Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

Vol. 8	No. 4	pp. 673-676	2009

Original Research Paper

Screening of Potential Arbuscular Mycorrhizal Fungal Isolates for *Costus speciosus*, a Medicinal Plant in Unsterile Soil

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Key Words:
Costus speciosus sm
Arbuscular mycorrhizal fungi
AMF colonization
Rhizosphere

ABSTRACT

Selection of an efficient Arbuscular mycorrhizal (AM) species for inoculum production is an important step towards adapting mycorrhizal inoculum technology in crop production. In this direction six indigenous AM species were screened, isolated, identified and cultured on two promising host plants of maize and Johnson grass. When host plant had optimum AMF colonization with respective AMF species; *Glomus intraradices*, *Glomus fasciculatum*, *Glomus microcarpum, Glomus mosseae, Gigaspora margarita* and *Sclerocystis dussii* were inoculated to *Costus speciosus* sm in unsterilized soil. Overall result revealed *that Sclerocystis dussii* was most efficient AM Fungus to *Costus speciosus* sm, a medicinal shrub. There was significant increase in plant height, leaf length, leaf number, total chlorophyll content in leaves and increased stem tubers length, tuber diameter and phosphorus content as compared to control uninoculated plants. However, this enhanced growth response was influenced by other promising AMF species, *Glomus fasciculatum* and *Gigaspora margarita*. The underground stem tubers and their phosphorus content increased with the inoculation of *Sclerocystis dussii* followed by *Glomus mosseae* and *Glomus microcarpum*. Therefore, an efficient indigenous *Sclerocystis dussii* may be made to inoculate on *Costus speciosus* sm tuber cuttings in an unsterilized soil to get healthy seedling stock.

INTRODUCTION

The improvement phase of medicinal plants starts from the time the domestication of plants is undertaken. It is certainly necessary to follow certain models like collection and evaluation, clonal selection hybridization, mutation, and other methods. Fertilizer consumption increased tremendously to maximise yield by cultivation of hybrid crops after green revolution. One more such step perhaps may be the use of AM fungi for growth improvement. Mycorrhizal plants are, therefore, adapted to cope up with nutrient deficient situations or prevent pathogenesis by other organisms. Mycorrhizal fungi will not prove effective with all plants when the plants have little mycorrhizal dependency. Kumar & Mahadevan (1984) have reported the absence of AM fungal association in medicinal plants and attributed this to the presence of various secondary metabolites in the host plants. However, there are many reports of medicinal plants with secondary substances harbouring AM fungal associations in their root systems (Abbott & Robson 1982, Lakshman & Raghavendra 1992, Prasad & Reddy 1998). Kumar & Muragesh (2002) have tested dependency of the three AM fungal species *Glomus mosseae, Glomus fasciculatum* and *Glomus monosporumon* on ten medicinal plants and showed clearly that mycorrhizal inoculation is beneficial in improving their growth. Basu & Srivastava (1998) have tested more then ten species of AM fungi and observed that AM fungal association had enhanced the growth of medicinal compounds. Therefore, there is a need for research in improving the quality and quality of drugs produced from native medicinal plants in relatively shorter period and a

lower expense by using selective AM fungi. This paper reports inoculation of different AM fungal species to understand the efficacy of *Costus speciosus* in pot experiments with unsterilized soil.

MATERIALS AND METHODS

Costus speciosus is an important medicinal plant. Its leaf juice is used for treatment of skin diseases, and tuber juice for diabetes. Leaf and tuber juices are proportionately mixed and taken along with milk to reduce hypertension. Stem tuber cuttings measuring 6cm were surface sterilized in 2% potassium hypochlorite and washed thrice in distilled water. Earthen pots (20 × 30cm) were filled with 4kg of unsterilized soil with E.C. 0.04 mhos/cm, organic matter 0.19%, total nitrogen 0.05%, available phosphorus 5.3 ppm and available potassium 110.6 ppm. Before planting the stem cutting, 10g of mixed inoculum of all the six AMF inocula was spread uniformly in each earthen pot except control or noninoculated one. Maize-root based cultures of three AM species (*Glomus intraradices*, *Glomus fasciculatum*, *Glomus microcarpum*), and John son grass-root based cultures of the three AM species (*Glomus mosseae*, *Glomus margarita*, *Sclerocystis dussii*) were developed with the species isolated from rhizosphere soil of grass hosts in fallow alluvial soil of the university farm. The mother cultures were maintained in steam-sterilized sand-soil mixture in PVC pots in isolation under transparent plastic sheet cover in a green house. Infected mother culture roots were maintained in green house with watering once in two days. Plants were harvested after 120 days. The roots were cleaned by dipping them in water for several times till the adhering soil particles were removed. Both inoculated and uninoculated root samples were cut into 1cm bits and fixed in standard FAA (formalin acetoalcohol) and processed further for the assessment of AMF colonization. Root pieces were mixed in 10% KOH and autoclaved for 30 minutes and washed in distilled water and stained in 0.05% try pan blue and mounted on clean slides in lactophenol (Phillips & Hayman 1970). For every

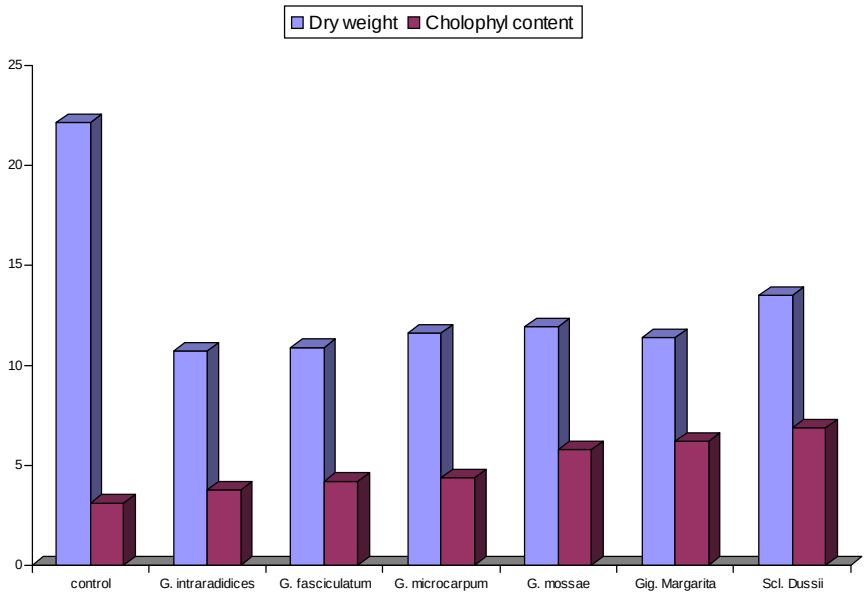

Fig. 1: Effect of AM fungi on *Costus speciosus* showing plant dry matter, with total chlorophyll content in leaves after 90 days.

harvested plant, parts were removed and oven dried for 72 hrs at 70ºC. Roots and shoot systems were then weighed. MD (mycorrhizal dependency) of plants were determined following the formula proposed by Gerdemann (1975). Mycorrhizal efficacy was calculated according to the formula of Sing & Tilak (1990). Of all the six AMF species inoculated plants, height, biomass production, number of tubers, chlorophyll content of leaves and phosphorus content were estimated by standard methods.

RESULTS AND DISCUSSION

It is clearly evident from the data that root system of *Costus speciosus* grown in both control and different species of AM inoculated was infected in unsterilized soil, and invariably found to harbour AMF association. This clearly brought enhanced plant height, stem diameter, leaf length, number of leaves and total chlorophyll content of leaves (Table 1, Fig. 1). Similarly, underground tubers with root system were found to be significantly increased in number, length, diameter, dry weight and P content in AM fungi inoculated plants over the control or uninoculated plants (Table 2). Overall results indicate that *Sclerocystis dussii* was the most efficient AM fungus which influenced the plants by increasing plant height, leaf length, leaf number, chlorophyll content, enlarged tube diameter, tuber length and phosphorus content of tuber as compared to uninoculated plants. However, the variation among AM fungal species inoculation on *Costus speciosus* exhibited vide variation. Plant height was increased with the inoculation of *Sclerocystis dussii* followed by *Glomus fasciculatum* and *Gigaspora margarita*, whereas chlorophyll content increased in plants inoculated with *Sclerocystis*

Table 1: Effect of AM fungi on growth characters in *Costus speciosus* after 90 days.

Treatment	Plants height (cm)	Stem diameter (cm)	Number of leaves/plant	Leaf length (cm)	Total chlorophyll
Glomus intraradices	46.2 a	2.5 a	12.6 b	4.7 c	0.37 a
Glomus fasciculatum	64.5 b	2.6 d	28.1 de	5.7 c	0.43 c
Glomus microcarpum	57.6 c	2.4 a	22.9 a	5.6 bc	0.42 b
Glomus mosseae	53.2 bc	2.3 b	19.7 bc	5.5 d	0.54 e
Gigaspora margarita	66.1 d	2.7 bc	26.3 d	6.2 de	0.56 cd
Sclerocystis dussii	78.3 e	2.9 d	31.2 e	6.7 a	0.58 b
Control	22.4 e	1.98 c	7.3 b	4.8 b	0.31 d

Mean values followed by the same letter within a column do not differ significantly at p = 0.05 by DMRT.

Table 2: Effect of AM fungi on tuber yield content in *Costus speciosus* after 90 days.

Treatment	Number of Tuber/plant	Length of tuber/plant (cm)	Diameter of tuber/plant (cm)	Tuber dry weight (mg/plant)	Phosphorus content/tuber (mg/plant)
Control	10.1b	8.5 c	1.2b	11.2a	0.143a
Glomus intraradice	10.1	9.2a	1.3e	13.5d	0.448b
Glomus faciculata	10.1a	11.3d	1.4d	16.3e	0.382b
Glomus microcarpus	11.6de	11.3c	1.3ab	18.4bc	0.418cd
Glomus mosseae	11.9c	11.5e	1.2a	20.5de	0.426b
Gigaspora margarita	10.4bc	11.7d	1.6dc	19.6b	0.453de
Sclerocystis dussii	12.5 d	13.4a	1.8b	25.4c	0.612a

Mean values followed by the same letter within a column do not differ significantly at p = 0.05 by DMRT.

dussii followed by *Gigaspora margarita* and *Glomus mosseae*. The increased leaf length and leaves number was recorded in plants inoculated with *Sclerocystis dussii* followed by *Glomus fasciculatum* and *Gigaspora margarita*. However, the underground parts such as stem tuber length, tuber diameter, tuber dry weight and phosphorus content was increased by *Sclerocystis dussii* followed by *Glomus mosseae* and *Glomus microcarpum.* The present investigation on the effect of inoculation of six AM fungal species showed growth response when grown in unsterile soil. These results confirm the earlier report by Rao et al. (1989) and Lakshman & Raghavendra (1992), who have shown that medicinal plants are known to contain several secondary metabolites, which response to AMF colonization when inoculated artificially. A pronounced growth response was recorded in the present study following the inoculation with *Sclerocystis dussii* in all the parameters.

In conclusion, for improvement of seedlings of the medicinal plant *Costus speciosus,* there needs to be selection of the most efficient indigenous AM fungi to improve underground stem tubers before transplantation these to nursery stocks.

REFERENCES

Abbott, L.K. and Robson, A.D. 1982. The effect of root density, inoculum placement and infectivity of inoculum on the formation of vesicular mycorrhiza. New Phytologist, 97: 285-299.

Basu, M. and Srivasatava, N. K. 1998. Root endophytes in medicinal plants: Their population and effect. Mycorrhiza News, 1(3): 2-3.

Gerdemann, J.W. 1975. Vesicular arbuscular mycorrhizae. In: Development and Function of Roots, edited by J. G. Torrey and D. T. Charkson, pp. 575-591, Academic Press, London.

Kumar, G.S. and Murugesh, S. 2002. Studies on the VAM fungi to improve growth of some medicinal plants. Advances in Plants Science, 15(1): 43-46.

Kumar, V. M and Mahadevan, A. 1984. Dose secondary substance inhibits mycorrhizal association. Current Science, 53: 377-378.

Lakshman, H.C. and Raghavendra 1992. Vesicular arbuscular mycorrhizal fungi in medicinal plants. Current Research, 21: 21-23.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Transaction of the British Mycological Society, 55: 158-161.

Prasad, V. and Reddy, N. 1998. VA-myceotrophy in some important ornamental and medicinal plants of Gulbarga, India. Bulletin of Pure and Applied Science, 17B(1): 23-29.

Rao, Y.S.G., Suresh, C.K., Suresh, N. S., Mallikarjunaiah, R. R. and Bagyaraj, O. F. 1989. Vesicular arbuscular mycorrhizae in medicinal plants. Indian Phytopathology, 92(3).

Sing, M. and Tilak, K.V.B.R. 1990. Current trends in mycorrhizal research. In: Proceeding of National Conference on Mycorrhiza, Hisar, 14-16 February 1990. Edited by B. L. Jalali and H. Chand, pp. 70-72.