Growth and yield response of chick pea (*Cicer arietinum*) to seed inoculation with *Rhizobium* sp.

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Abstract: The present study was conducted to evaluate effect of seed inoculation by using *Rhizobium* sp. as a biofertilizer on nodule formation and growth of chick-pea plant and to study the efficiency of seed inoculation for nitrogen fixation. *Rhizobium* effect was studied using *Cicer arietinum* and controlled condition. Soil analysis was done for all physico-chemical and microbiological parameters. *Rhizobium* sp. was isolated from root nodules of *cicer arietinum,* sterilized, prepared serial dilutions, incubated then applied on *Cicer arietinum* seeds and also on controlled seeds, pot experiments were conducted to evaluate the effect of bacterized seeds and controlled seeds. The results obtained from the study using observation and data reveals that, the bacterized seeds showed, 14.06% in total length over control, Increase of 10.83% in total weight over control and an increase of 9.0% on germination over control in pot experiment. The results indicate that *Rhizobium* inoculation is a promising fertilizer because it is cheap, easy to handle and improves plant growth and seed quality. [Nature and Science 2010;8(9):232-236]. (ISSN: 1545-0740).

Keywords: seed inoculation; bacterized seeds; nitrogen fixation; fertilizer

1. Introduction

The increasing demand for production of crops and food for such a vast population has led to an interest and necessity for the use of biofertilizers for the betterment of these crops and even for the health of soil. Biofertilizers can be a very good complimentary to the chemical pesticides as they not only kill the harmful insects but also the beneficial insects such as pollinators.

By the turn of this century, India's estimated food requirement will be about 240 million tons. One of the key limiting factors in crop productivity is the availability of nitrogen. Because of the constraints on the production, availability and use of chemical nitrogenous fertilizers, biologically fixed nitrogen will play an important role in increasing the crop production. India is the largest producer and importer of the leguminous crop (Shakya *et al*., 2008). Amongst the leguminous crops, chick-pea (*Cicer arietinum* L.) occupies an important position due to its nutritive values (17-23% protein) in large vegetarian population of the country (Ali and Kumar, 2006). According to Akhtar and Siddiqui, 2009, during last decade the production of chick-pea has been declined, and it has been argued that usual native soil rhizobial populations are inadequate and are ineffective in biological nitrogen fixation. To ensure an optimum rhizobial population in the rhizosphere, seed inoculation of legumes with an efficient rhizobial strain is necessary. This helps improve nodulation, N_2 -fixation solicit crop growth and yield of leguminous crops (Henzell, 1988). The process of nitrogen fixation is quite complex requiring interaction between bacterium and the host plant, *Rhizobium* sp. invade the root hairs of chick-pea and result in the formation of nodules, where free air nitrogen is fixed. These bacteria, although present in most of the soils vary in number, effectiveness in nodulation and N-fixation.

By land area planted to chick-pea, the country ranks second in the world. But, until recently, inadequate attention has been paid to increasing its production, which is either stagnant or declining. Major constraints in increasing production of chick-pea are poor soils, inadequate moisture, harsh climatic conditions, weeds and inadequate or even no fertilizer supply (Khan *et al.,* 1989; Aslam *et al*., 1996). Uncertain rains, low incentives, and poor economic factors of the farmers restrain them to supply any plant nutrition. Usually, most of the farmers do not use chemical fertilizers at all in growing chick-pea and other grain legumes. Moreover, due to the harsh climate, *Rhizobium* population is very low resulting in less yields (Aslam *et al*., 1997). It is possible to increase chick-pea yield by inoculation with *Rhizobium* strains even in fields where chick-pea have been grown for many years.

Chick-pea (*Cicer arietinum*) is a major pulse crop in India. There is a good possibility to increase its production by exploiting better colonization of the roots and rhizospheres through application of effective nitrogen fixing bacteria to the seed or to the soil. This can minimize uses of nitrogenous fertilizer, which is very costly in this country. Using high yielding varieties of chick-pea along with use of effective rhizobial strains can enhance the yield (Bhuiyan *et al*., 2008).

Some micro organisms are able to form close association with plants that can be either detrimental or beneficial to the plants. *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). The group of beneficial free living soil bacteria that stimulate the growth of plant is referred as plant growth promoting rhizobacteria (PGPR). The present studies were conducted to evaluate effect of seed inoculation on nodule formation, growth of chick-pea plant and to compare the efficiency of seed inoculation for nitrogen fixation.

2. Material and methods

2.1 Site Description

To evaluate the response of chick-pea to *Rhizobium* inoculation and use of N-fertilizer, a pot experiment was conducted in Department of Zoology and Environmental Sciences, Gurukula Kangri University, Haridwar, Uttarakhand during rabi season of 2008-09.

2.2 Analysis Method

For seed inoculation, seeds were coated with paste of *Rhizobium* inoculum and sown in the pots. Then seeds were sown by using thirty seeds per pot with three replications. The soil was sandy clay loam having a pH of 6.9, moisture content of 12.4%, organic matter of 1.69 mg g^{-1}; total of N 0.16%; available P of 14.2 mg g^{-1} and exchangeable K of 6.0 mg g^{-1}, available sodium of 32.0 mg g^{-1}, and available nitrate of 16.4 mg g^{-1}. All agronomic practices were kept uniform and normal for all treatments. A control without inoculation was included for comparison.

3. Results and Discussion

Results showed that inoculated plants gave significantly higher nodule number, nodule weight, root weight, shoot weight, and seed yield compared to non-inoculated plants. It was observed that inoculated seeds after germination gave higher biological yield (9.0%) compared to non-inoculated seeds cultivars as shown in Table -1.

Data on number of nodules per plant and root length at maturity, plant height at maturity, were recorded during the course of study by following standard procedures. During the present study, the plant vigour of *Cicer arietinum* was found 247.5 which is related to health of plant, defines about the ratio of root/shoot length and it also defines tolerance power of plant against any kind of stress.

After this study it has been found that the inoculation of *Rhizobium* as biofertilizer was very significant in production of number of nodules, growth, root/shoot length, root/shoot weight and in germination also. Effects of rhizobial inoculated plants gave significantly higher shoot/root length of 13.04% and 15.07% (as shown in Table 2) and shoot/root weight also increased by 10.26% and 11.42%, compared to un-inoculated control (as shown in Table 3).

These results are in line with the findings of Patra and Bhattacharyya (1998), Khalequzzaman and Hossain (2007), Ali *et al* (2008), who reported that seed inoculated plants, exhibited significantly greater root and shoot length as compared to un-inoculated control plants. Hoque and Haq (1994) reported that inoculation of seed with *Rhizobium* significantly increase plant height of lentil. Also the effect of combined inoculation with VAM-fungi and *Rhizobia* have been reported to further increase the growth and yield of some crops including soybean (Azcon, 1979; Bagyaraj *et al*., 1979; Young *et al*., 1988).

Ramaswami and Oblisami (1986) and Yadegari *et al.* (2008) reported the increase in nodules and yield due to inoculation application. Increase in nodules per plant due to application of inoculation in combination with nitrogen fertilizer was also reported by Rashid *et al*., (1999). Khalil *et al.* (1989) observed that nitrogen alone or in combination with inoculation gave the maximum plant height in mungbean as compared to control. Khanam *et al.* (1994) reported that inoculation with *Rhizobium* strains gave higher nodule number, nodule dry weight, stover yield and seed yield compared to uninoculated plants. Bhuiyan *et al.* (1998) found that *Rhizobium* inoculation increased nodulation and seed yields upto 35%. Gupta and Namdeo (1996) found that seed inoculation with *Rhizobium* increased chick-pea seed yields by 9.6-27.9%. Better effects of Inoculation with *Rhizobium* on seed yield and Yield components of Common Vetch (*Vicia sativa* L.) were also shown by Albayrak *et al*, (2006). Zarrin Fatima *et al*, in 2006 also found that the effect of growth was highly significant (α 0.05) with an increase in root/shoot dry and fresh weight in plants with mixture of *Rhizobium* inoculums with phosphorus on soybean.

The *Rhizobium* legume symbiotic relationship is highly specific and most legume plants form association with only a limited number of the *Rhizobium* strain (Subba Rao., 1999). The rhizobacteria involved in such interaction belong to several genera e.g. *Acetobacter*, *Actinoplanes, Agrobacterium*, *Alcaligens*, *Bacillus*, *Arthrobacter*, *Pseudomonas, Seretia and Xanthomonas*. (Triplett and Sadowsky, 1992).

Some species of *Rhizobium* are highly specific for one or a few leguminous host e.g. *Rhizobium trifoli* is specific for clover (*Trifolium-trifoli*) while *Rhizobium leguminosarum* is mainly for pea. *Rhizobium* in the rhizosphere of plants is chemo-attracted to various exudates, attractants released by legume roots (Hunter and Fahring., 1960).

Fig. 02. Tubes showing the increase in shoot/root length of bacterized seeds over control (after 30 days of incubation).

Fig. 01. White smooth colonies of Rhizobium sp. Apeeared on YEMA plate.

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Table 1: Percent increase of germinated in triplicate pots (values showing number of germinated seeds).

Treatment	Shoot length(cm)	% increase	Root length(cm)	% increase	Total length	% increase
Control	11.5 ± 0.16	13.04	12.6 ± .14	15.07	24.1	14.06
Rhizobium	13 ± 0.14		14.5 ± 19		17.5	

Table 2: Percent increase in shoot/root length of plants in triplicate tubes (values are the mean of three observations each).

Treatment	Germinated seeds			Mean	% increase
	I	II	III		
Control	21	22	22	21.67 ± 0.33	9.00
Rhizobium	22	25	24	23.67 ± 0.88	

Table 3: Percent increase in shoot/root weight of plants in triplicate tubes (values are the mean of three observations each).

Treatment	Shoot weight (gm)	% increase	Root weight (gm)	% increase	Total weight (gm)	% increase
Control	0.341 ± .032	10.26	0.324 ± 0.039	11.42	0.665	10.83
Rhizobium	0.376 ±.032		0.361 ± 0.052		0.737	

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