

ARBUSCULAR MYCORRHIZAL FUNGI STATUS OF SOME CROPS IN THE CROSS RIVER BASIN OF NIGERIA

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ABSTRACT

The incidence of arbuscular mycorrhizal fungi (AMF) colonization and rhizospheric spore prevalence of ten crops was studied in relation to their foliar concentration of nitrogen, phosphorus and potassium in the Calabar area of the Cross River Basin of Nigeria in order to determine their mycorrhizal status. All crops studied had an incidence of mycorrhizal colonization including *Talinum triangulare* a member of Portulacaceae family. The highest percentage AMF colonization of 97.60% and rhizospheric spore density of 112 spores/5g soil were found in *Arachis hypogea* while *Talinum triangulare* and *Telfairia occidentalis* had the lowest percentage AMF colonization of 30.90% and rhizospheric spore density of 10 spores/5g soil respectively. There was no correlation between the percentage AMF colonization and the rhizospheric spore density of the crops sampled. Foliar phosphorus concentration was highest in *A. hypogea*, (1.20%), for potassium it was in *Colocasia esculenta* (9.3%) while *T. occidentalis* had the highest foliar nitrogen concentration (4.9%). There was no significant relationship between foliar nutrient concentration and AM fungi colonization or rhizospheric spore density.

KEYWORDS: AMF, Colonization, Spore density, Foliar nutrients, Cross River Basin of Nigeria.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are found in most agricultural soils (Ryan and Angus, 2003). They are broad-host ranged and colonize the roots of most crops and weeds. About 90% of terrestrial plants species have been found to be colonized by mycorrhizal fungi (Gadkar *et al.*, 2001). This high percentage notwithstanding, non-mycorrhizal plants have been found in some species belonging to Araceae, Caryophyllaceae, Compositae, Cruciferae, Gramineae, Onagraceae, Polygonaceae, and Portulacaceae families (Berch *et al.*, 1998). Being obligate symbionts, AMF depend solely on the host plants for their carbon needs (Fitter and Merryweather, 1992). The host plant benefits immensely from arbuscular mycorrhizal association in the areas of nutrient and water uptake (Okon *et al.*, 1996; Fagbola *et al.*, 1998). Arbuscular mycorrhizal fungi are very important in increasing the ability of the host plant to absorb immobile or fixed ions such as phosphorus, zinc and copper in acidic or alkaline soils as well as in the absorption of water and nitrogen (Sharif and Moawad, 2006). Besides, arbuscular mycorrhizae have been implicated with the production of acid and alkaline phosphatase for hydrolysis of organic phosphates thereby making them available to the plant roots (Tarafdar and Marschner, 1994). This enhanced nutrient and water uptake results in improved nutrition, increased plant growth and crop yield. Nowadays much

emphasis is laid on sustainable agriculture. Arbuscular mycorrhizal fungi association with crops is considered to be of great significance in sustainable agriculture (Gianinazzi and Scheuepp, 1994; Sunilkumar *et al.*, 2012).

Abelmoschus esculentus, *Arachis hypogea*, *Capsicum annum*, *Colocasia esculenta*, *Ipomea batatas*, *Manihot esculenta*, *Talinum triangulare*, *Telfairia occidentalis*, *Vigna unguiculata* and *Zea mays* are all agricultural crops commonly grown in Calabar in the Cross River basin of Nigeria. Although the basin has produced much of these crops to the satisfaction of its human inhabitants for a long time, there has never been any investigation on their mycorrhizal status.

The objective of this work was therefore to investigate the mycorrhizal status of these crops in relation to their foliar nutrient (nitrogen, phosphorus and potassium) concentration and available arbuscular mycorrhizal fungi spores in their respective rhizosphere with a view to using mycorrhizal inoculation to increase their production.

MATERIALS and METHODS

Experimental site

Samples of roots and rhizosphere soil were collected within Calabar in the Cross River Basin area located between latitude 4.96°N and longitude 8.31°E at an elevation of 700 km above the sea level (Tageo.com 2013). Physicochemical analysis of the soil revealed that

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it is characteristically sandy loam consisting of 75.6% sand, 7.7% silt and 16.7% clay. The chemical composition of the soil was 0.10% total N; 35.5ppm P; 0.30 cmol kg⁻¹ K; 1.18% organic carbon and 2.04% organic matter.

Sampling procedure and species selection

Ten most commonly grown crops of the Cross River Basin were selected for this study. These were *Arachis hypogea*, *Vigna unguiculata*, *Zea mays*, *Capsicum annum*, *Colocasia esculenta*, *Ipomea batatas*, *Manihot esculenta*, *Abelmoschus esculentus*, *Talinum triangulare*, *Telfairia occidentalis*. This list comprised of two legume, one cereal, one spice, three tuber and three vegetable crops respectively. These crops which were all between 4-5 months old on the farms were carefully uprooted with the surrounding rhizospheric soil, transferred immediately into labeled polyethylene bags and taken to the laboratory. Sampling was carried out in four randomly selected locations within the experimental site.

Estimation of arbuscular mycorrhizal fungi (AMF) colonization of plant roots

Representative samples of fine terminal feeder roots were collected from each species harvested. These were washed clear of soil particles in running tap water and fixed in 50% ethanol. Clearing and staining of roots was done following the modified procedure of Koske and Gemma (1989). Percentage AMF colonization was assessed using the gridline intersect method of Giovanetti and Mosse (1980). Stained roots were scanned under a dissecting microscope along vertical and horizontal gridlines at a magnification of ×45. Presence of colonization was recorded at each point where a stained root with hyphae, vesicles or arbuscules intersected a line. Percentage root colonization was calculated as:

$$\frac{\text{No. of root/gridline intersects with colonization}}{\text{Total no. of root/gridline intersects counted}} \times \frac{100}{1}$$

Recovery and enumeration of AMF spores from the rhizosphere

Rhizospheric soil of each crop was air dried and homogenized. AMF spores associated with each crop were then extracted from their respective rhizosphere using the wet sieving technique of Daniels and Skipper (1982). The soil fractions of between less than 106 µm and 53 µm were collected from 100 g of air dried soil and the spores were then further concentrated by 70 % sucrose solution centrifugation at 3000 rpm for 4

minutes. The number of spores per 100 g of soil was determined by counting under a dissecting microscope at ×45 magnification. This value was then presented per 5 g of soil through simple proportion calculation.

Plant foliar nutrient analyses

Leaves from the crops sampled were oven dried, ground into powder and analyzed for total foliar nitrogen, phosphorus and potassium concentrations. Total foliar nitrogen was determined by Micro-Kjeldal digestion method while total foliar phosphorus was determined colorimetrically by Vanado-Molybdate method and total foliar potassium was determined by flame photometry all as described by Juo (1979).

Statistical Analysis

All data were analyzed by combined analysis of variance using the Windows version of Statistical Analysis System (SAS 1996). Mean values were compared using Duncan's Multiple Range Test (DMRT) at 0.05 level of probability when the F-ratio was significant.

RESULTS

Arbuscular mycorrhizal fungi spore prevalence and root colonization

Rhizospheric soil samples of the ten crops contained AMF spores. The lowest number of spore count, 10 spores/5g soil, was obtained from *Telfairia occidentalis* rhizosphere while the highest 112 spores/5g soil, was from *Arachis hypogea* rhizosphere. Rhizospheric soil spore densities were not significantly different in *C. annum* and *M. esculenta* or among *I. batatas*, *T. triangulare*, *C. esculenta* and *Z. mays* (Table 1).

The highest percentage AMF colonization of 97.6%, was observed in *Arachis hypogea* while the least of 30.9%, was observed in *Talinum triangulare*. There was however no significant difference between the percentage AMF colonization of roots in *C. annum* and *T. occidentalis* or among *V. unguiculata*, *I. batatas*, *M. esculenta* and *Z. mays* respectively (Table 1).

Foliar nutrient concentration

Arachis hypogea gave the highest foliar phosphorus concentration, the highest foliar nitrogen concentration was obtained from *Telfairia occidentalis* while *Colocasia esculenta* had the highest foliar potassium concentration followed by *Capsicum annum* and *Ipomea batatas* respectively. It is worthy of note that no detectable trace of potassium was found in the leaves of *A. hypogea* leaves.

Table 1: AMF colonization and foliar NPK concentration (%) of some crops in the Cross River Basin of Nigeria.

Crop	Phosphorus	Nitrogen	Potassium	% AMF Colonization	Spore count /5g soil
<i>A. hypogea</i>	1.20a	4.90b	0.00i	97.60a	112a
<i>V. unguiculata</i>	0.21cd	4.06d	6.10d	40.70d	58b
<i>A. esculentus,</i>	0.38b	3.43e	0.60h	32.00e	41d
<i>C. annum</i>	0.25c	5.25ab	8.80b	64.00c	48c
<i>I. batatas,</i>	0.24c	3.05f	7.70c	38.40d	24e
<i>M. esculenta</i>	0.26c	4.27c	3.90f	37.86d	49c
<i>T. triangulare</i>	0.24c	4.06d	3.10g	30.90e	29e
<i>T. occidentalis</i>	0.36b	5.36a	5.10de	62.00c	10f
<i>C. esculenta</i>	0.26c	3.50e	9.30a	67.63b	32e
<i>Z. mays.</i>	0.14d	0.34g	5.60d	40.00d	25e

*Values are means of four replicates. Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

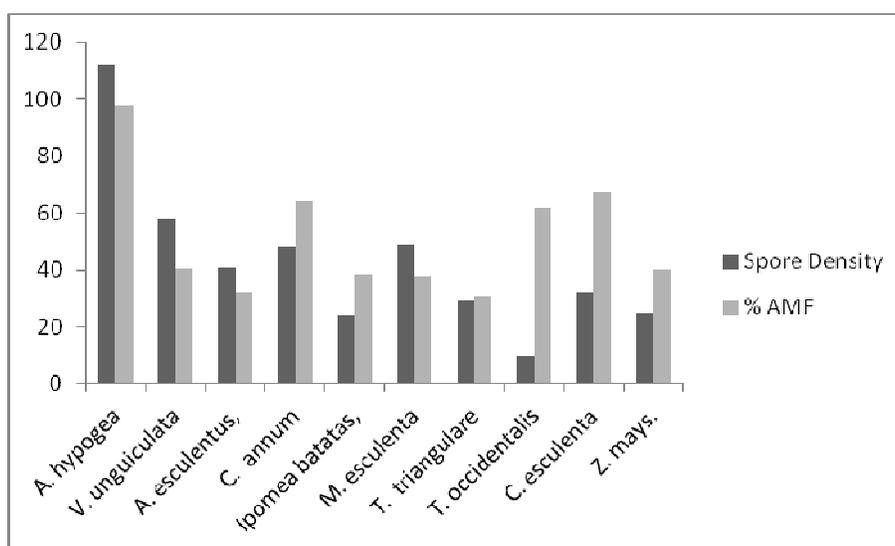


Fig. 1: % AMF colonization and rhizospheric spore density

DISCUSSION

From our investigation, we have established that *Arachis hypogea*, *Vigna unguiculata*, *Abelmoschus esculentus*, *Capsicum annum*, *Ipomea batatas*, *Manihot esculenta*, *Talinum triangulare*, *Telfairia occidentalis*, *Colocasia esculenta* and *Zea mays* are all associated with arbuscular mycorrhizal fungi colonization (Table 1). This is a confirmation that most agricultural crops are colonized by mycorrhizal fungi. It is interesting to observe mycorrhizal colonization in *Talinum triangulare* roots which is a member of Portulacaceae family which is known to be among the unusual few families where only isolated species form mycorrhizae (Sieverding, 1991; Berch *et al.*, 1998). The highest percentage colonization in *Arachis hypogea* may be connected with its rooting morphology as well as being a legume. *Arachis hypogea* has thick roots with few branches which according to Baylis (1975) have greater dependence on mycorrhizal association. Isobe and Tsuboki (1998), have found higher AMF colonization in leguminous than in graminaceous crops. Thus as a

legume, *A. hypogea* probably favoured mycorrhizal colonization to assist the symbioses with *Rhizobium* (Bethlenfalvay and Newton, 1991).

Our findings also showed that all the rhizospheric soil samples contained spores. This confirms that arbuscular mycorrhizal fungi are found in all agricultural soils. However, apart from the case with *Arachis hypogea* where there was relationship between the high percentage of AMF colonization and spore prevalence, there was no such relationship in other crops. This is an indication that root colonization may not necessarily be a function of spore number in the soil but a reflection of the response of the host plant to the inoculum. It has been suggested that different plant species differ in their degree of AMF support (Bagyaraj and Manjunath, 1980) and also their dependency on arbuscular mycorrhizal fungi (Plenchette *et al.*, 1993).

Foliar phosphorous concentration was correspondingly highest in *A. hypogea*. This could have possibly resulted from the increased amount extracted from the soil by the abundant AMF colonization. A higher colonization of the roots must have led to a

higher number of hyphae and a larger area of soil explored for nutrient extraction. Nitrogen, was not however as high in *A. hypogea* as in *T. occidentalis*. A possible explanation for this could be the need for protein formation in the seeds of the former whereas in the latter most of it might have been stored in the leaves where plenty of chlorophyll was probably being built up. The high foliar potassium concentration in *C. esculenta* and *I. batatas* could have resulted from the demand of these crops for this element for sugar translocation and starch synthesis being tuber crops (Reis Jr. and Fontes, 1996). The high foliar potassium concentration in *Capsicum annum* is also to be expected as previous findings have shown that this crop usually takes up more potassium than other elements (Santiago and Goyal 1985; Marcussi *et al.*, 2004.)

CONCLUSION

Our study has shown that the soil of the Cross River Basin of Nigeria is rich in arbuscular mycorrhizal fungi spores and that these ten commonly cultivated crops of the area are all mycorrhizal fungi colonized. If this association is effectively managed the positive benefits of AMF can be used to maximize the production of these crops.

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