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Research Article

Effect of co-inoculation of AM fungus *Scutellospora* sp. and fluorescent *Pseudomonas* on *Coleus forskohlii*

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ABSTRACT: An investigation was carried out with the aim to study the interactive effect of Arbuscular Mycorrhizal (AM) fungi and Plant Growth Promoting Rhizobacteria (PGPR), *Pseudomonas* on growth and yield of *Coleus forskohlii*. The inoculation of AM fungus, *Scutellospora* sp. and PGPR organisms (*Pseudomonas* sp.) showed increase in plant enzyme activities along with enhancement in tuber yield and alkaloid content, than the individual treatments. The combined inoculation of the AM fungus with PGPR recorded maximum shoot and root length with 40.4 and 203.1 per cent increase over control and the total dry matter content increased by 104.5 percent over control. The enzyme activities (acid, alkaline and dehydrogenase) were found to have enhanced upto 200 per cent due to the combined inoculation of SCL1+PFC1 which was double than single inoculations. The AM colonization (45-90 per cent) and spore load (155–330 spores) were also higher due to these treatments while, about 6-7, 1-2 and 5-6 fold increase in N, P and K uptake was noticed respectively. The tuber yield (0.73 g plant^{-1}) and forskohlin content (0.03-0.06 per cent) were also found to be doubled due to the inoculation of AM fungi with PGPR.

KEY WORDS: Arbuscular Mycorrhizal (AM) fungi, Plant Growth Promoting Rhizobacteria (PGPR), *Coleus forskohlii*, forskohlin content

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INTRODUCTION

Since the dawn of civilization, medicinal plants are known to be used for treatment of a number of diseases of man and animals. Plants continue to be a major source of medicines, as they have been existing throughout human history and in addition providing the basic ingredients for 30 and 40 per cent of today's conventional drugs. The medicinal and curative properties of various plants are also employed in herbal supplements, botanicals, nutraceuticals and tea. Most of the medicinal plants are used in Ayurveda and Unani system of medicines and by tribal healers among which, *Coleus forskohlii* Briq., holds an important position due to its incredible effect in treating many dreadful diseases like cancer, congestive heart failure, hypertension and asthma (Shah *et al*., 1980). The tubers of this plant seem to be the economically important, mostly due to the presence of the alkaloid called forskohlin that is responsible for curing diseases.

Arbuscular mycorrhizae (AM) are symbiotic associations, formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. There are reports providing evidence that infection with mycorrhizal fungi facilitates better nutrient uptake (Cantrell and Linderman, 2001) and the plant growth promoting rhizobacteria like *Pseudomonas* sp. appear to be a better option to enhance the plant growth. Hence to exploit these biological tools a pot experiment was carried out and the response on growth and alkaloid yield of *Coleus forskohlii* was studied.

MATERIALS AND METHODS

Inoculants

The soil samples collected from the rhizosphere of *Coleus forskohlii* were taken for isolating native species of AM fungi and PGPR organisms. Among the various microorganisms, the AM fungal population, *Scutellospora* sp. showed predominance and among the total bacteria, *Pseudomonas* sp. were dominant. These two organisms after isolation and purification were further coded as SCL 1 (*Scutellospora* sp.), PFC 1 and PFC2 (*Pseudomonas* sp.) for to be used as inoculants. A pot experiment was carried out in the Department of Agricultural Microbiology, TNAU during 2007 to study the effect of AM inoculation of (*Scutellospora*) with two isolates

(*Pseudomonas* sp.) on *Coleus forskohlii*. Pots of 30 x 28 cm size were filled with 10 kg pot mix soil (red soil:sand:farm yard manure – 2:1:1) with pH-8.2, EC–0.89 dSm^{-1}, available N–219 Kg/ha, P_2O_5–14.3 Kg/ha, K_2O–293 Kg/ha) and the AM inoculation was done at 2.5 – 5 cm depth @ 50 g per pot (containing 250-300 spores/100g inoculum). The *Scutellospora* sp. isolate SCL 1 was multiplied and the vermiculite based inocula was used along with *Pseudomonas* isolate (10^9 cells/ ml broth culture) @ 50 ml of per pot as per the treatment in the planting hole. Local variety of *Coleus forskohlii* was planted in the pots and irrigated periodically. Following treatments were replicated thrice in completely randomized design.

Treatments

T_1 – *Scutellospora* sp. SCL 1
T_2 – *Pseudomonas fluorescens* (PFC 1)
T_3 – *Pseudomonas fluorescens* (PFC 2)
T_4 – SCL 1+ PFC 1
T_5 – SCL 1 + PFC 2
T_6 – Uninoculated control

Sampling

Periodical sampling were made at 90, 120 and 150 DAP from the pots. Lab analysis were carried out with Borosil glasswares using chemicals and reagents made from SIGMA, HIMEDIA, FISCHER and MERCK. After plant sampling, the plants as well as the tubers were removed off for soil particles, weighed fresh and taken for laboratory analysis. Dry weight of the samples and tubers were registered after shade dry and documented.

Root samples were used for estimation of enzyme activities. Acid and alkaline phosphatase activities were estimated as per the method described by Morton, 1952. Total dehydrogenases in soil and roots were estimated using Triphenyl Tetrazolium chloride as per the method of Casida *et al.*, 1964. AM colonization was assessed in the roots as per Phillips and Hayman (1970) and spore count was made following the method of Gerdemann and Nicolson, 1963.

RESULTS AND DISCUSSION

Plant samples were taken at 90, 120 and 150 days after planting (DAP) for estimating yield parameters, root colonization and spore load of *Scutellospora* sp. (SCL 1), as well as enzyme activities and the results at 150 DAP are alone discussed here. On perusal of the results, it was evident that AM and *Pseudomonas* sp. inoculation exhibited significant increase in growth of *Coleus forskohlii*. Among the treatments, SCL 1 + PFC 1 combination was superior which influenced the plant height, number of leaves and dry matter production. It recorded the maximum shoot length of 45.30 cm the root length of 56.0 cm per plant with 40.8 and 203.1 per cent increase over control respectively at 150 DAP (Table 1). The results were supported by the work of Boby and Bagyaraj (2003), who found the enhanced growth of *Coleus forskohlii* with the dual inoculation of AM fungi and PGPR organisms. Enhanced dry matter production was observed with individual as well as combined inoculation treatments and the combination of SCL1 + PFC 1 registered 104.5 per cent increase over control (Table 1). Increased dry matter production with AM inoculation was observed in grapevine (Bavaresco *et al.*, 2000; Kendra and Xiaomei, 2004) and cassava which was 15 – 30 fold higher than control is in line with the results obtained in the present study.

AM root colonization

AM root colonization was found to be 60-80 per cent in *Scutellospora* sp. SCL 1 inoculated plants and the spore load was 17-200 spores/50g soil (Fig. 1). In T2 and T3 inoculated plants, AM colonization as well as spore load was observed high which showed the stimulatory effect of *Pseudomonas* isolates. In combined inoculation, significant increase in spore numbers (210-330 spores) as well as root colonization was noticed (80-93 per cent) than single inoculations of SCL1/PFC isolates. PGPR can influence the growth of hyphae from germinating arbuscular mycorrhizal spores (Barea *et al.*, 1998), colonization of plant roots by AM fungi and growth of external hyphae. It was also hypothesized that *Pseudomonas* (MHB – Mycorrrhiza Helper Bacterium) could soften the cell wall and the middle lamella between the cells of

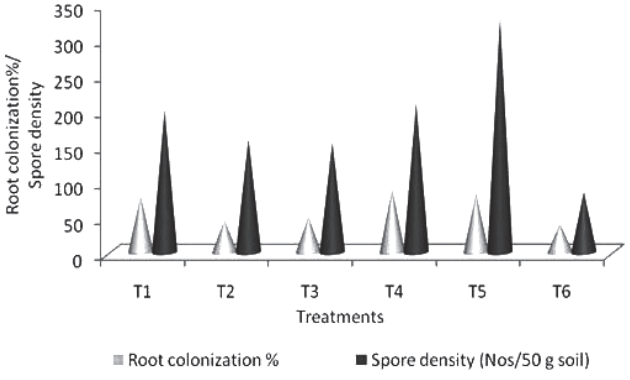

Fig. 1. Effect of combined inoculation of AM fungus and PGPR organisms on AM root colonization and spore count in *Coleus forskohlii*

Table 1. Effect of combined inoculation of AM fungus and PGPR organisms on growth and dry matter production of *Coleus forskohlii* at 150 DAP

No.	Treatments	Shoot length (cm plant^{-1})	Per cent increase over control	Root length (cm plant^{-1}) control	Per cent increase over	Dry matter production (g plant $^{-1}$)	Per cent increase over control
1.	*Scutellospora* sp. SCL 1	34.20	18.8	28.50	80.0	27.64	38.8
2.	*Pseudomonas fluorescens* PFC 1	35.40	24.3	36.10	111.1	32.85	63.3
3.	*Pseudomonas fluorescens* PFC 2	35.00	21.9	30.00	86.3	19.83	15.5
4.	SCL 1 + PFC 1	45.30	40.4	56.00	203.1	46.28	104.5
5.	SCL 1 + PFC 2	43.70	38.0	46.80	142.1	42.25	94.5
6.	Uninoculated Control	30.20	–	14.50	–	18.05	–
	S Ed	1.24		1.322.82		1.07	
	CD (P = 0.05)	2.67				2.03	

the root cortex by producing enzymes and thus making fungal penetration easier (Duponnois, 1992). These results tend to indicate some sort of tropic stimulation of the fungal growth by the bacteria is the main mechanism involved. Maximum spore count of 330 spores 50 g per soil, observed with dual inoculation highlighted the existence of synergistic interaction in the rhizosphere. It was explained that the fungus could be suffering from auto inhibition by producing some fungistatic compounds which could be metabolized by the accompanying microbes and this allows the growth of mycelium and spore production (Azcon-Aguilar and Bago, 1994).

Enzyme activities

AM inoculation showed significant increase in phosphatase as well as dehydrogenase enzyme activities over control, followed by inoculation of *P. fluorescens* at every sampling where the acid phosphatase activity was higher comparatively. The inoculation effected 3-4 fold increase in acid phosphatase activity and 1.5-2 fold increase in alkaline phophatase activities over control. Combined inoculations showed the maximum activity up to 212 and 253 µg PNPP release at 150 DAP in the case of acid phosphatases which were 2-3 fold higher than individual inoculations (Fig. 2). This combination also recorded the maximum alkaline phosphatase activity of 138 µg PNPP release at 150 DAP which was 80-100 per cent higher than individual inoculations (Fig. 2). Several reports showed the enhanced activity of phosphatases with AM inoculation as with *Glomus intraradices* (Erik and Johansen, 2000) which supports the results of this study. The increased concentration of acid phosphatases in AM plants was attributed to the direct fungal secretion or an induced secretion of the enzyme by the plants. Increase in alkaline phosphatase activity was positively correlated with extraradical and intraradical mycelium of AM fungus (Ingrid *et al*., 2002). Increased fungal colonization in roots might have resulted in more activity of phosphatases than poorly colonized plants.

Total dehydrogenases were observed higher in inoculated plants which was higher in PFC2 inoculation at 150 DAP followed by AM inoculation among the single culture treatments. Combined inoculation of SCL1 + PFC2 registered 67 µg TPF/min/g root which was double than single inoculation and 2-4 fold higher than control. Increase in enzyme activities was observed over the ageing of the plant (Fig. 2). The activity of the enzyme dehydrogenases in an organism or tissue serve as an index of the metabolic activity. Substrate specific dehydrogenases function as oxido-reducto enzymes transferring electrons from one substrate to the other. The dehydrogenases are a source of generating more electrons and hence more energy for the tissue. Increased activity with AM inoculation might have triggered up many dehydrogenases directly or indirectly by enhancing the rhizosphere microorganisms. Enhancement in the dehydrogenase activities to the tune of 15 – 19.9 per cent over control in roots as well as the soils of mulberry due to inoculation of AM fungi *G. fasciculatum* MGf 3 has been reported by Kumutha *et al*. (2006).

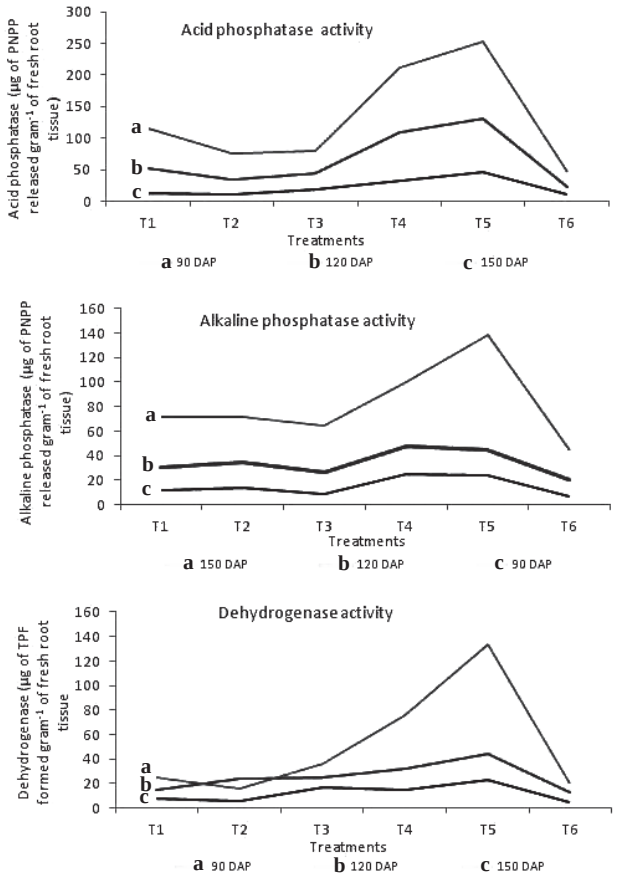

Fig. 2. Effect of combined inoculation of AM fungus and PGPR organisms on enzyme activities in roots of *Coleus forskohlii*

T1 – *Scutellospora* spp. SCL1 T4 – SCL 1 + PFC 1
T2 – *Pseudomonas fluorescens* PFC 1 T5 – SCLI + PFC 2
T3 – *Pseudomonas fluorescens* PFC 2 T6 – Uninoculated control

PNPP – para – nitrophenol phosphate
TPF – Triphenyl formazon
DAP– Days after planting

Nutrient uptake

With reference to the uptake of nutrients, AM inoculation had exhibited significant influence on uptake of N, P & K and it was further improved by the co-inoculation of *Pseudomonas*. Combinations of *Scutellospora* sp. SCL 1 + *Pseudomonas* PFC 1 registered 7-8 fold increase in N uptake, 1.5 fold increase in P uptake and 2-6 fold increase in K uptake of *C. forskohlii* over a period of 90-150 DAP (Table 2). Mycorrhizal fungi may affect mineral nutrition of the host plant either directly or indirectly. In many cases AM association usually enhanced the growth of plants solely by enhancing the uptake of nutrients. The increase may be attributed to the contribution of hyphal transport of N in the form of NO_3 or NH_4 (Taylor *et al*., 1995), P_2O_5 (Reinhardt, 2007; Meier *et al*., 2011; Meier *et al*., 2012) and K_2O. These results suggest two scenarios; one may be due to the increased absorption through extramatical hyphae of AMF and the second may be the enhanced growth of AM roots which facilitates in the absorption of more nutrients from soil than non AM roots.

Tuber and alkaloid production

Not only the growth but also a significant enhancement in tuber yield was resulted with the inoculation of AM fungi (by 68.5 % over control) and the tuber yield was doubled by the combined inoculations (Table 3). Improvement in the alkaloid forskohlin content (50%) was observed by AM inoculation over control and further a 2 fold increase was observed when AM was combinedly inoculated with *Pseudomonas* – PFC 2 (Table 4). This may be explained by the stimulation of specific metabolic pathways as reported in wheat (Walter *et al*., 2000) by the combined inoculations.

Table 2. Effect of combined inoculation of AM fungus and PGPR organisms on uptake of nutrients in *Coleus forskohlii* at 150 DAP

No.	Treatments	Nutrient uptake (mg plant $^{-1}$)					
		N	Per cent increase over control	P_2O_5	Per cent increase over control	K_2O	Per cent increase over control
1.	*Scutelliospora* spp. SCL 1	737.0	497	116	81.2	610.0	496.8
2.	*Pseudomonas fluorescens* PFC 1	481.0	289	98	53.0	399.0	290.4
3.	*Pseudomonas fluorescens* PFC 2	529.0	329	70.0	9.4	438.0	328.6
4.	SCL 1 + PFC 1	891.0	622	164.0	156.2	738.0	622.1
5.	SCL 1 + PFC 2	876.0	610	118.0	84.4	725.0	609.4
6.	Uninoculated Control	123.4	–	64.0	–	102.2	–
	SEd	14.3		1.90		11.8	
	CD (P = 0.05)	30.7		4.08		25.5	

Table 3. Effect of combined inoculation of AM fungus and PGPR organisms on tuber yield of *Coleus forskohlii* at 150 DAP

No.	Treatments	Tuber production			
		Fresh weight (g plant^{-1})	Per cent increase over control	Dry weight (g plant^{-1})	Per cent increase over control
1.	*Scutellospora* sp. SCL 1	4.21	41.3	0.59	68.5
2.	*Pseudomonas fluorescens* PFC 1	3.68	23.4	0.45	28.5
3.	*Pseudomonas fluorescens* PFC 2	3.08	3.3	0.39	11.4
4.	SCL 1 + PFC 1	6.36	113.4	0.72	105.7
5.	SCL 1 + PFC 2	5.18	73.8	0.73	108.5
6.	Uninoculated Control	2.98	–	0.35	–
	SEd	0.14		0.02	
	CD (P = 0.05)	0.31		0.04	

Table 4. Effect of combined inoculation of AM fungus and PGPR organisms on forskohlin content in the tubers of *Coleus forskohlii*

Sl. No.	Treatments	Forskohlin content (%)
1.	*Scutellospora* sp. SCL 1	0.03
2.	SCL 1 + PFC 1	0.06
3.	SCL 1 + PFC 2	0.05
4.	Uninoculated control	0.02
	SEd	5.80
	CD (P = 0.05)	12.30

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