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Influence of different AM-fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*

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A glasshouse investigation was conducted to study the effectiveness of 11 arbuscular mycorrhizal (AM) fungi on the medicinal plant *Coleus forskohlii*. Coleus plants raised in presence of most of the AM fungi in polythene bags showed an increase in plant growth (height, number of branches and biomass), P, and forskolin contents over those grown in the absence of soil inoculation with AM fungi. The extent of growth, P, and forskolin status varied with the AM fungi used. Based on the plant biomass, P uptake and forskolin content per plant, *Glomus bagyarajii* was found to be the best AM symbiont for inoculating *C. forskohlii*, the next being *Scutellospora calospora*.

INTRODUCTION

Inoculation with arbuscular mycorrhizal (AM) fungi is known to increase the growth of many plant species. This is attributed to increased uptake of nutrients, production of growth promoting substances, tolerance to drought, salinity, transplant shock, resistance to plant pathogens and synergistic interaction with other beneficial soil microorganisms such as N_2-fixers, P-solubilizers etc. (Jeffries 1987; Bagyaraj & Varma 1995). It has been established that mycorrhizal plants grow better in infertile soils because of improved mineral nutrition through hyphae which help in exploring greater volume of soil, beyond root hairs (Kormanik, Schultz & Bryan 1982, Rajan, Reddy & Bagyaraj 2000).

Coleus (*Coleus forskohlii*, syn. *C. barbatus*) is an important medicinal plant. The crop rose into prominence by virtue of its alkaloid, forskolin, a diterpine present in the roots (which are nothing but swollen primary roots), which is extensively used in the treatment of heart diseases, glaucoma, asthma and certain types of cancers (Shah *et al.* 1980). This alkaloid has the unique property of activating all hormones sensitive to adenylate cyclase enzyme in biological systems. The cost of 1 g of forskolin is US $ 85, showing the value of this plant (Gowda 2000). Because of the continuous collection of roots from wild sources, this plant has been listed as an endangered species, and farmers have started raising this crop (Vishwakarma *et al.* 1988). Information on the mycorrhizal symbiosis in Coleus is meagre as it is a newly cultivated economic plant (Boby & Bagyaraj 2003). This study was undertaken to investigate the influence of different AM fungi on the growth, phosphorus nutrition and root forskolin content of *C. forskohlii*.

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MATERIALS AND METHODS

The study was carried out under glasshouse conditions. The potting mix used in this study was a mixture of unsterilised sand: soil: compost (1:1:0.25 v/v) in black polythene bags 25 × 15 cm each filled with 2.5 kg of the mixture. The soil was an alfisol of the kaolinitic type, isothermic typical 'kanhalplustalf' with pH 5.8. The substrate used in the study had an available P of 5.6 ppm ($NH_4F + HCl$ extractable; Jackson 1973), organic C content of 0.35 %, and indigenous AM spore count of 4 50 g^{-1} substrate (Gerdemann & Nicolson 1963).

Root pieces and the substrate from pot cultures of Rhodes grass raised on sand: soil 1:1 by volume were used as the mycorrhizal inoculum. The selected fungi and their sources are given in Table 1.

To each planting hole, 12 500 infective propagules of AM fungi based on the MPN estimation (Porter 1979) was added according to the treatments. *C. forskohlii* rooted cuttings about 12 cm tall were obtained from

Table 1. Selected AM fungi and their original source.

AM fungi	Source
Acaulospora laevis	University of Western Australia, Nedlands, Australia.
Gigaspora margarita	ICRISAT, Hyderabad.
Glomus bagyarajii[a]	Centre for Mycorrhiza Collection, Tata Energy Research Institute, New Delhi.
G. monosporum	University of Western Australia, Nedlands, Australia.
G. fasciculatum	University of California Riverside, USA.
G. mosseae	University of Agricultural Sciences, Bangalore.
G. leptotichum	University of Agricultural Sciences, Bangalore.
G. macrocarpum	University of Agricultural Sciences, Bangalore.
G. intraradices	University of Agricultural Sciences, Bangalore.
G. etunicatum	Native Plants Institute, Salt Lake City, USA.
Scutellospora calospora	University of Agricultural Sciences, Bangalore.

[a] This species was isolated from revegetated coal mine spoils around Allahabad, India, and produces abundant bright yellow to yellow-brown spores, (36–)101(–125) μm, with four wall layers in two groups. Group A consists of an outer, hyaline evanescent wall layer, (0.5–)3(–3.5) μm thick and an inner yellow to yellow brown laminated layer, (1.5–)3.5(–4) μm thick. Group B consists of an outer hyaline unit wall layer, (0.5–)1(–1.5) μm thick and an inner hyaline, membranous wall layer, (0.25–)0.5(–1) μm thick. Reaction of wall layers to Melzer's regent is negative. Subtending hypha hyaline to pale yellow, cylindrical or funnel shaped (0.7–)11(–14) μm wide at the spore base. Germination is by regrowth of the subtending hypha. The fungus is deposited in the Centre for Mycorrhiza Culture Collection, Tata Energy Research Institute, New Delhi, as accession AM 1019. For a more detailed description see Mehrotra (1997).

the Department of Horticulture, University of Agricultural Sciences, Bangalore, and planted in polybags. One rooted cutting was planted in each bag. Each treatment was replicated 20 times. The plants were maintained in a glasshouse for 150 d and watered whenever necessary.

Plant height and number of branches were recorded every 30 d for 150 d. However, only observations made 150 d after planting (DAP) are presented here. The plants were harvested 150 DAP. The length and thickness of fresh tuber (primary roots) was measured using vernier callipers. Dry weight was determined after drying the shoot and root separately at 60 °C to a constant weight. Phosphorus content of the shoot and root was determined by vanadomolybdate yellow colour method as outlined by Jackson (1973).

Percentage mycorrhizal root colonization was estimated following grid line intersect method (Giovanetti & Mosse 1980) after staining the roots by the method of Philips & Hayman (1970), but using acid fuchsin as the stain. The mycorrhizal spore numbers in the root zone soil was determined by the wet sieving and decantation method (Gerdemann & Nicolson 1963).

Forskolin concentration in the root was determined by high performance thin layer chromatography (HPTLC). Standard forskolin and other samples were spotted on precoated silica plates as narrow bands 4 mm wide at a constant rate of 8 μl s^{-1} using Camag Linomat IV model applicator under nitrogen atmosphere. A mixture of benzene and ethyl acetate (85 : 15 v/v) was used as the mobile phase. The length of the chromatogram was 90 mm, and 15 min were required for each run. The plates were sprayed with anisaldehyde sulphuric acid reagent (0.5 ml anisaldehyde, 1 ml H_2SO_4, and 50 ml acetic acid) and heated at 110 ° for 5 min. Orange fluorescence observed at 366 nm was optically detected and quantified at 315 nm using Camag TLC scanner with CATS 3.17 software for quantification of the separated compounds in the chromatogram (Malathy & Pai 1999). Forskolin content per plant was obtained by multiplying dry weight of the root by its forskolin concentration.

The statistical analysis for all plant and mycorrizal parameters was by a one-way analysis of variance. The fungi were ranked for each character and compared pair wise using Duncan's Multiple Range Test at 5% level of significance (Little & Hills 1978).

RESULTS AND DISCUSSION

Coleus seedlings varied in their response to inoculation with different mycorrhizal fungi. Most of the AM fungi resulted in significant increase in plant height, shoot and root biomass, and the phosphorus content of *C. forskohlii*. Host preference among AM fungi has been reported by earlier workers (McGraw & Schenck 1981, Bagyaraj, Byra Reddy & Nalini 1989), hence, the need for inoculating different mycotrophic plants has been stressed (Jeffries 1987, Rajan *et al*. 2000).

Regarding plant height and number of branches, inoculation with *Glomus bagyarajii* showed maximum increase during the crop growth period, which was significantly different from all other treatments (Table 1). The next best fungus was *Scutellospora calospora*. This was followed by *G. fasciculatum* and *G. mossese* both behaving more or less similar. The length of the fresh root and dry weight of shoot and root (tuber + secondary roots) are presented in Table 2. There was no significant difference in the thickness of the fresh roots, but the length of the fresh roots differed significantly between treatments. Maximum length was observed in plants inoculated with *G. bagyarajii*, followed by *S. calospora*, both being statistically on par with each other. The next best treatments were *G. fasciculatum* and *G. mosseae*, both not differing significantly. The dry weight of the root followed a more or less a similar trend, except that the difference between *G. bagyarajii* and *S. calospora* was significant. The least dry weight was encountered in uninoculated plants. The increase in the root biomass because of inoculation with *G. bagyarajii* and *S. calospora* was 51.25