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The effect of bacterial fertilizers on seedling development of *Cajanus cajan* and *Vigna radiata*

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Biofertilizers are an alternative to mineral fertilizers for increasing soil productivity and plant growth in sustainable agriculture. The objective of this study was to evaluate possible effects of three plant growth promoting rhizobacteria (PGPR) as biofertilizers on the growth of *Cajanus cajan* and *Vigna radiata*. The application treatments included the control (without bacterial inoculation) and plant growth promoting rhizobacteria (*Rhizobium*, *Phosphobacterium*, and *Azotobacte*r) in sterilized soil. Data suggested that seed inoculation of *Cajanus cajan* and *Vigna radiata* with PGPR strains increased root length, shoot length, leaf surface area and plant height when compared with control. Present results showed that PGPR strains stimulated plants growth and could be used as an alternative to chemical fertilizer.

Key words : *Rhizobium*, *Phosphobacterium*, *Azotobacte*r, Biofertilizer, *Cajanus cajan*, *Vigna radiata*

INTRODUCTION

Modern agriculture technologies based on balanced fertilization, irrigation, pest management and mechanization are required for good crop productivity. The use of expensive chemical fertilizers is a limiting factor for the low income of farmers and increases the cost of crop production. Biofertilizers are ecofriendly and have been proved to be effective and economical alternate of chemical fertilizers with lesser in put of capital and energy (Hafeez *et al*., 2002). It is defined as a substance which contains living microorganisms when applied to seed, plant surface, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003).

Biofertilizers are well recognized as an important component of integrated plant nutrient management for sustainable agriculture and hold a great promise to improve yield (Narula *et al*., 2005; Wu *et al*., 2005). A group of biofertilizer contains beneficial rhizobacteria have been termed as plant growth promoting rhizobacteria (PGPR). Among them the strains from genera such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium*. Several mechanisms have been suggested by which PGPR can promote plant growth, including phytohormone production, N_2 fixation, stimulation of nutrient uptake and biocontrol of pathogenic microorganisms (Rodriguez and Fraga, 1999).

Red gram (*Cajanus cajan*) and green gram (*Vigna radiata*) are one of the major pulse crops in India that has considerable importance as food, feed and fodder. It also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. These are drought resistant crops and suitable for dry land farming and predominantly used as an intercrop with other crops (Hshvanchen, 2008). The objective of this study was to evaluate the effects of a symbiotic bacterium (*Rhizobium*) and some strains of nonsymbiotic rhizobateria from two genera, including *Azotobacter* and *Pseudomonas* on the growth and yield of red gram (*Cajanus cajan*)and green gram (*Vigna radiata*).

MATERIALS AND METHODS

Collection of seeds :

The seeds of *Cajanus cajan var*.Co_6 and *Vigna radiata* $CoRC_7$ were procured from Tamil Nadu Agricultural University, Coimbatore (T.N.).

Isolation of microorganisms :

The bacterial fertilizers *Rhizhobium, Azotobacter* and *Phosphobacterium* were isolated from root nodules and rhizosphere soil region of the respective plants by dilution plate method (Waksman, 1992). Bacterial strains were characterized using standard procedures. Selective medium were employed for the cultivation of *Rhizhobium, Azotobacter* and *Phosphobacterium* using Yeast extract mannitol agar, Jensen's medium, Trypticase soy agar, respectively. The inoculum concentration of

microorganisms used as biofertilizers are shown in Table 1.

Table 1: Biofertilizers and their inoculum concentration

Strains	Inoculum concentration (CFU/g)
Rhizhobium	$2.0x10^6$
Azotobacter	$1.70x10^7$
Phosphobacterium	$1.70x10^8$

CFU/g: Colony Forming Units per 1 g of peat based carrier

Experimental design :

The experimental design was randomized with 3 replications and 8 treatments include uninoculated control (T_1) *Rhizobium* (T_2), *Phosphobacterium* (T_3), *Azotobacter* (T_4), *Rhizobium* + *Phosphobacterium* (T_5), *Rhizobium*+*Azotobacter* (T_6) *Azotobacter* + *Phosphobacterium* (T_7) and *Rhizobium*, *Phosphobacterium*, *Azotobacter* (T_8). Sowing was performed by hand after mixing seeds of green gram and red gram with 20% sugar solution and bacterial inoculants at the rate of 2g peat based inoculants per each 100g seeds. The uninoculated control pots were sown before in hand and in order to prevent cross inoculation between other treatments, new sterile medical gloves were used for sowing each pot.

Seedling development :

After 30 days, the seedlings were carefully plucked out without damaging the root system for measuring the root length and the shoot height. Fresh biomass of root and shoot systems were determined.

***Estimation of biochemical parameters of plants* :**

The biochemical parameters such as protein estimation (Lowry *et al.*, 1958), extraction of chlorophyll (Sadasivam and Manikam,1996) and extraction of carbohydrate (Dinitro Salicylic Acid method) were performed.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below:

Biomass yield :

The results (Table 2) showed that the amount of fresh biomass yield has been found to be increased progressively irrespective of treatments over control. However, the magnitude of such changes varied with treatments, being recorded highest (18.6, 19.1) in treatment T_8 at 30 days of plant growth. The total biomass production was highest which might be due to combined application of biofertilizers that caused maximum fixation of atmospheric nitrogen, increased uptake of soil phosphorus and potassium by the both *Cajanus cajan* and *Vigna radiata* plats as compared to their respective sole applications (Kuntal *et al*., 2007).

Table 2 : Effect of bacterial fertilizers on the biomass yield of *Cajanus cajan* and *Vagna radiata*

Treatments	*Cajanus cajan*		*Vagnia radiata*	
	Total biomass (g)	Response of biomass yield (%)	Total biomass (g)	Response of biomass yield (%)
Control(T_1)	12.6	-	14.50	-
Rhi (T_2)	14.0	11.1	15.20	4.8
Phos (T_3)	14.9	18.3	15.90	9.7
Azo (T_4)	15.5	23.0	16.50	13.8
Rhi + Phos (T_5)	16.7	32.5	17.00	17.2
Rhi+Azo (T_6)	15.2	20.6	15.80	9.0
Azo + Phos (T_7)	17.0	34.9	18.60	28.3
Rhi+ Phos +Azo(T_8)	18.6	47.6	19.10	31.7

The total biomass yield was followed in the order of Rhi+phos+Azo (T_8) › Azo + Phos (T_7) › Rhi + Phos (T_5) › Azo (T_4) › Phos (T_3) › Rhi (T_2) › Control (T_1). The per cent response of microbial inoculants towards the total biomass yield of both *Cajanus cajan* and *Vigna radiata* was recorded highest (47.6%, 31.7%, respectively) in the treatment where Rhi+phos+Azo (T_8) were inoculated together which was closely followed by Azo + Phos (T_7) (34.9%, 28.3%, respectively) and Rhi+phos (T_5) (32.5%,17.2%, respectively) (Table 2).

The inoculation of seed with bacterial fertilizers namely *Rhizobium*, *Phosphobacterium* and *Azotobacter* and their combinations were significant. The combination of *Rhizobium* +*Phosphobacterium* +*Azotobacter* (T_8) showed rapid increase in the growth and yield of the plant. The leaf size, shoot length, and root length at 30days after sowing were more when compared to other combinations with control (Table 3).

The amount of chlorophyll, protein and carbohydrates were increased in the combination of *Rhizobium* +*Phosphobacterium*+ *Azotobacter* (T_8) than the other treatments in the pulse crop namely *Vigna radiata* and *Cajanus cajan* (Table 4). The morphological parameters of *Cajanus cajan* and *Vigna radiata* were more promising in T_8.

Conclusion:

Biofertilizers are widely used in crop production. Therefore, the application of biofertilizers and their

Table 3: Effect of bacterial fertilizers on the seedling development of *Cajanus cajan* and *Vigna radiata*

Biofertilizers	*Vigna radiata* ($CoRC_7$)			*Cajanus cajan* (Co_6)		
	Leaf length (cm)	Shoot length (cm)	Root length (cm)	Leaf length (cm)	Shoot length (cm)	Root length (cm)
T_1	6.0	11.7	12	6.7	12.2	13.2
T_2	7.3	12.5	14.8	8.2	13.5	15.3
T_3	6.4	12.7	13.6	8.4	14.2	14.8
T_4	7.6	12.9	14.6	8.3	14.6	14.4
T_5	7.4	13.4	15.2	8.9	14.8	15.6
T_6	7.5	13.6	15.6	9.2	15.2	15.7
T_7	7.8	14.2	15.8	8.5	15.4	16.2
T_8	8.2	14.8	16.3	9.4	15.8	17.2

Table 4 : Effect of bacterial fertilizers on the total concentration of carbohydrate, protein and chlorophyll

Biofertilizers	*Vigna radiata* (Co Rc_7)					*Cajanus cajan* (Co_6)				
	Carbohydrate	Protein	Chlorophyll (mg/g of leaf)			Carbohydrate	Protein	Chlorophyll (mg/g of leaf)		
			663	645	Total			663	645	Total
T_1	6.42	0.16	0.04	0.8	0.22	5.47	0.12	0.09	0.11	0.2
T_2	8.75	0.23	0.08	0.14	0.18	7.23	0.17	0.08	0.11	0.21
T_3	8.64	0.36	0.06	0.12	0.16	7.43	0.22	0.07	0.11	0.19
T_4	8.66	0.33	0.05	0.11	0.15	7.31	0.24	0.06	0.1	0.16
T_5	9.21	0.42	0.06	0.09	0.13	8.23	0.32	0.05	0.08	0.13
T_6	9.43	0.40	0.05	0.08	0.16	8.45	0.36	0.06	0.09	0.15
T_7	9.64	0.44	0.06	0.1	0.13	8.78	0.40	0.07	0.7	0.14
T_8	10.23	0.49	0.04	0.9	0.12	9.67	0.42	0.1	0.06	0.16

combinations were undertaken in the present study. The results showed that an inoculation of single biofertilizer significantly increased the biomass yield as well as nutrient content in plants. However, such increased effects have been found to be further enhanced due to dual or other compatible mixtures of inoculants resulting from their strong synergistic relationships among themselves.

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