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Synergistic Effect of *Rhizobium* Inoculation with Co-inoculants on Growth and Yield of Lentil (*Lens culinaris* Medikus)

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Abstract

A three-year field study was conducted during *rabi* seasons of 2005-06, 2006-07 and 2007-08 at the Pulses and Oilseeds Research Sub-station, Beldanga, Murshidabad, West Bengal, India to study the effect of seed inoculation with *Rhizobium* (LLR 22) alone and in combination with PGPR (CRB 1, CRB 2, RB 1, RB 2, PUR 34 and KB 133) on growth, nodulation and seed yield of lentil. The results revealed that significantly higher seed yield was obtained when *Rhizobium* is inoculated with PGPR strain (KB 133) as compared to uninoculated control. Yield advantages due to inoculation with *Rhizobium*+PGPR strain KB 133, *Rhizobium*+PGPR strain PUR 34, *Rhizobium*+PGPR strain RB 1 and *Rhizobium*+PGPR strain CRB 1 were 50.65, 29.41, 28.81 and 26.80%, respectively, over uninoculated control and 30.30, 11.93, 11.41 and 11.06%, respectively, over *Rhizobium* inoculation only

1. Introduction

Lentil (*Lens culinaris* Medikus) fixes atmospheric nitrogen and improves the soil fertility. *Rhizobium* inoculation to the legumes not only increases the yield but also shows many beneficial effects. Sometimes indigenous rhizobial population may not be able to form effective symbiosis in field conditions due to strain competition between introduced and native rhizobia. Use of plant growth promoting rhizobacteria (PGPR) is often associated with increased rates of plant growth, development and yield. Further, co-inoculation with *Rhizobium* and PGPR is even more effective for improving nodulation and growth of legumes (Goel et al., 2001; Zahir et al., 2004). Keeping this in view, the present study was taken up to study the co-inoculation effect of PGPRs on lentil-*Rhizobium* symbiosis.

2. Material and Methods

A consecutive three-year field experiment was conducted at the Pulses and Oilseed Research Sub-station, Beldanga, Murshidabad, West Bengal, India during *rabi* seasons of 2005-06, 2006-07 and 2007-08. The soil of the experimental site was sandy loam having pH 7.6, organic carbon 0.26%, available P_2O_5 67 kg ha^{-1} and available K_2O 109 kg ha^{-1}. The crop variety Subrata (WBL-58) was sown on November 24, December 13 and November 22 during 2005-06, 2006-07 and 2007-08, respectively. The experiment was laid out in RBD with four replications. Besides an uninoculated control, there were seven treatments of seed inoculation with *Rhizobium* (Rh.) strain LLR 22, Rh. + PGPR strain CRB 1, Rh.+ PGPR strain CRB 2, Rh. + PGPR strain RB 1, Rh. + PGPR strain RB 2, Rh. + PGPR strain PUR 34 and Rh.+ PGPR strain KB 133. The crop was fertilized with a uniform basal dose of N: P_2O_5: K_2O at 20-40-40 kg ha^{-1} applied through urea, single super phosphate and muriate of potash, respectively. Seeds were inoculated with *Rhizobium* and PGPR prior to sowing as per treatments using 60 g culture kg^{-1} seed. Efficient strains of both *Rhiobium* and PGPR were obtained from All India Coordinated Pulse Improvement Project (AICPIP). Those carrier based cultures were mixed with sterilized neutral charcoal in 1:2 (v/v) ratios. The crop was raised following all the recommended agronomic practices and harvested on March 31, 18 and 17 during 2005-06, 2006-07 and 2007-08, respectively. In order to study the nodulation in lentil, five plants from each plot were uprooted, their roots were gently washed with water, nodules were removed and counted at 60 DAS (days after sowing). The dry weight of root nodules and crop plants was recorded after drying in the hot air oven at 80°C to constant weight. Plant height was recorded at periodic interval whereas observations were made on dry matter accumulation (DMA) at 60 DAS and harvest. Seed yield in kg ha^{-1} and its attributes were also recorded after crop harvest.

3. Results and Discussion

3.1. Effect on crop growth

Rhizobium (LLR 22) alone increased the DMA of crop plants (0.84-1.80 g plant^{-1}) as compared to the uninoculated control (0.82-1.65 g plant^{-1}) at different growth stages. These results corroborated with the findings of Mondal et al. (2004) and Roy and Rahaman (1992). Co-inoculation of *Rhizobium* and PGPR (KB 133) recorded the highest mean values of DMA, being 1.23 and 2.21 g plant^{-1} at 60 DAS and harvest, respectively. It was 1.04-1.46 and 1.02-1.23 fold increase in DMA due to co-inoculation with Rh. + PGPR as compared to only *Rhizobium* inoculation at 60 DAS and harvest, respectively. Irrespective of strains, co-inoculation with PGPR always recorded more plant height in comparison to the uninoculated control both at 60 DAS and harvest (Table 1). Increase in dry matter production of co-inoculated plants might be attributed to earlier and enhanced nodulation, higher N_2-fixation rates and a general improvement in root development (Sanoria and Malik, 1981).

3.2. Effect on nodulation

Seed inoculation with *Rhizobium* strain LLR 22 alone in-

Table 1: Effect of different Rhizobium inoculants on crop growth

Inoculated treatments	Plant height (cm)						Dry matter accumulation (g plant^{-1})					
	Harvest			60 DAS			Harvest			60 DAS		
	1	2	3	1	2	3	1	2	3	1	2	3
Uninoculated	25.73	29.00	30.63	36.25	35.83	36.43	0.75	1.31	0.41	1.12	1.80	2.03
Rh. strain (LLR 22)	28.75	29.50	30.51	40.00	39.68	40.06	0.75	1.37	0.41	1.35	1.88	2.18
Rh.+PGPR (CRB 1)	29.50	28.75	30.99	41.75	40.25	40.61	0.90	1.87	0.41	1.62	2.05	2.30
Rh.+PGPR (CRB 2)	28.25	29.16	30.46	40.25	41.59	41.12	0.79	1.41	0.42	1.37	2.02	2.12
Rh.+PGPR (RB 1)	29.25	29.83	31.42	40.88	40.00	41.49	0.83	1.64	0.46	1.45	2.09	2.35
Rh. PGPR (RB 2)	29.75	29.25	30.81	42.00	41.08	41.41	0.97	1.53	0.43	1.70	2.11	2.30
Rh.+PGPR (PUR 34)	29.38	29.67	31.99	41.50	39.50	41.54	0.80	1.58	0.51	1.48	1.98	2.36
Rh.+PGPR (KB 133)	31.50	31.17	32.35	44.88	42.08	42.95	0.97	2.14	0.59	1.78	2.28	2.57
SEm±	1.39	0.74	0.88	2.15	1.67	1.79	0.03	0.10	0.03	0.05	0.12	0.11
CD (p=0.05)	4.06	2.16	2.58	6.28	4.87	5.25	0.08	0.30	0.10	0.14	0.35	0.31
CV (%)	9.6	5.0	5.6	10.5	8.3	8.8	6.2	12.6	14.4	6.6	11.7	9.3

DAS=Days after sowing; Rh.=*Rhizobium*; 1: 2005-06; 2: 2006-07; 3: 2007-08

Table 2: Effect of different Rhizobium inoculants on nodulation

Inoculated treatments	Nodule number plant^{-1}			Nodule weight (mg plant^{-1})		
	60 DAS			60 DAS		
	1	2	3	1	2	3
Uninoculated	12.83	14.43	15.14	11.98	14.50	18.75
Rh. Strain (LLR 22)	14.78	17.63	14.87	15.70	16.05	14.33
Rh.+PGPR (CRB 1)	15.70	16.57	17.45	13.83	16.30	19.75
Rh.+PGPR (CRB 2)	16.23	16.53	17.54	14.43	16.76	19.88
Rh.+PGPR (RB 1)	18.70	18.57	19.69	16.05	20.12	21.21
Rh. PGPR (RB 2)	20.88	21.81	23.16	19.18	17.72	20.75
Rh.+PGPR (PUR 34)	16.65	16.78	23.27	15.15	18.90	21.83
Rh.+PGPR (KB 133)	24.30	23.67	25.14	19.73	22.12	23.57
SEm±	0.64	0.93	1.08	0.62	0.76	0.52
CD (p=0.05)	1.88	2.72	3.17	1.82	2.24	1.53

DAS=Days after sowing; Rh.=*Rhizobium*; 1: 2005-06; 2: 2006-07; 3: 2007-08 creased the number and dry weight of nodules as compared to uninoculated control. Co-inoculation with PGPR (KB 133) gave the highest mean nodule number (24.37 number plant^{-1}) and weight (21.81 mg plant^{-1}) followed by Rh. + PGPR strain RB 2 (21.95 number of nodules weighing 19.23 mg plant^{-1}). Co-inoculation with PGPR in all the treatments increased the mean nodule number and weight at 60 DAS in comparison to the inoculation with *Rhizobium* alone (Table 2).

It indicated that PGPR favored the *Rhizobium* inoculum to form more nodules either by favoring its survival in the rhizosphere or by synthesis of plant growth promoting substances in developing more root hairs leading to more infection (Yahlom et al., 1988). The combined inoculation of PGPR and *Rhizobium* could remarkably increase the mean nodule number by 1.05 to 1.55 times and dry weight by 1.08 to 1.42 times over *Rhizobium* inoculation alone (19.71 number weighing 15.36 mg plant^{-1}). It was reported (Pareek et al., 2002) better and effective nodulation might have resulted in better nitrogen fixation and growth of legumes.

3.3. Effect on seed yield

Rhizobium inoculum alone increased the mean seed yield by 15.62% over uninoculated control (894 kg ha^{-1}). Dual inoculation with *Rhizobium* and PGPR strains recorded more seed yield over single inoculation with *Rhizobium*. These results were in agreement with earlier works of Chandra and Pareeek (2002). Significantly highest mean seed yield was obtained due to co-inoculation with Rh. + PGPR strain KB 133 (1347 kgha^{-1}), followed by Rh. + PGPR strain PUR 34 (1157 kg ha^{-1}), Rh. + PGPR strain RB 1 (1152 kg ha^{-1}) and Rh. + PGPR strain CRB 1 (1148 kg ha^{-1}). Compared with uninoculated control, yield advantages under these treatments were 50.65, 29.41, 28.81 and 28.40 %, respectively (Table 3).

Such increase in seed yield might have been attributed to better crop growth (plant height 39.91-43.30 cm and DMA of 1.80-2.21 g plant^{-1} at harvest), nodulation (15.76-24.37 number of nodules weighing 15.36-21.81 mg plant^{-1} at 60 DAS) and improvement in yield attributes. These results corroborated with the report of Singh and Chauhan (2005) and Zahir et al. (2004). The beneficial effect of PGPR on *Rhizobium* had probably induced the synthesis of growth promoting substances which could stimulate the root growth and elongation, thereby bringing about more nodulation, nitrogen fixation and crop yield (Rautela et al., 2001). The increase in seed yield due to PGPR inoculation might also have been attributed to antagonistic interaction with various soil-borne pathogens, production and release of secondary metabolites for plant growth, or increased uptake of certain nutrients from the root environment (Jalali and Chand, 1991; Zahir et al., 2004).

Table 3: Effect of *Rhizobium* inoculants on seed yield and its attributes

Inoculated treatments	Branches plant^{-1}			Pod plant^{-1}			Seed pod^{-1}			100 seed wt (g)			Seed yield (kg ha^{-1})			Pooled
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Uninoculated	4.65	2.82	2.91	34.15	32.01	53.14	1.80	1.73	1.74	1.83	1.87	1.89	825	877	981	894
Rh. strain (LLR 22)	4.95	3.34	3.03	44.50	37.75	56.23	1.85	1.78	1.80	1.87	1.98	2.04	1079	998	1025	1034
Rh.+PGPR (CRB 1)	6.45	3.34	3.01	50.35	40.09	55.95	1.88	1.78	1.79	2.10	2.06	2.02	1231	1204	1010	1148
Rh.+PGPR (CRB 2)	5.80	3.05	3.12	46.33	34.47	58.71	1.83	1.68	1.81	1.89	2.24	2.10	1121	1052	1056	1076
Rh.+PGPR (RB 1)	6.25	3.00	3.17	51.30	42.08	62.56	1.85	1.76	1.84	2.03	2.03	2.12	1219	1154	1083	1152
Rh. PGPR (RB 2)	6.63	3.49	3.16	50.63	40.78	61.21	1.88	1.80	1.83	2.13	2.15	2.19	1233	1104	1065	1134
Rh.+PGPR (PUR 34)	6.13	3.09	3.28	47.20	38.37	63.84	1.85	1.76	1.86	1.91	2.12	2.18	1142	1138	1192	1157
Rh.+PGPR (KB 133)	6.73	3.42	3.42	59.08	49.23	71.87	1.90	1.88	1.90	2.23	2.27	2.31	1427	1369	1246	1347
SEm±	0.22	0.22	0.14	1.85	1.94	4.15	0.04	0.05	0.04	0.05	0.08	0.05	49.17	38.33	40.00	43.01
CD (p=0.05)	0.64	0.64	0.40	5.44	5.64	12.21	0.11	0.16	0.11	0.15	0.23	0.15	143.6	111.7	116.7	121.26
CV (%)	7.3	13.7	8.6	7.7	9.8	13.7	4.1	6.1	4.1	5.1	7.6	4.9	8.4	6.9	7.3	

DAS=Days after sowing; Rh.=*Rhizobium*; 1: 2005-06; 2: 2006-07; 3: 2007-08

3.4. Economics

The economic evaluation of the results revealed that the net returns and gross returns were higher under dual inoculation with *Rhizobium* and PGPR strains (KB 133) over single inoculation and uninoculated control (Table 4). The highest net returns and B: C ratios were recorded with the treatments *Rhizobium* and PGPR strains (KB 133) in the years of study. The B: C ratio ranges from 2.34 to 2.83 as compared to uninoculated control.

Table 4: Economics of *Rhizobium* inoculation with co-inoculants

Inoculated treatments	d	2005-06			2006-07			2007-08		
		a	b	c	a	b	c	a	b	c
Uninoculated	11,100	25740	14,640	1.32	27,362	16,262	1.46	30,607	19,507	1.76
Rh. strain (LLR 22)	11,400	33773	22,373	1.96	31,237	19,837	1.74	32,082	20,682	1.81
Rh.+PGPR (CRB 1)	11,700	38653	26,953	2.30	37,685	25,985	2.22	31,613	19,913	1.70
Rh.+PGPR (CRB 2)	11,700	35199	23,499	2.00	33,033	21,333	1.82	33,158	21,458	1.83
Rh.+PGPR (RB 1)	11,700	38277	26,577	2.27	36,236	24,536	2.09	34,006	22,306	1.91
Rh. PGPR (RB 2)	11,700	38716	27,016	2.31	34,666	22,966	1.96	33,441	21,741	1.86
Rh.+PGPR (PUR 34)	11,700	35859	24,159	2.06	35,733	24,033	2.05	37,429	25,729	2.2
Rh.+PGPR (KB 133)	11,700	44808	33,108	2.83	42,987	31,287	2.67	39,124	27,424	2.34

Rh.=*Rhizobium*; a: Gross returns (₹ ha^{-1}); b: Net returns (₹ ha^{-1}); c: Benefit:cost ratio; d: Cost of cultivation (₹ ha^{-1})

4. Conclusion

The study suggested that though the PGPR had favorable effect on lentil-*Rhizobium* symbiosis, selection of effective strains which were more compatible to *Rhizobium* would be necessary for obtaining the meaningful benefits from co-inoculation. However, further studies need to be made for confirmation of the present findings at other locations under different soil and agro ecological situations.

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