under different level of saline alkali soil stress. Mean followed by same letter are not significantly different ($P<0.05$) for a particular trait in two cultivars at all the level of alkali stress.

Rice cultivars	Inoculants	Total Chlorophyll			Carotenoids			Electrolyte leakage (%)			MDA Content (µg/gm fresh weight)		
		Stress Level III	Stress Level II	Stress Level I	Stress Level III	Stress Level II	Stress Level I	Stress Level III	Stress Level II	Stress Level I	Stress Level III	Stress Level II	Stress Level I
CSR-36	control	4.75 a	5.40 bc	5.63 c	12.14 ab	12.34 b	12.62 bc	91.31 de	86.27 cd	80.30 b	81.72 e	58.71 cd	53.98 c
	YB1	5.44 bc	5.86 cd	6.25 de	12.25 ab	12.91 c	13.04 cd	85.86 cd	80.60 bc	74.48 a	44.41 bc	39.78 b	29.46 a
	YB3	5.83 cd	6.19 d	6.31 de	12.49 bc	12.79 c	12.88 c	87.56 cd	80.93 bc	72.81 a	66.24 d	58.06 cd	41.72 b
	P2	5.74 cd	5.99 cd	6.16 d	12.51 bc	12.76 c	12.86 c	84.34 c	83.50 bc	76.48 a	68.82 d	41.29 b	36.77 ab
	R62+R81	6.59 e	6.64 ef	6.82 ef	12.72 c	12.85 c	12.87 c	86.73 cd	84.93 cd	80.00 bc	58.28 cd	42.37 b	36.34 ab
IR-64	control	5.22 b	6.57 e	6.83 ef	12.07 ab	12.92 cd	13.25 d	92.24 e	88.38 d	84.73 cd	95.05 f	59.14 cd	45.81 bc
	YB1	5.21 b	6.17 d	7.00 f	12.00 a	12.87 c	13.51 d	86.40 cd	83.20 bc	75.66 a	70.32 d	52.04 c	45.81 bc
	YB3	6.10 d	6.35 de	6.73 ef	12.49 bc	12.35 b	13.24 d	87.92 cd	86.27 cd	81.61 bc	68.60 d	52.04 c	41.51 b
	P2	5.65 c	6.14 d	7.19 fg	12.39 b	12.49 bc	13.46 d	88.97 de	87.16 cd	84.32 c	69.68 d	55.27 c	34.84 ab
	R62+R81	6.15 d	6.83 ef	7.41 g	12.49 bc	12.88 c	13.56 d	91.12 de	89.45 de	74.23 a	65.81 d	57.42 c	42.37 b

Electrolyte leakage (EL)

Most of the treatments showed the greater reduction in electrolyte leakage as compare to their respective control in the entire stress level (Table 4), however their effect varied according to stress level and genotype used. At higher level of saline alkali soil stress (Level III) in CSR-36 only *P. fluorescence strain* P2 treated plants showed significantly reduced level of EL as compare to control plants, while in IR-64 three bacterial strains YB1, YB3 and P2 significantly reduced EL as compare to their respective control plants. Irrespective of treatments and soil ratio IR-64 showed higher (3.47%) EL leakage as compared to CSR-36. Salinity stress leads to significant increase in the level of EL in many crops [45]. Under salt or alkali stress leaf electrolyte leakage rate is a good physiological index as it reflects the degree of plant injury. Under intensified stress plasma membranes injured more seriously leading to an increase in the electrolyte leakage rate [46]. In present study reduced level of electrolyte leakage was observed in inoculated plants as compare to uninoculated plants. Similar to present study, PGPRs mediated reduced level of electrolyte leakage under stress condition has also been observed [47, 17].

Malondialdehyde (MDA) content

All the inoculated plants significantly reduced the MDA contents in both the varieties of rice under higher level of saline alkali soil stress (Level III) as compared to control plants (Table 4). Strain YB1 (1.84 fold) in CSR-36, while R62+R81 (1.44 fold) in IR-64 maximally reduced MDA content as compare to uninoculated control. Level II stress all the treated plants except YB3 in CSR-36 while in IR-64 none of the treatments showed the significant effect in MDA content as compare to uninoculated plants. In low level of soil stress (Level I) all the treatments in CSR-36 while in IR-64, bacterial strain P2 showed the significant reduction of MDA content as compared to control plants. High MDA content in plants can be correlated with higher stress condition in plants. Changing pH due to salt or alkali stress usually cause oxidative burst in plants, which can be demonstrated by increase MDA content [48]. Similar to present study reduced level of MDA content in PGPR inoculated plants have also been observed under salinity [49] and alkalinity [12] stress conditions. The lesser MDA content at stress level III in control plants of CSR-36 as compare to control plants of IR-64 might be an attribute of tolerant variety to with stand the saline alkali stress condition.

Proline content

All the PGPRs treated plants significantly reduced the proline contents of both varieties of rice under level III stress as compared to control plants. Among all the treatments strains R62+R81 with 1.85 fold in CSR-36 while in IR-64 strain YB3 with 1.89 fold maximally reduced the proline content over control. In Level II stress strain YB1 (1.22 fold) and YB3 (1.41 fold) in CSR-36, whereas in IR-64 only YB3 (1.53 fold) significantly decreased the proline contents as compared to uninoculated plants. In level I of stress none of the treatments showed significant effect on proline contents over control (Fig.1a). Irrespective of treatments and stress level CSR-36 showed 16.99% higher proline content as compare to IR-64. Proline accumulation may be considered a sensitive physiological marker of salt and other stresses that facilitate free radical scavenging and stabilization of sub cellular structures [50]. Reduced level of proline content in PGPRs inoculated plants as compare to their respective control under salinity stress condition have also been reported [43, 44, 51]. Present study indicating the PGPRs mediated amelioration of saline alkali soil stress in rice.

SOD activity

Pseudomonas strain P2 (1.56 fold) and strains R62+R81 (1.22 fold) in CSR-36, while in IR-64 all the treatments significantly reduced the SOD activity as compared to control under Level III soil stress. In Level II, strain P2 and strains R62+R81 in CSR-36 whereas in IR-64 strain YB3 and R62+R81 showed significant reduction in SOD activity as compare to uninoculated plants. Under Level I, strain P2 and strains R62+R81 significantly reduced SOD activity in CSR-36 while none of the treatments showed significant effect in IR-64. In overall stress level irrespective of treatments IR-64 variety of rice showed 40.62% higher SOD activity as compared to CSR-36 (Fig. 1b). Present study indicated that with increasing stress SOD activity increased in plants. However the PGPR treated plants showed the reduced level of SOD activity as compared to uninoculated plants. Reduced level of antioxidants in PGPR inoculated plants as compared to non inoculated plants under salinity stress condition have also been reported by the authors [52, 53, 17]. The reduced level of SOD in PGPRs treated plants indicated that PGPRs helps plants to maintain their internal homeostasis under saline alkali soil stress condition and may help plants to generate low level of reactive oxygen species (ROS) within cell and thus reduced its consequences in plant cell.

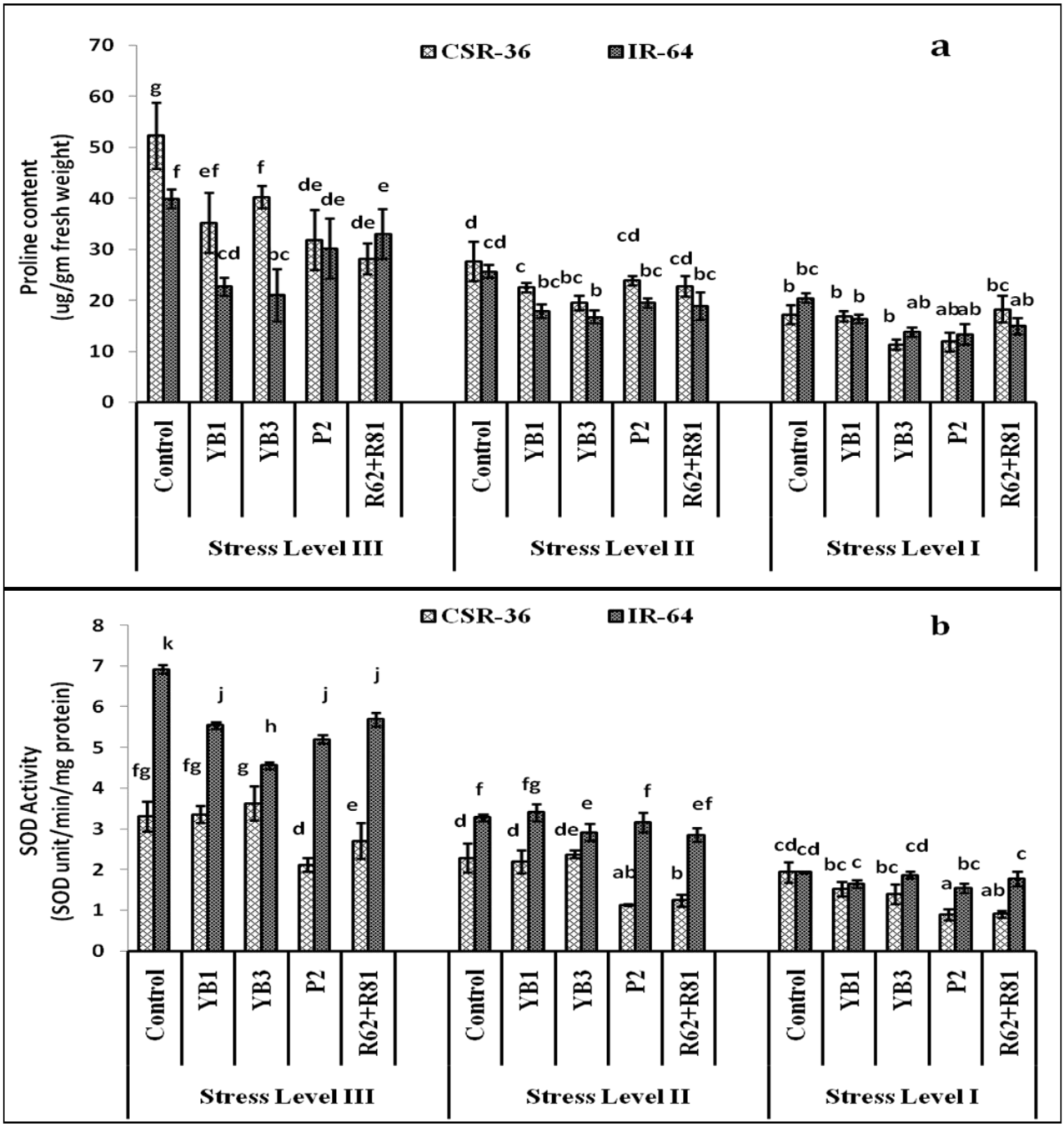

Fig 1: (a) Proline content and (b) SOD activity of two cultivars of rice inoculated with PGPRs under different level of saline alkali soil stress. Mean followed by same letter are not significantly different ($P < 0.05$) for a particular trait in two cultivars at all the level of stress.

Phosphorus uptake

Majority of the inoculated plants of both the varieties showed the higher phosphorus uptake as compared to uninoculated plants under all the level of soil stress. In CSR-36, YB1 and R62+R81, while in IR-64 all the inoculated plants showed the significant effect on phosphorus uptake as compared to untreated plants under stress level III. Under stress level II all the treatments in both the varieties, except YB1 in CSR-36, significantly increased the phosphorus uptake over control. Under stress level I all the treatments significantly increased the phosphorus uptake in both the varieties of rice. Irrespective of treatments and soil ratio, IR-64 showed slight high phosphorus uptake as compared to CSR-36 (Fig. 2c). Salinity decreased phosphorus accumulation in plants, which can cause phosphorus deficiency symptoms [45]. It is generally accepted mechanism that soil microorganisms synthesized the organic acids acted as mineral phosphate solubilizer. The production of organic acids has been well documented for different PGPR genera [54]. Similar to present study increased P- uptake in bacterial inoculated plants as compare to uninoculated plants under saline stress have also been observed [16].

Potassium and sodium ion uptake

All the inoculated plants of CSR-36 showed increased level of potassium uptake however the differences were not significant, whereas in IR-64 only R62+R81 showed the significant effect as compared to control plants under stress level III. In stress level II all the inoculants except P2 in CSR-36, while in IR-64, P2 and R62+R81 showed the significant effect as compared to uninoculated plants. Under stress level I none of the treated plants in CSR-36, whereas strain P2 and R62+R81 in IR-64, showed the significant effect on potassium uptake over control (Fig. 2b). In over all stress level irrespective of treatment salt tolerant CSR-36 accumulate 11% higher potassium uptake as compared to salt

sensitive IR-64. In case of sodium uptake all the inoculants except YB3 in CSR-36 significantly reduced the sodium uptake of both the genotype of rice under stress level III. In stress level II, *Pseudomonas* strain P2 significantly reduced the sodium uptake in CSR-36 whereas in IR-64 none of the treatments showed the significant effect over control. In stress level I none of the treatment in CSR-36, whereas Pseudomonas strain P2 and R62+R81, in IR64 significantly reduce sodium uptake as compare to uninoculated plants. In overall stress level irrespective of treatments, IR-64 uptake 31.86% more sodium as compared to

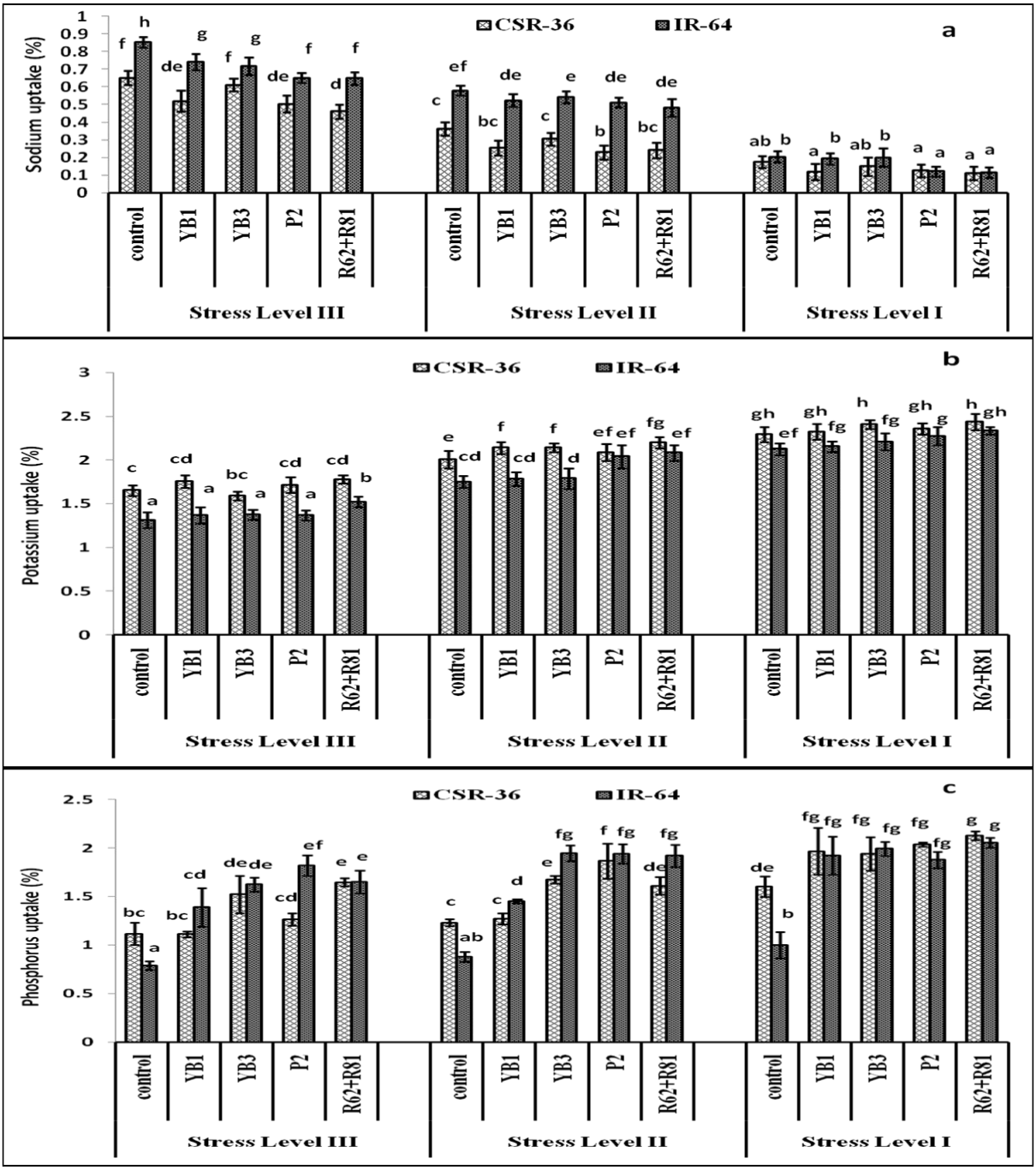

Fig 2: (a) Sodium (b) Potassium and (c) Phosphorus uptake by two cultivars of rice inoculated with PGPRs under different level of saline alkali soil stress. Mean followed by same letter are not significantly different ($P<0.05$) for a particular trait in two cultivars at all the level of stress.

CSR-36 (Fig. 2a). The metabolism of Na+ and K+ is an important component of salt stress. Usually, Na+ increases and K+ decreases in plants stressed by salt [46]. Na+ is the main poisonous ion in salinized soil. Low Na+ and high K+ in the cytoplasm are essential to maintain a number of enzymatic processes [50]. In present study**,** K+ decreases and Na+ increases with increase the saline alkali soil stress. PGPRs can alleviate the salinity stress by producing the bacterial exopolysacharides (EPS) which can bind with cations such as Na^+ and decrease the content of Na^+ available for plants [55] thus help to increased the ratio of K^+/Na^+. Selected PGPRs remarkably reduced the level of Na^+ and increased the concentration of K^+ in plants as compare to their respective control under all the level of saline alkali soil stress. Similar to the present study various authors have found decreased level of Na^+ and increased level of K^+ in PGPRs inoculated plants under saline stress condition [16, 56, 43]. In present study higher K^+ and lower Na^+ in CSR-36 as compare to IR-64 might be an attribute of tolerant variety to ameliorate the consequences of saline alkali soil stress.

Conclusion

In present study PGPRs inoculated plants frequently reduces the sodium absorption, increases potassium and phosphorus absorption and thus might ameliorate the saline –alkali soil stress in both the genotype of rice. This might be one of the reasons that most of the inoculated plants of both the genotype of rice showed reduced level of MDA, EL, Proline and SOD activity as well as higher chlorophyll, carotenoid content and enhanced growth parameters. In overall stress level, irrespective of treatments the lower level of Na+, MDA, EL and SOD activity as well higher K+ and proline in salt tolerant CSR-36 as compare to salt sensitive IR-64 might be an attribute of tolerant variety which could help it to alleviate the higher level of saline stress.

Acknowledgements

The first author is very thankful to International Rice Research Institute (IRRI), Phillipience for providing financial support through the STRASA project.

References

1. Kukavica B, Morina F, Janjic N, Boroja M, Jovanovic LJ, Jovanovic SV. Effects of mixed saline and alkaline stress on the morphology and anatomy of *Pisum sativum* L.: the role of peroxidase and Ascorbate oxidase in growth regulation. Archives of Biological Science Belgrade. 2013; 65(1):265-278.
2. Hu G, Liu Y, Zhang X, Yao F, Huang Y, Ervin EH, Zhao B. Physiological Evaluation of Alkali-Salt Tolerance of Thirty Switchgrass (*Panicum virgatum*) Lines. PLoS ONE. 2015; 10(7):e0125305.
3. Yang Guo-Hui. Alkali stress induced the accumulation and secretion of organic acids in wheat. African Journal of Agricultural Research. 2012; 7(18):2844-2852.
4. Zhang JT, Mu CS. Effects of saline and alkaline stresses on the germination, growth, photosynthesis, ionic balance and anti-oxidant system in an alkali-tolerant leguminous forage *Lathyrus quinquenervius*. Soil Science and Plant Nutrition. 2009; 55:685-697.
5. Li R, Shi F, Fukuda K, Yang Y. Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (*Medicago sativa* L.). Soil Science and Plant Nutrition. 2010; 56:725-733.
6. Zhu J, Fu X, Koo YD, Zhu JK, Jenney Jr FE, Adams MWW *et al*. An enhancer mutant of Arabidopsis salt overly sensitive 3 mediates both ion homeostasis and the oxidative stress response. Molecular and Cellular Biology. 2007; 27(14):5214-5224.
7. Miller G, Suzuki N, Ciftci-Yilmazi N, Mittler R. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. Plant Cell & Environment. 2010; 33:453-467.
8. Foyer CH, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant and Cell. 2005; 17:1866-1875.
9. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 2002; 7:405-410
10. Amos Ouma Onyango. Exploring Options for Improving Rice Production to Reduce Hunger and Poverty in Kenya. World Environment. 2014; 4(4):172-179.
11. Miransari M, Bahrami HA, Rejali F, Malakouti MJ. Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biology and Biochemistry. 2008; 40:1197-1206.
12. Abd-Alla MH, Elsadek El-Enany AW, Nafady NA, Khalaf DM, Morsy FM. Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* andarbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiological Research. 2014; 169:49-58
13. Pourbabaee AA, Bahmani E, Alikhani HA, Emami S. Promotion of Wheat growth under Salt Stress by Halotolerant Bacteria Containing ACC deaminase. Journal of Agricultural Science and Technology. 2016; 18:855-864.
14. Dimkpa C, Weinand T, Asch F. Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell & Environment. 2009; 32:1682-1694.
15. Vejan P, Abdullah R, Khadiran T, Ismail S, Boyce AN. Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability-A Review. Molecules. 2016; 21:573.
16. Kohler J, Hernandez JA, Caravaca F, Roldan A. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuces to severe salt stress. Environmental and Experimental Botany. 2009; 64:207-216
17. Kang SM, Khan AL, Waqas M, You YH, Kim JH, Kim JG *et al*. Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. Journal of Plant Interactions. 2014; 9(1):673-682.
18. Younesi O, Moradi A. Effects of plant growth-promoting rhizobacterium (PGPR) and arbuscular mycorrhizal fungus (AMF) on antioxidant enzyme activities in salt-stressed bean (*Phaseolus vulgaris* L.). Agriculture (Pol'nohospodárstvo). 2014; 60(1):10-21.
19. Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A *et al*. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Frontiers in Plant Science. 2017; 8:172
20. Singh YP, Nayak AK, Sharma DK, Gautam RK, Singh RK, Singh R *et al*. Varietal selection in sodic soils of Indo-Gangetic plains through farmers' participatory approach. African Journal of Agricultural Research. 2013; 8(23):2849-2860.
21. Sarhadi E, Bazargani MM, Sajise AG, Abdolahi S, Vispo NA, Arceta M *et al*. Proteomic analysis of rice anthers under salt stress. Plant Physiology and Biochemistry. 2012; 58:280-287.
22. Jackson ML. Soil Chemical Analysis. Prentice-Hall of India Private Limited, New Delhi, 1973, 1-498.
23. Subbiah BV, Asija GL. A rapid procedure for the estimation of available nitrogen in soils. Current Science. 1956; 25(8):259-260.
24. Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture Washington, DC Circular. 1954; 939:1-19.
25. Jackson ML. Soil Chemical Analysis. Prentice-Hall, Inc. Edgewood Cliffs, New Jersey. 1958, 84-133.
26. Mader P, Kaiser F, Adholeya A, Singh R, Uppal HS, Sharma AK *et al*. Inoculation of root microorganisms for sustainable wheat-rice and wheat black gram rotations in India. Soil Biology and Biochemistry. 2011; 43:609-619.
27. Gaur R, Shani N, Kawaljeet K, Johri BN, Rossi P, Aragno M. Diacetylphloroglucinol- producing

Pseudomonads do not influence AM fungi in wheat rhizosphere. Current Science. 2004; 86:453-457.
28. Roesti D, Gaur R, Johri BN, Imfeld G, Sharma S, Kawaljeet K *et al*. Plant growth stage, fertiliser management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in rain-fed wheat fields. Soil Biology and Biochemistry. 2006; 38:1111-1120.
29. Gusain YS, Kamal R, Mehta CM, Singh US, Sharma AK. Phosphate solubilizing and indole-3-acetic acid producing bacteria from the soil of Garhwal Himalaya aimed to improve the growth of rice. Journal of Environmental Biology. 2015; 36:301-307.
30. Arnon DI. Copper enzymes in isolated chloroplast polyphenol oxidase in Beta vulgaris. Plant Physiology. 1949; 24:1-15.
31. Dionisio-Sese ML, Tobita S. Antioxidant responses of rice seedlings to salinity stress. Plant Science. 1998; 135(1):1-9.
32. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 1968; 125(1):189-198.
33. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water–stress studies. Plant and Soil. 1973; 39:205-207.
34. Zhang J, Kirkham MB. Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytologist. 1996; 132:361-373.
35. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Chemistry. 1976; 72:246-254.
36. Jackson ML. Soil Chemical analysis. Prentice Hall of India, Pvt. Ltd, New Delhi. 1967, 111-203.
37. Ashraf M, Bashir A. Salt stress induced changes in some organic metabolites and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. Flora. 2003; 198:486-498.
38. Rabie GG, Almandini AM. Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. African Journal of Biotechnology. 2005; 4:210-223.
39. Mayak S, Tirosh T, Glick BR. Plant growth-promoting bacteria that confer resistance in tomato and pepper plants to salt stress. Plant Physiology and Biochemistry. 2004; 167:650-656.
40. Omar MNA, Osman MEH, Kasim WA, Abd El-Daim. Improvement of salt tolerance mechanisms of barely cultivated under salt stress using *Azospirillum brasilense*. In: M. Ashraf *et al*. (eds). Salinity and water stress, Springer, Netherlands. 2009; 44:133-147.
41. Habib SH, Kausar H, Saud HM. Plant Growth-Promoting Rhizobacteria Enhance Salinity Stress Tolerance in Okra through ROS-Scavenging Enzymes. BioMed Research International. 2016, 1-10.
42. Sairam RK, Rao KV, Srivastava GC. Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science. 2002; 163:1037-1046.
43. Tapias RD, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera D, Bonilla R. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). Applied Soil Ecology. 2012; 61:264-272.
44. Hamdia MAES, Shaddad MAK, Doaa MM. Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions. Plant Growth Regulation. 2004; 44:165-174.
45. Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety. 2005; 60:324-349.
46. Shi D, Wang D. Effects of various salt-alkaline mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. Plant and Soil. 2005; 271:15-26.
47. Yildrim E, Turan M, Donmez MF. Mitigation of salt stress in radish (*Raphanus sativus*) by plant growth promoting rhizobacteria. Romanian Biotechnological Letters. 2008; 13:3933-3943.
48. Dogan M. Investigation of the effect of salt stress on the antioxidant enzyme activities on the young and old leaves of salsola (*Stenoptera*) and tomato (*Lycopersicon esculentum* L.). African Journal of Plant Science. 2012; 6(2):62-72.
49. Han HS, Lee KD. Physiological responses of soybean-inoculation of *Bradyrhizobium japonicum* with PGPR in saline soil conditions. Research journal of agriculture and biological sciences. 2005; 1(3):216-221.
50. Yang J, Kloepper JW, Ryu CM. Rhizosphere bacteria help plants tolerate abiotic stress. Trends in Plant Science. 2009; 14:1-4.
51. Nadeem SM, Zahir ZA, Naveed M, Arshad M. Preliminary investigation on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC-deaminase activity. Canadian Journal of Microbiology. 2007; 53:1141-1149.
52. Han HS, Lee KD. Plant growth promoting rhizobacteria: effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Research journal of agriculture and biological sciences. 2005; 1:210-215.
53. Baniaghil N, Arzanesh MH, Ghorbanli M, Shahbazi M. The effect of plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress. Journal of Applied Environmental and Biological Sciences. 2013; 3(1):17-27.
54. Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances. 1999; 17:319-339.
55. Ashraf M, Berge SH, Mahmood OT. Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biology and Fertility of Soils. 2004; 40:157-162.
56. Fu Q, Liu C, Ding N, Lin Y, Guo G. Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas* sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. Agricultural Water Management. 2010; 97:1994-2000.