Pak. J. Bot., 43(3): 1643-1650, 2011.

GROWTH PROMOTING ACTIVITIES OF DIFFERENT *RHIZOBIUM* SPP., IN WHEAT

IJAZ MEHBOOB[1], ZAHIR AHMAD ZAHIR[1*], MUHAMMAD ARSHAD[1], ASIF TANVEER[2] AND FAROOQ-E-AZAM[3]

[1]*Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan*
[2]*Department of Agronomy, University of Agriculture, Faisalabad-38040, Pakistan*
[3]*Nuclear Institute for Food and Agriculture, Peshawar, Pakistan*

Abstract

In the present study, large number of rhizobial strains were isolated from chickpea, lentil and mung been nodules and twenty fast growing colonies of each host legume, were selected. A series of jar experiments were executed and three most efficient isolates of each rhizobium species were screened on the basis of their growth promoting activities under axenic conditions. Results revealed that, in general, most of the isolates showed growth promoting effect but deleterious effect by some of the isolates was also observed on different parameters. The nine screened isolates were further evaluated for their growth promoting behavior in pots. The results indicated positive and increasing impact on growth and yield attributes by all the inoculants. However, in some parameters the increases were statistically same as with un-inoculated control. The isolates improved plant height (up to 18.66%), tillers per plant (up to 68.76%), straw yield (up to 35.14%), grain yield (up to 30.29%), 1000-grain weight (up to 28.40%), root length (up to 51.72%), % N in grains and straw (up to 15.07 and 33.16%), % P in grains and straw (up to 23.39 and 66.66%) and % K in grains and straw (up to 51.72 and 21.80%) compared with un-inoculated control. Finally, it is suggested that the procedure adopted for the selection of fast growing isolates and test of their growth promoting potential with wheat seedlings under axenic conditions could be an effective approach for screening the efficient and effective isolates. Also, the selected rhizobial strains could be used as PGPR for non-legumes.

Introduction

World's major source of food for human nutrition comes from the most important grain crops such as wheat, rice and maize. As the food demand is increasing day by day because of increasing world population, so the growers need to use additional nutrient inputs to increase their yields. Mineral fertilizers and farmyard manure are the traditional source of nutrients. Farmers are averse to use these resources due to their unaffordable prices and their related environment hazards. There are number of approaches among which the best approach is to improve the uptake of nutrients and their use efficiency which can be engineered via using beneficial microbes (deFreitas & Germida, 1990) as they are cost effective and environment friendly and can reduce the dependence on the synthetic resources and can enhance the crop yield.

Soil bacteria of the family *Rhizobiaceae* are called Rhizobia (Werner, 1992), which wraps an array of bacterial genera; containing *Rhizobium, Bradyrhizobium*, *Allorhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Azorhizobium*. From more than a century it has been known that growth of legumes can be promoted by rhizobia via formation of nitrogen-fixing nodules but the rhizobial interaction with non-legumes erstwhile ignored as an investigational system. Efforts on the interaction of rhizobia with non-legumes have increased during the previous couple of decades, and it has been established that roots of non-legumes could also be associated with rhizobia, devoid of effective nodule formation. Also, several mechanisms e.g., phytohormones production (Zahir *et al*., 2010), siderophore production (Meyer, 2000), enzymes generation (Yang & Hoffman,1984) and increased availability of insoluble phosphorus (Fatima *et al*., 2006; Pandey & Maheshwari, 2007) have been proposed by which *Rhizobia* can stimulate the growth of non-legumes directly and indirectly via suppressing or eliminating deleterious microbes by producing antibiotics (Antoun & Prevost, 2000), HCN (Antoun *et al*., 1978), and by producing exopolysaccharides (EPS) (Alami *et al*., 2000).

On the contrary, few investigations have explained neutral or deleterious effects of rhizobial inoculation on growth and yield of non-leguminous plants (El-Tarabily *et al*., 2006). Natural variations in environment, cultivar, soil and indigenous micro-flora of a specific area (Mehboob *et al*., 2008) are the major challenges in the use of bio-inoculants. So, selection of efficient and effective strains of rhizobia for specific cereal crop is a critical aspect. Hence a study has been conducted on the potential use of rhizobia as a plant growth promoter for increasing yield of wheat.

Materials and Methods

Isolation of rhizobial isolates: Several strains of rhizobia were isolated from chickpea (*Cicer arietinum* L.), mung bean (*Vigna radiata* L.) and lentil (*Lens culinaris* M.) root nodules. Host plants were uprooted from the field with some non-rhizospheric soil and brought to laboratory in polythene bags. Non-rhizosphere soil from the roots was removed by gentle shaking while rhizosphere soil was removed by dipping and gentle shaking in water. From the roots of each legume host, nodules were separated with sterilized razor blade and placed separately in Petri plates. The surface disinfection of nodules was achieved by dipping for 20 seconds in ethanol (95%) and then for three minutes in $HgCl_2$ (0.2%) solution followed by 6 washings with sterilized distilled water (Russell *et al*., 1982). Surface disinfected nodules of each host were crushed by means of sterilized glass rod in sterilized test tube containing sterilized distilled water. The suspension acquired was used to inoculate Petri plates having autoclaved and solidified yeast extract mannitol (YEM) media which was incubated at 28 ± 1^0C for bacterial growth. The procedure was repeated 3-4 times in order to get pure culture. A total of sixty fast growing colonies of rhizobia, twenty from each host crop, were selected. The isolated strains were coded (Table 1, Mehboob *et al*., 2008) and stored.

*Corresponding author: E-mail: zazahir@yahoo.com

Table 1. Coding of the isolated rhizobial strains (Mehboob *et al*., 2008).

S. No.	Mung bean isolates (*Rhizobium phaseoli*)	Lentil isolates (*Rhizobium leguminosarum*)	Chickpea isolates (*Mesorhizobium ciceri*)
1.	A_2	LSI_{14}	CRI_{19}
2.	A_3	LSI_{15}	CRI_{21}
3.	A_{13}	LSI_{16}	CRI_{22}
4.	A_{18}	LSI_{17}	CRI_{23}
5.	A_{22}	LSI_{18}	CRI_{24}
6.	A_{23}	LSI_{19}	CRI_{25}
7.	S_6	LSI_{20}	CRI_{26}
8.	S_9	LSI_{21}	CRI_{27}
9.	S_{17}	LSI_{22}	CRI_{28}
10.	S_{24}	LSI_{23}	CRI_{29}
11.	S_{25}	LSI_{24}	CRI_{30}
12.	S_{43}	LSI_{25}	CRI_{31}
13.	N_8	LSI_{26}	CRI_{32}
14.	N_{11}	LSI_{27}	CRI_{33}
15.	N_{12}	LSI_{28}	CRI_{34}
16.	N_{15}	LSI_{29}	CRI_{35}
17.	N_{16}	LSI_{30}	CRI_{36}
18.	N_{18}	LSI_{31}	CRI_{37}
19.	N_{41}	LSI_{32}	CRI_{38}
20.	N_{42}	LSI_{33}	CRI_{39}

Screening rhizobial isolates for growth promoting potential (Jar experiment): A series of experiments were conducted in the growth room under controlled conditions to screen rhizobial strains for their growth promoting potential by using wheat as test crop. Sterilized YEM broth was prepared and 50 mL were poured in 100 mL sterilized conical flasks and was inoculated with a selected rhizobial isolate. The flasks were incubated at 28 ± 1ºC for 72 h in a shaking incubator at 100 rpm. Surface disinfection of wheat seeds was done by momentarily dipping in ethanol (95%) and for 3 minutes in $HgCl_2$ (0.2%) solution followed by six rinses with sterilized water. Three seeds of each isolate were sandwiched between two sheets of sterilized filter paper soaked in suspension of the same rhizobial culture. The sheets were placed in sterilized jars after rolling as described by Asghar *et al*., (2004). In the case of control, sheets were soaked with sterilized broth. Hoagland half-strength solution (Hoagland & Arnon, 1950) was applied in jars to meet water and nutritional requirements of the seedlings. The jars were placed randomly following completely randomized design in growth room adjusted with 10 and 14 hour day and night period. After twenty days of germination, the seedlings were measured for root/shoot length, fresh and dry weight. The data regarding all the parameters for each treatment were first analyzed for percent increase over control and then all the parameters were given point score. The point score for all the six parameters of each individual treatment were cumulated and on behalf of the acquired point scores, the top three strains from each host group i.e. chickpea, lentil and mung bean (total of nine) were selected.

Preparation of inocula and seed inoculation: Inoculum preparation was completed by growing the selected isolates of rhizobia in 250 mL conical flask having 100 mL YEM broth by incubating at 28 ± 1^0C in the orbital shaking incubator at 100 rpm for three days. To attain uniform cell density (10^8 – 10^9 CFU mL^{-1}), an optical density of 0.5 recorded at a wavelength of 535 nm was achieved by dilution. Wheat seeds were inoculated by mixing with peat based slurry containing 3-day-old inoculum of respective isolate and sterilized sugar solution (10%) at 100 mL kg^{-1} sterilized peat whereas, the seeds for control were mixed with sterilized peat containing sterilized broth and sugar solution. Inoculated seeds were air dried under shade for 6-8 h.

Pot experiment: Pot study was conducted with sandy clay loam soil having pH, 7.8; EC_e, 2.3 dS m^{-1}; organic matter, 0.96%; total nitrogen, 0.06%; available phosphorus, 7.5 mg kg^{-1} and extractable potassium, 110 mg kg^{-1} in the wire house of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. The pots were filled with this soil at 12 kg pot^{-1}. Seeds of wheat (*Triticum aestivum*) cv. Seher after inoculation according to the treatment plan were introduced in pots at 5 seed pot^{-1}. The pots were arranged randomly and each treatment was replicated thrice according to completely randomized design. Thinning was done after two weeks of germination to retain two seedlings in each pot. Pots were supplied with NPK at 120, 90, 60 kg ha^{-1} (using urea, DAP, and MOP as source of NPK). The whole PK was supplemented as basal dose whereas N was added in three splits at sowing, tillering and booting stage. All the pots were soaked with canal water. The information concerning growth and yield attributes were recorded at maturity. Nitrogen, phosphorus and potassium contents in grain and straw samples were determined.

Characterization of the selected isolates: The selected isolates were characterized for auxin production which was determined by colorimeter method according to the procedure described by Sarwar *et al*., (1992), phosphate solubilization by using National Botanical Research Institute, Rana Pratap Marg (NBRI-RPM) medium as described by Mehta & Nautiyal (2001), root colonization as described by Simons *et al*., (1996), chitinase activity according to the method described by Chernin *et al*., (1998), EPS production by growing the isolates on RCV mineral media enriched with mannitol, sucrose and with or without NaCl (Ashraf *et al*., 2004) and production of EPS was assessed visually, and for siderophore production the procedure described by Schwyn & Neilands (1987) was followed.

Statistical analysis: Data were subjected to statistical analysis by following CRD using standard procedure (Steel *et al*., 1997). The differences among the treatment means were compared by applying the Duncan's multiple range tests (DMR) (Duncan, 1955).

Results

According to the study plan, a total of sixty fast growing colonies of rhizobia isolated from root nodules, twenty from each host crop i.e. mung bean, chickpea and lentil were screened for their ability to prop up growth of wheat seedlings under controlled conditions. Three most efficient isolates from each group were chosen, characterized and further assessed for their growth promoting activities in pots using wheat as test crop. The results of the experiments are described below:

Jar experiments

***Rhizobium phaseoli*:** Different mung bean isolates had variable effects on root length of wheat seedlings whereas; the results concerning shoot length showed increase by every isolate in comparison with the un-inoculated control (Table 2). Sixteen isolates enhanced the root length up to 59.52% while rest of the isolates reduced it compared with un-inoculated control. In case of shoot length, the isolate N_{16} proved to be the most promising while N_{11} and A_{23} remained the least effective but still caused an increase of 7.25% over un-inoculated control.

Concerning root and shoot fresh weight, the inoculation exhibited both positive and negative effects (Table 2). Twelve isolates improved the fresh weight of roots and shoots up to 48.36 and 78.44%, respectively whereas, eight isolates such as A_3, A_{18}, S_9, S_{24}, S_{25}, N_{11}, N_{18}, N_{42} and A_3, A_{23}, S_6, S_{17}, S_{25}, N_{11}, N_{15}, N_{41} caused a reduction up to 41.80 and 34.90% in root and shoot fresh weight, respectively in comparison with un-inoculated control. The isolates N_{18} and N_{41} proved to be the most deleterious by causing maximum reduction in root and shoot fresh weight, respectively.

Likewise, both positive and negative effects of inoculation were also recorded on root and shoot dry weight of wheat seedlings (Table 2). Half of the isolates yielded positive response while the remaining half affected the root dry weight negatively whereas in case of shoot dry weight, thirteen isolates improved the shoot dry weight of wheat seedlings efficiently while seven reduced it compared with un-inoculated control. Maximum of 51.76 and 82.35% increase in dry weight of root and shoot was obtained with the isolates N_{16} and S_{43}, respectively.

***Mesorhizobium ciceri*:** Potential of different chickpea rhizobial isolates (*Mesorhizobium ciceri*) for improving root and shoot length of wheat seedlings varied among each other (Table 3). Out of twenty, 12 isolates (60%) increased root and shoot length up to 47.69% while the rest of the eight decreased root and shoot length up to 24.43% except the isolate CRI_{24} which exhibited no effect on shoot length with respect to un-inoculated control.

In case of root and shoot fresh weight, majority of the chickpea rhizobial isolates affected the root and shoot fresh weight positively yet three isolates in case of root fresh weight (i.e. CRI_{32}, CRI_{33} and CRI_{36}) and eight isolates in case of shoot fresh weight (i.e. CRI_{22}, CRI_{25}, CRI_{27}, CRI_{28}, CRI_{29}, CRI_{32}, CRI_{33} and CRI_{35}) gave negative response in comparison to un-inoculated control (Table 3). Maximum increases up to 172.71% by CRI_{38} and 27.18% by CRI_{31} in root and shoot fresh weight were recorded by inoculation with isolate CRI_{38} and $CRI_{31,}$ respectively.

Similarly, effect of inoculation on dry weight of root and shoot exhibited similar trend as of fresh weight of root that large number of isolates improved the root and shoot dry weight and lesser number caused reduction in these parameters compared with un-inoculated control (Table 3). The isolates CRI_{38} and CRI_{31} proved to be the most efficient, which caused maximum increases of 160.81 and 26.76% in dry weight of root and shoot, respectively. The isolate CRI_{33} and CRI_{35} remained the most deleterious which suppressed the dry weight of root and shoot by 45.50 and 56.18%, respectively, in comparison with un-inoculated control.

***Rhizobium leguminosarum*:** Both negative and positive results regarding root length of wheat seedlings were produced by inoculation with the *R. leguminosarum* while improvement in shoot length was recorded by the entire twenty rhizobial isolates under controlled conditions (Table 4). Root length of wheat seedlings was enhanced by 80% of the isolates, which ranged from 7.14 to 59.52% while the remaining 20% isolates decreased the root length compared with un-inoculated control. Highest increase of 59.52% in root length was produced by the isolate LSI_{15} whereas maximum enhancement of 40% in shoot length was given by the isolate $LSI_{30.}$

Upon inoculation, different isolates of *R. leguminosarum* affected root and shoot fresh weight of wheat seedlings with different degree of efficacy; however, a greater number (60%) of isolates proved their potential for improving the root fresh weight while less number (40%) of isolates exhibited deleterious effects (Table 4). The positive response observed in root and shoot fresh weight was up to 48.36 and 52.51% increase, while negative response up to 37.70 and 34.90% decrease, respectively, was recorded compared with the un-inoculated control.

Greater number of isolates improved the dry weight of root and shoot of wheat seedlings while some isolates reduced them when compared with the un-inoculated control (Table 4). Eleven isolates i.e. LSI_{14}, LSI_{16}, LSI_{18}, LSI_{19}, LSI_{20}, LSI_{22}, LSI_{23}, LSI_{26}, LSI_{28}, LSI_{30} and LSI_{32} were the promoters of dry weight of root and improved the same up to 51.76% compared with un-inoculated control whereas the remaining nine isolates i.e. LSI_{15}, LSI_{17}, LSI_{21}, LSI_{24}, LSI_{25}, LSI_{27}, LSI_{29}, LSI_{31} and LSI_{33} remained deleterious and suppressed the root dry weight up to 43.09% in comparison with un-inoculated control. But in case of shoot dry weight, thirteen isolates stimulated the shoot dry weight positively and increased it up to 63.14% while only seven isolates caused reduction up to 31.37% compared to un-inoculated control.

Pot experiment: Nine most efficient isolates (three each from mung bean (N_8, $N_{16,}$ $S_{43,}$), chickpea (CRI_{31}, CRI_{37}, CRI_{38}) and lentil (LSI_{19}, LSI_{23}, LSI_{30}) crop were chosen on the basis of their growth promoting results in jar experiments conducted under axenic conditions and were further assessed for their capability to augment the growth and yield of wheat plants grown in pots. The results from pot experiment are described below:

Influence of inoculation on the height of wheat plants by all the rhizobial isolates was found significantly higher when compared with the un-inoculated control (Fig. 1). Maximum height of 59.33 cm was attained by the wheat plants where the isolate CRI_{38} was used as inoculant in relation to un-inoculated control. Least increase of 8.0% in wheat plant height over un-inoculated control was recorded with the isolate LSI_{19}.

Table 2. Effect of inoculation with *Rhizobium phaseoli* on the growth parameters of wheat seedlings under axenic conditions.

Strain	(Average of 3 repeats ± SE)					
	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	14.00 ± 0.58	23.00 ± 0.58	0.2440 ± 0.016	0.3497 ± 0.0224	0.0123 ± 0.0008	0.0170 ± 0.0006
A_2	16.67 ± 0.67	29.00 ± 0.58	0.2483 ± 0.017	0.4220 ± 0.0106	0.0122 ± 0.0009	0.0210 ± 0.0005
A_3	22.33 ± 0.88	29.67 ± 0.88	0.2333 ± 0.010	0.2770 ± 0.0144	0.0113 ± 0.0004	0.0140 ± 0.0007
A_{13}	20.08 ± 0.79	32.00 ± 0.58	0.2970 ± 0.009	0.4267 ± 0.0156	0.0153 ± 0.0004	0.0213 ± 0.0010
A_{18}	20.67 ± 0.67	29.33 ± 0.33	0.2083 ± 0.009	0.3657 ± 0.0190	0.0103 ± 0.0005	0.0183 ± 0.0010
A_{22}	19.50 ± 0.87	31.67 ± 0.88	0.2997 ± 0.006	0.4173 ± 0.0298	0.0173 ± 0.0010	0.0220 ± 0.0005
A_{23}	15.00 ± 0.58	24.67 ± 0.88	0.2473 ± 0.021	0.3110 ± 0.0115	0.0127 ± 0.0009	0.0157 ± 0.0010
S_6	15.00 ± 0.58	27.33 ± 0.88	0.3497 ± 0.016	0.3063 ± 0.0135	0.0147 ± 0.0009	0.0153 ± 0.0010
S_9	8.67 ± 0.88	30.00 ± 0.58	0.2203 ± 0.012	0.3970 ± 0.0139	0.0110 ± 0.0006	0.0200 ± 0.0010
S_{17}	18.67 ± 0.67	27.33 ± 0.88	0.2597 ± 0.017	0.3013 ± 0.0144	0.0130 ± 0.0007	0.0150 ± 0.0009
S_{24}	13.67 ± 0.67	28.33 ± 0.88	0.1943 ± 0.009	0.4183 ± 0.0176	0.0097 ± 0.0003	0.0210 ± 0.0006
S_{25}	18.67 ± 0.67	26.67 ± 0.67	0.1333 ± 0.003	0.3217 ± 0.0197	0.0067 ± 0.0003	0.0163 ± 0.0010
S_{43}	20.33 ± 0.67	31.33 ± 0.88	0.2987 ± 0.011	0.6240 ± 0.0217	0.0153 ± 0.0005	0.0310 ± 0.0009
N_8	21.00 ± 0.58	30.67 ± 0.88	0.3287 ± 0.014	0.4123 ± 0.0195	0.0167 ± 0.0012	0.0215 ± 0.0010
N_{11}	15.67 ± 0.67	24.67 ± 0.33	0.1797 ± 0.011	0.3157 ± 0.0137	0.0090 ± 0.0007	0.0157 ± 0.0011
N_{12}	13.67 ± 0.67	30.33 ± 0.88	0.2847 ± 0.010	0.3647 ± 0.0214	0.0143 ± 0.0009	0.0183 ± 0.0010
N_{15}	16.33 ± 0.88	27.67 ± 1.20	0.2660 ± 0.015	0.3413 ± 0.0206	0.0103 ± 0.0003	0.0173 ± 0.0011
N_{16}	19.00 ± 0.58	32.67 ± 0.33	0.3620 ± 0.015	0.5070 ± 0.0220	0.0187 ± 0.0011	0.0253 ± 0.0009
N_{18}	17.33 ± 0.33	30.00 ± 0.58	0.1420 ± 0.011	0.3793 ± 0.0184	0.0070 ± 0.0007	0.0190 ± 0.0011
N_{41}	17.33 ± 0.88	28.00 ± 1.15	0.3040 ± 0.010	0.2277 ± 0.0188	0.0150 ± 0.0008	0.0117 ± 0.0008
N_{42}	11.33 ± 0.67	26.00 ± 0.58	0.2267 ± 0.012	0.4013 ± 0.0142	0.0117 ± 0.0004	0.0203 ± 0.0013

Table 3. Effect of inoculation with *Mesorhizobium cicerii* on the growth parameters of wheat seedlings under axenic conditions.

Strain	(Average of 3 repeats ± SE)					
	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	14.00 ± 0.58	23.00 ± 0.58	0.2440 ± 0.016	0.3497 ± 0.0224	0.0123 ± 0.0008	0.0170 ± 0.0006
CRI-19	18.83 ± 0.60	29.33 ± 1.20	0.2391 ± 0.007	0.4394 ± 0.0102	0.0133 ± 0.0006	0.0219 ± 0.0006
CRI-21	19.67 ± 0.88	29.67 ± 1.45	0.2227 ± 0.013	0.4330 ± 0.0076	0.0107 ± 0.0005	0.0183 ± 0.0007
CRI-22	18.33 ± 0.67	27.50 ± 0.76	0.2100 ± 0.009	0.3860 ± 0.0061	0.0107 ± 0.0009	0.0225 ± 0.0008
CRI-23	15.00 ± 0.58	29.33 ± 1.45	0.1500 ± 0.017	0.4540 ± 0.0172	0.0077 ± 0.0007	0.0227 ± 0.0008
CRI-24	16.00 ± 0.58	28.67 ± 0.88	0.2240 ± 0.009	0.4640 ± 0.0202	0.0113 ± 0.0004	0.0243 ± 0.0010
CRI-25	16.33 ± 0.88	25.67 ± 1.45	0.1540 ± 0.009	0.3343 ± 0.0035	0.0076 ± 0.0006	0.0157 ± 0.0008
CRI-26	22.83 ± 0.73	31.17 ± 0.73	0.3243 ± 0.013	0.4897 ± 0.0085	0.0163 ± 0.0009	0.0233 ± 0.0010
CRI-27	16.33 ± 0.88	28.33 ± 0.33	0.1497 ± 0.011	0.2880 ± 0.0129	0.0077 ± 0.0006	0.0143 ± 0.0009
CRI-28	17.67 ± 0.88	32.00 ± 1.00	0.1777 ± 0.009	0.4267 ± 0.0148	0.0090 ± 0.0006	0.0217 ± 0.0008
CRI-29	20.33 ± 0.88	27.33 ± 0.88	0.2427 ± 0.005	0.2357 ± 0.0066	0.0137 ± 0.0007	0.0188 ± 0.0002
CRI-30	15.33 ± 0.88	30.33 ± 0.33	0.2217 ± 0.010	0.4397 ± 0.0224	0.0110 ± 0.0006	0.0220 ± 0.0010
CRI-31	23.00 ± 1.15	32.00 ± 0.58	0.3550 ± 0.015	0.5427 ± 0.0227	0.0180 ± 0.0006	0.0270 ± 0.0011
CRI-32	20.33 ± 0.88	27.00 ± 0.58	0.1360 ± 0.015	0.3360 ± 0.0236	0.0067 ± 0.0007	0.0167 ± 0.0008
CRI-33	17.33 ± 0.88	21.67 ± 1.20	0.0787 ± 0.006	0.2243 ± 0.0072	0.0040 ± 0.0004	0.0137 ± 0.0022
CRI-34	26.33 ± 0.88	31.50 ± 0.76	0.2527 ± 0.015	0.4477 ± 0.0148	0.0126 ± 0.0004	0.0238 ± 0.0014
CRI-35	18.00 ± 1.15	31.33 ± 0.88	0.2357 ± 0.020	0.1933 ± 0.0090	0.0117 ± 0.0006	0.0093 ± 0.0006
CRI-36	14.33 ± 0.88	27.00 ± 1.73	0.1350 ± 0.016	0.4300 ± 0.0049	0.0067 ± 0.0006	0.0197 ± 0.0013
CRI-37	22.67 ± 0.88	33.33 ± 0.67	0.2940 ± 0.010	0.4653 ± 0.0491	0.0147 ± 0.0009	0.0253 ± 0.0025
CRI-38	22.33 ± 1.20	31.58 ± 0.51	0.4063 ± 0.008	0.4787 ± 0.0217	0.0193 ± 0.0004	0.0260 ± 0.0021
CRI-39	20.67 ± 1.20	29.00 ± 1.00	0.2403 ± 0.006	0.4370 ± 0.0133	0.0130 ± 0.0007	0.0187 ± 0.0013

Table 4. Effect of *Rhizobium leguminosarum* on the growth parameters of wheat seedlings under axenic conditions.

Strain	(Average of 3 repeats ± SE)					
	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	14.00 ± 0.58	23.33 ± 0.88	0.244 ± 0.005	0.3497 ± 0.0052	0.0123 ± 0.0008	0.0170 ± 0.0001
LSI-14	16.67 ± 0.88	29.00 ± 1.15	0.248 ± 0.017	0.4220 ± 0.0057	0.0126 ± 0.0007	0.0200 ± 0.0005
LSI-15	22.33 ± 0.88	29.67 ± 0.88	0.233 ± 0.004	0.2770 ± 0.0040	0.0113 ± 0.0004	0.0146 ± 0.0005
LSI-16	20.08 ± 0.79	24.67 ± 1.15	0.343 ± 0.006	0.4267 ± 0.0043	0.0150 ± 0.0008	0.0215 ± 0.0003
LSI-17	20.67 ± 0.67	29.33 ± 0.88	0.208 ± 0.005	0.3657 ± 0.0032	0.0103 ± 0.0005	0.0183 ± 0.0003
LSI-18	19.50 ± 0.87	31.67 ± 0.88	0.300 ± 0.006	0.4173 ± 0.0023	0.0173 ± 0.0010	0.0210 ± 0.0006
LSI-19	20.00 ± 0.58	32.00 ± 0.88	0.247 ± 0.004	0.3110 ± 0.0058	0.0127 ± 0.0009	0.0177 ± 0.0004
LSI-20	15.00 ± 0.58	27.33 ± 0.88	0.273 ± 0.005	0.3063 ± 0.0052	0.0147 ± 0.0008	0.0153 ± 0.0004
LSI-21	8.67 ± 0.88	30.00 ± 0.58	0.220 ± 0.002	0.3970 ± 0.0081	0.0110 ± 0.0006	0.0200 ± 0.0005
LSI-22	18.67 ± 0.88	27.00 ± 0.58	0.297 ± 0.009	0.3013 ± 0.0029	0.0133 ± 0.0004	0.0150 ± 0.0007
LSI-23	18.67 ± 0.67	28.33 ± 0.88	0.294 ± 0.009	0.5333 ± 0.0058	0.0197 ± 0.0003	0.0207 ± 0.0009
LSI-24	18.67 ± 0.88	26.67 ± 0.88	0.198 ± 0.010	0.3237 ± 0.0078	0.0100 ± 0.0035	0.0163 ± 0.0004
LSI-25	20.33 ± 0.67	31.33 ± 0.88	0.299 ± 0.006	0.4177 ± 0.0023	0.0150 ± 0.0008	0.0277 ± 0.0004
LSI-26	21.00 ± 1.00	30.67 ± 1.45	0.329 ± 0.005	0.4210 ± 0.0053	0.0167 ± 0.0012	0.0213 ± 0.0002
LSI-27	15.67 ± 0.67	24.67 ± 0.88	0.180 ± 0.006	0.3197 ± 0.0058	0.0090 ± 0.0007	0.0157 ± 0.0011
LSI-28	13.67 ± 0.67	30.33 ± 0.88	0.285 ± 0.004	0.3717 ± 0.0079	0.0143 ± 0.0009	0.0183 ± 0.0010
LSI-29	16.33 ± 0.88	27.67 ± 0.88	0.266 ± 0.004	0.3373 ± 0.0086	0.0103 ± 0.0003	0.0173 ± 0.0011
LSI-30	19.00 ± 0.58	32.67 ± 0.33	0.362 ± 0.004	0.5070 ± 0.0050	0.0187 ± 0.0011	0.0254 ± 0.0005
LSI-31	17.33 ± 0.33	30.00 ± 1.15	0.152 ± 0.006	0.3793 ± 0.0072	0.0070 ± 0.0007	0.0190 ± 0.0011
LSI-32	17.33 ± 0.88	28.00 ± 1.15	0.304 ± 0.005	0.2277 ± 0.0052	0.0143 ± 0.0004	0.0117 ± 0.0004
LSI-33	11.33 ± 0.67	26.00 ± 0.58	0.227 ± 0.006	0.4113 ± 0.0045	0.0117 ± 0.0004	0.0203 ± 0.0005

Results pertaining to the average number of tillers per plant presented in Figure 2 indicated significant increase by all the isolates except LSI_{19} and LSI_{30} which had non-significant effect when compared with un-inoculated control. Among the isolates, CRI_{37} was at the top which yielded 68.76% more tillers per plant compared with un-inoculated control. The isolate LSI_{19} remained at the bottom when its effect on number of tillers per plant (18.8% increases over un-inoculated control) was evaluated.

The values for grain yield presented in Figure 3 revealed that all the tested isolates had the capability to increase the grain yield of wheat significantly in comparison with un-inoculated control. Overall, the increase in grain yield as result of inoculation with all the rhizobial isolates ranged from 7.81 to 30.29% over un-inoculated control.

In case of straw yield, out of nine, six isolates increased the straw yield significantly while the effects of the remaining three isolates were statistically non significant (Fig. 4). The isolate S_{43} emerged as highest producer of wheat straw yield (35.14% more over un-inoculated control). On the other hand, isolates LSI_{19}, LSI_{30} and LSI_{23} although improved the straw yield by 9.00, 11.33 and 16.25%, respectively, over un-inoculated control yet the improvement was statistically non-significant compared with un-inoculated control.

Positive significant effect on 1000 grains weight in comparison with un-inoculated control was recorded with different rhizobial isolates in the pot trial (Fig. 5). Overall, all the tested isolates augmented the 1000 grains weight of wheat ranging from 10.12 to 28.40% over un-inoculated control. The most promising isolate was CRI_{38} which gave 28.40% more 1000 grain weight of wheat over un-inoculated control.

Improvement in root length obtained as a result of inoculation with the test isolates is presented in Figure 6. Greatest root length (19.83 cm) was recorded due to inoculation with the isolate CRI_{38} which was 51.72% greater than un-inoculated control and differed significantly from the un-inoculated control. The lowest of 16.07% boost in root length was recorded with the isolate LSI_{23} compared with un-inoculated control.

Percentage of NPK in wheat grains and straw revealed that although the results of some of the isolates remained non significant in comparison with control yet overall there was an increase in N, P and K % in relation to un-inoculated control (Table 5). The range of increase in N, P and K % was from 4.07 to 66.66% produced by the rhizobial inoculants in comparison with un-inoculated control.

Characterization of the selected isolates: Characterization of the selected wheat isolates showed variable ability in colonization of wheat roots and in the production of IAA (Table 6). The isolate N_{16} possessed highest ability to produce IAA (6.53 and 31.34 mg L^{-1} in the absence and presence of L-Tryptophan, respectively) and colonized wheat roots (4.8×10^4 cfu g^{-1}) compared to the other isolates. Greater number of isolates (CRI_{37}, LSI_{19}, LSI_{30}, N_8 and N_{16}) had chitinase activity while few (CRI_{31}, CRI_{38}, LSI_{23} and S_{43}) showed absence of this activity. Similarly, majority of the isolates exhibited phosphate solubilization ability except the isolate LSI_{30}, N_8 and S_{43}, siderophore production ability except the isolates CRI_{38}, LSI_{19}, LSI_{23} & N_{16} and exopolysaccharide production ability except the isolates CRI_{38}, LSI_{30} and N_8. In general, characterization of the rhizobial isolates selected for wheat crop reflected that all have multiple characteristics and were able to promote the growth and development of wheat plants upon inoculation.

Table 5. Effect of inoculation with different species of rhizobia on N, P and K% in grains and straw of wheat plants grown in pots.

Strain	(Average of 3 repeats)					
	% N in grains	% N in straw	% P in grains	% P in straw	% K in grains	% K in straw
Control	1.573 D	0.633 F	1.043 C	0.410 E	0.387 E	1.303 C
CRI_{31}	1.703 B	0.843A	1.153 B	0.583 BC	0.510 BC	1.453 B
CRI_{37}	1.787 A	0.763 BC	1.247 A	0.507 CD	0.443 DE	1.587 A
CRI_{38}	1.687 BC	0.693 DE	1.167 B	0.683 A	0.587 A	1.467 B
LSI_{19}	1.637 C	0.477 CD	1.153 B	0.477 DE	0.467 CD	1.453 B
LSI_{23}	1.660 BC	0.720 CDE	1.130 B	0.447 DE	0.420 DE	1.430 B
LSI_{30}	1.660 BC	0.687 EF	1.143 B	0.483 DE	0.440 DE	1.443 B
N_8	1.690 BC	0.750 CD	1.170 B	0.607 B	0.527 B	1.470 B
N_{16}	1.810 A	0.807 AB	1.287 A	0.487 DE	0.423 DE	1.557 A
S_{43}	1.760 A	0.837 A	1.257A	0.523 CD	0.473 BCD	1.547 A

Means sharing the same letter (s) do not differ significantly at $p \leq 0.05$ according to Duncan's Multiple Range (DMR) Test.

Table 6. Characterization of the selected rhizobial isolates.

Strain	Root colonization (cfu g^{-1})	Chitinase activity	Phosphate solubilization	Siderophore production	Exopoly-saccharide production	IAA production (mg L^{-1})	
						(without L-Tryptophan)	(with L-Tryptophan)
CRI_{31}	2.7 x 10^4	-	+ ve	+ ve	+ ve	1.41	8.24
CRI_{37}	2.8 x 10^4	+ ve	+ ve	+ ve	+ ve	0.92	4.02
CRI_{38}	2.3 x 10^4	-	+ ve	-	-	3.18	7.41
LSI_{19}	4.2 x 10^4	+ ve	+ ve	-	+ ve	1.17	12.67
LSI_{23}	4.0 x 10^4	-	+ ve	-	+ ve	0.75	4.02
LSI_{30}	4.5 x 10^4	+ ve	-	+ ve	-	1.19	24.11
N_8	4.7 x 10^4	+ ve	-	+ ve	-	4.18	21.45
N_{16}	4.8 x 10^4	+ ve	+ ve	-	+ ve	6.53	31.34
S_{43}	4.0 x 10^4	-	-	+ ve	+ ve	5.36	29.59

Note: The isolates CRI_{31}, CRI_{37} and CRI_{38} are of *Mesorhizobium ciceri sp*., LSI_{19}, $LSI_{23}$and LSI_{30} are of *R. leguminosarum sp*. and N_8, N_{16} and S_{43} are of *R. phaseoli* sp.

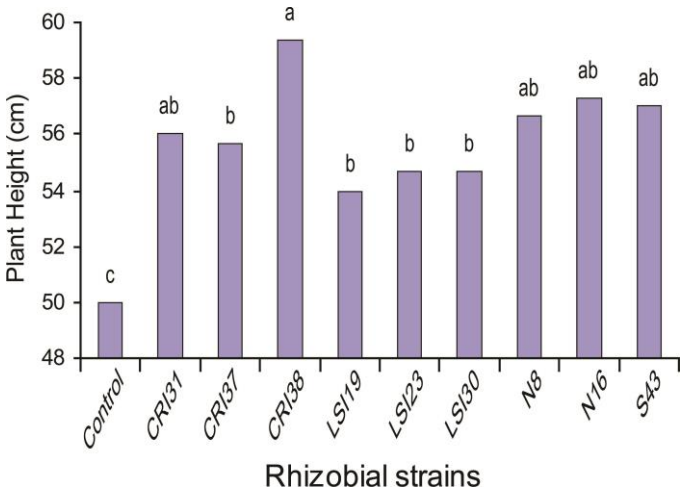
Discussion

Results of jar experiments revealed that, in general, most of the isolates showed growth promoting effects in wheat; may be because of one or more growth promoting mechanisms which may imply that the ability of rhizobia to produce different metabolites like phytohormones, organic acids, siderophores, enzymes and exopolysaccharides in the rhizosphere could be responsible for evoking the growth stimulating response in the inoculated non-leguminous plants. Similar to our findings, Biswas *et al*., (2000) found increased level of IAA in the peripheral rice rooting medium cultivated with rhizobia gnotobiotically and reported that the phytohormone IAA produced by the rhizobium may be a contributing factor for observed growth promotion of the inoculated rice plants, under gnotobiotic conditions. Moreover, Peix *et al*., (2001) assessed the effectiveness of *Mesorhizobium mediterraneum* in a growth chamber to improve the barley growth and demonstrated a considerable improvement in dry matter of barley plants by PECA21 inoculation. Even so, other mechanisms by means of which rhizobia affect the growth of plant can not be neglected.

But deleterious effects by some of the isolates on different parameters might be due to the excessive production of compounds that are useful to plant growth in small quantity, like IAA and similar substances (Antoun *et al*., 1998). Deleterious effects because of the production of high concentration of HCN have also been documented by Alstrom & Burn (1989). Moreover, El-Tarabily *et al*., (2006) reported that the inhibition of growth by rhizobial inoculation of non-legumes could also be connected to the generation of growth inhibitors by the rhizobial strain used.

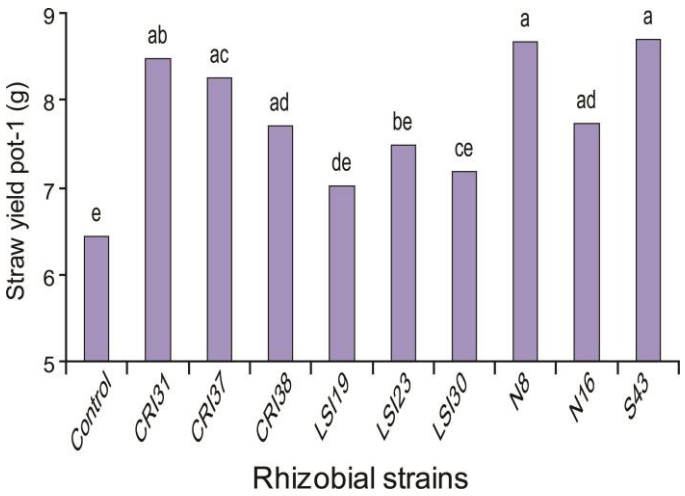
The results of the axenic experiments also indicated that the effectiveness of the isolates varied among the species against a common host which may be due to difference in their characteristics especially in root colonization ability with common host as well as with different host, which is evident from our results regarding characterization of the selected wheat isolates (Table 6). Moreover, the difference in the efficacy of the isolates could be because of difference in their natural potential. The findings of present study are supported by the results of Piesterse *et al*., (2001) who have also described differential behavior of different plant growth promoting rhizobial strains against common host.

It was observed from the pot experiments that the selected isolates of rhizobia had positive and increasing impact on growth and yield attributes of wheat crop however; in some parameters the increases caused by few isolates were statistically same as of un-inoculated control. These improvements in growth and yield attributes might be a result of one or more mechanisms of action the inoculated isolates possessed which is evident from their characterization data presented in table 6. Moreover, comparable outcomes have been explained by a number of workers who studied rhizobial activities with non-legumes (Matiru & Dakora, 2004; Mehboob *et al*., 2008).



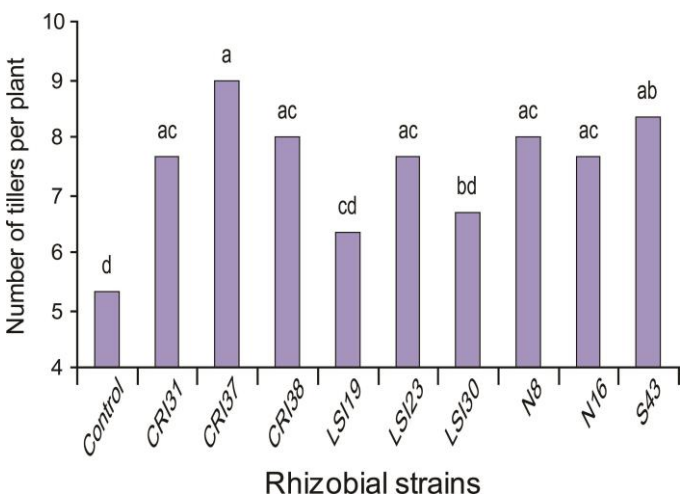
Bars containing similar letter (s) do not differ significantly at p≤0.05 according to DMR Test

Fig. 1. Effect of inoculation with different species of rhizobia on plant height of wheat plants grown in pots.



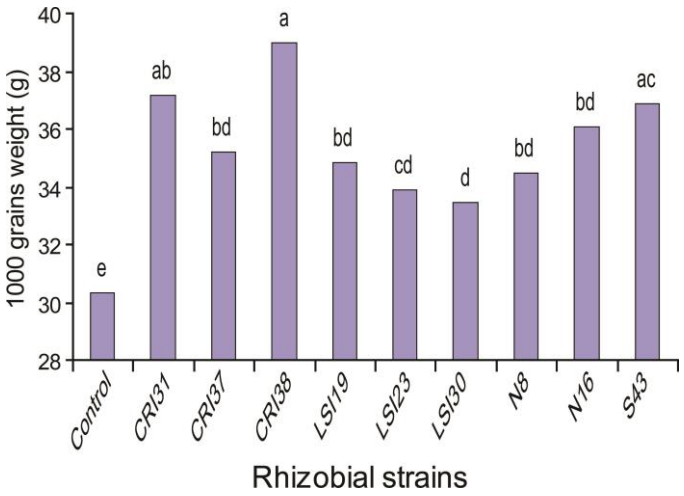
Bars containing similar letter (s) do not differ significantly at p≤0.05 according to DMR Test

Fig. 2. Effect of inoculation with different species of rhizobia on number of tillers per plant of wheat plants grown in pots.



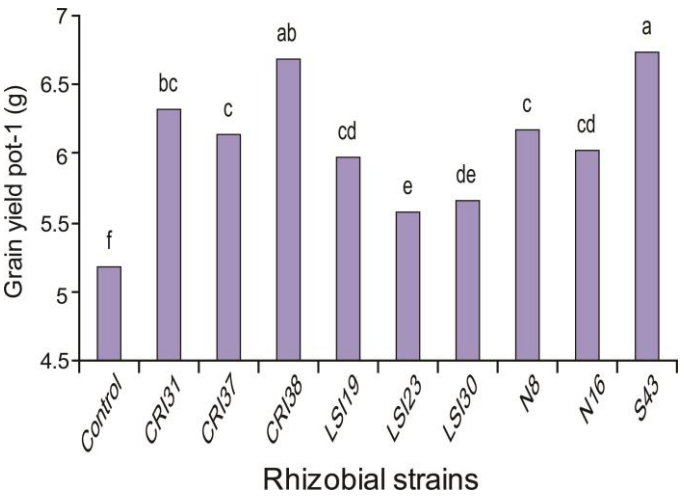
Bars containing similar letter (s) do not differ significantly at p≤0.05 according to DMR Test

Fig. 3. Effect of inoculation with different species of rhizobia on grain yield of wheat plants grown in pots.



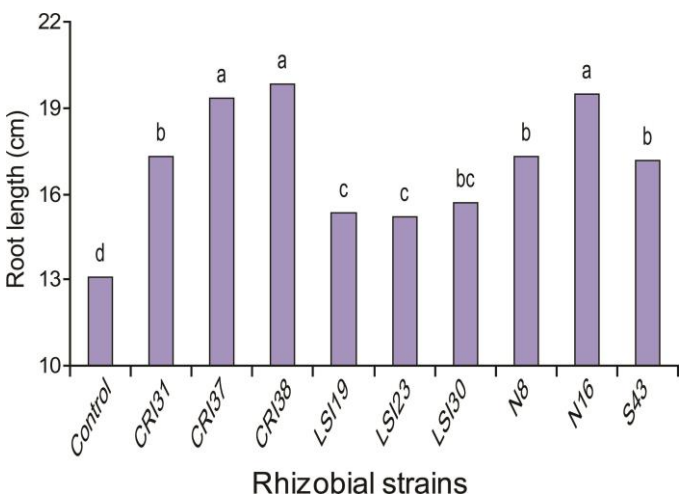
Bars containing similar letter (s) do not differ significantly at p ≤ 0.05 according to DMR Test

Fig. 4.. Effect of inoculation with different species of rhizobia on straw yield of wheat plants grown in pots.



Bars containing similar letter (s) do not differ significantly at p≤0.05 according to DMR Test

Fig. 5. Effect of inoculation with different species of rhizobia on 1000 grain weight of wheat plants grown in pots.



Bars containing similar letter (s) do not differ significantly at p≤0.05 according to DMR Test

Fig. 6. Effect of inoculation with different species of rhizobia on root length of wheat plants grown in pots.

In pot experiment, the selected isolates improved plant height (up to 18.66%), tillers per plant (up to 68.76%), straw yield (up to 35.14%), grain yield (up to 30.29%), 1000-grain weight (up to 28.40%), root length (up to 51.72%), %N in grains and straw (up to 15.07 and 33.16%), %P in grains and straw (up to 23.39 and 66.66%) and %K in grains and straw (up to 51.72 and 21.80%) compared with un-inoculated control. These results are supported by many greenhouse/pot studies which showed similar increases in different attributes of growth and yield of various non-legumes inoculated with different rhizobial strains by various researchers such as Singh *et al*., (2006) who applied three strains of rhizobia to rice and demonstrated increased plant height (5.62%), dry weight of root (20.91%), dry weight of shoot (19.95%), 1000-grain yield (4.85%), N in straw (17.06%), and P in straw (27.49%) compared to un-inoculated control. Similar to our findings, Hoflich (2000) also noted shoot growth of wheat and maize promoted by about 19-33% via inoculation with R39 (a *Rhizobium leguminosarum bv. trifolii* strain).

Conclusively, on the basis of the results of axenic study, it is suggested that the procedure adopted for the selection of fast growing isolates, acquired from the nodules of three local legumes and test of their growth promoting potential with wheat seedlings under axenic conditions could be an effective approach for screening the efficient and effective isolates. Also, further evaluation in pots of the selected isolates suggests that all were effective although their efficacy varied among the species. Moreover, characterization data revealed that although all the isolates were having more than one mechanism of action yet the isolates of *R. phaseoli* superseded the rest two species in root colonization, auxin production and in maximum grain yield induction.

Acknowledgement

We are thankful to the Higher Education Commission (HEC), Pakistan for funding this research project. Funding was provided by HEC under Indigenous 5000 Ph.D Fellowship phase-III scheme.

References

Alstrom, S. and R.G. Burns. 1989. Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. *Biol. Fertil. Soils*, 7: 232-238.

Antoun, H. and D. Prevost. 2000. PGPR activity of *Rhizobium* with nonleguminous plants. Available on :http://www.ag.auburn.edu/argentina/pdfmanuscripts/tableofcontents. pdf. (Assessed on: 30-09-2010).

Antoun, H., C.J. Beauchamp, N. Goussard, R. Chabot and R. Lalande. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). *Plant Soil*, 4: 57-68.

Asghar, H.N., Z.A. Zahir and M. Arshad. 2004. Screening rhizobacteria for improving growth, yield and oil contents of canola (*Brassica napus* L.). *Aust. J. Agric. Res*., 55: 87-194.

Ashraf, M., S.H. Berge and O.T. Mahmood. 2004. Inoculating wheat seedling with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biol. Fertil. Soils*, 40: 57-162.

Biswas, J.C., J.K. Ladha and F.B. Dazzo. 2000. Rhizobial inoculation improves nutrient uptake and growth of lowland rice. *Soil Sci. Soc. Am. J.*, 64: 1644-1650.

Chernin, L.S., M.K. Winson, J.M. Thompson, S. Haran, B.W. Bycroft, I. Chet, P. Williams and G.S.A.B. Stewart. 1998. Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by Quorum sensing. *J. Bacteriol.*, 180: 4435-4441.

de Freitas, J.R. and J.J. Germida. 1990. Plant growth promoting rhizobacteria for winter wheat. *Can. J. Microbiol.*, 36: 265-272.

Duncan, D.B. 1955. Multiple range and multiple F-test. *Biometrics*, 11: 1-42.

El-Tarabily, K.A., A.A. Soaud, M.E. Saleh and S. Matsumoto. 2006. Isolation and characterization of sulfer-oxidising bacteria, including strains of *Rhizobium* from calcareous sandy soils and their effects on nutrient uptake and growth of maize (*Zea mays* L.). *Aust. J. Agric. Res.*, 57: 101-111.

Fatima, Z., M. Zia and M.F. Chaudhary. 2006. Effect of Rhizobium strains and phosphorus on growth of soybean (G*lycine max*) and survival of Rhizobium and P solubilizing bacteria. *Pak. J. Bot*., 38: 459-464.

Hoagland, D.R. and D. Arnon. 1950. The water culture methods for growing plants without soil. Calif. Agric. *Exp. Sta. Circ.*, No. 347. pp. 39

Hoflich, G. 2000. Colonization and growth promotion of non-legumes by *Rhizobium* bacteria. p. 827-830. In: *Proceedings of the 8th International Symposium on Microbial Ecology. Microbial Biosystems: New Frontier*. C.R. Bell, M. Brylinsky and P. Johnson-Green (Eds.), Atlantic Canada Soc. Microbial Ecol., Aug. 9-14, 1998, Halifax, Canada.

Matiru, V.N. and F.D. Dakora. 2004. Potential use of rhizobial bacteria as promoters of Plant growth for increased yield in landraces of African cereal crops. *Afr. J. Biotechnol.*, 3:1-7.

Mehboob, I., Z.A. Zahir, A. Mahboob, S.M. Shahzad, A. Jawad and M. Arshad. 2008. Preliminary screening of Rhizobium isolates for improving growth of maize seedlings under axenic conditions. *Soil Environ.*, 27: 64-71.

Mehta, S. and C.S. Nautiyal. 2001. An efficient method for screening phosphate solubilization bacteria. *Curr. Microbiol.*, 43: 57-58.

Meyer, J.M. 2000. Pyoverdines: pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas* sp. *Arch. Microbiol.*, 174: 135-142.

Pandey, P. and D.K. Maheshwari. 2007. Two-species microbial consortium for growth promotion of *Cajanus cajan*. *Curr. Sci.*, 92: 1137-1141.

Peix, A., A.A. Rivas-Boyero, P.F. Mateos, C. Rodriguez-Barrueco, E. Martinez-Molina and E. Velaqez. 2001. Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. *Soil Biol. Biochem.*, 33: 103-110.

Piesterse, M.J., J.A.V. Pelt, S.C.M.V. Wees, J. Ton, K.M. Leon-Kloosterziel, J.J.B. Keurenties, B.W.M. Verhagen, M. Knoester, I.V. Sluits, P.A.H.M. Bakker and L.C. Van. 2001. Rhizobacteria mediated induced systemic resistance: triggering, signaling and expression. *Eur. J. Plant. Pathol.*, 107: 51-61.

Sarwar, M., M. Arshad, D.A. Martens, W.T. Frankenberger, Jr. 1992. Tryptophan dependent biosynthesis of auxins in soil. *Plant Soil*, 147: 207-215.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*, 160: 47-56.

Simon, M., A. van der Bij, I. Brand, L.A. de Weger, C.A. Wijffelman and B.J.J. Laugtenberg. 1996. Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *Mol. Plant-Microbe Interact.*, 9: 600-607.

Singh, R.K., R.P.N. Mishra, H.K. Jaiswal, V. Kumar, S.P. Pandey, S.B. Rao and K. Annapurna. 2006. Isolation and identification of natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. *Curr. Microbiol.*, 52: 345-349.

Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. *Principles and Procedures of Statistics-A Biometrical Approach* (3rd Ed.) McGraw-Hill Book International Co., Singapore. p. 204-227.

Werner, D. 1992. *Symbiosis of Plants and Microbes*. Chapman and Hall, London.

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annu. Rev. Plant Physiol.*, 35: 155-189.

Zahir, Z.A., H.M. Yasin, M. Naveed, M.A. Anjum and M. Khalid. 2010. L-Tryptophan application enhances the effectiveness of Rhizobium inoculation for improving growth and yield of mung bean [Vigna radiate (L.) Wilczek]. *Pak. J. Bot.*, 42: 1771-1780.

(Received for publication 17 March 2010)