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Preliminary screening of rhizobium isolates for improving growth of maize seedlings under axenic conditions

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Abstract

Experiments were carried out to examine the activities of rhizobia, isolated from three legumes (Mungbean, chickpea and lentil) for improving growth and yield of maize under axenic conditions. Twenty strains of rhizobia were isolated from root nodules of each, host legume by using dilution plate technique. These isolates were screened separately by conducting three jar experiments under axenic conditions. Most of the isolates had the potential to modify the growth of maize seedlings under axenic conditions. Results of the mungbean isolates (Rhizobium phaseoli) showed that inoculation with all the rhizobial isolates increased the root/shoot length and seedling biomass (up to 48.53, 21 and 35.03%, respectively over uninoculated control) except three isolates which reduced the biomass of maize seedlings. In the experiment with chickpea isolates (Mesorhizobium ciceri), only eight isolates increased the root/shoot length and seedling biomass (up to 27.69, 8.9 and 60.56%, respectively over uninoculated control) whereas three isolates did not show any effect while nine isolates showed negative effect and reduced the shoot length of maize seedlings. In the experiment with lentil isolates (Rhizobium leguminosarum), only one isolate increased the biomass and two increased the root length whereas in case of shoot length, seven strains showed positive effect. It is suggested that screening and extending the potential of these bacteria would be a useful approach for enhancing growth and yield of maize among resource-poor farmers in developing countries like Pakistan.

Key words: Plant growth, screening, rhizobia, axenic conditions, maize

Introduction

Altering the rhizosphere microflora by seed or root inoculation with specific organisms has long been recognized as a practical possibility. The objective of this practice is to modify plant growth in a desired direction as a result of beneficial interaction between the plant and the inoculated/introduced microorganisms. All those bacteria inhabiting plant roots and influencing the plant growth positively by any mechanism are referred to as plant growth promoting rhizobacteria (PGPR) (Arshad and Frankenberger, 1998). During the last couple of decades, the use of PGPR as inocula has been dramatically increased. Rhizobia which are Gram-negative chemo-organotrophic or chemolitotrophic soil bacteria belonging to the family Rhizobiacea can also act as PGPR as reported by many researchers (Noel *et al*., 1996). Rhizobial inoculation of legume seeds is well studied. However, much less information is available regarding the association and growth promoting activity of rhizobia with non-legumes. In nature, rhizobia do associate with roots of non-legumes without forming true nodules (Ladha *et al*., 1989) and can promote the growth of non-legumes upon inoculation (Yanni *et al*., 1997). The degree to which this association benefits plant growth varies with variety, cultural conditions, and inoculant strains (Dobbelaere *et al*., 2003). The mechanisms of growth promotion by rhizobia on non-legumes include production of phytohormones and/or phosphate-solubilizing activity (Chabot *et al*., 1996), inhibition of fungal growth (Nautiyal, 1997), and antagonism of the indigenous soil microflora (Schloter *et al*., 1997).

Root colonization is an important first step in the interaction of beneficial bacteria with plants (Kloepper and Beauchamp, 1992). A number of workers have experimentally demonstrated the ability of rhizobia to colonize roots of certain cereal crop plants, promoting their growth and grain yield at harvest while reducing their dependence on chemical fertilizer inputs, independent of root nodulation and biological N_2 fixation (Lupwayi *et al*., 2004). Chi *et al*. (2005) in their findings revealed that rhizobial inoculation with non-legumes increased the root and shoot biomass, their photosynthetic rate, stomatal conductance, transpiration velocity, water utilization efficiency and flag leaf area and accumulated higher levels of indole acetic acid and gibberellins, growth regulating phytohormones. However, some scientists have reported that rhizobium strains have both plant growth promoting

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and deleterious effects on non-legumes and concluded that only specific strains of rhizobium and bradyrhizobia have the potential to be used as PGPR on non-legumes (Antoun *et al*., 1998).

Keeping this in view, selection of effective plant growth promoting strains of rhizobia for specific cereal crop is a critical aspect. Hence, a study was conducted for the preliminary screening of rhizobial strains on the basis of growth promoting activities in maize under axenic conditions.

Materials and Methods

Isolation of rhizobial strains

Several bacterial strains were isolated from the root nodules of chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* M.) and mungbeen (*Vigna radiata* L.) grown at different locations of Faisalabad, Pakistan by using yeast mannitol agar (YMA) media. Plants of each host were uprooted along with non-rhizospheric soil and brought to laboratory in polythene bags. The non-rhizosphere soil was removed from plant roots by gentle shaking while rhizospheric soil adhered to roots was removed by dipping and gentle shaking in water under aseptic conditions. The nodules were removed from the roots of each legume host and placed separately in Petri plate. Then the nodules were surface-sterilized by dipping momentarily in 95% ethanol and 0.2% $HgCl_2$ solution for 3 minutes followed by 6 washings with sterilized distilled water. Surface-sterilized nodules from each host crop were crushed in sterilized test tube having sterilized distilled water with sterilized glass rod. The suspension obtained was used to inoculate YMA media and pure cultures were obtained by further streaking 3-4 times on fresh medium. A total of sixty fast growing colonies of bacteria, twenty from each host crop, were selected. The isolated rhizobial strains were coded (Table 1) and stored in slants at -20°C for subsequent use.

Screening rhizobial strains for growth promoting activity (Jar experiments)

A series of experiments were conducted on maize in the growth room under controlled conditions to screen rhizobial strains for their growth promoting ability. Sterilized glass jars were used for the experiment. Sterilized broth was prepared by using YMA medium and poured in sixty 100 mL sterilized conical flasks. Each conical flask containing 50 mL sterilized broth was inoculated with selected strains of rhizobia. Rhizobial strains in the conical flask were incubated in a shaking incubator (Firstek Scientific, Tokyo, Japan), at 28 ± 1 ^{0}C for 72 h at 100 rpm. An optical density of 0.5 recorded at a wavelength of 535 nm, was achieved by dilution to achieve uniform cell density (10^8-10^9 CFUmL^{-1}).

Maize (*Zea mays* L.) cv. Neelum seeds were surface-sterilized by dipping momentarily in 95% ethanol and 0.2% $HgCl_2$ solution for 3 minutes followed by 6 washings with sterilized distilled water. Surface-sterilized seeds were inoculated by dipping for five minutes in the broth of respective rhizobial culture. Surface-sterilized maize seeds were sown on sterilized filter paper sheets placed in autoclaved Petri plate which were incubated at 28 ± 1 ^{0}C for 4 days. Sterilized distilled water was used for maintaining optimum moisture for germination. Three uniformly germinated seedlings were sandwiched between two sterilized filter paper sheets soaked in suspension containing desired inoculum. These sheets were rolled and placed in sterilized jars as explained by Asghar *et al.* (2004). In the case of uninoculated control, sheets were soaked with sterilized broth. Hoagland solution (Hoagland and Arnon, 1950) was applied in jars to provide nutrition to the plants. Experiment was conducted for twenty days in growth room at 28 ± 1 ^{0}C with 10 hours light and 14 hours dark period and light intensity was set to 275 µmol m^{-2} s^{-1}. Data regarding the growth parameters (root/shoot length and biomass yield) were recorded. Standard error of means was calculated (Steel *et al*., 1997).

Results

Twenty strains of rhizobia isolated from root nodules each of mungbean, chickpea and lentil were tested separately by conducting three jar experiments under axenic conditions to assess their potential for improving growth of maize seedlings. The results are described below:

Rhizobium phaseoli

Results of inoculation with mungbean rhizobial isolates (*Rhizobium phaseoli)* indicated that all the isolates had the potential to increase the root length (Figure 1). Among the twenty isolates tested, a maximum of 48.53% increase in root length was shown by the isolate N_{42} followed by S_{43} which showed 39.71% increase in root length over un-inoculated control. The next effective group of isolates was N_{16}, A_3, S_{17}, S_{25}, N_8, A_{18}, A_{13}, S_{24}, A_{22}, S_9, A_{23}, N_{11} and S_6 which caused an increase in the range of 23.53-36.76% over uninoculated control. Rest of the isolates were also able to promote root growth however; they were relatively less effective in root elongation. The least effective isolate was N_{15} which showed only 2.94% increase over uninoculated control.

Shoot length in response to rhizobial inoculation was also monitored (Figure 2). Overall, shoot length was higher

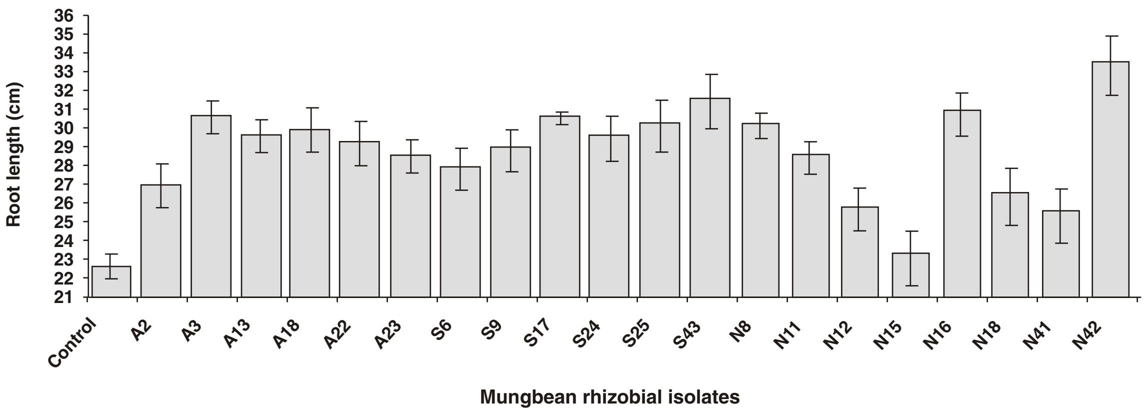

Figure 1. Effect of rhizobial inoculation on root growth of maize

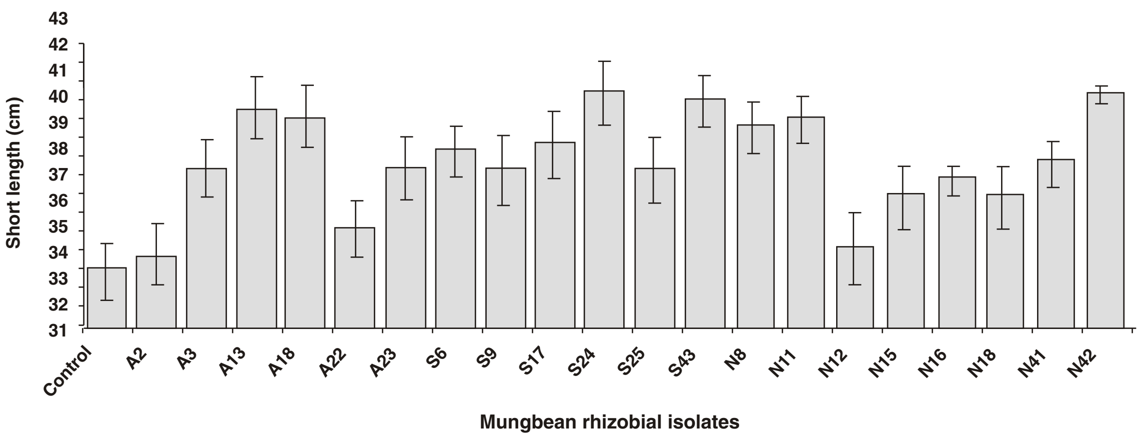

Figure 2. Effect of rhizobial inoculation on root growth of maize

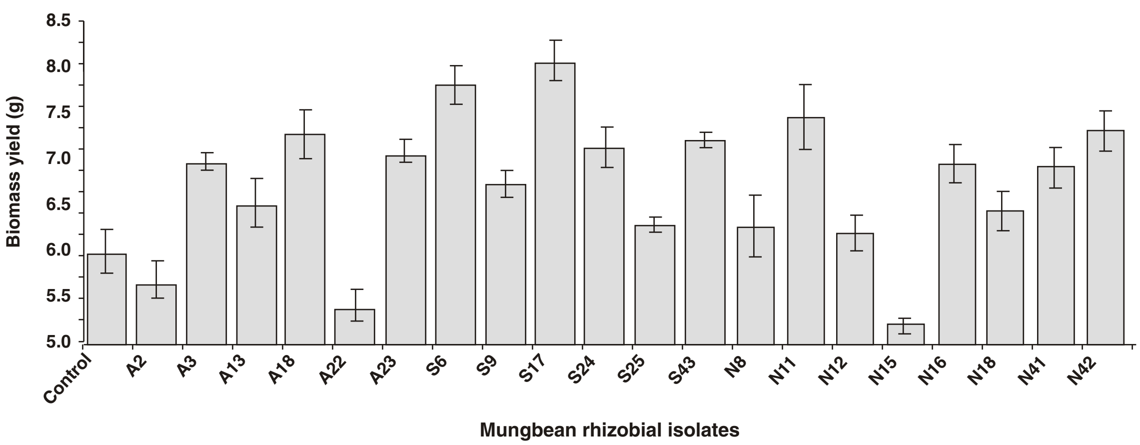

Figure 3. Effect of rhizobial inoculation on root growth of maize

Table 1. Coding of the isolated rhizobial strains

Serial No.	Mungbean 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

(varied from 2 to 21%) in all the inoculated treatments as compared to uninoculated control. Highest increment of 21% in shoot length was produced by the isolates N_{42} and S_{24} while lowest increment of 2% was caused by the isolate A_2 compared with uninoculated control.

Data regarding seedling biomass revealed that most of the rhizobial isolates had positive effect while few had negative effect on seedling biomass (Figure 3). The most promising isolate was S_{17} showing an increment of 35.03% in seedling biomass followed by isolate S_6 which showed 30.53% higher yield in seedling biomass over uninoculated control. Whereas, other bacteria increased the seedling biomass in the range of 4.10-25.05% except three isolates (A_2, A_{22} and N_{15}) which showed negative effect compared with uninoculated control and decreased the seedling biomass. The maximum decrease of 12.84% in seedling biomass was observed where rhizobial isolate N_{15} was used as inoculant.

Mesorhizobium ciceri

After twenty days, it was observed that the extent to which all the inoculated isolates affected the root length of maize seedlings appeared to have higher increase than uninoculated control (Figure 4). The isolate CRI-37 remained superior by yielding a maximum of 27.69% increase in root length over uninoculated control. The increase in the root length caused by the other strains varied from 1.54 to 23.08% over uninoculated control. The minimum increase in root length was 1.54% produced by the isolate CRI-29.

In case of shoot length, inoculation with chickpea rhizobial isolates showed negative, positive and even no effects (Figure 5). Only eight isolates showed positive effect, among which maximum increase in shoot length was 8.9% produced by CRI-22 and the minimum was 0.68% produced by CRI-24, 27 and 28. There were three isolates which showed no effect whereas nine isolates showed reduction in shoot length up to 6.85% upon inoculation compared with uninoculated control.

Regarding seedling biomass, the data demonstrated that growth stimulating activities of all the strains were positive (Figure 6). Maximum increase (60.56%) in seedling biomass was observed where CRI-28 was used as inoculant and minimum increase (3.86%) was produced by CRI-38 compared with uninoculated control.

Rhizobium leguminosarum

In all the three parameters i.e. root length, shoot length and seedling biomass, all the lentil rhizobial inoculants (*R. leguminosarum*) showed negative effect except 2, 7 and 1 isolates, respectively, which were having potential to increase these growth parameters (Figure 7, 8, 9). Maximum of 9.72, 12.5 and 2.34% increase in root length, shoot length

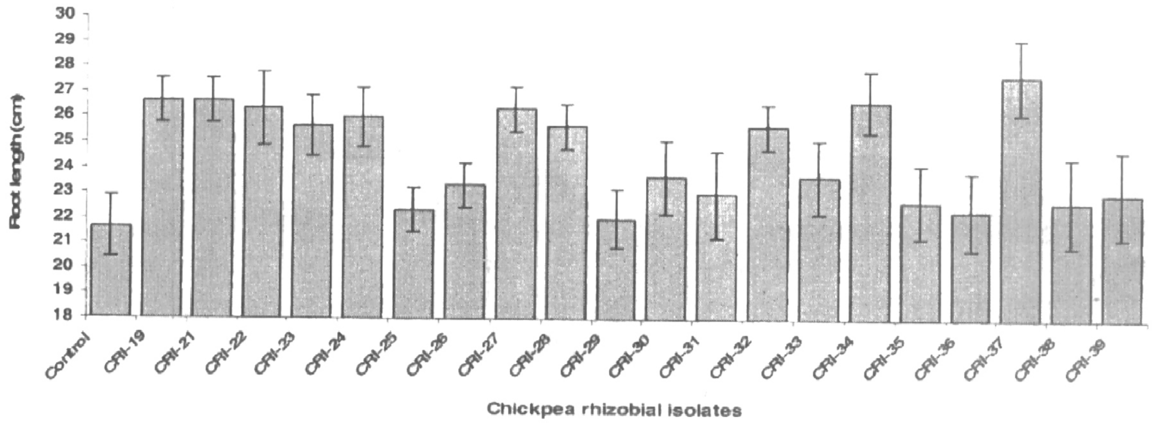

Figure 4. Effect of rhizobial inoculation on root growth of maize

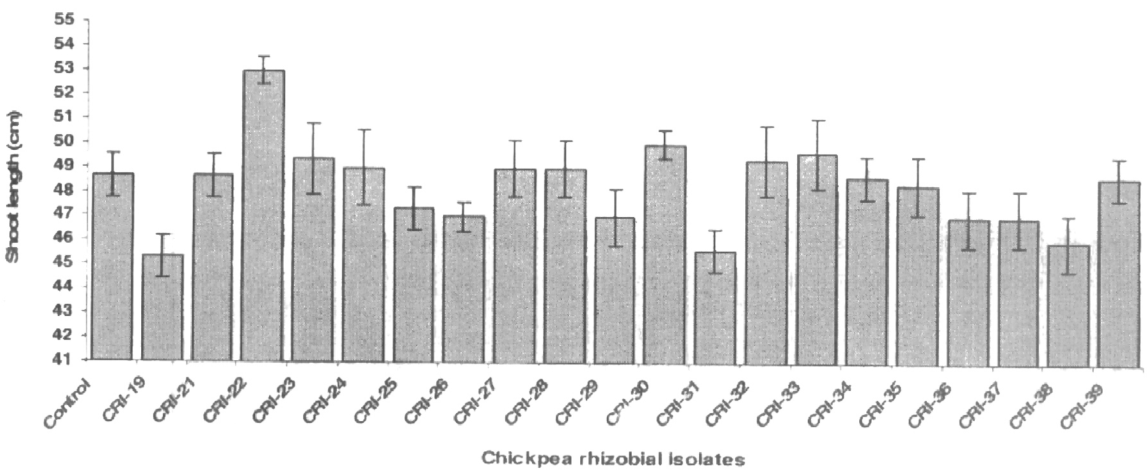

Figure 5. Effect of rhizobial inoculation on shoot growth of maize

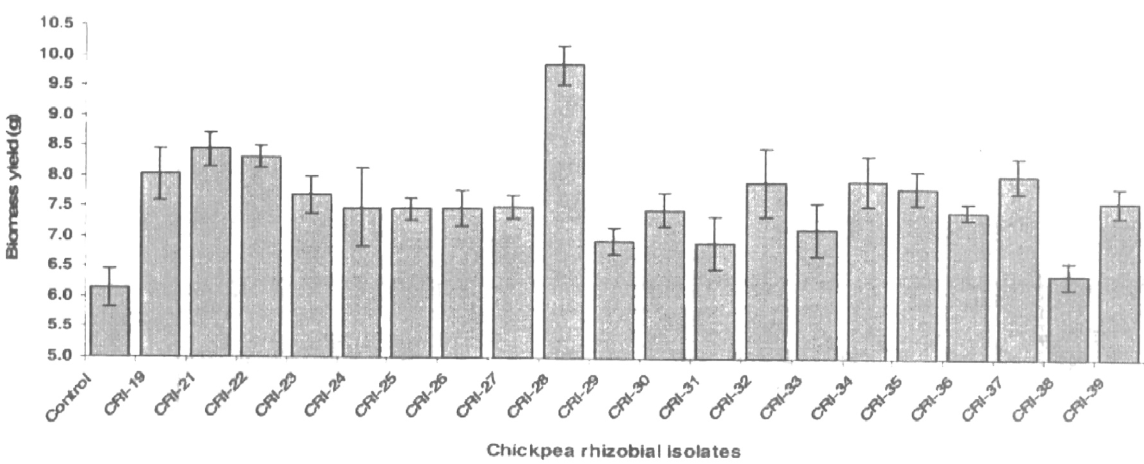

Figure 6. Effect of rhizobial inoculation on biomass yield of maize

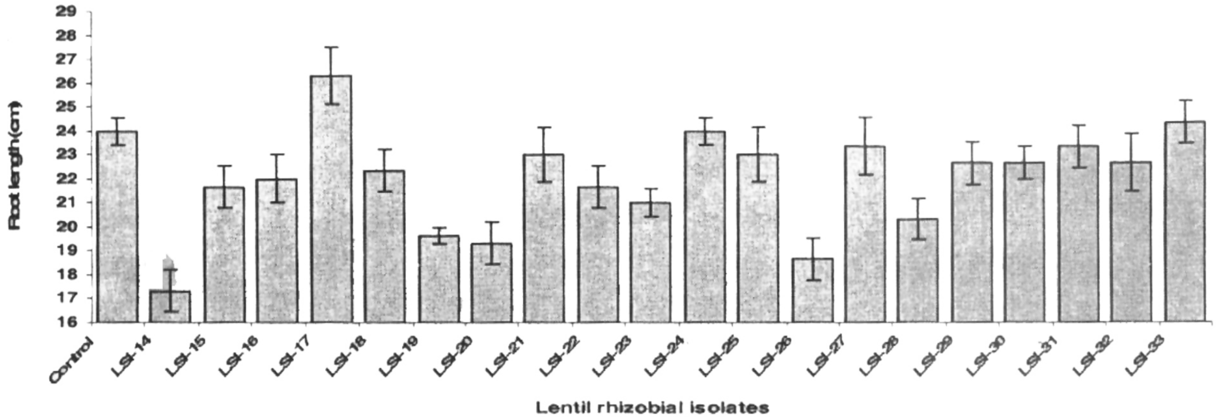

Figure 7. Effect of rhizobial inoculation on root growth of maize

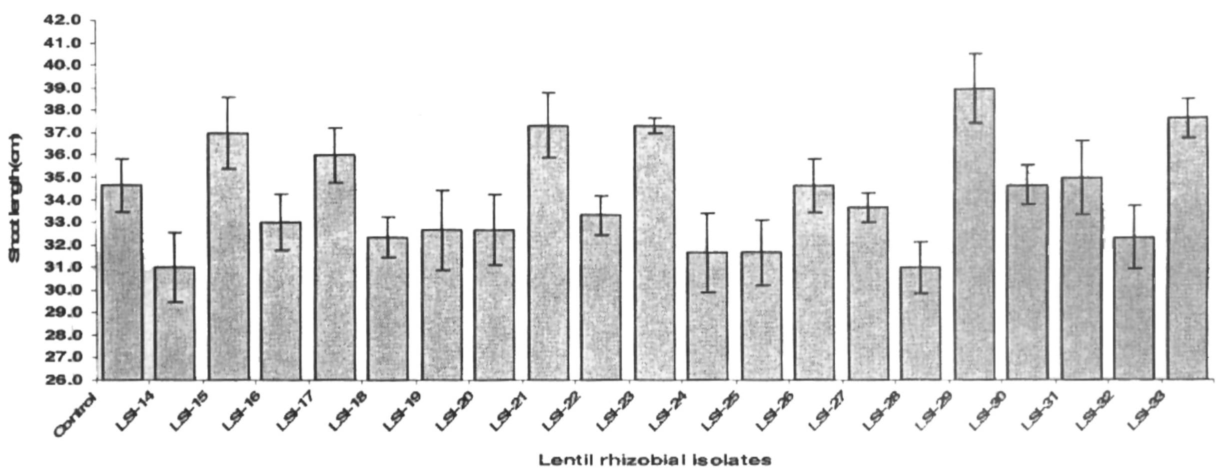

Figure 8. Effect of rhizobial inoculation on shoot growth of maize

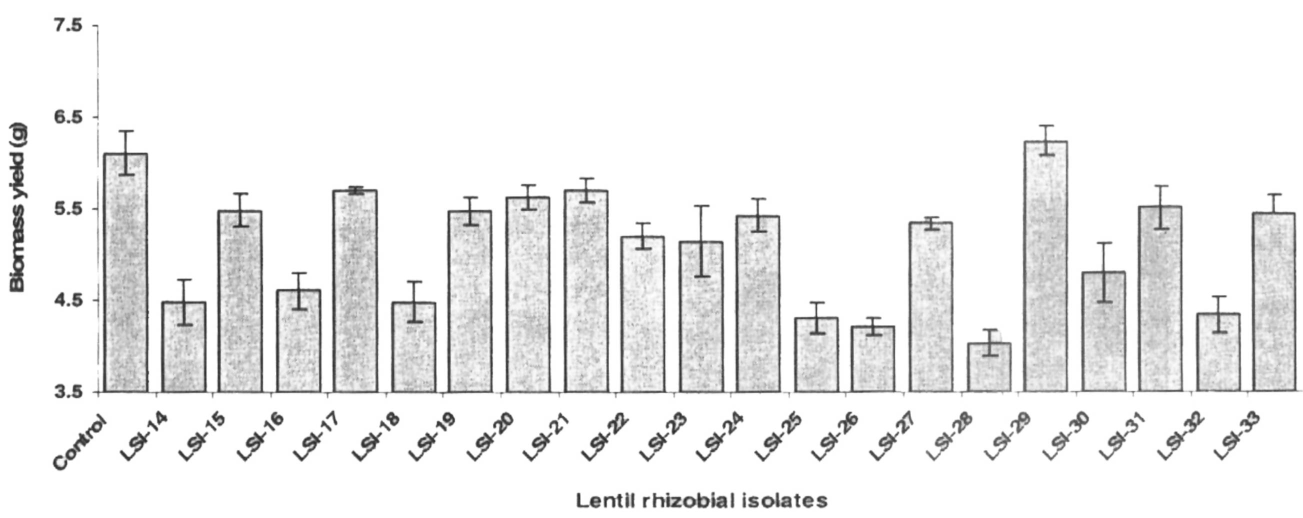

Figure 9. Effect of rhizobial inoculation on biomass yield of maize

and seedling biomass was recorded where LSI-17, LSI-29 and LSI-29, respectively, were used as inoculant.

Discussion

This study demonstrates the preliminary screening of rhizobial strains on the basis of growth promoting activities in maize under axenic conditions. The results showed that most of the mungbean (*Rhizobium phaseoli)* and chickpea (*Mesorhizobium ciceri*) rhizobial isolates were able to improve the growth parameters i.e. root/shoot length and seedling biomass. The beneficial effects of these rhizobial isolates may be attributed to one or more of the mechanisms including direct stimulation by the production of phytohormones (Humphry *et al*., 2007), siderophores (Meyer, 2000), increase in supply of nutrients (Biswas *et al*., 2000) by enhanced solubilization of P (Richardson *et al*., 2001) and indirectly by modifying the microbial balance in the rhizosphere through suppression or elimination of deleterious microbes by modulating competitiveness (Essalmani and Lahlou, 2002) or by antibiosis (Handlesman and Stabb, 1996) or by inducing systemic resistance (ISR) towards pathogenic microorganism (Liu *et al*., 1995). The results are in conformity with the findings of Humphry *et al*. (2007) who have also reported a successful usage of rhizobial culture for the promotion of barley seedling growth, in terms of better root and shoot length due to the production of phytohormones. Also an increase of 75 and 332% in biomass and root length of cotton cultivar was recorded, respectively (Hafeez *et al*., 2004) due to inoculation with *Rhizobium leguminosarum bv. trifolii* E11 under growth room conditions through efficient nutrient uptake mechanism**.** Similarly, Anyia *et al*. (2004) also observed an increase of 49% in total biomass of wheat upon rhizobial inoculation and hypothesized that the observed effects may be caused by changes in root morphology that may increase nutrient use efficiencies as growth promoting mechanism.

When the results of mungbean and chickpea isolates were compared with each other, it was observed that the increase in the growth parameters i.e. root/shoot length and seedling biomass was more where inoculation was done by *R. phaseoli* isolates compared with *Mesprjozbium ciceri* isolates. The differences in the relative efficiency of different host isolates suggest variation in their ability to react against a common plant. These results are in line with Piesterse *et al*. (2001) who have already reported differential behavior of different plant growth promoting rhizobial strains against common host.

Regarding *R. leguminosarum* isolates, the results showed positive effects; may be because of one or more growth promoting mechanisms already mentioned above, negative effects; may be due to the over production of chemicals i.e. Auxin (Perrine *et al*., 2005), HCN (Antoun *et al*., 1998), toxic accumulation of nitric oxide (Perrine *et al*., 2007) and enzymes in the rhizosphere i.e. urease (El-Tarabily *et al*., 2006) and no/neutral effect upon inoculation may be due to the non competitiveness of the strains with the maize root rhizosphere as explained by Rosenblueth and Martinesz-Romero (2004) that only those rhizobial strains which had the tolerance to 6-methoxy-2-benzoxazolinone (MBOA), a maize antimicrobial compound released by maize roots in the rhizosphere that was inhibitory to some bacteria could colonize the maize root and affect the plant.

Conclusion

Rhizobial isolates could be used for improving growth of maize crop. However, their effectiveness varies with the rhizobium species.

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