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EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON MICROPROPAGATED BANANA

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ABSTRACT

Micropropagated plants of banana cultivar (*Musa acuminata* x *Musa balbisiana* AAAB) were inoculated with two species of arbuscular mycorrhizal fungi [*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Glomus fasiculatum* (Thaxter & Gerd.) Gerd. & Trappe] during the initiation of acclimatization phase. Inoculated plants exhibited significantly greater biomass as compared to the non-inoculated control plants. However, *Glomus mosseae* was more effective in stimulating growth of micropropagated banana plantlets as compared to *Glomus fasciculatum*.

INTRODUCTION

Nowadays, *in vitro* micropropagation techniques are being increasingly applied to production of fruit trees. This procedure results in disappearance of natural microflora of the micropropagated plants. Thus, during relatively long periods of their development, the plants are without arbuscular mycorrhizal fungi. Inoculation of micro propagated plantlets with arbuscular mycorrhizal fungi have shown to induce positive influence on plant acclimatization and growth (Ravolanirina *et al.*, 1989; Schubert *et al.*, 1992; Vestberg & Estaum, 1994; Brazanti *et al.*, 1992). Therefore, it is interesting to explore the effects of mycorrhizal inoculation on early stages development, with the aim of obtaining better establishment and growth of micro-propagated plantlets (Fortuna *et al.*, 1998).

Banana (*Musa* sp.) has been reported to exhibit positive response to inoculation with arbuscular mycorrhizal fungi and its growth and P-uptake capacity has been reported to increase significantly as compared to non-inoculated plants (Declerck *et al.*, 1995). Banana micropropagation is a

well established technique with potential application for the production of virus-free plants. Micropropagated banana plants are grown in sterile media *invitro* and transplanted thereafter in substrate which, even when not sterilized often lack arbuscular mycorrhizal fungal propagules. At this stage plants can be easily stressed by unfavourable nutritional and environmental conditions. The presence of well developed mycorrhizal system, absorbing nutrients and water from the substrate by attached net work of external hyphae may be an important factor to plant growth (Schubert *et al.*, 1990).

Previous work reported that micropropagated plants could be successfully colonized by arbuscular mycorrhizal fungi *invitro* (Kiernan *et al.*, 1984; Declerck *et al.*, 1995). However, *invitro* inoculation is a lengthy and cumbersome practice requiring isolation and sterilization of fungal spores or colonized root fragments. Further, after transplanting, micropropagated plants replace majority of their roots grown *invitro* with new ones and as a consequence most of the mycorrhizal roots would be lost at this stage. Therefore, in *vivo* arbuscular mycorrhizal inoculation seems more suitable or commercial application than *invitro* inoculation (Schubert *et al.*, 1990). The response of micropropagated banana to inoculation with arbuscular mycorrhizal fungi under controlled environmental conditions has been studied by Declerck *et al.*, 1995. The objective of this study was to evaluate the influence of mycorrhizal inoculation on post-vitro acclimatization and growth of micropropagated banana plantlets.

MATERIALS AND METHODS:

Pot cultures of arbuscular mycorrhizal fungi - Peat moss based cultures of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Glomus fasciculatum* (Thaxter & Gerd.) Gerd. & Trappe were procured from Depar-tment of Microbiology, UAS, G.K.V.K Cam-pus, Bangalore, India. The purity of the inoculum was checked (Gerdemann & Nic-olson, 1963) and it contained 350 spores^{-1} 50g soil.

Plant material - Six weeks old micropropagated plantlets of banana *Musa acuminata* x *Musa balbisiana* AAAB) were procured from nursery farm prior to hardening stage.

Experimental set up - Garden soil (pH 6.1; E.C 0.06 mm hos/cm) of low available phosphorus status (6 Kg/Ha) was used. The macronutrient and the micronutrient content of the soil were analyzed in soil testing laboratory, which is recorded as follows.

- Macronutrients- Total Nitrogen (51 Kg/ Ha); Available phosphorus (89 Kg/ Ha).
- Micronutrients- Zn (2.67µg/g), Cu (3.84 µg/g). Fe (2.63µg/g) & Mn (19.5µg/g)

Soil was sterilized for 2hrs at 15 lbs for three consecutive days to eliminate naturally occurring endophytes and other contaminants. Clean pots of 12.5cm diameter were taken and filled with sterilized soil up to ¾ th of its volume. 1.5g of inoculum were spread in a layer. It was covered with 2cm of soil. Tissue cultured plantlets of banana procured from the nursery farm, just prior to hardening stage (*i.e.* with out any AM colonization) were placed in the pots. 0.5g of the inoculum

were spread around the root zone and again it was layered with sterilized soil. The experiment consisted of three treatments with 15 replications as given below.

- Control (un-inoculated plantlets)
- Inoculated with *Glomus fasciculatum*
- Inoculated with *Glomus mosseae*

The pots were placed in glass house and watered daily to field capacity with distilled water. At weekly intervals the plants received Hoaglands nutrient solution from which phosphorus had been excluded. After two months, the plants were transferred to 35-cm diameter earthen pots containing unsterelized garden soil. The nutrient status of the soil is the same as mentioned above. The pots were maintained outside the glass house for another 2 months. Growth measurements were recorded at the end of 120 days. The parameters carefully selected to examine the effect of arbuscular mycorrhizal fungi were plant height, stem girth (measured 2cm above the soil), leaf area (third leaf from top), root length and fresh weights of the root and shoot system. Further, under ground and the aerial parts of the plants of three treatments were oven dried at 80°C for 6 hrs and constant dry weights were recorded.

RESULTS

Arbuscular mycorrhizal fungi inoculated plants showed distinct morphological differences such as profuse branching of roots over un-inoculated (control) plants. Results obtained are recorded in table 1 and 2. The plant height and leaf area ranged from 18.00 cm (control plants)-30cm (inoculated plants) and 40cm^2 (control plants)- 147cm^2 (inoculated plants) respectively (Table1). The stem girth and root length ranged from 2.00 cm (control plants)- 4.70 cm (inoculated plants) and 12.0 cm (control plants) - 28.0 cm (inoculated plants) respectively (Table 1).

Table 1 : Influence of arbuscular mycorrhizal fungi on growth attributes of micro-propagated banana

Treatment	*Plant height (cm)	*Leaf Area (cm^2)	*Stem girth (cm)	*Root length (cm)
Control	18 ± 1.25	40 ±2.68	2.4 ±0.30	12 ± 0.35
Glomus facsiculatum	24 ± 1.75	82.5 0 ± 1.26	3.6 ± 0.15	15.5 ± 0.32
Glomus mosseae	30 ± 3.85	147±2.44	4.7± 0.07	28 ± 9.52
C.D.$_{0.05}$	5.97	2.34	0.23	0.52

*Mean = n ± 1 S.D; F test significant at 0.05 level of probability.

The fresh shoot and root weight, ranged from 4.57 g (control plants) - 13.3 (inoculated plants) and 2.50g (control plants) - 5.25g (inoculated plants) respectively (Table 2). The dry shoot and root weight, ranged from 1.8g (control plants) - 2.51 (inoculated plants) and 1.64g (control plants) - 4.84g (inoculated plants) respectively (Table 2).

DISCUSSION

Studies on the response of *Glomus mosseae* and *Glomus fasciculatum* on growth of micropropagated banana revea-

led definite stimulation of root and shoot growth in mycorrhizal plants as compared to un-inoculated controls. The beneficial effects of arbuscular mycorrhizal colonization on plant growth has been mostly attributed to improved uptake and concentration of nutrients in plant tissue especially phosphorus (Declerck *et al.*, 1995). In the present study, at the final harvest, leaf area of all the inoculated plants were significantly larger than controls. Similar observations were reported by Schubert *et al.* (1990) who studied the effect of arbuscular mycorrhizal fungi on micropropagated grapevine at the beginning of acclimatization phase in a glass house experiment.

Table 2: Influence of arbuscular mycorrhizal fungi on biomass of micropropagated banana.

Treatment	Fresh weight		Dry weight	
	*Shoot (g)	*Root (g)	*Shoot (g)	*Root (g)
Control	4.57 ± 0.23	2.5 ± 0.32	1.8 ± 0.04	1.64 ± 0.09
Inoculated with *Glomus fasciculatum*	8.72 ± 0.05	3.5 ± 0.22	2.15 ± 0.09	1.76 ± 0.03
Inoculated with *Glomus mosseae*	13.3 ± 0.55	5.25 ± 0.13	2.51 ± 0.03	1.84 ± 0.03
$C.D._{0.05}$	0.45	0.18	0.07	0.02

*Mean = n ± 1 S.D; F test significant at 0.05 level of probability

Furthermore, plants inoculated with arbuscular mycorrhizal fungi recorded significantly higher plant height, shoot and root fresh weight as compared to control plants. These observations are in accordance with the findings of Sivaprasad *et al.* (1995) on *exvitro* establishment of tissue-cultured plantlets of jackfruit through arbuscular mycorrhizal fungi. Fortuna *et al.* (1998) also reported higher shoot length, shoot and root fresh weight in micropropagated plum inoculated with arbuscular mycorrhizal fungi *in vivo* as compared to control in a green house experiment.

In the present study, inoculated plants also recorded significantly higher shoot and root dry weight as compared to control plants. These observations support the earlier findings of Declrek *et al.* (1995) where they reported increased shoot dry weight in mycorrhizal banana plant (*Musa acuminata* AAA) over the un-inoculated controls in a green house experiment.

Many fungal species are able to form arbuscular mycorrhizae on the same host but they vary in their efficiency in increasing plant growth (Plenchette *et al.*, 1983). These differences may depend on genetically controlled physiological characters of the fungus which play a role in the uptake of nutrients from the soil and in their transfer to the host root cells such as extraradical mycelium (Abbot & Robson, 1977). In the present study, *Glomus mosseae* was the most effective species in stimulating growth of micropropagated banana as compared to *Glomus fasciculatum.* Similarly, Kiernan *et al.* (1984) reported that micropropagated strawberry plants inoculated with *Glomus mosseae* exhibited better growth as compared to those with *Glomus constrictum* or *Glomus epigaeum.* Declerck *et al.* (1995) also

reported that *Glomus macrocarpum* resulted in highest relative mycorrhizal dependency as compared to other species in micropropagated banana grown on sterilized media. In another glass house experiment, Schubert *et al.* (1990) reported that *Glomus mosseae* and *Glomus occultum* significantly increased the leaf area of micropropagated grapevine at all levels of P-fertilization as compared to *Glomus versiforme* in sterilized medium.

In the present study, the plants were inoculated in sterilized substrate and at the end of two months, they were transplanted to the unsterile media containing indigenous population of arbuscular mycorrhizal fungi. Thus, our studies supports the earlier findings that arbuscular mycorrhizal fungi can be introduced into soils having considerable population of indigenous mycorrhizal fungi and can stimulate much more plant growth than indigenous fungi (Mosse, 1977).

Our study confirms the contention that inoculation of micropropagated banana plants with arbuscular mycorrhizal fungi is beneficial if appropriate species and soil conditions are employed and that the knowledge of the best conditions for growth and activity of arbuscular mycorrhizal fungi is of paramount importance if these organisms are to be commercially exploited.

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