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EFFECT OF MYCORRHIZAL INOCULATION AND PHOSPHORUS FERTILIZER ON MAIZE ROOT INFECTIVITY IN THREE SOIL SERIES

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Abstract: An experiment was carried out to examine the influence of mycorrhizal inoculation and phosphorus fertilizer on maize root infectivity in three soil series at the Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. The treatments included three soil series (Gambari, Itagunmodi and Iwo series), three levels of phosphorus fertilizer (0, 30 and 60 kg P_2O_5/ha) and three levels of mycorrhizal inoculation (0, 10 and 20 g per 15 kg soil). The experimental design used was a 3×3×3 factorial trial in a completely randomized design with three replications. Root infectivity was evaluated by the grid line intersect method. After harvesting, the phosphorus (P) uptake was determined by multiplying the P concentration in the plant by the total dry weight. The data were analyzed using analysis of variance and the significant means of the treatment were compared using the Duncan's multiple range test at the 5% significance level. The results revealed that P uptake was influenced significantly ($p < 0.05$) by the soil series. The order of decrease in P uptake across the soil series was as follows: Itagunmodi (0.54 mg/kg) < Iwo (0.90 mg/kg) < Gambari (2.52 mg/kg). Root infectivity significantly enhanced the uptake of P. The highest root infectivity and the highest P uptake were achieved at 20 g inoculation. Mycorrhizal infection levels were reduced by moderate to high rates of soluble phosphorus fertilizer. Thus, increasing levels of phosphate fertilizer stimulate maize root growth but significantly reduce root infection levels.

Key words: mycorrhizal inoculation, soil series, root infectivity, phosphorus uptake.

Introduction

Arbuscular mycorrhizal fungi (AMF) create a key functional group of soil organisms as a result of their effect on soil properties (Dal Cortivo et al., 2018). The phylum Glomeromycota comprises the AMF, which also belongs to the class

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Glomeromycetes (Brundrett and Tedersoo, 2018); and easily forms symbiotic associations with most terrestrial plants. Bushy, brush-like hyphae known as arbuscules are formed in the root cell of the host plant, enabling the exchange of carbohydrates and the absorption of water and nutrients between the host and fungi (Gutjahr and Parniske, 2013). However, the relationship is significant for the fungi because the plants serve as their only source of energy. The importance of maize cannot be overestimated, as it serves both as food for humans and animals and as an industrial raw material for the production of corn starch, beer, etc.

Phosphorus (P) is one of the three crucial nutrients required for plant growth. However, it is not readily available to plants, thus limiting biomass production in ecosystems (Rodrigues et al., 2021). Phosphorus as a principal component of deoxyribonucleic acid (DNA), and a genetic inheritance of ribonucleic acid (RNA) is essential for protein synthesis in both plants and animals. Plants require a significant amount of phosphorus in the early stages of growth for optimum crop production (Zhu and Whelan, 2018), and this element is highly mobile within the plant. If the plant does not contain enough phosphorus, the older leaves turn purple and premature senescence occurs, signifying phosphorus deficiency. However, the availability of phosphorus is also influenced by the type of soil. However, the quantity of available phosphorus and mycorrhizal inoculation are independent of root infectivity.

Maize (*Zea mays* L.) is an essential cereal crop worldwide (Ashraf et al., 2016). It is limited by many factors such as soil fertility, imbalanced nutrition, disturbed soil properties, cultivars, and weed infestation. Maize has some advantages compared to other cereals: high production, easy processing, easy digestibility and lower price. The high net energy content, fiber content and high protein level of maize make it an important food for humans and feed for livestock (Oladejo and Adetunji, 2012).

Different soil series have been used for maize cultivation in southwestern Nigeria, but little or no attention has been paid to the influence of the different soil series on mycorrhizal infectivity and phosphorus availability. Therefore, this study is crucial to examine the phosphorus level at which mycorrhizal infectivity is maximized and to determine the most suitable quantity of mycorrhizal propagules and soil series that significantly enhance mycorrhizal root infectivity in maize.

Material and Methods

Description of the sample locations and soil sample collection

Three soil series (Itagunmodi, Iwo and Gambari) were used for the study. The top soil samples were collected using shovel at the 0–15-cm depth from three study areas. These were the areas near Ilesha (Itagunmodi series), Ede – Iwo Area (Iwo series) and a series break of slope before the valley bottom location within the

Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Teaching and Research Farm (Gambari series). The soil types and potential land use patterns were described by Charles et al. (2004).

Experimental design

The experiment was carried out in pots at the Teaching and Research Farm, LAUTECH Ogbomoso. The experiment was a 3×3×3 factorial trial comprising: 3 types of soil series (Gambari, Itagunmodi and Iwo series); 3 levels of phosphorus application: 0 kg P_2O_5/ha, 30 kg P_2O_5/ha and 60 kg P_2O_5/ha and 3 levels of mycorrhizal application (*Glomus mossae*), 0 g/15 kg soil, 10 kg/15 kg soil and 20 g/15 kg soil. The trial was arranged in a completely randomized design (CRD) with three replications.

Field preparation and planting

The pots were placed at a distance of 0.75 m from each other. Three seeds of the maize variety SUWAN -1- SR-Y were sown in the middle of the pots and later, one week after sowing, thinned out to one plant per pot. Before sowing, three levels of the mycorrhizal *Glomus mossae* strain ([0 g/15 kg soil], [10 g/15 kg soil] and [20 g/15 kg soil]) were applied at a depth of 3 cm in the center of the pots. Phosphorus in the form of single superphosphate was applied at a rate of 0 kg P_2O_5/ha (0 g/15 kg soil), 30 kg P_2O_5/ha (1.3 g/15 kg soil) and 60 kg P_2O_5/ha (2.5 g/15 kg soil). Weeding was carried out manually three weeks after sowing (3 WAS) and six weeks after sowing (6 WAS).

Laboratory analyses of the soil samples

The particle size of each soil sample was determined by air-drying, crushing and sieving the soil samples through 2-mm and 0.5-mm meshes. Thereafter, pH (H_2O), total nitrogen (N), organic carbon, available phosphorus (P), iron (Fe), copper (Cu), zinc (Zn), the exchangeable cations (Ca, Na, Mg and K) and exchangeable acidity were determined using standard methods. Soil pH was determined in a 1:1 soil-water suspension using a digital pH meter (Bentop pH meter, pH-B200E/PH-B200EM by Infitek), while particle size distribution was determined by the Bouyoucos hydrometer method (Bouyoucos, 1962) using sodium hexametaphosphate as a dispersant. The exchangeable cations (Ca, Mg, K and Na), available phosphorus, Cu, Zn, Fe and Mn were also determined by extracting the soil with the Mehlich-3 extractant in a ratio of 1:10 soil/extractant. Potassium and Na were determined in the extract using the flame photometry method while Ca, Mg, Mn, Zn, Fe and Cu were determined by the atomic absorption spectrophotometer (AAS, 325-1100Nm, India). The available

phosphorus was determined colorimetrically by the ascorbic acid method. The exchangeable acidity (H^+, Al^{3+}) was determined by titrating 0.01N HCl with the 1N KCl extract of the soils using the phenolphthalein indicator (Kumar et al., 2014). Total nitrogen was determined from the concentrated sulphuric acid digestion using the Adapted Technicon Salicylate/Sodium Nitroprusside colorimetric method. Organic carbon was analyzed by the colorimetric complete oxidation method using a spectrophotometer (UV-Vis spectrophotometer, Hach company, Danahar) (Heanes, 1984).

Botanical data collection and plant analyses

At the twelfth week after sowing (12 WAS), cobs, roots and shoots were harvested. After harvesting, all shoots and roots were oven-dried at a temperature of 80^0C to a constant weight for five days. These were later used for the determination of dry weight and total biomass production. The plant samples were milled in a Wiley mill to pass through a 1-mm sieve and the plant samples were analyzed for phosphorus according to the plant analysis procedure outlined in Selected Methods for Soil and Plant Analysis Manual of International Institute of Tropical Agriculture, Ibadan (IITA, 1979). A portion (4.0 ml) of nitric/perchloric acid mixture (3:1 ratio) was added into 0.2 g of each sample in a 25-ml conical flask and left overnight. The content was heated until white fumes were formed, then 1.0 ml of hydrochloric acid/distilled water mixture (1:1 ratio) was added. It was heated further for 30 minutes before the heat was removed. Distilled water was added to the digestion and shaken before cooling to room temperature to avoid the formation of insoluble perchlorate compounds. The digestion was washed into a 50-ml volumetric flask. It was then made up to the mark with distilled water. Total phosphorus was determined by the colorimetric vanadomolybdate method on a spectrophotometer. The nutrient accumulated in the plant parts was calculated as follows: nutrient uptake = % nutrient concentration X sample dry weight, according to Ombo (1994) and Gungunla (1999).

The determination of mycorrhizal root infectivity was carried out by the grid line intersect method. After harvesting, root samples were cut into 1-cm length and stored in 50% ethanol. The root samples were later carefully rinsed with slow running tap water to remove the ethanol (before the commencement of the root staining procedure). The root particles were put in sample bottles with 10% KOH overnight. The root particles were placed in a water bath and steamed at 80°C for 30 minutes (Philip and Hayman, 1970). They were then poured into a sieve, rinsed under running water and returned to the sample bottles. They were then bleached in alkaline H_2O_2 for 10 minutes, rinsed with water and soaked in 1% HCl acid for 10 minutes. Trypan blue staining solution was used for the roots, containing 0.05% trypan blue; 10 ml of 50% glycerol was added to each bottle, shaken well and left for 24 hours for proper staining. The stained root particles were poured inside Petri

dishes with glycerol to prevent desiccation. The degree of mycorrhizal infectivity was assessed by spreading 25 root pieces per slide and observing them under a dissecting microscope at low magnification. The total number of roots and the infected roots intersecting the grids were counted using the grid line intersect method (Vierheilig et al., 2005). The percentage mycorrhizal root infectivity was calculated by the ratio between the number of intersects with infection and the total number of intersects multiplied by 100 (Fagbola et al., 2001).

$$\% \text{ root infectivity } = \frac{EP}{N \times Mp} \times \frac{100}{1}$$

where,
EP = estimated population,
N = number of lines per slide,
Mp = maximum root particles per slide.

Statistical analysis

The experimental design was a 3×3×3 factorial trial in a completely randomized design with three replications. The data were subjected to analysis of variance. Comparison of the various treatment means was done using the Duncan's multiple range test at the 5% level of significance.

Results and Discussion

Table 1 shows the physico-chemical properties of the soil series used. The Itagunmodi series had the lowest pH value and available phosphorus, while the Gambari series had the highest pH value and available phosphorus.

Table 1. Physical and chemical properties of the soil samples of the three studied soil series.

Properties	Gambari series	Itagunmodi series	Iwo series
Soil pH	5.00	3.60	4.40
Available P (mg/kg)	2.52	0.54	0.90
Fe (mg/kg)	63.30	42.50	69.80
Total N (g/kg)	1.00	1.50	0.70
Organic C (g/kg)	12.20	13.60	7.60
Ex. Ca (Cmol/kg)	0.67	0.21	0.05
Ex. Mg (Cmol/kg)	0.48	0.50	0.27
Ex. K (Cmol/kg)	0.17	0.26	0.09
Ex. Na (Cmol/kg)	0.01	0.06	0.02
Base saturation (%)	88.67	76.30	57.44
Sand (g/kg)	853.00	563.00	753.00
Silt (g/kg)	117.00	227.00	157.00
Clay (g/kg)	30.00	210.00	90.00

The Itagunmodi series had the lowest Fe and sand value, while the Iwo series had the highest value of Fe and the Gambari series had the highest sand value. The Itagunmodi series also had the highest value of silt and clay, while the Gambari series had the lowest value of silt and clay. The Iwo series had the lowest values of exchangable Ca, exchangeble Mg, exchangeable K, exchangeable Na and base saturation.

Phosphorus uptake

Table 2 shows that the soil series had a significant effect on phosphorus uptake. The Itagunmodi series had the highest mean phosphorus uptake (4.27 g/plant), followed by the Iwo series (3.44 g/plant), while the Gambari series had the lowest phosphorus uptake (2.28 g/plant). Mycorrhizal inoculation at 20 g/15 kg soil had a significant effect on phosphorus uptake, which was significantly ($p<0.05$) higher (4.58 g/plant) than 2.61–3.01 g/plant observed at lower doses of mycorrhizal inoculation. The combination of the Itagunmodi series with 20 g of mycorrhizal dose resulted in the highest phosphorus uptake (Figure 1).

Table 2. The effect of mycorrhizal inoculation, soil series and P fertilizer on phosphorus uptake (g/plant).

Factor	Treatments	Phosphorus uptake (g/plant)
P fertilizer		
	0gP_2O_5/15 kg	3.51a
	1.3gP_2O_5/15 kg	2.88a
	2.5gP_2O_5/15 kg	3.81a
Mycorrhiza		
	0g/15 kg soil	2.61b
	10g/15 kg soil	3.01b
	20g/15 kg soil	4.58a
Soil series		
	Gambari	2.28b
	Itagunmodi	4.27a
	Iwo	3.44ab

For each factor, means followed by the same alphabet in the column are not significantly different by the Duncan's multiple range test at the 5% level of probability.

Root infectivity

Table 3 shows that mycorrhizal root infectivity had a significant effect on maize root. The highest root infectivity (36%) was recorded at 20 g inoculum/15 kg soil and the lowest root infectivity (11.9%) was recorded at 0 g inoculum/15 kg soil.

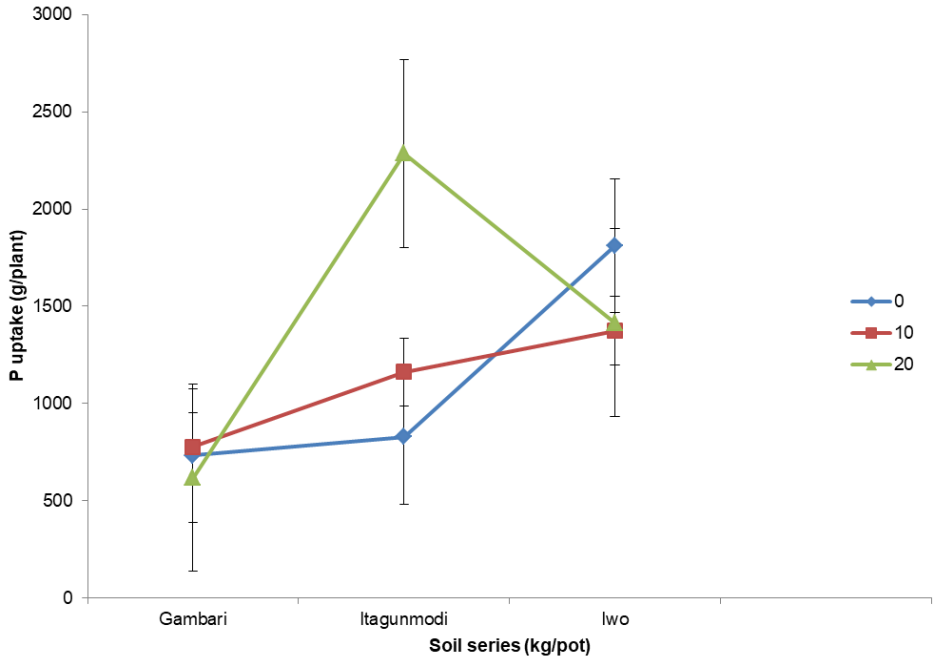

Figure 1. The interactive effect of P fertilizer, mycorrhizal inoculation and soil series on P uptake (g/plant) in maize.

Table 3. The effect of mycorrhizal inoculation, soil series and P fertilizer on maize root infectivity.

Factor	Treatments	Root infectivity (%)
P fertilizer		
	0gP_2O_5/15 kg	25.1a
	1.3P_2O_5/15 kg	26.7a
	2.5P_2O_5/15 kg	28.0a
Mycorrhiza		
	0g/15 kg soil	11.9c
	10g/15 kg soil	31.4b
	20g/15 kg soil	36.0a
Soil series		
	Gambari	27.4a
	Itagunmodi	25.3a
	Iwo	27.4a

For each factor, means followed by the same alphabet in each column are not significantly different by the Duncan's multiple range test at the 5% level.

The results showed no response of root infectivity to P fertilizer application, irrespective of the levels while mycorrhizal inoculation responded to root infectivity and the effect was significant with increasing quantity of inoculum. The result agrees with Cheng et al. (2013). Arbuscular mycorrhizal fungi increased the root length in all root diameter classes, although the proportion of medium and coarse roots was increased to a higher degree than that of fine roots. The interactions are dependent on plant-fungus compatibility, as some arbuscular mycorrhizal fungus-plant combinations are more beneficial than others (Kandhasamy et al., 2020). In this study, the Itagunmodi series gave the highest phosphorus uptake with 20 g mycorrhiza per 15 kg soil.

Phosphorus uptake was the highest in the crops on the Itagunmodi series. This was due to the low amount of available P and Fe from Table 1 compared to the other two soil series. Kowalska et al. (2015) have also reported that soil P plays the most significant role in regulating plant mycorrhizal infectivity. High P application was also found to have negative effects on root infectivity (Cheng et al., 2013). However, low fertilizer application optimizes the plant mycorrhizal infectivity (Rana et al., 2020). According to Ma et al. (2019), fertilizer application has been shown to have negative effects on AMF infectivity traits and thus affects plant responses to AM fungi.

The results therefore show that root infectivity responded to mycorrhizal inoculation but not to P fertilizer application.

Conclusion

This paper summarizes the influence of mycorrhizal inoculation and phosphorus fertilizer on root infectivity in three soil series. Root infectivity was only recorded with mycorrhizal inoculation and no significant infectivity was observed with fertilizer application. However, root infectivity was significantly enhanced with 20 g inoculum, irrespective of the soil series. The study therefore showed that high phosphorus levels had a negative influence on AMF infectivity of crops. However, phosphorus uptake was significantly enhanced by root infectivity. Therefore, excessive P application to maize would have negative impacts on maize growth. Apart from this observation, high P levels can also lead to incremental costs of production, which should be discouraged to promote farmer profitability. The results show that mycorrhizal inoculation significantly improved root infectivity and P uptake. Therefore, it is recommended to promote cropping systems that maintain mycorrhiza in the root zones of maize.

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UTICAJ MIKORIZNE INOKULACIJE I FOSFORNOG ĐUBRIVA NA INFEKTIVNOST KORENA KUKURUZA NA TRI ISPITIVANA PODRUČJA

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R e z i m e

Izveden je eksperiment kako bi se ispitalo uticaj mikorizne inokulacije i fosfornog đubriva na infektivnost korena kukuruza u tri ispitivana područja na Tehnološkom univerzitetu Ladoke Akintola (engl. *Ladoke Akintola University of Technology* – LAUTECH), Ogbomoso, Nigerija. Tretmani su obuhvatali tri lokacije (Gambari, Itagunmodi i Iwo), tri nivoa fosfornog đubriva (0, 30 i 60 kg P_2O_5/ha) i tri nivoa inokulacije mikorizom (0, 10 i 20 g na 15 kg zemljišta). Eksperiment je bio dizajniran kao trofaktorski ogled (3×3×3) u potpuno slučajnom dizajnu sa tri ponavljanja. Infektivnost korena procenjivana je metodom preseka linija mreže. Nakon žetve, usvajanje fosfora (P) određivano je množenjem koncentracije P u biljci sa ukupnom suvom masom. Podaci su analizirani primenom analize varijanse, a značajnost srednjih vrednosti tretmana upoređena je Dankanovim testom višestrukog opsega na nivou značajnosti od 5%. Rezultati su pokazali da je lokacija zemljišta značajno ($p < 0,05$) uticala na usvajanje fosfora (P). Redosled smanjenja usvajanja fosfora po lokacijama zemljišta bio je sledeći: Itagunmodi (0,54 mg/kg) < Iwo (0,90 mg/kg) < Gambari (2,52 mg/kg). Infektivnost korena značajno je povećala usvajanje fosfora. Najveća infektivnost korena i najveće usvajanje fosfora postignuti su pri inokulaciji od 20 g. Stepeni mikorizne infekcije smanjeni su pri umerenim do visokim dozama rastvorljivog fosfornog đubriva. Dakle, povećanje nivoa fosfornog đubriva podstiče rast korena kukuruza, ali značajno smanjuje nivo infekcije korena.

Ključne reči: mikorizna inokulacija, lokacije zemljišta, infektivnost korena, usvajanje fosfora.

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