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EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FOR THE GROWTH AND YIELD OF SWEET FLAG (*Acorus calamus* L.)

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Abstract

The effect of microbial for the improvement of growth consisting plant growth promoting rhizobacteria (PGPR) like *Azotobacter, Bacillus, Pseudomonas* and *Enterobacter* were tested separately and in combination on *Acorus calamus* for pot culture experiment. The plant height, number of rhizomes, rhizome length and rhizome yield of *Acorus calamus.* All the growth parameters were recorded on 60, 120, 180, 240, 300 and 330 DAP period of *Acorus calamus* when compared to the uninoculated control. Plant growth promoting rhizobacteria (PGPR) exhibit direct and indirect mechanisms as plant growth promoters and biological control agents. Direct mechanism by PGPR, include the provision of bio-available phosphorus for plant uptake, nitrogen fixation for plant. The results of this study suggest that the PGPR applied in combination have yield of *Acorus calamus*.

Key words: PGPR, Bio-fertilizer and Sweet flag.

1. Introduction

Medicinal plants are the richest resource of drugs for traditional system of medicine; therefore, human beings have been utilizing plant extracts to guard themselves against several diseases and also to maintain health. Medicinal plants contain several chemical constituents such as flavonoids, alkaloids, phenol and tannins, carboxylic acids, terpenes and amino acids and several other inorganic acids. In Ayurvedic medicine, it is used for the treatment of skin eruptions, epilepsy, mental ailments, chronic diarrhea, dysentery, rheumatic pains, neuralgia, cancer, dyspepsia, bronchial catarrh and intermittent fevers (Sabitha *et al*., 2003; Tank and Saraf, 2003; Prajapati e*t al*. 2003; Ajay Kumar *et al.*, 2012; Sandeep *et al.*, 2014). These phytochemical constituent gave definite individuality and properties to plants (Parekh and Chanda, 2007).

The WHO has also estimated 80% of world population meets their primary health care needs through traditionally medicinal plants only. Medicinal plants are those plants possessing secondary metabolites which are potential sources of curative drugs. India is eighth largest country having rich plant diversity with a total of around 47,000 species, of which more than 7500 species are being use as medicinal plants in India.

Rhizosphere is a dynamic environment which harbors diverse group of microbes. The bacteria which directly or indirectly stimulate plant growth have been referred to as plant growth promoting rhizobacteria (PGPR). PGPR promote growth of several annual crops by increasing uptake of nitrogen, iron (through siderophores), phosphorus, and synthesis of phytohormones by controlling plant diseases (Sayyed *et al.*, 2005).

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An intensive practice that warrants high yield and quality requires the extensive use of chemical fertilizers, which are costly and may create environmental problems. The recently environment friendly are sustainable and organic agricultural practices (Esitken *et al.,* 2005; O'Connell, 1992). Moreover, alternative medicinal plant cultivate has been found that exploiting these PGPR strains for the growth promotion could reduce that need for chemical fertilizers as well as the cost of cultivation.

Among different group of biofertilizers; nitrogen fixing and phosphorous solubilizing bacteria may be considered to be important since they improve plant nutrition. Plant Growth Prompting Rhizobacteria (PGPR) in the bio-fertilization of crops (Karlidag *et al.,* 2007) has been a well-known fact that these PGPR strains may promote growth either by fixation of atmospheric nitrogen or by solubilization, if minerals such as phosphorous (Safiullah Habibi *et al.,* 2014; Karthikeyan *et al.,* 2007; 2008; Rodriguez and Fraga, 1999; Sturz and Sudhakar *et al.,* 2000; Karlidag *et al.,* 2007) and they can also promote growth production of plant growth regulators (Lakshmi Priya *et al.,* 2015; Jaleel *et al.,* 2007).

2. Materials and Methods

2.1. Bacterial strains, Inoculation with Treatments

All bacterial strains used in the present study were isolated from the rhizosphere soil of *Acorus calamus, Azotobacter vinelandii*, grown in Waksman Base Medium for routine use and maintained in Waksman broth with 15 % glycerol. *Bacillus cereus* were grown on Picovskya medium for routine use and for long term storage they were maintained in nutrient broth with 15 % glycerol at 80ºC. *Pseudomonas fluorescens* King's B Agar Medium and *Enterobacter cloacae* grown in N free semisolid medium (NFb). They isolates were designated as *Azotobacter vinelandii* ACAzt-2, *Bacillus cereus* ACPb-3, *Pseudomonas fluorescens* ACPf-4 and *Enterobacter cloacae* ACEc-1. For each experiment a single colony was transferred to 500 ml flasks containing Waksman base no.77 broth, Picovskya medium, King's B broth and NFb broth grown aerobically in flasks on a rotating shaker (150 rpm) for 48 hrs. The bacterial suspensions were than diluted in sterile water to a final concentration of 10^9 CFU/ml, and the resulting suspensions were treated with *Acorus calamus* plants and control plants were dipped in sterile water.

2.2. Field trail condition

The seedling of *Acorus calamus* was raised in the pot culture yard, Department of Microbiology, Faculty of Agriculture, Annamalai University in the year experiment was conducted during March 2013 to February 2014. For planting, 15 to 20 cm long terminal transplanting in to two pairs of plant were dipped in the PGPR inoculums and planted in the field (Fig - 1). There were five treatments as given below,

T_1 – *Azotobacter vinelandii*-ACAzt-2

T_2 – *Bacillus cereus*- ACPb-3

T_3 - *Pseudomonas fluorescens*- ACPf-4

T_4 – *Enterobacter cloacae* - ACEc-1

T_5 – Consortium (ACAzt-2, ACPb-3, ACPf-4 and ACEc-1)

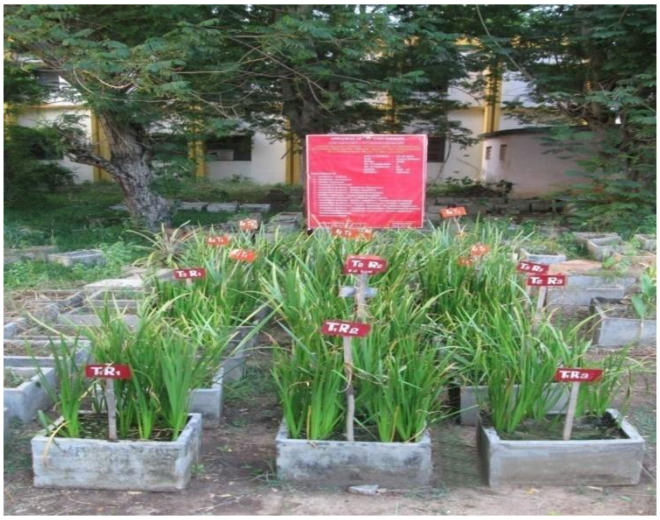

Fig - 1: Overall view on effect of PGPR inoculation on *Acorus calamus* Plant under pot culture

Five treatment plots (Four plants per pot) were prepared and irrigated immediately for a better accommodation. Three replications were maintained for each treatment subsequent irrigation was done daily water stored to keep the optimum moisture level of the soil. Growth promoting effects of bacterial treatments were evaluated by rhizome were recorded at final harvest 330 days.

3. Result and Discussion

The experiment was conducted to study the inoculation effect of single inoculants and consortium of inoculants preparations of PGPR isolates *viz.*, *Azotobacter* ACAzt-2, *Bacillus* ACPb-3, *Pseudomonas* ACPf-4 and *Enteobacter cloacae* ACEc-1 on the growth and yield of *Acorus calamus*. The plant height, number of rhizome, rhizome length and yield of *Acorus calamus.* All the growth parameters were recorded on 60,120, 180, 240, 300 and 330 DAP period of *Acorus calamus.*

3.1. PGPR strain inoculation on the growth and plant height of *Acorus calmus*

There was significant variation ($P \leq 0.05$) in plant height of *Acorus calamus* transplant treated with *Azotobacter, Bacillus, Pseudomonas* and *Enterobacter* obtained the maximum plant height at all sampling periods. The plant height of *Acorus calamus* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_5) recorded in at 330 DAP the maximum plant height of 95.05 cm plant^{-1} followed by treatments T_1, T_3, T_4 and T_2 respectively.

Among the single inoculant treatments of T_1 recorded the higher plant height of 90.07 cm plant^{-1} respectively followed by T_3 recorded the plant^{-1} height of 88.09 cm plant^{-1}, T_4 recorded the plant height of 87.00 cm plant^{-1}, T_2 recorded the plant height of 85.09 cm plant^{-1} on 330 DAP. The uninoculated treatment 68.01 cm plant^{-1}recorded the minimum plant height (Table - 1).

3.2. PGPR strain inoculation on the growth and number of rhizome in *Acorus calmus*

There was significant variation ($P \leq 0.05$) in number of rhizome per plant of *Acorus calamus* transplant treated with *Azotobacter, Bacillus, Pseudomonas* and *Enterobacter* obtained the maximum number of rhizome per plant at all sampling periods. The plant height of *Acorus calamus* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_5) recorded in at 330 DAP the maximum number of rhizome per plant of 16.0 cm plant^{-1} followed by treatments T_1, T_3, T_4 and T_2 respectively. Among the single inoculant treatments of T_1 recorded the higher number of rhizomes 13.5 per plant^{-1} respectively followed by T_3 recorded the number of rhizomes 12.9 per plant^{-1}, T_4 recorded the number of rhizomes 12.4 per plant^{-1} and T_2 recorded the number of rhizomes 11.2 per plant^{-1}. The uninoculated control treatment (T_6) recorded the minimum number of rhizomes of 8.4 per plant^{-1} in *Acorus calamus* (Table - 2).

3.3. PGPR strain inoculation on the growth and rhizome length in *Acorus calmus*

There was significant variation ($P \leq 0.05$) in rhizome length per plant of *Acorus calamus* transplant treated with *Azotobacter, Bacillus, Pseudomonas* and *Enterobacter* obtained the maximum number of rhizome per plant at all sampling periods. The rhizome length of *Acorus calamus* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_5) recorded in at 330 DAP the maximum rhizome length per plant of 45.00 cm plant^{-1} followed by treatments T_1, T_3, T_4 and T_2 respectively. Among the single inoculant treatments of T_1 recorded the higher rhizome of 42.00 cm plant^{-1} respectively followed by T_3 recorded the rhizomes length of 40.09 cm plant^{-1}, T_4 recorded the rhizomes length of 38.09 cm plant^{-1} and T_2 recorded the rhizomes length of 37.01 cm plant^{-1}. The uninoculated control treatment (T_6) recorded the minimum rhizome length of 26.03 cm plant^{-1} in *Acorus calamus* (Table - 3).

Table – 1: Effect of plant growth promoting rhizobacteria (PGPR) inoculation on plant height or *Acorus calamus* under pot culture condition

S. No	Treatment	Plant height (cm)/plant^{-1}					
		60 DAP	120 DAP	180 DAP	240 DAP	300 DAP	330 DAP
1.	T_1 - *A. vinelandii*	29.00	38.00	54.08	66.04	89.03	90.07
2.	T_2 - *B. cereus*	24.03	34.02	48.02	60.06	83.05	85.09
3.	T_3 - *P. fluorescens*	27.05	37.05	52.04	64.08	88.05	88.09
4.	T_4 - *E. cloacae*	26.09	36.08	50.09	62.06	85.07	87.00
5.	T_5 – ACAzt + ACPb + ACPf + ACEc	30.08	42.03	57.02	69.04	93.02	95.05
6.	T_6 - Uninoculated (Control)	17.08	25.06	34.04	44.08	56.09	68.01

Table – 2: Effect of plant growth promoting rhizobacteria (PGPR) inoculation on number of rhizome per plant of *Acorus calamus* under pot culture condition

S. No	Treatment	Number of Rhizome/plant^{-1}			
		180 DAP	240 DAP	300 DAP	330 DAP
1.	T_1 - *A. vinelandii*	9.1	11.1	12.8	13.8
2.	T_2 - *B. cereus*	7.0	8.6	10.2	11.2
3.	T_3 - *P. fluorescens*	8.5	10.7	11.7	12.9
4.	T_4 - *E. cloacae*	8.5	9.8	11.0	12.4
5.	T_5 – ACAzt + ACPb + ACPf + ACEc	11.9	13.1	15.9	16.0
6.	T_6- Uninoculated (Control)	5.4	6.7	7.0	8.4

Table – 3: Effect of plant growth promoting rhizobacteria (PGPR) inoculation on number of rhizome length per plant of *Acorus calamus* under pot culture condition

S. No	Treatment	Rhizome length (cm) / plant^{-1}			
		180 DAP	240 DAP	300 DAP	330 DAP
1.	T_1-*A. vinelandii*	19.09	29.03	39.08	42.00
2.	T_2-*B. cereus*	15.01	24.04	34.04	37.01
3.	T_3-*P. fluorescens*	17.09	26.04	37.05	40.09
4.	T_4-*E. cloacae*	16.05	25.07	35.08	38.09
5.	T_5- (ACAzt+ ACPb+ ACPf+ ACEc)	22.07	35.04	43.08	45.00
6.	T_6- Uninoculated (Control)	11.06	17.06	25.08	26.03

3.4. Effect of PGPR inoculation on the growth rhizome biomass yield of *Acorus calamus*

There was significant variation ($P \leq 0.05$) in rhizome wet weight and dry weight per plant of *Acorus calamus* transplant treated with *Azotobacter*, *Bacillus*, *Pseudomonas* and *Enterobacter* obtained the maximum number of rhizomes per plant at all sampling periods. The rhizome wet weight and dry weight of *Acorus calamus* significantly increased due to the inoculated PGPR strains. The PGPR consortium treatment (T_5) recorded in at 330 DAP, the maximum rhizome wet weight and dry weight per plant of 75.011 and 37.893 g plant^{-1} followed by treatments T_1, T_3, T_4 and T_2 respectively. Among the single inoculant treatments of T_1 recorded the higher rhizome wet weight and rhizome dry weight of 69.289 and 29.975 g plant^{-1} respectively, followed by T_3 recorded the rhizome wet weight and rhizome dry weight of 63.900 and 25.088 g plant^{-1}, T_4 recorded the rhizome wet weight and rhizome dry weight of 59.353 and 21.537 g plant^{-1} followed by T_2 recorded the lower rhizome wet weight and rhizome dry weight of 56.444 and 18.591 g plant^{-1}. The uninoculated control treatment (T_6) recorded the minimum rhizome wet weight and rhizome dry weight of 29.007 and 10.219 g plant^{-1} in *Acorus calamus* (Fig - 2).

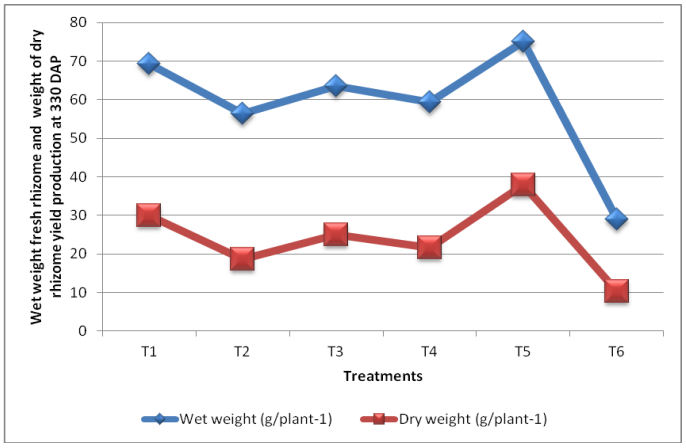

Fig-2 Effect of plant growth promoting rhizobacteria (PGPR) inoculation on rhizome biomass yield (g/plant^{-1}) of *Acorus calamus* under pot condition

Root inoculation with PGPR promoted significant increase in growth and yield content but the growth response varied between different rhizobacterial strains. However in general the growth response was found to be enhanced when the PGPR strains were applied in combination. This growth response was more effective in terms of an increased plant growth and yield compared to control. Earlier reports had shown that combined inoculation of sorghum with, *Azotobacter vinelandii*, *Bacillus cereus*, *Pseudomonas fluorescens* and *Enteobacter cloacae* significantly increased grain yield. The stimulatory effects of this PGPR strains on the yield and growth of these crops were attributed to the N_2 fixation ability, plant growth regulator production and phosphate solubilizing capacity (Priya, 2010; Rajasekar and Elango, 2011).Therefore, the rhizobacteria are the dominant deriving forces in recycling the soil nutrients and consequently, theyare crucial for soil fertility (Glick, 2012).Currently, the biological approaches for improving crop production are gainingstrong status among agronomists and environmentalists followingintegrated plant nutrient management system (Munees Ahemad and Mulugeta Kibret. 2014).

4. Conclusion

From the result of this experiment, it has been found that wider spacing had positive influence on plant height, rhizome length, number rhizome and wet weight and dry weight/ plant^{-1}. The combination of microbial consortium strains was found to have a great potential for use as bio-inoculants to increase production in medicinal plants and other crops.

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