Combined Phosphorus and Water stress Conditions Induce Negative Mycorrhizal Response in Maize (*Zea mays* L.)

Askangmi Timothy Ricwuskebnde [1], Ibrahim Abubakar Aliyu [2], Aisha Abdulkadir [3], Christogonus Kayode Daudu [4], Ado Adamu Yusuf [5]

[1]Department of Soil Science, Ahmadu Bello University, Zaria, Nigeria.
[4]National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria, Nigeria.
[1]Corresponding author: askangmitimothy@gmail.com

Article history:

Received: 25 October 2022
Accepted: 27 February 2023
Published: 30 June 2023

Keywords: *Maize; AMF; Biomass accumulation; Phosphorus rate; Water stress*

Abstract

Arbuscular mycorrhizal fungi (AMF) confer both positive and negative effects on the plant symbionts, depending on the prevailing growth condition. We investigated the effect of concurrent variations in phosphorus and soil moisture on percentage root colonization (%RC), mycorrhizal growth response (MGR) and drought response index (DRI) of SAMMAZ-16 maize variety in timescale. The experimental factors were AMF inoculation (addition or no addition), P_2O_5 applications (30, 60 or 90 kg ha^{-1}) and water regimes (100% and 50% of the soil's field capacity introduced after 4WAS). The result shows that the overall %RC was 62.22% at 8 weeks after sowing (WAS) and 71.33% at 12 WAS. With 30 and 60 kg P_2O_5 ha^{-1} application a rate, %RC was significantly higher at 12 WAS than that of similar application rates at 8 WAS. However, %RC was not different between 8 WAS and 12 WAS in 90 kg P_2O_5 ha^{-1} application. AMF inoculation tended to equilibrate the shoot growth of the inoculated plants to that of non-inoculated plants that received 50% higher doses of P_2O_5 under amply watered conditions. Increasing phosphorus application progressively alleviated the negative mycorrhizal response of the plants at the early stage of growth (week 4) and in 50% FC category at the other sampling times. Higher doses of P_2O_5 application improved the DRI of the maize in both samplings but the trend was more consistent in AMF-inoculated plants. We conclude that AMF inoculation would be detrimental to the growth of SAMMAZ-16 when there is combined phosphorus and water stress factors.

https://dx.doi.org/10.52951/dasj.23150107

Introduction

Arbuscular mycorrhizal fungi source and deliver resources beyond the root depletion zone to plant symbionts in return for photosynthates they obtain from the plants (Smith and Read, 2008; Ishaq *et al*., 2021). Usually, AMF-colonized plants show improved adaptation or resilience to abiotic stresses and better growth. It is widely believed that the enhanced physiological and growth response of AMF-colonized plants is due to additional nutrients and water absorption through AMF hyphae (Aliyu *et al*., 2019; Li and Cai, 2021; Cheng *et al*., 2022; Qi *et al*., 2022). Arbuscular Mycorrhizal symbiosis has the ability to alleviate water stress in plants (Cheng *et al*., 2022) by improving plant tissue hydration either through drought avoidance or drought tolerance.

The mycorrhizal status of a plant is often quantified by examining the degree of root colonization (RC) by the AMF. Root colonization is considered as the presence of at least, one of the features including arbuscule, vesicle or hyphae), or AMF-specific biochemical marker in the root of a host plant (Vierheilig *et al*., 2005). The percentage of root colonization is more often reported to decrease with an increase

in P availability (Aliyu *et al*., 2019; Ishaq *et al*., 2021). However, there are studies where mycorrhizal effects are observed even with high soil P nutrients (Chu *et al.*, 2013). Although convincing evidence from research suggests that RC is largely modulated by P availability in maize, this does not exclude the possibility of the plant resisting colonization or being colonized due to growth factors other than the P status of the growing media. Root Colonization was found to decrease with an increase in the severity of water stress in AMF-colonized plants (Abdel-Salem *et al.*, 2017; Cheng *et al*., 2022) and increased with prolonged drought (Bitterlich *et al.,* 2018). In a similar study, species richness of AMF was observed to improve with progressive drier Agro-ecology (Tchabi *et al*., 2008).

The degree of root colonization and mycorrhizal responsiveness to growth parameters are closely related to the genetics of the symbionts, the soil characteristics and Argo-ecology. Even plants that can form mycorrhizal symbiosis may become facultative symbionts under certain edaphic and climatic conditions (Walder, 2014; Pyšek *et al*., 2019). Based on growing conditions, plants have demonstrated a spectrum of mycorrhizal responses to growth parameters including negative, neutral and positive (Yin *et al*., 2021; Säle,*et al*., 2022). Aliyu *et al.* (2019) observed a decrease in the mycorrhizal responsiveness of cassava to root biomass with an increasing phosphorus application rate in the Northern Guinea Savanna (NGS) of Nigeria. The fact that AMF confers both benefits and costs on its plant symbionts presents a challenge that requires critical evaluation of the multifactor effects of growth conditions on mycorrhizal associations, and in time scale. Phosphorus and water are growth-limiting factors in the semi-arid ecology of the (NGS) of Nigeria. The soils are rich in ferrous oxide which easily forms complexes with phosphorus and constraint its uptake by the crops. Growers are, therefore, compelled to apply high amounts of phosphorus fertilizer in order to compensate for this inherent P fixation. Recently, the rainfall pattern of the region is characterized by late onset and erratic diurnal distribution with a mid-season drought episode that usually occurs in August-September and affects the productivity of moisture-sensitive crops such as maize which is the major crop grown in the region. Although research has reported the benefit of AMF in resource acquisition and growth responses of the plant symbionts in the region (Aliyu *et al*., 2019), whether or not this benefit is sustained at all levels of phosphorus application under both sufficient and deficit moisture regimes of the soil is not known. This is particularly important considering the role of phosphorus and moisture availability in root colonization by AMF. The knowledge of the temporal dynamics of mycorrhizal response under combined phosphorus and water availability in the soil for specific productivity determinants may be useful to synchronize AMF-inoculated maize growth with the growing conditions that best benefit the maize in this ecosystem. This study, therefore, was designed to answer the following research question: Would a functional mycorrhizal association be beneficial to maize plants at all phosphorus and moisture status at any moment of growth in the Alfisols of the NGS? To address this question, a screen house experiment was set up to determine the per cent root colonization of maize and mycorrhizal responsiveness of the plants to growth parameters in the Alfisols of the NGS of Nigeria at 4 weeks' frequency.

Materials and Methods

Soil Sample Collection and Preparation

The soil, classified based on USDA Soil as Acrisols in FAO-UNESCO Legend (Uyovbisere *et al*., 2000), was collected from the Institute for Agricultural Research (IAR) experimental field located in NGS of Nigeria. The sampling plot has a coordinate of 11^0 09' 96.5" N, 7^0 37' 96.2" E, and an altitude of 684 m above sea level. The

antecedent available phosphorus of the soil is 1.7 mg kg^{-1}. Because of the role of phosphorus availability in mycorrhizal development, the low phosphorus content was desirable to ensure that the phosphorus nutrient of the soil can be conveniently maintained at a deficient level for maize plants to assess the response of maize roots to colonization by AMF under inadequate phosphorus availability. The soil properties are shown in Table 1 below.

Table 1. Hydro-physical and Chemical properties of the Soil used for the Experiment

Physical Properties								
Soil Texture (%)			Textural Class	BD	ρ_s	F		
Sand	Silt	Clay		(Mg m^{-3})	(Mg m^{-3})	(cm^3cm^{-3})		
56.8	28.3	14.9	Sandy Loam	1.440	2.54	0.321		
Hydraulic Properties								
K_s ($cm\ s^{-1}$)	θ_{SC} ($cm^3\ cm^{-3}$)	θ_{FC} ($cm^3\ cm^{-3}$)		Θ_{PWP} ($cm^3\ cm^{-3}$)	PAWC ($cm^3\ cm^{-3}$)			
0.98	0.321	0.270		0.173	0.097			
Chemical Properties								
SOC (g kg^{-1})	pH (H_2O)	pH ($CaCl_2$)		Avail. P (mg kg^{-1})	TN (g kg^{-1})	EC (dS m^{-1})	Exch K (cmol kg^{-1})	Exch Na (cmol kg^{-1})
6.8	6.07	5.63		1.70	1.12	0.4	0.62	0.4

BD = bulk density, ρs = particle density, F = total porosity, Ks = hydraulic conductivity, θSC = moisture at saturation capacity, θ_{FC} = moisture at field capacity, θPWP, moisture at permanent wilting point, PAWC = plant available water capacity, SOC = soil organic carbon, TN = Total nitrogen, Avail. P = available phosphorus, Exch K = exchangeable potassium, Exch Na = Exhangeable exchangeable Sodium, EC = electrical conductivity.

The same quantities of disturbed soils were collected from 6 random points in a 15 m x 15 m plot at 0 - 0.15 m depth. The soils were mixed thoroughly to form a composite sample, after which the homogenized soil was air-dried and sieved through a 4 mm mesh sieve. The 4 mm soil was sterilized at about 110^o c for 90 minutes in an electric sterilizer.

Experimental Design and Procedure

The experiment was laid in a randomized complete block design (RCBD) in the screen house at the Department of Soil Science, Ahmadu Bello University Zaria. The average temperature of the greenhouse ranged between 37^oC – 38.6^oC during the experimentation. The experiment has three factors; Arbuscular Mycorrhiza Fungi (AM) at two levels (inoculated or not-inoculated), Irrigation (I) at two levels (50% FC and 100% FC) and Phosphorus (P) application at three levels (30, 60 and 90 kg P_2O_5 ha^{-1}) making a total of 12 treatment combinations. This was replicated 9 times to give a total of 108 experimental units. The test crop was maize plant (SAMMAZ-16 variety sourced from the IAR seed unit). The sterile 4 mm sieved soil was packed into cylindrical 9 L pots. Each pot held 7 kg of soil repacked at the bulk density of the field (1.44 Mg m^{-3}) which was pre-determined from undisturbed cores collected from the field by the oven-drying method.

For pots with mycorrhiza treatments, 40 g of mycorrhiza fungi inoculum of G. *mosseae* obtained from Soil Microbiology Laboratory, University of Ibadan was mixed with the soil that filled the uppermost 0.08-0.1m layer. The repacked soil was assigned to treatment combinations accordingly. Four seeds per hole were sown and were thinned to two seedlings stand per pot 10 days after germination by removing the weaker seedlings. The same doses of N in form of urea (46% N) and K in form of Muriate of Potash (60% K_2O) were given to each pot

based on the fertilizer recommendation of maize (120:60:60 kg NPK ha^{-1}). Nitrogen was split into 3 parts and applied at sowing, week 2 and week 6 at the rate of 20 kg ha^{-1} per application. Potassium was applied only at sowing. Phosphorus in form of single superphosphate (20% P_2O_5) was applied according to the treatments (i.e. 30, 60 and 90 kg P_2O_5 ha^{-1}). All pots were watered to the moisture at field capacity (FC) of the soil on the first day of sowing and maintained at the soil FC in the first 2 weeks to ensure proper seedling development. The pots were maintained at either 100% FC or 50% FC after the two weeks by applying volume equivalents of water. A moisture probe was used to measure the moisture of the soil (θ) at two depths (0-0.05 m and 0.07-0.12 m) at instants just before any irrigation. The access tube installed from the depth of 7 cm to the surface of the soil allowed for the 0.05 m long sensory prongs of the probe to reach 0.07-0.12 m depth of the soil. Irrigation scheduling was at 3 days intervals with water sourced from boreholes.

Data Collection

Three (3) replicates or 36 experimental units were destructively sampled at 4, 8 and 12 weeks after sowing. The following parameter was measured with the plant and soil samples collected:

Measurement of Plant biomass

Above- and below-ground biomasses were collected by destructive harvests on the 4th, 8th and 12th week, and oven-dried to constant masses. The biomasses were weighed. Root bits were also collected and stored in 50% ethanol in vials at 8 and 12 weeks after sowing for determination of root colonization by AMF.

Percentage Root Colonization

The degree of colonization of roots by AMF was quantified according to the procedure described by Brundrett *et al*. (1994). Washed root bits collected on the 8th and 12th week were cleared with 10% KOH at 90^{o}C for 30 min. The cleared roots were washed under running tap water before the addition of 5% hydrogen peroxide. The root bits were stained with a mixture of Trypan Blue containing lactoglycerol and water for 48 hrs. The stained roots were distained in 50% glycerol to improve stain quality. Percentage root colonization was estimated as follows:

$$RC = \frac{\text{Number of root bits colonized}}{\text{Number of root bits observed}} * 100 \ldots (1)$$

Mycorrhizal Growth Response of Plants (MGR)

Mycorrhizal responsiveness of plants to growth parameters was calculated as thus (Hetrick *et al.*, 1996):

$$MGR = \frac{(\text{parameter of the mycorrhizal plant}) - (\text{parameter non-mycorrhizal plant})}{\text{Mean growth parameter of mycorrhizal plant}} * 100 \ldots\ldots (2)$$

Drought Response Index (DRI)

The drought response index was estimated as thus (Osonubi

et al., 1991)

$$DRI = \frac{\text{Total Weight of plants under water stress condition}}{\text{Total Weight of plants under well-watered condition}} \quad \ldots\ldots (3)$$

Based on the definition in eqn. (3), DRI can assume values in the following range: $0 \leq DRI \leq 1$, such that the higher the value of DRI, the lesser the effect of water stress on the plants at a designated deficit soil moisture regime.

Statistical Analyses

All data collected for each sampling phase were subjected to Analysis of Variance (ANOVA). The means of the determined parameters were compared within sampling

phases using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. Windows-compatible Statistical Analysis System (SAS, 1996) was used for all the statistical analyses.

Results and discussion

Effect of Inoculation, Phosphorus and Irrigation on Root Colonization by AMF

Figures 1 (a-c) show %RC across sampling durations: as influenced by inoculation (1 a); as influenced by phosphorus addition (1 b); and as influenced by irrigation regimes (1 c). The percent root colonization (%RC) due to AMF inoculation was 62.22% at 8 WAS, which is significantly lower than the 71.33% observed at 12 WAS (Figure 1a). These high %RC demonstrates that G. *mosseae* and maize plant (SAMMAZ-16) can establish a functional AM symbiosis in the Alfisols used for this trial. AMF-inoculated plants supplied with 30 and 60 kg P_2O_5 ha^{-1} had statistically similar, but higher %RC at 12 WAS than they do at 8 WAS (Figure 1 b). This can be ascribed to P nutrient deficiency at 30 and 60 kg P_2O_5 ha^{-1} rates likely intensified by higher plant demand for P with increasing growth. It is expected under such circumstances that plants would invest more in arbuscular mycorrhizal fungi proliferation as a mechanism to maximize P uptake. However, when plants were supplied with 90 kg P_2O_5 ha^{-1}, there was no significant difference between %RC of AMF-inoculated plants sampled at 8 WAS and at 12 WAS (Figure 1 b). It is widely known that plants resist colonization by AMF under high phosphorus availability where uptake by roots alone could meet their P demands. This way, the plants could optimize the returns of their investment by shifting resources to developing other structures rather than AMF hyphae which would have little or no impact on their P uptake. The roots of inoculated plants were more severely colonized by AMF at 12 WAS than at 8 WAS in both irrigation regimes (Figure 1 c). Our experiment was a screen house trial with the possibility of more severe and frequent water depletion with increasing plant size, especially under the scheduled 3 days' irrigation during the growth episode. Therefore, it is not unlikely that the plants partitioned more energy into the development of mycorrhizal association in order to scale up water absorption to meet their water requirement as the plants grew bigger. However, this morphological adjustment may sometimes be at the expense of the biomass accumulation of the plants. In a similar study, Bitterlich *et al.* (2018) noticed an increase in %RC with prolonged drought stress in tomatoes. There was no root colonization by AMF in non-inoculated soils.

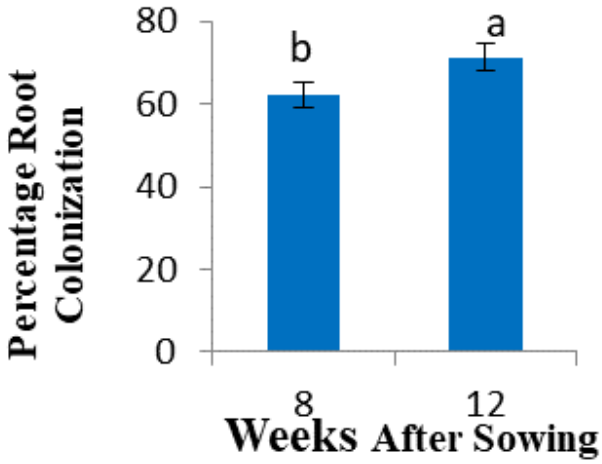


Figure 1. (a) Percentage Root Colonization of AM-roots across sampling times as influenced by inoculation

Means ±SE are shown (t-test, $p < 0.05$, n = 18).

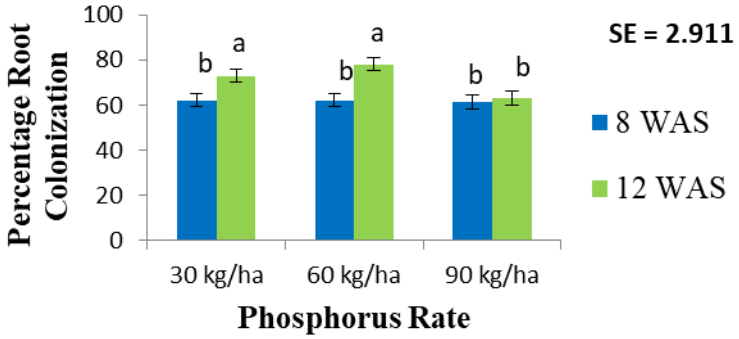


Figure 1. (b) Percent Root colonization by AMF across sampling times as influenced by Phosphorus

Values with the same letter are not significantly different (DMRT, $\alpha = 0.05$, n = 6)

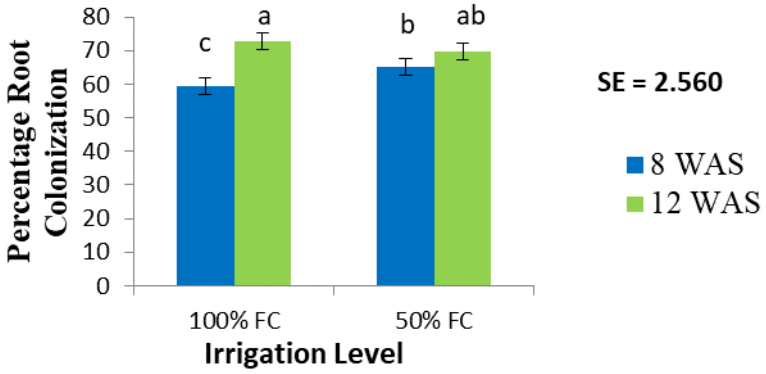


Figure 1. (c) Percentage Root colonization (%RC) by AMF across sampling times as influenced by Irrigation regimes

Values with the same letter(s) across sampling times are not significantly different (DMRT, $\alpha = 0.05$, n = 4).

Effect of AMF inoculation, Phosphorus and Soil Moisture Levels on the Allometric Scaling of the Maize Plants

Dry biomass

Table 2 shows the effects of AMF inoculation, phosphorus and irrigation treatments on plant biomass. A slightly suppressed, though invariable host's growth is noticed at the early stage of development which is captured in the sampling at 4 WAS, pointing to the tendency that AMF was taking more resources than they could supply to the plant symbionts *via* hyphal pathway. However, shoot growth was improved significantly due to the inoculation with AMF at 8 WAS by 11% ($P = 0.022$) and 12 WAS by 22% ($P < 0.001$) despite the growth suppression noticed at 4 WAS. The practical implication of this result is that the aerial growth of G. *mosseae*-inoculated SAMMAZ-16 growing in Alfisols of NGS of Nigeria could be suppressed at an early stage upon their association but may gradually improve with time. AMF inoculation suppressed root growth after 8 WAS as revealed by the examination of the samples conducted at 12 WAS but the negative impact of AMF on the root development translated to the improvement in shoot growth observed in the sampling at 12 WAS. This would have been a paradox except that the function of the roots was substituted by the fungal hyphae which appear to have played a better role than the replaced roots in the shoot development. On the premise that plants would allocate more resources to the structures that acquire their most limiting growth materials (Zheng *et al.*, 2015), it is

logical to infer that the presence of AMF might have prompted the plants to invest more resources in developing hyphae mass rather than the root mass in consonant with the availability of the resources during the plants' growth.

Table 2. Effect of AMF, Phosphorus and Irrigation on Biomass Accumulation and Water Use Efficiency

	Dry shoot mass (g pot^{-1})				Dry root mass (g pot^{-1})		
Treatment	4 WAS	8 WAS	12 WAS		4 WAS	8 WAS	12 WAS
Mycorrhiza (AM)							
AM^+	3.60	23.99a	40.83a		1.104	5.69	10.41b
AM^-	4.39	21.52b	33.48b		1.187	5.57	12.33a
LOS	NS	*	***		Ns	Ns	*
SE±	0.33	0.71	1.08		0.13	0.38	0.60
Phosphorus (P)							
30 kg P_2O_5 ha^{-1}	2.56c	17.62c	27.31b		0.95	3.87b	7.07b
60 kg P_2O_5 ha^{-1}	3.97b	24.03b	40.71a		1.06	5.98a	13.41a
90 kg P_2O_5 ha^{-1}	5.46a	26.62a	43.43a		1.42	7.05a	13.65a
LOS	***	***	***		Ns	***	***
SE±	0.402	0.871	1.280		0.15	0.46	0.72
Irrigation (I)							
100% FC	-	28.04a	43.56a		-	6.33a	12.09
50% FC	-	17.47b	30.74b		-	4.94b	10.66
LOS		***	***		-	*	Ns
SE±	-	0.71	1.04		-	0.38	0.59
AM x P	NS	*	**		NS	NS	NS
AM x I	-	***	*		-	NS	NS
P x I	-	NS	NS		-	NS	NS
AM x P x I	-	*	NS		-	NS	NS

Means followed by the same letter(s) within the same column are not significantly different at 0.05 level of probability as determined by DMRT, * = Significant at 0.05 level of probability, ** = Significant at 0.01 level of probability, *** = Significant at 0.001 level of probability NS = Not significant at 0.05 level of probability, MWD = Mean weight diameter, SE = Standard Error, WAS = weeks after sowing.

As expected, the incremental P_2O_5 application rate significantly improved shoot and root growth progressively, across the sampling times except for root growth at 4 WAS. Conversely, irrigating the samples at half of the moisture at field capacity reduced the shoot and root mass across the sampling phases except for the root mass at 12 WAS which was not affected. In this study, variation in irrigation was introduced after 4 WAS. If root mass can be reduced by AMF under water stress as suggested by Zheng *et al.* (2015), one may argue that a statistically significant reduction in belowground biomass by AMF was not detected at 4 and 8 WAS but was obvious at 12 WAS because of the time frame.

The interaction between AMF inoculation, phosphorus application rates, and irrigation regimes on shoot mass was significant at 8

WAS (Figure 2). For samples under 100% FC, AMF-inoculated plants had significantly higher dry shoot mass than the non-inoculated counterparts in the 30 and 90 kg P_2O_5 ha^{-1} applications. In the 100% FC-irrigated category, 30 kg P_2O_5 ha^{-1} application to AMF-inoculated plants produced the same shoot biomass as 60 kg P_2O_5 ha^{-1} application (whether or not they were inoculated) and 90 kg P_2O_5 ha^{-1} application to non-inoculated plants. When samples were maintained at 50% FC, AMF-inoculated plants produced statistically similar shoot mass to non-inoculated plants in all the levels of P_2O_5 application although shoot mass generally increased significantly with an increase in the P_2O_5 application level. There was a significant interaction between AMF inoculation and phosphorus treatment on dry shoot mass at 12 WAS (P = 0.002) (Figure 3). AMF-inoculated plants consistently had higher shoot mass than non-inoculated plants for any designated P_2O_5 level. The difference in shoot mass between AMF-inoculated and the non-inoculated counterpart was more pronounced in samples that received 60 kg P_2O_5 ha^{-1} application. The shoot mass of AMF-inoculated plants that received 30 kg P_2O_5 ha^{-1}, and that of non-inoculated plants that received 60 kg P_2O_5 ha^{-1} were statistically similar. However, AMF-inoculated plants that were supplied with 60 kg P_2O_5 ha^{-1} had significantly higher shoot mass than the plants that were supplied with 90 kg P_2O_5 ha^{-1} regardless of whether or not they were inoculated. AMF-inoculated plants accumulated higher dry shoot mass than non-inoculated plants in the samples that were irrigated at 100% FC at 12 WAS (Figure 4). Also, the samples that were irrigated at 100% FC had significantly higher shoot mass than those maintained at 50% FC in both inoculated and non-inoculated groups.

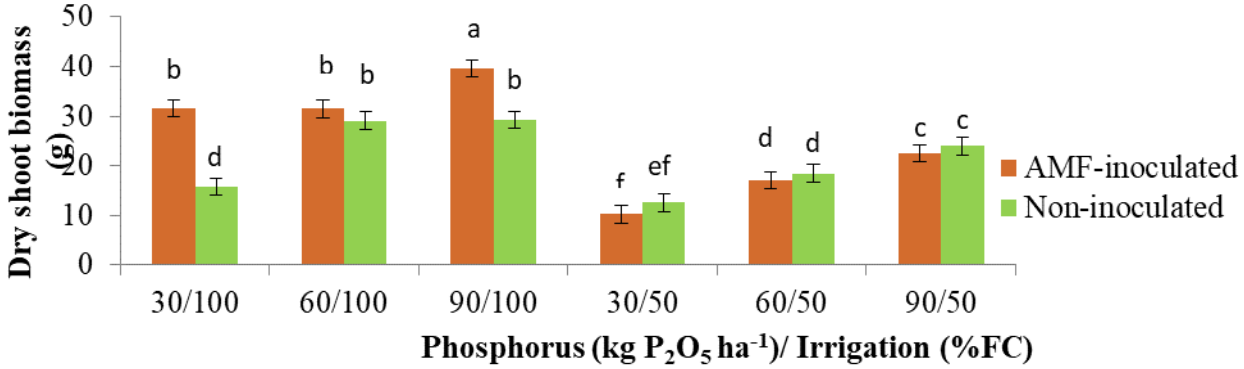


Figure 2. Interaction of AMF, phosphorus and irrigation on dry shoot biomass of plants at 8 WAS

Means (±SE) are shown and values with the same letter(s) are not significantly different (three-way ANOVA, DMRT, P < 0.05, n = 36).

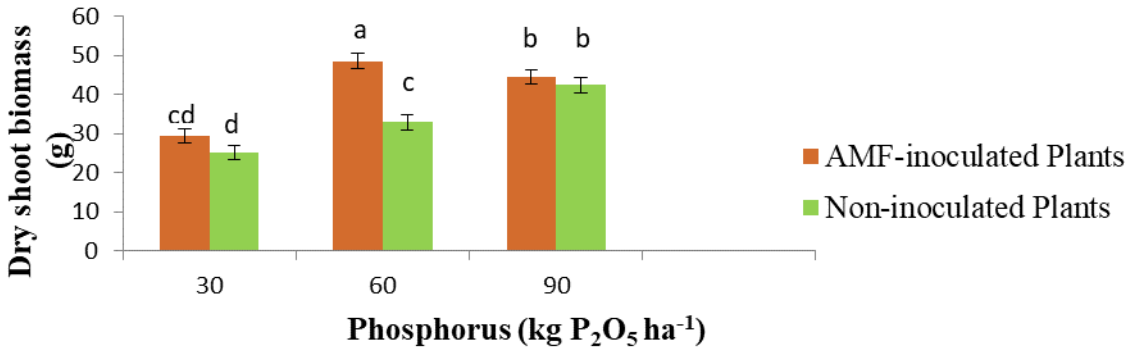


Figure 3. Interaction of AMF and phosphorus on dry shoot biomass of plants at 12 WAS

Means (±SE) are shown and values with the same letter(s) are not significantly different (two-way ANOVA, DMRT, P < 0.05, n = 36)

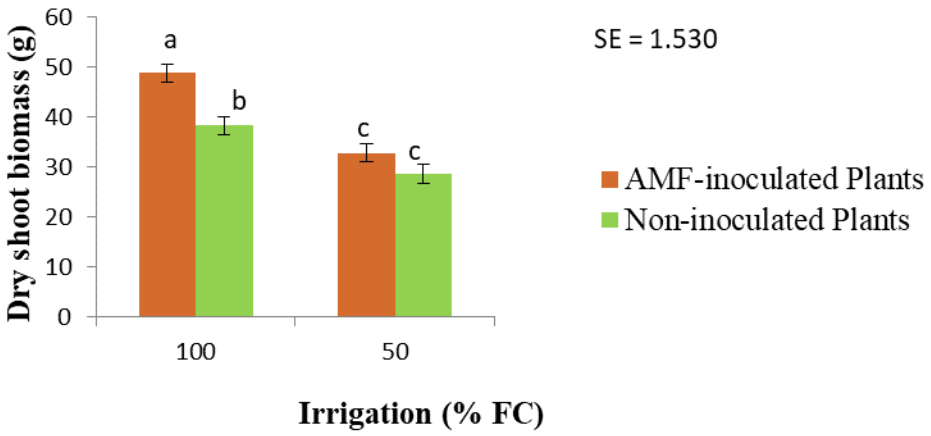


Figure 4. Interaction of AMF and Irrigation on Dry shoot biomass (g pot^{-1}) at 12 WAS

Means (±SE) are shown and values with the same letter are not significantly different (two-way ANOVA, DMRT, P < 0.05, n = 36).

The similar or even higher performance of the dry shoot biomass of the AMF-inoculated plants that received 50% lower doses of P_2O_5 compared to the non-inoculated plants under the amply watered condition in this study highlights the dual economic and ecosystem benefit of AMF inoculation. It indicates that not only would a 50% reduction in P fertilizer application in Agricultural production systems be feasible if AMF is used to complement the resource uptake of plants, but that environmental degradation associated with P application can be avoided or mitigated.

It is apparent that AMF became less responsive, mostly detrimental, to the growth of the plants at the water stress level. One reason for a reduction in biomass accumulation in plants is a reduction in stomatal conductance at the leaf surface to cope with the current water status of the soil (Blum, 2009). Even though plants showed higher root colonization at 12 WAS or in the 50% FC – irrigated plants at 8 WAS (Figure 1 c), the presence of AMF did not garner any benefit to the plants under this water-limiting condition (Figure 2); rather, it tended to depress the shoot growth. Veiga *et al*. (2011) observed that highly colonized plants showed a high degree of negative mycorrhizal responsiveness in a similar study. It has been proposed that interaction between environmental and growing conditions and the symbionts involved in AM symbiosis can shift the association along a parasitism-mutualism continuum (Johnson *et al.*, 1997).

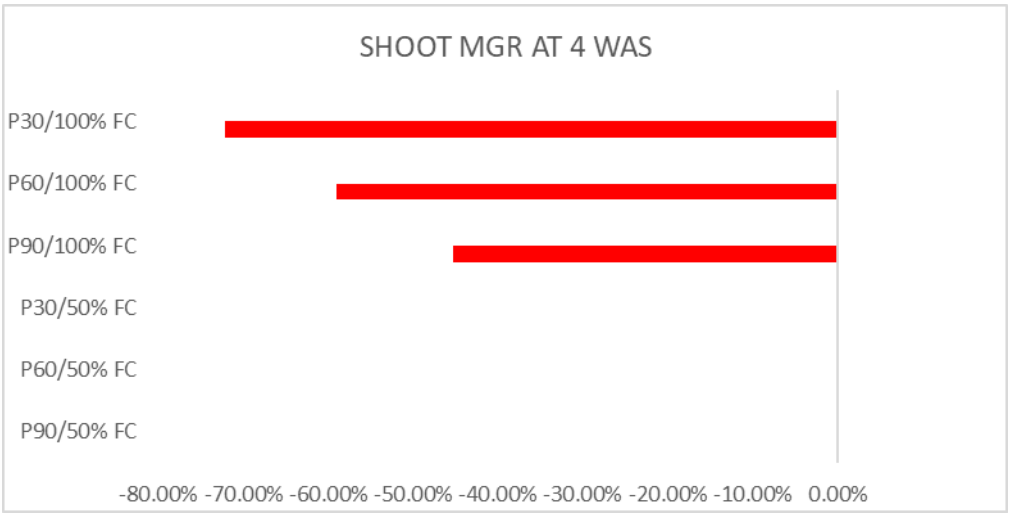
Mycorrhizal Responsiveness to Biomass Accumulation as Influenced by Irrigation

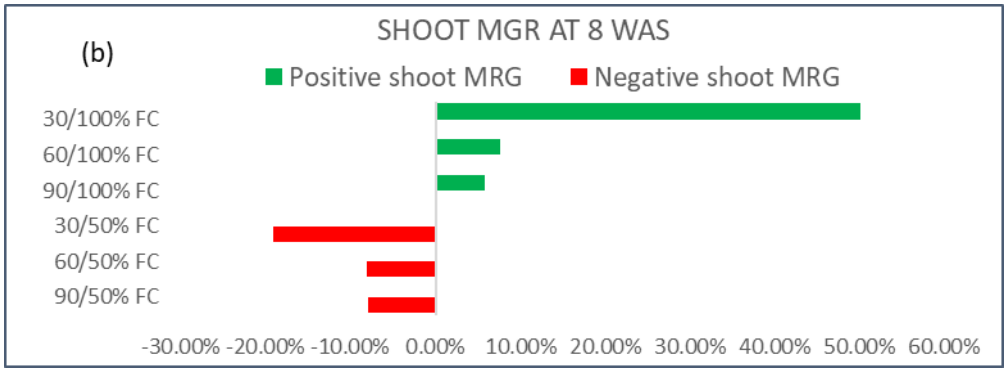
Mycorrhizal growth response (MGR) to biomass accumulation expressed in percentage is shown in Figure 5. The shoot mass response to mycorrhizal inoculation was negative in all application rates of P_2O_5 at 4 WAS, with -72, -59 and -45% in the experimental units that received 30, 60 and 90 kg P_2O_5 ha^{-1} respectively (Figure 5 a). At 8 WAS, the shoot mass of the samples that were irrigated at 100% FC of the growing media expressed a positive response to mycorrhizal inoculation in all P_2O_5 rates while those that grew on media maintained at 50% FC showed negative mycorrhizal responsiveness to shoot mass in all the P_2O_5 rates (Figure 5 b). The shoot responses at 8 WAS were 50.05% in the application of 30 kg P_2O_5 ha^{-1}, 7.62% in the application of 60 kg ha^{-1} and 5.81% in the application of 90 kg P_2O_5 ha^{-1} for plants that were amply watered at 100% FC while the experimental units that were susceptible to water stress indicated responsiveness to shoot mass of about -19% in 30 kg P_2O_5 ha^{-1} application rate and 8% in 60 and 90 kg P_2O_5 ha^{-1} application rates. In the amply watered category, shoot mass showed a positive

mycorrhizal response of 17.9%, 35.7% and 8.0% in the application rates of 30, 60 and 90 kg P_2O_5 ha^{-1} respectively, at 12 WAS (Figure 5 c). The responsiveness of shoot mass to mycorrhizal inoculation, however, showed a downward negative trend of -26.9%, -9.88% and -0.16% in the application rates of 30, 60 and 90 kg P_2O_5 ha^{-1} respectively, for samples that were maintained at 50% of the soil moisture at field capacity at this sampling stage.

At 4 WAS, the mycorrhizal responsiveness of plants to root mass was negative in all the designated P_2O_5 rates (Figure 5 d). However, an increase in the application rate of P_2O_5 tended to decrease the negativity of the root response to the AMF inoculation at the 4 WAS. At 8 WAS, the plants that were watered at 100% FC had a positive mycorrhizal response to root mass in all the levels of P_2O_5 application and the magnitude increased with a decrease in P_2O_5 rate i.e., the negativity increased with an increase in P_2O_5 rate (Figure 6e). For plants in 50% FC group, the responses of the root to the mycorrhizal inoculation were negative across the P_2O_5 application rates. The roots of the plants showed a negative mycorrhizal response at 12 WAS in all P_2O_5 rates regardless of the irrigation status except in fully irrigated plants in 90 kg P_2O_5 ha^{-1} application where mycorrhizal responsiveness to root mass was slightly positive (Figure 6 f). The negative response of the mycorrhizal association on root mass was particularly noticeable in the 30 kg P_2O_5 ha^{-1} application rate under amply watered condition and also, increasing P_2O_5 application rate generally improved the shoot growth response to the AMF inoculation at this sampling stage

.





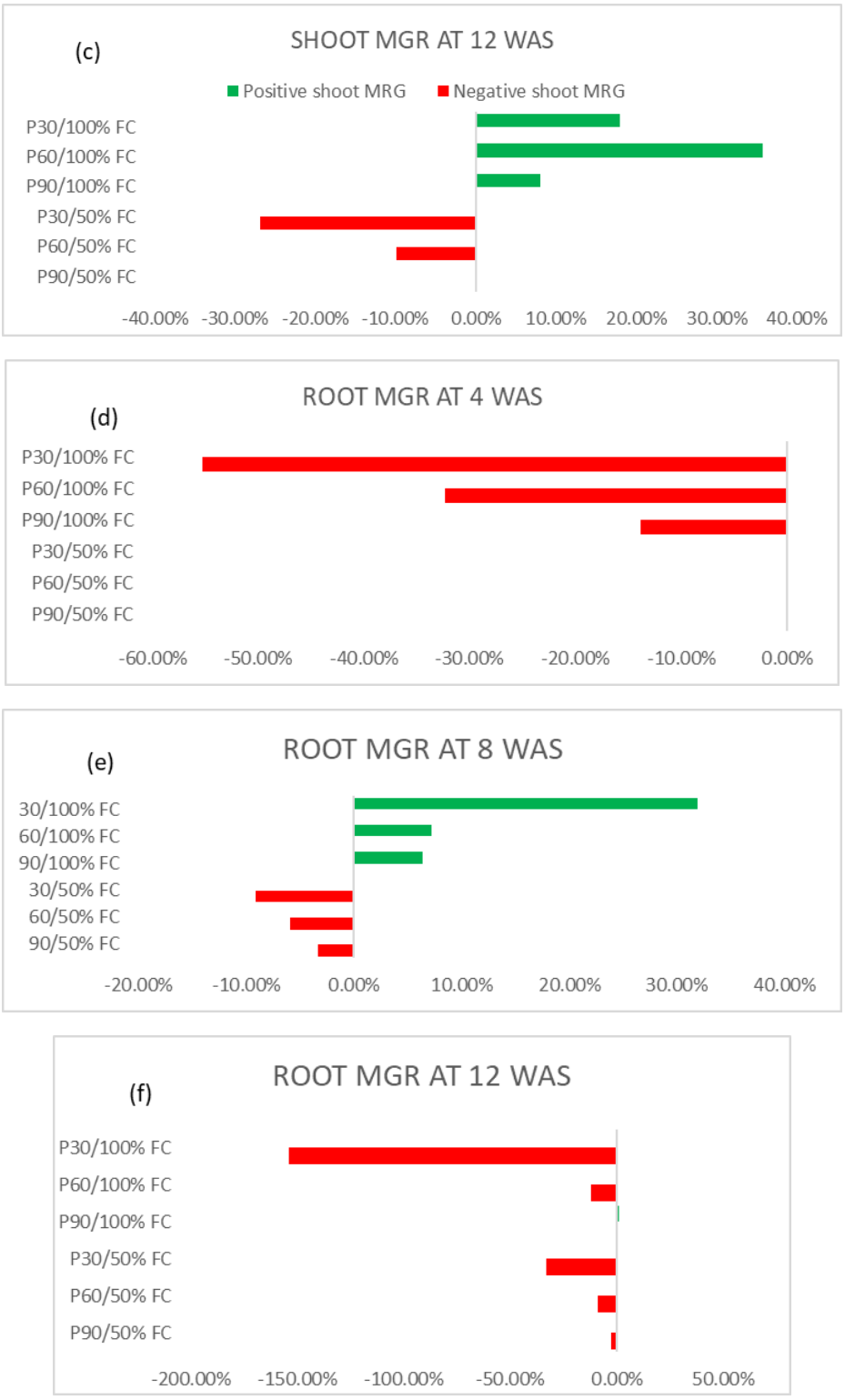


Figure 5. Mycorrhizal growth responses (%) of the (a) shoot mass at 4 WAS, (b) shoot mass at 8 WAS, (c) shoot mass at 12 WAS, (d) root mass at 4 WAS, (e) root mass at 8 WAS and (f) root mass at 12 WAS in the three phosphorus application rates (30, 60 and 90 kg P2O5 ha-1) under the two moisture regimes (100 and 50% FC). Bars are means of three replicates. Bars in green indicate samples with a positive mycorrhizal growth response while bars in red indicate samples with a negative mycorrhizal growth response. Asterisks represent significant differences (alpha = 0.05) in total biomass between the AMF-inoculated treatment and the non-inoculated control for each phosphorus application rate

Generally, the mycorrhizal responsiveness became less negative in 50% FC-irrigated samples as higher doses of P_2O_5 were given to the plants probably due to the role of phosphorus in root development which is key to moisture extraction from soils. Specifically, decreasing the phosphorus application rate increased the negative mycorrhizal response of the plants only at the early stage of growth (week 4) or in samples that were maintained at 50% FC at 8 WAS. This suggests that AMF hyphae were not compensating for the carbon cost provided by the maize plants for hyphal development at the initial stage of the association, and even when AMF became functionally useful to the maize plants at the later stage, the enhanced root colonization was detrimental under water deficit. This indicates an alternating parasitism-mutualism continuum along the growth cycle of the association depending on the prevailing status of the growth resources. Wang *et al.,* (2018) provided evidence of the possibility of parasitism in a mycorrhizal association. They noticed a lower N acquisition and subsequent lower grain yield in AMF-colonized maize compared to the non-colonized counterparts grown in N-deficient soil. Although the level of root colonization at week 4 was not measured, we supposed that the level of colonization of the roots by AMF would have the same trend as was observed at 8 and 12 WAS; relatively higher percent root colonization was noticed with decreasing doses of phosphorus application rate.

Drought Response Index of plants (DRI) and percentage reduction in yield due to water stress

AMF-inoculated plants had significantly lower DRI than non-inoculated plants at 8 WAS (Table 3). The lowest DRI was observed in the AMF-inoculated plants that received 30 kg P_2O_5 ha^{-1} while the highest DRI was observed in non-inoculated plants that received 30 kg P_2O_5 ha^{-1} or 90 kg P_2O_5 ha^{-1} (Figure 6). Table 4 shows the percentages by which the total biomass of plants was reduced under the designated P_2O_5 rates due to their exposure to water stress when the samples were irrigated at 50% FC. The total biomass reduction ranged between 11 – 59%. The water stress condition in the AMF-inoculated maize plants resulted in a yield reduction of 59, 43 and 31% at 8 WAS, and 22, 26 and 32% at 12 WAS in 30, 60 and 90 kg P_2O_5 ha^{-1} application rates respectively. The detrimental effect of AMF inoculation on the biomass of the plants signifies a possible deficit imbalance on the return of the carbon investment of the plant in the mycorrhizal association under the water stress condition. However, higher phosphorus application tended to play an ameliorative role in the adaptation of the inoculated plants to the water stress. It was suggested that phosphorus nutrients can improve the drought resistance of plants through various mechanisms including an improved root system that facilitates water uptake, and up-regulation of osmolytes and antioxidants (Begum *et al*., 2020). However, in the non-inoculated plants, the highest yield reduction was observed in plants supplied with 60 kg P_2O_5 ha^{-1} at 8WAS (34%), and in plants supplied with 30 kg P_2O_5 ha^{-1} at 12 WAS (33%) although no particular trend was noticeable. The non-inoculated plants may have shown a non-specific trend in yield reduction because the plants were largely deficient in P nutrient even at the highest phosphorus application rate (90 P_2O_5 ha^{-1}) since there was an absence of hyphae to complement the P uptake. The soil used for this experiment was highly deficient in P nutrient (1.7 mg kg^{-1}).

Table 3. Effect of inoculation and phosphorus on drought response index (DRI)

Treatment	8 WAS	12 WAS
Mycorrhiza (AM)		
AM^+	0.56a	0.72
AM^-	0.79b	0.77
LOS	***	NS
SE±	0.036	0.035
Phosphorus (P)		
30 kg P_2O_5 ha^{-1}	0.65	0.68
60 kg P_2O_5 ha^{-1}	0.62	0.79
90 kg P_2O_5 ha^{-1}	0.76	0.76
LOS	NS	NS
SE±	0.045	0.043
AM x P	*	NS

Lower values are ranked higher to indicate the severity of the effect of water stress with decreasing DRI. Values with different letters within the same column are significantly different (DMRT, α = 0.05, n = 6).

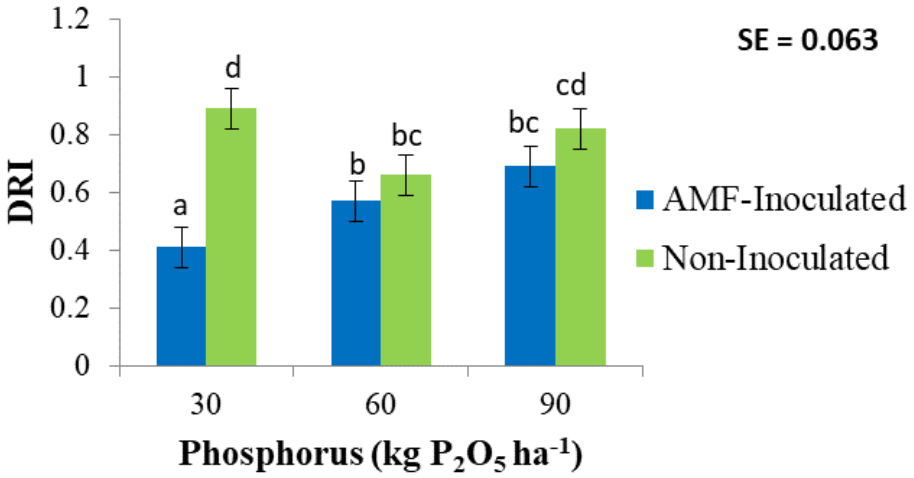


Figure 6. Interaction of AMF inoculation and phosphorus levels on drought response index (DRI) at 8 WAS

Lower DRI values are ranked higher to indicate the severity of the effect of water stress with decreasing DRI. Values with the same letter(s) are not significantly different (DMRT, α = 0.05, n = 6)

Table 4. Percentage reduction in total biomass due to 50% water stress

Phosphorus (kg P_2O_5 ha^{-1})	8 WAS			12 WAS	
	Inoculation Status			Inoculation Status	
	AM^+	AM^-		AM^+	AM^-
30	59	11		32	33
60	43	34		26	16
90	31	17		22	21

Conclusion

We assessed the root colonization percentage and the biomass partitioning of AMF-inoculated maize crops under phosphorus and water variations in time scale and found that the maize crop exhibited both positive and negative responses to mycorrhizal inoculation. Phosphorus and moisture deficiency encouraged root colonization by AMF. Although AMF suppressed the growth of the maize at 4 WAS, it enhanced biomass accumulation at the later stages as revealed by the examinations carried out at 8 and 12 WAS. While the exposure of the plants to water stress increased the chance of negative mycorrhizal responsiveness of the crops to biomass accumulation, higher phosphorus application rates alleviate this negative response. The AMF-inoculated plants that received 50% lower doses of P produced similar or in some cases, higher dry shoot biomass compared to the non-inoculated counterparts under the amply watered condition. The mixed responses of the crops to the AMF inoculation under phosphorus nutrient and soil moisture variations present the need for caution in harnessing the benefits of AMF in maize production in the NGS of Nigeria. As demonstrated by the results of this study, AMF inoculation would rather be detrimental to SAMMAZ-16 maize variety for example, when there is a concurrent shortage of phosphorus nutrients and soil moisture. It is, however, not very clear in this study how the combined factor effects can garner benefits or costs for the AMF-inoculated crop. Further research should explore the possibility of complementarity, additivity, antagony or synergy in multi-factor effects on the AMF-inoculated crops by involving molecular and biochemical assays. That way, the combined effects of abiotic stress on the responses of SAMMAZ-16 would be demystified.

Conflict of interest

Regarding the publication of this manuscript, the authors declare that there are no conflicts of interest.

Acknowledgement

The authors appreciate Mr. Shehu Al-hassan who is a technical staff of the Department of Soil Science, Ahmadu Bello University Zaria, for the soil sample collected from the field and its preparation before the setting up of this experiment. The benevolent effort of Mrs Juan Mutuah, the technitian who supervised the determination of root colonization by AMF in the Soil Microbiology Laboratory at Ahmadu Bello University is well acknowledged.

References

Aliyu, I. A., Yusuf, A. A., Uyovbisere, E. O., Masso, C. and Sanders, I. R. (2019). Effect of co-application ofphosphorus fertilizer and in vitro-produced mycorrhizal fungal inoculants on yield and leaf nutrient concentration of cassava. *PLoS ONE 14* (6): e0218969. https://doi.org/10.1371/journal.pone.0218969

Begum, N., Ahanger, M. A., & Zhang, L. (2020). AMF inoculation and phosphorus supplementation alleviates drought induced growth and photosynthetic decline in Nicotiana tabacum by up-regulating antioxidant metabolism and osmolyte accumulation. *Environmental and Experimental Botany*, *176*, 104088. https://doi.org/10.1016/j.envexpbot.2020.104088

Bitterlich, M., Sandmann, M. and Graefe, J. (2018) Arbuscular Mycorrhiza Alleviates Restrictions to Substrate Water Flow and Delays Transpiration Limitation to Stronger Drought in Tomato. *Front. Plant Sci. 9*:154. https://doi.org/10.3389/fpls.2018.00154

Blum, A. (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield

improvement under drought stress. *Field Crop Research, 112:* 119-123.

Brundrett, M., Melville, L. and Peterson, L. (1994). Practical Methods in Mycorrhizal Research. *Mycologue Publications Ltd*, Waterloo* (*see Kendrick, 1992)

Cheng, H. Q., Giri, B., Wu, Q. S., Zou, Y. N., and Kuča, K. (2022). Arbuscular mycorrhizal fungi mitigate drought stress in citrus by modulating root microenvironment. *Archives of Agronomy and Soil Science*, *68*(9), 1217-1228.

Chu, Q., X. Wang, Y. Yang,F. Chen, F. Zhang and G. Feng. (2013). Mycorrhizal responsiveness of maize (*Zea mays* L.) genotypes as related to releasing date and available P content in soil. *Mycorrhiza, 23*:497-505. https://doi.org/10.1007/s00572-013-0492-0

Hetrick, B.A.D, Wilson, G.W.T., Todd, T.C. (1996). Mycorrhizal response in wheat cultivars: relationship to phosphorus. *Canadian Journal of Botany, 74*: 19–25.

Ishaq, L., Tae, A. A., Airthur, M. A., and Bako, P. O. (2021). Effect of single and mixed inoculation of arbuscular mycorrhizal fungi and phosphorus fertilizer application on corn growth in calcareous soil. *Biodiversitas Journal of Biological Diversity*, *22*(4), 1920-1926. https://doi.org/10.13057/biodiv/d220439

Johnson, N. C., Graham, J. H. and Smith, F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist, 135*: 575–586

Li, M., and Cai, L. (2021). Biochar and Arbuscular mycorrhizal fungi play different roles in enabling maize to uptake phosphorus. *Sustainability*, *13*(6), 3244. https://doi.org/10.3390/su13063244

Osonubi, O., Mulongoy, K., Awotoye, O.O. et al. (1991). Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. *Plant Soil*, 136, 131–143. https://doi.org/10.1007/BF02465228

Pyšek, P., Guo, W. Y., Stajerova, K., Moora, M., Bueno, C. G., Dawson, W., Essl, F., Gerz, M., Kreft, H., Pergl, J., van Kleunen, M., Weigelt, P., Winter, M., and Zobel, M. (2019). Facultative mycorrhizal associations promote plant naturalization worldwide. Ecosphere 10(11), e02937. 10.1002/ecs2.2937

Qi, S., Wang, J., Wan, L., Dai, Z., da Silva Matos, D. M., Du, D., ... and Moles, A. T. (2022). Arbuscular mycorrhizal fungi contribute to phosphorous uptake and allocation strategies of Solidago canadensis in a phosphorous-deficient environment. *Frontiers in plant science*, *13*.

Säle, V., Sieverding, E., & Oehl, F. (2022). Growth responses of three European weeds on different AMF species during early development. *Plants*, *11*(15), 2020. https://doi.org/10.3390/plants11152020

SAS Institute, Inc., (1996). Statistical Analytical Package for Windows. SAS Institute, Cary: NE, USA.

Smith, S. E. and Read, D. J. (2008). *Mycorrhizal Symbiosis,* Ed. 3. Academic Press, New York

Tchabi, A., Coyne, D., Hountondji, F., Lawouin, L., Wiemken A. and Oehl, F. (2008). Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. *Mycorrhiza, 18*: 181-195.

Uyovbisere, E. O., Chude, V. O. and Bationo, A. (2000). Promising nutrient ratio fertilizer formulations for optimal performance of maize in Nigeria Savannah: The need for review of current

recommendations. *Nigerian Journal of Soil Research, 1:*29-34.

Veiga, R. S. L., Jansa, J., Frossard, E., and van der Heijden, M. G. A. (2011) Can Arbuscular Mycorrhizal Fungi Reduce the Growth of Agricultural Weeds? *PLoS ONE 6* (12):e27825. https://doi.org/10.1371/journal.pone.0027825

Vierheilig, H., Schweigerb, P. and Brundrett, M. (2005). An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum 125*: 393–404. https://doi.org/10.1111/j.1399-3054.2005.00564.x

Walder, F. (2014). *Common Arbuscular Mycorrhizal Networks: Trade of Carbon and Soil Nutrients between different Plant Species and their shared Fungal Symbiont*. (Doctoral dissertation, Botanical Institute, University of Basel).

Yin, Z., Zhang, Y., Hu, N., Shi, Y., Li, T., and Zhao, Z. (2021). Differential responses of 23 maize cultivar seedlings to an arbuscular mycorrhizal fungus when grown in a metal-polluted soil. *Science of The Total Environment*, *789*, 148015. https://doi.org/10.1016/j.scitotenv.2021.148015

Zheng, C., Ji, B., Zhang, J., Zhang, F., & Bever, J. D. (2015). Shading decreases plant carbon preferential allocation towards the most beneficial mycorrhizal mutualist. *New Phytologist*, *205*(1), 361-368. https://doi.org/10.1111/nph.13025