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Investigation Of Mycorrhizal Infection On Maize (Zea may [L]) Cultivated In Calabar

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Abstract: Mycorrhizal (fungus-root) infection on maize (Zea mays) was investigated. The samples – uprooted mature maize plants, the plant's rhizosphere soils and non-rhizosphere soils of the same area were randomly collected at 10 different locations in the Staff Village of the University of Calabar, Nigeria. The terminal feeder root samples were used for the determination of the fungal – root colonization by applying the method of root/gridline intersects. The recovery and enumeration of fungal spores in the rhizosphere soils was done through wet sieving, decantation and centrifuging in a sucrose density gradient column. The leaf samples were collected for the determination of total Nitrogen, Phosphorus and Potassium for both rhizosphere soil and non-rhizosphere soil. Organic Matter and the textural soil type were determined in the non-rhizosphere soils. The investigation revealed 40 % arbuscules in the feeder roots and 25 fungal spores from 5g of the rhizosphere soils both at high magnifications (X14) of the microscope. The soil analysis showed leaf samples had a high total Nitrogen level (3.36 %), low phosphorus content (14.3 $mgkg^{-1}$) and a high potassium content (5.6 $Cmolkg^{-1}$). The non-rhizosphere soil showed high level content of organic carbon (2.4 %), total Nitrogen (0.21 %) and available Phosphorus (78.6 $mgkg^{-1}$) while moderate content was shown in exchangeable potassium (0.48 $Cmolkg^{-1}$). The result of particle size showed that the soil contained 75.65 % Sand, 15.70 % clay and 8.70 silt, thus the textural class of the soil is Sandy Loam. Therefore, the soils can be conveniently used for cultivating crops without fertilizer application. Thus, there would be no need for inoculation of the soil with mycorrhizal fungi unless in a later depleted state of the soil, since it is observed that fertile soils are not responsive to mycorrhizal inoculation.
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Introduction

Maize (*Zea mays* L.) is a member of the grass family, Poaceae. It is one of the most important cereal crops in the world (Agbogidi, 2010). Maize is the most important cereal crop in Nigeria providing over 40% of the calories consumed in both rural and urban areas. The crop has increasingly become a staple food in many parts of the country due to changes in people eating habits. Small-scale farmers, who constitute the bulk (80%) of the rural people, also account for the largest share of maize producers. Starch from maize could be used as biofuel (corn ethanol), ornamental uses, and use as fodder among others (Rosenburg, 2014).

Generally, some of the major constraints identified to be responsible for low production of crops like maize include poor soil fertility, high cost, and unavailability of inorganic fertilizer, difficulty in obtaining adequate amount for large-scale agriculture and delay in the release of the essential mineral nutrients for immediate use of the plant (Olawuyi *et al*., 2010). Use of inorganic fertilizers is the traditional way of overcoming soil fertility depletion, and indeed it is responsible for a large part of food production increases in the world, including commercial farm sector in Africa. The use of inorganic fertilizers in most soils of the tropics has been reported to be accompanied by soil acidity problems (Kang *et al*., 1990).

Attempts to encourage this approach to smallholder farming in Africa (who are the backbone of agriculture) even with input subsidies have met limited success. The use is viewed as a recurring cost of production, which must be paid for by the increased crop yields. High costs and the associated ground water and stream pollution further limit their use. It is therefore imperative that a more viable way of alleviating P deficiencies in soils be sought within the reach of farmers. Arbuscular mycorrhiza fungi (AMF), reputed for their ability to enhance P uptake by plants would be such an alternative.

Arbuscular mycorrhizal fungi (AMF) have an important function in soil nutrient acquisition and mobilization, principally phosphorus. The role of AMF for nutrient acquisition is due to extending the root system by increasing the surface area for nutrient uptake and enhancing the ability of the plants to scavenge for scarce and immobile nutrients, particularly P (Gosling et al., 2006). As a symbiotic system, the fungi receive carbon sources from the host plant and provide nutrients to the plants (Grigera et al., 2007). AMF also improve the soil aggregate stability, as their extraradical hyphae can bind to soil particles mechanically and chemically through the exudation of glomalin (Wright et al., 2005). Therefore, these symbiotic associations are increasingly gaining popularity as a significant factor of sustainable agro-ecosystems. AMF are especially important for plant nutrition in organic and low input farming systems because these systems do not utilize synthetic fertilizers and fungicides, which can tremendously reduce levels of root colonization by AMF (Cavagnaro *et al.,* 2011).

However, in Nigeria particularly in Calabar Municipality, Cross River State; little is known about the influence of ecological site conditions on AMF community composition. Therefore, the aim of this study was to analyse the AMF communities composition in maize cultivated in Calabar Municipality, Cross Rivers State, Nigeria.

Materials and Methods

The study area

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)

Sample Collection

Matured maize plants of about two months old were uprooted at random from the different maize farm plots (a total of ten plants, one from each plot). Rhizosphere soil samples were collected by shaking the roots of the uprooted maize plants to release attached soil. The collected soil samples was bulked, packed into polythene bags, labelled and taken to the laboratory for analysis.

Root samples used for quantifying mycorrhizal infection were collected by severing-off the terminal feeder roots (unsuberized) from the uprooted maize plant's roots using a pair of Scissors. The roots were packed in polythene bags and taken to the laboratory for analysis. Leaves samples were collected from the uprooted maize plants using a sharp knife. The leaves collected were packed in polythene bags and taken to the laboratory. Non-rhizosphere soil samples were collected at random from ten different maize plots at depth of 0 – 15 cm using spades, bulked and carried to the laboratory using polythene bags (well labelled).

The bulked rhizosphere and non-rhizosphere soil samples were air-dried, ground and passed through a 2 mm sieve, labelled and stored for laboratory analysis. The leaves samples were washed, chopped into smaller fragments, packed in brown envelopes, dried in an oven for two days at 160^0 C, ground and stored for laboratory analysis.

Laboratory Analysis

Laboratory analysis carried out in this study were both soil chemical and physical analysis. Particles size distribution was determined using the hydrometer method as described by Bouyoucos (1962). Organic carbon was determined by the wet oxidation method (Walkley and Black, 1934). Total Nitrogen was by the micro-Kjeldhal digestion method. Available phosphorus was determined by Bray 1 method (Bray and Kurtz, 1945). Exchangeable bases (Ca, Mg, K and Na) were extracted in 1N NH_4OAC at pH 7. Sodium and K were determined with a flame photometer while Ca and Mg were determined with the atomic absorption spectrophotometer (AAS). Effective Cation Exchange Capacity was by summation of exchangeable bases (Ca, Mg, K and Na).

Arbuscular Mycorrhizal Fungi Colonization Assessment

Freshly harvested feeder roots were thoroughly washed in tap water and fixed in 50% ethanol. They were then cleared and stained after Koske and Gemma (1989) method. Colonization was determined by viewing under a dissecting microscope at × 45 magnifications using a grid intersection method of Giovannetti and Mosse (1980).

All data were subjected to a combined analysis of variance using the Windows version of Statistical Analysis System (1996) and Duncan's Multiple Range Test (DMRT) was used to separate the means at 0.05 level of probability when the F-ratio was significant.

Isolation and Estimation of Arbuscular Mycorrhizal (AM) Spore Population

The rhizosphere soil samples in three replicates were used to determine AM spore population from 50 grams portion of soil. The AM fungal spores were isolated from composite soil samples by employing "wet sieving and decanting technique" as described by Gerdemann and Nicolson (1963) and were quantified by using the "grid-line intersect method" as described by Gaur and Adholeya (1994). Results were ex-pressed as mean of three replicates for each sample. The abundance of spores determined for each sample was expressed as the number of AM fungal spores per 10 grams of soil for all the samples studied.

Identification of Arbuscular Mycorrhizal Fungal Species

Intact spores and sporocarps were mounted in lacto-phenol and identified according to their spore morphology by using taxonomic key [Schenck and Perez (1990) and Morton and Redecker (2001)]. The qualitative estimation was expressed as percentage frequency occurrence of AM fungal species.

Estimation of Arbuscular Mycorrhizal Root Colonization

Roots of the test plant species after thorough washing with tap water were cut into approximately 1 cm pieces and processed by employing "rapid clearing and staining technique" as described by Phillips and Hayman (1970). 50-stained root bits were examined under microscope for mycorrhizal root colonization. The observations were recorded from each sample containing 50 root segments according to the method suggested by Bierman and Linderman (1981). The relative abundance of mycelium, vesicles and arbuscules of mycorrhizal fungi in the host tissues were recorded as abundant, moderate and scanty. Percent root colonization was quantified based on the number of root segments colonized by AM fungi using the formula:

$$\text{Percentage root colonization} = \frac{\text{Number of root segments colonized}}{\text{Number of root segments observed}} \times \frac{100}{1}$$

Statistical Analysis

Statistical Packages for Social Sciences (SPSS) (Systat Co., http://www.spss.com) 16.0 version package were used for the statistical analysis of the data. Pearson Correlation analysis was performed to assess the relationship between AM fungal spore density of rhizosphere soils, percent root colonization by mycorrhizal fungi, fungal population of rhizosphere and non-rhizosphere soils and soil edaphic factors at 0.01% and 0.05% levels of significance and found a good association between them.

Results And Discussion

Maize was found to be mycorrhizal due to the presence of fungi spores and root colonization by vesicular arbuscular endomycorrhizal infection. Infection was confirmed to be caused by vesicular arbuscular endomycorrhizal because of the presence of a very high number of hyphae though vesicles and arbuscules were also observed in the stained roots. The percentage root colonization and spore count were 40% and 25 respectively (Table 1)

Table 1: Percentage root colonization by mycorrhizal infection of maize plant and spore count per five grams rhizosphere soil

Root Colonization (%)	Spore count per five grammas rhizosphere soil
40	25

The correlation analysis between rhizosphere phosphorus with plant phosphorus and non-rhizosphere phosphorus with plant phosphorus of crops established to be mycorrhizal gave positive values: 0.92 and 0.87 respectively (Table 2). This implies that a relationship existed between the two variables and consequently, an increase in the rhizosphere or non-rhizosphere phosphorus level will equally increase the plant's phosphorus level. Similarly, since the correction value is higher (0.92) in rhizosphere phosphorus with plant phosphorus than (0.87) for non-rhizosphere phosphorus with plant phosphorus (Table 2). It therefore implies that plants depend more on the rhizosphere for their nutrient uptake.

The statistical results showed that Coefficient of linear correlation (r) between rhizosphere P and Plant P = 0.92. Coefficient of linear correlation (r) for non-rhizosphere P and plant P = 0.87. The mean separating between rhizosphere soil P and non-rhizosphere soil P = 0.18 n.s (not significant). A non-significant result from mean separation analysis between rhizosphere and non-rhizosphere soil P levels implies that though the plant is mycorrhizal, the mycorrhizal infection does not contribute to enhancing an increase in the nutrient status of the plant.

Similarly, the high soil fertility probably affected the beneficial effects of mycorrhizal relationship in making nutrient (mostly P) available for plants uptake because dependency on mycorrhizae depends on the nutrient status of the soil (Marx, 1997). The findings of Marx et *al.,* (1997) reported that high P and N concentrations in the soil caused enhanced roots growth and protein synthesis causing a decrease in carbohydrate in feeder roots, thereby causing a dramatic reduction in their susceptibility to mycorrhizal infection.

The organic Carbon content in the non-rhizosphere soil was high (2.4 %) as seen in Table 3 which exceeded the critical value of 0.20 % established for ecological zone of the study area, 'lowland rainforest' (Holland *et al.,* 1989). This high Organic Carbon content could be attributed to high concentration of organic matter in the soil.

The total N was generally high. The TN value were 0.21 % in non-rhizosphere soil and 0.34 % in

plant (Table 3) which exceeded the critical value of 0.20 % established for the ecological zone of the study area, lowland rainforest (Enweozor *et al.,* 1981). However, the value of P in plant was low (14.3 $mgkg^{-1}$).

Potassium level was moderate in non-rhizosphere soil (0.48 $CmolKg^{-1}$) and high in plant (5.6 $CmolKg^{-1}$) as shown in Table 3. The Particle size distribution showed that soil had 75.6 % sand, 15.7 % clay and 8.7 % silt. Hence, the textural class of the non-rhizosphere soil is Sandy loam.

Table 2: Rhizosphere, non-rhizosphere and plants' phosphorus levels of established mycorrhizal infected crops

Crops	Rhizosphere P levels ($mgkg^{-1}$)	Non-Rhizosphere P Level ($mgkg^{-1}$)	Plant P levels (%)
Calopogonium	39.8	35.50	0.071
Ipomea batatas	18.8	30.50	0.238
Centrosema	46.6	36.60	0.190
Pepper	23.1	12.87	0.252
Telfairia	50.1	61.50	0.258
Cocoyam	41.3	28.25	0.258
Cassava	22.2	17.37	0.258
Maize	33.9	78.62	0.143
Cowpea	37.1	38.25	0.205
Okra	45.3	62.88	0.375
Water leaf	42.2	44.13	0.241
Total	400.40	443.70	2.589
Mean	36.4	40.31	0.235

Table 3: Physical and chemical properties of Non-rhizosphere, Rhizosphere and Maize Plant Samples

Soil Physicochemical properties	Non-Rhizosphere soil	Rhizosphere soil	Maize Plant
TN (%)	0.21	-	0.34
Avail. P ($MgKg^{-1}$)	78.62	33.90	14.30
K ($CmolKg^{-1}$)	0.48	-	5.6
OC (%)	2.43	-	-
Sand	75.6	-	-
Silt	8.7	-	-
Clay	15.7	-	-

TN = total nitrogen, Avail. P = Available Phosphorus and OC = Organic carbon

Conclusion

Maize (Zea mays) is a mycorrhizal crop, thus has a beneficial dependency upon mycorrhizal fungi for normal root functioning including increase in the absorption rate of phosphorus and other micro element present in the soil.

The rhizosphere is influenced by the activities of the root, including the release of organic substances which would stimulate the growth of fungi leading to increased number of spore in the rhizosphere. The type of exudate by plant root can influence the level of infection by the fungi and even the colonization of the rhizosphere.

The area under research (Calabar) is made up of depleted soils with a greater of phosphorus being in a fixed form, thus a greater need for mycorrhizal establishment to make the phosphate in available form to plant. The area also depends on maize as one of the major stable foods. Proper management of soil including artificial inoculation of soil with mycorrhizal fungi and guarding against systems and practises that can encourage mycorrhizal deficiency like fumigation can help to increase the production of a healthier, drought and disease resistant; and high yield maize in the area.

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