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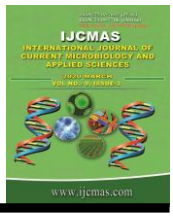

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Synergism of *Rhizobium* and Rhizobacteria on Growth, Symbiotic Parameters, Soil Quality and Grain Yield in Summer Mungbean (*Vigna radiata* L. Wilczek)

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A B S T R A C T

The present investigation was studied to evaluate the synergistic effect of *Rhizobium* and rhizobacteria consortium for improving growth, symbiotic efficiency, soil quality and yield in summer mungbean under field conditions during summer season 2015. Mungbean seeds of two varieties (SML668 and SML832) were inoculated with *Rhizobium* (M1, LSMR1 and LSMR2) singly and in combination with rhizobacteria (LSRB1, LSRB2 and LSRB3). Significantly high dry weight of shoot (4.22 and 5.29 g plant^{-1}) dry weight of root (0.411 and 0.604g plant^{-1}) total nitrogen (1.59 and 1.52%) and phosphorus content (0.109 and 0.129 %) of shoot were recorded with consortium of native *Rhizobium* sp. (LSMR1) and rhizobacteria (LSRB3) in SML668 and SML832 varieties, respectively as compared to *Rhizobium* sp. alone as well as un-inoculated control. On the basis of overall mean, symbiotic and soil quality parameters were significantly high *viz.* dry weight of nodules (105.3 mg), leghaemoglobin content (2.61 mg/g of nodules), nitrate reductase activity of nodules (13.86 µmNO^{-2}/hr/g of fresh nodules) and dehydrogenase activity (200 µg TPF/g/soil/hr) with LSMR1+LSRB3 treatment as compared to *Rhizobium* sp. alone as well as un-inoculated control. On an average, consortium of LSMR1+LSRB3 significantly improved the grain yield by 5.7% over *Rhizobium* sp. (LSMR1) and 9.2% over uninoculated control. Therefore present studies conclude that consortium of native *Rhizobium* sp. and rhizobacteria can be developed as a single delivery system biofertilizer for improving summer mungbean productivity.

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is an important source of protein (26%) for human diets (Keatinge *et al.*, 2011). Mungbean contains 51% carbohydrate, 26% protein, 10% moisture, 4% minerals and 3% vitamins (Afzal *et al.*, 2008). It increases soil fertility due to nitrogen fixing symbiotic rhizobia in root nodules thus adding large amounts of nitrogen to the soil after harvesting (Hosseini, 2008). It enriches the soil and breaks the soil fatigue caused by cereal–cereal rotations. *Rhizobium* is an excellent example of soil

bacteria engaged in symbiotic relationship with leguminous plants. They obtain their nutrients from the legume plants and produce nitrogen fixing root nodules through Biological Nitrogen Fixation (Datta *et al.*, 2015) and Rhizobia are known to fix nitrogen 50–100 kg/ ha in association with legumes only (Venkatashwarlu, 2008). *Rhizobium* inoculation can be demonstrated in summer mungbean as sustainable environment friendly agro-technological practice. Symbiotic effectiveness of rhizobial inoculants can be improved by co-inoculation with suitable non-rhizobial plant growth promoting bacteria (PGPB) (Lazdunski *et al.*, 2004). Various genera of bacteria, *Pseudomonas*, *Enterobacter*, *Bacillus*, *Klebsiella*, *Burkholderia*, *Azospirillum*, *Serratia* and *Azotobacter*, *Arthobacter*, *Hydrogenophaga* etc cause a pronounced effect on plant growth and are termed as plant growth promoting rhizobacteria (PGPR) (Verma *et al*., 2013). The PGPR may (i) promote the plant growth either by using their own metabolism (solubilising phosphates, producing hormones or fixing nitrogen) or directly affecting the plant metabolism (increasing the uptake of water and minerals), enhancing root development, increasing the enzymatic activity of the plant or "helping" other beneficial microorganisms to enhance their action on the plants; (ii) or may promote the plant growth by suppressing plant pathogens. These abilities are of great agriculture importance in terms of improving soil fertility and crop yield, thus reducing the negative impact of chemical fertilizers on the environment and for development of ecofriendly sustainable agriculture (Pérez–Montano *et al*., 2014; Gupta *et al*., 2015). Synergistic effects of *Rhizobium*–*Pseudomonas* co-inoculations have been reported at the level of different symbiotic and plant growth parameters and under different growth conditions (Yadav and verma, 2014). Co–inoculation also improved the nutrient balance and increased the phosphorus and protein concentration in grain of mungbean (Ahamd *et al.*, 2014). Similarly Co–inoculation studies with PGPR and *Rhizobium*/*Bradyrhizobium*/*Mesorhizobium* species have shown to increase root and shoot weight, plant vigor, nitrogen fixation and grain yield in various legumes (Valverde *et al.*, 2006; Yadegari *et al.*, 2008; Verma *et al.*, 2012). Co-inoculation of rhizobia with PGPR is therefore important for improving N and P availability in sustainable agriculture production systems (Samavat *et al.*, 2012).

Therefore, present study was carried out with the objectives to assess synergistic effect of plant growth promoting consortium of potential native PGPR with *Rhizobium* sp. for growth, symbiotic efficiency, soil quality and yield in summer mungbean.

Materials and Methods

Procurement of Bacterial cultures

Potential native isolates of *Rhizobium* (M1, LSMR1 and LSMR2) and rhizobacteria (LSRB1, LSRB2 and LSRB3) were obtained from the Pulses section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. Pure cultures of *Rhizobium* and rhizobacteria were maintained on Yeast Extract Manitol Agar (YEMA) and Nutrient Agar (NA) medium respectively, and further sub-cultured once a month throughout the period of investigation and stored at 4^0 C in refrigerator.

Evaluation of *Rhizobium* and rhizobacteria for growth, symbiotic parameters, soil quality and yield in summer mungbean

The present study was carried out at the Pulse Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India during summer

season in 2015. Field experiment was conducted in factorial randomised block design with three replication and thirteen treatments. Seeds of summer mungbean of two varieties (SML668 and SML832) were procured from the Pulses Section, Department of Plant Breeding and Genetics, PAU, Ludhiana.

Seed rate of 15 Kg/acre for SML 668 and 17 Kg/acre for SML 832 was used for sowing. The summer mungbean varieties SML668 and SML832 were sown on 10th April 2015 using 'kera' method at 22.5 cm row spacing, keeping a distance of about 7 cm between the seeds.

Mung been seeds of SML 668 and SML 832 varieties were inoculated with recommended culture of *Rhizobium* sp. (M1) and two native isolates of *Rhizobium* sp. (LSMR1, LSMR2) and PGPR (LSRB1, LSRB2 and LSRB3) as per treatment. Twenty g charcoal inoculants were used per kg of mung bean seeds for inoculation in monoculture treatment. In co-inoculation treatments, *Rhizobium* sp. and different PGPR were applied to mungbean seeds in ratio of 1:1. Before sowing, inoculated seeds were air dried at room temperature under shade and sown within two hours. Crop was sown on 10th April, 2015 following the recommended agronomic practice and harvested on 11 June, 2015. The observations were recorded on germination count at 10 days after sowing (DAS). Plant growth parameters *viz* plant height, dry weight of shoot and root, chlorophyll content of leaves, nodule number and dry weight of nodules were recorded at vegetative stage (40 DAS). Symbiotic parameters *viz* leghaemoglobin content of nodules, nitrate reductase activity of leaves and nodules, dehydrogenase activity (DHA) of soil were recorded at flowering stage while N-content from soot and soil and Phosphorous (P) content of shoot and grain yield was recorded at the harvesting stage.

Growth parameters

Emergence count was obtained by recording number of emerged seedlings per meter row length from central rows of each plot after leaving two border rows on each side. For Plant height three randomly selected plants were uprooted and roots were removed from shoots and the height of shoots was measured from the base in cm. Dry weight of shoot and root was observed by weighing the sun dried and then oven dried randomly selected uprooted plants at 60^0 C for 2 days in grams. Chlorophyll estimation was done by recording the optical density of the chlorophyll content on UV-Vis spectrophotometer using a solvent blank at 645 nm and 663 nm (Witham, 1971). Phosphorus content was estimated by digesting plant material (0.5g) with 20 ml of triacid mixture (HNO_3: $HClO_4$: H_2SO_4) and the volume was made up to 50 ml with distilled water; specific aliquots were used to estimate the P by reacting with 5 ml of ammonium molybdate reagent in nitric acid. The volume was made up to 50 ml and the intensity of yellow colour was estimated at 470 nm using spectronic 20 (Jackson, 1973). Grain yield from each plot (g/plot) was recorded and the final grain yield was expressed in Kg/ha.

Symbiotic parameters

The number of nodules per plant was recorded by taking average of nodules carefully detached from three randomly uprooted plants. The detached nodules were oven dried at 60^0 C for 2 days and the dry weight of nodules per plant was recorded in mg. Leghaemoglobin content was estimated by reading absorbance of clear nodule tissue extract with Drabkin's solution at 540 nm using UV-Vis spectrophotometer (Wilson and Reisenauer, 1963). Nitrate reductase activity of leaves and nodules was determined by the method of Jaworski, 1971 and the enzyme activity was expressed as µm of NO_2 $hr^{-1}g^{-1}$. Total N

content of shoot was determined by Kjeldahl's technique with slight modification of Mckenzie and Wallace.

Soil quality parameters

Dehydrogenase activity of soil was assayed at 40 DAS by the method of Tabatabai (1982). Total N content of soil was determined by Kjeldahl's technique with slight modification of Mckenzie and Wallace.

Analysis of data

Data was statistically analyzed using an analysis of variance (ANOVA) for factorial randomised block design. Further, mean separation of treatment effect was accomplished by Fisher's protected least significant difference test. All data analysis was carried out by using SAS- software.

Results and Discussion

Growth parameters

Germination is an index of dormancy and facilitate differentiation rate of germination among the varieties and treatments. Data on emergence count conclude that differences due to various treatments in both the varieties of mungbean were significant (Table 1). In co inoculation treatments germination was quite good and it varied from 90.6 to 96.0 % in SML668 and 91.7 to 98.3% in SML832. Significantly higher emergence count was observed with LSMR1+LSRB3 (96.0% and 98.3%) followed by LSMR1+LSRB2 (94.7 and 96.7 %) as compared with *Rhizobium* sp. LSMR1 alone treatment (88.3 and 89.0%) in SML668 and SML832 respectively, as well as uninoculated control. Improvement in seed germination with dual inoculation might be due to release of plant growth regulators which improve morphological characters of roots (Ashrafuzzaman *et al.,* 2009). The present study results are in harmony with the finding of Dasgupta *et al.,* (2015) and Bent *et al.,* (2001) who revealed that the use of PGPR with seed treatment improved seed germination; seedling emergence, seedling vigor and seedling stand over the control.

Significant difference for plant height was recorded between different dual treatments of rhizobacteria and *Rhizobium* sp. alone in SML 832 and SML 668 at 40 DAS. Significantly high plant height was recorded with dual inoculation treatment of LSMR1+LSRB3 (44.7 cm and 46.9 cm) followed by LSMR1+ LRRB1 (44.2 cm and 46.3 cm) in SML 668 and SML 832, respectively as compared to *Rhizobium* sp. alone treatment value and uninoculated control value. Improved plant height in dual inoculation can be attributed due to better establishment of *Rhizobium*–legume symbiosis due to production of plant growth regulator by PGPR in mungbean rhizosphere (Stajkovic *et al.,* 2011; Yadegari *et al.,* 2010). In earlial investigation mixed inoculation of *Rhizobium* sp., *Pseudomonas fluorescens* and *Bacillus megaterium* significantly increased the shoot and root growth compared to uninoculated control (Anandaraj and leema, 2010). Similarly Ahmad *et al., (*2014) revealed that co–inoculation reduced the effect of salinity on physiological parameters thus improving the photosynthetic rate which increased growth and yield of mung bean.

All the treatments and varieties differed significantly for shoot dry weight. On the basis of mean of both varieties, co-inoculation treatment LSMR1+LSRB3 showed significant increase in shoot dry weight (4.75g plant^{-1}) followed by LSMR1+LSRB1(4.63 g plant^{-1}) as compared to *Rhizobium* sp. alone as well as uninoculated control. Single and combined inoculation have shown positive response to the measured growth parameters that might be attributed to changes in endogenous ethylene

level by presence of PGPR containing ACC–deaminase on the roots of legumes (Shahroona *et al.,* 2006; Nadeem *et al.,* 2009; Ahmad *et al.,* 2011). Biologically fixed N_2 which might have contributed to enhancement of shoot dry weight in our study.

Significant increase in dry weight of root was observed with dual treatment of LSMR1+ LSRB3 (0.411g plant^{-1} and 0.604g plant^{-1}) followed by LSMR1+LSRB1 (0.403 g plant^{-1} and 0.483g plant^{-1}) in SML 668 and SML832, respectively as compared to *Rhizobium* sp. alone as well as uninoculated control treatment. Our results are in concurrence with the findings of Verma *et al.,* (2013) who revealed that the significant nodulation (62 and 86%), dry weight of root (44 and 57%) and shoot (26 and 45%) were recorded in co–inoculation of *Mesorhizobium sp*. and *Pseudomonas aeruginosa* over uninoculated control in pot and field conditions, respectively in chickpea. Inhibition of root length together with increase of root weight is a typical response to bacterial IAA production (Dobbelaere *et al.,* 1999). Hence the increase of root weight in present work might be the result of the high levels of IAA produced by combined treatment of *Rhizobium* and rhizobacteria.

Chlorophyll content indicates the amount of photosynthates that are present in plants. Numeric increase in chlorophyll content was observed in LSMR1+LSRB3 (0.845 and 0.867 mg/g fresh weight of leaves) followed by LSMR1+LSRB2 (0.802 and 0.822 mg/g fresh weight of leaves) in SML668 and SML 832 respectively. Nonsignificant difference existed among all treatments and the varieties for chlorophyll content. Results are well in accordance with Samavat *et al.,* (2012) and Bejandi *et al.,* (2012) who have reported that *Rhizobium* and *Pseudomonas fluorescens* treatment significantly improved leaves chlorophyll content of leaves in common bean and chickpea respectively, as compared with the control . Similarly, *Rhizobium* inoculation increased chlorophyll content and leaf area index by 5.43 and 6.99%, respectively compared to non–inoculated plants (Namvar *et al.,* 2013).

The data regarding phosphorus contents in shoot showed that co–inoculation significantly improved the parameter in comparison with *Rhizobium* sp. alone and there were significant difference among different treatment. Maximum increase in P content was recorded with co-inoculation of LSMR1+LSRB3 (0.243% and 0.259%) followed by LSMR1+LSRB2 (0.211% and 0.218%) in SML668 and SML832 respectively, as compared to *Rhizobium* sp. alone as well as un -inoculated control. Our results are supported by Yadav and Verma (2014) who reported that the combined inoculation of *R. leguminosarum* with *P. aeruginosa* showed significantly high P in grain (58.9%) and straw (80.6%) of chickpea over control. Similarly Stajkovic *et al.,* (2011) reported that shoot P content (0.90%) was highly affected by co–inoculation of *Rhizobium* with *Pseudomonas* sp. LG strain as compared to single inoculation of *Rhizobium* (0.59%). The increased concentration and uptake of N and P in plants treated with microbial inoculations suggest that a positive interaction exists between root colonization, N and P uptake, and growth promotion (Rudresh *et al.,* 2005).

Symbiotic parameters

Nodulation is one of important parameter indicating effective legume-Rhizobia symbiosis. Significantly high number of nodules was recorded with co–inoculation in both varieties of mungbean as compared to *Rhizobium* sp. alone treatment at 40 DAS (Table 2). The highest number of nodules was recorded with LSMR1+LSRB3 (20.9 and 22.5) followed by LSMR1+LSRB1 (18.0 and

20.1) in SML668 and SML832 respectively, as compared to *Rhizobium* sp. alone and uninoculated control treatment. Significant difference existed between both varieties for nodulation.

Significantly high nodule dry weight was recorded with LSMR1+LSRB3 (104.0 and 106.6 mg plant^{-1}) followed by LSMR1+LSRB2 (77.9 and 81.4 mg plant^{-1}) in SML668 and SML832 respectively, as compared to *Rhizobium* sp. alone treatment and uninoculated control. Difference for nodulation in both varieties was significant. Plant growth regulators (auxins) produced by PGPR play essential roles in nodule development. When co-inoculated with rhizobia resulting in improvement in symbiotic effectiveness (Sanchez *et al*.,2014; Yadav and Verma, 2014; Tariq *et al.,* 2012).

Leghaemoglobin content of the nodules is taken as the index of nodule efficiency as it regulates the oxygen supply to the bacteroid and hence the nitrogenase activity. Data on leghaemoglobin content depicted significant difference in both varieties. Leghaemoglobin content of nodules produced by introduced *Rhizobium* isolate (LSMR1) and rhizobacteria (LSRB3) was found to be significantly high compared to *Rhizobium* sp. alone and uninoculated control (Table 2). The nodules formed by dual inoculation of LSMRI and LSRB3 showed maximum leghaemoglobin content (2.27 and 2.31 mg g^{-1} fresh weight of nodules^{-1}) followed by LSMR2+LSRB3 (2.02 and 2.18 mg g^{-1} fresh weight of nodules) as compared to native isolate of *Rhizobium* sp. LSMRI (1.79 and 1.95 mg g^{-1} fresh weight of nodules^{-1}) in SML668 and SML832 respectively, as well as over un inoculated control.

Data was supported by Mishra *et al.,* (2012) who reported that co–inoculation of *Pseudomonas* sp. strain PGER17 with *R. leguminosarum*–PR1 and *R. leguminosarum*–PR1 treated plants resulted in 17.4 and 4.76 fold increase in leghaemoglobin content over control respectively. It was reported that the leghaemoglobin has a positive correlation with N_2 fixation and nitrogenase activity in nodules (Deka and Azad, 2006).

Nitrate reductase activity (NRA) provides a good estimate of the nitrogen status of plant and is correlated with growth and plant yield. Data revealed significant increase in NRA of leaves in both varieties of mungbean with single and dual treatments of different *Rhizobium* and PGPR. Dual treatment LSMR1+LSRB3 showed maximum increase in NRA of leaves (9.98 and 11.25µmNO^{-2} /hr/g of fresh leaf tissue) followed by LSMR2+LSRB3 (10.83 and 10.23µmNO^{-2}/hr/g of fresh leaf tissue) in SML668 and SML832, respectively as compared to single inoculation of *Rhizobium* sp. LSMR1 alone treatment. On the basis of pooled mean in both varieties the highest NRA of nodules was produced by LSMR1+LSRB1 (14.63µmNO^{-2}/hr/g of fresh nodule) followed by LSMR1+LSRB2 (13.86µmNO^{-2}/hr/g of fresh nodule) compared to *Rhizobium* sp. alone.

The increased NRA activity in inoculated plants could be explained by the increased efficiency of nitrogen fixation with dual inoculation of PGPR and *Rhizobium* sp. increased NRA directly related to increase in N content of shoot. Our results are in agreement with Mahmood *et al.,* (2010) who observed increased NRA with dual inoculation of *Bacillus sphaericus* UPMB10 and *Agrobacterium rhizogenes* strains AR9402 as compared to single inoculation.and uninoculated control in banana. Similarly Ahmad *et al.,* (2010) also reported higher NR activity in the leaves of *Ammi majus* L. grown with combined application of S and N when compared with N alone.

Table.1 Co-inoculation effect of *Rhizobium* and rhizobacteria on growth parameter in summer mungbean

Treatments	Emergence count (%)			Plant height (cm)			Dry wt. of shoot plant^{-1} (g)			Dry wt. of root plant^{-1} (g)			Total Chlorophyll content of leaves (mg/g fresh weight of leaves)			Total Phosphorus content of shoot (%)		
	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean
M1	88.8	89.1	88.9	35.2	36.0	35.6	3.33	3.87	3.60	0.312	0.382	0.347	0.590	0.770	0.680	0.129	0.148	0.139
LSMR1	88.3	89.0	88.7	38.0	41.6	39.8	3.23	4.13	3.68	0.310	0.401	0.355	0.650	0.778	0.714	0.135	0.162	0.149
LSMR2	88.7	87.3	88.0	37.3	40.2	38.7	3.25	4.25	3.75	0.324	0.424	0.374	0.670	0.740	0.704	0.130	0.157	0.144
M1+LSRB1	90.8	91.9	91.3	40.5	44.0	42.3	3.54	4.33	4.27	0.328	0.446	0.387	0.686	0.750	0.718	0.149	0.177	0.163
M1+LSRB2	90.6	91.7	91.2	40.8	41.0	40.9	4.10	4.70	4.40	0.329	0.443	0.386	0.725	0.740	0.728	0.158	0.170	0.164
M1+LSRB3	90.4	91.7	91.1	43.2	44.1	43.7	4.25	4.33	4.29	0.365	0.444	0.404	0.719	0.731	0.725	0.168	0.177	0.172
LSMR1+LSRB1	94.7	93.3	94.0	44.2	46.3	45.3	4.05	5.21	4.63	0.403	0.483	0.443	0.782	0.816	0.799	0.188	0.178	0.183
LSMR1+LSRB2	94.7	96.7	95.7	41.0	45.0	43.0	4.09	5.16	4.62	0.392	0.525	0.458	0.802	0.822	0.812	0.211	0.218	0.215
LSMR1+LSRB3	96.0	98.3	97.2	44.8	46.9	45.8	4.22	5.29	4.75	0.411	0.604	0.507	0.845	0.867	0.856	0.243	0.259	0.251
LSMR2+LSRB1	90.8	91.7	91.3	42.3	45.6	44.0	4.23	4.37	4.30	0.372	0.430	0.401	0.775	0.803	0.789	0.109	0.129	0.119
LSMR2+LSRB2	91.0	92.9	91.9	41.7	43.1	42.4	4.44	4.33	4.38	0.398	0.450	0.424	0.742	0.798	0.770	0.118	0.201	0.159
LSMR2+LSRB3	92.5	93.0	92.8	43.2	46.3	44.8	4.20	4.39	4.29	0.395	0.483	0.439	0.746	0.772	0.759	0.203	0.192	0.198
Uninoculated	87.5	88.4	87.9	32.1	35.3	33.7	2.99	3.28	3.13	0.297	0.347	0.322	0.645	0.659	0.652	0.103	0.107	0.105
Mean	91.13	91.86		40.3	42.7		3.84	4.43		0.356	0.451		0.711	0.784		0.157	0.175	
CD (p≤0.05)	T:0.91 V:0.35 TxV: 1.29			T:0.37 V:0.14 TxV:0.52			T:0.13 V:0.51 TxV: 0.19			T: 0.014 V: 0.034 TxV: 0.052			T:NS V:NS TxV: NS			T:0.044 V:0.018 TxV: NS		

Table.2 Co-inoculation effect of *Rhizobium* and rhizobacteria on symbiotic parameter in summer mungbean

Treatments	No. of nodules plant^{-1}			Dry wt. of nodules plant^{-1} (mg)			Leghaemoglobin content (mg/g of nodules)			Nitrate reductase activity of leaves (µm NO^{-2} /hr/g of fresh tissue)						Total N content of shoot (%)		
										Leaves			Nodules					
	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean	SML 668	SML 832	Mean
M1	15.3	17.8	16.5	53.5	56.1	54.8	1.63	1.59	1.61	3.39	3.65	3.52	5.78	5.27	5.53	1.25	1.27	1.26
LSMR1	14.5	17.8	16.2	60.9	65.3	63.1	1.79	1.95	1.87	3.98	4.26	4.11	8.89	13.75	11.32	1.29	1.40	1.35
LSMR2	15.5	18.3	16.9	57.0	62.2	59.6	1.72	1.76	1.74	3.43	3.61	3.52	8.88	10.15	9.47	1.28	1.30	1.30
M1+LSRB1	18.4	19.4	18.9	64.2	66.0	65.1	1.86	1.94	1.90	7.98	9.48	8.73	11.47	11.27	11.37	1.29	1.40	1.35
M1+LSRB2	15.8	18.21	17.0	68.3	71.2	69.7	1.97	2.01	1.99	6.49	6.59	6.54	11.92	13.03	12.47	1.34	1.35	1.35
M1+LSRB3	17.3	19.2	18.2	69.6	70.9	70.3	1.98	2.06	2.02	9.58	9.68	9.63	11.80	12.85	12.32	1.38	1.42	1.40
LSMR1+LSRB1	17.4	18.21	17.8	73.5	76.8	75.2	2.21	2.45	2.33	9.14	9.24	9.22	13.51	13.33	13.42	1.44	1.49	1.46
LSMR1+LSRB2	18.0	20.1	19.0	77.9	81.4	79.7	2.13	2.35	2.24	10.83	10.23	10.53	15.95	13.32	14.63	1.45	1.48	1.47
LSMR1+LSRB3	20.9	22.5	21.7	104.0	106.6	105.3	2.59	2.63	2.61	9.98	11.25	10.61	15.72	12.00	13.86	1.59	1.52	1.55
LSMR2+LSRB1	18.4	18.0	18.2	70.5	74.8	72.7	2.04	2.14	2.09	7.12	8.52	7.82	11.35	11.07	11.21	1.40	1.41	1.40
LSMR2+LSRB2	17.6	18.8	18.2	76.3	79.1	77.7	2.02	2.18	2.10	8.55	8.02	8.28	13.82	11.29	12.56	1.41	1.42	1.42
LSMR2+LSRB3	17.7	18.4	18.0	74.2	77.5	75.9	2.27	2.31	2.29	7.83	8.20	8.02	14.96	11.15	13.06	1.44	1.48	1.46
Uninoculated	13.36	16.2	14.8	38.0	42.0	40.0	1.25	1.49	1.37	2.02	3.02	2.52	4.82	3.01	4.92	1.25	1.22	1.24
Mean	16.9	18.8		68.31	71.50		1.96	2.06		6.94	7.36		11.45	11.12		1.37	1.40	
CD (p≤0.05)	T:1.13 V:0.45 TX V:NS			T:12.62 V:1.24 T X V:11.80			T:0.25 V:0.97 T X V: 0.35			T: 1.07 V: NS T X V: NS			T:0.92 V:0.36 TxV: 1.30			T:0.13 V: 0.51 TxV:0.19		

Fig.1 Co-inoculation effect of *Rhizobium* and rhizobacteria on dehydrogenase activity in soil of summer mungbean. Each bar represents the mean of triplicate values

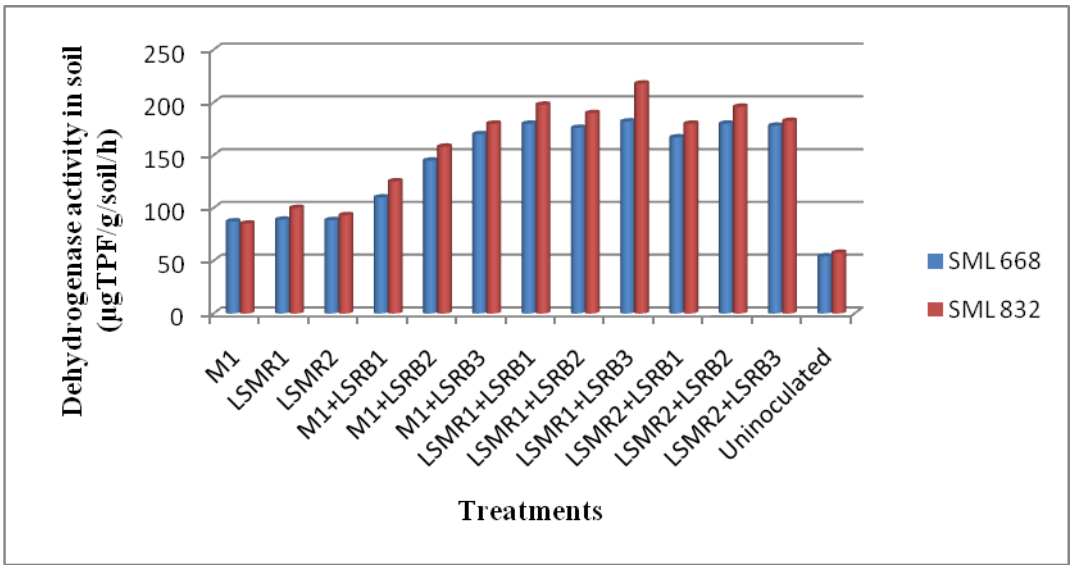

Fig.2 Co–inoculation of *Rhizobium* and rhizobacteria on N content from soil in the field of summer mungbean. Each bar represents the mean of triplicate values

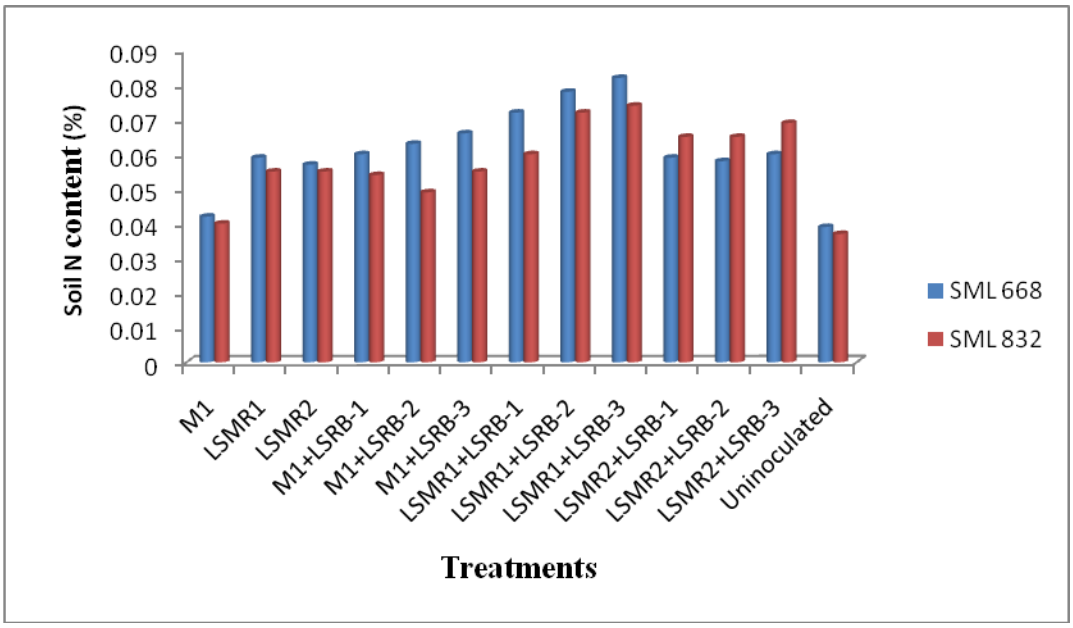

Table.3 Co-inoculation effect of *Rhizobium* and rhizobacteria on yield attributing traits in summer mungbean

Treatments	No. of pods plant^{-1}			No. of seeds pod^{-1}		
	SML 668	SML 832	Mean	SML 668	SML 832	Mean
M1	18.35	19.27	18.81	10.53	11.02	10.78
LSMR1	19.52	19.54	19.53	11.23	12.20	11.72
LSMR2	19.25	19.47	19.36	10.83	11.66	11.25
M1+LSRB1	19.56	19.64	19.60	11.26	12.23	11.75
M1+LSRB2	19.43	19.80	19.62	11.27	12.35	11.81
M1+LSRB3	19.53	19.67	19.60	11.29	12.46	11.88
LSMR1+LSRB1	19.90	21.23	20.57	11.46	12.8	12.13
LSMR1+LSRB2	20.75	20.97	20.86	11.86	12.57	12.22
LSMR1+LSRB3	20.8	21.7	21.25	13.13	12.73	12.93
LSMR2+LSRB1	19.48	19.84	19.66	11.6	12.2	11.63
LSMR2+LSRB2	19.02	19.7	19.36	11.09	11.83	11.46
LSMR2+LSRB3	19.35	19.81	19.58	11.24	11.89	11.57
Uninoculated	17.6	18.20	17.9	10.24	11.0	10.97
Mean	19.42	86.28		11.31	12.06	
CD (5%)	T:1.02	V: 0.40 TXV: NS		T:NS	V: NS TXV: NS	

Table.4 Co-inoculation effect of *Rhizobium* and rhizobacteria on grain yield in summer mungbean

Treatments	grain yield(Kg/ha)		
	SML 668	SML 832	Mean
M1	1272	1288	1280
LSMR1	1298	1305	1301
LSMR2	1287	1289	1288
M1+LSRB1	1298	1310	1304
M1+LSRB2	1318	1328	1323
M1+LSRB3	1325	1338	1332
LSMR1+LSRB1	1349	1359	1354
LSMR1+LSRB2	1358	1365	1361
LSMR1+LSRB3	1370	1380	1375
LSMR2+LSRB1	1333	1342	1337
LSMR2+LSRB2	1345	1356	1350
LSMR2+LSRB3	1355	1368	1361
Uninoculated	1234	1244	1259
Mean	1318.62	1328.62	
CD	T: 60 V: NS TxV:NS		

Nitrogen is a vital element for plant and soil microorganism's growth and activity. Data revealed significant increase in N content of shoot was observed with dual treatment of *Rhizobium* sp. and rhizobacteria (Table 2). Significant increase of total shoot nitrogen was observed with consortium of LSMR1+LSRB3 (1.59 and 1.51%) followed by LSMR1+LSRB2 (1.45 and 1.48%) as compared to *Rhizobium* sp. LSMR1 alone (1.29 and 1.40%) in SML668 and SML832 respectively, over uninoculated control treatment. SML 832 revealed significantly high shoot N content as compared to SML668. Increase in N content in shoot with co-inoculation of PGPR and *Rhizobium* was mainly due to significant enhancement in nodulation, it resulted in higher accumulation of N from atmospheric N_2 fixation. These results are in harmony with the finding of Stajkovic *et al.,* (2011) reported the increase in shoot N content (2.65%) with coinoculation of endophytic *Bacillus* sp. BX strain and *Rhizobium* as compared to single *Rhizobium* inoculation (2.34%).

Soil quality parameters

High soil Dehydrogenase activity indicates the number of microorganisms present in the soil. Co–inoculation treatment significantly increased soil DHA with LSMR1+LSRB3 (48.48 and 51.34µg/TPF/g/soil/h) and LSMR1+LSRB2 (43.69 and 46.47µg/TPF/g/soil/h) in SML668 and SML832 respectively, as compared to *Rhizobium* sp. alone treatment (Fig.1). Difference for DHA in both varieties was significant, Similar trend was followed for N content of soil. There was a significant increase in soil N content was observed with co–inoculation treatment of *Rhizobium* and

rhizobacteria (Fig.2). On the basis of pooled mean, significant increase was observed with consortium of LSMR1+LSRB3 (0.082 and 0.074% in SML668 and SML832, respectively) followed by LSMR1+LSRB2 (0.078 and 0.072% in SML668 and SML832, respectively) as compared to *Rhizobium* sp. alone and uninoculated treatment.

Rhizospheric microorganisms influence the community structure by facilitating plant nutrient uptake and release of root exudates. Soil dehydrogenase activity provides correlative information on biological activity and microbial population in soil. Our results are in agreement with Mader *et al.,* (2011) who showed that soil quality improved with single and dual inoculation of PGPR and arbuscular mycorrhizal fungi (AMF) with increased soil dehydrogenase activity in wheat, rice and blackgram. Similarly Meenakshi and Savalgi (2009) observed that dual treatment of *Methylobacterium* and *B. japonicum* increased soil dehydrogenase activity along with foliar spray in soybean. Microbial release of nutrients might have enhanced the N and P levels in soil due to increase in root hair density, more lateral roots, root surface area/ nodulation, thus more nitrogen fixation and phosphate solubilization. Our results are accordance with the work of Qureshi *et al.,* (2011) who showed that co-inoculation resulted in higher soil N content as compared to control.

Yield attributing traits and grain yield

Significantly high number of pods per plant was obtained with co–inoculation of LSMR1+LSRB3 (20.8 and 21.7 pods plant^{-1}) however numeric increase was recorded with LSMR1+LSRB2 (20.75 and 20.97 pods plant^{-1}) in SML668 and SML832, respectively as compared to *Rhizobium* and uninoculated control treatment.

The co-inoculation treatment of *Pseudomonas* + *Rhizobium* + *Azospirillum* significantly increased number of pods per plant as compared with control treatment (Hosseini *et al.,* 2014). Since the number of pod per plant is one of the factors related to grain yield, therefore any factor that increases yield also has significant affect on this trait.

Co–inoculation of *Rhizobium* and rhizobacteria increase the number of grain per pod (Table 3). The maximum increase in number of seeds per pod was exhibited by dual treatment of LSMR1+LSRB3 (13.13 and 12.73 grain pod^{-1}) followed by LSMR1+LSRB2 (11.86 and 12.57 grain pod^{-1}) in SML668 and SML832 respectively, as compared to *Rhizobium* sp. alone and uninoculated control. Non significant differences existed between varieties and treatments. These results are in harmony with findings of Hosseini *et al.,* (2014) and Shokuh *et al.,* (2008) who showed that *Azospirillum* + *Rhizobium* +*Pseudomonas* treatments had significant effect on the number of grain per pod as compared with control treatment in mungbean and soybean plant respectively . The sink capacity of plant is determine by the number of grain per pods.

Single inoculation of mungbean with different *Rhizobium* sp. increased the grain yield by 1.6 to 3.3% and dual inoculation increased grain yield by 3.57 to 9.21% as compared to uninoculated control (Table 4). On the basis of pooled mean of both varieties, significantly higher grain yield was recorded with consortium of LSMR1+LSRB3 (1375 Kg/ha) however numeric increase was recorded with LSMR1+LSRB2 (1361 Kg/ha) and LSMR2+LSRB3 as compared to *Rhizobium* sp. alone. Our results are in concurrence with Sanchez *et al.,* (2014) who showed that the effect of *Rhizobium –Pseudomonas* co–inoculation treatments was significantly better for grain yield compared to single *Rhizobium*

inoculation. The results were further supported by Yadav and Verma (2014) who showed the combined inoculation of *R. leguminosarum* with *P. aeruginosa* has shown significantly higher increase in yield of grain (31.8%) over control. Namvar and Sharifi (2011) also reported that *Rhizobium* inoculation increased grain yield per plant by about 9.04% as compared with the control. Positive results obtained in our study might be correlated to IAA production, phosphate solubilisation, ACC deaminase activity and *in vitro* compatibility of *Rhizobium* sp. with PGPR. More grains per pod recorded in our study might have leaded to more assimilates stored in grain and in turn increase in grain yield (Cheraghi *et al.,* 2011).

In conclusion, the present research aimed to investigate native potential strains of *Rhizobium* and rhizobacteria ability to adapt in prevailing environmental conditions for improving productivity in summer mungbean. It was concluded that consortium of native potential isolate *Rhizobium* (LSMR1) and rhizobacteria (LSRB3) emerged as effective strains for improving productivity and can be developed as a single delivery system biofertilizers in summer mungbean.

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