



Evaluation of synergistic potential of plant growth promoting rhizobacteria with *Rhizobium* in mungbean (*Vigna radiata* L.)

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Abstract: A pot experiment was conducted in glass house at PAU research farm, Ludhiana, Punjab, India to evaluate effect of co-inoculation of plant growth promoting rhizobacteria with *Rhizobium* on mungbean productivity. Co-inoculation showed a significant increase at 5% in nodule number, nodule dry weight, shoot and root dry and fresh weight, plant length, no. of pods, chlorophyll and leghaemoglobin content, over *Rhizobium* alone. Application of PGPRs R-4, R-6, S-5, S-9 and S-11 along with *Rhizobium* further enhanced the grain yield over *Rhizobium* inoculation alone. Rhizobacterial isolates R-6 and S-11 co-inoculated with *Rhizobium* showed better result than other isolates. These plant beneficial rhizobacteria may decrease the global dependence on various hazardous agricultural chemicals used in mungbean

Keywords: Co-inoculation, Mungbean, PGPR, *Rhizobium*

INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek) is an important pulse crop cultivated in Asia. India accounts about 1.94 million hectares area under mungbean cultivation of which Punjab occupied about 5.6 thousand hectare area with an average yield of 849 kg/ha (Anonymous, 2013). It is short duration crop and have better storage ability. Moreover, it is drought resistant that can withstand adverse environmental conditions, and hence successfully be grown in rain fed areas (Anjum *et al.*, 2006). Mungbean contains high amount of Vitamins A, B, C and niacin, and minerals such as potassium, phosphorus and calcium, which are necessary for human and commonly used as fodder and green manure.

The scarcity of food containing high levels of protein, micronutrients and various vitamins sources is an increasing problem affecting millions of people in developing countries (Burchi *et al.*, 2011). To improve the quality and yield of economically important legumes, particularly mungbean, farmers apply large quantities of chemical fertilizer, which has detrimental effects on the soil, such as the accumulation of toxic salts. Application of environmentally friendly and potentially cost effective microbial biofertilizer could be a better solution (Mia *et al.*, 2010). PGPR is a group of bacteria that facilitate the hydrolysis of a wide range of compounds leading to higher crop yields and reduce chemical hazards to the environment. The seed bacterization with the appropriate rhizobia at sowing is a recommended practice, but in recent years seed bacterization with both PGPR and *Rhizobium* or their

co-inoculation brought more balanced nutrition particularly under conditions of reduced nutrient inputs than single organism (Khanna and Sharma, 2011). The use of PGPR is steadily increasing in agriculture and offers an attractive alternative to chemical fertilizers, pesticides, and supplements (Fuzzaman *et al.*, 2009). Plant growth promoting rhizobacteria effect crop growth, nutrient uptake and yield of different agricultural crops (Parewa *et al.*, 2014). Combined application of *Rhizobium* and *Pseudomonas* strains can improve the productivity of mung bean and nitrogen concentration in grains of mungbean (99%), compared with the uninoculated control (Maqshoof *et al.* 2011; Jangra and Rajesh (2015) observed maximum growth in mungbean genotype MH-421 with dual inoculation of *Rhizobium* and PGPR. In pot experiment, it was reported that PGPR inoculation increased the growth of seedlings of Chickpea (Karnwal and Kumar, 2012) at 5% level of significance. The co-inoculation of PGPR and *Rhizobium* offers a novel prospective for future. Keeping in view the above discussion a pot study was conducted to evaluate the effect of co-inoculation on growth, nodulation, N content and yield in mungbean.

MATERIALS AND METHODS

Pot experiment was carried out in the experimental fields of Pulses Section, Department of Plant Breeding and Genetics, PAU, Ludhiana, during Kharif 2014 under glasshouse. The bacterial inoculums were prepared by inoculating them in respective broth

followed by incubation at 40 °C for 48 h at 150 rpm. Medium black clayey soil from local Mungbean field was collected and autoclaved 3x (1h 121°C) at 12 hrs interval. One Kg sterilized soil was filled per pot. Seeds of Mungbean variety PAU 911 were procured from the Department of Plant Breeding and Genetics. Mungbean seeds of variety PAU 911 were inoculated with recommended *Rhizobium* strain and PGPR, as per treatments.

Mungbean seeds were surface sterilised with 0.1% sodium hypochlorite solution for 10 min and then washed three times with distilled water. Previously prepared 1ml samples of each inoculant will be uniformly applied on seeds as single and co-inoculation and ten seeds will be sown in each pot. Before inoculation, equal volume of different organisms (1:1) were mixed and allowed to stand for 30 minutes at room temperature. Inoculated seeds were dried at room temperature under shade before sowing. Crop was sown on 21st July 2014 and harvested on October 14, 2014. The experiment was conducted in Randomized Block Design (RBD) with seven treatments (control, *Rhizobium*, *Rhizobium*+R-4, *Rhizobium* +R-6, *Rhizobium* + S-5, *Rhizobium* + S-9 and *Rhizobium* + S-11) having five replications each.

Observations for number and dry weight of nodules, Fresh and dry weight of shoot and root, Plant height were recorded at 35 DAS and harvesting. Chlorophyll content and leghaemoglobin content were recorded at 35 DAS. Observations N-content in grains and straw, grain yield was recorded after harvesting. Three randomly selected plants were carefully uprooted from each pot at 35 DAS and harvesting, with root system intact. The roots were washed in running tap water and nodules were detached carefully with forceps and total number of nodules was counted. The number of nodules per plant was recorded by taking average. The detached nodules were dried in oven at 60°C for 2 days and their dry weight per plant was recorded in mg.

Five randomly selected plants were uprooted from each pot and fresh weight of shoot and root per plant was measured in gm. These were then dried in oven at 60°C for 2 days and dry weight per plant was measured in gm. Chlorophyll content was estimated by method of Witham *et al* 1971 and Leghaemoglobin content by method of Wilson and Reisenauer 1963. Grain yield from each pot (gm/pot) was recorded and the final grain yield was expressed in gm/plant. Nitrogen content in straw and seed was estimated by method of McKenzie and Wallace 1954 and expressed in (%).

RESULTS AND DISCUSSION

Rhizobium co-inoculated with different PGPR cultures (R-4, R-6, S-5, S-9 and S-11) showed increase in number of nodules (17.6, 24.3, 20.0, 11.6, and 22.3 number of nodules/plant) than *Rhizobium* alone (17.3

nn/plant) and control (11.3 nn/plant) (Fig.1). The increase in nodulation may be credited to their abilities to produce IAA, as its role is reported to regulate formation of nodules in legumes, due to suppression of pathogens (Mahmoud and Abd Alla, 2001) or nutrient mobilization (Ahemad and Khan, 2011). Application of different PGPR cultures i.e R-4, R-6, S-5, S-9 and S-11 along with *Rhizobium* further increased nodule dry weight (130-230 mg/plant) as compared to *Rhizobium* alone (Table 1). Positive effect of inoculated *Rhizobium* on nodulation may be due to presence of either low or ineffective population of native rhizobia in soil (Chandra and Pareek, 2002).

At 35 days *Rhizobium* along with R-6 (10.1 gm/plant) showed maximum increase in shoot fresh weight as compared to *Rhizobium* along with R-4, S-5, S-9 and S-11. Increase in fresh weight of shoots was observed in *Rhizobium* along with R-6 (13.7 gm/plant) and S-11 (11.44 gm/plant) at 5% level of significance during harvesting. A similar trend with root fresh weight was observed with co-inoculation of *Rhizobium* along with R-6 (0.65 and 1.70 g/plant) and S-11 (0.63 and 1.59 gm/plant) at 35 DAS and harvesting (Table 1). Treatments with *Rhizobium* along with R-6 (2.3 gm/plant) showed increase in shoot dry weight as compared to *Rhizobium* (2.1 gm/plant) alone and control (1.8 g/plant) at 35 DAS. Significant increase (at 5%) was recorded in treatments with *Rhizobium* along with R-6 (4.01 gm/plant) and S-11 (3.79 gm/plant) at harvesting (Table 2). Dual inoculation of *Rhizobium* along with R-6 and S-11 showed higher root biomass at harvesting (0.80 and 0.77 gm/plant) which was on par than at 35 DAS (0.22 and .2 gm/plant) (Table 2).

Highest Chlorophyll content was recorded by *Rhizobium* + R-6 (.28 mg/g fresh wt. of leaves) and *Rhizobium* + S-11 (.23 mg/g fresh wt. of leaves). Mishra *et al* (2012) also reported that *Rhizobium leguminosarum*-PR1 inoculation showed significant increase at 5% in Chl a, Chl b and total Chl contents as compared to non-inoculated control plants in field pea. Maximum leghaemoglobin was recorded in *Rhizobium* with R-6 (3.47 mg/gm of nodule), followed by S-11, S-9, R-4 and S-5 (3.01, 2.54, 1.43 and 1.29 mg/gm of nodule) respectively to ensure the proper function of root nodules. Mishra *et al.* (2010) reported that co-inoculation of PGPR and *Rhizobium* resulted in maximum 45.9% increase in leghaemoglobin content as compared to *Rhizobium* inoculation alone in mungbean. Data regarding plant height revealed that *Rhizobium* inoculation and coinoculation significantly increased plant height over control, by 12%. Maximum increase in plant height (23.7%) over control and 10.8% over *Rhizobium* inoculation alone was recorded from coinoculation of *Rhizobium* + PGPR in mungbean under field conditions (Waseem *et al* 2004). *Rhizobium* co-inoculated with R-4, R-6, S-5, S-9 and S-11 showed an increase in shoot length (38.8, 42.4, 39.4, 38.4 and 40.4cm) respectively at 35 DAS. At harvesting *Rhizo-*

bium inoculated along R-6 showed further increase in shoot length (65 cm) followed by S-11 ,S-9,R-4 and S-5 (57,61,55 and 55 cm) respectively (Table 3).

Rhizobium along with R-4, S-5,S-9 and S-11 application showed increased root length (13.1,12.7,14.7 and 17.5 cm) respectively as compared to *Rhizobium* alone at 35 DAS. Maximum root length was recorded in co inoculation of *Rhizobium* along with R-6 (31cm) at 75 DAS. *Rhizobium* inoculation alone and in combination with PGPR increases number of pods/ plant compared

to uninoculated control at 5% level of significance. *Rhizobium* co-inoculated with R-4,R-6,S-5,S-9 and S-11 also increased the number of pods per plant (6,10,7,8 and 13) as compared to control and *Rhizobium* alone. Significant increase (5%) was observed in number of seeds per pod in *Rhizobium* co-inoculated with R-4, R-6, S-5, S-9 and S-11 (Table 4).

Nitrogen content is an important growth parameter, which has a direct bearing with biological Nitrogen fixation . Data recorded on N content in mungbean

Table 1. Effect of co-inoculation of *Rhizobium* and PGPR on nodulation and plant fresh weight.

Treatments	No. of nodules/ plant	Nodule dry weight (mg/ plant)	Shoot fresh weight (gm/ plant)		Root fresh weight (gm/ plant)	
	35 DAS	35 DAS	35 DAS	Harvesting	35 DAS	Harvesting
Control	11.3	90	4.81	6.20	0.36	1.0
<i>Rhizobium</i>	17.3	120	5.81	7.81	0.44	1.08
<i>Rhizobium</i> + R-4	17.6	130	8.6	9.44	0.52	1.46
<i>Rhizobium</i> + R-6	24.3	230	10.1	13.7	0.65	1.70
<i>Rhizobium</i> + S-5	20.0	180	8.0	8.24	0.52	1.47
<i>Rhizobium</i> + S-9	11.6	130	8.7	11.32	0.58	1.49
<i>Rhizobium</i> + S-11	22.3	220	8.7	11.44	0.63	1.59
CD @ 5%	1.2	7.6	.81	0.60	0.68	0.61

Table 2. Effect of co-inoculation of *Rhizobium* and PGPR on plant dry weight.

Treatments	Shoot dry weight (gm/plant)		Root dry weight (gm/plant)	
	35 DAS	harvesting	35 DAS	75 DAS
Control	1.8	1.89	0.16	0.54
<i>Rhizobium</i>	2.1	2.47	0.19	0.58
<i>Rhizobium</i> + R-4	2.1	3.04	0.18	0.60
<i>Rhizobium</i> + R-6	2.3	4.01	0.22	0.80
<i>Rhizobium</i> + S-5	1.5	2.07	0.12	0.69
<i>Rhizobium</i> + S-9	2.0	3.62	0.2	0.70
<i>Rhizobium</i> + S-11	2.5	3.79	0.2	0.77
CD @ 5%	0.67	0.13	0.23	0.18

Table 3. Effect of co-inoculation of *Rhizobium* and PGPR on chlorophyll , leghaemoglobin contents and plant height in mungbean.

Treatments	Chlorophyll content (mg/gm fresh weight of leaves)	Leghaemoglobin content (mg/gm fresh weight of nodules)	Shoot length (cm)		Root length (cm)	
	35 DAS	35 DAS	35 DAS	Harvesting	35 DAS	harvesting
Control	0.08	0.79	35.4	52	11.3	24
<i>Rhizobium</i>	0.20	1.04	39.6	56	14.1	23
<i>Rhizobium</i> + R-4	0.21	1.43	38.8	55	13.1	29
<i>Rhizobium</i> + R-6	0.28	3.47	42.4	65	18.2	31
<i>Rhizobium</i> + S-5	0.19	1.29	39.4	55	12.7	20
<i>Rhizobium</i> + S-9	0.21	2.54	38.4	61	14.7	28
<i>Rhizobium</i> + S-11	0.23	3.01	40.4	57	17.5	28.3
CD@5%	0.12	0.90	0.18	0.67	0.18	0.76

Table 4. Effect of dual-inoculation on yield attributing traits.

Treatments	No. of pods /plant	No. of seeds per pod	Yield (gm/plant)	N content % age	
				Grains	Straw
Control	4	7	0.83	3.31	0.82
<i>Rhizobium</i>	5	8	1.44	3.48	1.03
<i>Rhizobium</i> + R-4	6	9	1.32	3.53	1.26
<i>Rhizobium</i> + R-6	10	12	1.70	4.02	2.32
<i>Rhizobium</i> + S-5	7	9	1.23	3.55	1.17
<i>Rhizobium</i> + S-9	8	10	1.44	3.62	1.36
<i>Rhizobium</i> + S-11	13	10	1.60	3.75	1.39
CD @5%	0.30	0.18	0.15	0.62	0.76

grains in Table 4 depicts that all the treatments increased the N content over control which is beneficial in increasing the level of protein content. N content ranged from 3.31 to 4.02 % in grains and from 0.82 to 2.32 in straw. Treatments of R-6 and S-11 along with *Rhizobium* showed an increase in N content in grains and straw as compared to *Rhizobium*. Grain yield is the most important economic trait. The grain yield was recorded at harvesting stage to analyse best cultures that can be further used as biofertilizers in mungbean. Inoculation of *Rhizobium* and PGPR considerably enhanced the grain yield, while the effect was more pronounced when they are applied in combination as compared to un-inoculated ones. The highest grain yield was produced by R-6 along with *Rhizobium* (1.70 gm/plant) followed by *Rhizobium* alone (1.44 gm/plant). Combination of *Bradyrhizobium* sp. MN-S + *B. subtilis* M6 remarkably improved grain yield to 1478 kg/ha, which was 22% higher than the *Bradyrhizobium* sp. MN-S alone (1216 kg/ha) and 44% higher than the un-inoculated control (947 kg/ha) in mungbean (Mohsin *et al.*, 2012). Bansal (2009) reported that combined inoculation of mung bean seeds with *Rhizobium* + PGPR + PSB gave significantly higher (at 5 %) grain yield as compared to *Rhizobium* alone

Conclusion

During the present study native PGPR isolates R-6 and S-11 along with recommended *Rhizobium* improved plant growth, symbiotic parameters and grain yield over *Rhizobium* alone. The use of PGPR as inoculants is an efficient approach to replace chemical fertilizers, pesticides which have numerous side effects to sustainable agriculture. Further these rhizobacterial strains can be used as biofertilizer for a wide variety of legume

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