LR-4552
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RESEARCH ARTICLE Legume Research - An International Journal

Enhancing Mungbean (*Vigna radiata* L.) Productivity, Soil Health and Profitability through Conjoint use of *Rhizobium* and PGPR

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10.18805/LR-4552

ABSTRACT

Background: Grain legumes are considered as an important group of food crops that can play a vital role to address national food and nutritional security and also tackle environmental challenges. They are known as the basis for an ecologically-sound, farmer-focused agricultural development effort and an important component of sustainable farming systems. Biofertilizers, being a cheap and environment friendly source of plant nutrients assume a special significance in supplying the plant nutrients under the present context of indiscriminate use and high costs of chemical fertilizers. Biofertilizers could be a good alternative to supplement the chemical fertilizers.

Methods: A field experiment was carried out at Pantnagar in Mollisols during 2017-18 and 2018-19 to study the performance of *Rhizobium* sp. and plant growth promoting rhizobacteria (PGPR) inoculation in mungbean on productivity, soil health and economics.

Result: A pronounced effect of biofertilizers application in mungbean was observed on the root nodulation, yields, nutrient uptake and soil health. Co-inoculation of *Rhizobium* and PGPR significantly increased the nodule number, nodule dry weight, plant dry weight, grain yield, straw yield, N and P uptake in mungbean and improved soil health over the no inoculation. The study suggested that combined application of PGPR and *Rhizobium* sp. in mungbean is better than *Rhizobium* sp. alone in increasing crop yields, soil health and farmer's profit.

Key words: Biofertilizers, Co-inoculation, Economics, Mungbean, Productivity, Soil health.

INTRODUCTION

Among the cultivated crops of the world, grain legumes represent an important component of food crops with annual global production of 79 million metric tonnes (Joshi and Rao, 2017). They have long been considered as a good source of protein, thus play a crucial role in healthy diets, sustainable food production and in food security. Besides, legumes also have several positive impacts on soil health maintenance through processes of biological nitrogen fixation (BNF) and nutrient cycling. Mungbean (*Vigna radiata* L.) is one of the major grain legumes of India. It is a short duration crop and fits well in various cropping systems. It can also be grown as a catch crop during *kharif* season. It is cultivated on 4.25 million hectares area with the production of 2.41 million tonnes and average productivity of 567 kg ha^{-1} (Anonymous, 2019). Mungbean, like many other legumes, is capable of utilizing and fixing the atmospheric nitrogen (N_2) in association of soil bacteria of genus *Rhizobium* or *Bradyrhizobium* (Mondal *et al*. 2013). Combined inoculation of PGPR with rhizobia in grain legumes has received much attention in recent years mainly because of their positive effects on nodulation, N_2 fixation and yield of legume crops (Dudeja *et al.* 2011). The combined application of *Rhizobium* sp. with PGPR is advocated to obtain the benefits of both N_2 fixation by rhizobia and plant growth promotion through plant growth promoting (PGP) traits of PGPR (Bhattacharya and Jha, 2012; Kaur *et al.* 2015). However, the compatibility of these microorganisms needs to be evaluated because of the possibility of the antagonistic interactions among them. Present study communicates the findings of two years study on the performance of conjoint use of *Rhizobium* sp. and PGPR in mungbean on symbiotic performance, yield, nutrient uptake, soil fertility and economics with aim of making a practical recommendation for promotion of biofertilizers to minimize the use of costly chemical fertilizers and reducing the environmental pollution.

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How to cite this article: Neha, Chandra, R., Pareek, N., Raverkar, K.P. (2021). Enhancing Mungbean (*Vigna radiata* L.) Productivity, Soil Health and Profitability through Conjoint use of *Rhizobium* and PGPR. Legume Research. DOI: 10.18805/LR-4552.

Submitted: 25-11-2020 **Accepted:** 07-04-2021 **Online:** 29-04-2021

MATERIALS AND METHODS

A field experiment was conducted during *kharif* seasons of 2017-18 and 2018-19 at Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar (29.8°N, 73.9° E, 243.8 m above mean sea level). The experimental soil was Sandy loam of pH 7.43, E.C. 0.48 dS m^{-1} having 7.9 g kg^{-1} Organic C and 228.3, 23.4 and 218.1 kg ha^{-1} available N, P and K, respectively. Treatments consisted of seed inoculation with *Rhizobium* sp. (MR-14), PGPR (*Bacillus cereus*, NE-10), *Rhizobium* sp. + PGPR, RDF (20 kg N + 40 kg P_2O_5 ha^{-1}) and uninoculated control. The experiment was laid out in split plot design in plots of 2.4 m x 3.0 m size in 3 replications during both the years. The test crop variety was Pant Mung 5. Inoculation was done through seed treatment using carrier based inoculant at the rate of 20 g kg^{-1} seed at the time of sowing. Dual inoculation was done by mixing the required quantity of each *Rhizobium* sp. and PGPR inoculants. RDF treatment was executed through basal application of 20 kg N and 40 kg P_2O_5 ha^{-1} through urea and single super phosphate (SSP), respectively. The crop was raised following recommended agronomic practices.

Root nodulation was recorded at 30 and 45 days after sowing (DAS) from ten randomly selected plants. Plants were uprooted along with soil core of about 25 cm, the adhering soil on roots was gently washed off, root nodules were removed and counted. The dry weight of detached nodules and plants was recorded after drying in hot air oven at 70°C to constant weight. The grain and straw yields were recorded after harvesting the crop. The N and P content in grain and straw was estimated after finely grinding to 40 mesh following the methods as described by Page *et al*. (1982) and their uptake were computed. Soil samples of 0-15 cm depth were collected from individual plots after harvesting the crop. Soil samples were air-dried, processed to pass through 2-mm sieve and analyzed for organic C, mineral N (2 M KCl extractable), available N (0.32% alkaline $KMnO_4$ oxidizable), available P (0.5 M $NaHCO_3$ extractable) and available K (1*N* NH_4OAc; pH 7.0 extractable) following the methods described by Page *et al*. (1982). The experimental data were statistically analyzed by applying analysis of variance and treatments were compared using the F-test by calculating the critical difference at 5% level of significance.

RESULTS AND DISCUSSION

Nodulation

Seed inoculation with *Rhizobium* and PGPR in mungbean, individually and in combination, improved the number and dry weight of root nodules significantly (Table 1). Pooled mean of both the years indicated that *Rhizobium* inoculation alone increased the nodule number significantly, by 41.3 and 25.8%, and nodule dry weight, by 38.3 and 19.7% at 30 and 45 DAS over the uninoculated control, respectively. PGPR alone also increased nodule number and nodule dry weight of 18.7 and 30.5% at 30 DAS and 14.3 and 16.3% at 45 DAS over the uninoculated control, respectively. Combined inoculation of *Rhizobium* and PGPR further enhanced the number and dry weight of root nodules registering 20.7 and 16.7% more nodule number and 9.0 and 3.1% more nodule dry weight over *Rhizobium* alone inoculation at 30 and 45 DAS, respectively. The positive impacts of combined application of *Rhizobium* and PGPR in mungbean have also been reported by Bansal (2009). The increase in root nodules may be attributed to the enhancement in the effective rhizobial population in rhizosphere following inoculation. The PGPR (*B. cereus*) used in the present study was a potential P solubilizer (Rana *et al.* 2015), which possibly allowed the synthesis of more nodule tissue and N_2 fixation in comparison to uninoculated treatment. The results are in agreement with the report of Stajkovic *et al.* (2011) who reported that the enhancement in nodule number and nodule dry weight in common bean due to the co-inoculation was because of the expansion in root length and mass, thus responsible for more number of active sites for nodulation by the rhizobia.

Plant dry weight

Inoculation in mungbean with *Rhizobium* sp. and PGPR, alone and in combination, also increased the mean plant dry weight in both the years (Table 2). Sole inoculation of *Rhizobium* and PGPR produced significantly higher mean plant dry weight, by 35.3 and 23.7% at 30 DAS and 17.0 and 11.1% at 45 DAS over the uninoculated, respectively. The maximum mean plant dry weight of both the years was recorded with conjoint use of *Rhizobium* + PGPR, being 13.0 and 19.3% more over *Rhizobium* alone inoculation at 30 and 45 DAS, respectively. It could be attributed to the synergistic interaction between co-inoculated *Rhizobium* and PGPR leading to the increase in the plant nutrition and growth (Khanna and Sharma, 2011).

Grain and Straw yields

Although, treatment of recommended fertilizer dose produced the highest grain and straw yields in both the

Table 1: Effect of different inoculation treatments on number and dry weight of root nodules at different intervals.

Treatment	Nodule number $plant^{-1}$						Nodule dry weight (mg $plant^{-1}$)					
	30 DAS			45 DAS			30 DAS			45 DAS		
	2017	2018	Pooled mean	2017	2018	Pooled mean	2017	2018	Pooled mean	2017	2018	Pooled mean
Uninoculated control	7.4	7.6	7.5	14.6	15.0	14.7	7.87	8.11	7.99	15.47	15.76	15.61
Rhizobium (MR 14)	10.1	11.0	10.6	18.2	18.9	18.5	10.84	11.27	11.05	18.51	18.85	18.69
PGPR(NE 10)	8.5	9.2	8.9	16.6	17.2	16.8	10.22	10.64	10.43	17.98	18.32	18.15
Rhizobium + PGPR	12.3	13.3	12.8	21.1	22.1	21.6	11.74	12.35	12.05	19.22	19.58	19.40
RDF	9.6	10.4	10.0	18.2	18.8	18.6	8.54	8.86	8.70	16.08	16.38	16.23
C.D. (P=0.05)	**1.4**	**1.4**	**0.9**	**3.3**	**3.1**	**2.3**	**1.08**	**1.25**	**0.80**	**2.12**	**2.16**	**1.42**

years, however, it was statistically at par with dual inoculation of *Rhizobium* sp. + PGPR (Table 3). The latter treatment produced significantly more mean grain yield of 9.2% and numerically more mean straw yield of 11.1% over the uninoculated control. Individual inoculation of *Rhizobium* sp. and PGPR showed marginal increases in grain and straw yields. The symbiosis of rhizobia and legumes is well documented. It colonizes the plant root system and induces the nodule formation. However, when it was inoculated conjointly with PGPR, it allowed better nodulation and N_2 fixation due to the synergistic interaction among the inoculated microorganisms as reported elsewhere (Khanna and Sharma, 2011). The beneficial effects of PGPR is also attributed to increase in root volume and number, thus enhancing the action of *Rhizobium* sp. by supplying biologically fixed N to the plant resulting in increase in grain yield (Hungria *et al.* 2015).

N content and uptake

The different inoculation treatments in mungbean significantly increased the N content in grain ranging from 15.1 to 31.3% during 2017-18 and 16.4 to 33.7% during 2018-19 (Table 4). The increase in straw N content ranged from 16.7 to 49.5% during 2017-18 and 20.2 to 54.9% during 2018-19 over the uninoculated control, respectively. The highest N content in grain and straw was obtained with combined inoculation treatment of *Rhizobium* + PGPR. This treatment accumulated maximum N in plants. Mean N uptake by grain and straw being 45.3 and 69.2% more over uninoculated control and 7.6 and 9.3% over *Rhizobium* alone inoculation. It was followed by the sole inoculation of *Rhizobium* and RDF treatments in N uptake by grain. The favourable effects of rhizobia inoculation in legumes on N content and its accumulation are well known due to

Table 2: Effect of biofertilizer treatments in mungbean on plant dry weight (g plant^{-1}) at 30 and 45 days after sowing.

Treatment	Plant dry weight (g plant^{-1})					
	30 DAS			45 DAS		
	2017	2018	Pooled mean	2017	2018	Pooled mean
Uninoculated control	2.06	2.23	2.15	10.37	10.52	10.45
Rhizobium (MR 14)	2.76	3.06	2.91	12.13	12.34	12.23
PGPR (NE 10)	2.53	2.78	2.66	11.40	11.81	11.61
Rhizobium + PGPR	3.18	3.39	3.29	14.61	14.57	14.59
RDF	2.73	3.01	2.87	11.82	12.15	11.99
C.D. (P=0.05)	**0.56**	**0.67**	**0.45**	**1.35**	**1.40**	**1.09**

Table 3: Effect of biofertilizer treatments in mungbean on yields of mungbean.

Treatment	Grain yield (kg ha^{-1})			Straw yield (kg ha^{-1})		
	2017	2018	Pooled mean	2017	2018	Pooled mean
Uninoculated control	1191	1187	1189	1656	1637	1647
Rhizobium (MR 14)	1276	1256	1266	1800	1759	1779
PGPR (NE 10)	1214	1225	1219	1713	1704	1708
Rhizobium + PGPR	1294	1306	1299	1850	1810	1830
RDF	1324	1333	1328	1891	1803	1847
C.D. (P=0.05)	**NS**	**69**	**63**	**NS**	**NS**	**NS**

Table 4: Effect of biofertilizer treatments in mungbean on N content and uptake by mungbean grain and straw.

	N content (%)				N uptake (kg ha^{-1})					
	Grain		Straw		Grain			Straw		
	2017	2018	2017	2018	2017	2018	Pooled mean	2017	2018	Pooled mean
Uninoculated control	3.629	3.697	1.041	1.055	39.81	40.26	40.03	16.38	16.41	16.40
Rhizobium (MR 14)	4.533	4.691	1.469	1.536	53.80	54.31	54.06	25.10	25.65	25.38
PGPR (NE 10)	4.179	4.305	1.215	1.268	46.71	48.42	47.56	19.79	20.53	20.16
Rhizobium + PGPR	4.767	4.942	1.556	1.634	56.93	59.42	58.18	27.46	28.04	27.75
RDF	4.211	4.328	1.277	1.328	51.54	53.03	52.29	22.89	22.77	22.83
C.D. (P=0.05)	**0.688**	**0.659**	**0.225**	**0.189**	**10.86**	**8.05**	**6.35**	**3.70**	**3.09**	**2.41**

increased N_2 fixation (Singh and Singh, 2018). The enhancement in N content and its uptake could also be due to increased Biological N_2 fixation and nitrate reductase activities of PGPR, or to the uptake of NH_4^+ and amino acids produced by PGPR (Osman *et al.* 2010).

P content and uptake

P content due to different inoculation treatments was significantly higher, by 4.8 to 16.0% in grain and 14.4 to 28.8% in straw during 2017-18 and 5.6 to 18.6% in grain and 18.0 to 39.1% in straw during 2018-19, maximum being with the combined inoculation of *Rhizobium* + PGPR in both the years (Table 5). This trend of P content was also reflected in P uptake by grain and straw. The maximum mean P uptake by the grain and straw of both the years was recorded with the dual inoculation of *Rhizobium* + PGPR, which was significantly more, by 28.1 and 49.0% over the uninoculated control and 13.5 and 14.0% over the individual PGPR inoculation. Individual inoculation of *Rhizobium* and PGPR registered significant increases in mean P uptake of 11.7 and 12.8% by grain and 25.2 and 30.7% by straw over the uninoculated control, respectively. Verma *et al.* (2013) also reported similar increase in P content and uptake in chickpea following PGPR inoculation due to increased acquisition of P by the plant because of P solubilization in soil and increased root biomass due to PGP activities.

Soil properties

Different inoculation treatments indicated the non-significant impact on organic C content in soil after mungbean harvesting (Table 6). Dual inoculation treatment of *Rhizobium* + PGPR in mungbean resulted significant and highest increase in mineral N, available N, P and K in soil to the tune of 25.8, 18.8, 57.2 and 18.7% over the uninoculated control at crop harvest, respectively (Table 6 and Fig 1). An increase in available N in soil could be ascribed to enhanced N_2 fixation by the inoculated

Table 5: Effect of biofertilizer treatments in mungbean on P content and uptake by mungbean grain and straw.

Treatment	P content (%)				P uptake (kg ha^{-1})					
	Grain		Straw		Grain			Straw		
	2017	2018	2017	2018	2017	2018	Pooled mean	2017	2018	Pooled mean
Uninoculated control	0.331	0.338	0.125	0.133	3.63	3.69	3.66	1.97	2.07	2.02
Rhizobium (MR 14)	0.347	0.357	0.143	0.157	4.07	4.12	4.09	2.44	2.62	2.53
PGPR (NE 10)	0.362	0.374	0.153	0.171	4.05	4.21	4.13	2.50	2.78	2.64
Rhizobium +PGPR	0.384	0.401	0.161	0.185	4.57	4.82	4.69	2.84	3.18	3.01
RDF	0.352	0.361	0.147	0.158	4.28	4.43	4.35	2.65	2.70	2.68
C.D. (P=0.05)	**0.017**	**0.018**	**0.018**	**0.023**	**0.40**	**0.31**	**0.25**	**0.40**	**0.47**	**0.27**

Table 6: Effect of biofertilizer treatments in mungbean on soil organic C and mineral N at crop harvest.

Treatment	Organic C (g kg^{-1})			Mineral N (µg g^{-1} soil)		
	2017	2018	Pooled mean	2017	2018	Pooled mean
Uninoculated control	8.2	8.4	8.3	45.1	46.6	45.8
Rhizobium (MR 14)	8.3	8.3	8.3	53.0	55.9	54.5
PGPR (NE 10)	8.2	8.1	8.2	47.2	47.7	47.4
Rhizobium + PGPR	8.4	8.5	8.5	56.2	59.0	57.6
RDF	8.3	8.3	8.4	51.1	51.7	51.4
C.D. (P=0.05)	**NS**	**NS**	**NS**	**4.7**	**5.4**	**4.1**

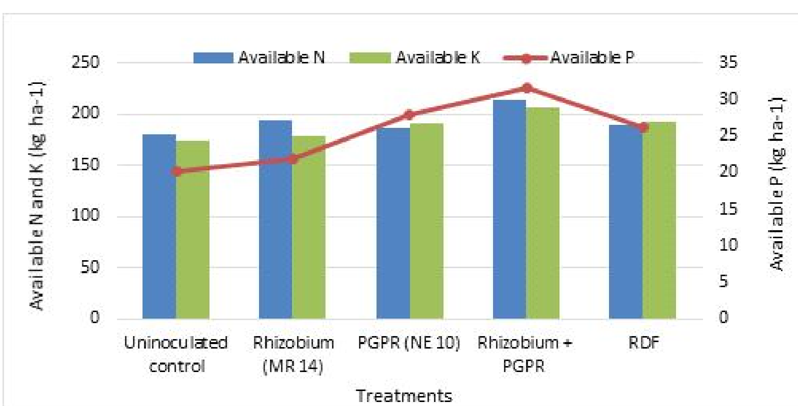

Fig 1: Effect of biofertilizer treatments in mungbean on mean available N, P and K in soil (kg ha^{-1}) at harvest.

Table 7: Effect of biofertilizer treatments on economics of mungbean cultivation.

Treatment	Gross return (Rs. ha^{-1})			Net return (Rs. ha^{-1})			B:C ratio		
	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean	2017-18	2018-19	Pooled mean
Uninoculated control	79652	97514	88583	57039	74354	65697	2.52	3.21	2.87
Rhizobium (MR 14)	85518	103419	94468	62805	80159	71482	2.77	3.45	3.11
PGPR (NE 10)	81403	100777	91090	58690	77517	68103	2.58	3.33	2.96
Rhizobium + PGPR	86918	107373	97146	64155	84063	74109	2.82	3.61	3.21
RDF	88960	109224	99092	63476	83164	73320	2.49	3.19	2.84
C.D. (P=0.05)	**NS**	**7916**	**4221**	**NS**	**NS**	**4219**	**-**	**-**	**-**

Rhizobium sp. and available P and K through solubilization in soil by the PGPR. Co-inoculation consequently led to greater N_2 fixation and P solubilization due to the release of protons by *Rhizobium* during BNF and also by synthesizing various organic acids resulted into lowering of the soil pH (Khan *et al.* 2006). Kaur *et al.* (2015) also reported similar positive effects of *Rhizobium* + PGPR inoculation on soil chemical properties due to their interactive effect and stated that these inoculants mediated the number of soil processes and thus enhanced the availability of various nutrients.

Economics

The mean gross and net returns of both the years varied significantly due to different treatments in mungbean (Table 7). The maximum gross return of Rs. 99,092 ha^{-1} was obtained with application of RDF treatment, however, it was at par with *Rhizobium* + PGPR treatment. In contrast, the maximum net return of Rs.74,109 ha^{-1} was observed with dual inoculation of *Rhizobium* + PGPR, which was Rs. 8,412 ha^{-1} more than the uninoculated control. This treatment also gave the highest B:C ratio of 3.21 in comparison to 2.84 of RDF treatment. These results are in agreement of the findings of Biswas *et al.* (2012) and Kumawat *et al.* (2019) and may be attributed to enhancement in the plant nutrient availability in soil resulting in improved growth and yield attributes leading to higher grain yield and gross and net returns.

CONCLUSION

It could be concluded from the study that combined application of *Rhizobium* and PGPR in mungbean is superior to individual inoculation of *Rhizobium* or PGPR in improving the productivity and profitability. It also had positive impacts on the soil fertility and soil biological properties. Results suggested that use of biofertilizers in grain legumes have promise to minimize the plant nutrient related shortcomings of grain legumes production.

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