PLANT GROWTH AND NODULATION OF MUCUNA (*MUCUNA PRURIENS*) IN RESPONSE TO *RHIZOBIUM* INOCULATION

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Abstract: A total 20 *Rhizobium* strains were isolated from nodules of *Pisum sativum*. Isolated strains were characterized on the basis of cultural staining and biochemical tests by standard methods. Further, Plant growth activities of characterized twenty *Rhizobium* strains were analysed. Only nine *Rhizobium* i.e. *Rhizobium* PMR-2, *Rhizobium* PMR-3, *Rhizobium* PMR-7, *Rhizobium* PMR-9, *Rhizobium* PMR-12, *Rhizobium* PMR-13, *Rhizobium* PMR-15, *Rhizobium* PMR-17, *Rhizobium* PMR-19 produced siderophore, HCN, IAA and solubilized phosphorous. *Mucuna pruriens* has some medicinal value as well as food –feed crop and selected for present study. Pot experiment had done to analyzed PGPR activity of *Rhizobium* strains. *Mucuna* seeds were surface-sterilized and bacterized with *Rhizobium* strain of density of 10^8 cfu ml^{-1}. Sterile earthen pots (24 cm × 12 cm × 12 cm) were filled with sterilized sandy loam soil. Total 10 treatment were prepared and these are *Rhizobium* PMR-2 + Seed; *Rhizobium* PMR-3 + Seed; *Rhizobium* PMR-7 + Seed; *Rhizobium* PMR-9 + Seed; *Rhizobium* PMR-12 + Seed; *Rhizobium* PMR-13 + Seed; *Rhizobium* PMR-15+ Seed; *Rhizobium* PMR-17 + Seed; *Rhizobium* PMR-19 + Seed and uninoculated seed (control). All bacterized *Rhizobium* strains produced more dry weight and plant height as compared to uninoculated seed (control). *Rhizobium* PMR-13 and PMR-19 increased plant dry weight by 181.7 and 181.9% respectively as compared to control. Maximum height has been observed in *Rhizobium* PMR-19 bacterized seed treatment and it was 122% as compared to control. *Rhizobium* PMR-13 bacterized seeds showed 52 nodules per plant. We concluded that use of rhizobia inoculant enhanced plant growth in Mucuna plant.

Keywords: *Rhizobium*, Siderophore, HCN, IAA, P-solubilization

INTRODUCTION

When natural fossil fuels finished up, ultimately the present practices of industrial production of N-fertilizer will suffer. As the productivity declines food and feeding will be affected. To stop such a disaster, Plant growth promoting rhizobacteria may properly be modulated to meet the demand of crop productivity on sustainable basis.

Plant growth promoting rhizobacteria represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of growth of their host (Deshwal *et al*., 2003, 2006). And few strains from genera such as *Pseudomonas*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* are well known PGPR (Rodriguez and Fraga, 1999; Misko and Germida, 2002).

Now a days rhizobial inoculant have some other quality with addition to nitrogen fixing capacity with enhanced nodulation, such as production of plant growth promoting hormones like Indole acetic acid (IAA), secretion of siderophores and solubilization of phosphates etc (Bashan and Holguin, 1997; Deshwal *et al*., 2003). Kumar and Chandra (2008) observed that *Rhizobium leguminosarum* bv. *viceae* (LB-4) significantly increased the nodule number at different intervals, nodule dry weight in Lentil plant. Recently, Deshwal and Vig (2010) mentioned that co-inoculation of *Pseudomonas* and *Rhizobium* enhanced plant growth in peanut (*Arachis hypogaea* L.). Such information showed the plant growth activity and symbiotic properties of rhizobia.

Mucuna pruriens belongs to the family Fabaceae. It has some medicinal value and it is also food –feed crop. The roots are bitter, sweet thermogenic emollient, stimulant, purgative, aphrodisiac and diuretic. The leaves are aphrodisiac. The seeds are astringent, laxative, anthelmentic, alexipharmic and tonic (Taylor, 2005). A clinical study confirmed the efficacy of the seeds of *Mucuna pruriens* in the management of Parkinson's disease by virtue of their L-DOPA content (Manyam *et al*., 1995). *M. pruriens* has been shown to increase male sex hormones - testosterone levels (Amin *et al*., 1996).

Eilitfa and Carsky (2003) have outlined efforts at increasing the potential of mucuna as a food and feed crop. This crop is known to accumulate large biomass (Carsky *et al*., 2001) and fixes large amount of nitrogen (Sanginga *et al*., 1996). So the objective of this study was the evaluate effect of *Rhizobium* on plant dry weight, plant height, number of nodule and nodule fresh weight per *Mucuna pruriens* plant.

Materials and Methods:

1. Isolation and Characterization of *Rhizobium* strains: Root-nodulating strains of *Rhizobium* were isolated from nodules of *Pisum sativum* by standard microbiological techniques (Deshwal *et al*., 2003). Rhizobia were maintained on yeast extract mannitol agar (YEMA) at 4°C. Strains were characterized according to *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994).

2. Evaluation of plant growth promoting activity of *Rhizobium* :

(a) Siderophore production: Siderophore production by *Rhizobium* strains was tested by using chrome-azurol S (CAS) assay medium. *Rhizobium* strains were spread over YEMA and incubated at 28°C for 24 h. Thereafter, a thin layer of CAS reagent in 0.7% agar was spread over the colonies of *Rhizobium* and the plates were re-incubated as earlier. Formation of yellow-orange halo around the colonies indicates siderophore production.

(b) HCN production: Exponentially grown bacterial cultures were streaked on YEMA plates supplemented with 4.4 g per liter glycine with simultaneous supplementation of a filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 in the upper lid of petri dishes. The plates were sealed with parafilm. Control plates did not receive inoculum. Plates were incubated at 28 ± 1°C for 2-3days. Observed change in colour from yellow to light brown, moderate (brown) or strong (reddish-brown) indicated HCN production.

(c) IAA production: For the indole production test, tryptone broth is prepared and transferred into test tubes. After sterilization, these test tubes were then inoculated with the culture and one tube was kept uninoculated as control. These inoculated tubes incubated at 28^0C for 24h. After 24h of incubation, added 1ml of kovac's reagent to each tube including control. Shaked the tubes gently after intervals for 10-15 mins. Allowed these tubes to standing position. Development of cherry red colour in the top layer of the tube indicated a positive result.

(d) P-solubilization: The plates containing Pikovskya's agar were spot inoculated by rhizobial strains and incubated at 28 ± 1°C for 3-5 days. Formation of clear zone around the colonies indicated phosphate solubilization.

3. Pot experiment:

(a) Seed bacterization: *Mucuna* seeds were surface-sterilized with 0.5% NaOCl solution for 1–2 min, rinsed in sterilized distilled water and dried under a sterile air stream. Cells of *Rhizobium* strains were grown under continuous shaking condition (150 rpm) on YEM broth at 28 ± 1°C for 24h. Each culture was separately centrifuged at 7000 rpm for 15 min at 4°C. The culture supernatant was discarded and the pellets were washed with sterile distilled water (SDW) and resuspended in SDW to obtain a population density of 10^8 cfu ml^{-1}. The cell suspension was mixed with 1% carboxymethylcellulose (CMC) solution. The slurry was coated separately on the surface of *Mucuna* seeds and allowed to air-dry overnight in aseptic condition. Care was taken to avoid clumping of seeds. The seeds coated with 1% CMC slurry without bacterial strains served as control.

(b) Pot size and sterilization of soil: Sterile earthen pots (24 cm × 12 cm × 12 cm) were filled with sterilized sandy loam soil (0.24% total organic matter, 0.096% total organic C, 37% water-holding capacity, pH 6.3).

(c) Treatments: Total 10 treatment were prepared and these are *Rhizobium* PMR-2 + Seed; *Rhizobium* PMR-3 + Seed; *Rhizobium* PMR-7 + Seed; *Rhizobium* PMR-9 + Seed; *Rhizobium* PMR-12 + Seed; *Rhizobium* PMR-13 + Seed; *Rhizobium* PMR-15+ Seed; *Rhizobium* PMR-17 + Seed; *Rhizobium* PMR-19 + Seed and uninoculated seed worked as control. Four seeds per pot were sown in each treatment. After 15 days, thinning was done to raise only single healthy plant in each pot. The plants were irrigated with sterilized water whenever required. Plant data such as plant dry weight, plant height, number of nodule and nodule fresh weight per plant were recoded after 45 days of sowing.

RESULTS AND DISCUSSION

Total 20 *Rhizobium* were isolated and characterized on the basis of gram staining and biochemicals tests. Similar observation has been mentioned in Bergey's Manual of Determinative Bacteriology by Holt *et al*., (1994). Further, these strains were screened on the basis of siderophore production, HCN production, Indole Acetic Acid (IAA) production and Phosphorous solubilisation. Only nine *Rhizobium* i.e. *Rhizobium* PMR-2, *Rhizobium* PMR-3, *Rhizobium* PMR-7, *Rhizobium* PMR-9, *Rhizobium* PMR-12, *Rhizobium* PMR-13, *Rhizobium* PMR-15, *Rhizobium* PMR-17, *Rhizobium* PMR-19, produced siderophore, HCN, IAA and solubilized phosphorous (Table 1). Similarly, Deshwal *et al*. (2003) reported that slow growing rhizobia produced siderophore, HCN, IAA and solubilized phosphorous and concluded that such rhizobia increased plant growth. PGPR strains enhance plant growth by direct promotion of plant growth has been assigned to gibberellins, IAA and biosynthesis (Upadhyay and Srivastava, 2010). Such reports supported our result that plant growth promoting rhizobacteria produced siderophore, HCN, IAA and P-solubilization.

Table 1. Production of siderophore HCN, IAA and phosphate solubilization by *Rhizobium* strains.

Rhizobium	Siderophore	HCN	IAA	P-solubilization
Rhizobium PMR-1	+	-	-	+
Rhizobium PMR-2	+	+	+	+
Rhizobium PMR-3	+	+	+	+
Rhizobium PMR-4	-	-	+	+
Rhizobium PMR-5	-	-	+	-
Rhizobium PMR-6	-	+	-	-
Rhizobium PMR-7	+	+	+	+
Rhizobium PMR-8	+	-	+	-
Rhizobium PMR-9	+	+	+	+
Rhizobium PMR-10	-	-	-	+
Rhizobium PMR-11	-	-	+	+
Rhizobium PMR-12	+	+	+	+
Rhizobium PMR-13	+	+	+	+
Rhizobium PMR-14	-	-	+	-
Rhizobium PMR-15	+	+	+	+
Rhizobium PMR-16	+	-	+	-
Rhizobium PMR-17	+	+	+	+
Rhizobium PMR-18	-	-	+	+
Rhizobium PMR-19	+	+	+	+
Rhizobium PMR-20	-	-	+	-

Table 2. Effect of bacterization with *Rhizobium* strains on plant dry weight, plant height, nodules per plant and fresh nodule weight of *Mucuna pruriens*.

Treatment number	Treatment	Plant		Nodule	
		Dry weight (gm)	Height (cm)	Number per plant	Fresh weight per plant
Treatment 1	*Rhizobium* PMR-2 + Seed	2.874	337	27	0.954
Treatment 2	*Rhizobium* PMR-3 + Seed	4.592	394	48	1.92
Treatment 3	*Rhizobium* PMR-7 + Seed	4.171	368	32	1.27

Treatment 4	*Rhizobium* PMR-9 + Seed	4.342	372	36	1.49
Treatment 5	*Rhizobium* PMR-12 + Seed	4.380	378	37	1.48
Treatment 6	*Rhizobium* PMR-13 + Seed	4.611	397	52	2.04
Treatment 7	*Rhizobium* PMR-15+ Seed	4.380	378	44	1.73
Treatment 8	*Rhizobium* PMR-17 + Seed	4.374	377	47	1.87
Treatment 9	*Rhizobium* PMR-19 + Seed	4.616	398	51	2.03
Treatment 10	Uninoculated Seed	2.537	326	-	-

In the present study *Rhizobium* PMR-13 and PMR-19 increased plant dry weight by 181.7 and 181.9% respectively as compared to control. Seed bacterization improved plant dry weight, plant height, nodules number and fresh nodule weight per mucuna plant. Maximum height has been observed in *Rhizobium* PMR-19 bacterized seed treatment and it was 122% as compared to control. *Rhizobium* PMR-13 bacterized seeds showed 52 nodules per plant (Table 2). Results indicated that plant was having high nodules per plant then plant significantly increased high plant dry weight as well as plant height. Similarly, Mai *et al*., (2010) reported that PGPR inoculation significantly increased the root properties (length, volume, mass) and shoot growth, the plant height (42-50%), leaf area (128-134%). Deshwal and Vig (2010) reported that PGPR strains increased plant growth in peanut. Such reports supported our results about beneficial effect of PGPR. Recently 2010, Anandaraj and Leema Rose delepierre, stated that Biofertilizer is natural product carrying living microorganisms derived from the root or cultivated soil so they donot have any ill effect on soil health and environment. Such information clearly indicated that PGPR are better due to non-toxic to environment and also improves the plant growth as well as productivity.

We know that *Mucuna pruriens* is nitrogen fixer and is having great medicinal value and our results indicated that *Rhizobium* strain produced siderophore, HCN, IAA and P-solubilization and bacterized seed increased the plant dry weight and plant height. Further, results showed that nodules are index of plant growth. So we concluded that use of rhizobia inoculant enhanced plant growth in mucuna plant.

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