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Short Communication

Screening of *Mesorhizobium ciceri* isolates from rainfed area for improvement in growth and nodulation of chickpea seedlings under controlled conditions

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Abstract

In Pakistan, about 18% of total cultivated area is rainfed. The production in these areas is entirely dependent on annual precipitation. The return from agriculture in these areas is low as compared with irrigated areas and efforts are therefore required to boost up the crop yields. So, this study was conducted to assess the potential of Rhizobium isolates for improving chickpea growth parameters under axenic conditions. Fifteen Mesorhizobium ciceri strains (JK-1 to JK-15) were isolated from the root nodules of chickpea grown in rainfed area for the study. Three chickpea seeds were grown in each jar after inoculation with Mesorhizobium ciceri cultures having uninoculated control. The jars were placed in the growth room under axenic condition. After eight weeks data for growth parameters and nodulation were collected. It is revealed from the data that Mesorhizobium ciceri isolates significantly improved the fresh and dry shoot/root weight and shoot/root length over the control but potential of improvement in these parameters varied with the isolates. The increase ranged between 35 to 60% in various isolates. The isolates Jk-10, Jk-8 and Jk-4 showed better results than other isolates. As far as nodulation is concerned, up to 13 nodules were found in plants treated with Jk-10 isolate.

Keywords: Screening, chickpea, *Mesorhizobium, nodulation, growth*

World demand for food production has increased enormously with the fast growing population. Chemical fertilizers are an important source of plant nutrition to maintain soil productivity. These have played a significant role in the green revolution throughout the world and have improved the crop yields. Since the excessive application of inorganic fertilizers has created the health and environmental problems, researchers in the world have focused their attention to alternative supplementary approaches that can ensure competitive yields without disturbing the soil health and environmental quality. In this regard, use of microbial inoculants in agriculture represents an environmental friendly approach for improving the yields and to reduce the dependence on expensive chemical fertilizers (Kantar *et al.,* 2007). The symbiotic nitrogen fixation can play an important role in improvement of soil fertility and productivity.

During the last century, biological nitrogen fixation has attracted the scientists working on plant nutrition. This has proved its efficacy in the improvement of crop yields in most parts of the world (Wakelin and Ryder, 2004). These are easy to use due to low cost and have less environment risk (Herridge *et al.,* 2002). Biofertilizers are an important component of organic farming in dry land areas where farmers mostly rely on low cost inputs. Soils in these areas are deficient in essential nutrients (Ali *et al*., 2002) and can be supplemented through the use of biofertilizers.

Legume inoculation is routine approach that has been followed for more than a century in agricultural systems (Brockwell and Bottomley, 1995). Legume-*rhizobium* symbiosis depends on the specificity of plant and bacterial species due to the presence of chemical signaling that results in formation of specialized structures i.e. nodules in which the bacteria are hosted and atmospheric nitrogen is reduced into ammonium (Bai *et al*., 2002). It is established that world supply of organic nitrogen is met via symbiosis between root nodulating bacteria and leguminous host plants (Postgate, 1998).

Chickpea is one of the most important legume crops grown in rainfed parts of the world. It is mostly grown in the semi arid regions of Asia and Africa. It has high protein and carbohydrate ranging between 25-28% and 57-60%, respectively (Hulse, 1991). It also provides high quality crop residues for animal feed and helps to maintain soil fertility through biological nitrogen fixation (Kantar *et al*., 2007). In Pakistan, 90% of total chickpea production is from rainfed area. In rainfed areas, soils are generally deficient in major plant nutrients that affect the yields.

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Inoculation of crop with rhizobium may assist plants in recovering these deficiencies. Nutrient deficiencies in chickpea can cause the yield losses of varying magnitude e.g. around 10-45% due to nitrogen and phosphorus deficiency (Ali *et al*., 2002). So, it is required that rhizobium inoculation may be practiced for nitrogen fixation and better crop yields in these areas. Besides inoculation, it is also needed to find out the best isolates through screening for improvement in crop growth. Many research workers have explained their findings regarding the screening of rhizobium isolates for positive effect on various crops. Bremer *et al*. (1990) conducted a study for screening of 24 *R. leguminoserum* isolates for improvement in lentil growth under controlled conditions for seven weeks and reported the significant increase in biomass yield, number of nodules plant^{-1} and shoot weight of lentil seedlings. Romdhane et al. (2007) concluded in a screening programme of different strains of *M. ciceri* that shoot yield and nodule number of chickpea plants were significantly increased with inoculation over uninoculated control. Zafar-ul-Hye *et al*. (2007) found an increase in root/ shoot weight, shoot/ root length and nodule numbers while conducting screening study of 60 rhizobium isolates on lentil plants under axenic conditions. The improvement in growth parameters was observed, while screening of *rhizobacteria* isolates for improvement in growth of sunflower seedlings under axenic conditions (Arif *et al.,* 2010).

So, this study was conducted to isolate the *Mesorhizobium* isolates from rainfed area and assess their beneficial effect on improvement of chickpea growth and nodulation under controlled conditions.

The study was conducted in the Laboratories of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad and growth room of Agricultural Biotechnology Research Institute, Ayub Agricultural Research Institute, Faisalabad during June- July, 2008. Following steps were taken for the experiment.

Isolation of Mesorhizobium

The root nodules were collected from chickpea crop grown at Barani Agricultural Research Institute (BARI), Chakwal situated in rainfed area. Roots were washed extensively in tap water. Nodules were then transferred to sterilized Petri dishes. The collected nodules were surface sterilized by dipping momentarily in 95% ethanol solution followed by dipping in 0.2% $HgCl_2$ solution for 3-5 minutes and thorough washing with sterilized/distilled water (Russell *et al*., 1982). The nodules were crushed in sterilized water to form a suspension. A loopful of suspension was streaked on sterilized yeast manitol agar (YMA) media (Yeast, 0.5 g; manitol, 10.0 g; K_2PO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; distilled water, 100 mL; pH 6.8) and incubated at 28±2 °C. The colonies were selected, isolated and purified.

Preparation of inoculum

Sterilized yeast manitol broth was inoculated with Mesorhizobium isolates and incubated at 28±1°C for 4 days in a shaking incubator at 100 rpm. Fresh inoculum were prepared for each experiment.

Screening of Mesorhizobium strains under controlled conditions

Jar experiments were conducted in the growth room of the Agricultural Biotechnology Research Institute, Ayub Agricultural Research Institute, Faisalabad for screening of the *Mesorhizobium* strains under controlled conditions. Broth cultures were prepared by using YMA medium. The plastic jars of 500 g capacity were filled with autoclaved sand. Three inoculated seeds of chickpea cv. AZRI- 2000 were placed in each autoclaved growth jar. Nitrogen free sterilized Hoagland solution of half strength was applied during growth for supply of essential nutrients (Fahraeus, 1957). The jars were arranged in completely randomized design (CRD) and replicated thrice. These jars were placed in growth room under controlled temperature and humidity conditions. After eight weeks, data regarding root length and shoot length, fresh/dry root weight, fresh/dry shoot weight, and number of nodules per plant were recorded.

Statistical Analysis

The data were analyzed statistically according to Steel *et al*. (1997). Means were compared according to Duncan's Multiple Range Test (Duncan, 1955).

Mesorhizobium ciceri isolates were evaluated for their potential of improving chickpea seedlings growth under controlled conditions. The isolates were applied to chickpea seeds and grown in growth jars. It was observed during the study that all the isolates had positive effect on growth parameters as compared with control.

The data presented in Figure 1 reveals the effect of *Mesorhizobium* strains on the total biomass production plant^{-1} of chickpea seedlings. The highest value of 0.780 g plant^{-1} was recorded for isolate JK-10 which was closely followed by JK-8 and JK-4 with 0.740 g and 0.725 g plant^{-1} biomass production, respectively. The isolates JK-9, JK-5, JK-11 and JK-12 also exhibited encouraging results. The lowest biomass production was observed in uninoculated control plants. All the isolates showed better yield in comparison with the control seedlings.

The fresh shoot weight of chickpea seedlings showed significant increase with inoculation of *Mesorhizobium* isolates of rainfed area compared with the control (Figure 2). The shoot weight with isolates ranged from 0.32 g to 0.48 g compared with the 0.24 g in control. Maximum shoot weight (0.48 g) was recorded for JK-10 isolate followed closely by that obtained with JK-8 and JK-4 which were statistically at par with each other. These results suggested that inoculation of cultures resulted in the improvement of fresh and dry shoot weight of seedlings as compared with the control. The data regarding the shoot dry weight is given in Figure 3. As for as dry soot weight is concerned, it was also improved significantly with all isolate inoculation over control. The increase in dry shoot weight with culture inoculation ranged between 29 to 68%. However, best results were obtained by the JK-10 isolate.

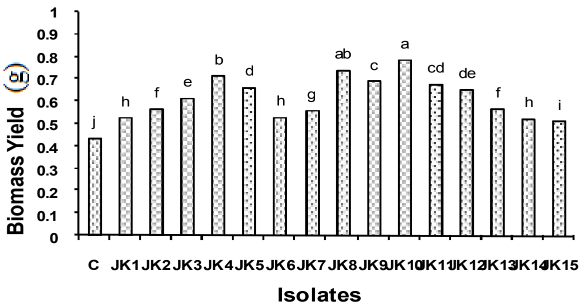

Figure 1: Effect of *Mesorhizobium ciceri* isolates on biomass yield g plant^{-1} of chickpea Seedlings

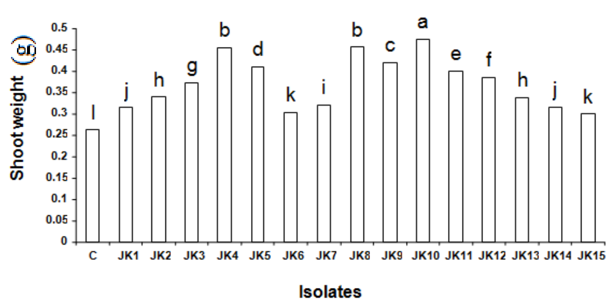

Figure 2: Effect of *Mesorhizobium ciceri* isolates on shoot weight (g) of chickpea seedlings

The data given in Figure 4 and 5 revealed the fresh/ dry root weight of chickpea plant grown under growth room conditions. These parameters were also improved significantly with seed inoculation of rhizobial isolates compared with the uninoculated treatment. As in case of biomass yield and shoot weight, JK-10 produced the highest fresh and dry root weight. It showed 65% greater root weight than the control plants. The isolates JK-8, JK-4, JK-5, JK-9 and JK-11 also performed well in terms of fresh/dry root weight. The increase in root weight (fresh/dry) by *Mesorhizobium* isolates varied between 25% and 65%.

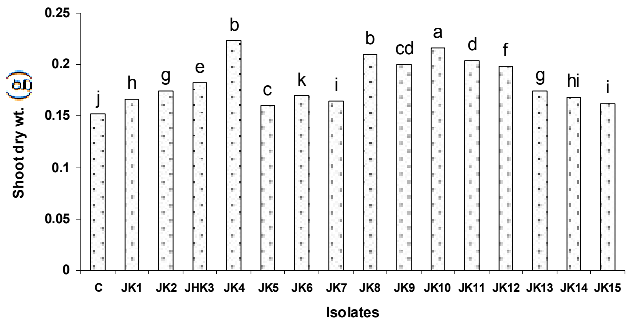

Figure 3: Effect of *Mesorhizobium ciceri* isolates on dry shoot weight (g) of chickpea seedlings

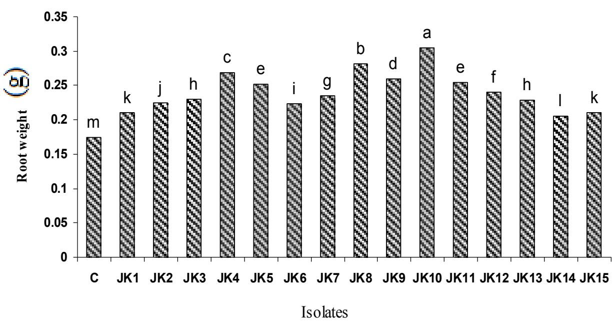

Figure 4: Effect of *Mesorhizobium ciceri* isolates on root weight (g) of chickpea seedlings

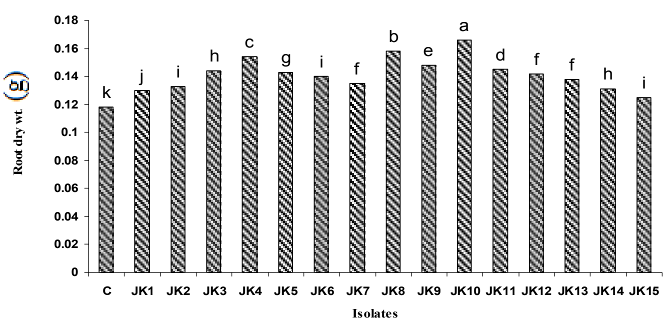

Figure 5: Effect of *Mesorhizobium ciceri* isolates on dry root weight (g) of chickpea seedlings

The results presented in figure 6 demonstrated the beneficial role of *Mesorhizobium* isolates in the development of shoot length of chickpea plants. Same *Mesorhizobium* isolates (JK-10) proved its efficacy in the improvement of shoot length and produced 21.2 cm shoot length. Other best isolates were JK-8, JK-4, JK-9, JK-11, JK-5 and JK-12, respectively, which also showed good results among the tested isolates. However, all the isolates were found better as compared with the control. The increase in shoot length of chickpea seedlings due to inoculation was recorded between 19.5 to 71.3%.

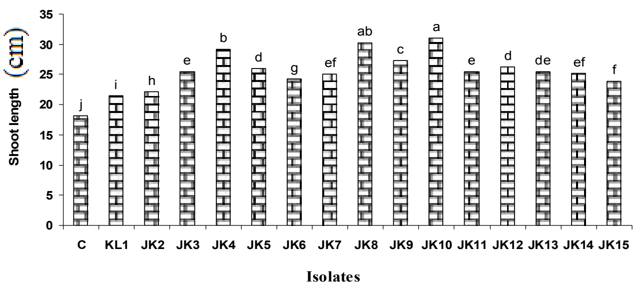

Figure 6: Effect of *Mesorhizobium ciceri* isolates on shoot length of chickpea seedling

The data regarding the effect on root length are presented in Figure 7. The data showed that all the rhizobial isolates were found effective in improving root length. The root length increase was observed with the range of 35-60% over control. Similar trend, as in other parameters, was observed here and isolates JK-10 and JK-8 was the best isolates with root length of 22.5 cm and 22.2 cm, respectively. The other isolates, which also showed a good response were JK-4, JK-9, JK-11 and JK-12 that produced the root length of 21.5, 20.2, 20.0 and 19.8 cm, respectively.

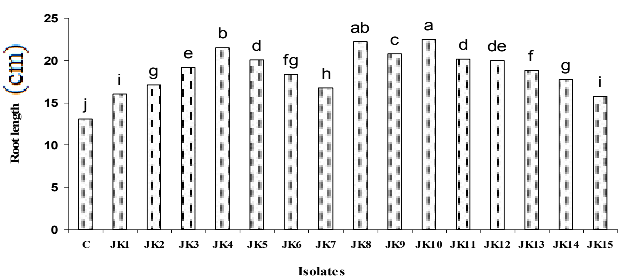

Figure 7: Effect of *Mesorhizobium ciceri* isolates on root length of chickpea seedlings

The data presented in Figure 8 shows that the inoculation wiht *Mesorhizobium* isolates were effective for nodule production in chickpea seedlings. With the treatment of *Mesorhizobium ciceri* cultures, nodules were formed in all chickpea seedlings. The uninoculated plant failed to produce any nodule. These nodules are necessary for atmospheric nitrogen fixation having beneficial effect on plant growth. The data revealed that isolates had variable potential for nodule formation in treated plants with the range of 5 to 13 nodules plant $^{-1}$. The maximum number of nodules (13) was demonstrated by JK-10 isolates. The results were also better by JK-8, JK-4, JK-9, JK-5 and JK-11 isolates which produced 12, 11, 11 and 10 nodules plant^{-1}, respectively.

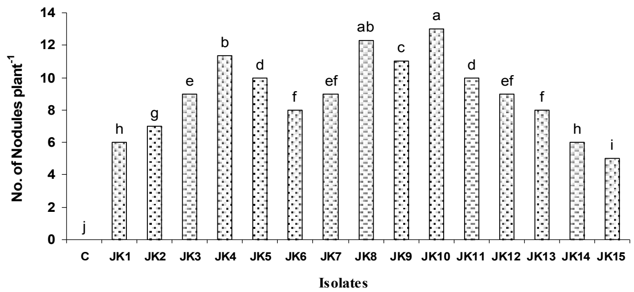

Figure 8: Effect of *Mesorhizobium ciceri* isolates on number of nodules of chickpea seedlings

The legume-rhizobium symbiosis is a typical example of mutualism. Due to this symbiosis, the nodules are formed in which bacteria reduce N_2 to NH_4 and this fixed nitrogen is transformed to the plants (Jain and Gupta, 2003).

The effect of *Mesorhizobium* strains on the total biomass production plant^{-1} of chickpea seedlings is presented in figure-1 The data revealed that all the isolates improved the biomass yield significantly over the uninoculated control. The maximum yield was recorded with JK-10 isolate while minimum growth was found in the control. The improvement in yield ranged between 35-60% by all the isolates. Zafar-ul-Hye *et al*. (2007) reported the increasing trend in root/shoot weight and length while conducting a screening study of rhizobium isolates for improvement in lentil growth under axenic conditions. The increase in total plant weight was observed by Bremer *et al*. (1990) during the screening study of *R. leguminosarum* for effect on growth of lentil. These growth responses were primarily related to the bacterial production of phytohormones that resulted in increased nutrient and water uptake from the soil (Andrew *et al.,* 2003).

The effect of *Mesorhizobium ciceri* isolates on the fresh weight of shoot had been elaborated in Figure 2. It is evident from the data that all the isolates had beneficial effect on the improvement in shoot weight. The increase in shoot weight of chickpea seedlings with isolates ranged from 33 to 100% over the control value of 0.24 g. Isolate JK-10 yielded the maximum shoot weight value of 0.480 g closely followed by the isolates JK-8 and JK-4 which did not differ statistically. The other isolates that were also better in terms of shoot weight were JkK-9, JK-5 and JK-11. Figure 3 indicated the data regarding the shoot dry weight as affected by the rhizobial isolates. As for as dry shoot weight is concerned, it was also improved significantly with all isolate inoculation over control.

However, best results were indicated by the isolates JK-10, JK-8, JK-4, JK-9, JK-5 and JK-11, respectively as in the case of fresh weight. Romdhane *et al*. (2007) reported the increase in shoot fresh/ dry weight of chickpea seedlings after inoculation with *Mesorhizobium ciceri* isolates.

Good root production is very important for the plant growth. Healthy roots help the plant for intake of water and essential plant nutrients from the soil. In this way, plant growth is enhanced resulting in good yields. The data given in figure 4 and 5 revealed the fresh/dry root weight of chickpea plant grown under gnotobiotic conditions. The results indicated that fresh/dry root weight of chickpea seedlings were significantly increased with rhizobial cultures in comparison uninoculated plants. The isolate JK-10 produced the highest values in terms of fresh and dry root weight. The increase in root weight varied by *Mesorhizobium* isolates within range of 25 to 65%. Bermer *et al*. (1990) reported the similar results of increase in root fresh/dry weight in lentil plants by inoculation with *Rhizobium leguminoserum* in a screening programme. Zafar-ul-Hye et *al*. (2007) also concluded the similar trend of increase in fresh/dry root weight results during a rhizobium screening experiment on lentil plants.

Good shoot length is an indication that required nutrients has been supplied to plant in sufficient quantities. The results quoted in Figure 4 are showing the beneficial role of rhizobium isolates in the development of shoot length of chickpea plants. Same *Mesorhizobium* isolates JK -10 improved the shoot length in a dominant manner with highest values. Good response was also observed from isolates JK-8, JK-4 and JK-9, respectively, while isolates JK-11, JK-5 and JK-12 also showed good results among the tested isolates. The other isolates also had positive effect on shoot length development as compared with the control. The increase in shoot length of chickpea seedlings due to inoculation were recorded between 19.5 to 71.3%. Similar findings were noted by Zafar-ul-Hye *et al.* (2007) and Bermer *et al*. (1990) who concluded the increase in shoot length of lentil and chickpea plants in a screening study of rhizobial isolates. The rhizobium- plant association increased plant growth at different growth stages such as enhanced seed germination, increased shoot length, total dry matter yield and grain yield (Prayinto *et al*., 1999).

Long roots can penetrate deeps and plant can absorb water and nutrients from the soil solution with better approach. Plants with long roots are suitable to the areas of water shortage or having deep water table. It was clear from the data given in Figure 7 that root length had been significantly affected with the culture inoculation. All the *Mesorhizobial* isolates improved the root length with the range of 35-60% over control. These beneficial effects require a successful colonization of *Mesorhizobia* on the roots of the host plant to create a conducive environment for increased interaction between bacteria and the roots. The attachment of rhizobia to the root is essential for the establishment of an efficient association with the plants (Verma *et a*l., 2004). Similar trend of other parameters was observed here and isolates JK-10 and JK-8 was the best isolates. The other isolates that also showed a good response were JK-4 and JK-9 that produced the root length of 21.5 cm and 20.2 cm respectively. Zafar-ul-Hye *et al*. (2007) observed the increase in root length of lentil plants during screening of *Mesorhizobium* isolates under controlled conditions. Root development is clearly influenced by the phytohormone auxin which are known to effect a number of plant functions including promotion of cell division and elongation, root initiation and ethylene biosynthesis (Chasan, 1993).There are evidences that Rhizobium can enhance plant growth through the changes in root physiology and morphology along with nitrogen fixation (Biswas et al,. 2000).

The data referred to Figure 8 had shown the nodule production with the treatment of *Mesorhizobium ciceri* cultures. The nodules are formed as a result of symbiosis between microbes and host plants. The atmospheric nitrogen is fixed through these nodules and these are called nitrogen factories. It was obvious from the data that nodules were formed by all the isolates. The maximum number of nodules (13) was produced by JK-10 isolates. The results were also better by JK-8 and JK-4 isolates. It indicated that *Mesorhizobium* isolates were helpful in the nodule formation by chickpea plants for the fixation of atmospheric nitrogen. The better growth of chickpea seedlings might be due to the presence of these nodules. Nitrogen fixation in legumes depends on the formation of nodules by rhizobium- plant symbiosis. Without sufficient nodules mass filled with an efficient strain of rhizobium, nitrogen fixation will be inadequate. Inoculation of legume seeds assures the growth of specific rhizobium population in the root environment (Verma *et al*., 2004).

Zafar-ul-Hye *et al*. (2007) reported the significant improvement in nodule production with rhizobium culture inoculation in lentil plants in contrast to the uninoculated plants in control. Romdhane *et al*. (2007) found that nodule number was increased significantly in chickpea plants treated with *Mesorhizobium ciceri* isolates for screening purpose over control. Rhizobia form intimate symbiotic relationship with legumes by responding chemotactically to flavonoid molecules released as signals by the legume host. These plant compounds induce the expression of nodulation (nod) genes in rhizobia, which in turn produce signals that trigger mitotic division in roots, leading to nodule formation (Dakora, 2004).

Conclusion

All the isolates proved their efficacy in improvement of growth parameters in chickpea seedlings as compared with control but potential varied with different isolates. The isolates JK-10, JK-8 and JK-4 were found better in terms of biomass plant^{-1}, shoot fresh/dry weight, root fresh/ dry weight and nodulation. Further studies are needed to confirm the findings.

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