Nigerian Journal of Soil Science

Journal homepage:www.soilsjournalnigeria.com

Research Communication

INVESTIGATION OF MYCORRHIZAE POPULATIONS OF ROOTS AND RHIZOSPHERE SOIL OF PLANTAIN *(MUSA SPP)*AT THE VARIOUS AGRO-ECOGICALZONES IN CROSS RIVER STATE

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ABSTRACT

The study was carried out to investigate the prevalence of Arbuscular Mycorrhizae (AM) infection in plantain*(Musa spp)* roots and population of spore in rhizosphere soils. Samples of roots and rhizophere soils were collected from the various ecological zones in Cross River State, Nigeria with locations at Lishikwel and Ablesang in Obanliku Local government area. Others include Boki, Etung, Obubra, Biase and Odukpani Local government areas. The representative roots were dug and immediately collected into polythene bags separately and rhizosphere soils were collected from the vicinity around the root zones into poly bags and labeled accordingly. The analysis of infection was carried in the laboratory. Assessment of root colonization was also done. Rhizophere soil samples were analyzed for spore counting using wet sieving and decanting technique. Percentage colonization of AM infection in plantain feeder roots at Lishikwel, Ablesang (Obanliku), Boki, Etung, Obubra, Blase and Odukpani were 11.5%, 6.79%, 4.71%, 12.01%, 7.50°/o, .40% and 15.20%, respectively. The AM spore counted per 50g dry soil for Lishikwel, Ablesang (Obanliku), Boki, Etung, Obubra, Blase and Odukpani were: 250, 117, 144, 85, 65, 470 and 60 respectively. The highest infection was recorded in odukpani, while the highest spore count value was recorded in Biase. These differences could be attributed to the ecological differences of the locations.

Key words*:* Arbuscular Mycorrhizae, Prevalence, Rhizophere soils, Infection, Root colonization

INTRODUCTION

Plantain is a species of the genus Musa and belongs to the family Musaceae and is usually cooked, in contrast to the soft, sweet banana (which is sometimes called the desert banana. Plantain is among the most important staple food crops in Humid Forest Zones of West and Central Africa. In Nigeria, it is grown in mostly South-east and South-western parts of the country. Plantain *(Musa spp)* tends to be firmer and lower in sugar contents than desert banana and used either when green or unripe or overripe.

Due to the increase of soil-related constraint on crop production and with the increase in human population, alternative sources apart from inorganic fertilizers or that will supply at low cost, need to be studied and developed. Research findings have shown that arbuscular mycorrhizas, (AM) enhance nutrient (N and P) and water uptake and root longevity.

Mutualistic or symbiotic associations between plants and arbuscular mycorrhiza fungi (AM) have beneficial effects on plants by ensuring increased absorption of nutrients, especially of phosphorus. Furthermore, mycorrhizas contribute to the increased resistance of plants to droughts, pathogens and insect attack (Ayres, et al., 2006). Allen (1991) highlights the role of mycorrhizas in ecology and in stimulating plant growth. Mycorrhiza benefits cannot be neglected in terms of resource conservation in agriculture, because mycorrhizal plants absorb more nutrients and soil or substrate water.

Arbuscular mycorrhiza fungi (AMF) form mutualistic or symbiotic relationships with plants. Over 90% of plant species associate with AMF, which are important for accessing and recycling nutrients.

AMF can also increase plant access to water (Gavito and Varela, 1995), play a role in the formation of soil aggregates, influence plant biodiversity help protect against pests and diseases (Azcon• Aguilar and Barea, 1996) and improve fitness of plants in polluted environments (Hildebrandt et al., 1999). Banana and plantain *(Musa spp.)* are important food crops across Africa, particularly in the Western and Central Africa and in the East African highlands. Much of these production areas are faced with poor soil fertility and significant pest and disease pressure (Akyeampong and Escalant, 1998). A number of studies reported on the protective effects of AMFon banana against nematodes (Gera Hol and Cook, 2005). AMF occur naturally in the rhizosphere, and banana and plantain plants appear to be highly mycorrhizal (Jaizme-Vega et al., 1991).

This work study estimated the populations of AMF in feeder roots, the spores and colonization of the rhizosphere soil in plantain in three ecological zones in Cross River State Nigeria. The specific objectives were to estimate the mycorrhiza infection population of feeder roots and, the spore populations in plantain rhizosphere soils.

MATERIALS AND METHODS

Location and Description of Research Sites

The research was carried out in six local government areas of Cross River State, covering the three agro-ecological zones. These Local Government Areas include Obanliku, Boki, Etung, Obubra, Biase and Odukpani. Obanliku and Boki local government area (Northern Senatorial Districts) are located between latitudes $6^0 46^1$ and $6^0\ 43^1$N and longitudes $9^0 31^1$ and $9^0 24^1$ East. Etung and Obubra local government areas (Central Senatorial Districts) are located between latitudes $05^0\ 5^0 22^1$ and $5^0 45^1$ N and longitudes $08^0\ 31^1\ 08^1$ and $7^0 55^1$ East. Biase and Odukpani local government areas (southern senatorial districts) are located between latitudes $5^0\ 43^1$N and $5^o\ 32^1$N and longitudes $4^0\ 54^1$ and $7^0\ 5^0$East.

The vegetation of Obanliku local government area is Derived Guinea Savanna with tall trees tuff, rough and wire grassed and scattered trees at the top of the hills. At the bottom of the hills are gallery forests and thick vegetation. The soil is a typical cateria soil distribution. Soils are reddish at the top of the hill and at the ground level, they are brownish. The temperature at the hill top can be as low as 11^0C, while at the bottom hills may be 32^0C.

Boki local government area is a forest vegetation zone which could be swampy or tropical high forest. Boki has a tropical type of climate with high temperature and high relative humidity of about 80%. Soil is ferruginous in a tropical region composing of sandy, clay, and loamy soil. The variation of soil occurs in about 2-3 kilometers climate.

Etung Local Government has a tropical rain-forest vegetation where different species of plants are found. Obubra local government has similar vegetation initially but due to human

activities, these have been removed. Obubra environment lies within the Basement complex Rock zone consisting of metamorphic Rock with intrusive granites bodies overlain by a series of younger strata. Most of the soils therefore are derived from granitic break-down. Weathering is favoured by the distinct laterite-oxidation and formations of durricrast materials. Blase and Odukpani local Government Areas (Souther Senatorial Districts) have similar climate which is described as humid tropical. The annual rainfall in these Southern Climatic Zones of Cross River State ranges from 2500-3000 mm. The rainy season extends from March to November while dry season extends from December to February. The mean annual temperatures and relative humidity are 26.25^0C and 85.42% respectively. The vegetation is primary rainforests with bush re-growth. Soils are derived from coastal plain sands of alluvial origin. The soil texture is generally loamy, sandy loam in the surface, but changes to clay in some localities. The soils are deep, well drained, well weathered, porous and of weak structure.

Collection of Plantain Roots

Samples of plantain *(Musa spp)* feeder roots were collected from seven locations within the six local government areas. Young healthy plantain plants were uprooted with the suckers after digging round them with cutlass. Two samples were collected at Ablesang and Lishikwel communities within Obanliku local government area, while one sample each was then collected from Boki, Etung, Obubra, Blase and Odukpani local government areas. Each sample was placed in different poly bags, properly labeled and carried to the laboratory for analysis of mycorrhizal infections.

Collection of Rhizosphere Soils

Rhizosphere soil samples were collected from around the plantain stands where the root samples were uprooted. Samples were collected using a hand trowel placed in poly bags and labeled. The soil samples were then taken to the laboratory for analysis of Abuscular Mycorrhizal spores.

Characterizations and Analysis of Vesicular Abuscular Mycorrhizal Infection in Plantain

The representative root samples were carefully picked (preferably root hairs) into plastic containers and were thoroughly washed clean to remove soil particles or dirt. The washed roots were then placed in beakers and heated distilled water was added and the beakers were then transferred into a water bath and heated further at 90^0 for 1 hour.

Subsequent clearing and staining of the roots were carried out. The roots were placed in 10% aqueous solution of KOH for 24 hrs at room temperature in freshly prepared solution of alkaline hydrogen peroxide (10% H_2O_2) until root tissues appear clear to the naked eye. After cleaning, the roots were washed using tap water, after which they were acidified in 2% HCI. The acidified roots were then stained in 0.05% aniline blue in 75% glycerol. The cleansed root samples were immersed in the stains for 30 minutes, excess stain was then washed off and the roots destained in acidified glycerol at room temperature. Assessment of root colonization was done using the ride line technique.

Stained root samples were evenly spread in a Petri dish with grid lines of uniform distances apart on the bottom to form 12.7cm squares. Vertical and horizontal grid line were scanned at 15-45 x magnification with a dissecting microscope. The presence of colonization was recorded at each point where a stained root with hyphae, vesicle or arbuscules intersect a line. Percentage root colonization was then calculated as follows:

$$\frac{\text{No. of roots/grid line intersect with colonization}}{\text{Total No. of roots/grid line intersect counted}} \times 100$$

Recovery and enumeration of Fungal spores from rhizosphere soil

The representative rhizosphere soil samples were air-dried under room temperature, ground and sieved using 2mm-sized sieve. Portion of 50 grammes of homogenized soil samples were taken for analysis. Recovery of spore from the soil was carried out by wet sieving and decanting techniques. Fifty (50g) grammes of the homogenized soil samples were weighed and poured into beakers, tap water was added and suspended for 10-20 seconds to allow for sedimentation of coarse sand.

The suspension was decanted over a series of sieves arranged on top of each other in a descending mesh order of 500, 250, 106, and 53µM. Suspending and decanting were repeated and then the contents of the medium and fine sieve were transferred with some distilled water into l00mI centrifuge tubes. These contents were suspended in 70% sucrose solution and centrifuge at 3,000 rpm for 4 minutes. The spores were extracted from the l000 ml centrifuge tubes using a syringe from the gradients and transferred to a Petri dish with parallel lines ruled 0.5cm apart. Counting under a dissecting microscope at 45 x magnification was done.

RESULTS AND DISCUSSION

Mycorrhizal Infection in Plantain Roots

Table 1 shows the prevalence of my corrhizal infection, AMF spores and average yields of plantain in the aeroecological zones of Cross River State, Nigeria. The highest mycorrhizal infection in plantain roots was observed in Odukpani with 15.20% followed by Etung local government area with12.10%. Likshiwel (Obanliku) had 11.50%, Blase 9.40%, Obubra 7.50%, Ablesang in Obanliku 6.70%, and the lowest infection was recorded in Boki with a total of 4.71%.

The result of AM spores showed that Biase and Lishikwel in Obanliku had the highest sporulation of 470 and 250 per 50g of dry soil. Ablesang in Obanliku arid Kanyang in Boki had 117 and 114 per 50g of dry soil respectively. Etung and Obubra had 85 and 65 spores per 50g of dry soil. Odukpani had the lowest with 60 spores per 50g of dry soil.

It has been shown that there is AMF infection and sporulation in plantain in all locations. This confirms the report of Agrios (1988) that AM type of endomycorrhizae are not visible to the naked eye but their effects are observed in cortical cells of the feeder roots of plants.

These include the major food crops of tropical regions such as plantain *(Musa spp).* This result also shows that colonization intensifies at Odukpani, Lishkwel (Obunliku) and Etung local government areas. The enhanced infections in the three areas might be due to greater moisture content because of higher rainfall. Since water is an enhancer to the vegetative growth of AM (i.e. fungal growth). Investigation also showed that in these areas with high colonization and infection of AM, the plantain plants were observed to have good vegetative growth and yield, and disease infestation was not observed. This confirms that AMF enhance disease resistance.

The highest spore numbers were found in Ibogo (Biase) with 470, Lishikwel (Obanliku) 250, Ablesang (Obinliku) 117, Kanyang (Boki) 144 while the lowest spore number occurred in Etung, Obubra and Odukpani with sporulation of 85, 65 and 60 per 50g soil respectively. The high spore number at Biase with sporulation of 470 showed that there was high AM host prevalence and this might have accounted for the high yield of plantain in this location. Lishikwel and Ablesang in Obahliku with sporulation of 250 and 117 per 50g soil indicated medium rate of sporulation, which could be as a result of dry nature of this area being drier than the former. Drought is known to favour colonization and sporulations than excessive moisture. Though there is a prevalence of AM host, but production in terms of yield was observed to be low.

Kanyang in Boki had spore number of 144 per 50g dry Boki local government area is one of the major producers of plantain and banana in Cross River State, but from the result obtained, sporulation is high due to the fact that the area has less rains than in Central and Southern zones of the State.

Relationship between AM Spore and Mycorrhizal Infection

Spores are survival structures of AM fungi which in certain circumstance represent the only infective VAM propagules in the field. This may be the case after a long period without vegetation, or several months after harvest where no crop or weed re-growth has been allowed through application of herbicide, or after a long dry season. In this case, the number of spores per unit dry weight or volume of soil revealed the extent of fungal populations in the field. However, spore numbers are often used to quantify the VAM populations.

CONCLUSION

With the available information and data obtained from percentage colonization of plantain roots and spore counting in rhizosphere soil, it is concluded that plantain *(Musa spp)* crops are mycorrhizal sensitive. However, the level of infections and sporulation of AM in some locations were not encouraging.

Table 1: Prevalence of Mycorrhizal Infection, Abuscular Mycorrhizal Spores and Average Yield of Plantain at the Various Agro-ecological Zones.

S/N	Location	% Infection/ Colonization	Spore count per 50g Dry Soil	Average Yield in each location (t/ha)
1.	Ablesang (Obanliku)	6.79	117	0.81
2.	Lishikwel (Obanliku)	11.50	250	2.61
3.	Kanyang (Boki)	4.72	144	0.76
4.	Etung (Bendghe-Nkim)	12.10	85	2.75
5.	Ochon (Obubra)	7.50	65	0.85
6.	Ibogo (Biase)	9.40	470	2.46
7.	Odukpani	15.20	60	3.00
Mean(x)		9.60	170.14	1.30
Standard Deviation(ð)		3.59	147.33	1.03
Coefficient of Variation (%)		37.40	86.60	54.50

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