African Journal of Biotechnology Vol. 8 (21), pp. 5762-5766, 2 November, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L.

Irum Naz, Asghari Bano and Tamoor-Ul-Hassan

Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan.

Accepted 5 October, 2009

Present investigation was made to isolate and characterize plant growth promoting rhizobacteria (Rkh1 - Rkh4) from rhizosphere of four weeds: *Chrysopogon aucheri*, *Lactuca dissecta*, *Solanum surratense* and *Sonchus arvensis* thriving under high salinity (EC: 2.3 dS/m) of Khewra salt range and the results were compared with strain Rak isolated from rhizosphere (EC: 0.2 dS/m) of *Solanum surratense* grown in arid area of district Attock. The tolerance of all the strains was checked against the salt present in culture media in the form of rhizospheric soil filtrate of weeds collected from Khewra salt range. The nutrient contents of rhizospheric soil of weeds were measured. All the strains were capable to produce phytohormones indole-3-acetic acid (IAA), gibberellic acid (GA_3), trans-zeatin riboside (t-zr) and abscisic acid (ABA) in the culture media. Inoculation of strains on soybean seedlings treated with or without 20 dS/m NaCl resulted in better growth and higher proline contents than control plants. The strains isolated from weeds of Khewra salt range particularly Rkh3 appears more promising for potential biofertilizers in saline fields.

Key words: Plant growth promoting rhizobacteria, nutrient contents, salt tolerance, phytohormones production, proline contents.

INTRODUCTION

Soil salinity is an important limiting factor in agriculture economy. In addition to traditional breeding and genetic modification of plants (Van der straeten et al., 1990; Blumwald, 2000), recent focus of research involves implication of plant growth promoting rhizobacteria to combat salt stress. The plant growth promoting rhizobacteria increase water use efficiency, fresh and dry weight of plants (Mayak et al., 2004) and render the plants more tolerant to salt stress by improving antioxidant status and physiological response (e.g proline used as osmoregulant) of plants (Han and Lee, 2005). PGPR also produce several other growth promoting substances including IAA, GA3, zeatin and ABA (Perrig et al., 2007).

*Corresponding author. E-mail: asgharibano@yahoo.com. Tel: 0092-51-90643096.

IAA producing PGPR have been isolated from Kallar grass (*Leptochloa fusca* (L.) Kunth) grown in salt effected soil of Pakistan and their growth promoting effects have been documented on rice (Mirza et al., 2006). The salt tolerant plants/halophytes usually have higher concentration of total salt and salt ions: Cl^-, Mg, Na, K and HCO_3^- in their rhizosphere (Liangpeng et al., 2007).

Khewra salt mine is the World second largest salt mine, situated in the foothills of the Salt Range, has plethora of valuable salts including halite (NaCl), sylvite (KCl) and gypsum ($CaSO_4$. $2H_2O$) salts. In order to simulate natural condition of Khewra salt range [32°56′00″N (North altitude); 73°44′00″E (East longitude); annual rain fall: 900 mm; maximum temperature in June: 45.7°C; minimum temperature in January: 1.8°C; Soil type: Sandy loam], present attempt has been made to isolate the PGPR from weeds growing in natural saline soil of area surrounding Khewra salt mine and to check their

tolerance to salt present in culture in the form of filtrate of rhizosphere soil of Khewra salt range. This is the first report regarding the isolation of PGPR from rhizosphric soil of weeds of Khewra salt range. The capability of these strains to biosynthesize phytohormones IAA, GA, t-zr and ABA have been evaluated. Furthermore implication of these PGPR strains as bio-inoculant on soybean has been demonstrated.

MATERIALS AND METHODS

Collection of soil samples

In the present study five weeds (four from Khewra salt range and one from district Attock)/ three replicates were collected at their vegetative stage from 0-5 cm depth in three different locations. The rhizospheric soils were obtained by gentle shaking of roots and stored at 4°C for further analysis. The four weeds collected from Khewra salt range are listed below.

1 *Chrysopogon aucheri* (Boiss.) Stapf. (golden beared grass)
2 *Lactuca dissecta* D. Don. (wild lettuce)
3 *Solanum surratense* Burm. F. (yellow berried night shade)
4 *Sonchus arvensis* L. (sow thistle).

Only one weed: *Solanum surratense* was collected from district Attock.

Nutrient analysis of rhizospheric soil

The Cl^- and HCO_3^- contents of rhizosphere were quantified by Reitemeir (1943) method. The available nutrients viz, Na^+, K^+, Mg^{2+} and Ca^{2+} of rhizosphere soil were measured adopting the method of Kingston (1994).

Bacterial growth media and isolation of PGPR

There are many reports regarding the free existence of Rhizobia in the rhizosphere of non-legumes. In present experiment Yeast extract mannitol agar medium (YMA) was used to isolate PGPR colonies similar to *Rhizobium* from rhizosphere of weeds.

Yeast extract mannitol agar media (YMA) comprising: 0.5 g K_2HPO_4, 10 g mannitol, 0.12 g Yeast extract, 1 g NaCl, 0.2 g $MgSO_4$. $7H_2O$, 20 g agar (all dissolved/litre of distilled water) and autoclaved at 121°C for 15 min. For the isolation of PGPR 10 g rhizospheric soil was transferred to 250 ml flask containing 90 ml distilled water and kept on shaker (100 rpm) for 30 min. Serial dilutions (10X) were made and aliquot (50 µl) from decimal dilutions (10^5 - 10^7) was used to inoculate YMA media. The culture plates were incubated at 30ºC for 24 - 72 h, total five colonies (one from rhizosphere of each weed) showed resemblance with rhizobia were selected and further purified by streaking.

Naz (unpublished 2008) preliminary identified the isolated PGPR as rhizobia on the basis of C/N source utilization pattern based on QTS (Quick testing system, DESTO laboratories Karachi Pakistan), colony morphology and gram staining. The isolates were gram -ve and were positive to catalase and oxidase tests. The isolated five PGPR colonies were morphologically similar to each other but fall in to five different groups (five different strains) on the basis of QTS kits results. The results were compared with Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Further more a band of almost 1500 bp was obtained during PCR specific for 16S-rRNA gene (Naz, unpublished 2008) of α-proteobacteria.

Tolerance of PGPR to salt of rhizospheric soil filtrate present in media

The tolerance of PGPR was demonstrated on YMA media which was supplemented with rhizospheric soil filtrate of weeds collected from Khewra salt range. The rhizospheric soil (50 g) was taken separately in three flasks (500 ml) each suspended in 250 ml of distilled water. The suspension was filtered using Whatman filter paper No. 42. The EC of each of the three soil filtrates was 2.3 dS/m. Two of the filtrates were diluted to have an EC of 1.3 and 1.8 dS/m. Soil filtrate (250 ml) was added to YMA media to replace distilled water which was added in case of control. The media were autoclaved at 121°C for 15 min. An aliquot (50 µl) from decimal dilutions (10^5 - 10^7) used for the isolation of PGPR was inoculated on YMA plates. The plates were incubated at 30ºC for 3 - 5 d. The tolerance of PGPR was measured by counting the number of colonies using the formula:

Viable cell count (CFU/g soil) = (number of colonies/volume of inoculum) × dilution factor

Extraction, purification and quantification of phytohormones from bacterial culture

Extraction, purification and quantification of phytohormones from bacterial culture were made to understand the mechanism of growth promotion of these microbes used as inoculants. YMA broth media supplemented with and without tryptophan (10 mg/100 ml) was inoculated with 24 h old bacterial cultures and kept on a shaker (ECELLA E24, USA) at 100 rpm for 5 d. Thereafter, the bacterial cells were harvested by centrifugation at 10,000 rpm for 15 min at 4ºC and supernatant (cell-free liquid culture medium) was used for extraction of phytohormones following the method described by Tien et al. (1979). The samples were analyzed on HPLC (Agilent 1100) equipped with variable UV detector and C_{18} column (39 x 300 mm) [BondaPack Porasil C-18, 37/50 µm, Waters, Eschborn, BRD). Methanol and water in the ratio of 30:70 v/v were used as mobile phase @ of 1500 µl/min. with a run time of 20 min/sample. For identification of hormones, 100 µl of sample filtered through a 0.45 millipore filter, were injected into column. The growth hormones were identified on the basis of retention time of phytohormone standards (commercially grade, Sigma Chemical USA Company). IAA was eluted at 280 nm wavelength while GA_3, t-zr and ABA were eluted at 254 nm respectively. The culture media without microbial inoculation were taken as blank and processed for phytohormones extraction as described above.

Inoculation studies of soybean [*Glycine max* (L.) Merr] by PGPR strains

Inoculation studies with PGPR strains were made using soybean as host. The seeds of the soybean (cv. NARC-1) were surface sterilized with 95% ethanol for 2 min and then with 10% chlorex for 2 - 3 min. After it, successive washing was done with distilled water to completely remove the chemicals. The seeds were soaked overnight in distilled water prior to soaking in 5 d old cultures of PGPR strains (10^6 - 10^7 cfu/g of soil) for 2 - 3 h. The seeds soaked in culture media (without microbial inoculation) were treated as control. The sandy loam soil and sand in a ratio of 2:1 was selected for germination of seeds. The native microbes of soil and sand were killed by autoclaving (at 121°C for 15 min) three times with gap of 24 h. The pots of 19 x 17 cm size were filled with soil/sand mixture, seeds were sown in them and they were kept in growth room. The temperature of growth room was maintained at 25 ± 2°C with 16 h light period and 8-h dark period; light intensity 5.5 W/m^2 and the humidity varying from 75 - 80%. The plants were irrigated with

Table 1. Concentration of salt ions in the rhizospheric soil of weeds collected from Khewra salt range and district Attock.

Rhizospheric soil	Concentration (g/Kg)					
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-
C. aucheri (Khewra)	3C ± 0.08	4C ± 0.04	4.6B ± 0.6	3.5B ± 0.04	1B ± 0.1	0.8C ± 0.08
L. dissecta (Khewra)	3.2B ± 0.04	4.5B ± 0.09	5.3A ± 0.2	4.3A ± 0.4	1.1A ± 0.06	0.9A ± 0.3
S. surratense (Khewra)	3.5A ± 0.2	4.6A ± 0.01	5.6A ± 0.3	4.5A ± 0.4	1.1A ± 0.04	0.9A ± 0.09
S. arvensis (Khewra)	3C ± 0.03	4.2BC ± 0.02	5.6A ± 0.02	3.8B ± 0.4	1B ± 0.02	0.88BC± 0.3
S. surratense (Attock)	0.3D ± 0.07	1D ± 0.001	0.33C ± 0.3	0.2C ± 0.01	0.25C ± 0.03	0.4D ± 0.2

Values showed mean ± standard error of means (SEM). All means which share different letters (DMRT values) are significantly different at p ≥ 0.05. Standard of means (SEM) have been.

Hogland nutrient solution added 4-5d of germination. Hogland nutrient solution (300 ml) was added to each pot containing five plants and the process was repeated after 4 days. After 2 weeks of inoculation seedlings were gradually exposed to NaCl by adding 5 dS/m NaCl (50 mM NaCl) per day until the final concentration of 20 dS/m (200 mM NaCl) was reached. Measurements were made 40 d after inoculation. Three replicates (five plants/replicate) were used.

Proline contents of shoots and roots of inoculated plants

Proline contents of shoots and roots were estimated by the method of Bates et al. (1973). Statistical analysis was done by factorial analysis of variance (ANOVA) and two factor completely randomized design test (CRD) with least significance difference (LSD) using MSTAT C program, version 4.0.

RESULTS AND DISCUSSION

Four PGPR strains were isolated from rhizospheric soil of weeds growing in Khewra salt range and they were named as: Rkh1 from rhizosphere of *Chrysopogon aucheri*, Rkh2 from rhizosphere of *Lactuca dissecta*, Rkh3 from rhizosphere of *Solanum surattense* and Rkh4 from rhizosphere of *Sonchus arvensis*. Rak was isolated from rhizosphere soil of *S surattense* grown in arid soil of district Attock.

During the present study high accumulation of salt ions: Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and HCO_3^- were detected in the rhizospheric soil of weeds collected from Khewra salt range (Table 1). The rhizospheric soil of *S. surattense* had higher concentration of salt ions as compared to other weeds collected from Khewra salt range. The content of Na^+ in the rhizosphere of weeds collected from Khewra salt range were 90 - 91% higher while Ca^{2+} and Mg^{2+} ions were 94 - 95% higher than the rhizospheric soil of weed collected from district Attock. The K^+ contents were 75 - 78% higher, Cl^- contents were 75 - 77% higher and HCO_3^- ions were 50 - 55% higher in rhizospheric soil of weeds of Khewra salt range over that of weed collected from district Attock. Presence of higher concentration of these salt ions may helps the weeds to physiologically adapt themselves to salty environment of Khewra salt range and enhances their tolerance in stress environment as reported by Feng et al. (2000). The accumulation of salt ions in the rhizosphere soil is positively correlated with the salt tolerance, the more tolerance the plant had the higher degree of salt accumulation they had in their rhizosphere (Liangpeng et al., 2007).

The number of colonies of isolates on culture media supplemented with rhizospheric soil filtrate (with three different EC levels: 1.3 dS/m; 1.8 dS/m; 2.3 dS/m) of weeds of Khewra salt range is indicative of their degree of tolerance to the salt of the soil filtrate. The tolerance was greater in Rkh2 and Rkh3 strains as compared to Rkh1 and Rkh4 strains (Figure 1) as indicated by cfu/g of soil. The number of viable colonies of PGPR strains declined with increasing concentration of soil filtrate. Least number of colonies of PGPR strains were observed on media having EC: 2.3 dS/m while maximum colonies were formed on media having EC 1.3 dS/m. A decrease in colony counts of PGPR has been reported with increasing concentration of salt (Zahran, 1999; Bano and Fatima, 2009). Omar et al. (2009) isolated PGPR strain from hypersaline soil and it was able to survive up to 1,800 mM NaCl present in media. The absence of any colony formed by the strain Rak isolated from rhizospheric soil of weed collected from district Attock represent the sensitivity of the strain to the salt present in the soil filtrate in culture media.

In present study all the PGPR strains showed differential capacities to produced phytohormones Indole-3-acetic acid (IAA), Gibberellic acid (GA_3), trans-zeatin-riboside (t-zr) and Abscisic acid (ABA) in culture media supplemented with and without tryptophan (Table 2). The highest amount of IAA was produced by the strain Rak in culture media from which tryptophan was omitted. The other four strains Rkh1-Rkh4 possessed similar magnitude to produce IAA. PGPR strains have been reported to produce IAA either with or without the tryptophan supplement in culture media (Horemans and Vlassak, 1985; Fallik et al., 1989; Mehnaz et al., 2001; Fatima et al., 2009). In our experiment the level of IAA produced in culture media from which tryptophan omitted was higher than those reported by Mehnaz et al. (2001). The level of IAA production increased several folds in culture media supplemented with tryptophan. The ability of the strains Rak and Rkh4 was similar to convert added tryptophan to IAA but possessed 3 - 8 folds lower efficiency in contrast

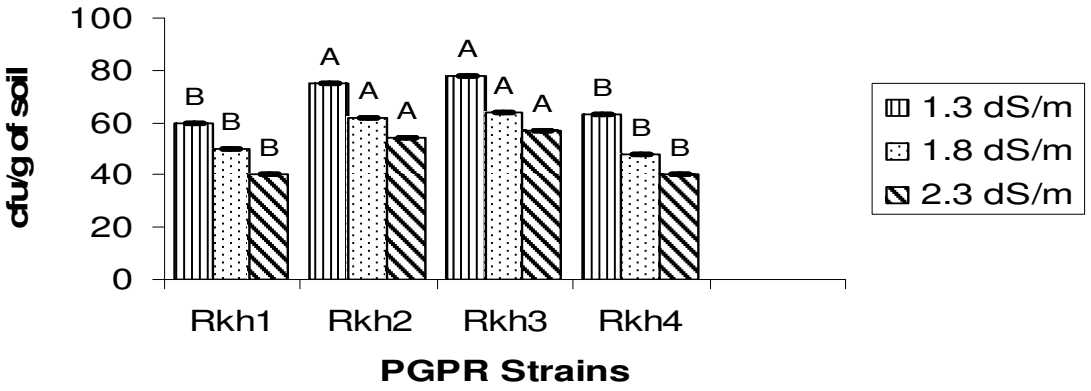

Figure 1. Measurement of colony counts of PGPR strains to check their salt tolerance in the culture media supplemented with salt of rhizospheric soil filtrate of weeds collected from Khewra salt range. Bars showed DMRT values along with standard error of means (SEM). All means which share different letters (DMRT values) are significantly different at $p \geq 0.05$.

Table 2. Production of phytohormones by PGPR strains in culture media supplemented with and without tryptophan.

PGPR Strains	Concentration (µg/ml)							
	IAA		GA3		t-zr		ABA	
	-Trp	+Trp	-Trp	+Trp	-Trp	+Trp	-Trp	+Trp
Rkh1	0.2E ± 0.01	2.5C ± 0.08	2D ± 0.07	0.2F ± 0.01	1.2C ± 0.08	0.1E ± 0.01	0.45D ± 0.04	0.1F ± 0.01
Rkh2	0.4E ± 0.01	4.7B ± 0.2	3.4C ± 0.01	0.1F ± 0.01	1.35BC ± 0.2	0.1E ± 0.01	0.6B ± 0.02	0.1 ± 0.001
Rkh3	0.3E ± 0.01	4.8B ± 0.2	3.9B ± 0.01	0.2F ± 0.01	1.4BC ± 0.2	0.1E ± 0.02	0.9A ± 0.02	0.1F ± 0.01
Rkh4	0.3E ± 0.2	2.5C ± 0.2	1.7E ± 0.9	0.1F ± 0.01	0.9D ± 0.1	0.1E ± 0.01	0.55C ± 0.02	0.1F ± 0.003
Rak	0.8D ± 0.01	6.9A ± 0.08	6.3A ± 0.1	0.3F ± 0.01	1.7A ± 0.08	0.2E ± 0.009	0.2E ± 0.1	0.06G ± 0.003

Values showed mean ± standard error of means (SEM). Rkh1-Rkh4 represents the strains isolated from rhizosphere soil of weeds of Khewra salt range and Rak from rhizosphere soil of *S. surratense* growing in Attock. –Trp = without tryptophan, +Trp= with 0.1 mg/ml tryptophan.

to conversion efficiency of strains Rkh1 - Rkh3. Among the strains Rkh1 - Rkh4 isolated from rhizospheric soil of weeds of Khewra salt range the strain Rkh3 was most efficient in the conversion of added tryptophan to IAA. The increased level of IAA production using tryptophan as precursor has been documented in PGPR by various authors (Tien et al., 1979; Park et al., 2005).

In contrast to increased level of IAA, the production of other phytohormones GA3, t-zr and ABA was decreased several folds in culture media supplemented with tryptophan. The positive effect of tryptophan addition on the production of IAA with corresponding decrease in GA3, ABA and t-zr may reveal interaction among hormones to regulate the endogenous level of phytohormones. Both types of synergistic and antagonistic interaction have been reported between CKs, ABA and IAA (Woodward and Bartel, 2005; Polanska et al., 2006). In the present study the strain Rak exhibited significantly (at $p \geq 0.05$) higher GA_3 and t-zr production than that of Rkh1 - Rkh4 strains in absence of tryptophan but in presence of tryptophan all the strains excreted same amount of GA_3 and t-zr (Table 2). Among the strains Rkh1 - Rkh4, the strain Rkh3 was significantly most active in the production of GA_3 in absence of tryptophan but its capability to synthesize t-zr was insignificantly different from other strains. GAs and CK synthesis have been reported in several species of PGPR (Tien et al., 1979; Akiyoshi et al., 1987; Cacciari et al., 1989). Cassan et al. (2009) identified and quantified GA_3 on gas chromatography-mass spectrometry with selective ion monitoring (GC-MS.SIM) produced by *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109. The strains Rkh1 - Rkh4 released significantly higher amount of ABA than that of strain Rak isolated from rhizospheric soil of weed of district Attock. Among the strains Rkh1 - Rkh4, the strain Rkh3 released higher magnitude of ABA production in absence of tryptophan but in presence of tryptophan there was no difference in the amount of ABA produced by these isolates. There are few reports about the biosynthesis of ABA by PGPR strains but there is still no report available about the synthesis of ABA by PGPR isolated from natural salt stressed area. The amount of ABA produced by the strains Rkh1 - Rkh4 in present study was higher than the level of ABA produced by PGPR strains reported by various authors (Boiero et al., 2007; Perrig et al., 2005; Perrig et al., 2007).

Table 3. Inoculation effects of PGPR strains on shoot and root dry weight and length of 40 days old soybean (cv. NARC-1) plants growing under induced salt stress and normal conditions.

Treatments	Shoot length (cm)		Root Length (cm)		Shoot weight (g)		Root weight (g)	
	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl
T1	33.3D ± 0.8	25.7H ± 0.1	20.6E ± 0.1	16.3I ± 0.15	4.8D ± 0.03	3.7FG ± 0.08	2.6D ± 0.05	1.5G ± 0.01
T2	38.7B ± 0.08	29F ± 0.2	23.8C ± 0.2	18.6G ± 0.15	5.1C ± 0.08	4.1E ± 0.1	3C ± 0.06	1.7F ± 0.06
T3	39.7B ± 0.1	31.7E ± 0.8	24.4B ± 0.06	19.7F ± 0.1	5.6B ± 0.09	4.2E ± 0.08	3.2B ± 0.07	2E ± 0.08
T4	37.2C ± 0.4	26.4GH ± 0.4	22.4D ± 0.3	17.4H ± 0.2	5CD ± 0.007	4EF ± 0.06	2.9C ± 0.06	1.5G ± 0.03
T5	43.8A ± 0.1	23.1I ± 0.06	26.7A ± 0.1	14.5J ± 0.14	6.2A ± 0.1	2.9H ± 0.07	4A ± 0.07	1H ± 0.03
T6	27.6G ± 0.8	20.5J ± 0.3	17.8H ± 0.1	11.2K ± 0.08	3.7G ± 0.1	2.2I ± 0.1	1.8F ± 0.03	0.6I ± 0.03

+NaCl = With 20 dS/m (50 mM) NaCl, -NaCl = Without 20 dS/m (50 mM) NaCl. T1-T4 represents the inoculation of soybean plants with Rkh1-Rkh4 strains while T5 represents the inoculation of soybean plants with Rak strain. T6 represents control (un-inoculated) plants. All such values which share different letters are significantly different at $p \geq 0.05$.

Table 4. Inoculation effects of PGPR on proline contents of shoots and roots of 40 days old soybean plants growing under induced salt stress and normal conditions.

Treatments	Concentration (mg/g)			
	Shoots		Roots	
	-NaCl	+NaCl	-NaCl	+NaCl
T1	11FG ± 0.2	14CDE ± 1.1	15.5FG ± 0.26	20.6C ± 1.7
T2	14.3CD ± 0.08	18.3B ± 1.3	16.7EF ± 0.1	24.2B ± 0.4
T3	18B ± 0.3	25.5A ± 2.1	18.9CD ± 0.3	28.6A ± 0.9
T4	11.5EFG ± 0.2	15.7BC ± 0.9	15.4FG ± 0.11	20.8C ± 0.8
T5	9.7GH ± 0.2	12.9DEF ± 1.1	13.4H ± 0.14	18.4DE ± 0.6
T6	8H ± 0.3	10.1GH ± 0.5	10.2I ± 0.11	14.4GH ± 0.3

+NaCl = With 20 dS/m NaCl, -NaCl = Without 20 dS/m NaCl. T1-T4 represents the inoculation of soybean plants with Rkh1-Rkh4 strains while T5 represents the inoculation of soybean plants with Rak strain. T6 represents control (un-inoculated) plants. All such values which share a common letter are insignificantly different at $p \geq 0.05$.

In present study soybean plants were selected for inoculation due to its economic importance and sensitivity to salt stress. A significant increase was observed in the shoot and root length and dry weight of soybean plants inoculated with PGPR strains both under induced salt stress (20 dS/m NaCl) and unstressed conditions as compared to control (un-inoculated) (Table 3). The stimulation of root length, shoot length, dry weight of root and shoot was greater by Rak strain under unstressed condition. The inoculation effect of Rkh1 - Rkh4 strains was more pronounced under induced salt stress than that of Rak strain might be due to high production of ABA by these strains that provide tolerance to plants to ameliorate adverse effects of salinity. Among the Rkh1 - Rkh4 strains, the strain Rkh3 was more effective in enhancing shoot and root length and root dry weight but the shoot weight of soybean plants inoculated with this strain was significantly indifferent than other strains. A decrease in growth and yield of plant has been reported under salinity stress (Ashraf and Bashir, 2003; Rabie and Almadini, 2005). Under such conditions inoculation of plants (particularly soybean) with beneficial micro-organisms promotes the plant growth (El-Mokadem et al., 1991; Omar et al., 2009). Elsheikh and Wood (1995) demonstrated an increase in dry matter of shoot and roots of soybean plants inoculated with the salt tolerant strain of rhizobia in saline conditions compared to inoculation effects of salt sensitive strains in non-saline conditions. PGPR enhance growth of plants through modulation of hormone-linked phenomenon in inoculated plants (Saubidet et al., 2000; Catroux et al., 2001).

Our results revealed a significant increase in the proline contents of shoots and roots of soybean plants growing under NaCl stress in contrast to plants growing under unstressed conditions. Inoculation with PGPR isolates further augmented the proline content of plants growing under salt stress (Table 4). The strains Rkh1 - Rkh4 isolated from rhizospheric soil of weeds of Khewra salt range were more stimulatory in increasing proline contents under both salt stress and unstressed conditions in comparison to strain Rak. The maximum shoot and root proline was observed in soybean plant inoculated with Rkh3 strain. The proline contents were more prominent in roots as compared to shoots. Induction of proline has been documented in legumes in response to salt stress (Sharma et al., 1990; Rabie and Almadini, 2005). In pre-

sent experiment the proline contents were more prominent in roots as compared to shoots. Microbial inoculation enhanced proline accumulation in the roots and provides tolerance to plants under salinity stress. The higher proline contents in roots might be due to the fact that roots are the primary sites of water absorption and must maintain the osmotic balance between the water absorbing cells and external media as reported by Sharifi et al. (2007). The putative mechanism of salt tolerance of PGPR have been reported to high ABA contents (Aziz et al., 1997), better scavenging system of free radicals (Han and Lee, 2005), better proline production, maintenance of water budget of plants and increase in diameter over plant height (Fatima and Bano, 2009).

Conclusion

This is the first report regarding the production of GA, t-zr and ABA from PGPR isolated from weeds growing in dry salty environment of Khewra salt range. Secondly, the strains exhibited their tolerance when tested on saline media simulated by rhizosphere soil filtrate. Noteworthy, the isolates produced ABA in a concentration much higher than that of previous reports. Furthermore production of proline, shoot/root length and dry weight was also higher in soybean plants inoculated with these isolates under induced salt stress. These results suggested the tolerance of PGPR to salt stress. Among the strain isolated from Khewra salt range, the strain Rkh3 appears promising for potential biofertilizers in saline fields.

ACKNOWLEDGEMENTS

We thank Dr. Mushtaq Ahmad (Herbarium Curator, Department of Plant Sciences, QAU Islamabad) for his help in the taxonomic identification of weeds. We are also grateful to Tanvir Ahmad (Technical Officer, QAU Islamabad) for providing technical assistance.

REFERENCES

Akiyoshi DE, Regier DA, Gordon MP (1987). Cytokinin production by *Agrobacterium* and *Pseudomonas* spp. J. Bacteriol. 169(9): 4242-4248.

Ashraf M, Bashir A (2003). 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, 198: 486-498.

Aziz A, Martin-Tanguy J, Larher F (1997). Plasticity of polyamine metabolism associated with high osmotic stress in rape leaf discs and with ethylene treatment. Plant Growth Regul. 21: 153-163.

Bano A, Fatima M (2009). Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. Biol. Fertil. Soils. 45: 405-413.

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.

Blumwald E (2000). Sodium transport and salt tolerance in plants. Curr. Opin. Cell. Biol. 12: 431-434.

Boiero L, Perrig D, Masciarelli O, Penna C, Cassan F, Luna V (2007). Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl. Microbiol. Biotechnol. 74: 874-880.

Cacciari I, Lippi D, Pietrosanti T, Pietrosanti W (1989). Phytohormone-like substances produced by single and mixed diazotrophic cultures of *Azospirillum* and *Arthrobacter*. Plant Soil, 115: 151-153.

Cassan F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V (2009). *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). Eur. J. Soil. Biol. 45: 28-35.

Catroux G, Hartmann A, Revellin C (2001). Trends in rhizobial inoculants production and use. Plant Soil. 230: 21-30.

El-Mokadem MT, Helemish FA, Abdel-Wahab SM, Abou-El-Nour (1991). Salt response of clover and alfalfa inoculated with salt tolerant strains of *Rhizobium*. Ain. Shams. Sci. Bull. 28B: 441-468.

Elsheikh EAE, Wood M (1995). Nodulation and N2 fixation by soybean inoculated with salt-tolerant Rhizobia or salt-sensitive bradyrhizobia in saline soil. Soil Biol. Biochem. 27(4/5): 657-661.

Fallik E, Okon Y, Epstein E, Goldman A, Fischer M (1989). Identification and quantification of IAA and IBA in *Azospirillum brasilense*-inoculated maize roots. Soil Biol. Biochem. 21: 147-153.

Fatima Z, Saleemi M, Zia M, Sultan T, Aslam M, Riaz-Ur-Rehman, Chaudhary MF (2009). Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. Afr. J. Biotechnol. 8(2): 219-225.

Feng F, Zhang FS, Yang XQ (2000). Plant nutriology-Advances and prospects. Beijing: China Agricultural University Press, pp. 12-21.

Han HS, Lee KD (2005). Physiological responses of soybean-Inoculation of *Bradyrhizobium japonicum* with PGPR in saline soil conditions. Res. J. Agric. Biol. Sci. 1(3): 216-221.

Holt JG, Kreig NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore, USA, pp. 40-169.

Horemans S, Vlassak K (1985). Production of indole-3-acetic acid by *Azospirillum brasilense*. In: Klingmuller W (eds) *Azospirillum* III, Genetics, Physiology, Ecology. Springer-Verlag, Berlin, pp. 98-108.

Kingston HMS (1994). Microwave assisted acid digestion of sediments, sludges, soils and oils: EPA method 3051. Duquesne University, Pittsburg, PA, USA.

Liangpeng Yi, Jian Ma, Yan Li (2007). Soil salt and nutrient concentration in the rhizosphere of desert halophytes. Acta. Ecologica. Sinica. 27(9): 3565-3571.

Mayak S, Tirosh T, Glick BR (2004). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem. 42: 565-572.

Mehnaz S, Mirza MS, Haurat J, Balley R, Normand P, Bano A, Malik KA (2001). Isolation and 16S-rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. Can. J. Microbiol. 47: 110-117.

Mirza MS, Mehnaz S, Normand P, Combaret CP, Loccoz YM, Balley R, Malik KA (2006). Molecular characterization and PCR detection of a nitrogen fixing *Pseudomonas* strain promoting rice growth. Biol. Fertil. Soils, 43: 163-170.

Naz I (2008). Physiological and Molecular characterization of Rhizobia and *Azospirillum* from rhizospheric soil and roots of halophytes of Khewra salt range and Attock. M.Phil Dissertification, Quaid-i-Azam University Islamabad, Pakistan.

Omar MNA, Osman MEH, Kasim WA, Abd El-Daim (2009). Improvement of salt tolerance mechanisms of barely cultivated under salt stress using *Azospirillum brasilense*. In: Ashraf M, Ozturk M, Athar HR (eds). Salinity and water stress, Springer, Netherlands, 44: 133-147.

Park M, Kin C, Yang J, Lee H, Shin W, Kim S, Sa T (2005). Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiol. Res. 160: 127-133.

Perrig D, Boiero ML, Masciarelli O, Penna C, Ruiz OA, Cassan F, Luna V (2007). Plant growth promoting compounds produced by two strains of *Azospirillum brasilense*,and implications for inoculant formation. Appl. Microbiol. Biotechnol. 75: 1143-1150.

Perrig D, Masciarelli O, Perticari A, Cassan F, Luna V (2005). Characterizacion de la capacidad promotora y biocontroladora de *Azospirillum brasilense* Az39, la cepa mas utilizada en la formulacion de inoculantes para gramineas en Argentina. Actas de la V Reunion

Nacional Cientifico Tecnica de Biologia del Suelo y FBN, Sociedad de Biologia de Suelos, Jujuy, Argentina, p. 70.
Polanska L, Vicankova A, Novakova M, Jiri M, Dobrev PI, Bretislav B, Vankova R, Machackova I (2006). Altered cytokinin metabolism affects cytokinin, auxin, and abscisic acid contents in leaves and chloroplasts, and chloroplast ultra structure in transgenic tobacco. J. Exp. Bot. pp. 1-22.
Rabie GG, Almandini AM (2005). Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress, Afr. J. Biotechnol. 4: 210-223.
Reitemeir RF (1943). Semimicroanalysis of saline soil solutions. Indus. Engin. Chem. Analyt. 15: 393-402.
Saubidet MI, Fatta N, Barneix AJ (2000). The effects of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization wheat plants. Plant Soil, 245: 215-222.
Sharifi M, Ghorbanli M, Ebrahimzadeh H (2007). Improved growth of salinity-stresses soybean after inoculation with salt pre-treated mycorrhizal fungi. J. Plant Physiol. 164: 1144-1151.
Sharma KD, Datta KS, Verma SK (1990). Effect of chloride and sulphate type of salinity on some metabolic drifts in chickpea (*Cicer arietinum* L). Indian. J. Exp. Biol. 28: 890-892.
Tien TM, Gaskins, MH, Hubbell DH (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L). Appl. Environ. Microbiol. 37: 1016-1024.
Van der straeten D, Van Wiemeersch L, Goodman HM, Van Montagu M (1990). Cloning and sequence of two different cDNAs encoding 1-aminocyclopropane-1-carboxylase synthase in tomato, Proc. Natl. Acad. Sci. USA, 87: 4859-4863.
Woodward AW, Bartel B (2005). Auxin: Regulation, action, and interaction. Anal. Bot. 95: 707-735.
Zahran HH (1999). *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in arid climate. Microbiol. Mol. Biol. Rev. 63: 968-989.