CHAPTER 5

PLANT INOCULATION STUDIES WITH FLUORESCENT PSEUDOMONADS AND RHIZOBIA ON *VIGNA RADIATA* AND *CAJANUS CAJAN*

5.1 INTRODUCTION:

5.1.1 PGPR interactions in rhizosphere and plant growth stimulation:

The rhizosphere is an ecological environment where numerous microorganisms colonize around the roots of growing plants and diverse groups of bacteria were found to be associated with the root systems of higher plants (Khalid et al., 2006). These bacteria are considered as efficient microbial competitors in the root zone and it was found that microbe associations with plant can be positive, neutral or negative (Kennedy, 2005; Nadeem et al., 2006; Patel et al., 2008; Khalid et al., 2009). Plant growth promoting rhizobacteria (PGPR) act in association with roots and stimulate the plant growth (Kloepper et al., 1986; Asghar et al., 2004; Khalid et al., 2004; Zahir et al., 2004; Kremer, 2006; Bohm et al., 2007) as discussed in earlier chapters of this thesis. PGPR includes species of *Rhizobium, Bradyrhizobium, Pseudomonas, Azospirillum, Azotobacter, Bacillus, Klebsiella, Enterobacter, Xanthomonas, Serratia* and many others as they have shown to have positive effect when co-inoculated and facilitate plant growth by various mechanisms (Lugtenberg et al., 2002; Raj et al., 2003; Guo et al., 2004; Khan, 2005; Greenberg et al., 2006; Akhtar and Siddiqui, 2009). In the recent years, the PGPR have received worldwide importance for agricultural benefits as they are the potential tools for sustainable agriculture and have shown significant increases in growth and yield of agricultural crops both under greenhouse and field conditions (Kennedy, 2004; Khalid et al., 2004, 2006; Kumar et al., 2007; Gravel et al., 2007; Zhuang et al., 2007; Arshad et al., 2008; Banchio et al., 2008; Contesto et al., 2008; Figueiredo et al., 2008; Mubeen et al., 2008; Naiman et al., 2008). Stimulation in root growth and biomass production of different plant species by inoculation with PGPR has been well documented (Belimove et al., 2001, 2005; Van Loon and Glick, 2004; Safronova et al., 2006; Nadeem et al., 2006; Arshad et al., 2008; Patel et al., 2008). Rapid growth and establishment of roots, whether by elongation of primary roots or development of lateral roots, is

advantageous for young seedlings as this increases the ability of the plant to obtain more water and nutrients from the surrounded environment. However, obtaining maximum benefits from microbial inoculants under field conditions requires a systematic strategy to fully utilize all beneficial factors for increasing crop yields.

5.1.2 Effect of coinoculation of other PGPR and rhizobia on plant growth:

Combinations of biocontrol agents could be more effective in controlling soil-borne pathogens than a single agent (D.P. Roberts et al. / Crop Protection 24 (2005) 141–155). The challenge is to obtain good isolates that colonize required host plants but they do not possess any deleterious traits for plant growth. These can be obtained through plant tests as a strategy to screen the best isolates for further tests in their growth promotion activities. It has been reported that there is an increase in the nodulation and nodule health under co-innoculation with various phosphate solubilizers (Wasule et al., 2002). It has also been reported that there is a mutualism between mineral phosphate solubilizing bacteria and plants growing in phosphate limiting conditons (Goldstein et al., 1999). Co- inoculation studies of *Rhizobia* with fluorescent *Pseudomonas* strain increases the beneficial effect of rhizobia (Kumar et al. , 2001). Table 5.1 summarizes the co inoculation studies by different researchers using several different rhizobia and other PGPR strains. Table 5.2 describes the coinoculation effect of PGPR strains on the nodulation efficiency of rhizobial strains.

The use of rhizobacteria containing ACC-demainase, IAA production, P-solubilization and siderophore production in co-inoculation with rhizobia could be more beneficial due to their multiple effects on plant through different growth enhancing mechanisms. Inoculation with PGPR containing ACC-deaminase is very effective in improving growth as the bacterial enzyme ACC-deaminase lowers the level of ethylene in roots; and nodulation of mung bean. Furthermore inoculation benefits can be enhanced by maintaining high densities of effective bacteria around the roots. Thus, integrated use of rhizobia and PGPR could be highly effective in improving yield and nodulation of mung bean. The combined inoculation of rhizobia and phosphate solubilizing bacteria has been shown to increase nodulation, growth and yield parameters in *Vigna radiata* (Kloepper and Schroth, 1981; Gupta et al, 1997, Jain, P.C. et al, 1999). Several workers have attempted to develop inorganic carrier based (talc, vermiculite, perlite, ground rock phosphate, and calcium sulphate) and organic carrier based (charcoal, peat) powder formulation of fluorescent pseudomonad to promote plant growth and control their disease (Bashan, et al 1988; Bennett and Lane, 1992; DeFreitas and Germide, 1992; Glick, 1995).

Table 5.1 Effect of co inoculation of rhizobia and PGPR strains on host plants

Rhizobial strain	Co-inoculated with PGPR	Host plant	Effect of co-inoculation on host plant	References
Rhizobium leguminosarum	*P.syringae/ P.putida*	*Pisum sativum*	Increased shoot, root and total plant weight	Freitas et al, 1992
R.phaseoli	*P.putida*105	*Phaseolus vulgaris*	No effect	
R.phaseoli	*P.syringae* R25		Severe deleterious effect on seedling emergence plant biomass and nodulation	
R.tropici CIAT899 *R.etli* TAL182	*A.brasilense* strain Cd ATCC29729	*P.vulgaris* cv *Bulgarian*	Coinoculation with Azospirillum at lower concentration of mineral N2 fertilizer slightly increased nodulation but high concentration of Azospirillum inhibited plant growth in terms of shoot height, dry wt.etc	Burdman et al,1996
Rhizobium sp. cicer strain ca181	*P.fluorescens*	*Cicer atrienum* L.	Enhanced nodulation and decreased wilt incidences due to production of β1-3 glucanase and siderophores	Khot et al, 1996
R.tropici CIAT899	*Bacillus* sp. CECT450	*P.vulgaris*	Improved nodulation efficiency	Camacho et al, 2001
B.japonicum A1017	*P.fluorescens* 2137	Soybean plant	Increased colonization of B.japonicum, nodulation, acetylene reduction activity	Chebotar et al,2001
R.leguminosarum bv *viceae*	Fluorescent *Pseudomonas*	*P.sativum* L.cv capella	Increased shoot height, root length and root dry wt.	Kumar et al, 2001
B. japonicum	*P.putida* *P.fluorescens*	Maize	Increased root and shoot length, Increased fresh biomass of seedling.	Shaharoona et al, 2006
Isolated rhizobial strains	*Enterobacter* sp.	Chickpea (NIFA88 &Parbat)	Increased plant biomass and Nodulation	Mirja et al, 2007
R.tropici CIAT899 *R.etli* ISP42	*Azospirillum brasilense*	*P.vulgaris*	Acetylene reduction activity favored due to co inoculation. No increase in nodule no.	Dardanelli et al, 2008
Mesorhizobium ciceri	*Azotobacter chroococcum*	*Cicer arietinum* L.	Increased biomass and grain yield, high nodule no. per plant, increased nodular mass, high N and P content in plant as well as soil	Qureshi et al, 2009
Bradyrhizobium japonicum SB1	*Bacillus thurengiensis* KR1	Soybean plant variety VL Soya2	Increase in shoot wt., root wt., nodule no., root volume and total biomass of plant	Mishra et al, 2009

Table 5.2: Effect of coinoculation of PGPR strains with rhizobia on nodulation (Kumar et al., 2001)

Treatment	Nodule occupancy (%)
Uninoculated control	–
Rhizobium alone	50
Rhizobium + *Azotobacter chroococcum*	55
Rhizobium + *Azospirillum brasilense*	56
Rhizobium + *Pseudomonas fluorescens*	75
Rhizobium + *Pseudomonas putida*	85
Rhizobium + *Bacillus cereus*	65

Mung bean (*Vigna radiata*) is model legume plant and also one of the most important crops of western India. Its per hectare yield is much lower than yield potential, because of suboptimal growth conditions and phytopathogen attack faced during different growth stages. Pigeon pea (*Cajanus Cajan)* is also an important pulse crop in India and cultivated to a large extent in Gujarat. In this chapter, plant inoculation studies were performed to see the, a) the effect of biocontrol strains of fluorescent pseudomonad on the *Vigna radiata,* b) the effect of combination of effect of model PGPR strain *Pseudomonas fluorescens* CHA0, standard rhizobial bioinoculants and model P-solubilizer *E. asburiae PSI3* on the growth of *Cajanus cajan* under pot conditions, c) Plant growth promotion effect under biocontrol supportive nutritional combination supplemented in synthetic MS medium.

5.2 MATERIALS AND METHODS:

5.2.1 Microorganisms and the plant materials used:

Table 5.3 describes the microorganisms and plants used in this study. *Rhizobium* isolates IC3123, IC3109, ST1 were grown in yeast extract mannitol (YEM) broth and *Pseudomonas fluorescens* CHA0 (*Pf* CHA0) and *Enterobacter asburiae* PSI3 were grown in the Luria-Bertanni broth (LB) till CFU reached 10^9 cells/ml. Cells were collected by centrifugation at 5000 RPM for 5 min, washed twice with 0.85% NaCl to remove media traces.

Table 5.3 Rhizobial strains, fluorescent pseudomonad strains and leguminous plant used in the present study

Sr. no.	Bacterial Strains/Plants	References/Source
1	Rhizobial strains IC3123, IC3109 (pigeon pea nodulating)	IARI, New Delhi, Rajendran et al., 2007; 2008; Arif et al., 2012
2	Rhizobial strain ST1(pigeon pea nodulating)	Laboratory isolate , Rajendran et al., 2007; Geetha et al., 2009; Arif et al., 2012.
2	*Enterobacter asburiae* PSI3	Laboratory isolate (P-solubilizing), (Gyaneshwar et al,1999, Kavita et al., 2008)
3.	Bacterial isolates G2, G3, G8, G16,G19, G20,G22, G26, G28, G35,G36, G46, and M3	Fluorescent pseudomonads isolated from local soils (Chapter 2)
3	*Pseudomonas fluorescens* CHA0	Model biocontrol and PGPR strain
5	*Vigna radiata K831*	Pulse research station, Anand Agriculture University,Vadodara
6	*Cajanus cajan*	Pulse research station, Anand Agriculture University,Vadodara

Yeast extract mannitol (YEM) agar

Ingredients	(g/L)
Mannitol	10.0
Sodium glutamate	0.2
KH_2PO_4	0.5
$MgSO_4$	0.2
NaCl	0.1
Yeast extract	1.0
Distilled Water	1000 ml

5.2.2 Plant inoculation study for studying the effect of fluorescent pseudomonad on plant growth:

5.2.2.1 Preparation of synthetic media for plant assay:

Both micro and macro elements were added as given below and 0.8% agar was added in each sugar tube containing 75 ml of the medium. The medium was autoclaved at 15 psi for 15 min. After autoclaving the media was immediately transferred to cold room for solidification.

Linsmaier and Skoog (microelements):

Ingredients	(mg/l)
$CoCl_2.6H_2O$	0.025
$CuSO_4.6H_2O$	0.025
Na EDTA	37.30
$FeSO_4.7\ H_2O$	27.80
H_3BO_4	6.20
KI	0.83
$MnSO_4.\ H_2O$	16.90
Sodium molybdate.2 H_2O	0.28
$ZnSO_4.7\ H_2O$	8.60

Murashige and Skoog (macroelements):

Ingredients	(g/l)
$CaCl_2.2H_2O$	0.44
KH2PO4	0.17
KNO3	0.19
$MgSO_4.7H_2O$	0.37
NH_4NO_3	1.6

5.2.2.2. Seed sterilization and germination:

Vigna radiata seeds (variety K831) were thoroughly washed 3-4 times by sterile distilled water subsequently treated with 0.1% $HgCl_2$ for 2 min followed by 2 min, treatment of 70% ethanol with vigorous shaking under laminar flow hood. Four washes of seeds by sterile distilled water were carried out to remove traces of $HgCl_2$. Surface sterilized seeds were transferred to sterile petri plates containing moistened filter paper and kept for germination after wrapping it in dark paper around. Sterile distilled water was again added on the second day to maintain humidity inside for proper germination. Seeds were allowed to germinate upto the time when radical size reached to 1 cm.

5.2.2.3 Treatment of fluorescent pseudomonad strains to germinated seedling:

Sterile seedlings were dipped in overnight grown fluorescent pseudomonad suspension (10^8cells/ml) for 45 min and transferred aseptically to tubes containing semisolid Murashige and Skoog (MS) media (1% agar). The seedlings were allowed to grow at 28-30°C under natural dark-light time period. After 7 d the plants were monitored for their shoot and root lengths and weights.

5.2.3 Plant study for the co inoculation effect of *Pf* CHA0, *E.asburiae* PSI3, and rhizobial strains on *Cajanus Cajan*

Soil (alluvial, loamy sand, pH 7.7 with moisture holding capacity 40%) was obtained from fields of Anand Agriculture University, Gujarat. For the plant assay experiment, 1kg air-dried and double sterilized soil was placed in plastic autoclavable bags (2 kg capacity) and a 70 ml cell suspensions of ST1, IC3123, IC3109, *E.asburiae* PSI3, *Pf* CHA0 and sterile distilled water (–ve control) was added (as described in Table 5.4). The soil was mixed thoroughly to ensure an even distribution of inoculants and filled in 10-cm diameter plastic pots at 400 g per pot. For the double inoculums combination, 200 g of each treatment from 1kg master mix was mixed thoroughly and for triple combination 133 gram of each treatment from master mix was mixed properly and filled in 10-cm diameter plastic pots at 400 g per pot. Six germinated mungbean seeds sown and sprinkled with sterile distilled water. Each treatment was replicated three times and randomized. Inoculations resulted in 10^7- 10^8 CFU of introduced pseudomonad per gram of dry soil. The soil was maintained at 30^{o}C with sufficient moisture content throughout the experiment. The plants were harvested and soil samples collected after 32 d.

Table 5.4 Experimental set up of pot inoculation study for single, double and triple combinations of PGPR strains

Treatment type	**Culture used**
No treatment	-ve control
Single treatment	ST1, IC3123, IC3109, *E. asburiae* PSI3, *Pf* CHA0
Double treatment	*Pf* CHA0 + ST1, *Pf* CHA0 + IC3109 *Pf* CHA0+IC3123, *E. asburiae* PSI3 + IC3123 *E. asburiae* PSI3 +IC3109, *E. asburiae* PSI3 +ST1 *E.asburiae* PSI3 + *Pf* CHA0
Triple treatment	*E.asburiae* PSI3+ *Pf* CHA0 +ST1 *E.asburiae* PSI3+ *Pf* CHA0+ IC3123 *E.asburiae* PSI3 + *Pf* CHA0+ IC3109

5.2.4 Antibiotic sensitivity of bacterial strains using octadiscs:

After bacterial application (8 h), the upper 1 cm soil surface was removed from each pot and from each treatment; 5 g of soil was taken and used for checking the presence of inoculated strains based on the pattern of antibiotic sensitivity using antibiotic octadisc experiment. Soil (1g) was added to the test tube (five replicates) containing 9 ml sterile distilled water and shaken vigorously for 2–3 min. Serial dilutions of the suspension was prepared in saline and 100 ul aliquots from appropriate dilutions were plated onto King's B agar medium supplemented with appropriate quantities of tetracycline. Antibiotic sensitivities of bacterial strains were checked using commercially available antibiotic octadiscs (Himedia Ltd, India) series type G1, G2, and G3 containing different antibiotic combinations were used for study. Cultures were spread using pour plate method and plates were incubated at 28^0C, and after 48 h inhibition zones were measured.

5.2.5 Plant protection assay under biocontrol supportive medium:

The plant growth promoting effect of *Pf* CHA0 on *Vigna radiata* under biocontrol trait supporting combinations (5, 8 and 11 as described in Table 3.2 of Chapter 3) that resulted in high antifungal activity.Controls includes non treated and treated with *R.bataticola*.

5.2.6 Seed germination assay:

Bacterial isolates were grown in 10 ml LB. After reaching active growth phase, 1 ml culture suspension was centrifuged at 8000 rpm for 10 min and washed with sterile 0.85 % Nail (N-saline). The cell pellet was re-suspended in 1 ml N-saline and was used for different experiments such as plant inoculation for protection of *Vigna radiata* from fungal phytopathogen *R.bataticola* by fluorescent *Pseudomonas* isolates.

5.3 RESULTS AND DISCUSSION:

5.3.1 Effect of biocontrol strains of fluorescent pseudomonad on plant growth:

Plant studies were performed with fifteen fluorescent *Pseudomonas* isolates on growth of *Vigna radiata,* with *Pf* CHA0 was used as positive control (Fig. 5.1). Isolates C2, G2, G8, G16, G19, G20, G22, G26, G28, G31, and M3 have shown more positive effect on plant growth than *Pf* CHA0 whereas isolates G28, G35, G36 and G46, have shown effect equal to that of *Pf* CHA0 (Fig. 5.2). M3 showed the good effect on root while G8 on shoot (Fig. 5.3).

Fig. 5.1 Plant growth promotion effect of fluorescent pseudomonad on *Vigna radiata* in synthetic MS medium (a) Uninoculated control (b) G8 (c) M3 (d) *Pf* CHA0 (e) G1

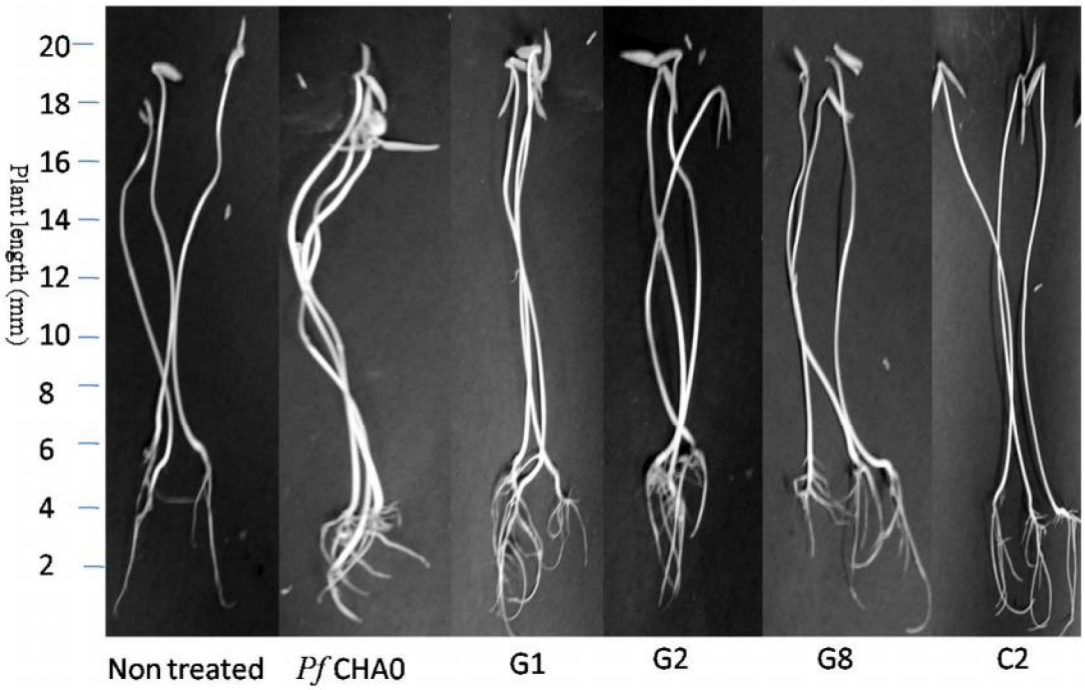


Fig. 5.2 Effect of fluorescent pseudomonad on *Vigna radiate growth*

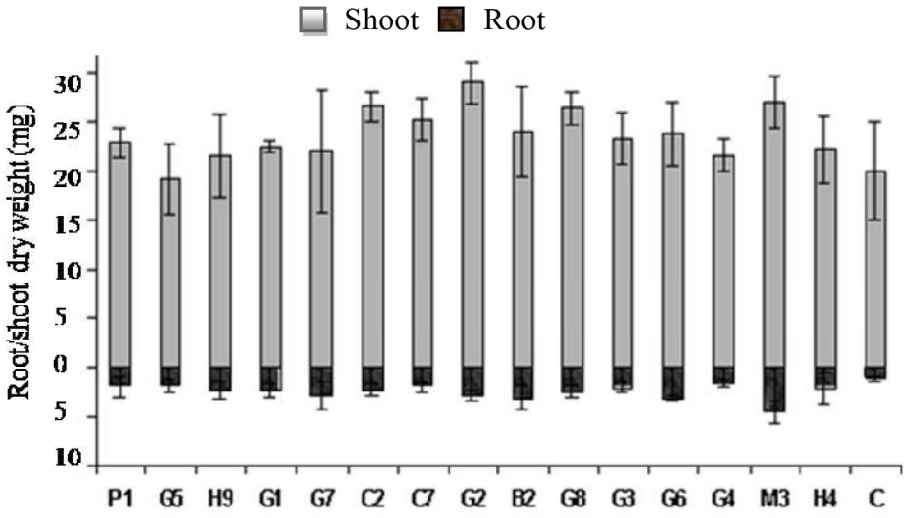


Fig. 5.3 (A)

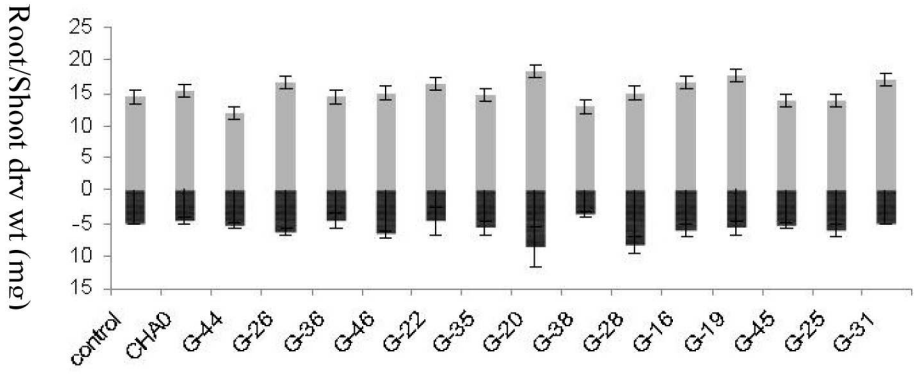
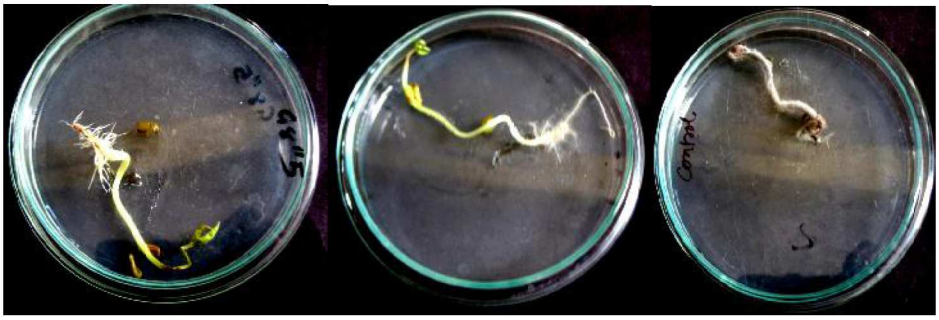


Fig. 5.3 (B)

Fig.5.3 Effect of fluorescent pseudomonad on root /shoot dry weight of *Vigna radiata*

5.3.2 Plant assay for the protection of *Vigna radiata* from *R.bataticola* by fluorescent pseudomonas strains:

Seed germination assay under pathogen challenge was performed to check the biocontrol potential of fluorescent pseudomonad. Strain G20 and G26 have shown protection of *Vigna radiata* from *R.bataticola* (Fig. 5.4). Strains G8, G20, G26 have shown good capacity to control the infection of *R.bataticola* and protect *Vigna radiata* from infection as shown in Fig. 5.5. Model biocontrol strains *Pf* CHA0 has shown the biocontrol activity against *R.bataticola* but at relatively lower extent. Pf-5 and Q-287 did not shown effective plant protection biocontrol traits in plant assay (Fig. 5.5).



(a) (b) (c)

Fig. 5.4 Seed germination assay for protection against fungus *R. bataticola.*by representative fluorescent pseudomonad strains (a) Strain G26+*R.bataticola* (b) Strain G20+*R.bataticola* (c) *R.bataticola* (fungal control)

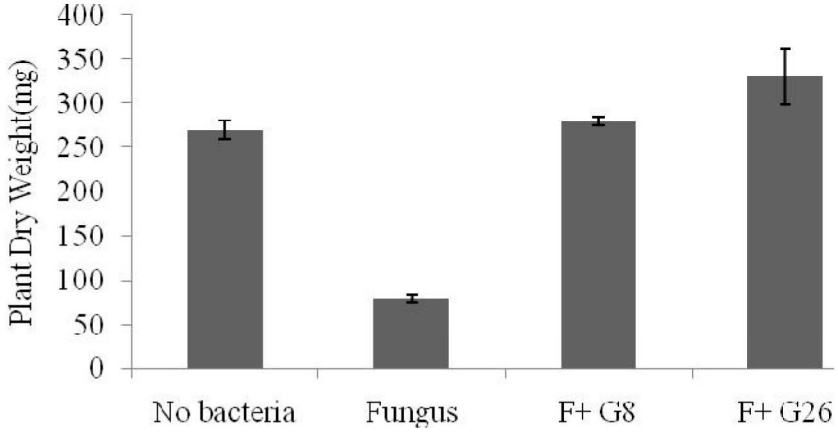


Fig. 5.5 Plant assay for the protection of *Vigna radiata* from *R.bataticola* by fluorescent pseudomonad

5.3.3 Coinoculation effect of *Pf* CHA0, *E.asburiae* PSI3 and rhizobia on *Cajanus Cajan*:

It has been reported that there is an increase in the nodulation and nodule health in co- innoculation studies of various phosphate solubilizers (Wasule et.al 2002). It has been also reported that co-innoculation studies of *Rhizobia* with flourescent *Pseudomonas* has increased PGPR activities of *Rhizobia* (Dileep et.al 2002). Here co-inoculation studies of different combinations of biocontrol strain *Pseudomonas fluorescens* CHA0, P solubilizing strain *E.asburiae* PSI3 with various rhizobial strains were performed. Since the rhizobial strains were specific for pigeon pea, these experiments were performed with this plant. In single treatments all three rhizobial isolates ST1, IC3123, IC3109 as well as *E.asburiae* PSI3 and *Pf* CHA0 showed positive effect on the growth of plant as shown in Table 5.5 and Fig. 5.6. Similar effect was found in double and triple combinations (Figs. 5.6-5.11).

However double combinations showed only slight better effect than the corresponding singlet counterparts while the triple combinations were found to have best effect. The root and shoot lengths and dry weights of above single, double and triple combinations were also increasing in the same order. The triple combination of ST1 was showing the highest root shoot lengths and dry weights (Table 5.5). The control pot which did not receive any pretreatment was showing least growth. Highest positive effect on root was observed in triple combination of PSI-3,Pf CHA0 and ST-1.

Statistical analysis of pot result shows that positive effect of co inoculation was more on shoot than the root (Fig. 5.12 & 5.14). Similarly box plot analysis has shown more effect on shoot than root as shown in Fig. 5.13 & Fig. 5.15. Determination of shoot N and P concentrations would help discern the beneficial effects of the bacterial inoculants on these parameters.

Table 5.5 Effect of co inoculation of fluorescent pseudomonad and other PGPR strains

Culture used	Root length(cm)	Shoot length(cm)
Control	6.90±0.361	8.50±0.5
IC3123	8.37±0.723	9.03±1.7
IC 3109	9.00±1	12.07±0.9
PSI3	11.27±0.643	9.87±1.85
CHA0	12.67±0.577	13.00±1.0
ST1	14.93±3.71	15.33±0.61
Combination with moderate effect on plant growth		
CHA0+ 3109	8.867±0.808	15.133±1.02
PSI3+3109	9.333±2.36	11.433±1.02
CHA0+IC3123	10.167±1.06	14.000±2.64
Combinations with highest positive effect on plant		
CHA0 + ST1	12.567±1.15	12.70±1.47
PS13+ CHA0+3109	13.00±2	14.60±2.11
PSI 3+ IC3123	14.333±3.2	16.667±1.52
PSI3+CHA0	14.667±4.163	13.33±2.082
PSI3+ CHA0+ IC3123	14.67±1.15	13.90±2.53
PSI3+ST1	17.667±1.52	17.33±3.05
PSI3+ST1+CHA0	19.83±1.04	16.33±3.215

5.3.4 Detection of treated strains in the pot soil using antibiotics sensitivity based analysis:

Cotrimoxazole was used to characterize the presence of ST1 and Pf CHA0, oxytetracycline and Gentamycin for the Pf CHA0, cindamycin for the *E. asburiae* PSI3, tetracycline for IC3123, Cephalexin for IC3109 (Table.5.6)

Table 5.6 Antibiotic sensitivity of IC 3123, IC 3109, ST1, *Pf*CHA0 and *Ea*PSI3

Antibiotic	**Symbol**	**Conc(ug)**	**IC 3123**	**ST1**	**IC 3109**	***E.asburiae* PSI3**	***Pf*CHA0**
Amoxyclav	Ac	10	-	21	-	-	-
Cephalexin	Cp	30	-	16	-	-	-
Ciprofloxacin	Cf	30	25	18	22	27	12
Cindamycin	Cd	2	21	-	16	-	-
Cloxacillin	Cx	1	18	-	16	-	-
Co-trimoxazole	Co	25	15	-	12	23	-
Tetracycline	T	30	-	20	-	11	-
Ampicillin	A	10	-	-	-	-	-
Carbecillin	Cb	100	-	-		17	-
Cephotaxime	Ce	30	-	-	-	12	-
Chloremphenicol	C	30	8	7	4	22	11
Co-trimazine	Cm	25	-	-	-	12	-
Gentamycin	G	10	19	19	15	15	-
Norfloxacin	Nx	10	35	30	35	29	26
Oxacillin	Ox	5	-	-	-	-	-
Amikacin	Ak	10	-	18	-	17	-
Amoxycillin	Am	10	-	-	-	--	-
Bacitracin	B	10	-	-	-	-	-
Cephalothin	Ch	30	-	-	-	-	-
Erythromycin	E	15	20	10	19	-	-
Novobiocin	Nv	30	19	12	15	-	-
Oxytetracycline	O	30	17	22	21	16	-
Vancomycin	Va	30	-	-	-	-	-

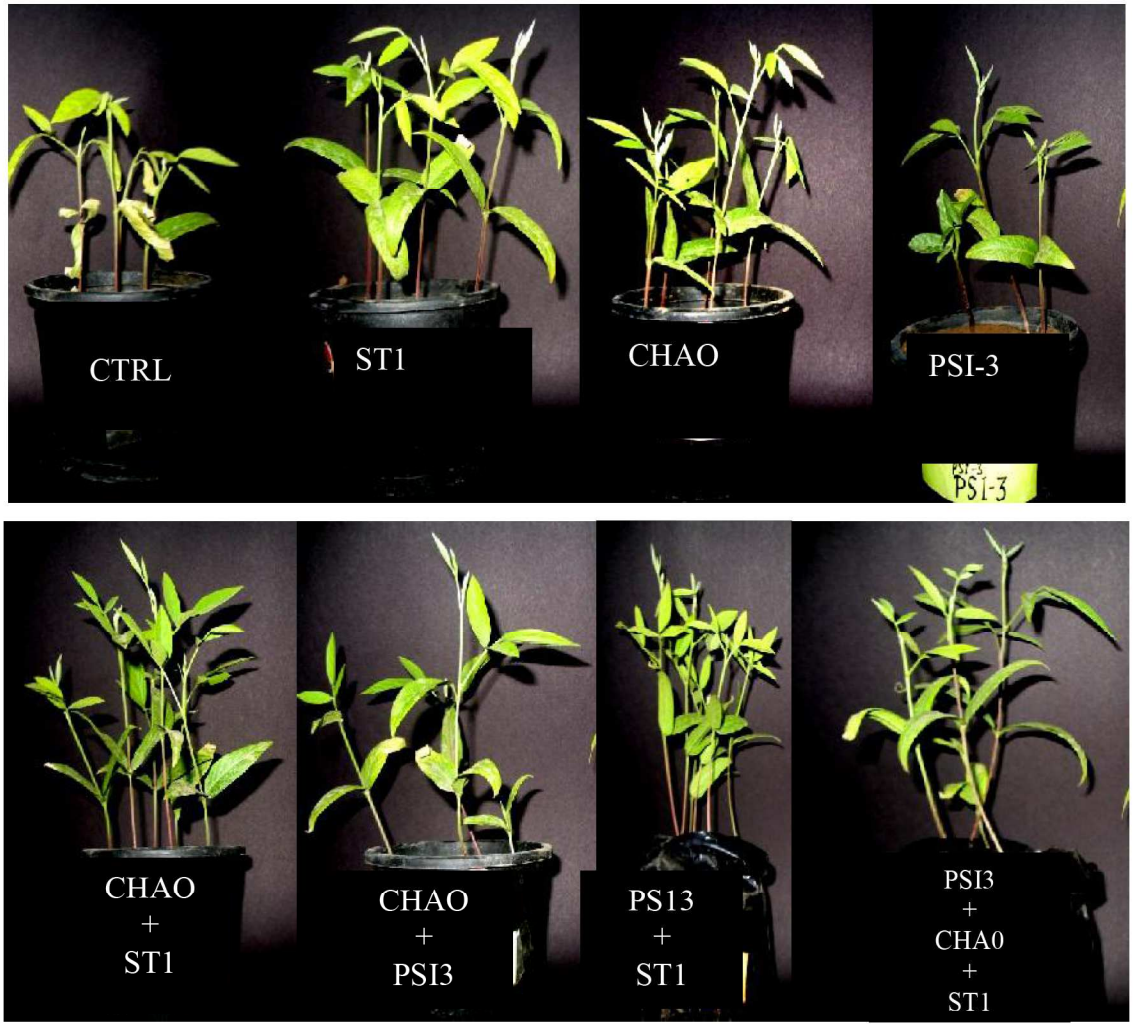


Fig. 5.6 Pot study for the effect of ST1,Pf CHA0 and *E.asburiae* PSI3 on *Cajanaus Cajan*

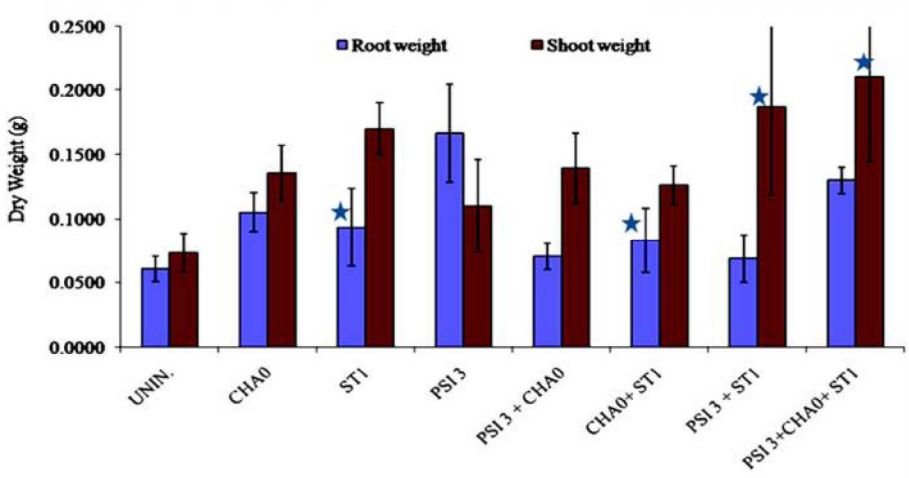


Fig. 5.7 Plant growth promotion effect of ST1,Pf CHA0 and *E.asburiae* PSI3 on *Cajanaus Cajan* (*P>0.05)

Fig. 5.8 Pot study for the effect of ST1,Pf CHA0 and *E.asburiae* PSI3 on *Cajanaus Cajan*

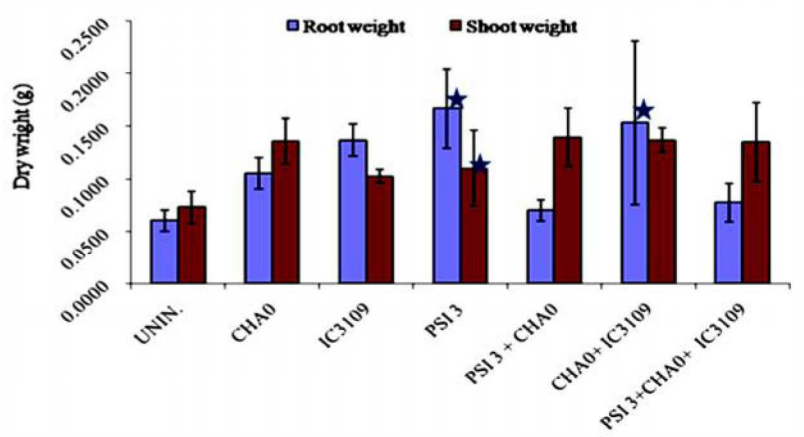


Fig. 5.9 Plant growth promotion effect of IC3109,Pf CHA0 and *E.asburiae* PSI3 on *Cajanaus Cajan* (*P>0.05)

Fig. 5.10 Pot study for the effect of IC3123,Pf CHA0 and *E.asburiae* PSI3 on *Cajanaus Cajan*

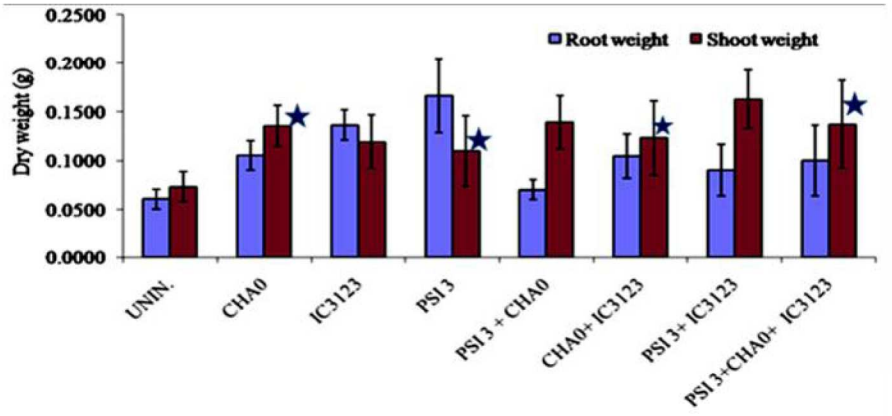


Fig. 5.11 Plant growth promotion effect of IC3123, *Pf* CHA0, *E.asburiae* PSI3 on *Cajanus Cajan* *(★P>0.05)*

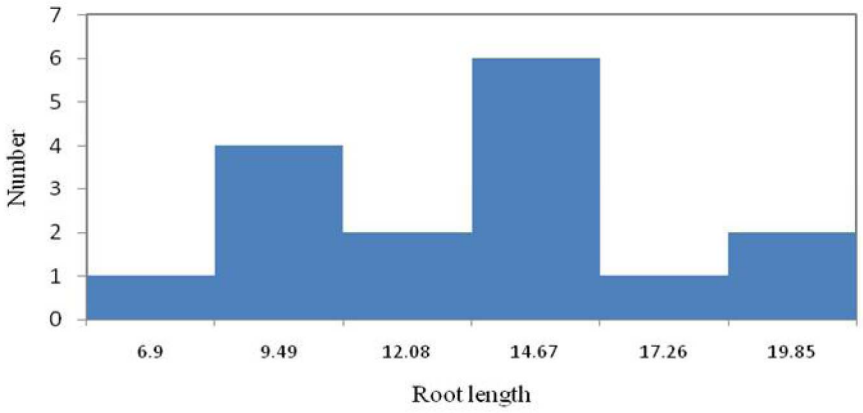


Fig. 5.12 Histogram for the effect of PGP strains on root length

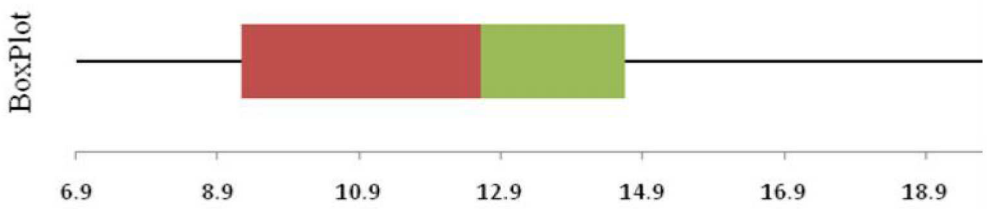


Fig. 5.13 Box plot for the effect of different combination of PGP strains on root length

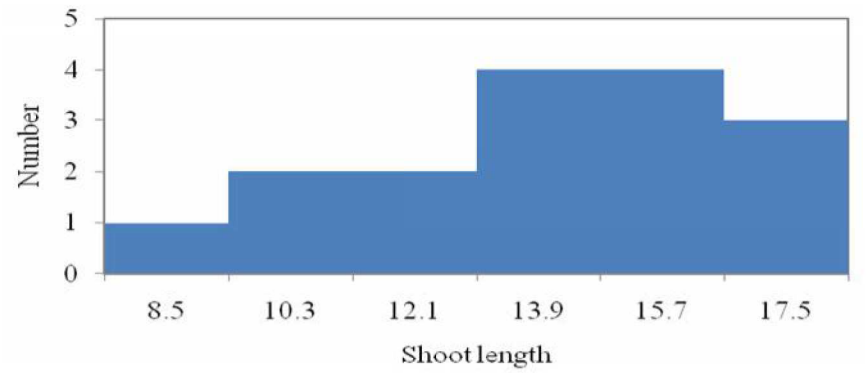


Fig. 5.14 Histogram for the effect of PGP strains on shoot length

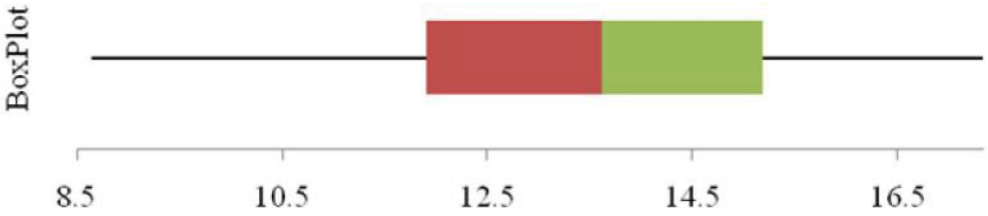


Fig. 5.15 Box plot for the effect of different combination of PGP strains on shoot length

5.3.5 Plant protection under biocontrol supportive medium:

Non-supplemented combination supported plant growth in uninoculated control and growth suppression of mung bean in *R.bataticola* treated set. *Pf* CHA0 has shown highest plant growth promotion out of all combination and MS medium control. The plant growth promoting effect of *Pf* CHA0 on *Vigna radiata* under biocontrol trait supporting combinations was studied. Combination with glucose, $FeSO_4$, KNO_3 and $ZnSO_4$ supplemented to MS medium and combination with glucose,citrate, nitrate,Pi, $ZnSO_4$, $FeSO_4$ and Mo^{2+} have shown inhibition of plant growth as compare to non-supplemented combination while inhibitory effect was minimized in inoculated with *Pf* CHA0 (Fig. 5.16). *Pf* CHA0 showed an effective inhibition of *R.bataticola* and plant protection in combination supplemented with glucose, $FeSO_4$, KNO_3 and $ZnSO_4$ supplemented to MS medium and combination with glucose, citrate, and nitrate, Pi, ZnSO4, FeSO4 and Mo^{2+} as compared to un-inoculated control. In biocontrol supportive combination with Mo and Zn it could be deleterious effect of metals on plant growth which could be the possible reason of less growth as compared to non-supplemented combination. In biocontrol supportive combination the effective suppression of *R.bataticola* by *Pf* CHA0 was observed. *Pf* CHA0 has shown highest plant growth promotion out of all combinations and uninoculated medium control. *Pf* CHA0 showed effective bio-control against *R.bataticola* in biocontrol supporting combinations.

Plant inoculation study for the antifungal activity of *Pf* CHA0 under antifungal traits supportive combinations has been shown in Table 3.2 of Chapter 3. Combination 5 have three metals so it has retorted the plant growth effectively in compare to combination 8 which have Fe^{2+} and Zn^{2+}.Combination 8 retardation effect was minimized by *Pf* CHAO inoculation compare to uninoculated. In bio control supportive combination the effective suppression of *R.bataticola* by *Pf* CHA0 was observed. Non Supplemented combination have supported to plant growth in uninoculated control and bio control support against *R.bataticola*. *Pf* CHA0 have shown highest plant growth promotion out of all combination and MS medium control. Possible reason could be that this combination does not have any metal which is inhibitory to plant growth and it is also supportive to *Pf* CHA0 growth in compare to MS medium.

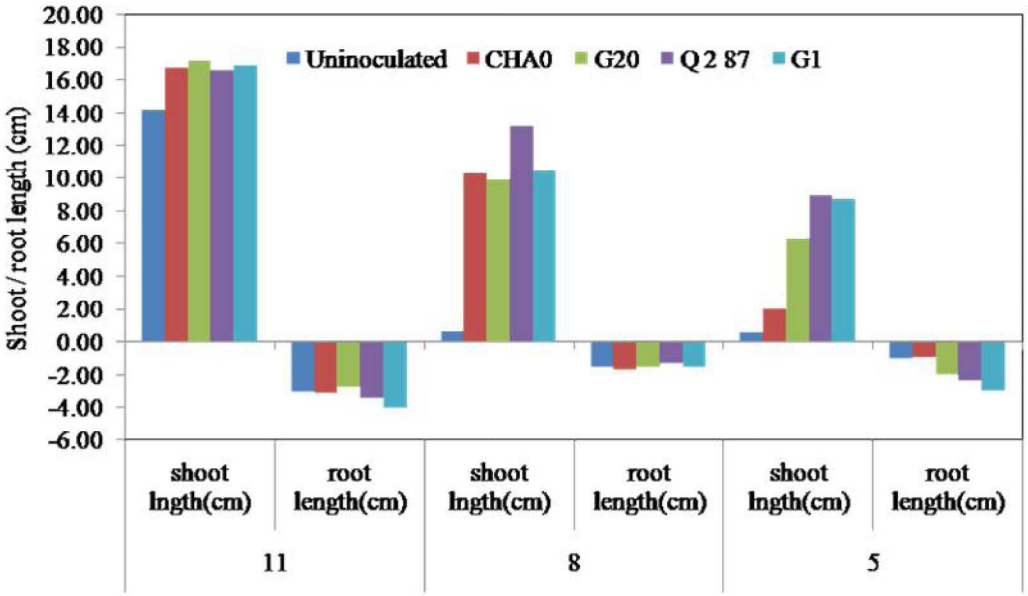


Fig. 5.16 Effect of plant inoculation of fluorescent pseudomonad strains under biocontrol supporting nutritional combinations

5.4 CONCLUSIONS:

In this chapter, effect of plant inoculations with PGPR strains of fluorescent pseudomonads singly and in combination with other plant beneficial strains such as rhizobia and P-solubilizing *E. asburiae* PSI3 was studied. Many fluorescent pseudomonad isolated and characterized in this work (such as C2, G2, G8, and M3) showed good effect on plant growth. M3 showed more positive effect on root while G8 showed on shoot. Isolates such as G16, G19, G20, G22, G26, G28 and G31 have shown better plant growth promotion than *Pf*CHA0 whereas isolates G28, G35, G36, and G46 have shown effect on the growth equal to that of *Pf*CHA0. Strains G8, G20 and G26 have good capacity to control the infection of *R.bataticola* and save the *Vigna radiata* from infection. Model biocontrol strains *Pf* CHA0 has also shown the biocontrol against *R.bataticola* but at lower extent. But Pf-5 and Q-287 did not showed any biocontrol traits in plant assay under the conditions tested. In plant assay during single treatments all three rhizobial isolates ST1, IC3123, IC3109 as well as *E. asburiae* PSI3 and *Pf* CHA0 showed good activity on the growth of plant. However double combinations showed a

slight better effect than the corresponding singlet counterparts while the triple combinations were found to be best. The root and shoot lengths and dry weights of above single, double and triple combinations were also increasing in the same order. The triple combination of ST1, *E. asburiae* PSI3 and *Pf* CHA0 was showed highest root shoot lengths and dry weights. *Pf* CHA0 showed an effective inhibition of *R.bataticola* and plant protection in MS medium supplemented with glucose, $FeSO_4$, KNO_3 and $ZnSO_4$ and combination with glucose, citrate, and nitrate, Pi, ZnSO4, FeSO4 and Mo^{2+} as compared to un-inoculated control. Combination 16 of media amendments has three metals so it has retorted the plant growth effectively in compare to combination 9 which has Fe^{2+} and Zn^{2+}. Medium combination 9 had retardation effect which was minimized by *Pf* CHAO inoculation as compared to uninoculated. The present study indicates that consortia of PGPR can work effectively in plant growth promotion. Amendment of certain nutrients might improve the performance of the consortia. Thus, this study provides a evidence to the factors that can be manipulated to improve bacterial inoculants and expand the application prospect of the biocontrol strains based on the rhizosphere and soil type for better bio-control potential. The study of combining PGPR organisms in double and triple combination is of great potential value in order to minimize use of fertilizers and pesticides.

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- Presented at the 1st Asian PGPR congress organized by Auburn University, USA and ANGRU, Hyderabad (21-24 June ,2009). **Best Poster Award out of posters from India, USA, China, Indonesia, Holland and other countries across Asia** for the Work "Nutritional factors affecting the bio-control traits of fluorescent *Pseudomonas* in *Vigna radiata* rhizosphere".
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- ❖ Two times letter of appreciation received from MS University, Vice chancellor for Research work and research achievements

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