Soil Environ. 31(1): 37-46, 2012
www.se.org.pk
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Comparative effectiveness of different Rhizobium sp. for improving growth and yield of maize (*Zea mays* L.)

Ijaz Mehboob[1], Zahir Ahmad Zahir[*1], Muhammad Arshad[1], Asif Tanveer[2] and Muhammad Khalid[1]
[1]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan
[2]Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

Abstract

During the last couple of decades, it has been demonstrated that rhizobia can associate with roots of non-legumes also without forming true nodules, and can promote their growth by using one or more of the direct or indirect mechanisms of actions. This work examines the growth and yield responses of maize to inoculation with different species of rhizobia, isolated from the root nodules of chickpea (Cicer arietinum L.), lentil (Lens culinaris M.) and mung bean (Vigna radiata L.) in pots and fields. Twenty isolates of rhizobia were isolated from root nodules each of mung bean, lentil and chickpea and were screened under axenic conditions. On the basis of their promising performance under axenic conditions, nine most efficient isolates (three from each legume host) were selected, characterized and further evaluated for their growth promoting activities by conducting pot and field experiments. Results of pot experiment revealed that maximum increase in grain yield, 1000 grain weight, N, P and K uptake (up to 47.89, 54.52, 73.46, 84.66 and 59.19% by CRI_{28}, respectively, over un-inoculated control) was produced by the isolate of Mesorhizobium ciceri. Whereas, maximum improvement in rest of the parameters was caused by the isolates of Rhizobium phaseoli (i.e. fresh biomass, straw yield and root length up to 36.30% by A_{18}, 25.46% by S_6 and 81.89% by A_{18}, respectively over un-inoculated control). Rhizobium leguminosarum isolates came out to be the least effective among the species tested. Similarly, all the selected isolates improved the growth and yield attributing parameters in fields as well but with varying capacity compared with un-inoculated control. The selected isolates of Mesorhizobium ciceri and Rhizobium phaseoli again remained superior compared to the isolates of Rhizobium leguminosarum under field conditions. The results of this study imply that rhizobium species had potential to promote growth and yield of maize but this technology should be employed after appropriate site specific investigations of particular rhizobial specie with respect to specific non-leguminous crop variety to get maximum benefit in terms of better growth and yield.

Key words: *Rhizobium phaseoli*, *Mesorhizobium ciceri*, *Rhizobium leguminosarum*, growth, yield, maize

Introduction

The most studied and longest exploited plant growth promoting rhizobacteria (PGPR) are the rhizobia (including the *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium*) for their ability to fix nitrogen (Werner, 1992) in only their legume host plants. But the interaction of rhizobia with non-legumes has been neglected as an experimental system. During the last couple of decades, work on rhizobial interaction with non-legumes has been done progressively and there has been increasing evidence that rhizobia can also play an important role in the growth promotion of non-legumes (Yanni *et al*., 2001; Lupwayi *et al*., 2004) by using one or more of the direct or indirect mechanisms of actions. Phytohormone production like IAA (Roy and Basu, 2004), cytokinins (Upadhyaya *et al*., 1991), abscisic acid (Frankenberger and Arshad, 1995) and gibberellins (Chi *et al*., 2005), secretion of chemicals like lipo-chito-oligosaccharides (Smith *et al*., 2002), lumichrome (Dakora *et al*., 2002), solubilization of precipitated phosphorous through releasing organic acids (Alikhani *et al*., 2006) and mineralization of organic P through releasing phosphatase enzymes (Abd-Alla, 1994), improvement in uptake of plant nutrients (Biswas *et al*., 2000) by altering root morphology (Anyia *et al.*, 2004), production of siderophores (Meyer, 2000) and lowering of ethylene level through ACC deaminase enzyme (Madhaiyan *et al*., 2006), are some examples of rhizobial mechanisms that directly influence plant growth. While indirectly, rhizobia improve the growth of non-legumes through biocontrol of pathogens via antibiosis (Hoflich, 2000), parasitism (Ozkoc and Deliveli, 2001) or competition (Arora *et al*., 2001) with pathogens for nutrients and space, by inducing systemic resistance (Liu *et al.*, 1995) in host plant and through increasing root adhering soil by releasing exopolysaccarides (Alami *et al*., 2000).

*Email: zazahir@yahoo.com

© 2012, Soil Science Society of Pakistan (http://www.sss-pakistan.org)

On the other hand, there are evidences for no influence or even inhibitory effects of rhizobial inoculation in non-legumes (Perrine *et al*., 2001; El-Tarabily *et al*., 2006). Some researchers have also indicated specificity of rhizobial inoculants with soil (Hilali, 2001), crop variety and environmental conditions (Chelius and Triplett, 2000).

Keeping in view these facts, pot and field trials were conducted to assess the efficacy of nine promising isolates of rhizobia (which were previously found most efficient under axenic conditions) for their ability to promote growth and yield of maize (*Zea mays* L.) under natural environmental conditions.

Materials and methods

Isolation and Screening of rhizobial isolates

Nine rhizobial isolates used in this study were isolated from the root nodules of chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* M.) and mung bean (*Vigna radiata* L.) collected from different sites and screened on the basis of their promising performance on maize seedling growth in a series of experiments conducted under axenic conditions (Mehboob *et al*., 2008).

Preparation of inocula and seed inoculation

Inocula were prepared by growing the selected rhizobial isolates in 250 mL conical flask containing 100 mL yeast extract mannitol (YEM) broth which were incubated in the orbital shaking incubator at 28 $\pm$ 1^{0}C with 100 rpm for three days. An optical density of 0.5 recorded at a wavelength of 535 nm, was achieved by dilution for uniform cell density (10^8–10^9 CFU mL^{-1}). The seeds of maize were inoculated by mixing with peat based slurry containing 3-day-old inoculum of respective isolate and 10% sterilized sugar solution at 100 mL kg^{-1} sterilized peat. The seeds for control were treated with sterilized peat having sterilized broth and sugar solution. Inoculated seeds were dried for 6-8 h under shade.

Pot experiment

Nine most efficient isolates (3 each from mung bean i.e. A_{18}, S_6, S_{17}, chickpea i.e. CRI_{28}, CRI_{34}, CRI_{35} and lentil i.e. LSI_{21}, LSI_{29}, LSI_{32}) selected on the basis of their promising performance in jar experiments (Mehboob *et al*., 2008) conducted under axenic conditions were evaluated for their potential to enhance the growth and yield of maize plants grown in pots. Pot study was conducted with sandy clay loam having pH, 7.8; ECe, 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, (situated at longitude 72°0′ and 73°45′ E and latitude 30° 30′ and 32°0′ N), Pakistan. The inoculated and uninoculated seeds of maize (*Zea mays* L.) cv. Neelum were sown in pots at 5 seeds per pot having 12 kg soil. The pots were arranged randomly at ambient light and temperature according to completely randomized design and each treatment was replicated thrice. Thinning was done after two weeks of germination and one seedling was maintained in each pot. The pots received NPK at 180, 140, 90 kg ha^{-1} in the form of urea, di-ammonium phosphate (DAP) and muriate of potash (MOP). The whole PK was applied as basal dose while N was applied in splits. The pots were irrigated with good quality canal water. Data regarding growth and yield parameters were recorded at maturity. Nitrogen, phosphorus and potassium contents in grain and straw samples were determined (Ryan *et al*., 2001).

Field experiments

Field experiments were conducted at two sites (site-I and site-II) in order to further evaluate and confirm the results of pot trials at the research area of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. The soil at site-I was sandy clay loam and had pH, 7.8; ECe, 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} whereas the soil at site-II was sandy clay loam and had pH, 8.0; ECe, 2.23 dS m^{-1}; organic matter, 0.75%; total nitrogen, 0.05%; available phosphorus, 8 mg kg^{-1} and extractable potassium, 121 mg kg^{-1}. Inoculation of the seeds was completed in a similar fashion as described in case of pot experiments. Experiments were laid out in randomized complete block design with three repeats. Dibbling method was followed for the sowing of maize crop. NPK were applied at180, 140, 90 kg ha^{-1} using urea, DAP and MOP as source, respectively. Full dose of P and K was applied as basal dose while N was applied in two splits. Fields were irrigated with good quality canal water. Data regarding growth and yield parameters were recorded at maturity. Grain and straw samples were analyzed for NPK using standard protocol.

Characterization of the selected isolates

The selected isolates were characterized for auxin production, phosphate solubilization, root colonization, chitinase activity, exopolysaccharides and siderophore production.

Auxin production by the selected isolates in the presence and absence of L-tryptophan (L-TRP) was determined by colorimeter method as described by Sarwar *et al*. (1992). Ability to solubilize inorganic phosphate of the selected isolates was measured qualitatively by using National Botanical Research Institute, Rana Pratap Marg

(NBRI-RPM) medium as described by Mehta and Nautiyal (2001). For exopolysaccharides activity (qualitative) isolates were grown on RCV mineral media enriched with mannitol, sucrose and with or without NaCl (Ashraf *et al*., 2004) and production of EPS was assessed visually. Root colonization of selected isolates was studied under axenic conditions as described by Simons *et al*. (1996). For siderophore production culture of the selected isolate was added in to the well made on Chrome azurol S (CAS) agar media taken in Petri plate prepared by following the procedure described by Schwyn and Neilands (1987). The plates were incubated at room temperature for 48 hours after which change in colour in the medium was recorded and characterized as positive for siderophore production. Chitinase activity of the selected isolates was determined according to the method described by Chernin *et al*. (1998). The isolates were maintained at -20^{0}C in liquid broth containing 20% (*v/v*) glycerol.

Statistical analysis

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

Results

Pot Trial

The plant height of maize grown in pots was stimulated significantly by all the isolates except LSI_{29} in comparison with un-inoculated control (Table 1). The increase in plant height was between 3.23 to 23.44% over un-inoculated control. Maize plants attained maximum height when inoculated with CRI_{34} which was 23.44% more than un-inoculated control. Isolates CRI_{28}, A_{18}, S_6, and S_{17} yielded statistically same results and caused up to 13.74% increase in plant height. Isolate LSI_{29} was the least effective which caused statistically non-significant increase up to 3.23% in plant height compared with un-inoculated control.

Significant improvement in fresh biomass of maize plants was recorded upon inoculation with six isolates whereas three isolates gave non-significant improvement compared with un-inoculated control (Table 1). Maximum improvement of 36.30% in fresh biomass was obtained by inoculation with the isolate A_{18} compared with un-inoculated control. Moreover, significant improvement in fresh biomass was also noted by the isolates S_6 (24.72%), CRI_{35} (23.97%), S_{17} (21.87%), CR_{I34} (19.14%) and CRI_{28} (17.28%), in descending order compared with un-inoculated control. However, the isolates LSI_{21}, LSI_{29} and LSI_{32} also increased the fresh biomass up to 12.16% but non-significantly compared with un-inoculated control.

As observed in the maize pot experiment, 67% of the isolates had significant increasing effect while the others 33% had non-significant effect on straw yield of maize as compared to the un-inoculated control (Table 1). Isolate S_6 was superior among the isolates which enhanced the straw yield significantly up to 25.46% over un-inoculated control. The results of the five isolates i.e. A_{18}, CRI_{28}, CRI_{34}, CRI_{35} and S_{17} also indicated an increase in straw yield of maize ranging from 14.94 to 19.94% over un-inoculated control but were statistically similar in their effects when compared with each other. Likewise, the isolates LSI_{21}, LSI_{29} and LSI_{32} exhibited statistically similar results when compared with each other.

Except LSI_{29} and LSI_{32}, all the other isolates induced significant increase in grain yield of maize grown in pots over un-inoculated control (Table 1). The rhizobial isolates increased the grain yield up to 47.89% in comparison with un-inoculated control. The isolate CRI_{28} yielded maximum increases of 47.89% while the minimum of 5.83% increase in grain yield was induced by the isolate LSI_{29} which was at par with the isolate LSI_{32} and un-inoculated control. The isolates CRI_{34} and S_6 were found statistically similar in their results when compared with each other. The isolates S_{17} and CRI_{35} were the next better isolates which increased the grain yield up to 24.25% in comparison with un-inoculated control.

The effect of rhizobial inoculation on 1000 grain weight revealed that all the isolates performed positively and significantly (Table 1). The investigations demonstrated that the isolate CRI_{28} increased the weight of 1000 grains maximally up to 54.52% over un-inoculated control which was followed by 46.43% increase given by the isolate CRI_{34} compared to un-inoculated control. The effect of all the remaining isolates was positive ranging from 8.26 to 27.91% higher than un-inoculated control. The isolates A_{18}, LSI_{21} and S_6 appeared statistically similar in their effects when compared with each other. Likewise, the isolates $LSI_{29,}$ CRI_{35} and LRI_{32} remained at par with respect to each other.

Regarding root length of maize grown in pots, all the isolates showed significant increase with respect to un-inoculated control (Table 1). The longest roots (81.89% more over un-inoculated control) were obtained where the isolate A_{18} was used as inoculant. The next effective isolates were S_6 and S_{17} which increased the root length up to 67.39 % in comparison with un-inoculated control. The least effective isolate was LSI_{32} which showed 23.91% increased root length in comparison with un-inoculated

control. All the isolates differed significantly from each other as well as from control.

plants attained maximum height (26.51% over un-inoculated control) with the isolate S_{17} inoculation.

Table 1: Effect of inoculation with different species of rhizobia on plant height, fresh biomass, straw yield, grain yield, 1000 grain weight and root length of maize plants grown in pots

(Average of 3 repeats)

Strain	Plant Height (cm)	Fresh Biomass (g pot^{-1})	Straw yield (g pot^{-1})	Grain Yield (g pot^{-1})	1000 Grain Weight (g)	Root Length (cm)
Control	123.7 f	171.9 e	37.27 d	37.90 e	115.0 g	46.00 j
CRI_{28}	135.7 cd	201.6 bd	43.44 ac	56.05 a	177.7 a	68.00 d
CRI_{34}	152.7 a	204.8 bc	43.83 ac	45.14 bc	168.4 b	67.67 e
CRI_{35}	144.0 b	213.1 ab	44.70 ab	46.01 b	128.6 ef	66.00 f
LSI_{21}	132.7 de	182.8 ce	39.80 cd	42.44 cd	136.0 d	59.33 h
LSI_{29}	127.7 ef	192.8 be	40.61 bd	40.11 de	126.7 ef	60.67 g
LSI_{32}	133.7 de	178.4 de	40.74 bd	40.41 de	124.5 f	57.00 i
A_{18}	136.7 cd	234.3 a	42.84 ac	42.36 cd	137.1 d	83.67 a
S_6	137.3 cd	214.4 ab	46.76 a	44.31 bc	132.0 de	77.00 b
S_{17}	140.7 bc	209.5 ab	43.17 ac	47.09 b	147.1 c	74.00 c
LSD value	5.973	23.19	3.648	2.770	5.992	0.162

*Means sharing the same letter (s) do not differ significantly at $p < 0.05$ according to Duncan's Multiple Range Test.
CRI_{28}, CRI_{34}, CRI_{35}: Chickpea isolates (*Mesorhizobium ciceri*); LSI_{21}, LSI_{29}, LSI_{32}: Lentil Isolates (*Rhizobium leguminosarum)*; A_{18}, S_6, S_{17}: Mung bean isolates *(Rhizobium phaseoli)*

NPK uptake (mg pot^{-1}) by maize plants

Statistical behavior of NPK uptake by maize plants grown in pots revealed that all the rhizobial isolates increased the uptake of NPK significantly upon inoculation compared with un-inoculated control (Table 2). The range of increase in N, P and K uptake was from 19.92 to 84.66% caused by the rhizobial inoculants in comparison with un-inoculated control. Among the isolates, isolate CRI_{28} emerged as the most promising one which increased N, P and K uptake maximally by 73.46, 84.66 and 59.19%, respectively over un-inoculated control. The least effective isolate was LSI_{29} that gave an increase of 19.96, 24.13 and 19.92% in N, P and K uptake, respectively over un-inoculated control. However, non-significant differences were observed when the results of the isolates were compared with each other.

Field trials

Field trials were conducted at two sites of the farm of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad to evaluate the inoculation effect of different rhizobial isolates on growth and yield of maize and the results obtained are described as under:

At both the sites (site-I and site-II) rhizobial inoculation showed their growth promoting potential and increased the plant height significantly (Table 3). Maximum plant height (25.31% over un-inoculated control) was obtained by inoculation with the isolate S_6 at site-I whereas at site-II the

Moreover, both the isolates (S_6 and S_{17}) differed significantly from all the other isolates as well as from the un-inoculated control but non-significantly with one another at both the sites. Furthermore, the isolate LSI_{21} remained least effective at both the sites but still increased the plant height up to 14.17% over un-inoculated control.

Table 2: Effect of inoculation with different species of rhizobia on N, P and K uptake of maize plants grown in pots

(Average of 3 repeats)

Strain	N uptake (mg pot^{-1})	P uptake (mg pot^{-1})	K uptake (mg pot^{-1})
Control	445.8 e	267.3 d	393.5 d
CRI_{28}	773.3 a	493.6 a	626.4 a
CRI_{34}	640.9 bc	417.8 b	546.5 b
CRI_{35}	633.4 bc	437.7 b	546.0 b
LSI_{21}	579.1 cd	350.3 c	474.0 c
LSI_{29}	534.8 d	331.8 c	471.9 c
LSI_{32}	541.8 d	336.1 c	478.9 c
A_{18}	619.0 bc	367.1 c	536.3 b
S_6	638.8 bc	442.2 b	604.0 a
S_{17}	669.1 b	421.3 b	592.1 a
LSD value	65.09	43.85	42.61

*Means sharing the same letter (s) do not differ significantly at $p < 0.05$ according to Duncan's Multiple Range Test.
CRI_{28}, CRI_{34}, CRI_{35}: Chickpea isolates (*Mesorhizobium ciceri*); LSI_{21}, LSI_{29}, LSI_{32}: Lentil Isolates (*Rhizobium leguminosarum)*; A_{18}, S_6, S_{17}: Mung bean isolates *(Rhizobium phaseoli)*

Greater biomass of maize crop was obtained under field conditions in response to rhizobial inoculation compared to the treatments where no inoculum was applied (Table 3). At site-I, the isolate CRI_{28} yielded maximum fresh biomass up to 64.37% more than un-inoculated control. Whereas, at site-II, the most efficient isolate was S_6 which improved the fresh biomass up to 62.64% over un-inoculated control. The isolate A_{18} was statistically at par with the isolates S_6 and S_{17} at site-I and with isolate S_{17} at site-II when compared with one another. Moreover, non significant differences were also observed between some isolates at both the sites when compared with each other.

site-II these were between 18.61 and 63.37% over un-inoculated control. The most promising isolate at site-I was S_{17} whereas at site-II, it was isolate S_6. The next promising isolate CRI_{28} increased the grain yield by 47.79 % over un-inoculated control which was followed by two isolates (A_{18} & S_6) at site-I. At site-II, it was the isolate A_{18} which gave maximum grain yield of 54.59% over un-inoculated control after S_6 and was followed by the isolate CRI_{28}. The isolate LSI_{32} gave the lowest values of grain yield (6.950 and 6.757 t ha^{-1} at site I and II, respectively) in comparison with un-inoculated control.

Three isolates at site-I and seven at site-II had non-

Table 3: Effect of inoculation with different species of rhizobia on plant height, fresh biomass, straw yield and grain yield of maize in fields

(Average of 3 repeats)

Strain	Plant Height (cm)		Fresh Biomass (t ha^{-1})		Straw Yield (t ha^{-1})		Grain Yield (t ha^{-1})	
	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II
Control	245.0 f	235.0 g	21.64 f	24.57 g	5.74 e	6.60 f	5.993 g	5.697 h
CRI_{28}	278.3 d	273.7 de	35.57 a	33.42 c	7.01 cd	8.65 bc	8.857 b	8.333 c
CRI_{34}	294.7 b	289.0 b	29.29 c	30.49 d	8.34 ab	9.13 ab	7.820 d	7.397 ef
CRI_{35}	288.3 c	283.3 c	28.19 cd	31.33 d	6.38 de	7.07 ef	7.513 e	7.683 de
LSI_{21}	266.3 e	268.3 f	27.59 d	28.74 ef	6.31 de	7.93 cd	7.103 f	7.080 f
LSI_{29}	282.7 cd	277.7 d	27.17 d	30.33 de	7.14 cd	7.10 ef	7.710 de	7.387 ef
LSI_{32}	278.7 d	269.3 ef	25.77 e	28.20 f	6.36 de	7.07 ef	6.950 f	6.757 g
A_{18}	280.0 d	287.3 bc	33.58 b	36.69 b	7.86 bc	7.80 de	8.193 c	8.807 b
S_6	307.0 a	294.3 a	33.35 b	39.96 a	8.74 a	8.08 cd	8.193 c	9.307 a
S_{17}	305.0 a	297.3 a	32.82 b	35.69 b	7.29 c	9.53 a	9.343 a	7.903 d
LSD value	5.674	4.747	1.179	1.601	0.786	0.762	0.241	0.309

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

CRI_{28}, CRI_{34}, CRI_{35}: Chickpea isolates (*Mesorhizobium ciceri*); LSI_{21}, LSI_{29}, LSI_{32}: Lentil Isolates (*Rhizobium leguminosarum)*; A_{18}, S_6, S_{17}*:* Mung bean isolates *(Rhizobium phaseoli)*

As for as the straw yield of maize is concerned, most of the isolates produced significantly higher straw yield compared with un-inoculated control while few were non-significant in comparison with un-inoculated control (Table 3). Among the isolates, isolate S_6 gave maximum straw yield (52.26% more than the un-inoculated control) at site-I whereas at site-II, the isolate S_{17} behaved as the most efficient isolate producing 44.39% higher straw yield over un-inoculated control. Moreover, at site-I three isolates (CRI_{35}, LSI_{21} and LSI_{32}) which increased straw yield up to 11.15% over un-inoculated control and three isolates (CRI_{35}, LSI_{29} and LSI_{32}) at site-II that increased the straw yield of maize up to 7.58% over un-inoculated control failed to show statistical difference in comparison with one another and with un-inoculated control.

All the rhizobial isolates differed significantly in their effects on grain yield of maize compared with un-inoculated control at both the sites (Table 3). Increases in grain yield at site-I ranged from 15.97 to 55.90% whereas at

significant effect on 1000 grain weight compared to the un-inoculated control (Table 4). Maximum weight of 1000 grains (up to 14.99% increase) at site-I was obtained by inoculation with isolate CRI_{35} in comparison with un-inoculated control which was statistically similar to the isolates S_{17} and LSI_{32}. Rest of the six isolates at site-I remained statistically at par with each other and they increased the 1000 grain weight up to 8.36% over un-inoculated control. Results of the inoculation at site-II showed that the isolate CRI_{28} dominated all the other isolates by causing maximum improvement of 28.88% in the 1000 grain weight over un-inoculated control followed by the isolate S_6. At site-II, the remaining seven isolates yielded statistically similar results compared to un-inoculated control.

NPK uptake (kg ha^{-1}) by maize plants in fields

Although all the isolates increased N, P and K uptake by maize plants significantly in comparison with un-

inoculated control however; among the isolates, non-significant differences were also experienced at both the sites (Table 4). Improvement in N, P and K uptake was maximum up to 89.34 (caused by S_{17}), 93.76 (caused by CRI_{28}) and 81.23% (caused by S_6), respectively over un-inoculated control at site-I. Other isolates at site-I increased N, P and K uptake ranging between 24.58 to 90.29% over un-inoculated control. Whereas, at site-II, it was observed that the isolates S_6, A_{18} and S_{17} performed better among the isolates which increased N (up to 88.26%), P (up to105.27%) and K uptake (up to 77.40%), respectively, by maize plants over un-inoculated control. Moreover, the isolate LSI_{32} remained least effective in increasing N, P and K uptake at both the sites (except for P uptake at site-I).

Table 4: Effect of inoculation with different species of rhizobia on 1000 grain weight, N, P and K uptake by maize plants in fields

(Average of 3 repeats)

Strain	1000 Grain Weight (g)		N uptake (kg ha^{-1})		P uptake (kg ha^{-1})		K uptake (kg ha^{-1})	
	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II
Control	179.4 d	207.4 c	65.49 e	73.62 e	42.02 e	43.82 e	60.42 f	67.53 d
CRI_{28}	187.1 cd	267.3 a	114.1 ab	129.1 ab	81.42 a	80.45 ab	93.21 cd	110.2 ab
CRI_{34}	194.4 bc	215.7 bc	112.7 ab	122.6 abc	71.06 bc	78.43 bc	96.57 bc	106.9 b
CRI_{35}	206.3 a	212.8 c	99.23 c	112.1 bcd	63.36 cd	70.63 cd	81.91 e	91.18 c
LSI_{21}	187.7 cd	217.9 bc	93.95 cd	105.3 cd	55.43 d	65.13 d	76.76 e	92.92 c
LSI_{29}	189.4 cd	221.9 bc	97.35 c	100.6 d	62.47 cd	62.10 d	85.31 de	86.96 c
LSI_{32}	196.2 ac	213.3 c	84.40 d	94.48 d	58.78 d	61.72 d	75.27 e	84.84 c
A_{18}	191.8 bc	233.8 bc	103.8 bc	129.6 ab	79.96 ab	89.95 a	102.1 abc	108.3 b
S_6	190.8 bc	248.5 ab	113.7 ab	138.6 a	75.19 ab	84.44 ab	109.5 a	114.7 ab
S_{17}	201.3 ab	230.2 bc	124.0 a	125.7 ab	78.73 ab	80.96 ab	105.4 ab	119.8 a
LSD value	9.981	30.80	11.45	16.78	8.553	9.160	9.364	9.774

*Means sharing the same letter (s) do not differ significantly at $p < 0.05$ according to Duncan's Multiple Range Test.
CRI_{28}, CRI_{34}, CRI_{35}: Chickpea isolates (*Mesorhizobium ciceri*); LSI_{21}, LSI_{29}, LSI_{32}: Lentil Isolates (*Rhizobium leguminosarum)*; A_{18}, S_6, S_{17}*:* Mung bean isolates *(Rhizobium phaseoli)*

Characterization of selected Isolates

Data regarding characterization of the maize isolates revealed that all the isolates colonized the maize roots but with different degree of efficacy (Table 5). The isolates belonging to *Rhizobium phaseoli* i.e. A_{18}, S_6 and S_{17} showed higher root colonization (up to 6.77 x 10^5 cfu g^{-1}) followed by the isolates of *Mesorhizobium ciceri* i.e. CRI_{28}, CRI_{34} and CRI_{35} which exhibited up to 5.44 x 10^5 cfu g^{-1}. Rest of the isolate of the *Rhizobium leguminosarum* i.e. LSI_{21}, LSI_{29} and LSI_{32} remained poorer as a group than the isolates of above two groups as they caused root colonization up to 2.06 x 10^5 cfu g^{-1}. For chitinase and phosphate solubilization activities, the isolates of *Mesorhizobium ciceri* were positive whereas the isolates of *Rhizobium leguminosarum* varied in their behavior for these characters as chitinase activity was absent in all the three isolates (LSI_{21}, LSI_{29} and LSI_{32}) but for phosphate solubilization except $LSI_{32,}$ the other two isolates had this ability. Likewise, except A_{18} isolate, the other two isolates of *Rhizobium phaseoli* i.e. S_6 and S_{17} had chitinase activity but in case of phosphate solubilization ability, only the isolate S_6 was positive. Moreover, for exopolysaccharide production ability, all the isolates of the three groups were found positive while for siderophore production ability, only the isolates CRI_{35}, LSI_{21}, LSI_{32} and A_{18} were positive and this ability was absent in all the remaining isolates. Furthermore, all the isolate showed the ability to produce IAA in the presence and absence of L-tryptophan. The isolates CRI_{28} of *Mesorhizobium ciceri* stood first by showing its higher auxin production in the presence and absence of L-tryptophan compared to rest of the isolates. Overall, the data revealed that the isolates, very likely, had more than one mechanism of action for the modification in growth and yield of maize plants.

Discussion

In the present study, large number of rhizobia were isolated from the nodules of three legumes (chickpea, lentil and mung been) and a total of sixty fast growing colonies of rhizobia, twenty from each host plant, were selected. A series of jar experiments were carried out to screen three most efficient isolates from twenty of each rhizobium species (i.e. *Rhizobium phaseoli*, *Mesorhizobium ciceri* and *Rhizobium leguminosarum*) on the basis of their growth

promoting potential with maize seedlings in growth room under axenic conditions (Mehboob *et al*., 2008). The selected nine promising isolates of rhizobia were further evaluated for their growth promoting activities in pots and fields, and were characterized for various characters.

more promising results compared to the results of the isolates of *Rhizobium leguminosarum*. The variation in results of different species could be due to their specificity or due to difference in their compatibility potential towards a common host (which is evident from the root colonization

Table 5: Characterization of the selected isolates of rhizobia for maize crop

Strain	Root Colonization (cfu g^{-1})	Chitinase Activity	Phosphate Solubilization	Siderophore Production	Exopolysaccharide Production	IAA Production (mg L^{-1})	
						(Without L-Tryptophan)	(With L-Tryptophan)
CRI_{28}	4.57 x 10^5	+ ve	+ ve	-	+ ve	4.67	37.82
CRI_{34}	3.69 x 10^5	+ ve	+ ve	-	+ ve	0.80	28.36
CRI_{35}	5.44 x 10^5	+ ve	+ ve	+ ve	+ ve	0.72	30.87
LSI_{21}	1.05 x 10^5	-	+ ve	+ ve	+ ve	0.91	22.37
LSI_{29}	2.05 x 10^5	-	+ ve	-	+ ve	0.96	13.78
LSI_{32}	2.06 x 10^5	-	-	+ ve	+ ve	0.96	25.31
A_{18}	6.19 x 10^5	-	-	+ ve	+ ve	2.3	28.37
S_6	6.77 x 10^5	+ ve	+ ve	-	+ ve	2.1	28.60
S_{17}	6.04 x 10^5	+ ve	-	-	+ ve	3.57	27.69

CRI_{28}, CRI_{34}, CRI_{35}: Chickpea isolates (*Mesorhizobium ciceri*); LSI_{21}, LSI_{29}, LSI_{32}: Lentil Isolates (*Rhizobium leguminosarum)*; A_{18}, S_6, S_{17}*:* Mung bean isolates *(Rhizobium phaseoli)*

Results indicated that the selected isolates of rhizobia had increasing effect on growth and yield of maize however; in some parameters the increases caused by few isolates were statistically at par with un-inoculated control. In general, inoculation improved growth and yield parameters of maize crop in pots and fields. This may imply that the ability of rhizobia to produce different metabolites like phytohormone, organic acids, siderophores, and exopolysaccharides in the rhizosphere could be responsible for evoking the growth stimulating response in the inoculated maize plants. However, other mechanisms of action through which rhizobia influence plant growth cannot be ruled out. It is highly likely that the observed effects of rhizobia on plant growth could be the result of more than one mechanism of action other than N_2 fixation; the inoculated isolates possessed which is evident from our characterization data. Similar results have been reported by some workers while studying rizobial activities with non-legumes (Sheikh *et al*., 2006; Chandra *et al*., 2007; Mehboob *et al*., 2008).

The results of the present study also revealed that the effectiveness of the isolates varied among the species against a common host e.g. the isolates of *Rhizobium ciceri* increased grain yield in pots and fields up to 47.89%, whereas, the isolates of *Rhizobium phaseoli* improved up to 63.37% and *Rhizobium leguminosarum* isolates caused increment up to 29.66%, respectively in grain yield over respective un-inoculated control. Overall, the isolates of *Rhizobium phaseoli* and *Mesorhizobium ciceri* exhibited data clearly reflecting the superiority of the isolates of *Rhizobium phaseoli* and *Mesorhizobium ciceri* over the isolates of *Rhizobium leguminosarum* (Table 5). Our results are supported by the findings of Piesterse *et al*. (2001) and Hafeez *et al*. (2004) who have also reported differential behavior of different plant growth promoting rhizobial strains against a common host.

Moreover, the data of pot and field trials indicated that the impact of rhizobial isolates also varied among the isolates within a specie (e.g. the isolates of *Rhizobium ciceri* i.e. $CRI_{28,}$ CRI_{34} and CRI_{35} increased grain yield up to 47.89, 30.49 and 34.86% while *Rhizobium phaseoli* isolates i.e. $A_{18,}$ S_6 and S_{17} increased up to 54.59, 63.37 and 55.90% and *Rhizobium leguminosarum* isolates i.e. LSI_{21}, LSI_{29} and LSI_{32} increased grain yield up to 24.28, 29.66 and 18.61%, respectively over un-inoculated control) which might be due to difference in their natural potential. Similarly, the difference in efficiency of different strains against a common host was also seen by Yanni *et al*. (1997) who used two rhizobial strains i.e. E11 and E12 and reported an increase in grain and straw yield by 45.7 and 14.8% with E11 and 42.0 and 10.3%, respectively by E12 when inoculated with rice. Furthermore, they also recorded different increases in N content in rice grain by E11 (53%) and E12 (34.3%).

The results of our field trials also showed that the effect of the selected rhizobial isolates on growth and yield of maize varied from site to site. The isolates of *Rhizobium ciceri* increased grain yield at site-I and II up

to 47.79 and 46.27% while *Rhizobium phaseoli* isolates increased up to 55.90 and 63.37% and *Rhizobium leguminosarum* isolates increased up to 28.65 and 29.66%, respectively over un-inoculated control. This variability in the effect of rhizobial inoculants could be explained on the basis of changing soil-plant and micro floral components at any given experimental site. Thus different biotic and abiotic factors e.g., soil pH, EC, organic matter, mineral composition, fertility status and population density as well as diversity of the indigenous microbes can modify the response of the inoculants. Similar to our findings, Lynch, (1990) reported that sometimes the effect of a particular bacterium may vary as a consequence of soil conditions. Likewise, Khalid (2006) while observing the effect of PGPR on the growth and yield of wheat and rice at two different sites revealed that the effect of PGPR strains on growth and yield of wheat and rice varied from site to site.

In the present scenario of severe need of food because of rapid population growth and agricultural limitations for meeting the world food demand, it is difficult to compromise on real potential of crop productivity; thus all the efforts should be focused on maximizing the crop production along with the judicious exploitation of all the available resources wisely, efficiently and in an integrated manner, which is also a need of the day for achieving sustainability in agriculture. Use of bio-fertilizers is an emerging cost effective and environment friendly technology which reduce the dependence on the synthetic resources as well as their cost. But major challenges in the use of bio-inoculants are natural variations in environment, soil, crop and indigenous micro flora of a specific area because of which the recognized inoculants could not show the desired results. This situation demands the selection of the PGPR for specific crop variety under specific soil and environmental condition. Likewise, the results of this study also imply that use of general PGPR strains of rhizobium spp. could be avoided to a significant extent without knowing the particular information about the compatibility potential of the strains toward specific crop variety, soil and environmental conditions for which it could be used. In other words, it could be inferred that rhizobium spp. recognized for the growth and yield promotion of non-legumes could be used efficiently and effectively only against specific host under specific set of soil and environmental conditions for maximum benefits. Hence, it could be concluded that rhizobium species had the potential to promote growth and yield of maize but the PGPR technology should be employed after appropriate site specific investigations of particular rhizobial specie with respect to specific non-leguminous crop variety to get maximum benefit in term of better growth and yield.

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

Abd-Alla, M.H. 1994. Use of organic phosphorous by *Rhizobium leguminosarum* bv. *viciae* phosphatases. *Biology and Fertility of Soils* 8: 216-218.

Alami, Y., W.A. Achouak, C. Marol and T. Heulin. 2000. Rhizosphere soil aggregation and plant growth promotion of sunflower by an exopolysaccharide producing *Rhizobium* sp. strain isolated from sunflower roots. *Applied and Environmental Microbiology* 66: 3393-3398.

Alikhani, H.A., N. Saleh-Rastin and H. Antoun. 2006. Phosphate solubilization activity of rhizobia native to Iranian soils. *Plant and Soil* 287: 35-41.

Anyia, A.O., D.J. Archambault and J.J. Slaski. 2004. Growth promoting effects of the diazotroph *Azorhizobium caulinodans* on Canadian wheat cultivars. Available on: www.cropscience.org.au [Accessed: 04/09/2009].

Arora, N.K., S.C. Kang and D.K. Maheshwari. 2001. Isolation of siderophore-producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Current Science* 8: 673-677.

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. *Biology and Fertility of 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 Science Society of America Journal* 64: 1644-1650.

Chandra, S., K. Choure, R.C. Dubey and D.K. Maheshwari. 2007. Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica csmpestris*). *Brazilian Journal of Microbiology* 3: 128-130.

Chelius, M.K. and E.W. Triplett. 2000. Immunolocalization of dinitrogenase reductase produced by *Klebsiella pneumoniae* in association with *Zea mays* L. *Applied and Environmental Microbiology* 66: 783-787.

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. *Journal of Bacteriology* 180: 4435-4441.

Chi, F., S.H. Shen, H.P. Cheng, Y.X. Jing, Y.G. Yanni and F.B. Dazzo. 2005. Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Applied and Environmental Microbiology* 71: 7271-7278.

Dakora, F.D., V. Matiru, M. King and D.A. Phillips. 2002. Plant growth promotion in legumes and cereals by lumichrome, a rhizobial signal metabolite. p. 321-322. *In:* Nitrogen Fixation: Global Perspectives. T.M. Finan, M.R. O'Brian, D.B. Layzell, K. Vessey and W.E. Newton, (eds.). CABI Publishing, Wallingford, U.K.

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.). *Australian Journal of Agricultural Research* **57:** 101-111.

Frankenberger, W.T. Jr. and M. Arshad. 1995. Phytohormones in soil: Microbial Production and Function. New York, Marcel Dekker.

Hafeez, F.Y., M.E. Safdar, A.U. Chaudhry and K.A. Malik. 2004. Rhizobial inoculation improves seedling emergence, nutrient uptake and growth of cotton. *Australian Journal of Experimental Agriculture* 44: 617-622.

Hilali, A., D. Przrost, W.J. Broughton and A. Antoun. 2001. Effects de I'inoculation avec des souches de *Rhizobium leguminosarum* bv. *trifolii* sur la croissance du bl'e dans deux sols du Marco. *Canadian Journal of Microbiology* 47: 590-593.

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 Society of Microbial Ecology. Halifax, Canada.

Khalid, M., M. Arshad and Z.A. Zahir. 2006. Phytohormones: Microbial production and application. p. 207-220. *In:* Biological Approaches to Sustainable Soil Systems. N. Uphoff, A.S. Ball, C. Palm, E. Fernandes, J. Pretty, H. Herren, P. Sanchez, O. Husson, N. Sanginga, M. Laing and J. Thies (eds.) CRC Press, Taylor & Francis, Boca Raton, New York, USA.

Liu, L., J.W. Kloepper and S. Tuzun. 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology* 85: 695-698.

Lupwayi, N.Z., G.W. Clyton, K.G. Hanson, W.A. Rice and V.O. Biederbeck. 2004. Endophytic rhizobia in barley, wheat and canola roots. *Canadian Journal of Plant Sciences* 84: 37-45.

Lynch, J.M. 1990. The Rhizosphere. Wiley-Interscience, Chichester, UK.

Madhaiyan, M., S. Poonguzhali, J.H. Ryu and T.M. Sa. 2006. Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase containing *Methylobacterium fujisawaense*. *Planta* 224: 268-278.

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 and Environment* 27: 64-71.

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

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

Ozkoc, I. and M.H. Deliveli. 2001. *In vitro* inhibition of the mycelial growth of some root rot fungi by *Rhizobium leguminosarum* biovar *phaseoli* isolates. *Turkish Journal of Biology* 25: 435-445.

Perrine, F.M., J. Prayito, J.J. Frank, B. Dazzo and B.G. Rolfe. 2001. *Rhizobium* plasmids are involved in the inhibition or stimulation of rice growth and development. *Australian Journal of Plant Physiology* 28: 923-937.

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. *European Journal of Plant Pathololgy* **107:** 51-61.

Roy, M. and P.S. Basu. 2004. Studies on root nodules of leguminous plants bioproduction of indole acetic acid by a *Rhizobium sp.* from a twiner *Clitoria ternatea* L. *Acta Biotechnology* 12: 453-460.

Ryan, J., G. Estefan and A. Rashid. 2001. Soil and Plant Analysis: Laboratory Mannual. International Centre for Agricultural Research in Dry Areas (ICARDA) Aleppo. 172p.

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

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

Sheikh, L.I, S. Dawar, M.J. Zaki and A. Ghaffar. 2006. Efficacy of *Bacillus thuingiensis* and *Rhizobium meliloti* wth nursery fertilizers in the control of root infecting fungi on mung bean and okra plants. *Pakistan Journal of Botany* 38: 465-473.

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. *Molecular Plant-Microbe Interactions* 9: 600-607.

Smith, D.L., B. Prithiviraj and F. Zhang. 2002. Rhizobial signals and control of plant growth. p. 327-330. *In:* Nitrogen Fixation: Global Perspectives. T.M. Finan, M.R. O'Brian, D.B. Layzell, K. Vessey and W.E. Newton (eds.). CABI Publishing, Wallingford, UK.

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.

Upadhyaya, N.M., D.S. Letham, C.W. Parker, C.H. Hocart and P.J. Dart. 1991. Do rhizobia produce cytokinins? *Biochemistry International* 24: 123-130.

Yanni, Y.G., R.Y. Rizk, V. Corich, A. Squartini, K. Ninke, S. Philip-Hollingsworth, G. Orgambide, F. De Bruijn, J. Stoltzfus, D. Buckley, T.M. Schmidt, P.F. Mateos, J.K. Ladha and F.B. Dazzo. 1997. Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant and Soil* 194: 99-114.

Yanni, Y.G., R.Y. Rizk, F.K. Abd El-Fattah, A. Squartini, V. Corich, A. Giacomini, F. De Bruijn, J. Rademaker, J. Maya-Flores, P. Ostrom, M. Vega-Hernandez, R.I. Hollingsworth, E. Martinez-Molina, P. Mateos, E. Velazquez, J. Wopereis, E. Triplett, M. Umali-Gracia, J.A. Anarna, B.G. Rolfe, J.K. Ladha, J. Hill, R. Mujoo, P.K. Ng and F.B. Dazzo. 2001. The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. *trifolii* with rice roots. Australian Journal Plant Physiology 28: 845-870.

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