Response of Three Varieties of Maize (*Zeamays*) to Arbuscular Mycorrhizal Fungi (*Gigaspora Gigantea*) in the Humid Tropics

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Abstract
The influence of AMF (Gigaspora gigantea) on growth of three varieties of Maize (Oba super 2, Oba 4 and Oba 98 seeds) was investigated in the screen house of Department of Crop and Soil Science, University of Port Harcourt. The experiment was laid out in a complete randomized design with three replicates. Gigaspora gigantea was used to inoculate pots containing 10 kg of sterilized soil at 3 weeks before sowing of seeds at the rate of 0, 5, 10 and 15g respectively per pot. Results showed that AMF significantly improved the growth and nutrient uptake in inoculated pot over un-inoculated pots. There was significant increase (P<0.001) in P, N and C. Stem girth and plant height were also significantly (P<0.001) higher in AMF inoculated pots when compared with the control. Pot inoculated with 15g AMF produced the optimum result while the Oba Super 2 variety responded better than the other varieties. It could be concluded from the current study that the use of AMF as soil inoculants could improve maize production and the varying response of the different maize varieties to AMF emphasize the importance of fungi and plant host interactions in crop growth.

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).
Food security implies ensuring sustainable access, availability and quality food to all citizens to meet up with their physiological needs (Okuneye, 2014). When employed at the level of individual household members, food security implies an intake of food and absorption of nutrient sufficient to meet differential individual needs for activity, health, growth and development. Household food security is mostly dependent on maize yields as it is the staple food and the most widespread crop grown in Nigeria (FAO, 2010).
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).

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). Alternative to the use of organomineral fertilizer that is environmental friendly, readily available and cheap for a common farmer; have been advocated. This suggests the use of arbuscular mycorrhizal fungi (AMF), which are commonly available.

Symbiotic associations between AMF and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant (Gosling *et al*., 2006). Mycorrhizal fungi contribute to overcoming mineral element deficiencies, improve plant ability to use the soil resources and improve plant performance (Abbott and Robson, 1991). Although, some studies have been done to evaluate the effect of AMF on the growth and yield of maize, no such studies has been documented on comparative evaluation of AMF on different varieties of maize. Thus, the objective of this study was to investigate the effect of AMF (*Gigaspora gigantea*) applied at four levels on three varieties of maize commonly cultivated in the humid tropic of southern, Nigeria.

Materials and Methods

The experiment was conducted at the screen house, Department of Crop and Soil Science, University of Port Harcourt. The farm is situated at latitude 6° 45 N to 7° E with an average temperature of 27°C, relative humidity of 78% and an average rainfall ranging from 2500-4000mm (Nwankwo and Ehirim, 2010). Samples of top soil (0 to 30cm depth) were taken randomly from the experimental farm, air-dried, sieved through a 2 mm sieve and analyzed for their physical and chemical properties using standard procedures as described by Udo *et al* (2009) before use. The treatments consisted of four levels (0, 5, 10, 15 g/plant) of AMF. The AMF inocula were multiplied for increase using available inocula source collected from the Department of Soil Science, University of Calabar, Nigeria. This consists of mixture of chopped root of the trapping plant hyphae, spores and soil fragments. Seeds of maize varieties (Oba super 2, Oba 4 and Oba 98 seeds) were used for the study. The Oba 98 was gotten from the National seeds research Institute (Umudike) while Oba 4 and Oba Super 2 were obtained from Agro-tropic Industries, Port Harcourt, Nigeria. The choice of varieties used was based on the availability and acceptability. The variability of seeds was tested using the floatation method. The seeds were planted in 10litre plastic pots filled with sterilized soil (4 seeds per pots) at a depth of about 2.5cm below the soil surface, and later thinned to two seedlings per pot 2 week after germination. The pots were watered when necessary. Growth parameters such as stem girth, number of leaves and plant height were assessed. Data were collected at 2, 4, 6 and 8WAI. Soil samples were taken for post-harvest analysis to ascertain its nutrients contents. The experiment was laid out in a completely randomized design (CRD) with three replicate. Data was subjected to Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to test for significant different between means at 5% probability level.

Results and Discussion

Soil parameters

The physical analysis of the soil used for this experiment revealed that it contained mean value of 92.0% sand, 6.0% clay and 2.0% silt. The soil pH was neutral with value of 6.90 (Table 1). It was observed that the percentage sand was reduced in AMF inoculated plants (89.86) compared to its value in Soil before planting. (92.0%) and in Control (91.5%) (Tables 1 & 2). Probably, this observation could be due to the capability of AMF to bind the soil together, thus modifying the soil structure through its hyphae. This observation agrees with the findings of Rillings and Mummey (2006) who reported that AMF secrets Glomalin, glue-like substance which aids in aggregating the soil particles.

Table 1: Physico-chemical properties of soil before treatment with AMF (*Gigaspora gigantea)*

pH	Sand	Silt	Clay	TOC	TN	Avail. P	Ca	Mg	K	Na
	←		%	→		mg/kg	→	$Cmolkg^{-1}$	←	
6.9	92.0	2.0	6.0	0.86	0.025	33.34	0.78	1.30	0.79	0.80

TOC = total organic carbon, TN=total nitrogen, Available P=available phosphorus, Ca=calcium, Mg=magnesium, K=potassium, Na=sodium

Table 2: Physico-chemical properties of soil after inoculation of AMF (*Gigasporagigantea)*

AMF (g)	pH	Sand	Silt	Clay	TOC	TN	Avail. P	Ca	Mg	K	Na
		←		%	→		mg/kg	→	$Cmolkg^{-1}$	←	
0	6.9	91.5	1.9	6.6	3.4	1.0	82.3	0.84	0.7	0.30	0.76
5	5.9	91.2	6.0	2.8	3.12	0.98	79.0	1.01	0.60	0.29	0.42
10	6.0	89.2	8.0	2.8	2.76	0.92	78.5	0.97	0.48	0.20	0.65
15	6.0	89.2	8.0	2.8	2.05	0.96	74.4	1.22	0.59	0.28	0.69

TOC = total organic carbon, TN=total nitrogen, Available P=available phosphorus, Ca=calcium, Mg=magnesium, K=potassium, Na=sodium, Soil BP= soil sample analysis before planting, AMF=arbuscular mycorrhiza fungi.

The major limitation of tropical farmers is the inability to replenish nutrients lost due to continuous cultivation of land (Mokwunye *et al*., 1996). The pre-planting soil analysis results showed that the soil was low in Calcium (0.78 Cmolkg), Magnesium (1.30 Cmol/kg), Potassium (0.79 Cmol/kg) and Sodium (0.80 Cmolkg) below their critical level (Table 1). At the termination of the experiment at 6WAI, the result of the soil analysis showed increase in organic Carbon, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Sodium in AMF inoculated soil, above their critical values according to Enwenzor *et al.*, (1989). This indicated that cultivation of maize with the inoculation of mycorrhiza increased the soil organic matter and nutrient elements. Inoculation of AMF significantly increased the growth of maize compared to the treatments without mycorrhiza (Table 1 & 2). This was in agreement with earlier work done by Oyetunji *et al.*, (2009) and Olawuyi *et al.*, (2010) who reported that inoculation of AMF to maize significantly improved the growth and yield of maize compared to non-mycorrhizal maize. The increased in the nutrient elements could be due to the presence of Mycorrhizal fungi, which penetrate by forming a hyphal network in order to mineralize nutrient. This observation agrees with the findings of Rousseau *et al.*, (1994) who reported that mycorrhizal hyphae could increase the absorbing surface area of the root in soil deficient of nutrient. The hypha penetrates beyond the nutrient deficient zone, readily bridge this depletion zone, and grow into soil with an adequate supply of phosphorus and other nutrients thereby enriching the soil nutrient content.

The value of N in soil before planting (0.025%) increased to 0.65% (mean value) in AMF inoculated pots. This observation is in agreement with the findings of Muok et al., (2009) who reported that mycorrhizal fungi increased the root efficiency to absorb nutrient and in nutrient depleted soil, mycorrhizal fungi develop strand in the soil and absorb phosphorus through the root hairs, thereby increase nodulation and nitrogen. However, this character of the fungi enhanced plant growth of maize.

Phosphorus value in AMF inoculated pot increased to mean value of 77.3mg/kg compared to initial value (33.34mg/kg) of P in SBP (Table1 & 2). The increase in P concentration in AMF inoculated plants can be attributed to the extensive root development and hyphae that reduce the distance for diffusion of nutrients thus enhancing the nutrient absorption. This observation is in agreement with the work of Dodd *et al*., (1987) who reported that the enhanced acid phosphatase activities in mycorrhizal plants assist in releasing P, which in turn transported by the external mycelium (Jakobsen *et al*., 1992) and resulted in enhancement of nutritional status of the host plants.

The values of Nitrogen and Phosphorus increased in the AMF inoculated plants than in Control and soil before planting. This agrees with the findings of George *et al*., (1995) and Chen *et al*., (2005) who found that colonization of plant roots by AMF greatly increase the plant uptake of P and N.

Therefore, the trend towards higher soil organic C content (2.64%) upon AMF inoculation compared to SBP value (0.86%) could be due to the increased distribution of photosynthates in underground structures, since mycorrhizal roots could release more root exudates than non-mycorrhizal roots because of the larger root system and/or improved nutrition as earlier reported by Hu *et al.* (2010).

Table 3: Effect of Mycorrhiza on Plant Height (cm) of Maize Varieties.

Varieties	**AMF (g)**	**2WAI**	**3WAI**	**4WAI**	**5WAI**	**6WAI**
Oba4	0	65.83^{ab}	74.43^{bc}	84.3^{ab}	86.0b^{cd}	93.2^{abc}
	5	67.57^{a}	83.50^{a}	84.3^{ab}	97.4^{ab}	101.5^{ab}
	10	64.67^{ab}	77.80^{abc}	87.6^{a}	93.9^{abc}	98.2^{abc}
	15	59.20^{bcd}	73.20^{bc}	56.5^{b}	81.5^{cd}	87.4^{bc}
Oba 98	0	52.73^{d}	70.60^{cd}	78.1^{ab}	84.4^{bcd}	90.3^{bc}
	5	54.40^{cd}	73.40^{bc}	81.0^{ab}	89.1^{abc}	99.8^{abc}
	10	55.20^{cd}	77.60^{abc}	88.2^{a}	91.4^{abc}	101.0^{ab}
	15	62.10^{abc}	80.70^{ab}	90.1^{a}	99.3^{a}	106.0^{a}
Oba S2	0	52.30^{d}	61.50^{e}	71.7^{ab}	73.4^{d}	85.2^{c}
	5	52.00^{d}	63.30^{de}	74.5^{ab}	81.1^{cd}	90.0^{bc}
	10	55.90^{cd}	62.80^{e}	77.2^{ab}	89.8^{abc}	97.5^{abc}
	15	56.10^{cd}	65.70^{de}	82.3^{ab}	97.0^{ab}	105.8^{a}

Means with different superscript on the same row are significantly different from each other ($p<0.05$) according to the Duncan multiple range test., AMF= Arbuscular mycorrhizal fungi, WAI=weeks after inoculation.

Table 4: Effect of Mycorrhiza on Number of Leaves of Maize Varieties.

Varieties	AMF (g)	2WAI	3WAI	4WAI	5WAI	6WAI
Oba 4	0	5.00^{ab}	5.00^{ab}	5.00^{ab}	5.00^{bc}	5.00^{abc}
	5	5.00^{ab}	6.00^{a}	5.00^{ab}	6.00^{ab}	5.00^{abc}
	10	5.00^{ab}	4.00^{bc}	6.00^{a}	6.00^{ab}	5.50^{ab}
	15	5.00^{ab}	6.00^{a}	6.00^{a}	7.00^{a}	6.00^{a}
Oba 98	0	5.00^{ab}	3.00^{c}	4.00^{b}	5.00^{bc}	4.00^{bc}
	5	6.00^{a}	3.00^{c}	5.00^{ab}	4.00^{c}	5.00^{abc}

	10	5.00^{ab}	4.00^{c}	6.00^{a}	6.00^{ab}	6.00^{a}
	15	5.00^{ab}	5.00^{ab}	5.00^{ab}	5.00^{bc}	6.00^{a}
Oba super 2	0	4.00^{b}	5.00^{ab}	4.00^{b}	5.00^{bc}	5.00^{abc}
	5	5.00^{ab}	6.00^{a}	4.00^{b}	5.00^{bc}	4.00^{c}
	10	5.00^{ab}	5.00^{ab}	5.00^{ab}	6.00^{ab}	6.00^{a}
	15	6.00^{a}	5.00^{ab}	5.00^{ab}	7.00^{a}	6.00^{a}

Means with different superscript on the same row are significantly different from each other ($p<0.05$) according to the Duncan multiple range test., AMF= Arbuscular mycorrhizal fungi, WAI=weeks after inoculation.

Table 5: Effect of Mycorrhiza on Leaf Area (Cm^2) of Maize Varieties.

Varieties	AMF (g)	2WAI	3WAI	4WAI	5WAI	6WAI
Oba 4	0ml	78.50^{b}	107.3^{cd}	126.4^{b}	141.0^{abc}	159.0^{abc}
	5ml	90.20^{a}	131.1^{a}	154.7^{a}	136.6^{abc}	148.6^{def}
	10ml	65.10^{d}	107.6^{cd}	117.0^{cd}	134.7^{bc}	155.7^{bcd}
	15ml	61.80^{d}	117.0^{bc}	103.8f	125.4^{c}	149.1^{cde}
Oba 98	0ml	59.70^{d}	88.3^{e}	102.4^{f}	136.8^{abc}	140.7^{ef}
	5ml	73.30^{b}	100.2^{d}	118.6^{bcd}	156.9^{a}	156.0^{bcd}
	10ml	76.50^{b}	108.9^{cd}	112.9^{de}	135.1^{abc}	139.0^{f}
	15ml	77.50^{b}	122.1^{ab}	150.0^{a}	155.2^{ab}	160.6^{ab}
Oba super 2	0ml	63.33^{d}	87.7^{e}	99.9^{f}	139.7^{abc}	138.8^{f}
	5	74.30^{b}	99.7^{d}	114.4^{de}	143.4^{abc}	140.6^{ef}
	10	76.50^{b}	106.5^{cd}	107.1^{ef}	150.0^{ab}	153.3^{bcd}
	15ml	77.50^{b}	115.8^{bc}	124.5^{bc}	157.1^{a}	166.0^{a}

Means with different superscript on the same row are significantly different from each other ($p<0.05$) according to the Duncan multiple range test., AMF= Arbuscular mycorrhizal fungi, WAI=weeks after inoculation.

5.2 Plant parameters

The growth of maize varieties inoculated with Mycorrhiza was generally increased compared to the control. This was in agreement with the earlier work done by Hamel (2004) that inoculation of AMF contributes positively to growth and development of plant. The height of AMF inoculated maize was significantly higher ($p< 0.05$) compared to non-Mycorrhizal inoculated maize. At 2WAI, 5g AMF Oba 4 produced the highest height (67.57cm) and is not significantly different from 0ml Oba 4, 10g Oba 4 and 15g Oba 98 but significantly higher than other AMF treatments at $P<0.05$. This is supported by works of George (1995) who noted that mycorrhizal increased the phosphorus and micronutrient uptake and growth of their plant host resulting in significant increase in vegetative growth and yield of plant compared to when there was no mycorrhizal inoculation. Larger number of leaves was observed in Oba super 2 at 15g (7.00cm) and Oba 4 at 15g (7.00cm) as seen in 5WAI (Table 4), though not significantly different from Oba 4 at 5g, Oba 4 at 10g, Oba 98 at 10g and Oba Super 2 at 10g AMF inoculation but significantly higher than other AMF treatments ($p<0.05$)**.** This finding supported the view of Olawuyi *et al*., (2010) and Schippers, (2000) which reported the contribution of Nitrogen sources from AMF in promoting the vegetative portion of the plant, producing large green leaves.

Also, at 4WAI, Oba 98 produced the highest dry matter weight (2.15g) which is not significantly different from 15ml Oba Super 2, 10ml Oba Super 2 but significantly higher than other AMF treatments in other varieties at $P<0.05$. The dry matter weight of maize was significantly increased by the inoculation of AMF and this was in agreement with work done by Fagbola *et al*., (1998) who reported that AMF inoculation increases dry matter of maize plant. It was observed that Oba 4 variety did not follow a regular growth pattern as compared to other varieties (Table 4), probably due to environmental condition, crop growth pattern, or slow response to AMF inoculation. Further studies are yet to unravel this complex interaction

Conclusion

It could be concluded that AMF help in mineralization of nutrient, thereby making them available for plant absorption and utilization. They significantly improved soil nutrient status, hence the growth of maize. On the average, plants inoculated with 15ml of AMF showed significantly higher cumulative growth response than others. Therefore, growth of maize was optimized with 15ml AMF inoculation in this study. Based on comparative evaluation of the three maize varieties namely, Oba super 2, Oba 4 and Oba 98; Oba Super 2 outperformed others. Therefore, Oba Super 2 is the host of AMF and could be used to multiply the fungi under prevailing soil conditions. The farmers should be encouraged to use AMF as it is environmentally friendly, required no specialize skill for its application and there is no need of frequent application as it is in the case of chemical fertilizers.

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