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Influence of phosphate solubilizing bacteria for enhancement of plant growth and seed yield in lentil

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ABSTRACT

The ability of few rhizospheric microorganisms to convert insoluble form of phosphorous to an accessible form is an important trait of plant growth promotory rhizobacteria for increasing plant yield. Consequent to use of phosphate solubilizing bacteria as inoculants there is increase of P uptake by plant. In this context, the influences on plant growth promotion by three phosphate solubilizing bacteria (PSB) i.e. RMV1, RMV4 and RPB3 were evaluated through seed inoculation on plant growth, nodulation and seed yield in lentil. All the evaluated isolates had significant influence on plant growth in terms of field emergence, root length, plant height, number of branch and nodule number per plant along with seed yield contributing traits i.e. number of pod per plant, number of seed per pod and per plant, 1000-seed weight and ultimately seed yield ha^{-1} over un-inoculated control. Among these isolates, RPB3 exhibited greater influence on most of the characters studied followed by RMV4 and RMV1. The better performance of these three bacterial isolates was attributed to its greater P solubilization activity and positive correlation with plant growth and seed yield of lentil. However, RPB3 appear to be good phosphate solubilizing bacteria to be developed in to a P solubilizing bio-inoculant.

Key words: Lentil, PGPR, seed yield

Phosphorous (P) is the most limiting macronutrient for plant growth after nitrogen. 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. Furthermore, use of P fertilizers has become costly affair and there is a need for alternative sources.

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). Phosphate solubilizing microorganisms (PSMs) are ubiquitous in soils and could play an important role in supplying P to plants in a more environmental friendly and sustainable manner. This process not only compensates for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil (Rodriguez and Fraga, 1999). However, the success of introduced microorganisms for phosphate solubilization has been related to its capacity to readily colonize surrounding plant roots. Available nutrients will probably also affect the ability of an introduced plant growth promoting rhizobacteria (PGPR) to colonize root and to perform their beneficial activity. Several studies have been reported to increase plant growth and nodulation in Greengram (Vikram and Hamzehzarghani, 2008), yield and nutrient uptake of soybean (Sandeep *et al.*, 2008) by phosphate solubilizing microorganisms (PSMs). Inoculation of phosphate solubilizing bacteria like *Serratia marcescens*, *Pseudomonas fluorescens* and *Bacillus* spp. improved the phosphorous uptake of shoot and grain in maize and peanut plants (Dey *et al.*, 2004, Sahin *et al.*, 2004, Hameeda *et al.*, 2008).

The present investigation was carried out to screen phosphate solubilizing bacteria (PSB) from pea and mustard rhizosphere. The selected strains were further evaluated for plant growth and seed yield enhancement of lentil (*Lens culinaris* Medik) under field conditions.

MATERIALS AND METHODS

Soil samples were collected from the rhizosphere of mustard and pea field of 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. Out of these 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.

On the basis of phosphate solubilization ability three bacterial isolates *i.e.* RMV1, RMV4, RPB3 were screened (Singh *et al.*, 2010) and their effect on plant growth and seed yield were studied by inoculating surface sterilized seeds of *L. culinaris* cv. VL 4 with individual bacterial isolates along with un-inoculated control under field conditions. The experiment was conducted during two successive *rabi* seasons of 2007-08 and 2008-09. 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 was 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

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min with 0.1% mercuric chloride solution, and then disinfected again with 70% ethanol for 3 min. Seeds to be treated were weighed and moistened with sterilized distilled water for surface inoculation with talc based formulation and shade dried for two hours as described by Lokesha and Benagi (2007). The experiments were laid out in a randomized block design with four replications.

Proportion of emerged seed to the total number of seed sown in a row was counted after 20 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, sun dried and weighed to assess the seed yield (q/ha). For 1000-seed 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 pre-pod setting stage.

RESULTS AND DISCUSSION

The activity of these PSB inoculants exhibited a considerable influence on plant growth parameters i.e. field emergence, root length, plant height, number of branch per plant, number of nodule per plant and also on seed yield and its contributing characters over un-inoculated control under field condition (Table 1, 2). However, the influence on plant growth varied for different bacterial isolates. The higher P solubilization efficiency of bacterial strain RPB3 have shown maximum performance during field study and indicated that the isolate was established themselves and proliferated field emergence, root length, plant height, branching, nodulation number, pod per plant, number of seed per pod and plant, 1000-seed weight and finally seed yield by 15.52, 41.35, 23.00, 35.27, 47.67, 23.92, 5.33, 30.96, 16.47 and 30.31 per cent respectively over control in pooled values of two successive years (Table 1, 2). The performance of RPB3 bacterial isolate can directly be correlated with their higher P solubilization efficiency. However, other P solubilizing bacterial isolates RMV1 and RMV4 performed somewhat poorly as compared to RPB3. The mechanisms for plant growth promotion by bacteria 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-aminocyclopropane-1-carboxylate deaminase (Glick, 1999); and increased mineral, N and P availability of the soil (Kumar and Chandra, 2008). Similar results were also reported by Linu *et al.* (2009) in cowpea and Prasad *et al.* (2009) in wheat.

The pooled results of two consecutive years revealed the effectiveness of these PSB isolates over control. It is suggested that PSB had an ability of PGPR and able to enhance the plant growth and seed yield, and contribute to the protection of plants against certain pathogens (Herman *et al.*, 2008). Inoculation of these bacterial isolates also enhanced the number of root nodules per plant and increased by 22.17 - 47.67 per cent over the un-inoculated control (Table 1). Significant enhancement of seed yield and its contributing characters through bacterial isolates in lentil under field conditions in respect of number of pod per plant, number of seed per pod and plant by 8.58–23.92, 3.33–5.33 and 12.18–30.96 per cent respectively could be observed (Table 2). Higher seed yield per hectare and 1000 seed weight were also recorded for all the treatments over un-inoculated control and greatest influence had recorded for RPB3 (16.47%) for test weight and for seed yield by 30.31% in two year field studied (Table 2). Similar results were also reported by Biswas and Bhowmick (2009) in urd bean.

A correlation coefficient of pooled values of two successive years for different plant growth and seed yield contributing characters were studied with seed yield and found positive (Table 3). The value of correlation coefficient was varied from 0.997 to 0.972 that indicated these parameters were highly significant and inter correlated with seed yield except root length which exhibited non significant correlation (Table 3). The data revealed that plant height and seed yield showed greatest correlation coefficient (0.997), however least correlation coefficient (0.0938) was observed for root length.

Results of this study suggested that all the P solubilizing bacterial isolates were capable to enhance the plant height and other yield contributing characters by secreting plant growth promoting substances. Therefore, evaluated 'P' solubilized bacterial isolates were important contributor to improve plant growth and seed yield in lentil.

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Table 1: Effect of bacterial isolates on plant growth parameters of lentil

Treatments	Field emergence (%)			Root length (cm)			Plant height (cm)			Branch plant $^{-1}$			Nodule plant $^{-1}$		
	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled
RMV1	70.00	68.25	69.12	9.05	9.63	9.34	30.68	30.72	30.70	3.60	3.54	3.57	8.67	9.72	9.20
	(7.69)*	(9.63)	(9.28)	(7.73)	(13.42)	(10.66)	(10.71)	(13.48)	(12.08)	(20.80)	(23.77)	(22.26)	(18.76)	(25.09)	(22.17)
RMV4	71.25	71.50	71.37	9.66	10.21	9.93	32.23	31.92	32.08	3.63	3.60	3.62	9.60	10.05	9.82
	(9.61)	(14.85)	(12.18)	(15.00)	(20.25)	(17.65)	(16.31)	(17.91)	(17.12)	(21.81)	(25.87)	(23.97)	(31.50)	(29.34)	(30.41)
RPB3	74.75	72.25	73.50	11.58	12.28	11.93	33.75	33.64	33.69	3.97	3.94	3.95	11.22	11.02	11.12
	(15.00)	(16.06)	(15.52)	(37.85)	(44.64)	(41.35)	(21.79)	(24.27)	(23.00)	(33.22)	(37.76)	(35.27)	(53.69)	(41.82)	(47.67)
Control	65.00	62.25	63.62	8.40	8.49	8.44	27.71	27.07	27.39	2.98	2.86	2.92	7.30	7.77	7.53
SEm(±)	**1.08**	**1.15**	**0.74**	**0.15**	**0.22**	**0.14**	**0.49**	**0.73**	**0.55**	**0.10**	**0.10**	**0.08**	**0.30**	**0.32**	**0.25**
LSD (0.05)	**3.47**	**3.69**	**2.39**	**0.50**	**0.72**	**0.47**	**1.59**	**2.35**	**1.75**	**0.32**	**0.32**	**0.25**	**0.97**	**1.03**	**0.80**
CV	**3.09**	**3.37**	**2.15**	**3.23**	**4.44**	**3.00**	**3.20**	**4.77**	**3.55**	**5.80**	**5.88**	**4.61**	**6.65**	**6.68**	**5.34**

* *Values in parentheses indicate percent increase over control*

Table 2: Effect of bacterial isolates on yield and yield attributing factors of lentil

Treatments	Pods plant $^{-1}$			Seeds pod $^{-1}$			Seeds plant $^{-1}$			1000-seed weight (g)			Seed yield (q ha $^{-1}$)		
	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled
RMV1	69.56	65.50	67.53	1.56	1.53	1.55	109.08	100.71	104.89	22.91	22.02	22.46	11.85	13.00	12.42
	(9.33)*	(7.78)	(8.58)	(4.00)	(2.00)	(3.33)	(14.14)	(10.13)	(12.18)	(7.20)	(7.30)	(7.25)	(8.61)	(16.07)	(13.73)
RMV4	72.75	69.75	71.25	1.59	1.56	1.57	116.01	109.06	112.53	23.03	22.92	22.97	12.58	14.08	13.33
	(14.35)	(14.77)	(14.56)	(6.00)	(4.00)	(4.66)	(21.40)	(19.26)	(20.35)	(7.76)	(11.69)	(9.69)	(15.30)	(25.71)	(22.06)
RPB3	78.81	75.32	77.07	1.60	1.57	1.58	126.53	118.36	122.45	24.43	24.36	24.39	13.33	15.12	14.23
	(23.87)	(23.94)	(23.92)	(6.60)	(4.66)	(5.33)	(32.40)	(29.44)	(30.96)	(14.31)	(18.71)	(16.47)	(22.18)	(35.00)	(30.31)
Control	63.62	60.77	62.19	1.50	1.50	1.50	95.56	91.44	93.50	21.37	20.52	20.94	10.91	11.20	10.92
SEm(±)	**0.47**	**0.88**	**0.36**	**0.02**	**0.03**	**0.02**	**1.28**	**2.59**	**1.38**	**0.23**	**0.34**	**0.20**	**0.22**	**0.21**	**0.20**
LSD (0.05)	**1.51**	**2.83**	**1.16**	**0.06**	**0.10**	**0.06**	**4.12**	**8.29**	**4.44**	**0.75**	**1.09**	**0.66**	**0.72**	**0.68**	**0.64**
CV	**1.33**	**2.61**	**1.04**	**2.78**	**4.40**	**2.69**	**2.29**	**4.94**	**2.56**	**2.04**	**3.05**	**1.82**	**3.73**	**3.21**	**3.16**

* *Values in parentheses indicate percent increase over control*

Table 3: Correlation coefficient for plant growth parameters and yield attributing characters with seed yield of lentil

Characters	Seed yield (q ha $^{-1}$)	Field emergence	Root length	Plant height	Branch plant^{-1}	Nodule plant^{-1}	Pod plant^{-1}	Seeds pod^{-1}	Seeds plant^{-1}	1000-seed weight
Seed yield q ha^{-1}		0.99**	0.94ns	0.99**	0.97 *	0.99**	0.99**	0.98 *	0.99**	0.99 *
Field emergence			0.90ns	0.99**	0.99 *	0.99 *	0.97 *	0.99**	0.98 *	0.98 *
Root length				0.92ns	0.90ns	0.96 *	0.98 *	0.85ns	0.97 *	0.97 *
Plant height					0.99 *	0.99**	0.98 *	0.99**	0.99 *	0.98 *
No. of branch plant^{-1}						0.98 *	0.95 *	0.98 *	0.96 *	0.98 *
No. of nodule plant^{-1}							0.99**	0.96 *	0.99**	0.99**
No. of pod plant^{-1}								0.94ns	0.99**	0.99**
No. of seed pod^{-1}									0.96 *	0.95 *
No. of seed plant^{-1}										0.99**
1000-seed weight										

**Significant,* ***highly significant,* ^{ns} *non significant correlation with seed yield*

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