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Impact of Seed Bacterization with PGPR on Growth and Nutrient Uptake in Different Cultivable Varieties of Green Gram

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ABSTRACT

Mung bean (*Vigna radiata* L.), also popularly as green gram, is an ancient and well known legume crop in Asia. A little information is available about the impact of bio inoculants application for improving growth of different cultivable varieties of green gram. Thus, the aim of this study was to assess the influence of *Pseudomonas* and *Rhizobium* as single and dual inoculants to promote the growth and nutrient uptake in green gram. Evaluation of bacterial bioinoculants for plant growth promotion and nutrient uptake in six cultivable varieties of green gram (MGG347, LGG460, ML 267, MGG 296, LGG 40 and TM 96.2) by selecting two plant growth promoting rhizobacterial isolates of *Rhizobium*-IC 3195 and *Pseudomonas*-P17 was done. Preliminary screening for various PGPR traits showed both the isolates were positive for production of IAA, HCN, siderophore, ammonia, biofilm, phosphate solubilization. A pot experiment was conducted with single and double inoculants of test isolates on six varieties of green gram for 75 days. After 75 days of sowing, plants agronomical and nutrient parameters were recorded as indicative parameters of plant growth promotion. The outcome of this investigation showed that the combination of *Pseudomonas* and *Rhizobium* influenced plant growth promotion most efficiently in plant varieties viz., MGG347, LGG460, ML267, MGG296, LGG40 and TM96.2. No one particular treatment could promote the growth of a given variety in all aspects studied. It was observed with these studies that the ability of a strain or combination of bioinoculants to promote growth of green gram plants varied with change in cultivable variety. This could be due to variation in the rhizosphere dynamics of a given bacterial isolate which in turn could be because of the change in the root exudates of a given plant. However, it was found that the combination of *Pseudomonas* and *Rhizobium* can be a viable, low-cost, effective alternative for improved growth and nutrient uptake in green gram.

Key words: *Pseudomonas* sp., P17, *Rhizobium*-IC 3195, green gram, nutrient uptake, plant growth promotion

INTRODUCTION

Mungbean (*Vigna radiata* L.), also popularly known as green gram, is an ancient and well known legume crop in Asia, particularly in the Indian subcontinent. It is one of the important pulse crops of Bangladesh, as it is an excellent source of easily-digestible protein of low flatulence which complements the staple rice diet in the country. Since it is a short duration legume, it is cultivated under both rainfed and irrigated conditions. In India, it is grown over on area of 30,084 ha with a

production of 10,232 t. Maharashtra state is the largest producer of green gram accounting nearly for 23.05% of the total production followed by Karnataka (17.46%) Andhra Pradesh (17.39%), Bihar (14.69%) and Rajasthan (7.50%).

The negative impact of chemical fertilizers on the global environment and the cost of production have lead to research with objective of replacing chemical fertilizers with organic amendment completely or partially. The application of microbial products to replenish the reserve nutrients is now increasing throughout the world (Kumar *et al*., 2012). Soil bacteria are very important in biogeochemical cycling and have been used for crop production and they are determinants of plant health and soil fertility (Kumar *et al*., 2014). Plant growth promoting rhizobacteria have the potential to contribute to sustainable plant growth promotion. The PGPR retain more soil organic N and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients. Different cultivable varieties of a crop plant offer different advantages for the growers. The advantages or drawbacks offered could vary due to the variation in genotype of the plant. Likewise, the dynamic rhizosphere microflora is also known to exert difference in morpho-physiological traits because of the variation in quality and/or quantity of root exudates produced in the rhizosphere of a particular crop variety.

Biofertilizers play a main key role for selective adsorption of immobile (P, Zn, Cu) and mobile (C, S, Ca, K, Mn, Cl, Br and N) elements to plants (Tinker, 1984). The rhizosphere bacteria secrete growth substances and secondary metabolic which contribute to seed germination and plant growth (Bilgrami, 1989). The fertilizer input required for green gram production was relatively high for a poor dryland farmer. Developing eco-friendly, cost effective production system for green gram immensely helps the farming community in a significant way. Co-inoculation of *Pseudomonas* sp., with *Rhizobium* has been reported to enhance nodulation, plant dry matter and grain yield in many legumes like alfalfa (Knight and Langston-Unkefer, 1988) and clover (Derylo and Skorupska, 1993) important feature of the green gram crop is its ability to establish a symbiotic partnership with specific bacteria, setting up the biological N_2-fixation in root nodules that supply the plant's needs for N_2 (Mandal *et al.,* 2009).

A little information is available about the impact of use of bio inoculants for improving growth of different cultivable varieties of green gram. Thus, the aim of this study was to investigate the influence of *Pseudomonas* and *Rhizobium* as single and dual inoculants to influence the growth and nutrient uptake in green gram.

MATERIALS AND METHODS

Bacterial cultures: Bacterial strains of sp., *Rhizobium* IC 3195 from International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India and *Pseudomonas* P17 were selected from Central Research Institute for Dryland Agriculture, Hyderabad, India germplasm and were used for the current experiment. The PGPR traits of the two strains were evaluated under *in vitro* conditions. The PGPR traits studied were indole acetic acid, HCN, siderophores, ammonia production, phosphate solubilizing ability were assessed by the method described by Kaur and Sharma (2013). Antagonistic activity against selected fungal phytopathogen *Sclerotium rolfsii* was checked using the procedure described by Kumar *et al*. (2014).

Seed bacterization: Talc formulations of each bacterial strain containing 2×10^8 cfu g^{-1} was used for seed bacterization. Six varieties of green gram viz., MGG347, LGG460, ML267, MGG296, LGG40 and TM96.2 were used to evaluate varietal response of PGPR. Ten gram of each formulation were separately mixed with equal proportion of water and treated with 1 kg of seeds

of green gram and three treatments were derived to evaluate the PGP viz., *Rhizobium*, *Pseudomonas*, *Rhizobium+Pseudomonas*. In case of consortia, 5 g of each product was mixed together and treated with the seed accordingly. Similar treatments were maintained in all the six varieties of green gram.

Pot trial: The treated seeds were sown in the plastic bags of 7 kg capacity filled with 5 kg each of sterile soil. In each bag 5 seeds were sown and thinned to two plants seven days after sowing. The plastic bags were watered to their field capacity in regular interval. Six replications were maintained for each treatment (Kumar *et al*., 2012). After 75 days of sowing root length, shoot length, leaf area (measured by LI 3100 Lincoln Nebraska USA leaf area meter), total chlorophyll (measured by Minolta Spad chlorophyll meter-502), photosynthetic activity (measured by Li-Cor Biosciences instrument, USA) and dry mass of root and shoot were recorded. Analysis of the macronutrients like nitrogen (N), phosphorus (P) and potassium (K) and micro-nutrients like iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) was done in the experimental plant tissue and was carried out following the protocols as explained by Tandon (2001).

Statistical analyses: The pooled data was subjected to statistical analysis using two way ANOVA at a probability of 0.001. The means of the values among the treatments were compared with one another by applying Fisher's least significant difference test ($p<0.05$).

RESULTS AND DISCUSSION

Bacterial isolates and soil analysis: The isolates of *Pseudomonas* sp.-P17 from germplasm of CRIDA and green gram nodulating *Rhizobium* sp., IC-3195 from culture bank of ICRISAT, were previously evaluated for their PGPR traits and the results are presented in Table 1. The soil that was used for all pot experiments was evaluated for different physico-chemical parameters and was found suitable for potting experiments the data of which is presented in Table 2.

The present study revealed that under glass house conditions seed bacterization of green gram with PGPR strains improves nutrient uptake and plant growth. The common PGPR traits include production of plant growth regulators (auxin, gibberellin, indole acetic acid, ethylene etc.), siderophores, HCN and antibiotics (Arshad and Frankenberger, 1993). *Rhizobium* and *Pseudomonas* sp., were able to enhance plant growth by several mechanisms. Co-inoculation studies with PGPR and *Rhizobium*/*Bradyrhizobium* spp., have shown to increase root and shoot

Table 1: The PGPR traits of *Pseudomonas* sp.-P17 and *Rhizobium* sp. IC-3195 strains used in current study

PGPR traits	*Pseudomonas*-P17	*Rhizobium*-IC 3195
IAA (μg mL^{-1})	19.9	13.2
HCN	+	-
Siderophore	+	+
Phosphate solubilization (mm)	16	18
NH_3 production	Strong	Strong
*Antagonistic activity (%)	75	96
Biofilm	+	+

*Antagonistic activity against *Sclerotium rolfsii* evaluated by dual culture assay

Table 2: Physico-chemical properties of red soil used for present plant experiments

Parameters	Values
pH	7.75
E.C.	0.1568
Organic carbon (%)	0.9
Nitrogen (kg ha^{-1})	92.8
Phosphorus (kg ha^{-1})	23.1
Potassium (kg ha^{-1})	175.728

weight, plant vigor, N_2 fixation and grain yield in various legumes such as alfalfa (Knight and Langston-Unkefer, 1988), common bean (Grimes and Mount, 1984), green gram (Sindhu *et al.,* 1999).

Sometimes, rhizobacteria isolated from a particular crop show some variation in plant growth-promoting activity with other legume crops which may be due to different colonization potentials (Anderson *et al*., 1988), their response to different amounts and compositions of root exudates (Miller *et al*., 1995). The results of the investigation showed considerable differences among genotypes through plant growth promotion and production of plant growth regulators when the bio inoculants were treated individually or in combination to the crop system. Different kinds of compounds exist in root exudates which consist of amino acids, organic acids, sugars, vitamins, purines/nucleosides, enzymes, inorganic ions and gaseous molecules (Dakora and Phillips, 2002). Nowak (1998) reported that the benefits of bacterization depended on plant species, cv. and growth conditions. The degree to which the inoculation imparts benefits to plant growth can vary with variety, cultural conditions and PGPR strains. In this study also, the variable effects of seed bacterization on green gram could be attributed to the origin of the test isolates as well as differences in host species.

Pseudomonas has shown high IAA production as compared to *Rhizobium* and it could be a possible reason for its success in plant growth promotion in pot culture assay. Interestingly, it was observed that root length was found more in only *Rhizobium* treated plants (Table 3). *Pseudomonas* sp. produced higher zone of siderophore on CAS medium as compared to *Rhizobium*. Role of these compounds is to scavenge iron from the environment and to make the mineral which is almost always essential, not available to the near vicinity. Isolates of *Rhizobium trifolium* grow by using various siderophores (Skorupska *et al*., 1989). This could be the reason behind exerting the various traits of growth promotion in combination inoculated plants for higher growth of plants (Table 3).

Pot trials

Agronomical plant parameters: On 75 DAS, among all the six varieties, four varieties have shown highest root length in combination treated plants whereas in the other two varieties, *Rhizobium* treated plants has been recorded high when compared with uninoculated treatments (Table 3).

In MGG296 (36 cm), TM 96.2 (32.6 cm), MGG347 (35.75 cm) and LGG40 (42.9 cm), combination treated plants, has shown highest root length when compared to their respective controls (31.4, 29.6, 33.4 and 30.6 cm) whereas in LGG460 (41.9 cm) and ML 267 (35.1 cm), *Rhizobium* treated plants have shown highest root length when compared to their respective controls (36.3 and 30.6 cm) (Table 3).

Of all the six varieties, increase in shoot length was observed in five varieties with combination treated plants and one variety with *Rhizobium* treated plants on 75 DAS. In MGG296 (25.3 cm), LGG460 (25.8 cm), TM96.2 (27.1 cm), MGG347 (29 cm) and LGG40 (23.6 cm), plants treated with combination (*Pseudomonas+Rhizobium*) have shown increase in shoot length over respective controls. In case of ML267, *Rhizobium* showed a significant increase in the shoot length of about 25 cm when compared to the control which was only 20.9 cm (Table 3).

All the six varieties of green gram have shown increase in their dry mass in plants treated with combination (*Pseudomonas+Rhizobium*). On 75 DAS, MGG296 (2.38 g), LGG460 (2.82 g), ML267 (2.05 g), TM96.2 (1.92 g), MGG347 (2.33 g) and LGG40 (2.96 g), have shown increase in dry mass with combination treated plants in comparison with their controls (Table 3).

Table 3: Plant growth of green gram as influenced by seed bacterization with test bioinoculants (75 days after sowing)

Crop variety and treatments	Root length (cm)	Shoot length (cm)	Dry mass (m)	Leaf area sq. (cm)	Chlorophyll (SPAD units)	Photosynthetic activity (μmol CO_2 m^{-2} sec^{-1})
MGG296						
Control	31.4±1.43	22.1±1	1.45±0.07	135±6.16	34.8±1.59	18.2±0.83
Pseudomonas (P)	32.8±1.5	25±1.14	1.83±0.08	185.5±8.47	43.2±1.97	26.5±1.21
Rhizobium (R)	33.1±1.51	24.6±1.12	2.24±0.1	228.5±10.43[a]	45.1±2.06	29.2±1.33
P+R	36±1.64	25.3±1.15	2.38±0.11	226.8±10.35[a]	50.6±2.31	31.6±1.44
LSD	0.23	0.17	0.05	5.21	0.77	0.69
CV (%)	5.79	6.08	21.3	22.71	15.08	22.12
MGG347						
Control	33.4±1.5	26.3±1.2	1.49±0.07	103±4.7	40.8±1.9	22.9±1
Pseudomonas (P)	33.11±1.5	27.4±1.2	1.88±0.08[a]	122.2±5.6	48.9±2.2	24.9±1.1
Rhizobium (R)	33.8±1.5	27±1.2	1.92±0.09[a]	135.8±6.2	50.02±2.3	26±1.2
P+R	35.75±1.6	29±1.3	2.33±0.11	152.8±7	51.56±2.3	29.1±1.3
LSD	0.14	0.14	0.04	2.49	0.57	0.31
CV (%)	3.5	4.17	18.02	16.41	10.05	10.07
LGG460						
Control	36.3±1.6[a]	24.3±1.1	1.86±0.08	110.2±5	48.9±2.2	29.8±1.4
Pseudomonas (P)	36.3±1.6[a]	25.6±1.2	2.29±0.1	128±5.8	50.6±2.3	30.6±1.4
Rhizobium (R)	41.9±1.9	25±1.1	2.6±0.12	132±6	52.9±2.4[a]	31.7±1.4
P+R	39.3±1.8	25.8±1.2	2.82±0.13	154.5±7.1	52.8±2.4[a]	34.1±1.6
LSD	0.32	0.08	0.05	2.15	0.23	0.22
CV (%)	7.02	2.68	17.4	13.88	3.74	5.93
LGG40						
Control	30.6±1.4	21.1±1	1.92±0.09	127.2±5.8	43.3±1.9	26±1.2
Pseudomonas (P)	32.5±1.5	21.9±1[a]	2.36±0.11	152±6.9	51.7±2.3[a]	30.8±1.4
Rhizobium (R)	34.3±1.6	21.9±1[a]	2.64±0.12	216.1±9.9	51.9±2.4[a]	31.3±1.4
P+R	42.9±1.9	23.6±1.1	2.96±0.13	222.6±10.2	53.3±2.4	28.9±1.3
LSD	0.64	0.12	0.05	5.59	0.54	0.28
CV%	15.48	4.76	17.86	26.31	9.1	8.21
ML267						
Control	30.6±1.4	20.9±0.9	1.1±0.05	58.2±2.6	45.5±2.1	24.4±1.1
Pseudomonas (P)	31.8±1.4	21.8±1	1.54±0.07	80.4±3.7	51.9±2.4[a]	27.2±1.2[a]
Rhizobium (R)	35.1±1.6	25±1.1	1.76±0.08	92±4.2[a]	52±2.4[a]	26.9±1.2[a]
P+R	32.3±1.5	23.3±1.1	2.05±0.09	92.4±4.2[a]	51.2±2.3	30.6±1.4
LSD	0.23	0.21	0.05	1.9	0.37	0.3
CV (%)	5.87	7.9	24.83	19.85	6.22	9.34
TM96.2						
Control	29.6±1.3	19.8±0.9	1.13±0.05	100.6±4.6[a]	27.1±1.2	22.3±1
Pseudomonas (P)	29.8±1.4	25.5±1.2	1.19±0.05	101±4.6[a]	61±2.8	26.7±1.2
Rhizobium (R)	32.4±1.5	22.8±1	1.73±0.08	136.7±6.2	51.9±2.4	23.4±1.1
P+R	32.6±1.5	27.1±1.2	1.92±0.09	157.5±7.2	58.7±2.7	28.4±1.3
LSD	0.19	0.38	0.05	3.32	1.84	0.34
CV (%)	5.21	13.46	26.29	22.63	31.28	11.26

Values superscribed by same letter are not significantly different according to Fisher's LSD test ($p<0.05$), values are Mean±SEM of two independent experiments with six replicates each time

Among the six varieties, five varieties have shown good increase in leaf area with combination treated plants and one variety has shown with *Rhizobium* treated plants. After 75 DAS, combination treated plants of LGG460 (154.5 cm^2), ML267 (92.4 cm^2), TM96.2 (157.5 cm^2), MGG347 (152.8 cm^2) and LGG40 (222.6 cm^2) have recorded highest leaf area as compared to their respective uninoculated treatments (110.2, 58.2, 100.6, 103 and 127.2 cm^2), whereas *Rhizobium* treated plants showed good result in MGG296 (228.5 cm^2) when compared to its control (135 cm^2) (Table 3).

On 75 DAS, MGG296 (50.6), MGG347 (51.56) and LGG40 (53.3) varieties treated with combination have shown increase in their chlorophyll content (SPAD units) over their controls (34.8, 40.8 and 43.3), respectively. In case of LGG460 (52.9) and ML267 (52), *Rhizobium* treated

plants showed relative increase over controls (48.9 and 45.5). In TM96.2 (61) variety *Pseudomonas* treated plants have shown a tremendous increase in chlorophyll content (SPAD units) when compared to its control (27.1) (Table 3).

Of all the six varieties, five varieties showed increase in their photosynthetic activity when treated with combination (*Pseudomonas*+*Rhizobium*) and one variety with *Rhizobium* treated plants. After 75 DAS, MGG296 (31.6), LGG460 (34.1), ML267 (30.6), TM96.2 (28.4) and MGG347 (29.1) treated with combination, have shown increase in their photosynthetic activity when compared to their respective uninoculated treatments (18.2, 29.8, 24.4, 22.3 and 22.9). In LGG40 (31.3), *Rhizobium* treated plants showed highest photosynthetic activity compared to their control (Table 3).

Nutrient analyses: A clear distinction in assimilation of macro and micro-nutrients between the treatments and their respective controls of all the six varieties was observed.

It was noted that, after 75 DAS the nitrogen assimilation was recorded highest in MGG296 with 3.8% and LGG460 (3.7%) plants treated with combined inoculants (P+R). The LGG40 cv., was on par with the rest to varieties with 3.6%. However with TM 96.2 cv., *Pseudomonas* treated plants showed highest 'N' content of 3.4%. Similarly, in case of phosphorus, maximum assimilation was seen in MGG296 (3.5%) and ML267 (2.0%) plants treated with combined inoculants. Whereas, LGG460, LGG40 and MGG347 vars., the *Pseudomonas* inoculants were better performers than combination treatments for 'P' uptake. Likewise, combination treatments were also found to be promising for potassium assimilation in case of TM96.2 (0.89%) cv. In rest other treatments the 'K' was influenced by *Pseudomonas* treatments alone like ML267 (0.96%), MGG296 (1.48%) etc. But for LGG460 (1.02%) *Rhizobium*, treatment appeared to be a good one for 'K' assimilation (Table 4).

A similar trend was also noted in case of assimilation of micro-nutrients between the treatments and their respective un-inoculated controls. On 75 DAS, 'Mn' uptake was found to be high in MGG296 (291.6 ppm), MGG347 (101.2 ppm), LGG40 (79 ppm) plants treated with combination of bioinoculants where as in case of TM 96.2 single *Rhizobium* and P+R inoculants were on par with each other. The 'Fe' assimilation was found to be high with combined inoculants (P+R) treatment in MGG347 (1118 ppm), LGG460 (1290 ppm), LGG 40 (2156 ppm), ML267 (1381 ppm) and TM 96.2 (1582 ppm). If concentration of 'Cu' in plant tissue is observed, only in TM 96.2 var., P+R inoculant was better with an uptake of 23.2 ppm however in others like ML267 and MGG347 un-inoculated control were better with 10.2 and 7.2 ppm, respectively. But with LGG40 and LGG460 vars., *Pseudomonas* alone was better for uptake of 'Cu' with 8.2 and 13.6 ppm, respectively. Finally in case of, 'Zn' assimilation interesting results can be noted. In most of the varieties, *Pseudomonas* alone was better for the uptake of this element. The MGG296 (76.4 ppm), LGG460 (55.2 ppm), LGG40 (56 ppm), ML267 (53.2 ppm) and TM96.2 (51.4 ppm) have shown highest uptake of 'Zn' than their other counterparts. But in case of MGG347 combination of P+R has improved the uptake of 'Zn' with 48.4 ppm (Table 5).

Rhizobium and *Pseudomonas* could release phosphorus from by solubilizing tri-calcium phosphate amended medium up to a considerable level (Table 1). The release of organic acids that both sequester and acidify the micro-environment near root is thought to be a mechanism of solubilization of phosphorus as well as Mn, Fe and Zn (Cunningham and Kuiack, 1992). Solubilization mainly takes place due to the secretion of chelating agent such as citric acid, malic acid, oxalic acid, succinic acid, lactic acid and fumaric acid (Desai *et al*., 2012).

Table 4: Macro-nutrient concentrations (75 DAS) in green gram plants grown from seed coated with different inoculants of *Pseudomonas*, *Rhizobium* and their combination

Crop variety and treatment	N (%)	P (%)	K (%)
MGG296			
Control	2.8±0.13	3.0±0.14	0.84±0.04
Pseudomonas (P)	4.5±0.2	2.4±0.11	1.48±0.07
Rhizobium (R)	2.6±0.12	3.3±0.15	0.68±0.03[a]
P+R	3.8±0.17	3.5±0.16	0.68±0.03[a]
LSD	0.105	0.06	0.05
CV (%)	25.94	15.72	41.4
MGG347			
Control	1.9±0.09	0.3±0.01	1.09±0.05
Pseudomonas (P)	3.5±0.16	2.5±0.11	0.96±0.04
Rhizobium (R)	2.3±0.1[a]	1.7±0.08	0.72±0.03
P+R	2.3±0.1[a]	2.2±0.10	0.6±0.03
LSD	0.08	0.11	0.03
CV (%)	27.71	58.03	26.44
LGG460			
Control	3.4±0.15[a]	1.2±0.05	0.75±0.03[a]
Pseudomonas (P)	3.4±0.15[a]	1.9±0.09	0.78±0.03[a]
Rhizobium (R)	4±0.18	1.5±0.07[a]	1.02±0.05
P+R	3.7±0.16	1.5±0.07[a]	0.78±0.03[a]
LSD	0.03	0.03	0.01
CV (%)	7.92	18.83	15.11
LGG40			
Control	3.4±0.15	1.7±0.08[a]	0.63±0.03
Pseudomonas (P)	3±0.14	1.8±0.08	0.61±0.03
Rhizobium (R)	3.2±0.15	1.7±0.08[a]	0.58±0.03
P+R	3.6±0.16	1.3±0.06	0.47±0.02
LSD	0.03	0.026	0.008
CV (%)	7.82	13.25	12.46
ML267			
Control	3.4±0.15	1.1±0.05	0.73±0.03
Pseudomonas (P)	3.3±0.15	1.5±0.07[a]	0.96±0.04
Rhizobium (R)	2.8±0.13	1.5±0.07[a]	0.69±0.03
P+R	3.2±0.15	2.0±0.09	0.77±0.03
LSD	0.03	0.04	0.01
CV (%)	8.28	21.17	15.18
TM96.2			
Control	3.0±0.14	1.9±0.09	0.63±0.03
Pseudomonas (P)	3.4±0.15	2.1±0.09[a]	0.60±0.03
Rhizobium (R)	2.8±0.13	2.1±0.09[a]	0.81±0.04
P+R	2.9±0.13	0.8±0.04	0.89±0.04
LSD	0.03	0.07	0.02
CV (%)	8.69	36.16	19.12

Values superscribed by same letter are not significantly different according to Fisher's LSD test ($p<0.05$), values are Means±SEM of two independent experiments with six replicates each time

From the recorded data after 75 DAS, for all the tested plant parameters combination of *Pseudomonas* and *Rhizobium* was found to be more efficient to improve all the agronomical parameters like root and shoot length, dry mass, chlorophyll content and photosynthetic activity (Table 3). These findings are in line with the results of various previous studies where it was proved that symbiotic effect of bioinoculants were proved to be effective for plant growth. Phosphate Solubilizing Bacteria (PSB) enhanced the seedling length of *Cicer arietinum* (Sharma *et al*., 2007) while co-inoculation of PSB and PGPR reduced P application by 50% without affecting corn yield (Yazdani *et al*., 2009). Phosphate solubilizing *P. putida* was shown to enhance the rhizobium-legume symbiosis in a study by Rosas *et al*. (2006).

Similarly, in case of macro- and micro-nutrients also (N, P, K) uptake by the plant mixed response of inoculants was observed. No one particular treatment could promote the uptake of all

Table 5: Micro-nutrient concentrations (75 DAS) in green gram plants grown from seed coated with different inoculants of *Pseudomonas*, *Rhizobium* and their combination

Crop variety and treatment	Mn	Fe	Cu	Zn
MGG296				
Control	115.2±5.26[a]	820±37.43	9.0±0.41	50±2.28
Pseudomonas (P)	23.6±1.08	1831±83.57	11.4±0.52	76.4±3.49
Rhizobium (R)	105.6±4.82[a]	2060±94.02[a]	13.4±0.61	51.4±2.34
P+R	291.6±13.31	2001±91.33[a]	12.8±0.58	65.4±2.98
LSD	13.35	68.65	0.23	1.48
CV (%)	84.19	34.58	16.78	20.58
MGG347				
Control	14.4±0.66[a]	843±38.48[a]	7.2±0.33	41.2±1.88
Pseudomonas (P)	9.4±0.43[a]	589±26.88	6.6±0.3	42.6±1.94
Rhizobium (R)	50.6±2.31	839±38.29[a]	6±0.27	43.4±1.98
P+R	101.2±4.62	1118±51.03	5±0.23	48.4±2.21
LSD	5.01	25.57	0.11	0.37
CV (%)	96.54	25.5	15.13	7.14
LGG460				
Control	64.8±2.96	476±21.73[a]	8.8±0.4	52.6±2.4
Pseudomonas (P)	33.6±1.53	985±44.96	13.6±0.62	55.2±2.52
Rhizobium (R)	51.8±2.36	499±22.78[a]	5.6±0.25	45.6±2.08
P+R	58.4±2.66	1290±58.88	3.2±0.15	49.2±2.24
LSD	1.59	46.8	0.53	0.49
CV (%)	25.81	48.68	57.64	8.23
LGG40				
Control	42.8±1.95	610±27.84	3.6±0.16	36.4±1.66
Pseudomonas (P)	46.2±2.11	1382±63.08	8.2±0.37	56±2.56
Rhizobium (R)	67.8±3.09	1152±52.58	5.6±0.25	50±2.28
P+R	79±3.6	2156±98.41	4.4±0.2	31.6±1.44
LSD	2.05	75.91	0.238	1.35
CV (%)	29.44	48.42	36.87	26.23
ML267				
Control	8±0.36	624±28.48	10.2±0.46	47.2±2.15
Pseudomonas (P)	62.2±2.83	684±31.22	7±0.31	53.2±2.43
Rhizobium (R)	28.2±1.29	865±39.48	4.4±0.2	47.6±2.17
P+R	57.8±2.64	1381±63.03	8.6±0.39	46.2±2.11
LSD	3.03	40.7	0.293	0.373
CV (%)	65.61	38.71	32.76	6.5
TM96.2				
Control	60.4±2.76	74±3.38	8.2±0.37	10±0.46
Pseudomonas (P)	71.4±3.26	770±35.14	10.4±0.47	51.4±2.35[a]
Rhizobium (R)	104.4±4.76[a]	1578±72.05[a]	16.8±0.77	51.0±2.33[a]
P+R	102.4±4.67[a]	1582±72.21[a]	23.2±1.06	49.8±2.27[a]
LSD	2.62	85.95	0.8	2.41
CV (%)	26.14	72.57	46.19	50.25

Values superscribed by same letter are not significantly different according to Fisher's LSD test ($p<0.05$), values are Mean±SEM of two independent experiments with six replicates each time

nutrients. Combination of inoculants was found to be better for some varieties and single inoculants for others (Table 4-5). This again can be attributed to the fact that the concentration and variety of rhizosphere exudates could have influenced the growth of the inoculants.

A remarkable uptake of 'Zn' was noted down in case of plants inoculated with *Pseudomonas* sp., alone (Table 5). This is because as the strain used in the current study is a known isolate of good zinc solubilizer for maize and therefore, has performed in a similar manner in the rhizosphere of green gram as well (Kumar *et al*., 2013). The uptake of all essential macro and micro-nutrients significantly improved up on inoculation with the bacterial strains as these isolates are known for solubilization of TCP and zinc again this finding is in line with the observations of Kumar *et al*. (2014).

CONCLUSION

Present study was carried out with the objective of assessing the role of PGPR strains in promoting the growth of different cultivable varieties of green gram. However, it was observed with these studies that the ability of a strain or combination of bioinoculants to promote growth of green gram plants varied with change in cultivable variety. Again this may be due to the variation in the rhizosphere dynamics of a given bacterial isolate which in turn could be because of the change in the exudates pattern in the root region of a given plant variety. Further, no particular treatment of seed could improve the growth in multi-dimension. However, it was found that the combination of *Pseudomonas* and *Rhizobium* can be a viable, low-cost, effective alternative for improved growth and nutrient uptake in green gram.

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