

CHAPTER 5

MYCORRHIZATION OF MICROPROPAGATED GRAINDE NAINÉ (*Musa acuminata*, group AAA) WITH *GLOMUS MOSSEAE*

5.1 Introduction

Current developments emphasize the potential of plant tissue culture for the propagation, conservation and genetic improvement of bananas. A wide range of banana cultivars of all genomic constitutions has been found amenable to *in vitro* culture (Cronauer and Krikorian, 1984). However, micropropagated plants have problems concerning their survival and development, particularly in the acclimatization phase demonstrated by poor root, shoot and cuticle development (Varma and Schuepp, 1995). The sterile environment during the *in vitro* phase gives rise to pathogen free-plants, however, this technique presents the disadvantage of depriving plants of all soil microbiota which may include important rhizosphere commensals that maintain normal biological homeostasis as well as potentially crucial plant symbionts such as arbuscular mycorrhizal fungi (AMF) (Rizzardi, 1990).

The lack of exposure to rhizosphere organisms in micropropagated plants often continues in the nursery during the weaning phase and makes eventual outplanting stressful and/or unsuccessful.

The potting substrates are disinfected (either with steam or with general biocides) to eradicate or to prevent soil-borne plant pathogens and pests. General biocides are often applied to avoid weed problems in nursery soil substrates. It has been shown that general biocides greatly reduce the propagule density of indigenous AMF (Sieverding, 1991). Untreated potting material is derived from subsoils that have low natural content of soil-borne pathogens and these soils contain little in the way of AMF propagules. Composted organic materials and artificially produced media like vermiculite and perlite do not contain any AMF.

Non-mycorrhizal plants obtained from the micropropagation process eventually become mycorrhizal when they are planted in field soil. As demonstrated in chapter 3, AMF occur naturally in banana farm soils of Uganda. However, an early inoculation of these plants with selected AMF promises to improve plant survival and performance and allow lower chemical inputs (Varma and Schuepp, 1995) throughout the growth and productive phase of the plant.

Several workers have demonstrated that the acclimatization period of micropropagated plants can be shortened by the application of mycorrhizal technology (Lovato *et al.*, 1995). This reflects the potential gain in time and cost that is made possible through the use of mycorrhizal technology. Varma and Schuepp (1995) reported that mycorrhized apple plants were more uniform in size. From an industrial and economic point of view the uniformity of the plants is a desirable characteristic in any nursery, allowing for a homogenous

classification of the stock. Mycorrhization at the nursery level allows easy access to large numbers of plants, requires lower inoculum concentration and has an added advantage in that mycorrhiza and plant develop together.

This experiment was conducted to determine if a species of AMF, isolated from Ugandan banana farm soils, can colonize micropropagated banana plantlets (*Musa acuminata* cv. Grande Naine, group AAA).

(2) inoculated banana plantlets 5 cm in height from the weaning stage (30 replicates)

(3) control: micropropagated banana plantlets without inoculation for both stages of growth (10 replicates each) and,

(4) sterile sudan grass (*Sorghum sudanense* (Piper) Staph.) seedlings were inoculated to test viability of inoculum (10 pots) according to the method of Tommerup (1994)

5.2.2 Experimental plant material

Micropropagated banana plantlets (*Musa acuminata* cv. Grande Naine, group AAA) were supplied by African Biotechnologies, Tzaneen, South Africa. *In vitro* plantlets are derived from meristems tissue after multiple divisions on sterile modified banana growing medium. The weaning phase plantlets were allowed to grow to a 5-cm stage in plastic containers (60 ml) filled with peat moss in a

5.2 Materials and methods

5.2.1 Experimental design

The following treatments were established:

- (1) inoculated rooted banana plantlets from sterile modified banana growing medium (*in vitro* stage) (30 replicates),
- (2) inoculated banana plantlets 5 cm in height from the weaning stage (30 replicates),
- (3) control: micropropagated banana plantlets without inoculation for both stages of growth (10 replicates each) and,
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misting tunnel before inoculation. The experimental plants were washed free of the growing medium before inoculation.

5.2.3 Inoculum source

G. mosseae (Nicolson & Gerdemann) Gerdemann & Trappe spores collected from a monospecific culture as described in section 4.2.4 were surface sterilized according to the method used in section 4.2.3. The spores were then incubated in 0.02 % Streptomycin for 30 days in a fridge (4°C) to break any possible dormancy. Spores were washed several times with distilled water before inoculation.

5.2.4 Inoculation method and growth conditions

The experiment was carried out in May-July 1999 at African Biotechnologies, Tzaneen. Approximately 100 spores (per plant) were collected by pipette and placed directly unto the surface of the roots of the experimental plants prior to placing into growing substrate. The substrate consisted of a 1:1 sterilized soil/sand mixture obtained from the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI). The plants were maintained in a misting tunnel. Relative humidity was 60-70% and temperatures ranged between 25°C-27°C (day and night). The tunnel received a 12-hour day photoperiod and

had 40% shading. Plants were watered to field capacity once a day with no addition of nutrients.

5.2.5 Harvesting

At 3, 6 and 10 weeks after inoculation, ten plantlets were randomly harvested from treatments 1 and 2. At 3 and 6 weeks after inoculation, two plantlets were randomly harvested from treatment 3 and the remaining plantlets were harvested at 10 weeks. *S. sudanense* viability test plants were all harvested at 10 weeks.

5.2.6 Measurement of infection by AMF

Whole root systems of each plantlet were carefully washed under tap water, cut into 1 cm sections, cleared in 10% KOH and stained with trypan blue according to the method of Koske and Gemma (1989). The percentage root colonization by AMF was determined using the visual estimation technique (Kormanik and McGraw, 1982) under a dissecting microscope at a X400 magnification (Chapter 3; Section 3.2.2).

5.3 Results

Inoculated or not with *G. mosseae*

Inoculum produced for mycorrhization of banana plantlets consisted of a pure culture of *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe.

Photographs of wall structure and spore shape and colour are presented in chapter 4 (Plate 2). Inoculum produced was viability tested on *S. sudanense* in the greenhouse at the ARC-PPRI. Average root percentage colonization ranged between 6-26% of the test plant root system. This inoculum was subjectively considered to have a high infection potential.

*Class 1 = 0-5% infection.

Mycorrhization of both *in vitro* and weaning phase plantlets was demonstrated in the 10-week post-inoculation plantlets. All the other plantlets from the post-inoculation age groups were negative for the demonstration of infection by root staining using the methods of Koske and Gemma (1989). Examples of the infection are provided in Plate 3. Root percent colonization ranged between 0-5% infection of the test plant root system (Table 4). Thirty percent of the *in vitro* plants and fifty percent of the weaning phase plants harvested at 10 weeks were mycorrhizal.

Control plants consistently demonstrated no infection throughout the course of the experiment.

Table 4: Mycorrhizal colonization of micropropagated banana plantlets inoculated or not with *G. mosseae*

Micropropagated banana plantlets (both *in vitro* and weaning phase) exhibited

% colonization (Class)*	3 weeks	6 weeks	10 weeks
<i>In vitro</i> phase plantlets	0	0	1
Weaning phase plantlets	0	0	1
Non- inoculated control	0	0	0

*Class 0 = no infection.

*Class 1 = 0-5% infection.

occurred earlier in this experiment because the AMF were used. There is a dormancy period of approximately 6 weeks for *Glomus spp.* in wet soil and that no environmental condition will shorten this maturation period before germination.

In this experiment a low colonization level of the banana plantlets was recorded, ranging between 0-5% of the total root length. A high level of root colonization is in principle desirable, since it means that sites for the entrance of microorganisms into the roots are already occupied. This is the basis of potential resistance to pathogenic microorganisms (Gianinazzi *et al.*, 1990). Production of antimicrobial substances like phytoalexins increases with the level of arbuscular mycorrhizal infection (Morandi *et al.*, 1984). Considering the fact that Ugandan banana farming is under pressure from continuously declining production due to plant parasitic nematodes, banana weevil, leaf diseases, *Fusarium* wilt and, Banana Streak Virus, means that selection of efficient AMF requires a critical

5.4 Discussion

Micropropagated banana plantlets (both *in vitro* and weaning phase) exhibited AMF development 10 weeks after inoculation. It is possible that no infection occurred earlier in this experiment because the spores were under the influence of a dormancy period not broken in the conditions and time-span used. There is evidence that the germination of spores of some species of AMF is blocked by a dormancy factor for a certain period of time depending on the species of AMF (Tommerup, 1983; Bowen, 1987; Bagyaraj, 1991). Tommerup (1983) reported a dormancy period of approximately 6 weeks for *Glomus spp.* in wet soil and that no environmental condition will shorten this maturation period before germination.

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evaluation of infection levels. Several studies have shown that plant growth enhancement by AMF generally is related to colonization level and to the extent of external mycelium, the latter being necessary for P uptake (Sanders *et al.*, 1977; Abbott and Robson, 1981; Tommerup, 1994)).

ideal for increased AMF root colonization.

There is a range of factors that can affect the result of mycorrhizal inoculation in a micropropagation system. It has been shown that increased inoculum dosage results in increased colonization rate (Daft and Nicholson, 1969) and increased percentage root colonization (Johnson, 1977). It is possible that the amount of inoculum used in this experiment was low thereby resulting in the low percentage colonization observed. However, *S. sudanense* demonstrated high infection, which draws attention to possible host-dependent factors in receptivity to AMF infection.

Extent of root colonization of a fungal species varies with the host plant (Declerck *et al.* 1995). This may explain the difference in colonization levels between the banana plantlets and *S. sudanense*. It is possible that *S. sudanense* has a greater mycorrhizal dependency (MD) than Grainde Naine.

Both temperature and light have been shown to have a significant influence on colonization by AMF under greenhouse conditions. Higher temperatures generally result in greater root colonization (Hayman, 1974). According to Schenck and Schroder (1974), maximum arbuscule development occurs near

30°C, mycelial colonization of the root surface is greatest between 28°C and 34°C and vesicle development is greatest at 35°C. In this experiment, the banana plantlets were grown in a tunnel with temperatures ranging between 25°C-27°C (day/night). It is possible that the temperature settings of the tunnel were not ideal for increased AMF root colonization.

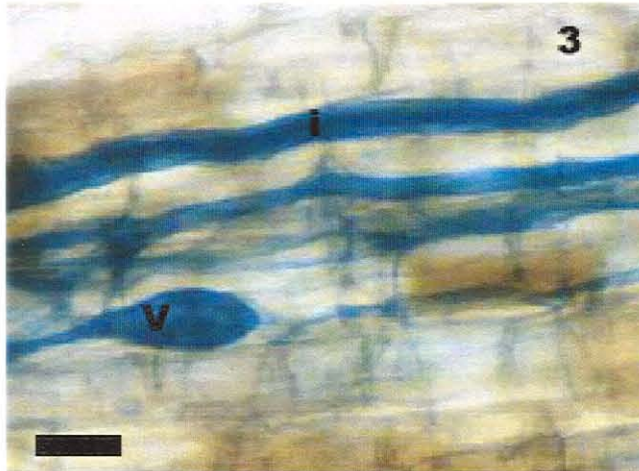
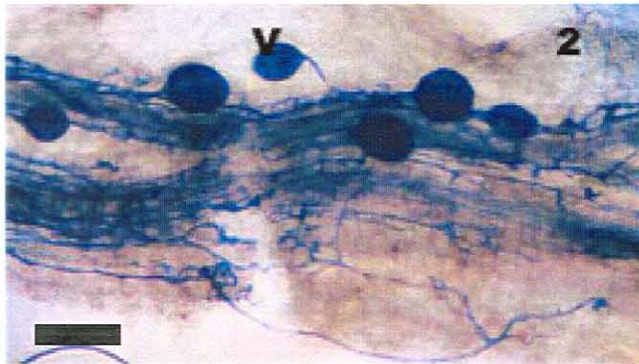
Low light intensity can significantly reduce root colonization (Hayman, 1974; Furlan and Fortin, 1977; Johnson *et al.*, 1982). In this experiment, the tunnel received a 16-hour photoperiod, however, it had 40% shading. It is therefore possible that the tunnel did not receive ideal light intensity for increased AMF root colonization. It is also possible that due to the diminished light intensity of the tunnel (40% shading), the banana plantlets carried out insufficient photosynthetic capacity to support high levels of colonization.

Gange *et al.* (1999) suggest that the level of mycorrhizal colonization recorded in any particular plant species at a particular time could be dependent on the stain employed. The differences in the ability of stains to reveal AMF is likely to be due to differential stain penetration of the roots of different plant species and the extent to which the stain is taken up by the fungus (Morton, 1985). The ability of a stain to penetrate a piece of plant tissue is affected by many factors including stain type, the vehicle in which it is dissolved, the concentration and pH of the staining solution, the temperature and duration of staining and the thickness of the plant material (Hayat, 1993). In addition, the nature and location of the target

material also has an effect, and it is possible that the depth at which arbuscules are located in the roots differs between plant species. This is because AMF hyphae grow through intercellular air spaces, and suberin in the cell walls regulate the growth direction of the fungus (Brundrett *et al.*, 1996), two factors which could vary between plant species. It would appear that trypan blue (used in this experiment) has different staining abilities in different plant species. It is possible that plants recorded as non-mycorrhizal or with low colonization are not so at all; the data simply reflect the inability of the stain used to detect the fungus.

The main hurdle in establishing strains of mycorrhizae on host plants is the starting point, the difficulty being two main points concerning the quality of spores: (1) ability of quick germination and (2) development of the mycelial network responsible for penetration of root cells (Rai, 2001). The results of this experiment suggest that *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe spores isolated from Uganda are of low quality with regards to the two points mentioned above. It is possible that the use of *G. etunicatum* Becker & Gerdemann or a combination of both species isolated from Uganda would have yielded different results. There is evidence that mixed inocula may interact with plants differently from single-species inocula (Daft, 1983). It is also possible that the use of whole inoculum (spores, roots, hyphae) as opposed to the use of spore-only inoculum for the inoculations would have also yielded different results.

It is conspicuous that *in vitro* inoculation of micropropagated banana plantlets at African Biotechnologies does not seem a feasible practice without changes to a number of factors such as maximizing greenhouse growth conditions conducive to AMF and root growth and screening for efficient mycorrhizal strains. Integrated technologies should be considered for future studies concerning the mycorrhization of micropropagated plants. Von (1998) emphasized the use of mycorrhizae helping bacteria (MHB) for enhancement of growth of plants. He reported that rhizosphere strains of *Bacillus mycoides* and *Pseudomonas fluorescens* promoted AMF formation in various crop plants by improving susceptibility of roots to AMF. The role of MHB was studied in detail by Garbaye *et al.* (1996). Evidently, MHB can also be inoculated into the roots for giving an opportunity to AMF for efficient, profuse and rapid colonization.



CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Consistent AMF colonization of *S. sudanense* trap plants by banana plantation soils from Uganda suggests that AMF are active biological components of this habitat. MIP assessment of the Ugandan banana farm soils showed that soils containing banana genotype *Musa* AAA had a higher mycorrhizal activity than

Plate 3

Figure 1 and 2. *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe in roots of weaning phase banana plantlets showing darkly staining vesicles (V).

Note the abundant root hairs (R) of Grainde Naine (*Musa acuminata*, group AAA). Bar = 21 μm .

Figure 3 and 4: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe in roots of *in vitro* banana plantlets showing intraradical spores (S) and intraradical foraging hyphae (i) growing parallel to each other and the root axis. Vesicles are seen in figure 3. Bar = 14.4 μm .

Bananas are known to form facultative symbiosis with AMF in the field (Rizzardi, 1990). However, mycorrhization of these plants in the farm soils would be unplanned and uncontrolled. This would result in association with unknown indigenous AMF of unknown beneficial properties and quantity. Therefore screening for AMF effective at addressing specific problems faced by Ugandan