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Potential of *Rhizobium* spp. for improving growth and yield of rice (*Oryza sativa* L.)

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Abstract

The ability of Rhizobium to colonize roots of certain cereals and promote their growth and yield at harvest has been proven experimentally involving a number of mechanisms which were independent of root nodulation and biological N_2-fixation. Various rhizobial strains i.e. Rhizobium phaseoli (A2, A3, S17, N8), Rhizobium leguminosarum (LSI-23, LSI-26, LSI-29, LSI-30) and Mesorhizobium ciceri (CRI-28, CRI-31, CRI-32, CRI-38) isolated from the nodules of mung bean, lentil and chickpea, respectively, were tested for their potential to promote growth and yield of rice grown in potted soil. Seedlings of rice cultivar "Super Basmati" were transplanted in pots after inoculation with test strains of rhizobium. The fertilizers, P and K were applied at 60-60 kg ha^{-1} as a basal dose while N was applied at 120 kg ha^{-1} in two splits. Good quality canal water was used for irrigation. Most of the parameters i.e. number of tiller[1] (46%); paddy yield (43%), plant biomass (18%), straw dry weight (45%) and 1000-grain (25%) improved maximally by the strain LSI-29 over un-inoculated control. Whereas, in case of plant height, and number of grains $panicle^{-1}$, strain A2 caused maximum increase up to 28 and 29%, respectively over un-inoculated control. Furthermore, both the strains i.e. LSI-29 and A2 gave significant increase in nitrogen, phosphorus and potassium contents of paddy. It is concluded that all the tested rhizobial strains have the potential to enhance the growth and yield of rice, however, LSI-29 and A2 may have better prospects. Moreover, further work is needed to explore the effectiveness of these strains under field conditions.

Key words: *Rhizobium*, potential, growth, yield, rice

Introduction

Rice is highly valued diet and cash crop that earns substantial foreign exchange. But the resource poor farmers can not afford costly chemical fertilizers and pesticides to achieve the potential. Hence there is a severe need to develop a technology which can fulfill crop production and protection requirements economically and on sustainable basis.

Rhizobium association has been extensively explored in the root nodules of legumes where they fix atmospheric nitrogen but recent studies also suggest that *rhizobium* can exhibit plant growth promoting (PGP) activities with non-legumes (Yanni *et al*., 1997). Researchers studied these minute living creatures in association with certain non-legumes (cereals) after isolating them from the root nodules of local legumes and reported their beneficial effects (Mehboob *et al*., 2008; Humphry *et al*., 2007). Therefore, altering the rhizosphere microflora by seed, soil or root inoculation with specific organism is considered a sensible opportunity. Certain mechanisms are attributed towards *rhizobium* which may be involved in their PGP activities i.e. mobilization and efficient uptake of nutrient (Biswas *et al*., 2000a, b), enhancement in stress resistance (Mayak *et al.*, 2004), solubilization of insoluble phosphates (Alikhani *et al*., 2006), induction of systemic disease resistance (Tuzun and Kloepper, 1994), production of phytohormones (Dakora, 2003), vitamins (Dobbelaere *et al*., 2003) and siderophores (Neiland and leong, 1986).

Research efforts made so far regarding the impact of rhizobial inoculation on non-legumes indicated specificity of different *rhizobium* strains towards cultivar, soil, and environment. Furthermore, investigations have also pointed out that indigenous microflora had a key role in the establishment of the introduced microbes. Therefore, a pot study was conducted to assess the potential of three *rhizobium* species isolated from the root nodules of three local legumes (four from each host) i.e. mung bean, lentil and chickpea for improving the growth and yield of local rice cultivar "Super Basmati" under wire house conditions.

Materials and Methods

A pot trial was conducted to assess the potential of *rhizobium* species for improving growth and yield of rice in the wire house of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, during Kharif season 2008.

Assortment of rhizobium strains

Twelve rhizobial strains (isolated and used by Mehboob *et al*., 2008) were collected from the Soil Microbiology and Biochemistry Laboratory of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Four strains, each of *Rhizobium*

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phaseoli, *Mesorhizobium ciceri* and *Rhizobium leguminosarum* were taken and stored for future experimentation (Table 1).

Table 1. Rhizobial strains used in experiment

Species	Strain	Host plant
Rhizobium phaseoli	A2	Mung bean
Rhizobium phaseoli	A3	---do---
Rhizobium phaseoli	S17	---do---
Rhizobium phaseoli	N8	---do---
Mesorhizobium ciceri	CRI-28	Chickpea
Mesorhizobium ciceri	CRI-31	---do---
Mesorhizobium ciceri	CRI-32	---do---
Mesorhizobium ciceri	CRI-38	---do---
Rhizobium leguminosarum	LSI-23	Lentil
Rhizobium leguminosarum	LSI-26	---do---
Rhizobium leguminosarum	LSI-29	---do---
Rhizobium leguminosarum	LSI-30	---do---

Inoculum preparation

Fresh inoculum was prepared by taking 25 mL of sterilized yeast mannitol broth in twelve 50 mL conical flasks. Each flask was inoculated with one rhizobial strain and incubated at 28 ± 1 ºC for four days in shaking incubator at 100 rpm. An un-inoculated control was also maintained. In order to attain uniform cell density, optical density (OD) of 0.5 of each broth was achieved using OD meter.

Seedling inoculation

Twelve day old rice seedlings were obtained from the Agronomy Experimental Farm of the University of Agriculture, Faisalabad. The seedling roots were inoculated by dipping in freshly prepared inoculum for two hours under shade (Gutiérrez-Zamora and Romero, 2001).

Pot experiment

Thirty nine pots were filled with air dried; sieved, well mixed and physico-chemically analyzed soil. The soil was sandy clay loam having a pH of 7.8; ECe, 1.2 dS m^{-1}, organic matter, 0.68%; total N, 0.044%; available P, 7.8 mg kg^{-1} and exchangeable K, 123 mg kg^{-1}. Recommended doses of N, P and K (120-60-60 kg ha^{-1}) were applied using urea, single super phosphate and muriate of potash as source of nutrient, respectively. Inoculated seedlings with each strain were transplanted into pots and grown until maturity to get final yield. Pots were arranged with three repeats according to completely randomized design and were irrigated with good quality canal water.

The data collected were subjected to analysis of variance (Steel *et al*., 1997). Duncan's multiple rang test (DMR) was applied at 5 percent probability to compare treatment means (Duncan, 1955).

Results

Data regarding the plant height revealed that seven strains (A2, S17, N8, LSI-30, CRI-28, CRI-31, CRI-38) exhibited significant increase in plant height while from rest of the strains, three (LSI-23, LSI-26, LSI-29) improved the plant height non-significantly while two (A3, CRI-32) caused reduction in plant height compared to un-inoculated control (Table 2). Maximum increase of 28.34% in plant height was given by the strains A2 whereas minimum increase of 10.18% was recorded with the strain N8 in comparison to un-inoculated control. The strains A3 and CRI-32 remained deleterious by causing reduction of 1.91 and 0.64%, respectively, in plant height compared to un-inoculated control.

Number of tillers per pot in response to rhizobial inoculation was also monitored and it was found that numbers of tillers pot^{-1} were increased by all the inoculants compared to un-inoculated control (Table 2). Among the group of seven strains (A3, S17, N8, LSI-23, LSI-29, CRI-28, CRI-32) the increase (up to 46.48%) in number of tillers pot^{-1} was significant compared to control whereas the increase (up to 5.62%) caused by rest of the five strains (A2, LSI-26, LSI-30, CRI-31 and CRI-38) was statistically at par with control. The most efficient strain was LSI-29 which increased the number of tillers up to 46.47% followed by 28.14 and 38.9% increase given by the strains N8 and LSI-23, respectively, over un-inoculated control.

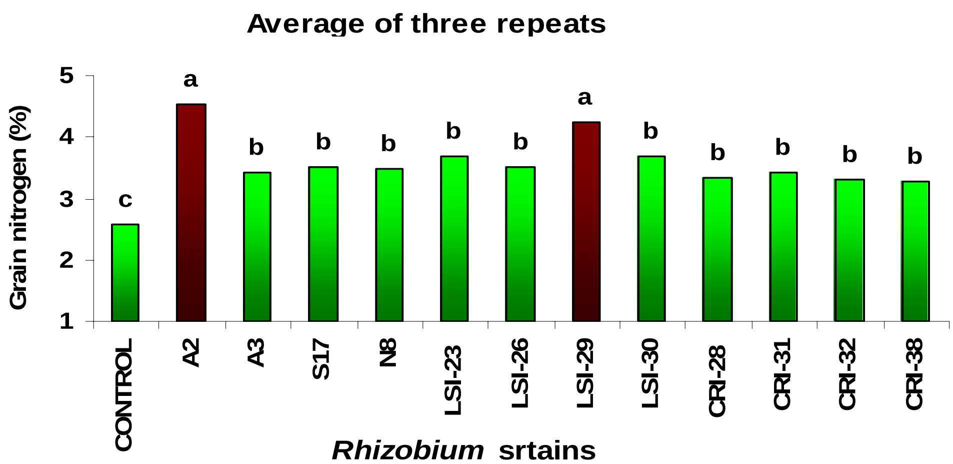
Regarding panicle length the data demonstrated that although all the test strains improved the panicle length of rice plants significantly with respect to un-inoculated control (Table 2) yet, most of the strains were at par with each other. Maximum of 54.11% increase in panicle length was recorded where the strain A2 was used as inoculant followed by the strains S17 and CRI-38 which showed 37.73 and 36.10% more panicle length than control. The least effective strain was A3 which yielded minimum increase of 18.05% compared to un-inoculated control.

In case of number of grains per panicle, nine strains i.e. A2, A3, S17, N8, LSI-23, LSI-26, LSI-30, CRI-28, CRI-32 yielded significant increase in number of grains per panicle whereas among the rest three, one strains (LSI-29) gave non-significant increment while rest of the two strains (CRI-31, CRI-38) showed reduction in number of grains per panicle compared to un-inoculated control (Table 2). Highest increase of 29.58% in number of grain per panicle was given by the strain A2 which was statistically similar in

Table 2. Effect of *Rhizobium* sp. on the growth parameters of rice

Treatment	Plant height (cm)	No. of tillers pot^{-1}	Panicle length (cm)	No. of grains panicle^{-1}
Control	104.67e	23.67e	20.33d	089.00de
A2	134.33a	24.33e	31.33a	115.33a
A3	102.67e	28.33bc	24.00c	098.33bc
S17	115.67cd	28.67bc	28.00b	099.33bc
N8	115.33cd	30.33b	26.00bc	104.33b
LSI-23	105.33e	31.00b	25.33bc	095.33cd
LSI-26	107.00e	25.00de	24.67c	096.67c
LSI-29	108.33de	34.67a	25.33bc	089.67de
LSI-30	121.67bc	24.67de	26.00bc	113.33a
CRI-28	125.33b	27.33cd	26.67bc	100.00bc
CRI-31	120.00bc	25.00de	26.00bc	087.33e
CRI-32	104.00e	29.67bc	26.33bc	101.33bc
CRI-38	122.33bc	24.37de	27.67b	088.67e
LSD value	007.7738	02.564	02.404	006.063

*Values sharing similar letter (s) do not differ significantly at $P < 0.05$, Duncan's Multiple Range Test



Bars sharing similar letter(s) do not differ significantly at $P < 0.05$, Duncan's Multiple Range Test

Figure 1. Effect of *rhizobium* inoculation on grain nitrogen content of rice under pot conditions

its effects to LSI-30 which showed an increase of 27.34% over un-inoculated control. The strain LSI-26 appeared as the least effective with a significant increase of 8.62% in number of grains per panicle compared to un-inoculated control. Whereas, negative effect was recorded in case of the strain CRI-31 and CRI-38 as they showed reduction of 1.88 and 0.37%, respectively, in number of grains per panicle with respect to control.

Results regarding fresh biomass per pot revealed that half of the strains i.e. A2, S17, N8, LSI-26, LSI-29, LSI-30 caused significant increase in fresh biomass per pot while the remaining half i.e. A3, LSI-23, CRI-28, CRI-31, CRI-32, CRI-38 exhibited non-significant increase compared to un-inoculated control (Table 3). Highest increase of 18.60% was caused by LSI-29 over un-inoculated control followed by an increase of 14.35% produced by S17. The strain A3 caused lowest increase (1.57%) in fresh biomass per pot in comparison with control.

Except two (LSI-26 and LSI-30) all the strains (A2, A3, S17, N8, LSI-23, LSI-29, CRI-28, CRI-31, CRI-32, CRI-38) significantly increased paddy yield per pot of rice in comparison with control (Table 3). The strain LSI-29 showed highest increase of 43.48% in paddy yield per pot over un-inoculated control followed by A2 which increased paddy yield by 25.52% over un-inoculated control. The lowest increase of 12.27% in paddy yield per pot was given by the strain A3 with respect to control. The most efficient group i.e. S17, CRI-38, CRI-31, N8, CRI-32, LSI-23 caused significant increase in paddy yield which ranged between 21.77 to 26.97% as compared to un-inoculated control. A non-significant increase of 5.16 and 4.22% in paddy yield was also recorded in case of the strains LSI-26 and LSI-30, respectively, compared to un-treated control.

The investigations on the potential of the test strains revealed that all were having the ability to significantly increase the straw dry weight per pot in comparison with control (Table 3). Overall the improvement in straw dry weight ranged between 10.43 to 45.51%. The strains LSI-29 and A2 were found to be the most efficient ones. The next efficient group was (CRI-28, LSI-23, A3, CRI-31, CRI-38, N8, S17) which improved straw dry weight by 22.39 to 29.10% compared with control. The strains LSI-30 and LSI-26 remained the least effective over un-inoculated control.

Rhizobial inoculation significantly increased the nitrogen contents of rice grains compared with un-inoculated control (Figure 1). Maximum increase of 76.26% in nitrogen contents of rice grains was recorded in case of inoculation with the strain A2 compared to un-inoculated control. The strain CRI-38 affected grain nitrogen content least efficiently and improved the nitrogen content of rice grain by 27.24% in comparison to un-inoculated control.

Most of the *rhizobium* strains exhibited significant increase in grain phosphorus content except few where increased phosphorus content was statistically similar to uninoculated control (Figure 2). The strain S17 showed maximum increase of 150% in grain phosphorus content over uninoculated control whereas minimum increase of 100% was recorded by inoculation with all of the other strains except three (N8, LSI-30 and CRI-31) which showed same non-significant increase of 50% in grain phosphorus over uninoculated control.

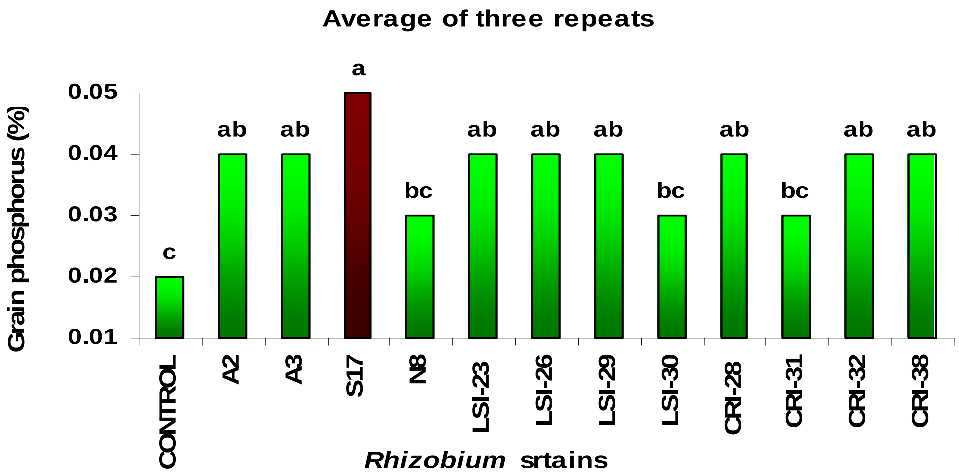
Table 3. Effect of *Rhizobium* sp. on the growth parameters of rice

Treatment	Plant biomass (g pot^{-1})	Paddy yield (g pot^{-1})	Straw yield (g pot^{-1})	1000-grain (g)
Control	148.67d	27.70f	44.67g	16.33d
A2	164.67bc	34.77b	61.00b	20.33b
A3	151.00d	31.10de	57.00bcd	18.90bc
S17	170.00b	35.17bc	54.67cde	19.73b
N8	167.00bc	33.73bc	55.33cde	19.40bc
LSI-23	151.00d	33.77bc	57.33bc	18.73bc
LSI-26	161.00c	29.13ef	52.67ef	18.63bc
LSI-29	176.33a	39.73a	65.00a	22.33a
LSI-30	160.33c	28.87ef	53.00def	19.93b
CRI-28	151.67d	33.00cd	57.67bc	18.63bc
CRI-31	153.33d	34.77bc	56.67cde	19.20bc
CRI-32	152.00d	34.33bc	49.33f	17.30cd
CRI-38	153.00d	34.87bc	56.33cde	19.53bc
LSD value	006.158	02.395	03.695	01.969

*Values sharing similar letter (s) do not differ significantly at $P < 0.05$, Duncan's Multiple Range Test

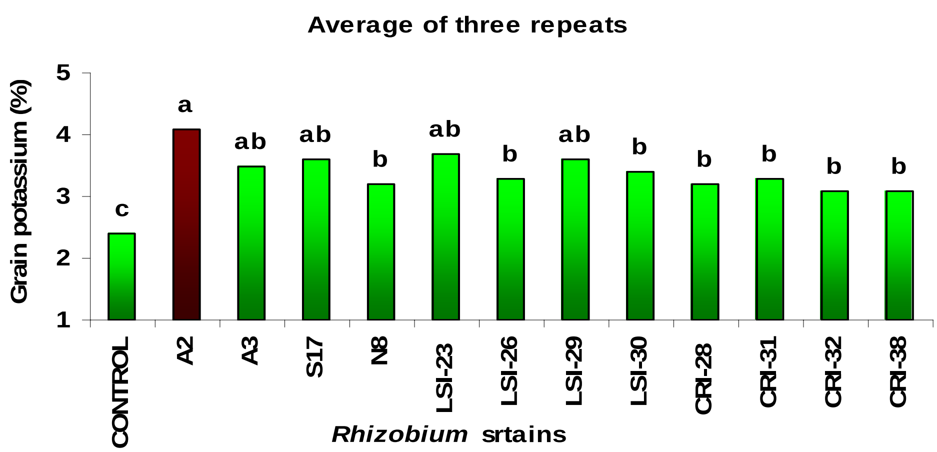
The 1000-grain weight of rice was significantly improved by all the test strains except CRI-32 which although increased 1000-grain weight but was statistically non-significant with respect to un-inoculated control (Table 3). The strain LSI-29 appeared as the most efficient as it showed maximum significant increase of 36.74% in the 1000-grain weight of rice in comparison to un-inoculated control which was followed by 24.49 and 22.05% increase caused by the strains A2 and LSI-30, respectively, over un-inoculated control. The strain LSI-26 and CRI-28 proved to be the least effective and both showed same minimum increase of 14.08% over un-inoculated control. Rest of the *rhizobium* strains i.e. A3, S17, N8, LSI-23, CRI-31 and CRI-38 increased the1000-grain weight which varied from 14.70 to 20.82% compared to un-inoculated control.

Data regarding potassium contents of rice grain showed significant increase in potassium contents upon inoculation with all the *rhizobium* strains as compared to uninoculated control (Figure 3). Maximum increase of 70.83% was observed where the strain A2 was used as inoculant followed by the strain LSI-26 which increased grain potassium content by 50% over uninoculated control. The strains CRI-32 and CRI-38 showed a minimum of 29.17% increase in grain potassium content of rice in relation to uninoculated control. Increase in potassium content of rice grain caused by rest of the strains i.e. A3, S17, N8, LSI-23, LSI-29, LSI-30, CRI-28 and CRI-31 varied between 33.33 to 45.83% compared to control.



Bars sharing similar letter(s) do not differ significantly at $P < 0.05$, Duncan's Multiple Range Test

Figure 2. Effect of *rhizobium* inoculation on grain phosphorus content of rice under pot conditions



Bars sharing similar letter(s) do not differ significantly at $P < 0.05$, Duncan's Multiple Range Test

Figure 3. Effect of *rhizobium* inoculation on grain potassium content of rice under pot conditions

Discussion

The results of the present study have established the effectiveness of the local *rhizobium* strains for improving the growth and yield of local rice cultivar "Super Basmati" in pots under natural conditions. The results showed that almost all the test strains of *rhizobium* caused improvement in all the parameters except plant height where two strains (A3 and CRI-32) and number of grains per panicle where two strains (CRI-32 and CRI-38) affected deleteriously and caused reduction up to 1.91% compared to control. The increment in the parameters in response to rhizobial inoculation endorsed the fact that the test strains were having one or more growth promoting mechanisms including mobilization and efficient uptake of nutrients (Biswas *et al*., 2000a, b), enhancement in stress resistance (Alami *et al*., 2000), solubilization of insoluble phosphates (Alikhani *et al.,* 2006), induction of systemic disease resistance (Tuzun and Kloepper, 1994), inhibition of fungal growth (Nautiyal, 1997), production of phytohormones (Dakora, 2003), vitamins (Dobbelaere *et al.,* 2003) and siderophores (Neiland and leong, 1986). These results are in line with Biswas *et al.* (2000b) who have reported 16% increase in number of panicles per plant of rice and suggested that the improvement was due to increased availability of nutrients and phytohormones like indol acetic acid and ethylene. Similarly, Chi *et al.* (2005) observed up to 23.63% increase in plant height of rice over un-inoculated control and argued indol acetic acid and gibberellins production as the key mechanism for that improvement. Also, an increase of 27.11% in number of panicles per plant of rice due to *rhizobium* inoculation over uninoculated control was recorded by Peng *et al*. (2002) who have suggested efficient nutrient and water uptake as important mechanisms. In the same way, Singh *et al*. (2005) showed significant increase in plant height up to 6.9% over un-inoculated control. Results from our study regarding fresh biomass and straw dry weight (g pot^{-1}) of rice showed up to 18.60 and 45.51% increase, respectively, over un-inoculated control. These results are also similar with those of Singh *et al*. (2005) who found significant increase of 29.09% in straw dry weight (g pot^{-1}) by rhizobial inoculation over control and proposed phosphate solubilization and plant growth regulators production as the key mechanisms. Similarly, 42.55% increase in straw dry weight (g pot^{-1}) was observed due to auxin and gibberellin production by Chi *et al*. (2005).

The results also indicated that effect of some strains on some parameters was deleterious compared to un-inoculated control which may be due to the over production of microbial metabolites like auxin (Perrine *et al*., 2005), cyanogens (Antoun *et al.*, 1998) or due to the toxic accumulation of nitric oxide (NO) in the rhizosphere (Francine *et al.*, 2007).

Comparison of the inoculated *rhizobium* species among each other demonstrated that the strains of *R. phaseoli* and *R. leguminosarum* performed better than the isolates of *Mesorhizobium ciceri*. The strains A2 (*R. phaseoli*), and LSI-29 (*R. leguminosarum*) proved most proficient compared to all other inoculants. This variation in relative efficiency of different strains explains their ability to cope with specific crop. These findings are in conformity with Mehboob *et al*. (2008) who have already verified this kind of behavior from different *rhizobium* strains used as inoculant in maize. Furthermore, differential behavior of different PGPR strains of *rhizobia* against common host has also been reported by Piesterse *et al*. (2001).

Conclusion

This study reveals that among the *rhizobium* strains tested, the strains A2 (*R. phaseoli*) and LSI-29 (*R. leguminosarum*) performed better than all other strains in improving the different growth and yield parameters which may imply that these could be used as PGPR for enhancing the growth and yield of rice. However, further field oriented investigations are needed to confirm their potential.

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