

Evaluation of Antagonistic Potential and Induction of Flowering in *Anthurium Andreanum* L. by Indigenous *Pseudomonastaiwanensis*

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Abstract – Bacteria belonging to *Pseudomonas* spp. from anthurium rhizosphere were studied for antagonistic potential of isolates against blight disease of anthurium caused by *Xanthomonas axonopodis* pv. *dieffenbachiae*. These rhizobacteria were also evaluated for growth promotion properties with the aim of increasing anthurium flower production. Two of the highly efficient isolates P1 and P9 were selected for pot studies to evaluate disease suppression and flowering induction properties. P1 and P9 were identified as *Pseudomonastaiwanensis* (Microbial type culture collection center: MTCC11631) with their respective gene bank accession numbers KM576801 and KM576802. Application of P9 and P1 resulted in disease reduction to 85.0% and 80.0% respectively compared to chemical controls mancozeb 57.91% and phytolan 54.16% at 35 days after inoculation. Treatments with these rhizobacteria improved flower production without any significant changes in biomass. Foliar application of P1 and P9 resulted in significantly higher number of flowers produced (1.6 and 1.25 respectively). Foliar spray of GA₃ 100 ppm resulted in the production of largest spathe size compared to all other treatments (10.58 cm). The results confirm the promising applications of the selected bacterial isolates in the biological control flower induction of *Anthurium andreanum* L. The isolates exhibited aminocyclopropane carboxylic acid (ACC) deaminase activity, gibberellic acid and biosurfactant production in addition to siderophore production and phosphate solubilization.

Keywords – *Anthurium Andreanum*, Bio Control, Flowering Induction, *Pseudomonas Taiwanensis*.

I. INTRODUCTION

Floriculture is a highly dynamic sector in world economy due to a steady demand on classic and cut flowers and essential oils. Indian floriculture economy is rapidly growing over the years and 22, 485.21 metric tonnes of flowers worth 4559 million were exported from India in 2013-14 [1].

Anthurium (Anthurium andreanum L.) belonging to the Araceae family are perennial herbaceous epiphytes with a climbing habitat. Among tropical flowers, its trade value is next to orchids and its commercial value has increased in recent years in Asian countries as well as in Europe. Possibility of a variety of shapes and colors of the spathe, candle shaped spadix covered with male and female flowers and longer vase life of the spathe are the main attractions in marketing. Production of anthurium in the world is threatened by bacterial and fungal diseases leading to a considerable decrease in production and

economic loss. Bacterial blight of anthurium (ABB), which is caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* remains the highest limiting factor in anthurium cultivation and production [5]. Blight affected flowers get deformed and fall off easily and the systemic infection leads to complete death of plants. ABB was first reported in Hawaii in 1972 and reached epidemic proportions in 1985-89 in Hawaii. The disease subsequently has been found in the different anthurium cultivating regions including India [31, 32]. First described as a pathogen of *Dieffenbachia* species, *X. axonopodis* pv. *dieffenbachiae* can infect a broad range of plants in the Araceae (aroids) family and is considered a quarantine organism in the European Union and in major anthurium-producing countries that are still free of the pathogen [16]. Other than chemical control using carbendazim® and copper based fungicides and antibiotics, the management strategies for ABB consist principally of prevention, sanitation, cultural practices and the use of axenically propagated plants.

The increased awareness on environmental concern over antibiotic and pesticides has resulted in a large upsurge of biological disease control. Fluorescent *Pseudomonas* and related species have been implicated in the control of several bacterial and fungal pathogens. Periodical reports of *Pseudomonas* spp. giving protection to plant diseases in green house environment are available [39, 18]. The bio control strategies adopted in greenhouse conditions differ considerably from that of field conditions as viewed by [27].

For uniformity of flower production, and reproduction purposes, qualitative and quantitative improvement as well as to meet the demand from the industry, application of new techniques especially phytohormone in crop plants and ornamental plants have been practiced. Application of GA₃ and varying light intensities were proved to improve flower production of anthurium in previous attempts [22, 21]. Potential phytohormone producing PGPR have been used to induce flowering in crop plants as well as ornamental plants [23]. In recent years the need of plant growth promoting rhizobacteria, found in rhizosphere soil and phyllosphere stimulating plant growth by various mechanisms have been well established [3].

The present study is an attempt to evaluate the potential of indigenous rhizobacterial *Pseudomonas* spp. isolates to decrease the severity of bacterial blight disease of anthurium and to enhance flower production and growth of *Anthurium andreanum* var. *tropicalred*. Since studies on

this aspect from *Anthurium andreanum* is very limited, the study is an insight into the future potential green house cultivation.

II. METHODOLOGY

2.1. Isolation and Identification of Bacterial Blight Pathogen *Xanthomonas Axonopodis* pv. *dieffenbachiae* (Xad).

Pathogenic bacteria were isolated from diseased anthurium leaves collected from various anthurium cultivating regions of Kerala. Samples were numbered as to sample location and cultivar and placed into individual plastic bags to avoid cross contamination. The pathogenic bacteria was isolated with standard isolation techniques using YDCA (Yeast extract, dextrose calcium carbonate) and YPGA (yeast extract, peptone, and glucose) selective medium. Secondary screening was performed using cellobiose starch methionine medium (CS) with 0.001% triphenyl tetrazolium chloride added [34]. Identification was achieved based on biochemical reactions, pigmentation, growth on differential and selective medium, and molecular characterisation.

2.2. Isolation of Rhizosphere Bacteria from Soil and Screening for Antagonistic and Plant Growth Promoting Characteristics

Rhizosphere soil samples collected from different anthurium cultivation areas were brought to laboratory. The roots were washed to remove adhering soil particles, cut into small pieces and surface sterilized using HgCl_2 and vortexed in a shaker for 20 min. at 250rpm in 250 ml flasks. The suspension was serially diluted and plated on nutrient agar and Kings B medium for specific detection of pigment producing *Pseudomonas* species. The plates were incubated at 28°C for 24-48 hrs. and isolates were transferred to Kings B agar and stored until further use.

2.2.1. Production of Siderophore

Each isolate was inoculated in King's B medium and incubated for 24 – 48hrs. at 28°C. Cultures were centrifuged at 1000rpm for 15min. To 1.0 ml of culture supernatant, a pinch of tetrazolium chloride was added [41] and observed for a change to deep red colour. Isolates showing positive siderophore production were further grown in succinate broth (KH_2PO_4 6.0 g/l, K_2HPO_4 3.0 g/l, $(\text{NH}_4)_2\text{SO}_4$ 1.0 g/l, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g/l, succinic acid 4.0g/l) for 48 hrs. at 28°C and absorbance was determined at 400nm (HITACHI UV 5100). Quantification of the pyoverdine was performed using $\epsilon = 20000$, $\lambda_{\text{max}} = 400\text{nm}$. High performance thin layer chromatography (HPTLC) was performed to analyze the siderophore produced by the isolates. The rhizo bacterial cultures were grown in iron free succinate medium for 48 hrs. at 28°C. The broth was centrifuged at 1000rpm for 15min, supernatant acidified to pH 2.0 and extracted with ethyl acetate. The ethyl acetate was evaporated and dried residue was dissolved in methanol. HPTLC was performed using toluene: ethyl acetate (9:1) with a saturation time of 30 min. After development up to 85mm, the plates were dried for 5 minutes and the spots were observed under Hitachi UV 5100 at 366nm [30].

2.2.2. Phosphatesolubilization

Phosphate solubilization ability of isolates was determined by inoculating on to Pikovskayas' medium and observing zone of clearance around the inoculated area. Phosphate solubilisation efficiency (SE %) of the selected isolates were calculated as percentage of phosphate solubilization [34].

2.2.3. Production of Gibberellic Acid

The gibberellic acid production by rhizobacterial isolates was determined by following [8]. 100 ml of King's B broth was inoculated with isolates and incubated at 37°C for seven days. After seven days of incubation, the culture was centrifuged at 8000 rpm for 10 min and 15 ml of the culture was pipetted out separately into the test tubes. 2.0 ml of zinc acetate solution (21.9 g of zinc acetate dissolved in 80 ml of distilled water, one ml of glacial acetic acid and the volume made up to 100 ml with distilled water) was added. After 2 min, 2 ml of potassium ferrocyanide solution (10.6 g of potassium ferrocyanide in 100 ml of distilled water) was added and centrifuged at 8000 rpm for 10 min. 5 ml of supernatant was added to 5ml of 30 % HCl and the mixture was incubated at 27°C for 75 min. The blank was prepared with 5% HCl. Absorbance was measured at 254 nm in a Hitachi UV 5100 spectrophotometer. From the standard graph prepared by using gibberellic acid solutions of known quantities, the amount of GA_3 produced by the culture was calculated and expressed as $\mu\text{g } 25 \text{ ml}^{-1}$ broth.

2.2.4. Production of Indole Acetic Acid

Isolates were grown in nutrient broth with 1% tryptophan. Salkowskys reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl_3 solution) was added to the supernatant to detect the presence of indole acetic acid [2].

2.2.5. Production of Biosurfactant

The isolates were grown in mineral salt medium for 8 days at 28°C and production of biosurfactant was detected using emulsification ability. The isolates showing emulsification were tested for emulsification index (E24) [10]. The culture was centrifuged at 1000rpm for 15 mins. 2.0ml of culture supernatant was mixed with 2.0ml of kerosene and vortexed for 2 min. The tube was left undisturbed for 24 hrs. and afterwards, observed for stable emulsification. Emulsification index was calculated as percentage of stable emulsification.

The presence of carbohydrate groups in the biosurfactant molecule was assayed by rhamnose test [13]. A volume of 0.5 ml of cell supernatant was mixed with 0.5 ml of 5% phenol solution and 2.5 ml of sulphuric acid, and incubated for 15 min before measuring absorbance at 490 nm (Hitachi UV 5100 spectrophotometer).

2.2.6. Production of ACC Deaminase

The ACC deaminase production of the isolates was screened [15]. For this, the isolates were inoculated on to DF minimal salt medium (KH_2PO_4 4.0 g, Na_2HPO_4 6.0 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g, H_3BO_3 10 μg , MnSO_4 10.0 μg , ZnSO_4 70.0 μg , CuSO_4 50.0 μg , MoO_3 10.0 μg , Glucose 2.0 g, gluconic acid 2.0 g, citric acid 2.0 g, agar 12.0 g, distilled water 1l) amended with 2 g/l ammonium sulphate. The presence of bacterial growth in

the media after incubation was considered as positive result.

2.3. Identification of the Screened Isolates

The selected isolated strains were subjected to identification based on morphological, biochemical characteristics and genotypic characteristics by 16S rDNA typing. Genomic DNA was isolated from the 24hr. bacterial culture in L.B broth [45] and the quality of the isolated DNA was evaluated on 0.8% Agarose Gel. 16S rDNA fragment was amplified by PCR using 16S rDNA universal primers 10-30 F: 5'-GAG TTT GAT CCT GGC TCA G-3', 530R: 5'-G(AT)A TTA CCG CGG CGG CTG-3' [46]. PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube. AGE analyse of the PCR product was done using 100BP ladders and purified using column purification. The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI3730xl Genetic Analyser (Applied Biosystems, USA). Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequence at the 3' and 5' ends (considering peak and Quality Values for each base) using the sequence analysis tools (Applied Biosystems). The sequence was analysed using the BLAST (www.ncbi.nlm.nih.gov) search algorithm and aligned to their nearest neighbours. The sequence was deposited in the NCBI GenBank database.

2.4. Screening for Antagonism

Pseudomonas taiwanensis isolates indigenous to anthurium rhizosphere were inoculated into 100ml Kings B broth and incubated for 24 hrs. at 28°C. The blight pathogen was inoculated into 100ml nutrient broth and incubated for 4-7 days at 28°C. In vitro antagonism was performed by dual plate assay and observed zone of inhibition. A filter paper disc soaked with pathogenic bacterial suspension was placed on the centre of nutrient agar medium seeded with rhizobacterial suspension.

Two *Pseudomonas* isolates P1 and P9 with highest in vivo antagonistic potential were tested for their efficiency to control bacterial blight of anthurium and growth promotion properties in the green house.

Seven month old tissue culture anthurium plants of Tropical red variety were used for the study. Plants grown in poly bags were transplanted into 10 kg pots filled with coarse gravel, and potting mixture. Soil amendments including N: P₂O₅:K₂O at 20:20:20 were performed at two weeks intervals. *Xanthomonas* isolate Xad was inoculated into nutrient broth to achieve a cell concentration of 10⁷-10⁸ cfu/ml. The rhizosphere isolates and standard culture of *Pseudomonas fluorescens* were inoculated into Kings B broth and incubated at 28°C for 2 days to allow a cell concentration of 10⁷ to 10⁸ cfu/ml. The suspensions were centrifuged and the cells dispensed in phosphate saline (pH7.0). The pots were arranged in the shade house adopting a completely randomised design with five replications with 8 treatments. Seven-month-old plants were spray inoculated with 24hr. old rhizobacterial suspensions (10⁶cfu/ml) followed by soil drenching. A commercial product of *Pseudomonas fluorescens* was used at a concentration of 0.3%. Dithiocarbamate fungicide

Mancozeb® (0.3%) and copper fungicide phytolan® (0.4%) were applied as soil treatments. The plants were spray inoculated with a suspension of the blight pathogen 2 days after soil treatment. The plants were covered with polythene to maintain humidity. Disease index (DI) and disease reduction were recorded at 35 days after challenge inoculation.

2.5. Green House Evaluation for Induction of Flowering

The plants were acclimatized and the pots were arranged in completely randomized manner with 8 treatments and 5 replications. 24hrs. old rhizobacterial suspensions of isolates P1, P9 and standard *Pseudomonas fluorescens* (10⁶cfu/ml) in phosphate buffer were applied as soil drenching followed by foliar spray. 1.0 ml of commercial formulation of gibberellic acid was diluted to 1.0 l and applied as single foliar spray. GA₃ 100 ppm solution was applied as single foliar spray. Plants sprayed with sterile phosphate buffer served as control. Observations including plant height, number of leaves in each plant, number of flowers in each plant and spathe size (cm) were recorded at 120 days after inoculation.

2.6. Statistical Analysis

Statistical analysis was performed using SAS system; Analysis of variance (ANOVA) means were separated by Duncan's multiple range test.

III. RESULT AND DISCUSSION

3.1. Isolation and Identification of Blight Pathogen *Xanthomonas Axonopodis pv. dieffenbachiae* (Xad)

Isolates obtained from anthurium rhizosphere were identified by 16S rDNA sequencing followed by BLAST analysis.

3.2. Isolation and Screening of Rhizobacteria for Antagonism and Growth Promotion Characteristics

Gram negative bacteria isolated from anthurium rhizosphere belonging to the fluorescent pseudomonads group were screened for antagonism against the pathogen and growth promotion ability. In vitro studies indicated that the rhizobacterial strains could inhibit the pathogen in solid phase (Table 1). 10 isolates inhibited the growth of Xad on nutrient agar. P1 and P9 showed highest (11cm and 18cm respectively).

Varying degrees of phosphate solubilization, production of siderophores, gibberellic acid, biosurfactant and IAA were shown by the isolates as recorded in Table II. Based on the plant growth promoting characteristics, two isolates P1 and P9 were selected for further studies. Biocontrol and growth promotion characteristics of the two isolates are shown in Table III. Hydroxamate nature of siderophore was determined by tetrazolium salt test which resulted in a red colour formation. HPTLC was performed to detect the fluorescent nature of the siderophore under UV illumination at 366nm where RF=0.44 and compared with a standard strain obtained from Tamilnadu Agricultural University (Fig.1).

Table 1. In vitro growth inhibition of *Xanthomonas axonopodis* pv. *dieffenbachiae* isolate by *Pseudomonas* spp.

Isolates	Zone of Inhibition (mm)
P 1	11.0±1.2
P 2	8.3±0.76
P 3	7.0±2.76
P 4	8.0±0
P 5	10.8±4.21
P 6	0.0±0
P 7	9.0±1.18
P 8	10.0±2.66
P 9	18.6±7.17
P 10	7.83±0.21

Values are represented as ±SD.

Table 2. Antagonistic and growth promoting characteristics of the isolates

Isolates	Siderophore	HCN	GA	IAA	Biosurfactant	Phosphate Solubilisation	ACC deaminase
P1	+	+	+	-	+	+	+
P2	-	-	-	+	+	-	+
P 3	+	+	-	-	-	+	+
P 4	+	+	-	+	-	+	-
P 5	+	+	-	+	+	+	-
P 6	+	+	-	+	-	+	+
P 7	+	+	-	-	-	+	+
P 8	+	+	-	-	-	+	-
P 9	+	+	+	-	+	+	+
P 10	+	+	-	-	-	+	-

The quantity of siderophore produced by P1 and P9 was estimated at 60 hours of incubation and found to be 137.97 and 120 µM respectively without any significant difference between the isolates. Phosphate solubilisation ability (ES %) of the isolates P1 and P9 were recorded as 36.51 and 24.54 respectively. Gibberellic acid production of P1 and P9 was estimated to 1.42µg/25ml and 1.56 µg/25ml respectively. There is no significant difference of gibberellic acid production between P1 and P9. Growth of both P1 and P9 were observed in medium with 0.2% ammonium sulphate, indicating ACC deaminase activity. Emulsification index (E24) was calculated after 8 days. P1 showed 55.53% and P9 showed 65.53% emulsification in kerosene. No significant change was observed with the isolates. The glycolipid nature of the biosurfactant was established by rhamnase assay and observing the absorption at 490nm.

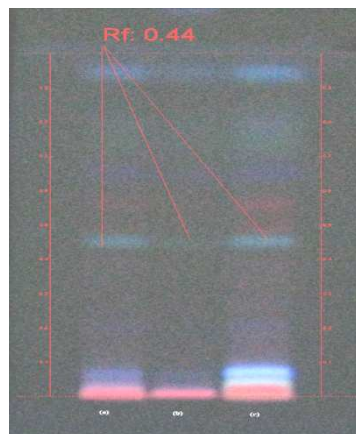


Fig. 1.

HPTLC of Siderophore components marked at Rf=0.44. (a)=P1, (b)=P9, (c)=*Pseudomonas fluorescens*

Table 3. Characteristics of the isolates P1 and P9

Isolates	Siderophore (400nm)	Phosphate solubilisation (SI)%	Biosurfactant production (EI %)	Gibberellic acid (µg/25ml)
P1	*135.97±0.66	36.51± 29.07	55.53± 4.79	6.08 ±0.33
P9	120.97± 0.47	24.54± 26.54	65.53± 5.23	6.9 ±0.72

* Values are ±SD

3.3. Identification of the Selected Isolates

The PCR amplification of the 16S rDNA of the isolated bacteria was confirmed by agarose gel electrophoresis. BLAST analysis was conducted for the sequences

obtained and the isolates P1 and P9 were 99.9 % (KM576801) and 99.8 % (KM576802) similar to *Pseudomonas taiwanensis*. The isolate P9 was deposited in Microbial Type Culture Collection Centre (MTCC11631)

[35, 26]. *Pseudomonasmonteilii*, *Pseudomonasmosselii* and *Pseudomonasplecoglossicida* are the most related species [15, 11, 28].

3.4. Greenhouse Evaluation of Disease Index and Disease Reduction

Physical properties of the soil used for the experiment were recorded in Table III. The soil had pH: 5.0, EC: 0.04, 0.28% organic carbon, 80.6 kg/ha K and 15 kg/ha P. Both the isolates reduced blight symptoms. Protection provided by P1 (80.0 %) and P9 (85.0%) was more than that of chemical controls. Mancozeb and phytolan reduced disease to 57.91% and 54.16%. Application of P1 prevented spreading of disease symptoms after 1 week of challenge inoculation whereas disease development in P9 applied plants was observed up to 3 weeks. Phytolan was able to prevent further infection after second week whereas disease development in mancozeb and commercial formulation applied plants was observed up to 5 weeks. All treatments were successful in preventing mortality of the diseased plants compared to control.

Table 4. Disease index and disease reduction on *Anthuriumandreanum* - tropicalred

Treatments	DI(%)	DR(%)
<i>P.taiwanensis</i> (P1)	16.00 ^{bc}	80 ^{ab}
<i>P.taiwanensis</i> (P9)	12.00 ^{bc}	85.00 ^{ab}
Mancozeb®	35.66 ^{bc}	57.91 ^{bc}
Phytolan®	36.66 ^{bc}	54.16 ^{bc}
Commercial <i>P.fluorescens</i>	55.00 ^{ab}	36.25 ^{cd}
<i>P.fluorescens</i>	47.05 ^{ab}	48.75 ^{bc}
Inoculated Control	84.00 ^a	0 ^d
Uninoculated Control	0 ^c	100 ^a

Means with the same letter are not significant at 5% level.

3.5. Green House Evaluation for Induction of Flowering

Growth promotion and flowering enhancement effect of the PGPR isolate were determined by measuring plant height (cm), number of leaves, number of flowers and spathe size (cm) and the data is represented in Table V.

Foliar spray of GA₃ 100ppm resulted in production of largest spathe size compared to all other treatments (FigIII). Significant increases in anthurium flower production were observed in greenhouse experiment followed by the application of the selected rhizosphere bacteria P1 and P9 (1.6 and 1.25) without significant effect on plant height and number of leaves (FigIII). Supplementation of commercially formulated gibberellic acid with P9 further improved flower production.

The group of fluorescent pseudomonads are reported to be the dominant of rhizobacteria associated with plants. This group of rhizosphere bacteria are well known for their diversity and rhizosphere effect [9]. Biological control of bacterial blight was previously reported [17]. A bacterial community isolated from the guttation fluids of anthurium consisting of *Sphingomonaspaucimobilis*, *Brevundimonasvesicularis*, *Microbacterium* spp. showed antagonism against the pathogen and prevented the entry of the pathogen when applied as foliar spray. In a short communication [12], usage of chemicals for blight disease control was reported. Use of turmeric powder and streptomycin after 5 rounds of spraying proved to be 100% effective in controlling blight disease. *Bacillus* spp. B014 was isolated from an anthurium plant with antagonistic effect on the blight pathogen and foliar application of the isolates reduced leaf lesions to 17.86% and 28.57% respectively [25]. *Bacillus subtilis* EPB14 reduced disease in greenhouse experiments [24]. The difference in antagonistic potential of P1 and P9 could be owing to their genetic variability. The efficiency of the antagonistic bacteria to inhibit the pathogen growth indicate that the cultures can effectively suppress disease by establishing themselves at the site of infection. Inhibition of the pathogen occurred due to the effect of antimicrobial metabolites including HCN and siderophore produced by the isolates P1 and P9. Hydroxamate type of siderophore produced by fluorescent

Pseudomonads contribute in disease suppression by depriving the pathogen of the available iron in the surroundings. Siderophores mediated immune response is a well-studied mechanism and more insight into the unique phenomenon of iron scavenging and immunity activation is provided by [6].

Table 5. Effect of PGPR on Growth and Flowering in *Anthuriumtropicalred*

Treatments	No: of flowers	Spathe size	No: leaves	Plant height
<i>P.taiwanensis</i> (P1)	*81.60 ^a	8.16 ^{abc}	3.80 ^a	23.46 ^{bc}
<i>P.taiwanensis</i> (P9)	1.25 ^{ab}	8.32 ^{bc}	4.40 ^a	22.52 ^{bc}
GA ₃ ®	0.80 ^{bc}	8.12 ^{bc}	4.80 ^a	20.48 ^c
P1+CGA®	1.60 ^a	7.23 ^{bc}	5.10 ^a	22.86 ^{bc}
P9+CGA®	1.60 ^a	8.80 ^{ab}	4.60 ^a	28.56 ^b
<i>P.fluorescens</i>	0.60 ^c	7.05 ^{abc}	4.00 ^a	17.98 ^c
GA ₃	1.25 ^{abc}	10.58 ^a	3.80 ^a	34.00 ^a
<i>P.fluorescens</i> +GA ₃	0.81 ^{bc}	7.53	4.80 ^a	28.96 ^{ab}
Uninoculated Control	0.60 ^c	7.80 ^{bc}	3.60 ^a	20.00 ^{ca}

*Values are means of 5 replications. Means with the same superscript letters are not significant at 5% significant level

The results of flowering experiment are in agreement with earlier observations [20]. Similar reports include regulation of flowering in *Arabidopsis* by gibberellic acid and in anthurium 'amazone' and 'rennate' varieties [7, 21].

Inability of the isolates to produce Indole acetic acid resulted in less growth or vegetative activity of the plants in terms of height and number of leaves, whereas

production of gibberellic acid resulted in higher reproductive effort of the plant.

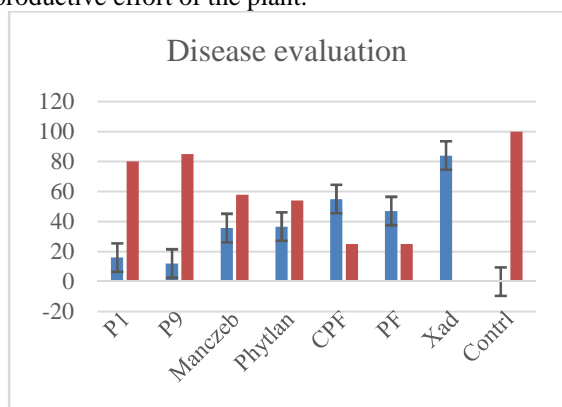


Fig. 2.

Disease index and disease reduction in *Anthurium andreanum*. Values are represented as \pm SE. P1= *P. taiwanensis*; P9=*P. taiwanensis*; CPF=commercial *P. fluoreescens* (CGa); PF=*P. fluoreescens*; Xad=*X. axonopodis pv. dieffenbachiae*

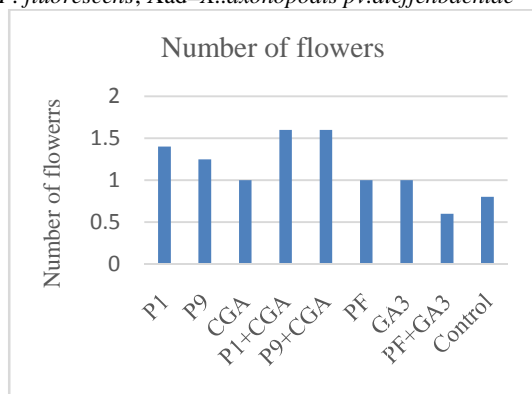


Fig. 3.

Flower production in *Anthurium andreanum* after pgpr and GA treatments. Values are represented as \pm SE. P1= *P. taiwanensis*; P9=*P. taiwanensis*; CGA =commercial gibberellic acid; PF=*P. fluoreescens*; GA3=Gibberellic acid

Root colonization and establishment in the phyllosphere of beneficial plants by microbial community belonging to *Pseudomonas*, *Bacillus* and other species require ability to adhere, establish and proliferate. Biosurfactant producing rhizobacteria effectively achieve root colonization which leads to improved plant nutrition, flowering and disease resistance [4].

Enhanced flowering observed in the current greenhouse experiment is attained by the availability of soluble phosphate, uptake of iron mediated by siderophores produced by the rhizobacteria and gibberellic acid. Phosphorus is the second important macronutrient required for plants and phosphate solubilization is considered as one of the most important requirement to be an effective PGPB. Enhanced flowering and growth was attained in *Gerbera jasmoni* after application of arbuscular mycorrhizal fungi, *Pseudomonasfluoreescens* and superphosphate [23]. Microbial production of ACC deaminase leads to an ethylene zinc which leads to reduced ethylene stress [19].

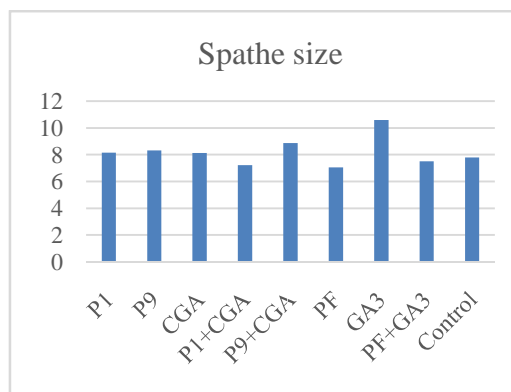


Fig. 4.

Spathe size in cm. in *Anthurium andreanum* after pgpr and GA treatments. Values are represented as \pm SE. P1= *P. taiwanensis*; P9=*P. taiwanensis*; CGA =commercial gibberellic acid; PF=*P. fluoreescens*; GA3=Gibberellic acid

Presence of ACC deaminase indicated by ability to grow in 0.2% ammonium sulphate by the selected isolates. The results of these experiments show that the rhizosphere isolates P1 and P9 are potential rhizobacteria which could be recommended as suitable for suppression of blight disease and induction of flowering in anthurium.

IV. CONCLUSION

The existence of plant beneficial *pseudomonas* community in the rhizosphere, their establishment, rhizosphere functioning and interaction with other microbial communities has led to a significant development in plant protection and growth. A detailed study of the interaction of the above described rhizobacteria with other crop plants will help us understand the potential of these bacteria in their commercial use.

V. ACKNOWLEDGMENT

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