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Effects of arbuscular mycorrhizal inoculation on growth performance of *Piper longum* L. (Piperaceae) under sterilized soil conditions

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Abstract

A green house study was carried out to investigate the effect of inoculation with four native arbuscular mycorrhizal fungi (AMF), *Glomus mosseae*, *G. fasciculatum*, *G. clarum* and *G. versiforme* on growth performance of a medicinally important plant "Long pepper" (*Piper longum* L.). Inoculation with all AMF species enhanced plant growth, however, significant variation in effectiveness of the four AMF species was observed in relation to both root and shoot growth. A significantly higher total biomass (0.84g/plant) was observed in *G. fasciculatum* and *G. clarum* inoculated plants. The performance of *G. fasciculatum*, *G. clarum* and *G. versiforme* were statistically on par to each other in increasing the chlorophyll content over the control plants. The root colonizing capacity of *G. fasciculatum* was found to be significantly higher, the next being *G. versiforme*.

Key words: Arbuscular mycorrhizal, *Piper longum*, total biomass, chlorophyll content, *Glomus*

Introduction

Since long time, long pepper has been used as a medicine in 'Ayurveda', the ancient Indian Science of Life. It is an important ingredient in Ayurvedic principles/preparations such as 'Trikadu' and 'Panchakolam'. Long pepper of commerce is the dried spikes of at least four different species of *Piper (P. chaba*, *P. longum*, *P. mullesua* and *P. peepuloides)*, out of which *Piper longum* L. is the most commonly used species. Fruits of *P. longum* known as "Pippali" in Sanskrit are used as carminative, liver tonic, abortifacient and for the treatment of joint pains. *P. longum* is also one of the main components used in formulations for the treatment of gonorrhea, menstrual pain, tuberculosis, respiratory tract infections and arthritic conditions. It is also used in combination with other digestive herbs for promotion of proper digestive, bowel movement. Decoction of the fruit is used extensively in acute and chronic bronchitis and the alcoholic extract of fruits shows the promising immunomodulatory and antitumor activity (Hullatti *et al.*, 2006). Besides the spikes, the roots also have medicinal value, which contains three alkaloids *viz.*, piperine, piperlongumine or piplartine.

India is the major producer, exporter and consumer of long pepper. The plant has an annual demand of 6280 tonnes (2004-05) with an annual increase in demand of 16.3% [National Medicinal Plant Board (NMPB) web site]. As a result, it has been included in the list of 32 prioritized medicinal plants by NMPB. The plant grows wildly in Western Ghats, North-East and Himalayan region. It is cultivated also in the states of Kerala, Andhra Pradesh and Maharashtra. The plant is commonly propagated through stem cuttings and grows well in the areas with hot, moist climate and sandy loamy soil with rich organic matter and good moisture holding capacity. One-year-old stem cuttings are planted in the well-prepared field and the first crop comes after 6 months of planting.

Due to its immense economic importance, the plant is being overexploited from the wild by indiscriminate fruit collection for trade resulting into its disappearance from wild habitat at an alarming rate. The disappearance of the plant from its natural habitat can be prevented by promoting large scale cultivation. However, the problems associated with large scale cultivation are lack of quality planting material, mortality in field, poor growth and yield. These problems can be overcome by application of efficient AM fungi as biofertilizer. These fungi form symbiotic relationship with plant roots and improve the nutrient uptake by the host plant from soil thereby stimulating their growth (Smith and Read, 1997). They also enhance rooting of stem cuttings, reduce transplantation shock of the seedlings (Singh, 2002), improve their resistance to environmental stresses (Augé, 2001; Bagyaraj, 1991) and protect the host plant from root pathogens (Jalali and Jalali, 1991). They also interact synergistically with other beneficial soil microorganisms such as nitrogen fixers and phosphate solubilizers (Bagyaraj and Varma, 1995; Jeffries,1987). Therefore, in the past few decades, these AM fungi have emerged as potential biofertilizers, a cheap, environment friendly alternative to expensive chemical fertilizers (Srivastava *et al.*, 1996). Though, growth enhancement of many agricultural and horticultural crops through application of AM fungi has been reported, very little is known about their potential to enhance the productivity of medicinal plants belonging to the genus Piper and particularly *P. longum*. Therefore, the present study was carried out to investigate the influence of different AM fungi on the growth performance of *P. longum* plant.

Materials and methods

A pot culture experiment was conducted in the green house of Rajiv Gandhi University, Arunachal Pradesh, Itanagar from July to October 2006. For AMF species belonging to the genus *Glomus* namely *G. mosseae* (GM)*, G. fasciculatum* (GF), *G. clarum* (GCl) and *G. versiforme* (GV) were taken for the experiment. For each AMF species, one inoculation bed was prepared in the green house. The bed was filled with autoclaved sand soil mix (2:1)

as the rooting medium. Each bed was inoculated with culture of individual AMF species by taking out 40 g of soil inoculum from AMF pure culture pots maintained by our lab and by distributing the propagules containing large numbers of spores/sporocarps and mycelium uniformly. The beds were 10 cm thick in which inoculum were placed as a layer at approx. 5 cm from the top surface of the bed so that the growing roots from each stem cutting pass through the inoculum layer. Large numbers of piper cuttings of uniform size were planted in the prepared inoculum beds for one month before being transferred to plastic pots for further growth for next three months. This was done to ensure that plants become mycorrhizal and five live replicates are kept maintained in the pots during the entire period of the experiment. Control plants were also grown in control inoculation bed (without AMF inoculation).

All the plastic pots (200 g capacity) were properly surface cleaned and filled with 200 g autoclaved sand: soil mixture (2:1) before transplanting the rooted stem cutting. Each seedling was transplanted along with rhizosphere soil without disturbing the rooting zone by making a small cylindrical hole in the centre of the pot. The experiment was a completely randomized block design with four treatments and five replications. Treatments consisted of plants inoculated either with (a) GM, (b) GF, (c) GCl or (d) GV. The soil used in the experiment was acidic with pH 5.5, organic carbon 1.54 -2.51 mg g^{-1} and available P 0.38-0.66 µg g^{-1}. Pots were maintained in the green house at a temperature of 22 ± 1°C with 12 h fluorescent illumination with 8000 lx light intensity, and water was supplied daily to maintain the soil moisture level close to field capacity. Plants were grown without any application of fertilizer or pesticides. After 90 days of growth period, the harvesting was carried out. The biomass production was recorded in the form of shoot length, shoot fresh weight shoot dry weight and root fresh weight and root dry weight and also the total biomass of the plant. Dry weight was determined after drying the shoot and root separately at 60 °C to a constant weight in hot-air oven for 48 hours. Percentage mycorrhizal root colonization was estimated following grid line intersect method (Giovanetti and Mosse, 1980) after staining the roots with Trypan blue (Philips and Hayman, 1970). The total leaf chlorophyll content was determined spectrophotometerically after extraction in 80% acetone (Arnon, 1949).

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

Result and discussion

AMF colonization significantly increased the shoot length, shoot fresh weight, shoot dry weight (Table 1) root fresh weight, root dry weight and the total biomass of the plant (P<0.05) (Table 2). A significant increase in chlorophyll content of AMF inoculated plants was also observed over the uninoculated control plants (Fig.1 A).

Inoculation with *G. fasciculatum* resulted into the maximum shoot length (31 cm) during the growth period and it was significantly different (P<0.05) from all other AMF inoculation. The shoot length of uninoculated plants was only 7.3 cm. The next best inoculant was *G. versiformae*, (22.8 cm) and the performance of this species was similar to *G. mossae* (20.7 cm). However, in case of both shoot fresh weight and shoot dry weight, the maximum increase was observed in *G. fasciculatum* inoculated plants followed by *G. clarum*, but both were statistically at par with each other. The minimum increase of both shoot fresh weight and dry weight over the control plants (0.26, 0.07 g respectively) was observed in plants inoculated with *G. versiformae* and *G. mossae* (1.90, 0.38 and 1.82, 0.37 g, respectively).

Table 1. Effect of AM fungal inoculation on shoot length, shoot fresh weight and shoot dry weight of *P. longum* seedlings

Treatments	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Uninoculated	07.3 ± 0.519a	0.26 ± 0.023a	0.07 ±0.008a
G. mossae	20.7 ± 0.707b	1.82 ± 0.308b	0.37 ± 0.038b
G. fasiculatum	31.0 ± 1.083c	2.77 ± 0.307c	0.54 ±0.024c
G. clarum	16.0 ± 0.651d	2.09 ± 0.098cd	0.47 ± 0.020cd
G. versiformae	22.8 ± 0.704b	1.90 ± 0.282bd	0.38 ± 0.053bd
LSD at 5%	2.247	0.693	0.093

Numbers followed by the same letter within a column are not significantly different (P<0.05) by Duncan's multiple range test. Values are the means of five replicates ± standard error.

Table 2. Effect of AM fungal inoculation on root fresh and dry weight, and total biomass of *P. longum* seedlings

Treatments	Root fresh weight (g)	Root dry weight (g)	Total biomass (g)
Uninoculated	0.62 ± 0.033a	0.17 ± 0.018a	0.23 ± 0.019a
G. mossae	0.66 ±0.033a	0.20 ± 0.13a	0.57 ± 0.039b
G. fasiculatum	1.46 ± 0.130b	0.30 ± 0.019b	0.84 ± 0.041c
G. clarum	1.03 ± 0.048b	0.24 ± 0.018b	0.71 ± 0.031d
G. versiformae	0.77 ± 0.031a	0.18 ± 0.017a	0.55 ± 0.044b
LSD at 5%	0.2	0.042	0.102

Numbers followed by the same letter within a column are not significantly different (P<0.05) by Duncan's multiple range test. Values are the means of five replicates ± standard error.

G. fasciculatum inoculated plants also showed superior performance in case of both root fresh and dry weight (1.46, 0.30 g, respectively) in comparison to control plants (0.626, 0.17 g, respectively). The second best AMF was *G. clarum*, its performance was also statistically at par with the former one and followed by *G. versiformae* and *G. mossae,* respectively. On the other hand, the performance of *G. versiformae* and *G. mossae* was statistically similar with that of control plants in increasing both the root fresh weight and dry weight. All the AMF inoculants tested significantly increased the total biomass production over the uninoculated control plants. The biomass was enhanced more due to inoculation with *G. fasciculatum* (0.84 g) followed by *G. clarum*. The efficacy of *G. versiformae* and *G. mossae* was found to be statistically similar.

The total chlorophyll content of both inoculated and uninoculated plants has been presented in Fig. 1 (A). The total chlorophyll content of leaves differed significantly and the content was highest in plants inoculated with *G. clarum* (1.163 mg g^{-1}), which was followed by *G. fasciculatum* (0.983 mg g^{-1}) and *G. versiformae* (0.885 mg g^{-1}). However, these three species did not differ significantly in this respect. The least amount of chlorophyll content (0.387 mg g^{-1}) was observed in uninoculated plant.

Piper seedlings showed varied response to the inoculation of different AM fungi. It has been reported that the effect of mycorrhization on plant development is influenced both by the

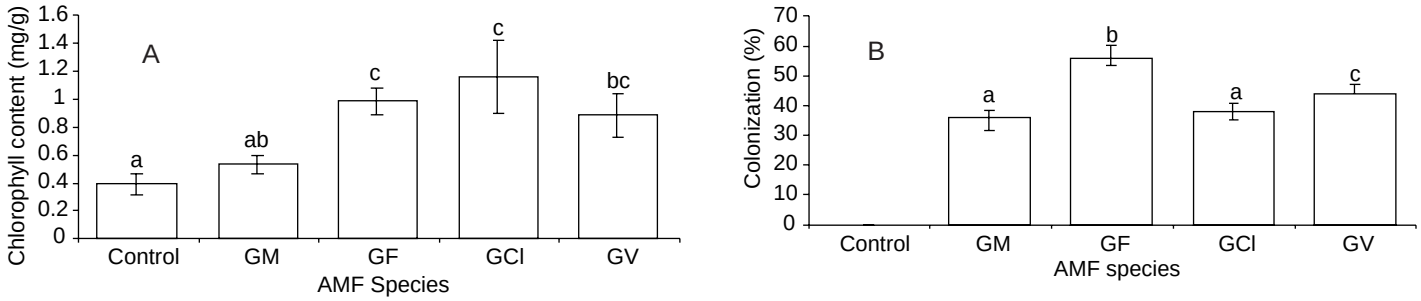

Fig. 1. Effects of inoculation with four different AM fungi on (A) chlorophyll content in plant leaves and (B) Colonization (%) in plant roots of *P. longum*. Histograms with a common letter are not significantly different (P<0.05) by Duncan's multiple-range test. Error bars indicate the standard error of the replicates

host plant and the fungal partner; hence different isolates of AM fungi can result in different effects on the same plant (Jackobsen *et al.*, 1992). Our study also supports this finding as all the four AMF species behaved differently in their growth promoting capabilities. Further, host preference among AM fungi has been reported by earlier researchers (Bagyaraj *et al.*, 1989; McGraw and Schenck, 1981). In present study, *G. fasciculatum* was found to be the most efficient AMF in increasing plant growth and at the same time it was also the most infective AMF colonizing the root cortical cells maximally. Therefore, it seems that this native AMF has some preference for the *P. longum* plant and their symbiotic relationship is most compatible. Enhanced plant growth due to mycorrhizal inoculation has been reported earlier in some medicinal plants also (Earanna *et al.*, 2002; Sailo and Bagyaraj, 2005; Sen and Das, 1998). This may be due to a combined effect of many processes contributing to improved P acquisition by mycorrhizal plants including increased absorption surface area of roots (Smith and Read, 1997) and increased exploration of soil micro sites by AMF hyphae (Cui and Caldwell, 1996). The enhancement of chlorophyll content due to AMF colonization has been reported by Morte *et al.* (2000). The increase of chlorophyll content in AM inoculated plant tissues may be due to an increase in stomatal conductance, photosynthesis, transpiration, enhanced plant growth (Hayman, 1983; Levi and Krikum, 1980), or due to the presence of larger or more numerous bundle sheath chloroplasts present in the inoculated leaves (Krishna and Bagyaraj, 1984).

The root colonization of *P. longum* by all four AMF species differed significantly (Fig.1B). Significantly maximum percentage root colonization was observed due to *G. fasciculatum* inoculation followed by *G. versiformae*. There was a positive trend between the intensity of mycorrhizal root colonization and growth response. This supports the observations made by earlier workers on other plants (Earanna *et al.*, 2002; Gracy and Bagyaraj, 2003; Kormanik *et al.*, 1982).

On the basis of the findings, the study suggests that AMF inoculation can effectively increase the growth of *P. longum*. Based on the growth performance of plants, *G. fasciculatum* was found to be the best AMF for inoculating *P. longum* followed by *G. clarum*.

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