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Original Research Article

Enhancement of plant growth, nodulation and seed yield through Plant Growth Promoting Rhizobacteria in Lentil (*Lens culinaris* Medik cv. VL125)

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A B S T R A C T

Rhizobacteria that exert beneficial effects on plant growth and development are referred to as Plant Growth Promoting Rhizobacteria (PGPR). Plant growth promoting rhizobacteria is a group of free living soil bacteria which have ability to promote growth and yield of crop plant by direct and indirect mechanisms. Some rhizospheric microorganisms have capable to convert the insoluble phosphorous to an accessible form is an important attributes of PGPR that exhibits to increase the plant growth, nodulation and yield of the plants. In order to consider the influence of phosphate solubilizing bacteria (PSB) i.e. RMV1, RMV4 and RPB3 were observed on the plant growth, nodulation, yield and yield contributing characters of lentil under two year field experiments. Among these, RPB3 exhibited greatest influence by 13.17, 19.77, 10.69 and 21.11 per cent for field emergence, root length, plant height and number of branches per plant, respectively in pooled value for two years. While, RMV4 and RMV1 reflected somewhat lower efficacy of magnitude than RPB3 for characters studied. However, RPB3, RMV4 and RMV1 showed 28.34, 25.99 and 18.33 per cent increment on nodulation, respectively over control. Furthermore, these bio-inoculants also exhibited the same pattern of efficiency over control for seed yield and its contributing traits. However, all these PSB had proved significant influence on plant growth, nodulation, yield and its attributing traits in lentil. Therefore, RPB3 appears to be a better PSB candidate to be utilized as phosphate solubilizing bio-inoculants.

Keywords

PGPR; Phosphate solubilization; Plant growth; Nodulation; Yield; Lentil

Introduction

Phosphorous is an important plant macronutrient, making up about 0.2 percent of a plant's dry weight. It is a component of key molecules such as nucleic acid, phospholipids, ATP and consequently, plants cannot survive without a reliable supply of this nutrient. Pi is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993). After nitrogen, phosphorous is the second most frequently limiting macronutrient for plant growth and development. Application of soluble forms of P fertilizers are easily precipitated into insoluble forms and are not efficiently taken up by the plant, which lead to an excess application of P fertilizer to crop land and ultimately leads to pollution due to soil erosion and runoff water containing large amounts of soluble P. Availability of phosphate in soil is greatly enhanced through microbial production of metabolites leading to lowering the pH and release of phosphate from organic and inorganic complexes (Haque and Dave, 2005). Optimization of a biological phosphate solubilizer in the form of rhizospheric microorganisms seems to be a suitable tool to release some of the soil-bound phosphate and to reduce the use of chemical fertilizers. In particular, soil microorganisms are effective in releasing phosphate from total soil phosphorus through solubilization and mineralization. Currently, the main purpose to manage soil phosphate is to optimize crop production and minimize phosphate loss from soils.

Plant growth promotory property of phosphate solubilizing bacteria can be considered as one of the most important trait associated with plant phosphate nutrition, which is advantageous in the sustainable agricultural practices. The role of microorganisms in solubilizing inorganic phosphates in soil and making them available to plants is well known. These microorganisms are called phosphate solubilizers and they convert the insoluble phosphates into soluble phosphate by acidification, chelation, ion exchange reaction and production of low molecular weight organic acids (Barroso *et al*., 2006). Phosphate deficiency in soil can severely limit plant growth productivity of legumes, where both the plants and their symbiotic bacteria are affected and this may have a deleterious effect on nodule formation, development and function (Alikhani *et al*., 2006). However, Phosphate solubilization is a complex phenomenon which depends on many factors such as nutritional, physiological and growth conditions of the culture (Reyes *et al.,* 1999). Interest has been focused on the inoculation of phosphate solubilizing bacteria (PSB) into the soil to increase the availability of native fixed phosphate and to reduce the use of fertilizers. Many phosphate solubilizing bacteria *viz., Pseudomonas, Bacillus, Rhizobium, Agrobacterium, Achromobacter, Micrococcus, Aerobacter, Enterobacter, Flavobacterium and Erwinia* genera have been isolated from soil (Rodriguez and Fraga, 1999; Gulati *et al.* 2007) and being used for plant growth promotion.

Application of some PGPR strains to seeds or seedlings has also been found to lead to a state of induced systemic resistance in the treated plant (Kloepper *et al*., 1999). In addition to improvement of plant growth, PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus, and production of siderophores that chelate iron and make it available to the plant root (Lalande *et al*., 1989; Glick, 1995).

Recently, there is a growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many crops (Thakuria *et al*., 2004). There is very little information regarding the use of PGPR as biofertilizers in lentil. Therefore, the present investigation has been designed to evaluate the effect of phosphate solubilizing bacteria (PSB) on plant growth, nodulation and seed yield in lentil under field conditions.

Materials and Methods

Bacterial isolates

The bacterial isolates were collected from the department of Basic Science, GBPUA&T, Hill Campus, Ranichauri, Uttarakhand, India. All the bacterial isolates were maintained on nutrient agar slants at 4^0C and glycerol stock at -20^0C. All bacterial isolates RMV1 and RMV4 from mustard and RPB3 from pea rhizosphere were isolated and identified as strong phosphate solubilizers by Singh *et al.* (2010).

Field experiment

The effect of all these three bacterial isolates i.e. RMV1, RMV4, RPB3 were studied by inoculating surface sterilized seeds of *Lens culinaris* Medik cv. VL125 with individual bacterial isolates along with un-inoculated control under field conditions. The experiments were laid out during 2010-11 and 2011-12 of two consecutive *rabi* seasons. The field soil was silt clay loam in texture, having pH- 6.2±0.2. Talc based formulation of RMV1, RMV4 and RPB3 were used for seed inoculation. Talc based formulation of the isolates were prepared according to Commare *et al*. (2002) and at the time of application, the population of phosphate solubilizing bacteria in the formulation was 3×10^8 cfu g^{-1}.

Lentil seeds were disinfected for 3 minutes with 0.1% mercuric chloride solution, and then disinfected again with 70% ethanol for 3 minutes. Seeds to be treated were weighed and moistened with sterilized distilled water for surface inoculation with talc based formulation and dried in shade for two hours as described by Lokesha and Benagi (2007). The experiments were laid out in a randomized block design with four replications. Each replication had four treatments in two rows. Treatments were planted and each rows of 3 meters length with row spacing of 30 cm and plant to plant spacing were kept of 5 cm. Standard cultural practices were adopted to raise normal healthy crop.

Proportion of emerged seed to the total number of seed sown in a row was counted after 21 days of sowing. Observations were recorded for every row of 10 randomly selected plants. Before harvesting randomly selected plants were safely uprooted for recording root length, plant height, number of branch and pod per plant, number of seed per pod and number of seed per plant. Crops were thrashed, cleaned the seed, sun dried up to 12 percent moisture content and weighed to assess the seed yield (q/ha). For test weight, random sample of 1000 well matured seed from the bulk produce of each plot was weighed. However, number of nodules per plant was counted from 10 randomly selected plants for each replication of every treatment at flowering stage.

Results and Discussion

The performance of bacterial isolates i.e. RMV1, RMV4 and RPB3 has considerable significant positive influence on plant growth, nodulation and seed yield and its contributing characters in lentil (Table. 1 & 2, Figure. 1 & 2). However, the different bacterial isolates exhibited their presence by varied level of influence on plant growth promotion, nodulation and seed yield. The maximum phosphate solubilization efficacy of bacterial isolate RPB3 have shown greatest performance in the field and exhibited that the isolate was established themselves and proliferated field emergence, root length, plant height, number of branch per plant, nodulation, pod per plant, number of seed per pod and per plant, 1000-seed weight and ultimately seed yield by 13.17, 19.77, 10.69, 21.11, 28.34, 24.17, 7.66, 21.23, 13.28 and 26.14 per cent respectively over control in pooled values of two consecutive years (Table. 1 & 2). The performance of RPB3 bacterial isolate can directly be correlated with their greater phosphate solubilization efficiency. While, other P solubilizing bacterial isolates RMV1 and RMV4 showed somewhat lower performance as compared to RPB3. There are several studies which indicate that seed inoculation with phosphate solubilizing bacteria (PSB) improve solubilization of fixed soil phosphorous and applied phosphates resulting in higher crop yields (Kumar *et al*., 2001; Katiyar and Goel, 2003; Nautiyal *et al*., 2003). These microorganisms also hold the potential of ecological amelioration of P and thereby improving growth and establishment of plants under low phosphorous availability. The beneficial bacteria enhanced plant growth by improving soil nutrient status, secreting plant growth regulators and suppressing soil borne plant pathogens (Gulati *et al*., 2007). This is fact that the P solubilization ability was accompanied by a decrease in pH of the medium. The decrease in pH clearly indicates the production of acids, which is considered to play a major role in P solubilization and subsequently plant growth promotion (Kpomblekou and Tabatabai, 1994; Reyes *et al*., 2001). The mechanisms for plant growth promotion by bacterial inoculation might be due to synthesis of the plant hormones indole-3-acetic acid (Barozani and Jacob, 1999), cytokinin (Timmusk *et al*., 1999), and gibberellin (Karakoc and Aksoz, 2006); breakdown of plant produced ethylene by bacterial production of 1-aminocyclo-propane-1-carboxylate deaminase (Glick, 1999); and increased mineral, N and P availability of the soil (Kumar and Chandra 2008). Such types of findings were also supported by Linu *et al*. (2009) in cowpea and Prasad *et al*. (2009) in wheat.

The pooled results of two consecutive years depicted the effectiveness of these tested PSB isolates in terms of field emergence by 6.27 – 13.17, root length by 7.53 – 19.77, plant height 6.43 – 10.69 and number of branch per plant by 7.68 – 21.11 percent over control. It was also suggested that PSB had capacity of PGPR ability and able to enhance the plant growth and seed yield, and contribute to the protection of plants against certain pathogens (Herman *et al*., 2008). These finding were also close confirmation with Ashrafuzzaman *et al.* (2009) and found efficiency of plant growth promoting rhizobacteria (PGPR) for the enhancement of rice growth. Enhancement in number of root nodules per plant was also increased through inoculation of these tested bacterial isolates by 18.33 - 28.34 per cent over the un-inoculated control (Table. 1 and Figure. 1).

Enhancement in seed yield and its contributing attributes through bacterial isolates were also statistically significant for lentil under field conditions with respect to number of pod per plant, number of seed per pod and plant by 12.08 – 24.17, 2.00 – 7.66 and 11.92 – 21.23 per cent respectively observed in pooled value of two consecutive years (Table. 2 and Figure. 2). Test weight of the seed in any crop is one of the prime factor that contribute and decide the yield of a particular crop and higher seed yield per hectare and 1000- seed weight were also recorded for all the treatments over un-inoculated control and maximum influence had observed for RPB3 (13.28%) for 1000 - seed weight and for seed yield by 26.14% in two year field studied (Table. 2 and Figure. 2). However, lower influence for test weight and seed yield per hectare was recorded for RMV1 by 9.12 and 13.95%, respectively. While, RMV4 had intermediate effect for these characters (Table. 2). Plant growth promoting microbes are an important contributor to biofertilization of agricultural crops. Ponmurugan and Gopi (2006) studied the production of growth regulators by phosphate solubilizing bacteria.

Table.1 Enhancement of plant growth and nodulation through bacterial isolates in Lentil under field conditions

Treatments	Field emergence (%)			Root length (cm)			Plant height (cm)			No. of branch/plant			No. of nodule / plant		
	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled
RMV1	68.50 (6.20)	67.00 (6.34)	67.75 (6.27)	8.80 (5.38)	8.90 (9.87)	8.85 (7.53)	28.80 (6.39)	28.63 (6.47)	28.71 (6.43)	3.04 (7.80)	3.13 (7.56)	3.09 (7.68)	8.41 (18.62)	8.63 (18.05)	8.52 (18.33)
RMV4	70.00 (8.52)	71.00 (12.69)	70.50 (10.58)	8.95 (7.18)	9.05 (11.72)	9.00 (9.45)	29.13 (7.60)	28.97 (7.73)	29.05 (7.67)	3.12 (10.63)	3.41 (17.18)	3.27 (13.91)	9.03 (27.36)	9.11 (24.62)	9.22 (25.99)
RPB3	72.50 (12.40)	72.00 (14.28)	72.25 (13.17)	9.90 (18.56)	9.80 (20.98)	9.85 (19.77)	29.82 (10.15)	29.91 (11.23)	29.86 (10.69)	3.41 (20.92)	3.53 (21.30)	3.47 (21.11)	9.18 (29.47)	9.30 (27.22)	9.24 (28.34)
Control	64.50	63.00	63.75	8.35	8.10	8.23	27.07	26.89	26.98	2.82	2.91	2.86	7.09	7.31	7.20
SEm±	1.10	1.18	1.19	0.14	0.20	0.22	0.51	0.59	0.53	0.11	0.13	0.16	0.32	0.33	0.34
C.D at 5%	3.43	3.75	3.78	0.44	0.75	0.76	1.63	1.70	1.73	0.34	0.31	0.38	0.93	0.97	0.92
CV	3.10	3.58	3.61	3.15	4.55	4.68	3.35	3.41	3.32	5.81	5.69	5.80	6.05	6.32	6.18

Table. 2 Enhancement of seed yield and its contributing characters through bacterial isolates in lentil under field conditions.

Treatments	No. of pod/ plant			No. of seed / pod			No. of seed/plant			1000-seed weight (g)			Seed yield/ hectare (q)		
	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled
RMV1	69.03 (13.33)	66.67 (10.83)	67.85 (12.08)	1.53 (2.00)	1.53 (2.00)	1.53 (2.00)	102.30 (11.96)	100.94 (11.86)	101.62 (11.92)	24.52 (9.90)	23.98 (8.35)	24.25 (9.12)	16.31 (13.58)	16.29 (14.32)	16.30 (13.95)
RMV4	71.15 (16.81)	71.10 (18.20)	71.13 (17.51)	1.58 (5.33)	1.59 (6.00)	1.58 (5.66)	104.75 (14.64)	105.03 (16.40)	104.89 (15.52)	24.73 (10.84)	24.61 (11.21)	24.67 (11.03)	17.05 (18.73)	17.25 (21.05)	17.15 (19.89)
RPB3	75.63 (24.16)	74.69 (24.17)	75.16 (24.17)	1.60 (6.66)	1.63 (8.66)	1.62 (7.66)	109.11 (19.41)	111.05 (23.07)	110.08 (21.23)	25.11 (12.55)	25.23 (14.01)	25.17 (13.28)	18.11 (26.11)	17.98 (26.17)	18.04 (26.14)
Control	60.91	60.15	60.53	1.50	1.50	1.50	91.37	90.23	90.80	22.31	22.13	22.22	14.36	14.25	14.30
SEm±	0.43	0.83	0.68	0.02	0.02	0.02	1.32	1.29	1.38	0.17	0.22	0.18	0.25	0.26	0.23
C.D at 5%	1.67	2.61	1.91	0.07	0.08	0.09	5.16	4.29	4.45	0.68	0.73	0.81	0.75	0.78	0.91
CV	1.61	2.43	3.81	2.81	2.89	3.01	2.73	2.17	2.25	2.01	2.10	3.26	2.15	2.18	3.11

Figure.1 Efficacy of plant growth promoting rhizobacteria on plant height, nodulaion and seed yield.

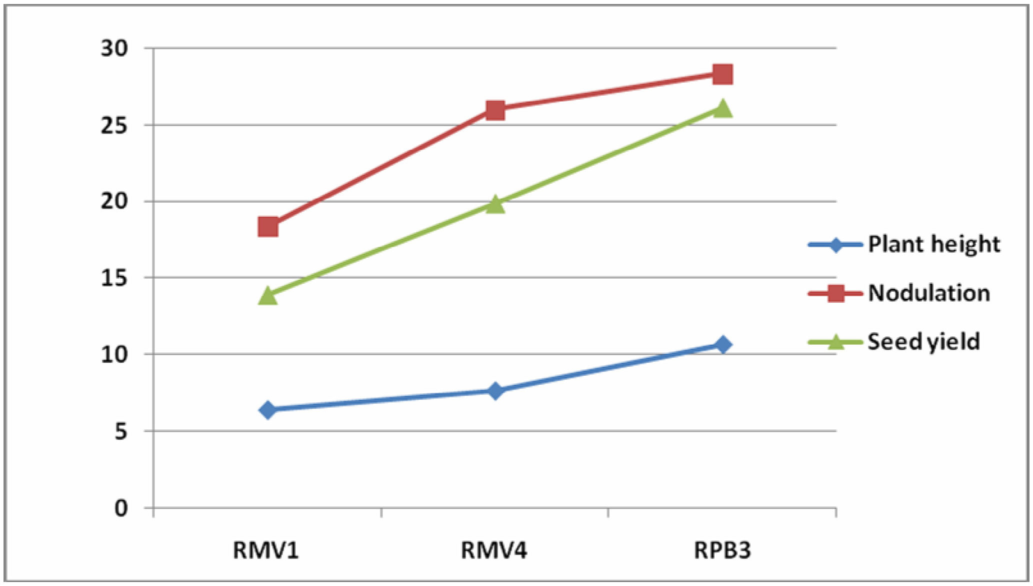
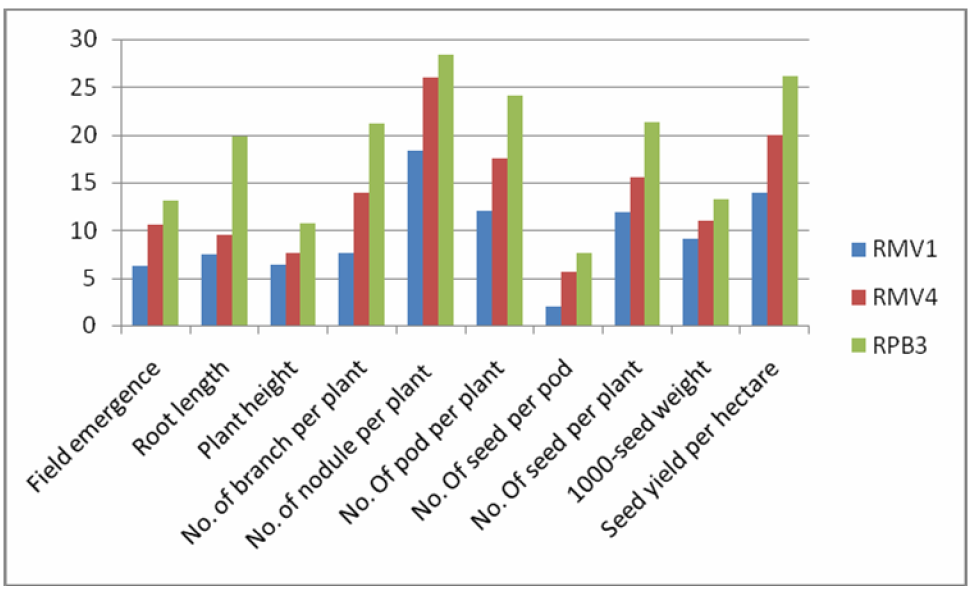


Figure.2 Enhancement of plant growth parameters, seed yield and its contributing traits through plant growth promoting rhizobacteria in lentil.



While, Chaykovskaya (2001) also reported that treatment with phosphate solubilizing bacteria resulted in increased yield of pea and barley. These results were close agreement with Biswas and Bhowmick (2009) and Singh *et al*. (2010) for plant growth promotion, nodulation and seed yield in urdbean with *Rhizobium* and soybean with PSB, respectively.

Therefore, on behalf of this study suggested that all the phosphate solubilizing bacterial isolates were capable to enhance the plant height, nodulation, seed yield and its contributing traits by secreting plant growth promoting substances. That's why the evaluated 'P' solubilizing bacterial isolates were important contributor to improve plant growth and seed yield in lentil. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable lentil cultivation. Further investigations including efficiency test under field conditions might be needed to clarify the role of PGPR as biofertilizers that exert beneficial effects on plant growth and development along with nodulation and yield.

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