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Detection and Observation of Arbuscular Mycorrhizal Fungus (AMF) in the roots of Shea Tree Seedlings Vitelleria paradoxa in Nigeria

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ABSTRACT: Shea tree seedling root noodles also known as black dots were detected three months after the application of Arbuscular Mycorrhizal Fungus (AMF) Acaulospora scrobiculatu in roots of Shea tree seedlings. The present study was carried out to detect the presence of mycorrhiza on the roots of Shea tree seedlings with the view to know whether it support the growth of the seedlings in the nursery The 300 gram application of AMF lead to the highest colonization rate observed with 59.00%. The 100 gram application also had colonization rate with 42.14% when compared with non application of AMF at 0.0 gram which recorded 0.00% colonization rate while the second non application of AMF also at 0.0 gram recorded 8.90% colonization rate. This suggests there were significant differences between treatment L with 300 g at 59.00% and treatment k with 100 g at 42.14% compared with treatment G with 0.0 g at 8.10%, A with 0.0 g at 0.00%, and D also with 0.0 g at 0.00% respectively. The application of AMF best supported the growth of Shea tree seedlings in the nursery with more leaves, thickness of the roots, black dots and influencing the growth of mycorrhizal hyphae. Non application of AMF was less suitable in supporting growth. In the absence of AMF, rich top soil of decomposed organic matters should be used.
Key words: Arbuscular mycorrhiza, Shea tree, Root, Colonization

Introduction

The shea tree is important as an economic crop because of the heavy demand for its butter both locally and internationally mainly as cocoa butter (Abigor et al., 2008). The natural tree populations are subjected to normal bush burning, competition from weeds and several species of epiphytes that drastically reduce yields and destroy many of the trees, thus resulting in long gestation period of growth (FAO, 1988).

Mycorrhizal fungi are normally root symbiotic inhabitants which aid plants primarily in uptake of water and mineral nutrients, The degree of exchange between the cortical cells of the host root and the fungal endophyte apparently depends largely on the amount of exchange surface and on the inherent efficiency of the endophyte in acquiring water and nutrients, especially phosphorus and zinc (Brenda, 1980).

Soil microorganisms are largely responsible for the recycling of nutrients within terrestrial ecosystems (van der Heijden et al., 2008). Root-associating fungi such as mycorrhizal symbionts can acquire nutrients directly from soil organic matter yet they vary widely in their ability to do so (Read et al., 2004). Emergent patterns among the types of mycorrhizal associations suggest a functional relationship between the leaf litter traits of plant hosts and the nutrient-acquiring traits of their fungal symbionts.

Arbuscular mycorrhizas (AM) are symbiosis between plant roots and fungi belonging to the Glomales. The mutualistic nutrient exchange in this symbiosis is characterized by the transfer of phosphorus from the mycosymbiont to the host plant and by the reverse transfer of carbon compounds derived from photosynthates (Smith and Read, 1997; Solaiman and Saito, 1997). It is now widely accepted that phosphate in the soil is taken up into the extraradical hyphae by a phosphate transporter, subsequently condensed into

polyphosphate and translocated by protoplasmic streaming into the intraradical hyphae. The arbuscular hypha is probably the main site for the nutrient exchange.

Arbuscular mycorrhizae (AM) are widespread mutualistic symbioses between most of the terrestrial plants and fungi of the phylum Glomeromycota (Schüßler et al., 2001). The mutualistic association sets up a strategy to improve the nutritional status of both partners. The fungi receive fixed carbon compounds, mainly in the form of glucose (Schüßler et al., 2007) from the host plant, whilst the plant benefits from the association by increased nutrient uptake, especially phosphate (Ezawa et al., 2002) and nitrogen (Govindarajulu et al., 2005), as well as water (Uehlein et al., 2007) and Improved tolerance to abiotic stress and resistance to pests have been also noted

AMF enlarge the soil volume from which nutrients can be taken up, via an extensive mycelium network, enabling host plants to access more resources (Finlay 2004). As a consequence, AMF enhance uptake of nutrients, particularly phosphorus (Hayman and Mosse 1971), and may allow for a reduction of the amount of fertilizers applied (Linderman and Davis 2004). Furthermore, AMF can protect the plant against biotic (diseases) and abiotic (drought) stress, and improve soil aggregation (Gosling et al., 2006).

One of the major problems against the successful establishment of Shea tree nursery is the problem of gestation period of seedlings from nursery to the field which include planting of seeds and grafting. Before it is finally being transferred to the field, it takes one and half year. This eventually results to acute shortage of seedlings and the upsetting of a long term planting. Elsewhere AMF has been applied to increase plant uptake of nutrients from the soil for growth (Ezawa et al., 2002). Literature review revealed that no work has been reported on detection and observation of AMF in roots of Shea tree seedlings in Nigeria. The study was carried out to detect the presence of AMF on the roots of Shea tree seedlings with the view to know whether it supports the growth of the seedlings in the nursery.

Materials and Methods

Root samples

Root samples were washed free of soil. It is imperative that KOH or staining solution volumes are sufficient for the amount of roots being processed. The roots were cut into 2-4 cm long segments before clearing them.

Staining roots to observe mycorrhizal colonization.

The KOH 10% w/v was used to clear roots. This require root samples that are not more than 1-2 g. Root clearing was done in an autoclave, which provides the most efficient means of processing samples. The KOH 10% W/V was autoclaved at 15-20 minutes at 121° C was used. Roots were removed from KOH and rinsed with water two to three times to remove to remove excess KOH. Roots that were too pigmented were soaked in alkaline H202 solution for two to three times. They were later soaked 1 per cent HCL for 5 minutes. Roots were removed from HCL before staining with in acidic glycerol solution containing trypan blue at 90 ºC for one hour. The stained solution was discarded and wee kept in acidic glycerol at room temperature.

Measuring mycorrhazal colonization

Stained roots were spread out evenly in the Petri dish under a dissecting digital Motic 230 camera microscope connected to the computer and scanned vertical and horizontal lines of the grid. %MycCol = (Total no of intersections with mycorrhizally infected roots / Total no of intersections between root and the gridline) x 100.

Statistical analysis

The experiments were repeated three times. They were kept in a randomized manner.
Data were analyzed as % MyCol = (Total no of intersections with mycorrhizal infected roots / Total no of intersections between root and the gridline) x 100 (Fatima et al., 2008), and student test analysis (t-test).

Results

Black dots representing mycorrhizal colonization was detected and observed on the roots of shea tree seedlings using staining method. Calculation of percentage of mycorrhizal colonization rate was based on the number of black dots on the roots. A good percentage of colonization rates of the roots were achieved with 300 g at 59%. (Table 1)

There was non significant difference between treatment L with 300 g at 59.00% and treatment k with 100 g at 42.20% when compared with the significant difference of treatment G with 0.0 g at 8.10%, A with 0.0 g at 0.00%, and D also with 0.0 g at 0.00% respectively (Table 1).

One out of the 0.0 gram application under light microscopic observation of the stained trypan blue on Shea roots seedling counterstained with acidic glycerol revealed that the roots did not contained root noodles (black dots) (Figure 1A). However, the second 0.0 gram with the same application revealed that the roots contained few and thin root nodules (Plate 2 A and Figure 1B) but with few number of leaves (Plate 1). However, the 100 gram and 300 gram applications also under light microscopic observations of the stained trypan blue on Shea roots seedling counterstained with acidic glycerol revealed that the roots contained root noodles (black dots) (Figure 1 C and D). Moreso, the 300 gram application had thick root noodles (Plate 2 B) and more number of leaves (Plate 1 B). This was evidenced in the presence of mycorrhizal hyphae on the root nodules (Figure 1 E and F).

Discussion

This study has shown that the high application of AMF lead to the highest colonization rate observed. The root nodules (black dots) described in this study was particularly noteworthy because they represented a specialized plant structure that evidences the presence arbuscular mycorrhzal fungus. The presence of AMF as black dots on Shea tree roots seedling plays a functional role. Obviously, the application of AMF best supported the growth of Shea tree seedlings in the nursery with more leaves, thickness of the roots, black dots and influencing the growth of mycorrhizal hyphae. It is not out of place to say mycorrhaza is a biofertilizer. This agrees with Gianinazzi and Schuepp (1994), who reported that Mycorrhiza is a symbiosis between plants and mutualistic soil fungi, is undoubtedly of extraordinary importance in plant production, plant and soil ecology, and plays a key role in what is generally nowadays called “sustainable agriculture“.

The presence of AMF in Shea root seedlings from natural soil with non application of AMF was an indication that rich top soil of decomposed organic matter could have AMF that promotes growth of plants. This supports the work of Frank (1885), who reported that there are several types of mycorrhiza, all defined by a fungal mycelium growing outwardly into the soil, which extends the root system of the associated plant. Thus, mycorrhizal fungi colonize not only plant roots but also soils by creating a crucial linkage between these two. Ectomycorrhiza (EM) which is formed by most trees and shrubs in the temperate zone with Basidomycotina and (few) Ascomycotina has been the subject of research since the last century.

The 300 gram application had thick root nodules and more number of leaves. This was evidenced in the presence of mycorrhizal hyphae on the root noodles. Govindarajulu et al., (2005), reported that extraradical hyphae take up inorganic nitrogen and transport it to intraradical hyphae in the form of amino acids There, it is released in the form of ammonia into the apoplast where it can be used by the cells of the root (Chalot et al., 2006). In addition, the transport of nitrate via mycorrhizal fungi to the host plant has been demonstrated preferentially under drought when nitrate diffusion in the soil is reduced (Tobar et al., 1994).

Conclusion

Both AMF and non AMF applications on Shea root seedlings supported seedlings growth. But better growth was experienced with AMF application. In the absence of both, top soil rich in decomposed organic matter should be used.

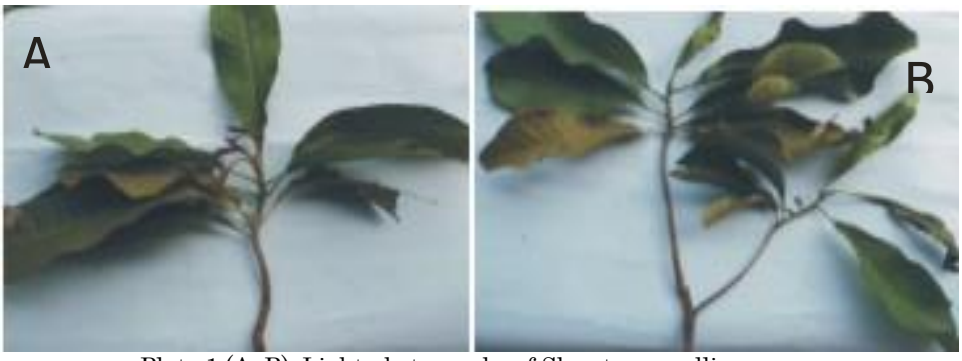

Plate 1 (A, B). Light photographs of Shea tree seedlings.

A. Shows Shea tree seedling three months after the application of 100g Mycorrhizal with 11 leaves
B. Shows Shea tree seedlings three months after the application of 300g Mycorrhizal with 16 leaves

Plate 2 (A, B). Light photographs of Petri dishes with stained roots evenly distributed to score mycorrhizal colonization by a line intersection method.

A. Shows stained roots of three months Shea tree seedling without mycorrhizal but few black dots.
B. Shows stained roots of three months Shea tree seedling with 300g Mycorrizal. Arrows head shows more mycorrhizal colonization with black dots.

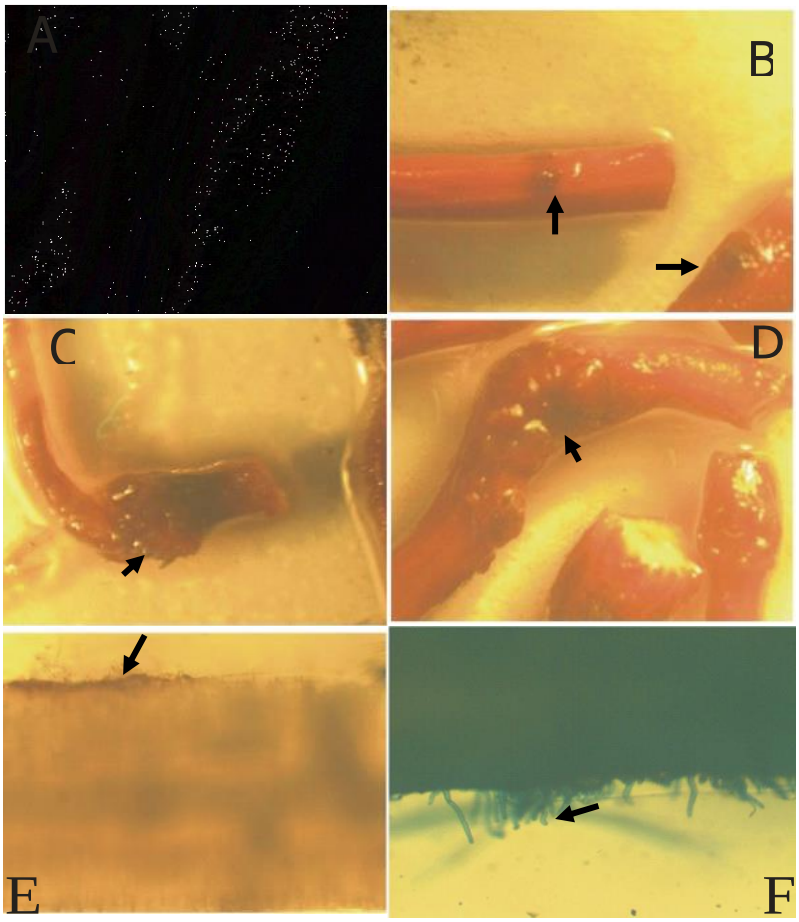

Figure 1. (A, B, C, D, E, F). Photomicrographs of stained roots of Shea tree seedlings to determine AMF / fungi colonization.

A. Stained root of Shea tree seedling, three months without the application of mycorrhizal shows no black dot indicating absence of mycorrhizal colonization
(x 6.4).
B. Stained root of Shea tree seedling three months without the application of mycorrhizal. Arrow head shows black dots indicating presence of mycorrhizal colonization (x 6.4).
C. Stained root of Shea tree seedling three months after the application of 100g mycorrhizal Arrow head shows few black dots indicating the presence of mycorrhizal colonization (x 6.4).
D. Stained root of Shea tree seedling three months after the application of 300g mycorrhizal. Arrow head shows more black dots indicating presence of mycorrhizal colonization (x 6.4).
E. Stained root of Shea tree seedling three months after the application of 300g mycorrhizal. Arrow head shows mycorrhizal hyphae at low magnification (x 10).
F. Stained root of Shea tree seedling three months after the application of 300g mycorrhizal. Arrows head shows mycorrhizal hyphae at high magnification (x 40).

Table 1. Arbuscular Mycorhiza Fungi Inoculated on Roots of Shea Tree Seedlings

Treatment	Gram/bag	AMF	Evidence of fungi in nodules	Mean % of colonization
L	300	+	+	59.00 ±1.20 a
K	100	+	+	42.14 ± 0.41b
A	0.0	-	-	0.00 ± 0.0
D	0.0	-	-	0.00 ±0.0
G	0.0	+	-	8.90± 0.17c

Mean with the same letters indicate non-significant differences while different letter indicate significant difference at p=0.05 using student test (t –test), to test for standard error of mean (SEM). (+/–) indicates the presence or absence of AMF / fungi in the nodules, respectively.

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