INFLUENCE OF *PSEUDOMONAS* VP-2 ON GROWTH OF SOYBEAN CROP

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Abstract: *Pseudomonas* VP-2 showed highest shoot, root dry weight, number of nodules per plant and nodules dry weight by 186.36, 283.33, 201.33 and 225% respectively as compared to control. All *Pseudomonas* strains showed improved shoot dry weight, root weight ranges between 147 to 186% and 194.66 to 201% respectively as compared to control. Although control plant also produced nodules but *Pseudomonas* bacterized seeds improved nodulation by 188 to 201% as compared to control. Similarly, nodules dry weight also got enhanced by 212.5 to 225% as compared to control. All the results suggested that *Pseudomonas* improves the plant growth and productivity in Soybean crop.

Keywords: *Pseudomonas*, PGPR, Soybean

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978) to describe soil bacteria that colonize the roots of plants following inoculation onto seed and they enhance plant growth. These biofertilizer is alternative source of chemical fertilizer (Deshwal et al., 2011a). Biofertilizer can promote plant growth and productivity. They have internationally been accepted as an alternative source of chemical fertilizer. These rhizobacteria effectively colonize plant root and increases plant growth by production of various plant growth hormones, P-solubilizing activity, N_2- fixation and biological control activity (Deshwal et al., 2003). 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).

Pseudomonas sp. is ubiquitous bacteria in agricultural soils and and has many traits that make them well suited as PGPR. The most effective strains of *Pseudomonas* have been *Fluorescent Pseudomonas* spp. Considerable research is underway globally to exploit the potential of one group of bacteria that belong to *Fluorescent pseudomonas* (Flops). FLPs help in the maintenance of soil health and are metabolically and functionally most diverse (Lata et al., 2002; Lugtenberg and Dekkers 1999). *Pseudomonas* sp has been reviewed for the biofertilizer, phytostimulator and phytopathogen biocontrol activities. Direct plant growth activities of *Pseudomonas* sp include the production of Indole Acetic Acid (IAA) (Vasanthakumar and McManus, 2004) and siderophore (Dey *et al*., 2004), phosphate solubilization (Wu *et al*., 2005), ACC deaminase production, root elongation, degradation of toxic compound (Bano and Musarrat, 2003), as biological control agent for phytopathogens (Dey *et al*., 2004) and residual effect of *Pseudomonas* has been observed in Rice crop (Deshwal et al., 2006). In present study, *Pseudomonas* strains were selected due to their plant growth activity and also evaluated there PGPR activity.

MATERIAL AND METHOD

Isolation of *Pseudomonas* strains: *Pseudomonas* strains were isolated from soil of cultivated field. 1g of rhizosphere soil dissolved in 9ml sterilized distilled water in test tube and mixed well. Sample was diluted up to $1/10^5$ dilution. 0.5ml sample of each tube was spread on separate King's B Agar plates. Plates were incubated at 28^oC for 24h. Fluorescent colonies on agar medium were purified.

Characterization of *Pseudomonas* strains: Twenty *Pseudomonas* strains were characterized on the basis of gram staining and bio-chemicals tests. These tests were done according to Bergey's manual of Determinative Bacteriology (Holt et al., 1994).

Screening of Plant growth promoting activity of *Pseudomonas* strains: These strains were screened on the basis of plant growth promoting activity such as IAA, HCN, siderophore production and P-solubilization.

(i) Indole production test: Tryptone broth was 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 and allowed tubes in standing position. Development of cherry red colour in the top layer of the tube indicated a positive result.

(ii) HCN production: *Pseudomonas* strains were streaked on TSM medium plates supplemented with 4.4g per litre glycine with simultaneously supplemented filter paper soaked in a 0.5% picric acid in 1% Na_2CO_3 in the upper lid of Petri plate. The plates were sealed with paraffin and control plates did not receive any *Pseudomonas* inoculum. Plates were incubated at 28 ± 1^0C for 1-2days. Change in colour of the filter paper from yellow to brown,

moderate brown to strong reddish brown indicated HCN production.

(iii) Siderophore production: Siderophore production was tested by using chrome-azurol S (CAS) assay medium. *Pseudomonas* strains were spread over tryptic soya agar medium and incubated at 28±1°C for 24 h. Thereafter, a thin layer of CAS reagent in 0.7% agar was spread over the colonies of *Pseudomonas* and plates were re-incubated at 28±1°C for 24-48h. Observation formation of yellow-orange halo around the colony shows siderophore production.

(iv) P- solubilization test: *Pseudomonas* strains were transferred on Pikovaskya's Agar medium and inoculated at 28±1°C for 3-5days. Observed clear zone around the colony showed P- solubilization.

Pot experiment

(a) Seed bacterization: Soybean 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 *Pseudomonas* strains were grown under continuous shaking condition (120 rpm) on King'B broth respectively, 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 soybean seeds and allowed to air-dry overnight in aseptic condition. The seeds coated with 1% CMC slurry without bacterial strains served as control.

(b) Pot size and soil: Sterile earthen pots (24 cm × 12 cm × 12 cm) were filled with unsterilized sandy loam soil (0.25% total organic matter, 0.096% total organic C, 38% water-holding capacity, pH 6.5).

(c) Treatments: Total 09 treatments were prepared and these are treatment I: *Pseudomonas* VP-2, treatment II: *Pseudomonas* VP-5, treatment III: *Pseudomonas* VP-7, treatment IV: *Pseudomonas* VP-11, Treatment V: Pseudomonas VP-15, Treatment VI: *Pseudomonas* VP-16, Treatment VII: *Pseudomonas* VP-19, Treatment VIII: *Pseudomonas* VP-20 and Treatment IX: control (non-bacterized seeds). 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 shoot dry weight; plant root dry weight, number of nodule per plant and nodule dry weight per plant were recorded after 60 days of sowing.

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

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

Table 2. Effect of *Pseudomonas* on plant growth of Soybean plant after 60DAS.

S. No	Treatment	Shoot dry weight (g/plant)*	Root dry weight (g/plant)**	Number of nodules / plant*	Nodules dry weight (g/plant)**
1	*Pseudomonas* VP-2	8.2	3.4	75.5	0.54

2	*Pseudomonas* VP-5	6.5	2.6	71.5	0.50
3	*Pseudomonas* VP-7	7.9	3.0	74.0	0.53
4	*Pseudomonas* VP-11	6.1	2.4	73.0	0.52
5	*Pseudomonas* VP-15	7.9	3.0	74.0	0.53
6	*Pseudomonas* VP-16	6.5	2.6	70.5	0.51
7	*Pseudomonas* VP-19	8.0	3.2	74.5	0.53
8	*Pseudomonas* VP-20	6.1	2.3	70.5	0.52
9	Control	4.4	1.2	37.5	0.24

Values are mean of four replicates. Ns- non-significant at 0.05 level of ANOVA *- significant at 0.05 level of ANOVA, **- significant at 0.01 levels of ANOVA.

RESULT AND DISCUSSION

Twenty *Pseudomonas* strains were characterized on the basis of different bio-chemicals tests. Our results were compared with Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Further, these strains were screened on the basis of plant growth activity such as HCN, Siderophore, IAA and P-solubilization.

Change in colour of filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 from yellow to brown, which showed that strains were HCN positive. *Pseudomonas* VP-1, VP-2, VP-3, VP-5, VP-6, VP-7, VP-10, VP-11, VP-12, VP-13, VP-14, VP-15, VP-16, VP-19 and VP-20 were HCN positive. In Siderophore positive strains showed yellow orange halo around the colony when grown on chrome-azurol S (CAS) assay medium. Such observation had been seen in *Pseudomonas* VP- 1, VP-2, VP-4, VP-5, VP-6, VP-7, VP-9, VP-10, VP-11, VP-13, VP-14, VP-15, VP-16, VP-17, VP-19 and VP-20. All *Pseudomonas* strain except *Pseudomonas* VP-1 and VP-10 produced Indole Acetic Acid (IAA) which is plant growth hormones. IAA reacts with ortho-phosphoric acid and produced pink colour. Clear zone around the colony of *Pseudomonas* strains on Pikovskya's agar medium showed P-solubilization. All *Pseudomonas* strains except *Pseudomonas* VP-3, VP-6, VP-13 were solubilised Phosphorous (Table 1). *Pseudomonas* VP-2, VP-5, VP-7, VP-11, VP-15, VP-16, VP-19, VP-20 improved shoot dry weight by 186.36, 147.72, 179.54, 138.63, 179.54, 147.72, 181.81, 138.63 % respectively as compared to control. *Pseudomonas* VP-2, VP-5, VP-7, VP-11, VP-15, VP-16, VP-19, VP-20 enhanced root dry weight by 283.33, 216.66, 250.00, 200.00, 250.00, 216.66, 266.66, 191.66% respectively as compared to control. *Pseudomonas* VP-2, VP-5, VP-7, VP-11, VP-15, VP-16, VP-19, VP-20 showed number of nodules per plant by 201.33, 190.66, 197.33, 194.66, 197.33, 188.00, 198.66, 188.00,% respectively as compared to control. *Pseudomonas* VP-2, VP-5, VP-7, VP-11, VP-15, VP-16, VP-19, VP-20 increased nodules dry weight by 225.00, 208.33, 220.83, 216.66, 220.83, 212.50, 220.83, 216.66% respectively as compared to control (table 2).

Results suggested that isolated *Pseudomonas* strains showed plant growth promoting activity such as HCN, Siderophore, IAA and P-solubilization. Deshwal et al., (2011b) reported that Plant growth promoting Rhizobacteria improved the plant growth by production of HCN, IAA, Siderophore and P-solubilization. Lippman *et al.* (1995) stated that PGPR could directly enhance plant growth by phytohormones production and enhanced nutrient uptake. Further, our data showed that these strains improved the plant growth activity in Soybean crop. Similarly, Deshwal et al., (2006) observed the same observation in *Pseudomonas* GRC_1 and mentioned the residual effect of *Pseudomonas* in Rice crop. Deshwal et al., (2011c) observed that *Pseudomonas* P3 improved dry shoot weight, dry root weight in *Mucuna* plant by 168, 132% respectively as compared to control. All the above data and information clearly indicated that isolated strains showed plant growth promoting activity in soybean crop.

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