INFLUENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON THE GROWTH OF CHICKPEA (*CICER ARIETINUM* L.)

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Abstract
Plant Growth Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by wide variety of mechanism these includes direct and indirect mechanisms like phosphate solubilization, phytohormone production, antifungal activity, etc. Six isolates of bacteria, designated as, VA1, VVA2, VA3, VA4, VA5 and VA6 were successfully isolated and characterized by morphological and biochemical methods as P. aeruginosa. Subsequently to investigate the effect of PGPR isolates on the growth of Cicer arietinum L., a pot culture experiment was conducted. Isolated PGPRs showed upto 62% seed germination power over to controls. Isolates VA3 and VA1 showed highest positive effect on the growth of experimental plant.
Most of isolates resulted in a significant increasing of shoot length, root length and dry matter production of shoot and root of Cicer arietinum seedlings. In our results, Shoot length of Chickpea after inoculation increased up to 92%, and dry matter increased up to 43% in comparison to controls. As same, bacterial inoculation also had the positive effect on the root length up to 35% and dry weight up to 40% in comparison to controls. These results show the potential to use PGPR in order to improve yields in Chickpea crop in fields.

Keywords: Seed inoculation, *P. aeruginosa*, Biofertilizer, PGPR, Rhizobacteria.

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1. INTRODUCTION

The use of PGPR is steadily increased in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Preparations of live microorganisms (bacteria, fungi) utilized for improving plant growth and crop productivity are generally referred to as biofertilizers or microbial inoculants (SubbaRao N.S., Dommergues Y.R., 1998; Vessey J.K., 2003) helps to promote growth by increasing the supply or availability of primary nutrient to the host plant (Narula N. *et al.,* 2005). A group of biofertilizers contains beneficial, free-living soil-borne bacteria that colonize the rhizosphere, and when applied to seed or crops enhance the growth of plants either by direct or indirect mechanisms, termed as plant growth-promoting rhizobacteria (PGPR) (Kloepper J.W. *et al.*, 1980; Glick B.R., 1995).
The use of PGPR is steadily increased in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. The concept of PGPR began to gain importance and a large number of bacterial strains have been isolated, screened (Chanway C.P., Holl F.B., 1993; Cattelan A.J. *et al.*, 1999; Bertrand H. *et al.*, 2001) and evaluated for plant growth promotion (Lifshtiz R. *et al.*, 1987; Chanway C.P. *et al.*, 1989; Abbas Z., Okon Y., 1993; Glick B.R. *et al.*, 1997; Zhang F. *et al.*, 1997; Bashan Y., Holguin G., 1998; Mayak S. *et al.*, 1999; Bent E. *et al.*, 2001; Salamone I.E.G., 2000).
Indirect stimulation includes the ability to reduce the deleterious effects of plant pathogens on crop yield such as suppression of phytopathogens by producing siderophores that chelate iron, making it unavailable to pathogens; the ability to synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens (Cook R.J., 1993; Glick B.R., 1995; Nelson L.M., 2004; Weller D.M., Cook R.J., 1986; Dunne C. *et al.*, 1993; Kloepper J.W. *et al.*, 1988; Liu L. *et al.*, 1995; Glick B.R. *et al.*, 1999) while the direct stimulation occurs due to the fixation of atmospheric nitrogen that is

transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Glick B.R. *et al.* , 1997; Timmusk S. *et al.*, 1999; Salamone I.E.G., 2000; Cartieaux F.P. *et al.*, 2003). Bertrand H. *et al.*, (2001) and many other researchers identified bacteria belonging to the genera *Pseudomonas, Varivorax*, *Agrobacterium* and *Phyllobacterium* as the most efficient PGPR associated with different plants or crops.

However, the amount of nitrogen fixed by *Pseudomonas* was minimal and the positive plant growth response observed may be due to other factors such as phytohormone production and enhance mineral uptake (James E.K., Olivares F.L., 1997). Chickpea is very often used as a crash diet due to its good taste and nutritive values. It is used in cosmetic widely, in preventing hair fall and hair related problems. Its paste is applied on the skin specially the face to enhance the shine and glow on the face. It is also used to open the pores and blocked pores. Its powder is used in improving the indigestion and digestive disorders. It is also used as body building agents by any body builders. In present research work *P. aeruginosa*, a gram negative rod shape bacteria belonging to the community of PGPR was used to study their effect on the growth of Chickpea (*Cicer arietinum* L.).

2. MATERIAL AND METHODS

Relevant soil chemical characteristics were as follows: soil pH 6.84; moisture 0.8mg/g, organic carbon 9.54%; InorganicPO_4^- 0.0048mg/l; Total organic content 21.095 mg/g;chloride 36mg/100g; sulphate 6.5mg/100g and calcium carbonate 0.075% in experimental soil.

Bacterial strains were originally isolated from the rhizosphere of different plants from garden soil of Bhojia Institute of Life Sciences. Isolated bacterial strains was tested for their PGPR activities and based on higher PGPR activities 6 isolates of *Pseudomonas aeruginosa* were selected for pot experiments. Identification and characterization of selected isolate were done by using morphological, physiological, biochemical testing methods as described in Bergey's manual of Systematic Bacteriology (Holt J.G. *et al.*, 1994).

Chickpea (*Cicer arietinum* L.) seeds were used as plant materials. A randomized complete block design was employed as the experimental design with three replications. Seeds of Chickpea were sterilized in 1% $HgCl_2$ for 2 minutes, for surface sterilization and then, washed with sterilized distilled water at least 10 times to remove traces of toxic $HgCl_2$. After air drying Chickpea seeds were sawn into sterilized pots (eight seeds per pot). For experiment, the bacterial strains were grown on nutrient agar. A single colony was transferred to 250 ml flasks containing nutrient broth, and grown aerobically in flasks on a rotating shaker (95 rpm) for 24 h at 27ºC. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 CFU ml^{-1}. 1ml of log culture (10^8 cells) of each bacterial isolates was transferred as inoculum in the corresponding treatments. Treated and non treated pots were irrigated with sterilized water daily. After every 7 days interval 2ml of microorganism inoculum was inoculated in the corresponding pot as booster dose. *Pseudomonas* strains along with their respective non-bacterized seeds as control were sown in following sets of treatments:

- **Treatment 2:** Sterilized soil+ *Pseudomonas aeruginosa* VA1 bacterized seeds.
- **Treatment 3:** Sterilized soil+ *Pseudomonas aeruginosa* VA2 bacterized seeds.
- **Treatment 4:** Sterilized soil+ *Pseudomonas aeruginosa* VA3 bacterized seeds
- **Treatment 5:** Sterilized soil+ *Pseudomonas aeruginosa* VA4 bacterized seeds.
- **Treatment 6:** Sterilized soil+ *Pseudomonas aeruginosa* VA5 bacterized seeds
- **Treatment 7:** Sterilized soil+ *Pseudomonas aeruginosa* VA6 bacterized seeds.

Plants from each replicate were randomly harvested, and data on plant growth variables, such as leaf numbers, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were collected. Dry weights (after

drying at 70°C) of root and leaf samples were measured.

3. RESULTS

Our experiments showed that PGPRs Inoculation significantly enhanced seed germination and seedling vigour of Chickpea. However, the rate of enhancement varied with bacterial strains. Shoot length of Chickpea after inoculation increased up to 92%, and dry matter increased up to 43% in comparison to controls. As same bacterial inoculation also had the positive effect on the root length up to 35% and dry weight up to 40% in comparison to controls (Table 1).

Table 1: Effect of isolated bacterial strains on the growth of Chickpea

Treatments*	Root		Shoot		No. of leaves
	Length (mm)	Dry weight (mg)	Length (mm)	Dry weight (mg)	
Treatment 1	100(35)	100(36)	100(93)	100(68)	27
Treatment 2	115	123	192	122	58
Treatment 3	128	104	177	115	48
Treatment 4	120	140	167	143	56
Treatment 5	115	102	94	93	26
Treatment 6	131	119	157	107	44
Treatment 7	135	102	132	82	35

*__Treatment 1:__ Sterilized soil + Sterilized seeds (without *Pseudomonas* inoculation) work as control.

Treatment 2: Sterilized soil + *Pseudomonas fluorescens* VA1bacterized seeds.

Treatment 3: Sterilized soil + *Pseudomonas aeruginosa* VA2 bacterized seeds.

Treatment 4: Sterilized soil + *Pseudomonas aeruginosa* VA3 bacterized seeds

Treatment 5: Sterilized soil + *Pseudomonas aeruginosa* VA4 bacterized seeds.

Treatment 6: Sterilized soil + *Pseudomonas aeruginosa* VA5 bacterized seeds

Treatment 7: Sterilized soil + *Pseudomonas aeruginosa* VA6 bacterized seeds

All bacteria strains except VA6 increased seed germination up to 25-62% over non-treated control (Table 2).

Table 2: Variation in Germination index of Chickpea seeds (8 seeds per pot)

S.No.	Treatment	Number of germinated seeds	Germination percentage
1	**Treatment 1**	3	37.50
2	**Treatment 2**	6	75.00
3	**Treatment 3**	5	62.50
4	**Treatment 4**	5	62.50
5	**Treatment 5**	8	100
6	**Treatment 6**	5	62.50
7	**Treatment 7**	3	37.50

The results of pot study showed that inoculation of Chickpea seeds with bacterial strains showed a positive effect on root and shoot length (Figure 1 and Figure 2).

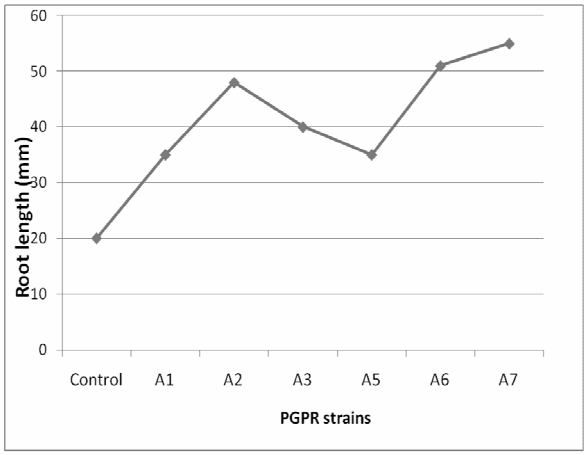

Figure 1: Effect of different PGPR strains on the roots length on *Cicer arietinum*

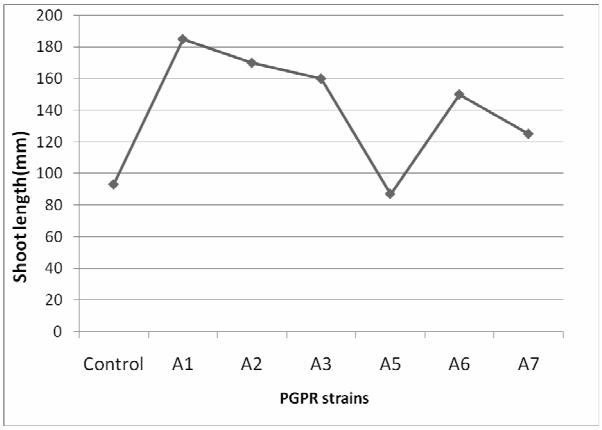

Figure 2: Effect of different PGPR strains on the shoots length on *Cicer arietinum*

In contrast, shoot and root dry weight significantly increased by inoculation in sterile soil (Figure 3 and Figure 4).

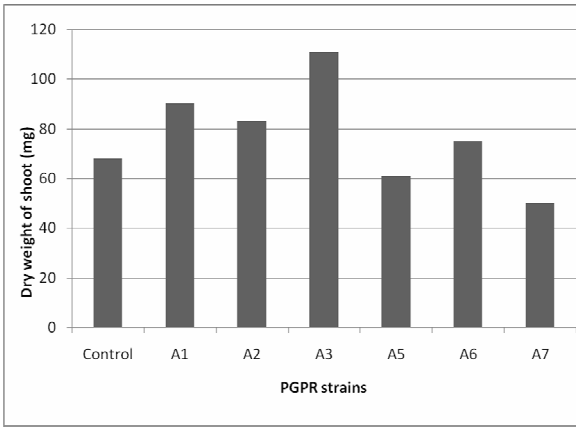

Figure 3: Effect of different PGPR strains on the dry weight of shoots on *Cicer arietinum*

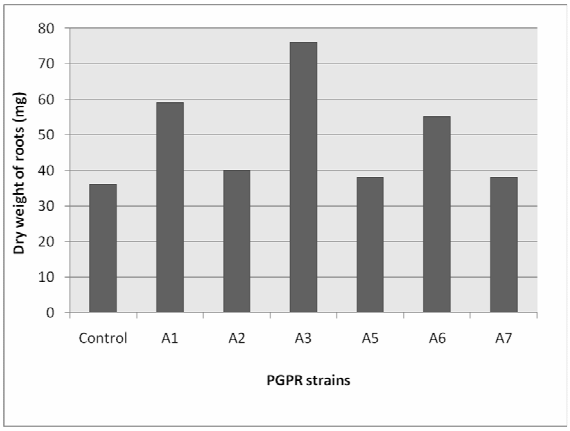

Figure 4: Effect of different PGPR strains on the dry weight of roots on *Cicer arietinum*

The highest shoot and root dry weight was recorded from treatment 4 inoculated with VA3 bacteria strain and from Treatment 2 inoculated with bacterial strain VA1 in sterile soil (Table 1). It is recorded that treatment 2 and 4 also show highest numbers of leafs in comparison to other treatments and controls (Figure 5).

In comparison to control, inoculation of VA4 and VA6 bacterial strain not showing effective results on the shoot dry weight and shoot length as shown in Figure 1.

4. DISCUSSION

It is experimentally proved that PGPR have positive effect on the growth of different crops and plants (Wu S.C. *et al.*, 2005).

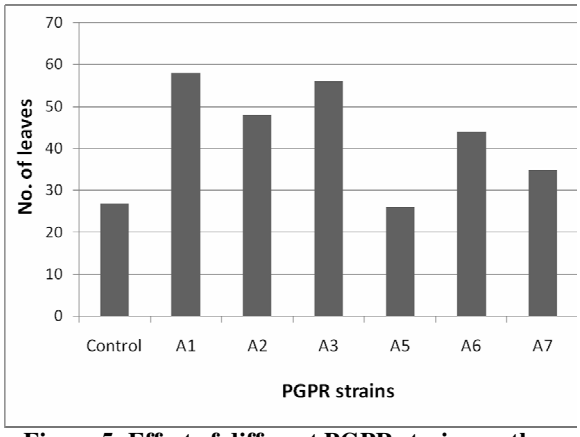

Figure 5: Effect of different PGPR strains on the number of leaves on *Cicer arietinum*

The impact of rhizobacteria generally on plant growth and health may be classified as neutral, deleterious or beneficial (Kloepper J.W. *et al.*, 1989). Many researchers (Lifshtiz R. *et al.*, 1987; Chanway C.P. *et al.*, 1989; Abbas Z., Okon Y., 1993; Glick B.R. *et al.*, 1997; Zhang F. *et al.*, 1997; Bashan Y., Holguin G., 1998; Mayak S. *et al.*, 1999; Bent E. *et al.*, 2001) records the ability of microbial inoculants, to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease. This present investigation confirms the earlier

works. It revealed that use of PGPRs with seed treatment improve seed germination, seedling emergence, seedling vigor and seedling stand over the control. Similar results have been reported in other crops such as potato, radish plants, sorghum and pearl millet (Burr T.J. *et al.*, 1978; Raju N.S. *et al.*, 1999; Niranjan S.R. *et al.*, 2004). The improvement in seed germination by PGPR was also found in work with wheat and sunflower (Shaukat K. *et al.* , 2006), where it was found that some PGPR induced increases in the seed emergence, in some cases achieving increases up to 100% greater than controls. Our results also show the higher seedling with isolated bacterial strains.

In pot experiment, it was observed that PGPR inoculation significantly increase the growth of seedlings of Chickpea. In general, inoculation resulted in early seedling growth and development. Similar findings were reported by Dobbelaere S. *et al.*, (2006) who assessed the inoculation effect of PGPR *Azospirillum brasilense* on growth of spring wheat. They observed that inoculated plants resulted in better germination, early development and flowering and also increase in dry weight of both the root system and the upper plant parts (Gravel V. *et al.*, 2007; Kozdroja J. *et al.*, 2004). Soil condition also influenced the growth promotion by bacterial strains. Martinez-Toledo M.V. *et al.*, (1988) showed that the numbers of *Azotobacter* decreased as plant growth continued in nonsterile agricultural soil, while the numbers of *Azotobacter* associated with maize roots grown in sterile agricultural soils remained similar to those of the original inoculum. This may imply rhizobacteria had a more competitive ability to survive and affect the growth of inoculated plants in the presence of indigenous micro flora (Khalid A. *et al.*, 2004). In this study, Inoculation of PGPR strains increased all parameters determined in-pot experiment. The present experiment revealed that seed inoculation with all isolated bacteria resulted in an increased plant height and leaf numbers. Similar increases in plant height and leaf area were observed in different crops such as potato, radish plants, sorghum and pearl millet inoculated with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains (Burr T.J. *et al.*, 1978; Raju N.S. *et al.*, 1999; Niranjan S.R. *et al.*, 2004).

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