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Yield of mung bean and rhizospheric microbial population as affected by inoculation of stress tolerant *Rhizobium* and PSB

Uttara Sahu, D Dash, Gayatri and Karthika P

Abstract

The experiment was being carried out in the summer season, 2020-2021 at College of Agriculture, Raipur, Chhattisgarh to study the dual inoculation of stress tolerant *Rhizobium* and Phosphorus solubilizing bacteria (PSB) on the growth and yield of mung bean crop. The experiment was laid out in RCBD with nine treatments replicated thrice. *Rhizobium* and PSB isolates collected from culture deposits of Department of Agricultural Microbiology, IGKV, Raipur were studied for screening of their stress tolerance ability.The effect of pH and temperature on *Rhizobium* and PSB isolates were tested by growing strains on YEMA and PVK medium respectively. Isolates were subjected to extreme acidic pH (pH: 4.5) and extreme temperature (55 ^{0}C), Two isolates each from *Rhizobium* (Mung 8 and Mung 3512) and PSB (PSB 5-69 and PSB 5- 71) were screened as stress tolerant isolates which found favourable colony growth at low pH (4.5) and high temperature, hence used for inoculation as single and dual inoculation in experiment with mung crop. Results showed that dry biomass of shoot as well as shoot N uptake were observed for significant increase i.e., 0.93 to 2.68g/plant and 16.00 to 70.22 mg N/ plant respectively in co- inoculation of isolates, Mung8 and PSB-5-69 over single inoculation and control at 40 Days after sowing. Grain yield also increased significantly (7.28 to 12.78 qha^{-1}) by application of treatment T_6(Mung8+PSB-5-69)followed by T_8 (Mung8+PSB-5-71) over uninoculated. Combined application of Mung8 and PSB-5-69 was considered to be the suitable treatment and can be recommended as nutrient management strategy for yield enhancement under stressed condition.

Keywords: *Rhizobium*, PSB, stress tolerant strains, mung crop, yield

Introduction

Mung bean (*Vigna radiata*) is a major pulse crop that belongs to family, Fabaceae, grown in an area of 8.40ha having an average production of 4.07MT and productivity of 485 kg ha^{-1} in Chhattisgarh (Comissioner L and Record 2017) [8]. Raigarh, Surguja, Durg, Bilaspur, and Rajnandgaon are the key pulse-growing districts in Chhattisgarh, Whenmung bean is being incorporated in cropping system, the soil microbiological properties were significantly higher in the soils (Kumar, 2014). It is extensively planted as a short-term catch crop between two main crops on the Indian subcontinent. Mung beans have a carbohydrate content of 51%, a protein content of 24-26%, a mineral content of 4%, and a vitamin content of 3%. In addition to being an important source of human food and animal feed, mung beans being legume crop has a remarkable ability to fix atmospheric nitrogen using symbiotic root rhizobia, hence enriching soil fertility (Mondal *et al*. 2012) [5]. Plants usually depend upon nitrogen, such as ammonia and nitrate, but it is not available by its most prevalent form such as atmospheric nitrogen. Most of nitrogen nutrient is provided to plants through Biological Nitrogen Fixation (Al-Mujahidy *et al.* 2013) [1]. Phosphorous is such an important macronutrient which is very often present in the soil in unavailable form. The soil fertility is also improved by these biofertilizers. Living microorganisms in biofertilizers enhance plant growth by improving the availability of primary nutrients (nitrogen and phosphorus) to the host plant (Painkra *et al.* 2019) [6].

However, the biological process efficiency is determined by various aspects relating to interaction of plant and microbes, edaphic conditions i.e., soil acidity, low soil fertility, extreme temperatures and drought frequently restrict nitrogen fixation's contribution. Plants are harmed by high temperatures at all phases of development, resulting in a significant loss in productivity (Rao *et al.* 2016) [3]. As a result, the goal of this study was to find stress-tolerant *Rhizobium* and PSB strains and to assess the performance of the mung bean crop by inoculating with these stress tolerant microbes.

It is seen that positive effect on the yield and nutrient uptake of legume crops as well as nodulation improvement due to combined inoculation of PSB and nitrogen fixing microbes (Khan *et al.*, 2007). Dual inoculation of nitrogen fixers and phosphate solubilizers in legumes may have synergistic effects resulting into better crop yield. Therefore, this study was taken to evaluate the potential role of dual inoculation of stress tolerant *Rhizobium* and PSB towards the nutrient management for mung bean crop to enhance the growth and productivity systems.

Material and Method

A field experiment was conducted at the Research farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during 2020-2021 with the mung bean (*Vigna radiata*) crop, Variety: (Hum). Seeds were collected from IGKV, Raipur. Raipur is situated in the plains of Chhattisgarh at 21°16' N latitude and 81°36' E longitude with an altitude of 298.56 metres above the mean sea level (MSL).Ten *Rhizobium* isolates of Mung bean and 8 PSB isolates were collected from the microbiology repository of the Department of Agricultural Microbiology, CoA, Raipur. These isolates were screened in the laboratory for their stress tolerant (low pH and high temperature) ability. The selected two promising stress-tolerant mung bean *Rhizobium* and PSB isolates were used as treatments with mung bean in field experiment.

The experiment was taken in RBD with 9 treatments and treatments were applied with three replications. The treatments include two promising stress tolerant *Rhizobium* isolates, two promising stress tolerant PSB isolates as sole inoculation and their dual inoculation including one un inoculated (control). *Rhizobium* and PSB were applied as seed treatments just before sowing @ 20 g *Rhizobium*/ PSB per kg seed.

Screening of isolates on basis of stress (temperature and pH) tolerance ability

Screening for pH stress tolerance the *Rhizobium* and PSB were tested at pH, i.e. 4.5pH and 7.5 pH in YEMA and Pikovskayas media respectively by adjusting the pH with dilute Hcl. All the broth of concerned pH were inoculated and incubated at 30 °C for 72 hours with shaking.After completion of 3 days incubation period, survival of *Rhizobium* and PSB were recorded by inoculating on agar plates and were observed for the colony formation.Screening for temperature tolerance of *Rhizobium* and PSB were tested at different temperatures. Loopful culture of each isolates was inoculated in 5 ml of YEMA broth and Pikovskayasbroth in test tubes and incubated for three days for growth. Then the bacterial culture tubes were placed in water bath and temperatures were maintained 35 °C, 45 °C and 55 °C. Tubes were kept in water bath for 30 minutes at respective temperature, after which they were taken out and cooled to room temperature. These bacterial cultures were then spot inoculated on plates containing YEMA and PVK medium and further observed for colony formation by them.

Shoot Biomass yield was also recorded and Shoot Nutrient (N) uptake was calculated at 40 DAS by Kjeldahl method (Jackson-1958) following digesting sample in conc.H_2SO_4 followed by distillation and titration.Yield for both grain and stover (kg ha^{-1}) were recorded and converted into kilos per hectare as per plot area.

Enumeration of the microbial population in rhizosphere soil

Following the completion of the experiment, rhizosphere soil samples from various treatments were collected for *Rhizobium* and PSB enumeration. The dilution plate method was used to estimate microbial counts with regard to PSB and rhizobial count in the soil (Subba Rao, 1988). *Rhizobium* cell count was done in Yeast Extract Mannitol Agar while PSB cell count was done in Pikovskaya‟s medium.

$$\textbf{Number of } \textbf{\textit{Rhizobium}}\textbf{/PSB per gram of oven dry soil} = \frac{\text{No of colony forming units (cfu) x dilution}}{\text{Dry weight of one g moist soil sample x aliquot taken}}$$

Mean data of each quantitative trait were statistically analysed by the technique of analysis of variance. The significant difference was tested by F test and difference between mean by using CD at 5% level (Panse and Shukhatme, 1978) [7].

Result and Discussion

Acid and temperature Tolerance of Rhizobial and PSB Isolates

The hypothesis that fast growth in yeast mannitol adjusted to acidic pH, indicates acid tolerant ones which is a little support for our observations. In view of the wide acceptance of this hypothesis (most recently Sanchez, 1977), we tested all isolates for their colony growth on the respective media with acidic pH(pH 4.5). The rhizobia varied greatly in growth rate and final pH of the medium at 4.5and 7.5. All the *Rhizobium* isolates showed growth in pH 4.5 except Mung9 and Mung125 isolates, whereas isolates Mung8 and Mung3512 showed more favourable growth at pH 4.5. While at pH 7.5 all *Rhizobium* isolates tested showed favourable colony growth. Screening test was done for obtaining low pH tolerance capacity of *Rhizobium* and PSB isolates. All the PSB isolates showed growth in pH 4.5, whereas isolates PSB-5-69and PSB-5-71showed more favourable growth at pH 4.5. Similarly at pH 7.5 all PSB isolates tested showed colony growth whereas isolates PSB-5-69and PSB-5-71showed more favourable growth. (Table no. 1)

Acidity depressed growth of plants with precisely the same frequency in abundantly and sparsely nodulating associations.As per acidity tolerance test of *Rhizobium* and PSB isolates i.e. Mung-8, and mung-3512 and PSB-5-69 and PSB-5-71 isolates found superior. The qualitative growth data was given in table 4.5(a) and (b). Segura (1995) [9] recorded similar observations while screening *Rhizobium* strain for acidity tolerance.

Temperature is an important factor which reduces the productivity and production of mung bean crop. All isolates of Rhizobium and PSB were exposed to high temperature in water bath for at least 30 minutes, after exposure, their colony growth were examined by inoculating on the agar plates. In higher temperature at 55 ºC growth is observed in isolates Mung-8, mung-3512 and PSB-5-69, PSB-5-71 showed favourable colony growth as compared to other isolates. Jimenez *et al*, (2011) also expressed *Rhizobium* isolates formed colonies at normal temperature, i.e., nearly up to 35 ^{0}C, with increasing temperature, growth decreased, but some promising strains were tolerant upto 50 °C temperature (Kumar *et al.* 2017) [8]. (Table no.2)

Table 1: Efficacy of *Rhizobium* and PSB isolates against stress (Low pH) tolerance test

S. No.	Name of isolates	Colony growth of *Rhizobium* isolates	
		4.5 pH	7.5 pH
1.	Mung-7	+	+
2.	Mung-8	++	++
3.	Mung-9	-	+
4.	Mung-10	+	+
5.	Mung-13	+	+
6.	Mung-15	+	+
7.	Mung-3512	++	++
8.	Mung-3494	+	+
9.	Mung-577	+	+
10.	Mung-125	-	+
Colony growth of *PSB* isolates			
1.	PSB-5-62	+	+
2.	PSB-5-68	+	++
3.	PSB-5-69	+++	++
4.	PSB-5-71	+++	++
5.	PSB-5-77	+	++
6.	PSB-5-91	+	+
7.	PSB-127	+	++
8.	PSB-129	+	+

Table 2: Efficacy of *Rhizobium* and Phosphate solubilizing bacteria isolates against stress (High Temperature) tolerance test

S. No.	Name of *Rhizobium* isolates	Colony growth of *Rhizobium* isolates			
		35 °C	40 °C	45 °C	55 °C
1.	Mung-7	++	+	+	-
2.	Mung-8	+++	++	+	+
3.	Mung-9	++	+	+	-
4.	Mung-10	++	+	+	-
5.	Mung-13	++	+	+	-
6.	Mung-15	+	+	+	-
7.	Mung-3512	+++	++	+	+
8.	Mung-3494	+	+	+	-
9.	Mung-577	+	+	+	-
10.	Mung-125	++	+	+	-
Colony growth of PSB isolates					
1.	PSB-5-62	+	+	+	-
2.	PSB-5-68	++	+	+	-
3.	PSB-5-69	+++	+++	++	+
4.	PSB-5-71	+++	+++	++	+
5.	PSB-5-77	++	+	+	-
6.	PSB-5-91	+	+	+	-
7.	PSB-127	+	+	+	-
8.	PSB-129	+	+	+	-

Dry biomass accumulation in shoot

Results of shoot dry biomass accumulation. Significantly highest shoot dry biomass at 40 days after sowing was observed in plants where inoculation was done with the isolates Mung8+ PSB-5-69(2.68 g plant-¹) followed by Mung8+ PSB-5-71 (2.54 g plant-1). In inoculated plants, shoot dry biomass was observed lowest i.e., 1.61 g plant-1 at PSB -5-71whereasit was maximum in the plant inoculated with Mung8+ PSB-5-69(2.68 g plant-1). These results are in agreement with the findings of Jha *et al.* (2012) [4]; Walpola and Yoon (2013) [10], who signified that increase in dry biomass due to inoculation of microbes. (Table no. 3)

Number of pods per plant

The data on the number of pods plant^{-1}at harvest under different treatments has been presented in table 4.10. Single and dual inoculation significantly affected the number of pods plant^{-1}. Number of pod/plant ranged from 11.24 to 16.94. T6 produced significantly higher pods/plant (16.94), which was though higher than other treatments Mung8+ PSB-5-69 (16.94) gave significantly maximum number of pods plant^{-1}followed by Mung8+PSB-5-71 (15.67). This observation was in close agreement with Gupta *et al.* (2006) [8]; Ghanem and Abbas (2009) who reported that number of pods per plant were increased than that of uninoculated control plant due to PSB seed inoculation.

Number of seeds per pod

With regards to native *Rhizobium* and PSB isolates number of seeds pod^{-1} increased significantly over control by the treatment, Mung8+PSB-5-69 (11.12) followed by Mung8+PSB-5-71 (11.07) and Mung3512+ PSB-5-69 (10.40). The lowest was found under control is (6.89). It was observed that *Rhizobium* inoculant in association with PSB led to increase the number of seeds per pod of mungbean.

100 Seed weight (g)

Result obtained on seed weight is presented in Table 2. Significant effect was observed on 100 seed wt. due to the application of *Rhizobium and* PSB inoculation. Significantly the highest 100-grain weight was seen with Mung8+PSB-5-69 (3.72g) followed by Mung8+PSB-5-71 (3.32g), Mung8 (3.31g). Whereas, minimum was seen in control. The minimum value of inoculated 100-grain weight (g) was observed in control is (2.01). (Table no.4).

Stover yield

The stover yield increased from 14.88 (control) to 25.59 qha-1 due to various treatments. The highest stover yield was recorded (25.59qha-1) in T_6 (Mung8+PSB-5-69) at harvest, which was statistically higher than other treatments. The lowest Stover yield per plant was recorded (14.88 qha-1) with control. The effect of dual inoculation on stover yield of mungbean was also significantly influenced at harvest.

This results were similar with the results of Bahadur and Tiwari (2014) [2] who mentioned increase in grain yield with *Rhizobium* and PSB dual inoculations as compared to single inoculation of *Rhizobium* or PSB or un-inoculation. (Table no.5). Significantly highest was recorded due to Mung8+PSB-5-69 (25.59) followed by Mung8+ PSB-5-71 (23.24).

Seed yield

Seed yield was significantly influenced due to the application of *Rhizobium and PSB* inoculants and are presented in Table 3. The grain yield increased significantly from (control) 7.28 to 12.78 qha-1 due to various treatments. The significantly maximum grain yield was recorded due to inoculation with Mung8+ PSB-5-69 (12.78 qha-1) followed by Mung8+PSB-5-71 (11.52 qha-1).

Table 3: Synergistic effect of stress tolerant *Rhizobium* and PSB on biomass accumulation by mung bean at 40 DAS

Treatment	Name of isolates	Dry biomass in shoot (g/plant)	N Content(%)	N uptake (mg/plant)
T_1	Control (Un-inoculated)	0.93	1.72	16.00
T_2	Mung8	1.90	2.45	47.69
T_3	Mung3512	1.84	2.42	44.53
T_4	PSB-5-69	1.75	2.24	39.20
T_5	PSB-5-71	1.61	2.16	34.78
T_6	Mung8+ PSB-5-69	2.68	2.62	70.22
T_7	Mung3512 + PSB-5-69	2.24	2.33	51.03
T_8	Mung8 + PSB-5-71	2.54	2.51	62.23
T_9	Mung3512+ PSB-5-71	2.19	2.30	51.52
	CD 5%	0.126	0.050	4.23

Nitrogen content and uptake by shoot

Nitrogen content and uptake by shoot was significantly influenced by inoculants. Nitrogen content in shoot varied from 1.72 to 2.62% at 40 DAS. The highest N content 2.62% was recorded with Mung8+PSB-5-69 which was significantly higher than other treatments (Table 3). Singh *et al.* (1992) reported that N content in plant tops increase due to inoculation. Effect of *Rhizobium* inoculant alone or in combination with PSB significantly higher N uptake by shoot compared to control.

N uptake per plant was increased significantly from (control) to 70.22, 62.23, 51.52, 51.03, 47.69, 44.69,39.20 and 34.78 mg plant^{-1} due to inoculation with Mung8+PSB-5-69, Mung8+PSB-5-71, Mung3512+ PSB-5-71, Mung3512+PSB-5-69, Mung8, Mung3512, PSB-5-69, and PSB-5-71. It was seen that maximum amount of N uptake was recorded in treatment Mung8+ PSB-5-69 (70.22mg plant^{-1}) and minimum was recorded in PSB -5-71 (34.78mg plant^{-1}) among inoculated treatments while lowest amount was observed in un inoculated control (16.00 mg plant^{-1}). (Table no.3)

Table 4: Synergistic effect of stress tolerant *Rhizobium* and PSB on Yield and Yield attributing characters of mung bean

Treatment	Name of isolates	Pods plant^{-1}	Seeds pod^{-1}	100 Grain weight (g)	Seed Yield (qha^{-1})	Stover Yield (qha^{-1})
T_1	Control (Un-inoculated)	8.99	6.89	2.01	7.28	14.88
T_2	Mung8	14.34	9.28	3.31	11.12	21.84
T_3	Mung3512	13.88	9.08	3.12	10.78	19.46
T_4	PSB-5-69	12.78	9.14	2.98	9.74	17.52
T_5	PSB-5-71	11.24	8.88	2.87	7.98	16. 78
T_6	Mung8+ PSB-5-69	16.94	11.12	3.72	12.78	25.59
T_7	Mung3512 + PSB-5-69	14.64	10.40	3.28	11.14	19.59
T_8	Mung8 + PSB-5-71	15.67	11.07	3.32	11.52	23.24
T_9	Mung3512+ PSB-5-71	14.54	10.39	3.11	10.72	18.41
	CD 5%	0.062	0.133	0.059	0.292	2.42

***Rhizobium* and PSB Population in rhizosphere soil**

Data presented in (Fig.1) revealed that *Rhizobium* population density at harvest increased significantly over control. At harvest stage highest *Rhizobium* population density in rhizosphere soil in mung bean crop performance was found with isolate Mung8+PSB-5-69 and Mung8+ PSB-5-71 were observed (8.68 X 10^{3}per g of soil) and (8.01 X 10^{3}per g of dry soil) respectively.

Data presented in (Fig.1) revealed that PSB population density at harvest increased significantly over control. At harvest stage highest PSB population density in rhizosphere soil in mung bean crop was found with dual inoculation of Mung 8+PSB-5-69 followed by Mung8+ PSB-5-71 which were observed as (8.92 X 10^{3}per g of soil) and (8.76 X 10^{3}per g of soil) respectively.

Results of present investigation are in confirmation with the findings of Saxena (2010) [4]; Rudresh *et al.* (2005); Walpola and Yoon (2013) [10] who reported that remarkable increase in them icrobialpopulation was observed in *Rhizobium*-inoculated rhizospheresoil when compared with uninoculated soil.

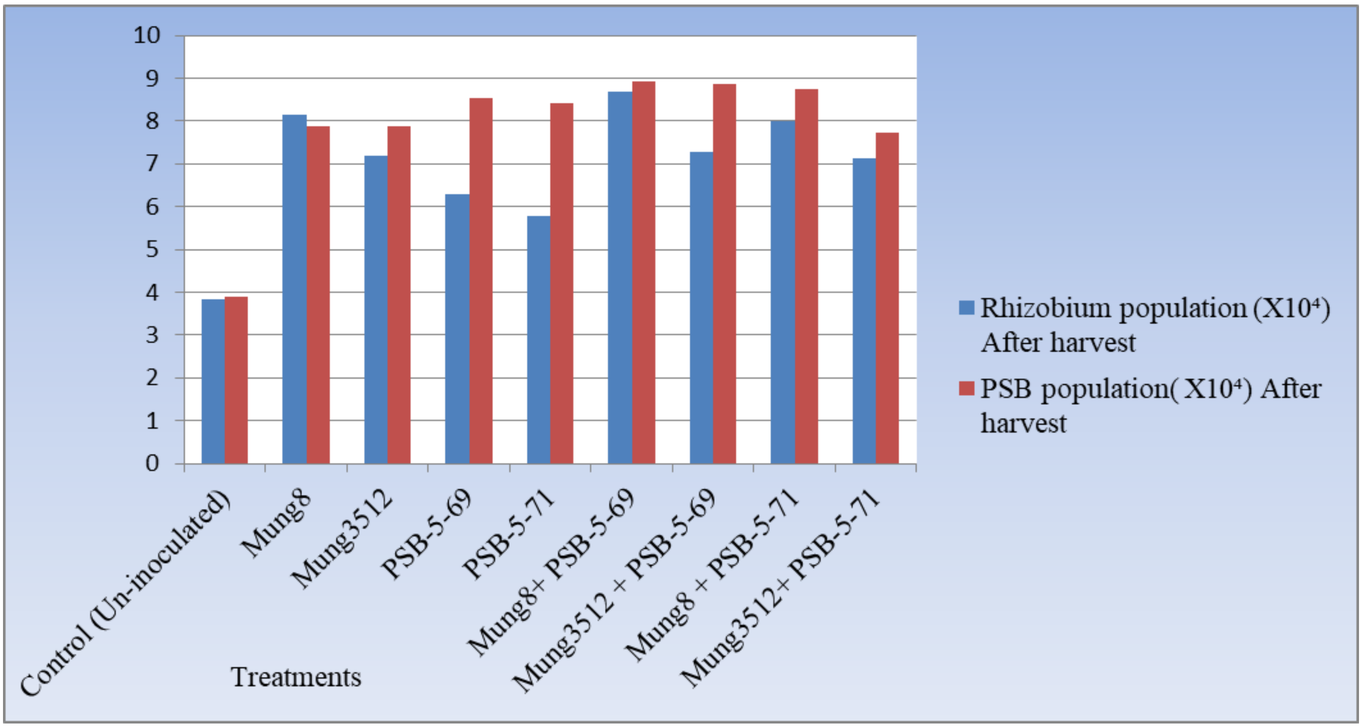

Fig 1: Inoculation effect on population dynamics of *Rhizobium*and PSB in rhizospher soils of mung bean at harvest.

Conclusion

Rhizobium isolate Mung 8 and PSB isolate PSB-5-69 were promising ones. Combined inoculation of N fixing microbes and PSB showed better improvement in yield of mung than either group of organisms alone due to synergistic effect, which was clearly observed in the present investigation. From experimental results it was found that dual inoculation effect with Mung 8+PSB-5-69 was superior followed by Mung8+ PSB-5-71 showing significant increase in yield over control, in addition improved growth parameters of mung as well as enhance the biological properties of soil.

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