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Potential for enhancing lentil (*Lens culinaris*) productivity by co-inoculation with PSB, plant growth-promoting rhizobacteria and *Rhizobium*

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ABSTRACT

Rhizobia in association with a legume host have the exceptional ability to form root nodules. Free living microorganisms, especially the plant growth-promoting rhizobacteria are known to promote *Rhizobium* efficiency by different mechanisms. Field experiments were conducted to assess the effect of co-inoculation of *Rhizobium leguminosarum* bv. viciae, phosphorus-solubilizing bacteria and plant growth-promoting rhizobacteria on symbiotic parameters, growth and yield of lentil. Seeds of lentil (*Lens culinaris* Medikus) variety LL 699 were treated with the three inoculants singly and in combination and sown following randomized block design in three replications. Results revealed that PGPR with rhizobial inoculation enhanced nodulation, growth and yield (19.8nodules/plant, 70.6mg nodule dry weight/plant, 1 605kg/ha) as compared to *Rhizobium* alone (17.8nodules/plant, 64.3mg/plant and 1 546kg/ha resp.) and non-inoculated control (13.9nodules/plant, 47.7mg/plant and 1 401kg/ha resp.). Treatment comprising *Rhizobium* +PSB was found to be at par with *Rhizobium* inoculation alone in terms of nodulation and yield.

However, the response was more prominent when all the three microorganisms were applied in combination, higher nodule biomass (16.7%) and yield (5%) was recorded over that with *Rhizobium* inoculation alone. Although PSB alone did not show any positive effect on symbiotic parameters and yield, however in conjunction it seemed to assert a synergistic effect. The results imply that co-inoculation with *Rhizobium*, PSB and PGPR is a beneficial approach for improving the nodulation, growth and yield of lentil.

Key words: Phosphorus-solubilising bacteria, Plant growth-promoting rhizobacteria, Synergism

Legume roots are colonized by numerous rhizospheric microorganisms and many of these are known to beneficially affect plant growth and assert synergistic influence on *Rhizobium* efficiency. The mechanisms by which PGPR promote plant growth are not fully elucidated, but are reported to include the ability to produce phytostimulators, siderophores, asymbiotic N_2 fixation, nutrient mobilization, production of antibacterial compounds effective against certain plant pathogens etc (Qureshi *et al.* 2009). Application of naturally occurring organisms possessing multiple growth-promoting activities hold greater potential for increasing the productivity of crops including legumes. Lentil (*Lens culinaris* Medikus) is an important food legume crop since times immemorial. Its protein-rich seed is an important dietary component. However, it is generally grown on marginal lands with low fertilizer inputs. Being a legume, lentil can fix its own nitrogen from the atmosphere in conjunction with its microbial symbiont *Rhizobium***.** The seed bacterization with the appropriate rhizobia at sowing is a recommended practice, but in recent years the potential of combined inoculation of N_2 -fixers, and plant growth-promoting rhizobacteria (PGPR) including phosphorus-solubilizing bacteria (PSB) being more effective than single organism for providing a more balanced nutrition particularly under conditions of reduced nutrient inputs has been reported (Ahmad *et al.* 2006). The application of synergistically interacting microbes is an effort to shift the microbiological equilibrium in favour of increased plant growth and productivity and is thus likely to help sustaining the legume productivity in different agricultural systems. Keeping this in view, the present study was conducted to evaluate the effect of co-inoculation of PGPR including PSB with the recommended inoculant in lentil under field conditions.

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MATERIALS AND METHODS

The effect of plant growth-promoting rhizobacteria (PGPR) including phosphate-solubilizing bacteria (PSB) and recommended *Rhizobium* inoculant on symbiotic parameters, growth and yield of lentil was studied during *rabi* season 2005–07 at research farms, Department of Plant Breeding

and Genetics, PAU, Ludhiana. The treatments comprised inoculation with *Rhizobium leguminosarum* bv. viciae from PAU, Ludhiana, *Pseudomonas* sp. (PSB) from CCSHAU, Hisar, *Pseudomonas* sp. (PGPR) from department of Soil Sciences, GBPUA&T, Pantnagar, individually, dually and in combination with each other arranged in random block design in triplicate. The soil of the experimental site was loamy, sand, low in organic carbon (0.22%) and available nitrogen (104 kg N/ha) and medium in phosphorus (12.5 kg/ha) and potash (197 kg/ha). Lentil variety LL 699 was sown on 4 November 2005 and 7 November 2006 during the two consecutive seasons and correspondingly the harvesting dates were 14 April 2006 and 10 April 2007 respectively. Sowing was done at 22.5 cm. row spacing and lentil seed was treated with the required inoculants (20g inoculant/kg seed) prior to sowing. Crop was raised as per the recommended practice. Symbiotic parameters were recorded at 50% flowering stage. Five plants from each treatment were uprooted, roots were washed and nodules detached and counted.

Dry weight of nodules and plant biomass was recorded after drying at 65ºC to constant weight. Grain yield was recorded at harvesting stage.

Evaluation of inoculants for plant growth-promoting traits: the three cultures, i e *Rhizobium* and the two *Pseudomonas* sp. isolates were screened for their potential for production of indole acetic acid(IAA) equivalents and siderophores and phosphorus solubilization as reported by Sarwar *et al.* (1992), Schwan and Neilands (1987) and Pikovaskya medium (1948) respectively.

RESULTS AND DISCUSSION

Inoculation with *Rhizobium* and PGPR individually enhanced the number of nodules as compared to uninoculated control. Similarly, significant increase in dry weight of nodules was observed with *Rhizobium* and PGPR alone. Positive effect of *Rhizobium* inoculation on symbiotic parameters is very well documented and this response has been attributed to the presence of either low and/or ineffective native *Rhizobium* population in soils. Thus, inoculations with effective isolates of *Rhizobium* have significant effect on growth and nodulation (Khanna *et al* 2006). Inoculation with PSB alone did not significantly influence the number or nodule biomass over the period under study. This may be attributed to the medium status of available P in soil, hence exhibiting little response to PSB. Dual inoculation of *Rhizobium* + PSB was also found to be statistically comparable to *Rhizobium* inoculation both in terms of number as well as dry weight of nodules. These results are in carroboration with Kumar and Chandra (2008) in lentil, and Bansal (2009) in mungbean. However, the co-inoculation of PGPR with *Rhizobium* solicited an improved response in terms of nodulation (Table 1) over *Rhizobium* inoculation alone. A number of reports clearly elucidate the effect of PGPR on stimulating *Rhizobium* efficiency and plant growth

Table 1 Synergism amongst *Rhizobium*, PSB and PGPR for nodulation in lentil

Treatment	No. of nodules/ plant			Dry weight of nodules (mg/plant)		
	2005–06	2006–07	Mean	2005–06	2006–07	Mean
Control	13.9	13.9	13.9	52	43.5	47.7
Rhizobium (R)	18.0	17.7	17.8	72.6	56.1	64.3
PSB	12.1	13.1	12.6	55.2	39.3	47.2
PGPR	16.0	14.4	15.2	68.6	47.0	57.8
R + PSB	19.0	18.9	18.9	75.4	60.6	68
R + PGPR	20.5	19.2	19.8	77.2	64.1	70.6
PSB + PGPR	14.2	14.8	14.5	60.6	46.5	53.5
R + PSB + PGPR	21.9	20.6	25.2	83.2	67.0	75.1
CD *P*=0.05	1.9	4.8		15.6	6.1	

Table 2 Synergism amongst *Rhizobium* PSB and PGPR for growth and grain yield in lentil

Treatment	Plant dry weight g/plant			Yield (kg/ha)		
	2005–06	2006–07	Mean	2005–06	2006–07	Mean
Control	.811	1.11	.96	1 475	1 333	1 404
Rhizobium (R)	1.08	1.633	1.356	1 660	1 432	1 546
PSB	.762	1.811	1.286	1 415	1 123	1 268
PGPR	1.01	1.644	1.327	1 500	1 345	1 422
R + PSB	1.08	1.929	1.504	1 690	1 407	1 548
R + PGPR	1.03	1.628	1.329	1 720	1 491	1 605
PSB + PGPR	.982	1.428	1.205	1 610	1 296	1 453
R + PSB + PGPR	1.205	1.814	1.509	1 748	1 500	1 624
CD *P*=0.05	NS	.154		105	136	

(Tilak *et al.* 2006, Yumming *et al.* 2003, Chandra and Pareek 2002). Inoculation of lentil with one or more rhizobacteria has been reported to improve nodulation, plant health and yield by production of phytostimulators, siderophores, flavonoid-like compounds which may promote nodulation. Most of the phytohormones are also implicated in nodule formation in one way or another (Mishra *et al* 2010, Egamberdieva 2008). A similar response to *Rhizobium* inoculation alone and *Rhizobium* in combination with PGPR and PSB was also noted in terms of plant biomass (Table 2) for both the years under study. *Rhizobium* inoculation alone itself enhanced plant dry weight (1.365g/plant) as compared to uninoculated control (0.96g/plant). Individual inoculation as well as combined inoculations significantly improved plant biomass. However, the composite inoculation of *Rhizobium* +PSB+PGPR recorded the highest number and dry weight of nodules as well as plant biomass (1.509g/ plant) average for both the years of study, indicating the

Table 3 Evaluation of plant growth-promoting traits of *Rhizobium* PSB and PGPR inoculants

Bacterial culture	IAA (µg/ml)	Siderophore production (dia in cm)*	P-solubilising potential
Rhizobium	24.5	–	–
Pseudomons sp (PSB)	–	2.2	+
Pseudomonas sp (PGPR)	12.5	–	–

* Diameter of yellow zone on chrome azurol assay plate after 96hr of incubation

potential of such consortia in enhancing crop productivity. This could be attributed to the varied functionality traits exhibited by these isolates (Table 3). Two of the inoculants, *Rhizobium* as well as *Pseudomonas* sp. (PGPR) showed the production of indole acetic acid (24.5 and 12.0µg/ml). The bacterial isolate *Pseudomonas* sp. (PSB) apart from possessing phosphorus solubilizing ability also produced siderophores as indicated by the production of yellow colour halo on CAS agar plates. When these mixed cultures having varied plant growth promoting traits become established their individual beneficial effects may be magnified in a synergistic manner. Such successful establishment of combined inoculants has been reported by many other workers (Mishra *et al*. 2010, Suneja *et al*. 2007 and Khanna *et al.* 2006)

A similar response in grain yield was recorded with various treatments. Significant increase in grain yield was recorded with *Rhizobium* inoculation alone (10.1%) as compared to uninoculated control. Dual inoculation with *Rhizobium* + PGPR further enhanced the yield (3.8%), over *Rhizobium* alone. However, maximum increase in grain yield (5%) was recorded with combined inoculation of *Rhizobium* + PSB+ PGPR (1 624 kg/ha) which was numerically higher than *Rhizobium* alone (1 546 kg/ha) and *Rhizobium* + PGPR (1 605kg/ha). Grain yield obtained with *Rhizobium* + PSB inoculation was comparable to that with *Rhizobium* alone. Such type of microbial interactions have been a subject of several investigations and reported as potential inputs for improving crop yields in a number of leguminous crops including lentil (Gupta *et al.* 2003). The findings of the present study clearly define the synergistic effect of co-inoculation of *Rhizobium*, PSB and PGPR in enhancing symbiotic efficiency and yield of lentil. The results imply that co-inoculation with *Rhizobium*, PSB and PGPR to be a beneficial strategy for improving symbiotic efficiency, growth and yield of lentil, however detailed experimentation at farmers' fields need to be carried out to confirm this approach for sustainable crop production.

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