World Journal of Agricultural Sciences 4 (3): 297-301, 2008
ISSN 1817-3047

Influence of PGPR and PSB on *Rhizobium leguminosarum* Bv. *viciae* Strain Competition and Symbiotic Performance in Lentil

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Abstract: In a field experiment conducted during *Rabi* 2002-03 at Pantnagar, seed inoculation with *Rhizobium leguminosarum* bv. *viceae* (LB-4), PSB (*Bacillus megaterium*) and PGPR LK-786 (*Kurthia* sp.) alone significantly increased the nodule number at different intervals, nodule dry weight at 45 and 90 DAS, plant dry weight at 70 and 90 DAS and N uptake by grain and straw in lentil over the uninoculated control. PGPR LK-884 (*Pseudomonas diminuta*) alone recorded only significantly more number and dry weight of nodule and N uptake by grain and straw. *Rhizobium* sp. alone recorded the highest occupancy of 34.4% in nodules at 70 DAS. Co-inoculation of PSB and both PGPRs further improved the nodule occupancy of inoculated *Rhizobium* sp. and recorded highest value at 90 DAS. Dual inoculation of *Rhizobium* sp.+PSB was statistically comparable to *Rhizobium* sp. and PSB alone in different parameter, but *Rhizobium* sp.+LK-786 and *Rhizobium* sp.+LK-884 recorded significantly more plant dry weight than their individual inoculation at 90 DAS. Combined inoculation treatment of *Rhizobium* sp.+PSB+LK-786 produced the highest and significantly more number and dry weight of nodules and plant dry weight than *Rhizobium* sp. alone at different intervals. It also gave highest and significantly more nodule occupancy at 45 and 90 DAS of 34.7 to 42.3% than *Rhizobium* sp. alone inoculation. This treatment recorded significant increases of 20.8 and 23.5% in grain yields and numerical increases of 13.2 and 14.0% in straw yields over *Rhizobium* sp. and PSB alone inoculation, respectively. N and P uptake by grain and straw with this treatment were also significantly more than individual inoculation of *Rhizobium* sp., PSB and LK-786. Combined inoculation treatment of *Rhizobium* sp.+PSB+LK-884 was also significantly superior to *Rhizobium* sp. alone in plant dry weight at different intervals and P uptake by grain and straw. Both the latter treatments were statistically comparable to application of 20 kg N+40 kg P_2O_5 ha^{-1} in most parameters, except nodule number at different intervals.

Key words: Inoculation · PSB · PGPR · *Rhizobium* · Nodulation · Nutrient uptake · Lentil

INTRODUCTION

Seed inoculation with appropriate *Rhizobium* culture at sowing is a recommended agronomic practice in pulse production technology. However, it has been observed that traditional pulse crops usually give poor and variable response to inoculation because of the presence of sufficient, but not necessarily efficient, native rhizobia population in soil nodulating these crops [1, 2]. The presence of fairly high population of rhizobia in soil nodulating these crops gives strong competition to seed inoculated rhizobia and many a times do not allow the inoculum to occupy nodule sites resulting in to low or failure of inoculation response [3]. Although, competitive ability and effectiveness of a *Rhizobium* sp. strain are genetic characters, nevertheless, soil biological environment influence the competitive ability of inoculum rhizobia [4]. Legumes root are colonized by numerous rhizospheric microorganisms and these organisms have definite influence on the survival and nodulation ability of seed inoculated rhizobia [5]. Rhizospheric microorganisms may not only influence the inoculated rhizobia adversely through saprophytic competition, but also help them in survival through synergism resulting in an increase in their nodulation ability and N_2 fixing efficiency [2, 6]. Several mechanisms such as alteration in the composition of rhizospheric microorganisms, production of plant signaling compounds, bacteriocins, siderophores, plant

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growth hormones and improving availability of nutrients by rhizospheric microorganisms have been reported for such synergism [5, 7, 8]. However, compatibility of these microorganisms needs to be evaluated because of the possibility of antagonistic interactions among them [9].

Lentil (*Lens culinaris* L.) is cultivated from time immemorial in Asian sub continent on poor and marginal lands with minimum or no fertilizer inputs. This crop has inherent capacity of atmospheric N_2 fixation in association with *Rhizobium leguminosarum* and generally gives poor response to inoculation [1] because of the build up of rhizobia population in the soils. The present study was, therefore, undertaken to enhance the symbiotic efficiency and strain competition of inoculated *Rhizobium leguminosarum* in lentil through co-inoculation of PSB (*B. megaterium*) and PGPRs (*Kurthia* sp. and *P. diminuta*) under field conditions.

MATERIALS AND METHODS

Microbial Cultures: Effective strains of *Rhizobium leguminosarum* bv. *viciae* (strain LB-4) having intrinsic resistance of 250 µg/ml streptomycin+150 µg/ml ampicillin and PGPRs LK-786 (*Kurthia* sp.) and LK-884 (*Pseudomonas diminuta*), isolated from lentil rhizosphere, were obtained from the culture collection of AICRP on Pulses in the Soil Science department. PSB (*Bacillus megaterium*) was obtained from Division of Microbiology, TNAU, Coimbatore. The obtained *Rhizobium* sp. was multiplied in YEM broth for 4 days, PSB in Pikovaskaya broth and PGPRs in succinate broth for 2 days and mixed with sterilized charcoal, neutralized with 12.5% $CaCO_3$, in 1:2 ratio to prepare their carrier based inoculants.

Field Study: Effect of PGPRs and PSB on competitive ability of inoculated *Rhizobium* sp. and symbiosis was studied during *Rabi* 2002-03 at Crop Research Centre of the G. B. Pant University of Agriculture and Technology, Pantnagar. The soil was sandy loam of pH 7.4 having 0.59% Organic C and 159.6, 15.2 and 298.6 kg ha^{-1} available N, P and K, respectively. Treatments consisted of inoculation with *Rhizobium* sp, PSB (*Bacillus megaterium*), PGPR LK-786 (*Kurthia* sp.) and LK-884 (*Pseudomonas diminuta*), either alone or in different combinations, two P levels (20 and 40 Kg P_2O_5/ha along with 20 kg N/ha) and an uninoculated control. Treatments were laid out in Randomized Block Design in plots of 2.4 m × 3.0 m size in 3 replications. Lentil seed (cv. PL-5) was treated with the required inoculant(s) @ 20 g inoculant /kg seed rate at the time of sowing. Crop was raised as per the recommended agronomic practices.

Strain Competition: Ten plants from the each plot were randomly uprooted along with a soil core at 45, 70 and 90 days after sowing (DAS). Soil cores with plants were placed in sieve and washed off with water jet. Five nodules from each plant, fifty in all for each replicate, were removed and each nodule was surface sterilized in separate test tube with 95 per cent ethyl alcohol for 2 to 3 minutes. The nodules were crushed in test tubes having 4 ml sterile distilled water with a flame sterilized glass rod. The nodule suspensions were streaked on YEMA plates containing 250 µg/ml streptomycin+150 µg/ml ampicillin. Each plate containing 10 streaks of 10 nodules was duplicated and incubated at 28±2°C for 4 days and per cent nodule occupancy of inoculum *Rhizobium* sp. strain was calculated [10].

Symbiotic Effectivity: Similarly, another 10 plants from each plot at the different intervals were uprooted, roots were washed, nodules were removed and counted. Dry weights of nodules and plants were determined after drying to constant weight. Grain and straw yields were recorded at final harvest. N and P concentrations in grain and straw were determined after grinding the samples to 40 mesh. N contents was determined by micro-Kjeldahl method and P after wet digestion in tri-acid mixture (HNO_3: H_2SO_4: $HClO_4$ in 9: 4: 1 ratio) by vanadomolybdophoshporic yellow colour methods [11] and N and P uptake by grain and straw were calculated.

RESULTS AND DISCUSSION

Competitiveness: The highest occupancy of inoculant *Rhizobium* sp. in nodules of 42.7% was recorded with combined inoculation of *Rhizobium* sp.+PSB+LK 786 at 90 DAS, being significantly higher, by 42.3%, than *Rhizobium* sp. alone at the same interval. Inoculation of *Rhizobium* sp. occupied 16.1 to 34.4% nodules at different intervals (Table 1) indicating the presence of highly competitive population of native rhizobia nodulating lentil in the soil. Co-inoculation of PSB with *Rhizobium* sp. did not influence the per cent nodule occupancy at different intervals. However, dual inoculation of *Rhizobium* sp.+LK-786 recorded significantly more nodule occupancy than *Rhizobium* sp. alone and *Rhizobium* sp.+PSB at 45 and 90 DAS. Similarly, *Rhizobium* sp.+LK-884 treatment gave significantly more nodule occupancy than *Rhizobium* sp. alone at 90 DAS. The favourable effect of PGPRs on competitiveness of inoculum rhizobia was probably

Table 1: Combined inoculation effect of PSB and PGPRs. on nodule occupancy of inoculated *Rhizobium* sp. at different days after sowing (DAS)

	Nodule occupancy (%)		
Treatments	45 DAS	70 DAS	90 DAS
Rhizobium sp.	16.1	34.4	30.0
Rhizobium sp.+PSB	17.2	32.2	35.0
Rhizobium sp.+LK 786	24.6	35.4	40.9
Rhizobium sp.+LK 884	22.1	32.4	34.7
Rhizobium sp.+PSB+LK 786	21.7	38.3	42.7
Rhizobium sp.+PSB+LK 884	18.3	30.7	38.6
C.D at 5%	5.5	NS	5.6

Table 2: Combined inoculation effect of *Rhizobium* sp., PSB and PGPRs on root nodule number and their dry weight at different intervals

	Nodule number plant^{-1}			Nodule dry weight (mg plant^{-1})		
Treatment	45 DAS	70 DAS	90 DAS	45 DAS	70 DAS	90 DAS
Uninoculated control	6.4	8.1	7.8	6.6	10.4	7.4
Rhizobium sp.	9.7	12.6	10.7	8.6	12.7	9.3
PSB	8.5	11.9	10.1	8.8	13.1	9.5
LK 786	8.6	11.1	9.9	8.3	11.1	9.1
LK 884	8.1	10.8	9.2	8.1	10.8	9.0
Rhizobium sp.+PSB	9.6	13.9	11.3	9.3	13.4	9.9
Rhizobium sp.+LK 786	10.0	13.6	11.0	9.2	13.5	9.5
Rhizobium sp.+LK 884	9.6	12.7	10.2	9.0	12.9	9.3
PSB+LK-786	10.0	13.2	10.5	9.6	13.8	9.2
PSB+LK 884	9.5	11.8	10.7	9.3	13.3	8.8
Rhizobium sp.+ PSB+LK-786	11.2	14.8	12.6	10.1	15.8	11.9
Rhizobium sp.+ PSB+LK-884	10.5	13.9	12.2	9.9	14.8	11.7
20 Kg N+40 Kg P_2O_5 ha^{-1}	8.1	10.7	9.0	8.9	14.2	11.1
20 Kg N+20 Kg P_2O_5 ha^{-1}	7.9	10.6	8.8	8.4	12.5	9.7
C.D at 5%	1.1	1.5	1.1	1.3	2.9	1.4

due to better survival of inoculated *Rhizobium* sp. in rhizosphere in presence of PGPRs as reported for urdbean rhizobia in culture medium [12] and for chickpea rhizobia in soil conditions [8].

Nodulation: Inoculation of *Rhizobium* sp., PSB and both PGPRs individually increased the number and dry weight of root nodules significantly, except nodule dry weight at 70 DAS, over the uninoculated control at all the intervals (Table 2). Similar effects to *Rhizobium* sp. inoculation on nodulation in lentil have also been reported earlier [13, 14] and may be attributed to presence of sufficient, but ineffective native *Rhizobium* sp. population in the soil. Dual inoculation of *Rhizobium* sp. with PSB was statistically comparable to *Rhizobium* sp. alone. These results corroborate with the findings of Khurana and Sharma [15] who also found statistically similar effects of *Rhizobium* sp.+PSB and *Rhizobium* sp. alone treatments in chickpea and may be attributed to medium status of available P in soil showing little response of PSB. The positive effect of PSB on nodulation was due to its more favorable effect on native than on inoculated *Rhizobium* sp.. Co-inoculation of both the PGPRs with *Rhizobium* sp. though showed an increase in per cent nodule occupancy, the benefits were not reflected in nodulation. Dual inoculation of *Rhizobium* sp.+LK-786 and *Rhizobium* sp.+LK-884 produced more nodule number and more nodule dry weight at different intervals which statistically comparable to *Rhizobium* alone inoculation. This indicated limited nodulation sites on lentil roots [16], at the same time and PGPRs may increased the proportion of effective nodules formed by inoculum as indicated by data on nodule occupancy. The combined inoculation of *Rhizobium* sp.+PSB+LK-786, by recording the highest number and dry weight of root nodules, was significantly better than *Rhizobium* alone. This treatment also gave significantly more nodule number dry weight than *Rhizobium* sp.+LK 786 and *Rhizobium* sp.+PSB, which could be due to additive effect of both PSB and PGPR. It was at par with *Rhizobium* sp.+PSB+LK-884 in number and dry weight of nodules and significant better than 20 kg N+40 kg P_2O_5 ha^{-1} in nodule number at all intervals and nodule dry weight at 45 DAS. Application of 20 and 40 kg P_2O_5 ha^{-1} together with 20 kg N ha^{-1}, improved the nodulation significantly over the uninoculated control at all growth stages, as reported by Tomer *et al.* [17]. However, both the levels of P fertilizer were statistically at par among themselves and to PSB, LK-786 and LK-884 alone inoculation in nodulation at 45 and 70 DAS due to medium P status of soil.

Plant Dry Matter and Yields: Compared to the uninoculated control, *Rhizobium* sp. alone inoculation recorded significant increases in plant dry weight of 30.7 and 47.4% at 70 and 90 DAS and non significant

Table 3: Combined inoculation effects of *Rhizobium* sp., PSB and PGPRs on plant dry weight at different intervals and grain and straw yields

Treatment	Plant dry weight per plant (g plant^{-1})			Yields (kg ha^{-1})	
	45 DAS	70 DAS	90 DAS	Grain	Straw
Uninoculated control	0.115	0.628	0.989	704	1213
Rhizobium sp.	0.124	0.821	1.458	874	1465
PSB	0.121	0.765	1.260	855	1455
LK 786	0.140	0.780	1.233	892	1472
LK 884	0.121	0.697	1.147	841	1450
Rhizobium sp.+PSB	0.142	0.860	1.927	956	1521
Rhizobium sp.+LK 786	0.176	0.825	1.859	1003	1647
Rhizobium sp.+LK 884	0.157	0.780	1.747	973	1589
PSB+LK-786	0.118	0.855	1.699	937	1573
PSB+LK 884	0.116	0.825	1.568	926	1562
Rhizobium sp. +PSB+LK-786	0.191	0.989	2.173	1056	1659
Rhizobium sp. +PSB+LK-884	0.170	0.924	2.040	977	1558
20 Kg N+40 Kg P_2O_5 ha^{-1}	0.182	0.941	2.245	970	1608
20 Kg N+20 Kg P_2O_5 ha^{-1}	0.131	0.912	2.092	926	1577
C.D at 5%	0.026	0.087	0.207	182	284

increases of 24.1 and 20.7% in grain and straw yields, respectively (Table 3). Combined inoculation of *Rhizobium* sp.+PSB+LK 786 produced the highest plant dry weight at different intervals and grain and straw yields and was at par with inoculation of *Rhizobium* sp.+PSB+LK 884. Both these two treatments were significantly superior to *Rhizobium* sp. alone by recording significantly more plant dry weight at different intervals ranging from 12.5 to 54.0% and numerical increases in grain yields, by 20.8 and 11.8% and straw yields, by 13.2 and 6.3%, respectively. Dual inoculation of *Rhizobium* sp.+LK 786 and *Rhizoibum* sp.+LK 884 were also significantly better than *Rhizobium* sp. alone in plant dry weight at 45 and 90 DAS, but gave grain and straw yields comparable to *Rhizobium* sp. alone. Such synergistic effect of inoculation of PSB and PGPRs (LK-786 and LK-884) on plant growth and yields was in agreement with the earlier reports in mungbean [6] and urdbean and lentil [14] due to the increase in proportion of effective nodules, supply of N and P and synthesis of plant growth hormones. Alike nodulation, both levels of P combined with 20 kg N ha^{-1} were at par. The 40 kg P_2O_5+20 Kg N ha^{-1} showed significant increase in plant dry weight over *Rhizobium* sp. and PSB at different intervals and numerical increases of 10.9 and 13.4% in grain yields and 9.7 and 10.5% in straw yields, respectively, which could be due to medium status of available P in soil.

Table 4: Combined inoculation with *Rhizobium* sp., PSB and PGPRs on N and P uptake by lentil grain and straw

Treatment	N uptake (Kg ha^{-1})		P uptake (Kg ha^{-1})	
	Grain	Straw	Grain	Straw
Uninoculated control	16.26	6.14	1.57	1.55
Rhizobium sp.	27.33	10.48	2.33	2.01
PSB	25.27	8.69	2.22	2.13
LK 786	27.62	9.12	2.19	2.08
LK 884	25.94	8.62	2.16	1.95
Rhizobium sp.+PSB	30.97	11.21	3.20	2.31
Rhizobium sp.+LK 786	32.89	11.02	2.93	2.43
Rhizobium sp.+LK 884	31.89	9.83	2.54	2.26
PSB+LK-786	26.16	9.59	3.12	2.89
PSB+LK 884	28.85	10.18	2.98	2.38
Rhizobium sp.+PSB+LK-786	36.10	12.77	3.17	2.86
Rhizobium sp.+PSB+LK-884	32.65	12.20	3.21	2.53
20 Kg N+40 Kg P_2O_5 ha^{-1}	31.37	12.06	2.60	2.71
20 Kg N+20 Kg P_2O_5 ha^{-1}	28.88	10.48	2.31	2.16
C.D at 5%	6.63	2.26	0.64	0.55

Nutrient Uptake: Individually, *Rhizobium* sp. and both PGPRs, significantly increased only N while PSB increased N and P uptake by grain and straw significantly over the uninoculated control (Table 4). However, dual inoculations of them (i.e *Rhizobium* sp.+PSB, *Rhizobium* sp.+LK-786 and *Rhizobium* sp.+LK-884) were comparable to their inoculation alone in N uptake by grain and N and P uptake by straw. P uptake by grain with *Rhizobium* sp+PSB was significantly better than the *Rhizobium* sp. and PSB alone inoculation. The highest N and P uptake by grain and straw was observed with the combined inoculation of *Rhizobium* sp.+PSB+LK-786, which was significantly better than single inoculation with *Rhizobium* sp., PSB, LK-786 or 20 Kg N+20 kg P_2O_5 ha^{-1} treatments. It was comparable to combined inoculation of *Rhizobium* sp.+PSB+LK 884 in N and P uptake. The better effect of all the three inoculants together was due to the fact that PSB and PGPRs in combination with *Rhizobium* sp. formed more proportion of effective nodules and resulting in to better N_2 fixation by facilitating the inoculated *Rhizobium* sp. [5, 18]. Besides, PSB and PGPR are reported to facilitate P supply to plant by solubilising insoluble P [2] and resulted in better P uptake following their inoculation. A non significant effect of dual inoculation of PSB and PGPR with *Rhizobium* sp. in comparison to their single inoculation in P uptake was due to medium P status of soil and probably *Rhizobium*, PSB and PGPR individually solublized sufficient P to meet the crop need. It is substantiated by the fact that application of 40 kg P_2O_5+20 kg N ha^{-1} was also statistically similar to individual inoculants, dual inoculation of *Rhizobium* sp.+LK-786, *Rhizobium* sp.+LK-884 and PSB+LK-884 treatments and significantly better than the uninoculated control in P uptake by grain and straw.

The study revealed that PSB and PGPRs could improve the competitive ability and symbiotic effectiveness of inoculated *Rhizobium* sp. in lentil under field conditions; however, their benefits depend on the co-inoculated organisms. Compatibility of these organisms with *Rhizobium* sp. needs to be evaluated for using such combinations in field.

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