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IMPACT OF CO-INOCULATION OF *PSEUDOMONAS* AND *RHIZOBIUM STRAINS* ON SEED GERMINATION AND PLANT GROWTH ACTIVITY IN MAIZE PLANT

Vishal Kumar Deshwal[1*] and Surya Bhan Singh[2]

[1]Dept. of Microbiology, Doon (P.G.) Paramedical College, Dehradun, India.
[2]Dept. of Microbiology Singhania University, Pachri Bari, Rajasthan, India.

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ABSTRACT

Rhizobium is well known nitrogen fixer and *Pseudomonas* is known for Biological control. In present study screen the impact of co-inoculation of *Pseudomonas* and *Rhizobium* strains on seed germination, seedling plant height and seedling biomass of Maize plant. Twenty–twenty Plant growth promoting *Rhizobium* and *Pseudomonas* were selected for present study. Interaction study of *Rhizobium* and *Pseudomonas* were analysed. Only *Pseudomonas* PSB2, *Pseudomonas* PSB17, *Rhizobium* RSB4, *Rhizobium* RSB16 did not inhibit growth of each other. Therefore, they were selected for pot experiment. Nine treatments such as Treatment 1: *Pseudomonas* PSB2, Treatment 2: *Pseudomonas* PSB17, Treatment 3: *Rhizobium* RSB4, Treatment 4: *Rhizobium* RSB16, Treatment 5: *Rhizobium* RSB4 + *Pseudomonas* PSB2, Treatment 6: *Rhizobium* RSB16 + *Pseudomonas* PSB2, Treatment 7: *Rhizobium* RSB4 + *Pseudomonas* PSB17, Treatment 8: *Rhizobium* RSB16 + *Pseudomonas* PSB17, Treatment 9: Control (without bacteria) were prepared for pot experiment. Results suggested that all bacterized enhanced seed germination and plant growth parameters such as root length, shoot length, root weight and shoot weight. Maximum root length and shoot length was observed in co-inoculation of *Rhizobium* RSB4 + *Pseudomonas* PSB2 by 83.87, 60.71% more as compared to control. We concluded that co-inoculation of *Rhizobium* and *Pseudomonas* improves plant growth activity in Maize.

INTRODUCTION

Plant growth promoting bacteria enhance plant growth and productivity. Literature suggested that few strains from genera such as *Pseudomonas*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* are well known PGPRs [1-3]. *Rhizobium* is well known biological nitrogen fixer and on the other hand *Pseudomonas* has known for its biological control activity. Interaction of *Rhizobium*-*Pseudomonas* showed that only *Rhizobium* SR-2, SR-9, SR-17 and *Pseudomonas* SP-8 did not affect the growth of each another [4]. Further, Co-inoculation of *Rhizobium* SR-9 and *Pseudomonas* SP-8 enhanced plant shoot dry weight and root dry weight by 185, 271% respectively as compared to control [4]. Co-inoculation with *Pseudomonas* spp. and *Rhizobioum* spp. has been shown to increase the degree of colonization of the legume rhizosphere by rhizobia resulting in enhanced plant nodulation [5]. This tripartite association composed of legume plant and two soil bacteria, i.e. *Rhizobium* spp. and *Pseudomonas* spp., has been reported to increase root and shoot weight, plant vigour, N-fixation and grain yield in various legumes such as alfalfa [6], Clover [7], common bean [8], Pea [5]. Co-inoculation effect of *Bradyrhizobium japonicum* A1017 and a gus A-marked strain of *Pseudomonas fluorescens*2137 on soybean (*Glycinemax* L.) [9]. Some studies indicated that co-inoculation of *Bradyrhizobium*

Corresponding Author

Vishal Kumar Deshwal
Email:-vishal_deshwal@rediffmail.com

and certain PGPR's can positively affect symbiotic nitrogen fixation by enhancing both root nodule number or mass [10] and increasing the nitrogenase activity [11]. The co-inoculation of PGPR's with *Bradyrhizobium* increased the nodulation and nitrogen fixation in Glycine max at a low root zone temperature [12]. All the above literature suggests that co-inoculation improve plant growth and yield. Therefore, this study was carried out to screen the effect of co-inoculation of *Pseudomonas* and *Rhizobium* strains on seed germination, seedling plant height and seedling biomass of Maize plant.

MATERIAL AND METHODS

(i) Selected strains: Twenty-twenty characterized and plant growth promoting *Rhizobium* and *Pseudomonas* strains were selected. These selected strains were showed plant growth activity such as siderophore, HCN, indole acetic acid (IAA) and P-solubilization.

(ii) Interaction study between *Rhizobium* and *Pseudomonas* strains: first, *Pseudomonas* strains were grown in King's B medium and filter with bacteriological filter. Different concentration (5, 10, 15%) of culture filtrate was added in sterilized YEM broth. 0.5 ml of log phase culture of *Rhizobium* strain was transferred in it and incubated at 28^{o}C for 24h at 120rpm. *Rhizobium* strain was inoculated in YEM broth without culture filtrate which worked as control. Growth was observed in term of OD (optical density) at 610nm. Similarly, observed the antimicrobial effect of culture filtrate of *Rhizobium* against *Pseudomonas* as mention above [4].

(iii) Pot experiment: This test was done to evaluate plant growth activity of *Rhizobium* and *Pseudomonas*.

(a) Seed bacterization: Maize 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 and *Pseudomonas* strains were grown under continuous shaking condition (120 rpm) on YEM and 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}. In co-inoculation treatment, 0.5ml of *Rhizobium* and *Pseudomonas* was mixed together so that total microbial count would be equal to individual strain. The cell suspension was mixed with 1% carboxy methylcellulose (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). Interaction study showed that only *Rhizobium* RSB4, RSB16 could grow in YEM broth contained culture filtrate of *Pseudomonas* PSB2, PSB17 and vice versa, which showed that such strain did not affect the growth of each another. All *Pseudomonas* strains were grown in broth medium containing CF of *Rhizobium*. Therefore, *Rhizobium* RSB4, RSB16 and *Pseudomonas* PSB2, PSB17 were selected for further plant growth experiment. Seed coated with *Rhizobium* and *Pseudomonas* strains were transferred in earthen pots containing around 500 g unsterilized soil. Nine treatments were prepared and these were Treatment 1: *Pseudomonas* PSB2, Treatment 2: *Pseudomonas* PSB17, Treatment 3: *Rhizobium* RSB4, Treatment 4: *Rhizobium* RSB16, Treatment 5: *Rhizobium* RSB4 + *Pseudomonas* PSB2, Treatment 6: *Rhizobium* RSB16 + *Pseudomonas* PSB2, Treatment 7: *Rhizobium* RSB4 + *Pseudomonas* PSB17, Treatment 8: *Rhizobium* RSB16 + *Pseudomonas* PSB17, Treatment 9: Control (without bacteria). Different plant growth parameters like seed germination, root length, shoot length, root weight, shoot weight were measure.

RESULTS

All bacterized seed showed 42.85% more seed germination as compared to control. These bacterized seed improved the fresh root weight, dry root weight, fresh shoot weight, dry shoot weight as compared to control. Maximum root length and shoot length was observed in co-inoculation of *Rhizobium* RSB4 + *Pseudomonas* PSB2 by 83.87, 60.71% more as compared to control and it was highest among all treatments. Second highest root length was observed in PSB17 and it was 80.6% more as compared to control. Maximum fresh root weight, dry root weight, fresh shoot weight, dry shoot weight was observed in treatment *Rhizobium* RSB4 + *Pseudomonas* PSB2 by 186.02, 329.03, 184.9 and 195.80% more respectively as compared to control (Table 1).

DISCUSSION

Selected strains were showed plant growth activity such as siderophore, HCN, Indole Acetic acid and P-solubilization. The interaction study showed that only few strains of *Rhizobium* and *Pseudomonas* did not inhibit the growth of each other. Four co-inoculation treatment such as RSB4 + PSB2, RSB16 + PSB2, RSB4 + PSB17, RSB16 + PSB17 were prepared and only co-inoculation of RSB4 + PSB2 significantly enhanced the plant growth parameter as compared to other co-inoculated treatments as well as individual strains. Further, our results suggested that bacterized strains improved the seed germination, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight. Previous reports suggested about the plant growth activity of rhizobacteria. Similarly, *Rhizobium* strains produced Plant growth promoting activity [13]. *Pseudomonas* strains showed Plant growth activity and showed residual effect in plant growth of mustard [14].

Table 1. Effect of bacterization with *Pseudomonas* and *Rhizobium strains* on seed germination, seedling biomass of Maize plant

S. No.	Treatment	Seed germination	Length (Inch)		Weight (g)			
					Root		Shoot	
			Root	Shoot	Fresh	Dry	Fresh	Dry
1	*Pseudomonas* PSB2	100	10.4±0.3*	20.3±0.5*	1.45±0.4*	0.72±0.3*	7.91±0.5*	6.35±0.2*
2	*Pseudomonas* PSB17	100	11.2±0.4*	20.4±0.4*	1.63±0.3*	0.81±0.2*	7.95±0.3*	6.38±0.3*
3	*Rhizobium* RSB4	100	10.6±0.4*	19.4±0.5*	1.48±0.2*	0.7±0.2*	7.56±0.2*	6.07±0.2*
4	*Rhizobium* RSB16	100	10.9±0.3*	19.7±0.3*	1.52±0.4*	0.76±0.3*	7.68±0.2*	6.16±0.3*
5	RSB4 + PSB2	100	11.4±0.2*	22.5±0.7*	1.59±0.3*	0.79±0.2*	8.77±0.5*	7.04±0.3*
6	RSB16 + PSB2	100	10.5±0.4*	19.6±0.4*	1.47±0.3*	0.73±0.2*	7.64±0.2*	6.13±0.4*
7	RSB4 + PSB17	100	10.6±0.4*	19.4±0.2*	1.48±0.4*	0.74±0.3*	7.56±0.4*	6.07±0.2*
8	RSB16 + PSB17	100	10.9±0.4*	21.3±0.5**	1.52±0.5*	0.76±0.2*	8.30±0.3*	6.66±0.2*
9	Control	70	6.2±0.4	14±0.3	0.55±0.3	0.18±0.2	3.08±0.3	2.38±0.2

Values are Means of 5 replicate; mean values ± SD; *=significant at 0.05 level of LSD; **= significant at 0.01 level of LSD

Pseudomonas coated seeds significantly enhanced seed germination and seedling vigour of maize and leaf, shoot dry weight, leaf surface area, plant height [15] and mentioned about findings that crop enhancement of cereals occur due to rhizobial inoculation [16]. Our finding showed that Co-inoculation of *Rhizobium* + *Pseudomonas* enhanced the plant growth. Further, *Rhizobium*, *Pseudomonas* strains were also increase plant growth as compared to control. These results showed that *Rhizobium* and *Pseudomonas* are good biofertilizer. Such microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants [17-20]. Other reports suggested that Co-inoculation improved plant growth activity [21]. Plant Growth Promoting Rizobacteria (PGPR) *Pseudomonas* has shown potential to improve nodulation of legumes when co-inoculated with *Rhizobium* [22]. Plant growth promoting *Pseudomonas* and *Rhizobium* isolates can improve common bean growth and yield production [23].

CONCLUSION

Our results and all above literature suggest that plant growth promoting rhizobacteria improves the plant growth. Only one co-inoculation out of four treatments was significantly improved plant growth as compared to other treatments. This is clearly indicated that only few combinations of *Rhizobium* and *Pseudomonas* successfully improve plant growth activity in maize plant. So we concluded that co-inoculation of *Rhizobium* and *Pseudomonas* may improves plant growth.

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