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Impact of Bacterial Dual Inoculation (PSB and *Rhizobium*) on Nodulation Behavior and Biomass of Sand Culture Grown Kulthi (*Dolichos biflorus*)

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A B S T R A C T

The present investigation was carried out in green house of Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh (India) during the year 2015-16. The co-inoculation of Rhizobium + PSB at 45 DAS significantly increased no. of root nodules per plant, biomass of nodules, shoot biomass accumulation and N accumulated by shoot over control plants. Plants were allowed to grow up to 45 days under sand culture, data of plant biomass revealed that the highest dry shoot weight was (0.156 gm/plant) associated with combination of Rhz-10+PSB-142 followed by isolate Rhz-10+PSB-184 (0.137 gm/plant). Similarly, highest dry wt. of nodules was observed 1.70 mg/plant due to combinations of Rhz-10+PSB-142 followed by isolate Rhz-10+PSB-118 (1.52 mg/plant), while the highest number of nodules (6.33 per plant) associated with combination of Rhz-10+PSB-142 followed by Rhz- 10+PSB-118 (5.66 per plant). The shoot N accumulation study of the same sand culture experiment indicated that highest shoot N-uptake associated with combination of Rhz- 10+PSB-142 (4.57 mg/plant) followed by Rhz-10+PSB-184 (3.94 mg/ plant). Hence, is concluded that combination of Rhz-10+PSB-142 is the most potent N2 fixer for kulthi cultivation in acidic soils of Chhattisgarh.

Introduction

Out of 135.00 lakh ha (1.35 lakh sq km) area of Chhattisgarh, about 46.02 lakh hectares is under *kharif* mostly with rice crop while about 12.45 lakh ha only under *rabi* crops. About 30.00 lakh ha area remains fallow during *rabi*. Horse gram (locally known as kulthi in Chhattisgarh) is one of the unexploited legumes of the tropics and subtropics grown mostly under dry-land agriculture. It is important pulse crop taken in tribal belt of the Chhattisgarh. In Chhattisgarh total cultivated area of this crop is about 75 thousand hectares, out of this, nearly 37 thousand hectares are situated only in Bastar Divison representing an important kulthi belt in the state. This crop is very popular among the marginal and poor farmers which includes mostly tribal population of Chhattisgarh. The important kulthi growing districts of Chhattisgarh are Jagdalpur, Kanker and Surguja. Total area, production and productivity of kulthi in Chhattisgarh is 17.70 thousand ha, 5.75 thousand ton and 3.25 quintal per ha respectively (Anonymous, 2009-10). The productivity per unit area of this crop is very low mainly because of sub optimal nutrition including low or ineffective native population of N fixing and P solubilizing microbes (Gupta *et al.*,; 2000a).

The poor productivity of kulthi in this region is mainly due to imbalance application of nutrients and use of traditional varieties. Under such situations, use of Rhizobium and phosphate solubilizing bacteria (PSB) had shown advantage in enhancing kulthi productivity. Microbial inoculants are cost effective, ecofriendly, and renewable sources of plant nutrients (Khan, M.S. *et al.*,, 2007). Rhizobium and PSB assume a great importance on account of their vital role in N2-fixation and P-solubilisation. The introduction of efficient strains of P-solubilizing species of Bacillus megaterium biovar phosphaticum, Bacillus polymyxa, Pseudomonas striata, Aspergillus awamori, and Penicillium digitatum in the rhizosphere of crops and soils has been reported to help in increasing phosphorus availability in the soil (Gaur, A.C. *et al.*,, 1990). Since the information on response of nodulation behavior of kulthi to inoculation with Rhizobium and phosphate solubilizing bacterial inoculants is meager under such situation, therefore, an experiment was designed to assess the productivity of kulthi.

Materials and Methods

Treatments Details and Crop Culture

The experiment was conducted during the kharif season, 2015-16. The experiment was laid out in CRD design with three replications. The experiment was conducted with nine treatments including one control plant which are thus (*Rhizobium*-2, *Rhizobium*-10, *Rhizobium*-2+ PSB-118, *Rhizobium*-2+ PSB-142, *Rhizobium*-2+ PSB-184, *Rhizobium*-10+ PSB-118, *Rhizobium*-10+ PSB-142 and *Rhizobium*-10+ PSB-184). For the purpose of nodulation study, seeds were sown in fine graded sterilized river sand with disposable cups. Timely and uniform irrigation were provided to all the cups by N free Mcknight seedling nutrient solution as required.

Nodulation, Shoot biomass and N accumulation

After 45 DAS the sand culture grown plants were uprooted, then number of nodules per plant and other observations were recorded. The nitrogen accumulated in the plant samples was estimated by Micro-Kjeldahl method as described by Jackson (1973) using auto digestion and distillation system.

Statistical Analysis

Statistical analysis of data was carried out using online statistical analysis pack- age (OPSTAT, Computer section, CCS HAU Hisar, Haryana) for calculation of ANOVA.

Results and Discussion

Nodulation Study

The data presented in (Table-1) indicated that at 45 DAS, the highest no. of nodules per plant, fresh weight of nodules (mg/plant) and dry weight of nodules (mg/plant) increased significantly due to all treatments over control plant.

No. of nodules

The number of nodules per plant increased significantly from 0 to 6.33, 5.66, 4.66, 4.33, 3.66, 3.66, 3.66, 3.33 and 3.33 per plant due to inoculation of seeds with the isolates Rhz-10+PSB-142, Rhz-10+PSB-118, Rhz-10+PSB-184, Rhz- 10, Rhz-2+PSB-118, Rhz-2+PSB-184, Rhz-2+PSB-142 and Rhz-2, respectively. This study revealed that highest value of nodules per plant was 6.33 associated with Rhz-10+PSB-142 and No nodule was observed in uninoculated control plant.

Prasad and Ram (1986), Alagawadi *et al.*, (1993) and Devi and Gupta (1996). clearly mentioned that number of nodules can be increased by inoculation with effective rhizobial strains.Similar finding was also reported by Bhattacharyya P.K. (2012), he observed that effect of inoculation with Rhizobium spp. and phosphate solubilizing bacteria (PSB) on mungbean with regards to nodulation.

Fresh weight of nodules

Fresh fresh weight of nodule increased significantly over control from 0 to 14.18, 13.83, 10.44, 8.95, 8.94, 8.20, 7.45 and 5.96 mg per plant due to plants raised from inoculation treatments Rhz-10+PSB-142, Rhz-10+PSB-118, Rhz- 10+PSB-184, Rhz-10, Rhz-2+PSB-184, Rhz-2+PSB-118, Rhz-2+PSB-142 and Rhz-2, respectively. This study revealed that highest value of nodules fresh wt. was 14.18 mg followed by 13.83 mg per plant associated with isolates Rhz-10+PSB-142 and Rhz-10+PSB-118, respectively amongst all the nodulated plants, under the study.

Dry weight of nodules

Dry weight of nodule was increased significantly over control from 0 to 1.70, 1.52, 1.25, 1.16, 0.99, 0.98, 0.89 and 0.88 mg per plant due seeds inoculated with treatments Rhz-10+PSB-142, Rhz-10+PSB-118, Rhz-10+PSB-184, Rhz-10, Rhz-2+PSB-184, Rhz-2+PSB-118, Rhz-2 and Rhz-2+PSB-142, respectively. The highest value of nodules dry wt. was 1.70 mg followed by 1.50 mg per plant associated with isolates Rhz-10+PSB-142 and Rhz-10+PSB-118 respectively, amongst all the inoculated plants. This observation was in close agreement with Wankhade *et al.*, (1992) who reported that number of root nodules and their weight per plant were significantly increased due to rhizobial seed inoculation.

Similar observations were also reported by Gupta *et al.*, (2000) and Gupta *et al.*, (2005), Kalita *et al.*, (2006); Garg (2010) and Sharma *et al.*, (2012).They mentioned that the *Rhizobium* isolate was most effective in respect to increase number of nodules, nodules fresh weight and nodules dry weight in legumes. Saleh *et al.*, (2013) clearly mentioned that *Rhizobium* inoculation improved nodulation in the varieties of urad bean than that of uninoculated control plant.

Shoot biomass accumulation study

At 45 DAS fresh weight of shoot (Table-2) increased significantly from 0.659 to 0.992, 0.984, 0.943, 0.877, 0.857 and 0.806 gm per plant due to plants raised from inoculation treatments Rhz-10+PSB-142, Rhz- 10+PSB-184, Rhz-2+PSB-118, Rhz-10+PSB-118, Rhz-2+PSB-142 and Rhz- 2+PSB-184, respectively.

The highest value of fresh shoot weight was 0.992 followed by 0.984 gm per plant amongst all inoculated plants. Dry shoot weight (Table 4.2 Fig. 4.2) increased significantly over control from 0.080 to 0.156, 0.137, 0.132, 0.131, 0.128, 0.125, 0.118 and 0.107 gm per plant due to seeds inoculated with treatments Rhz-10+PSB-142, Rhz-10+PSB-184, Rhz-10+PSB-118, Rhz-2+PSB-142, Rhz-2+PSB-118, Rhz-2+PSB-184,Rhz-10, and Rhz-2, respectively.

Table.1 Influence of different combinations of native *Rhizobium* and PSB isolates on nodulation behavior of sand culture grown kulthi at 45 DAS

Treatment no.	Treatments	No. of nodules per plant	Fresh nodules wt. (mg/plant)	Dry nodules wt. (mg/plant)
		45 DAS	**45 DAS**	**45 DAS**
T1	Control	0	0	0
T2	Rhz-2	3.33	5.96	0.89
T3	Rhz-10	4.33	8.95	1.16
T4	Rhz-2+PSB-118	3.66	8.20	0.98
T5	Rhz-2+PSB-142	3.33	7.45	0.88
T6	Rhz-2+PSB-184	3.66	8.94	0.99
T7	Rhz-10+PSB-118	5.66	13.83	1.52
T8	Rhz-10+PSB-142	6.33	14.18	1.70
T9	Rhz-10+PSB-184	4.66	10.44	1.25
CD (5%)		0.048	0.812	0.012

Table.2 Influence of different combinations of native *Rhizobium* and PSB isolates on shoot biomass and N accumulation by sand culture grown kulthi at 45 DAS

Treatment no.	Treatment	Fresh shoot wt. (gm/plant)	Dry shoot wt.	N- accumulation in shoot (gm/plant)	
				% N-content	**N-uptake (mg/plant)**
T1	Control	0.659	0.080	1.96	1.56
T2	Rhz-2	0.701	0.107	2.71	2.90
T3	Rhz-10	0.707	0.118	2.80	3.30
T4	Rhz-2+PSB-118	0.943	0.128	2.66	3.61
T5	Rhz-2+PSB-142	0.857	0.131	2.37	3.11
T6	Rhz-2+PSB-184	0.806	0.125	2.16	2.71
T7	Rhz-10+PSB-118	0.877	0.132	2.93	3.88
T8	Rhz-10+PSB-142	0.992	0.156	3.02	4.57
T9	Rhz-10+PSB-184	0.984	0.137	2.89	3.94
CD (5%)		0.074	0.015	0.248	0.365

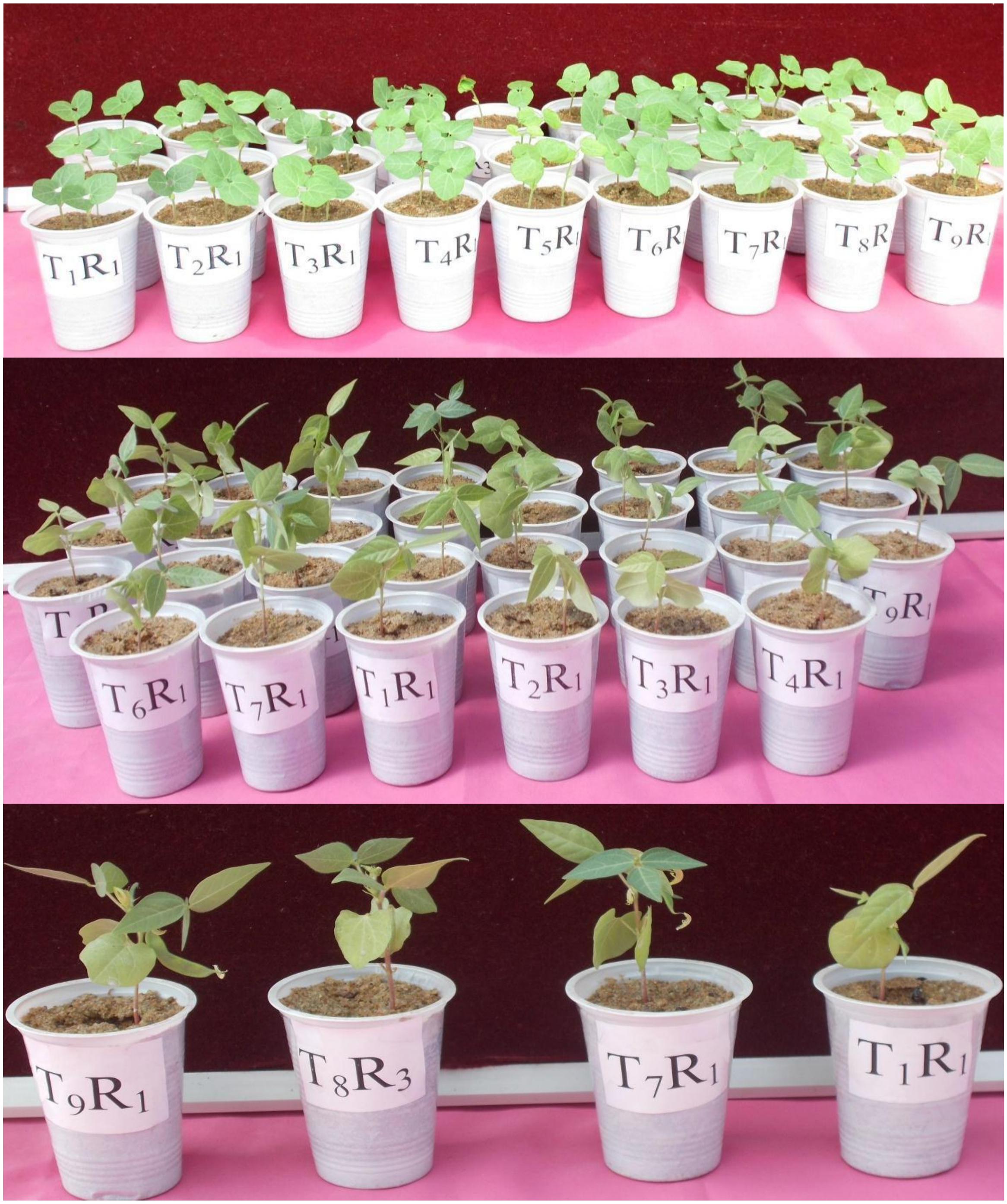

Plate.1 Performance of sand culture grown kulthi at different stages of plant growth

This observation was also supported by Egamberdiyeva *et al.*, 2004; Gupta *et al.*, 2005; Singh and Kumar 2007; Katiyar *et al.*, 2009, they recorded significantly higher plant dry weight due to inoculation of effective soybean *Rhizobium* isolates.

N-accumulation study

Data presented in (Table-2) clearly indicate that % N- content of shoot at 45 DAS increased significantly from 1.96 to 3.02, 2.93, 2.89, 2.80, 2.71, 2.66 and 2.37 due to seeds inoculated with treatments Rhz-10+PSB- 142, Rhz-10+PSB-118, 10+PSB-184, Rhz-10, Rhz-2, Rhz-2+PSB-118 and Rhz-2+PSB-142, respectively. In case of % N content of shoot was recorded 3.02 as highest followed by 2.93 associated with isolates Rhz-10+PSB-142 and Rhz- 10+PSB-118, respectively. The lowest % N content was 1.96, plant raised from uninoculation treatment. Value of N-uptake (Table 4.2, Fig. 4.2, Plate 4.4) by shoot at 45 DAS increased significantly from 1.56 to 4.57, 3.94, 3.88, 3.61, 3.30, 3.11, 2.90 and 2.71 mg/plant, associated with isolates 10+PSB-142, Rhz-10+PSB-184, Rhz- 10+PSB-118, Rhz-2+PSB-118, Rhz-10, Rhz-2+PSB-142, Rhz-2 and Rhz-2+PSB- 184, respectively. Lowest value of N-uptake was 1.56 and highest was 4.57 mg per plant amongst all plants. This findings are supported by Miyan *et al.*, 1989. They clearly mentioned that dry matter yield and N-uptake by rajma legume can be increased by use of specific *Rhizobium* strains.

Based on above results, it can be concluded that combination of Rhz-10+PSB-142 (T_9) is superior over the remaining treatments with respect to nodulation, shoot biomass and accumulated N by shoot. However use of Rhizobium and PSB inoculation had also shown advantage over no inoculation. Thus, co-inoculation of Rhizobium and PSB may be recommended to realize higher nodulation of kulthi.

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