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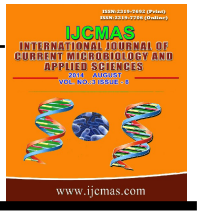

Original Research Article

Effect of Plant Growth Promoting Rhizobacteria (PGPR) on *Coleus forskohlii*

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A B S T R A C T

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive alternative to chemical fertilizers, pesticides, and supplements. The authors have isolated and characterized several PGPR strains from the rhizosphere soil of different medicinal plants from various locations in Andhra Pradesh. The present study pertains to the effect of three plant growth promoting rhizobacterial strains i.e., *Pantoea* sp. (Cf 7) and *Pseudomonas* sp. (Te 1, Av 30) in individual or in combinations treatments (T1–Cf7; T2–Te1; T3–Av30; T4–Cf7+Te1; T5–Te1+Av30; T6–Cf7+Av30; T7–Cf7+Te1+Av30; T8–Control) on *Coleus forskohlii*. The combinations of above mentioned PGPR strains significantly increased plant growth parameters (shoot length, number of branches, number of tubers, diameter of tuber and total biomass) of *Coleus* in field conditions in comparison to the control. The results of this study suggest that PGPR applied in combination have the potential to increase the plant growth of *C. forskohlii*. The present findings suggest that the PGPR isolates Cf7, Te1 and Av30 are beneficial for Coleus cultivation as they enhanced plant growth, total biomass; and as it provides an eco-friendly approach can be used as a biofertilizer.

K e y w o r d s

PGPR,
C. forskohlii,
Pantoea sp.,
Pseudomonas sp.,
Plant growth promotion

Introduction

Medicinal plants are known to be rich in secondary metabolites and are potentially useful to produce natural drugs. (Majid Pouryosef *et al.,* 2007). India has one of the richest plant medical cultures in the world. To protect these herbal medicinal plants in their natural habitat, systematic agrotechniques needs to be developed. The cultural practices have not been standardized for medicinal plants production and is being done as advocated in the report of Farooqi and Khan (1993).

Coleus forskohlii (Willd) Briq. [syn. *C. barbatus* (Andr.) Benth] is a plant that is being used since ancient times in Ayurvedic and traditional medicine. The root portion of the plant has been traditionally used for medicinal purposes and contains an active constituent, forskolin. Historically, it has

been used to treat hypertension, congestive heart failure, eczema, colic pain, respiratory disorders, painful urination, insomnia, and convulsions. Clinical studies of the plant and the forskolin constituent support these traditional uses, it also have therapeutic benefit in asthma, angina, psoriasis, and prevention of cancer metastases.

Plant growth promoting rhizobacteria (PGPR) are usually applied to a wide range of agricultural crops for the purpose of growth enhancement, including increased seed germination, plant weight, and harvest yields. PGPR colonization triggers plant growth by bacterial synthesis of plant hormones including indole-3-acetic acid, cytokinin, and gibberellins as well as by increased mineral and nitrogen availability in the soil. Some of them were also known to protect their host plant from pathogenic microorganisms. This PGPR activity is reported in species belonging to *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Alcaligenes*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Rodriguez and Fraga, 1999; Sturz and Nowak, 2000, Sudhakar *et al.,* 2000; Karlidag *et al.,* 2007) were reported to enhance plant growth. PGPR strains use one or more direct or indirect mechanisms to enhance the growth and health of plants. The use of microorganisms with the aim of improving nutrient availability for plants is an important practice and necessary for agriculture (Freitas *et al.,* 2007).

During the past couple of decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world. Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been repeatedly reported (Kloepper *et al.,* 1980; Seldin, 1984; Chen *et al.,* 1994; Zhang *et al.,* 1996; Amara and Dahdoh, 1997; Chanway, 1998; Pan *et al.,* 1999; Bin *et al.,* 2000, Figueiredo *et al.,* 2008). Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to certain plant species, cultivar and genotype (Bashan and Holguin, 1998; Gupta *et al.,* 2000; Lucy *et al.,* 2004). The role played by PGPR in relation to medicinal plants and their effect on the production of botanicals/alkaloids is an area remaining naive. This paper brings out the possible PGPR – medicinal plant interactions which could improve the potency of the medicinal plant, particularly the cultivated one.

However, reports are scarce regarding the bioinoculation effect of these PGPR strains in medicinal plants and particularly in *C. forskohlii*. Hence the present study was undertaken to investigate the growth promoting effects of *Pantoea* sp. and *Pesudomonas* sp. through *root* inoculation on plant height, root length, root girth and total biomass etc. in *C. forskohlii.*

Materials and Methods

Preparation of inoculums and culture conditions

All bacterial strains used in the present study were isolated from the rhizosphere of different medicinal plants (Malleswari and Bagyanarayana, 2013). The *Pantoea* sp. (Cf7) and *Pseudomonas* sp. (Te 1, Av 30) bacterial cultures were grown in King's B medium (KB) for routine use and maintained in King's B broth with 15% glycerol.

For each experiment a single colony was transferred to 500ml flasks containing KB grown aerobically in flasks on a rotating shaker (150 rpm) for 48h and centrifuged at 5,500 rpm for 7 min, the supernatant

discarded and the pellets containing bacterial cells were suspended in 250ml of 0.01M $MgSO_4$ solution. The colony-forming units of bacteria were 24×10^5 /ml inoculum (Boby and Bagyaraj, 2003). The resulting suspensions were treated with *Coleus* plants.

Bacterization of *Coleus forskohlii* cuttings and Growth conditions

Uniform sized, pencil-thick *Coleus* cuttings (12cm long) were selected and planted in poly bags containing 2 kg of 1:1 sterilized soil and sand. Before planting the cuttings bacterial inoculums 10 ml/bag was added to the planting hole as per the treatment. The cuttings with sterile water served as control.

The poly bags were kept on benches in moisturized conditions in glasshouse for 40 days for rooting and suitably watered. After 40 days, plants were removed and maintained in field conditions in order to harden the plants. Observations on plant height, number of branches and total biomass etc. of the Coleus plants were recorded at 60, 90, 150 and 180 days after planting (DAP).

Transplanting

Field experiment was conducted for *Coleus* at Botanical garden, Dept of Botany, Osmania University, Hyderabad. A completely randomized block design was maintained.

Hardened plants were transplanted to the main field. Before transplanting, the field was ploughed well and ridges were made 60cm apart. Plants were transplanted at 20cm spacing on the ridges. Planting was done with intact ball of earth without polythene cover. There were eight treatments as given below.

Treatments

PGPR treatments (Individual and Combinations; Cf 7, Te 1, Av 30)

T1 – Cf7
T2 – Te1
T3 – Av30
T4 – Cf7+Te1
T5 – Te1+Av30
T6 – Cf7+Av30
T7 – Cf7+Te1+Av30
T8 – Control

Cf 7 = *Pantoea* sp., Te 1= *Pseudomonas* sp., Av 30 = *Pseudomonas* sp.

A second dose of inoculum was added 1 week after transplanting. When only one organism was used for inoculation, it was inoculated on one side of the plant close to root by making a hole in the soil and subsequently closing it. When two or three organisms were used for inoculation, they were inoculated on either side of plant and close to the root system. Irrigation was given once in 3–4 days.

Plant parameters studied

Observations were made for plant growth parameters at different stages of growth period. Plant height and number of branches were recorded up to the day of harvest from days after planting (DAP) i.e. 60, 90, 150 and 180. Plant height, number of branches and at harvest, yield parameters i.e. weight of tubers, tuber length, number of tubers, thickness of roots fresh and dry weight of total biomass etc. were recorded. Fresh weight and dry weight of roots were also recorded. Dry weights of shoot and root were determined after chopping and shade drying to constant weight.

Statistical analysis

The data were subjected to ANOVA (analysis of variance) analysis for a completely randomized design for *in vitro* studies in accordance using SPSS statistical software to quantify and evaluate the sources of significance variances among the treatments and comparisons of treatment means were accomplished by least significance difference (LSD) test at 0.5% level of significance. Values represent mean ±CD for three samples in each group. P values <0.5 were considered as significant.

Results and Discussion

Microorganisms living in the plant rhizosphere interact with each other and with plant roots in several ways that affect plant growth and development (Asghar *et al.,* 2002; Glick *et al.,* 1995; Jetiyanon and Kloepper, 2002). In the present study, the PGPR strains which were isolated from medicinal plants were applied as bioinoculants on *Coleus forskohlii* Briq. (Family Labiatae). *Pantoea* sp. (Cf 7) and *Pseudomonas* sp. (Av 30, Te 1) is considered as a preferred plant growth promoting strategy.

The PGPR isolates significantly affected the growth of *C. forskohlii* plants (Table 1). Results reveal that plant height increased in PGPR treated plants over uninoculated control. There was a significant increase in plant shoot length (55.6, 89.76%), number of branches (12.6, 87.4%), number of tubers (11.3, 88.7%), tuberous root length (16.6, 78.49%), root girth (4.9, 133.3%), shoot weight (fresh (87.33, 54.12%), dry (15.50, 142.18%)), root weight (fresh (23.36, 143.33%), dry (10.23, 487.93%)) and total biomass (fresh (110.69, 67.05%), dry (25.73, 217.65%)) obtained with triple combined application of PGPR Cf7+Te1+Av30 for 180 days, plant height and number of branches studied at 45 days interval after transplanting showed an increasing trend from 45 DAP to the day of harvest.

The next best treatment was followed by dual inoculation of Te1+Av 30, Cf7+Av30, Cf7+Te1 showed maximum effect on growth and dry matter yield of coleus (Table 1) and single inoculation with Av30, Te1, Cf7 enhanced plant height, number of branches and tuber characteristics compared to uninoculated control. Among these three, inoculation with *Pantoea* sp. (Cf7) resulted in better response.

Pantoea is known to induce increased growth of various crops through production of growth-regulating factors and solubilization of phosphate (Dastager *et al.,* 2009) showed that bacterial treatments increased growth parameters compared to the control. Earlier similar type of studies were done on some other medicinally important plants *i.e. Withania somnifera* (Rajasekar and Elango, 2011; Mallesh *et al.,* 2009) *Ociumum basilicm* L. (Golpayegani and Tilebeni, 2011). They are improved tuber yield, alkaloid content and showed biochemical changes in the rhizosphere of *Coleus forskohlii* (Priya and Kumutha, 2009; Rakshapal Singh *et al.,* 2009; Rakshapal Singh *et al.,* 2011) etc.

Root inoculation with PGPR promoted significant increase in growth but the growth responses 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 compared to the control.

Table.1 Effect of PGPR strains on plant growth promoting activity in *Coleus forskohlii* under field conditions

PGPR strain treatments	Shoot length (cm) DAP				No. of branches	No. of tubers	Tuber length (cm)	Diam. of tubers (cm)	Shoot weight (gm)		Root weight (gm)		Total biomass (gm)	
	60	90	150	180					Fresh	Dry	Fresh	Dry	Fresh	Dry
Cf7	20.6	26.0	31.3	39.6	7.6	5.6	9.5	2.2	57.00	9.00	11.30	2.53	68.30	11.53
Te1	21.6	26.6	32.0	40.0	8.0	5.3	10.1	2.7	58.60	9.93	12.20	3.22	70.80	13.13
Av30	22.0	26.6	34.6	41.3	9.0	7.6	11.3	3.6	59.33	11.50	13.50	4.44	72.83	15.90
Cf7+Te1	24.3	29.3	37.0	43.3	10.3	7.6	11.6	2.9	62.33	12.53	15.30	5.33	77.63	17.86
Cf7+Av30	26.0	30.3	36.6	47.0	9.6	8.3	12.5	3.2	66.66	13.33	17.50	6.36	84.16	19.69
Te1 +Av 30	27.0	30.6	38.3	51.3	12.0	9.3	15.3	3.6	78.33	14.83	19.20	9.44	97.53	24.23
Cf7+Te1+Av 30	27.4	31.1	40.3	55.6	12.6	11.3	16.6	4.9	87.33	15.50	23.36	10.23	110.69	25.73
Control	20.0	25.6	27.6	29.3	6.0	4.3	9.3	2.1	56.66	6.40	9.60	1.74	66.26	8.10
Probability	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD	2.35	2.50	1.17	2.57	1.81	1.23	1.18	0.32	4.18	1.28	1.07	0.88	--	--
CV (%)	5.71	5.05	1.92	3.39	11.0	9.32	5.60	5.93	3.70	6.30	4.01	9.37	--	--

Values are mean of three replications ± CD, CV (%) for five replicates per treatment. There is a significant difference at P value of 0.5, as determined by SPSS (Statistical Package for the Social Sciences), DAP- Days after planting; No- Number; Diam- Diameters.

Earlier reports had shown that combined inoculation of sorghum with *A. brasilense* and phosphate solubilization bacteria; *P. striata* or *B. polymyxa* significantly increased grain yield and dry matter content, N and P uptake as compared with single inoculation of individual organisms (Alagawadi and Gaur, 1992). 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 (Cakmakci *et al.,* 2007; Kevinvessey, 2003). The present study reveals that the root inoculation of *Pantoea* sp. (Cf7) and *Pseudomonas* sp. (Te1, Av30) has enhanced biomass yield and dry matter content under field conditions for medicinally important Coleus.

In addition to these traits, plant growth promoting rhizobacterial strains must be rhizospheric component, able to survive and colonize in the rhizospheric soil. Unfortunately, the interaction between associative PGPR and plants can be unstable. The good results obtained *in vitro* cannot always be dependably reproduced under pot and field conditions. In the present studies it is expected that inoculation with *Pseudomonas* rhizobacteria containing PGP characteristics consequently promote root, shoot growth and yield. The pot and field experiments conducted in the present investigation on growth promotion in Coleus revealed significant increase in plant growth parameters, *viz*. plant height, shoot and root weight, tuber length, number of branches and total biomass on fresh weight and dry weight basis in plants treated with Cf7+Te1+Av30. The mechanisms of growth promotion by *Pantoea, Pseudomonas* sp. are complex and appear to compare both changes in the microbial balance in the rhizosphere and alteration in the host plant physiology (Glick *et al.,* 1999). The isolates exhibiting multiple plant growth promoting (PGP) traits on soil-plant system under pot and field conditions. Significant increases in plant growth parameters in the present study may be attributed to the production of plant growth regulators such as auxins, gibberellins and cytokinins (Frankenberger and Arshad, 1995). It has often been inferred that rhizobacterially produced auxins are responsible for growth promotion, indole acetic acid promotes ethylene production by stimulating the enzyme in the ethylene biosynthetic pathway (Kende, 1993).

As our understanding of the complex environment of the rhizosphere, of the mechanisms of action of PGPR, and of the practical aspects of inoculants formulation and delivery increases we can expect to see new PGPR products becoming available (Bowen and Rovira, 1999). The success of these products will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Rhizosphere management will require consideration of soil and crop cultural practices as well as inoculants formulation and delivery (McSpadden Gardener and Fravel, 2002).

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