Octa Journal of Environmental Research
International Peer-Reviewed Journal
Oct. Jour. Env. Res. Vol. 1(3): 173-176 **_July - Sept, 2013_**
Available online http://www.sciencebeingjournal.com ISSN 2321-3655
Research Article

Octa Journal of Environmental Research
(Oct. Jour. Env. Res.) ISSN: 2321-3655
Journal Homepage: http://www.sciencebeingjournal.com

ANATOMY OF ARBUSCULAR MYCORRIZA FUNGUS (AMF) *ACAULOSPORA SCROBICULATU* ON ROOTS OF THE SHEA TREE *VITELLARIA PARADOXA* IN NIGERIA

Odigie E.E.[a] and Eziashi E.I.[b]
a,b. Nigerian Institute for Oil Palm Research NIFOR, PMB 1030 Benin City, Edo state. Nigeria
Corresponding Author's Email: eziashius@yahoo.com
Received: 14th June, 2013 Revised: 29th August 2013 Accepted: 15th Sept. 2013

Abstract: The discovery of root nodules on healthy Shea tree seedlings revealed the mutualistic associations between root nodules, arbuscular mycorrhiza fungus *Acaulospora scrobiculatu* and the host plant. The root was transversally sectioned to illustrate its structure, detect the presence of mycorrhiza colonization, intact cells of healthy tissues, impact of the mycorrhiza on host tissue and changes resulting from presence of the colonization. The root with mycorrhiza application was thicker and bigger when compared to the control treatment. The anatomical study revealed that the root contains epidemis, exodemis, cortex, early metaxylem and the pith. The nodule contains fungus hyphae which were seen growing outward and inward of the host without causing damage to the host cells. This mycorrhiza colonization resulted in enlargement of the host cells possibly to facilitate and improve the nutritional uptake of both partners.
Key words: Colonization, Mycorrhiza, Root, Section, Shea tree.

INTRODUCTION

Shea tree *Vitellaria paradoxa* grows naturally throughout the Guinea savannah to Sahel savannah regions. It is perennial and deciduous. Mature tree heights vary considerably with some trees attaining heights of over 14m and a girth of over 1.75m (Yidana, 2004). The gestation period varies from 15-20 years depending on the variety from 15-20 years considerably from 1 kg of fresh fruit to over 60 kg. The natural tree populations are subjected to annual 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). Mycorrhiza 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). During the first steps of mycorrhizal colonisation, the extracellular fungal hyphae approach the root surface to form appressoria. During this process, several changes occur in the cellular organization of the underlying epidermal root cell (Reinhardt, 2007). The presence in roots of the arbuscular mycorrhizal fungi can facilitate the phosphorus and others nutrients uptake, and in turn the plant supplies carbon to the fungal partner. 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 (Schubler 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. Staining and microscopic methods not only provide reliable data on the degree of root colonization but also permit to visualize the presence of key features such as arbuscules, which are the morphological criteria that define AM associations (Brundrett, 2004). Anatomy and ultrastructure of root and mycorrhiza of Shea tree relatively unexplored Thus, for example, there are no ultrastructural studies on Shea tree roots In order to study AM fungal colonization, we carried out roots sectioning in such a way that the defining anatomical features could easily be detected. The aim of the present study was to investigate the anatomy and ultra structure of the symbiosis between the Shea tree roots and arbuscular mycorrhizal fungus, as well as some root characteristics.

MATERIALS AND METHODS

The arbuscular mycorrhizal fungus *Acaulospora scrobiculatu* was collected from International Institute of Tropical Agriculture (IITA) Ibadan. The Shea tree seedlings were collected from Nigerian Institute for Oil Palm Research (NIFOR) nursery garden in Bida Niger State. We inoculated roots with the 100g each of AM fungus *A. scrobiculatu*, The control treatment was prepared, except in absence of the fungus. The seedlings were watered regularly and kept in an opened nursery garden 30 ± 2 ºC.

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 H_2O_2 solution for two to three times. They were later soaked 1% HCL for 5 minutes. Roots were removed from HCL before staining with in acidic glycerol solution containing trypan blue at 90°C for one hour. The stained solution was discarded and wee kept in acidic glycerol at room temperature.

Sample preparations and microtome sections: Cross sections of roots were made by hand using a sharp razor blade processed with different staining and microscopy procedures according to the methods of Sass (1958).

RESULTS AND DISCUSSION

The mounted sections in Canada balsam from microtome sections were carefully studied under a light microscope. Observations were made with regards to intact cells of healthy tissues, impact of the mycorrhizal on host tissue and changes resulting from presence of the colonization. Photomicrographs were made where necessary with an attached camera (Motic MCCamera) to the microscope connected to a computer. The stained root without AM fungus application had absence of black dot and the cells of the healthy tissues were intact (Figure 1A). The cross sections of root of Shea tree seedling revealed that it was made up of epidemis, which gives mechanical protection and it is regarded as an absorbing tissue. The exodemis occurs beneath the epidemis and appeared to be thick (Figure 1B and C). The cortex is characterized with intercellular deposit. It is seen cast off together with early metaxylem (Figure 1C and D). The vascular structure was characterized with pith (Figure 1D). The root with AM fungus application had black dots or nodules indicating mycorrhiza colonization (Figure 2A). The cross sections of Shea tree root showed that slight discoloration noticed from the epidemis, enlargement of the cortex and early metaxylem represents the effect of AMF on the root of Shea (Figure 2B, C and D). The AM fungus was seen growing from the epidemis

(Figure C, D and E). It occurred in the root cortex of the host and in some cases of the root structures, mycorrhizal hyphae were observed (Figure 2E).

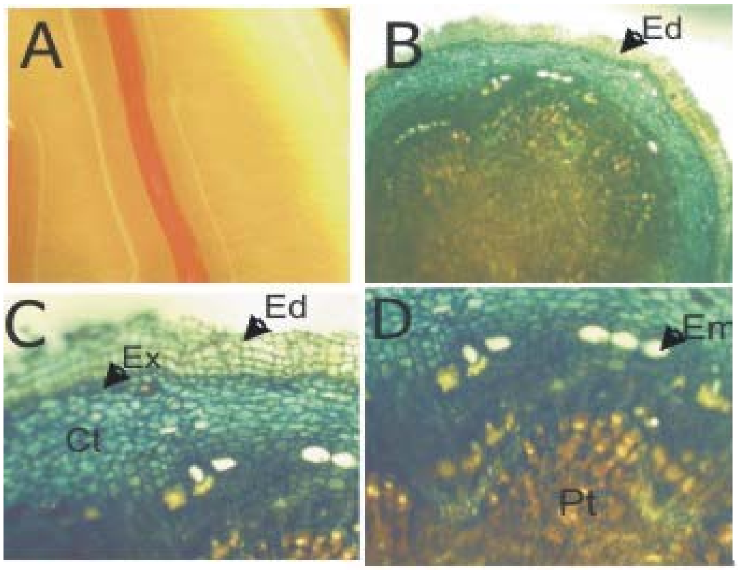

Figure 1 (A, B, C, D, E). Photomicrographs of stained root and cross sections of Shea tree seedlings to determine AMF colonization.
A. Stained root of Shea tree seedling, three months without the application of mycorrhizal shows no black dot indicating absence of mycorrhizal colonization (x 6.4).

B. Cross section of Shea tree seedling three months without the application of mycorrhizal. Arrows head shows epidermis (Ed) (x 10).

C. Cross section of Shea tree seedling three months after the application of mycorrhizal Arrows head shows epidermis (Ed), exodermis (Ex) and cortex (Ct) (x 40).

D. Cross section of Shea tree seedling three months after the application of mycorrhizal. Arrows head shows early metaxylem (Em) and pith (Pt) (x 40).

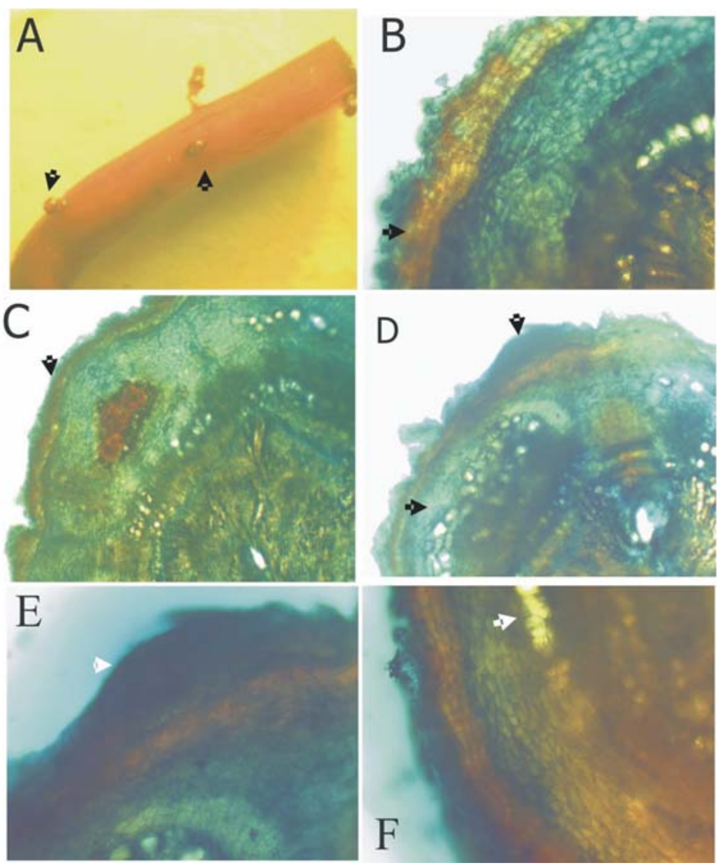

Figure 2 (A, B, C, D, E, F). Photomicrographs of stained roots of Shea tree Seedlings to determine AMF colonization.
A. Stained root of Shea tree seedling three months after the application of AMF. Arrows head shows black dots indicating presence of mycorrhizal colonization (x 6.4).

B. Stained root of Shea tree seedling three months after the application of mycorrhizal Arrows head shows discoloration indicating the presence of mycorrhizal colonization (x 40).

C. Stained root of Shea tree seedling three months after the application of mycorrhizal. Arrows head shows the enlargement of Exodermis indicating presence of mycorrhizal colonization (x 40).

D. Stained root of Shea tree seedling three months after the application of mycorrhizal. Arrows head shows the growth of AMF (*Acaulospora scrobiculata*) indicating presence of mycorrhizal colonization (x 80).

E. Stained root of Shea tree seedling three months after the application of mycorrhizal. Arrows head shows the growth of AMF (*Acaulospora scrobiculata*) indicating presence of mycorrhizal colonization (x 80).

F. Stained root of Shea tree seedling three months after the application of mycorrhizal. Arrows head shows the hyphae growth of AMF (*Acaulospora scrobiculata*) indicating presence of mycorrhizal colonization (x 80).

The root nodules (black dots) described in this study was particularly noteworthy because they represent a specialized plant structure that evidenced the presence of arbuscular mycorrhza fungus. The presence of AMF as black dots on Shea tree roots seedling plays a functional role in promoting growth of the plant. This agrees with Marx (1972) and Auge (2001) who reported that the presence of fungus within the root facilitates the uptake of nutrients and water, and may also have a protective effect against soil pathogens. The presence of fungi in the cortex of the nodules is important for establishing mycorrhizal interaction and plant growth. This was evidenced with the enlargement of the epidemis, cortex and early metaxylem. This supports the work of Frank (1885), who reported that there are several types of mycorrhizas, all defined by a fungal mycelium growing outwardly into the soil, which extends the root system of the associated plant. Thus, mycorrhiza fungi colonize not only plant roots but also soils by creating a crucial linkage between these two. The presence of fungi in the cortex of the fossil nodules is particularly important for establishing the mycorrhizal nature of the association. In extant podocarps, formation of nodules is known to be a constitutive developmental trait Kahn (1967) and (Russell et al., 2002).

CONCLUSION

The mycorrhizal nodules on the roots of Shea tree seedling illustrated the intact cells of healthy tissues, changes resulting from presence of the colonization and importance of mycorrhiza and microorganism mutualistic interactions.

REFERENCES

Auge R.M. (2001); Water relations, drought and VA mycorrhizal symbiosis. Mycorrhiza, 11:3–42.

Brenda B. (1980); Quantifying vesicular – Arbuscular Mycorrhizal: A proposed Method towards standardization. *New Phytol*, 87: 63-67.

Brundrett M.C. (2004); Diversity and classification of mycorrhiza associations. *Biol Rev*, 78: 473–495.

Ezawa T., Smith S.E and Smith F.A. (2002); P metabolism and transport in AM fungi. *Plant Soil,* 244:221–230.

FAO (1988); Traditional food plants. Food nutritional paper, FAO Rome. 42: 125-129.

Frank B. (1888); Über die physiologische Bedeutung der Mycorrhiza. *Ber. Dt. Bot. Gesellsch*, 6: 248-269.

Govindarajulu M, Pfeffer P.E., Jin H, Abubaker J., Douds D.D., Allen J.W., Bücking H, Lammers P.J. and Shacher-Hill Y. (2005); Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature,* 435(7043):819-823.

Kahn A.G. (1967); Podocarpus root nodules in sterile culture. Nature, 215:5106.

Marx D.H. (1972); Ectomycorrhizae as biological deterrents to pathogenic root Infections. *Annu Rev Phytopathol,* 10:429–454.

Reinhardt D. (2007); Programming good relations—development of the arbuscular mycorrhizal symbiosis. *Curr Opin Plant Biol*., 10:98–105.

Russell A.J., Bidartondo M.I., Butterfield B.G. (2002); The root nodules of the Podocarpaceae harbour arbuscular mycorrhizal fungi. *New Phytol,* 156:283–295.

Sass J.E. (1958); Botanical Microtechnique Jowa State University Press, Jowa pp 229.

Schubler A, Martin D, Cohen D, Fitz M, Wipf D (2007) Studies on the geosiphon symbiosis lead to the characterization of the first Glomeromycotan sugar transporter. Plant Signal Behav, 2:314–317.

Yidana A. Joshua (2004); Progress in developing technologies to demonstrate the cultivation of Shea tree (Vitellaria paradoxa L.) in Ghana. Agricultural and food science Journal of Ghana, 3: 249-267.

Uehlein N., Fileschi K., Eckert M., Bienert GP., Bertl A and Kaldenhoff R. (2007); Arbuscular mycorrhizal symbiosis and plant aquaporin expression. Phytochemistry 68:122–129.

CONFLICT OF INTEREST : Nothing