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Morpho-Physiological Responses of *Abelmoschus esculentus* (L.) Moench to Arbuscular Mycorrhizal Fungi Inoculation under Drought Stress

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Authors' contributions

This work was carried out in collaboration among all authors. Author OOG designed the study, wrote the protocol and wrote the first and final draft of the manuscript. Author UPP managed the analyses of the study. Author NEM managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess the potential impacts of arbuscular mycorrhizal fungi (AMF) (*G. geosporum*) inoculation on the survival of *A. esculentus* under drought stress.
Study Design: This experiment was set up in a completely randomized design (CRD) with all treatments replicated thrice. This gave a total of 7 treatments, 21 replicates.
Place and Duration of Study: The experimental soil used for this study was collected from the Botanical Garden of the Department of Biological Sciences, Ritman University (Latitude 5º11'44ºN and Longitude 7º42'12ºE), Akwa Ibom State, Nigeria. All analysis was carried out in Soil Science Laboratory and Botany Laboratory, Akwa Ibom State University, between January and march 2019.
Methodology: Soil samples were analyzed following the standard procedures outlined for wet acid digestions. Growth parameters were determined using standard methods. At Leaf chlorophyll meter

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was employed in the assessment of the photosynthetic pigments of the experimental plant. Biomass yield were calculated using standard formulas.
Results: The physicochemical analysis of the experimental soil used in this study revealed the physical and chemical properties of the soil; pH (6.12), EC (0.06dS/m), organic matter (2.90%), Av. P (44.62 mg/kg) and textural class of the soil was described as loamy sandy soil. Shoot length, petiole length, internode length, number of leaves and leaf area as well as the total photosynthetic pigments (TPP) contents of *A. esculentus* were significantly (*P* =.05) reduced (from 38.77±3.01 mg/kg to 29.83±1.89 mg/kg) by drought stress. There was also significantly (*P* = .05) reduction in N, P, K, Ca and Mg composition of *A. esculentus* as well as its biomass yield. However, the inoculation of *A. esculentus* roots with AMF (*G. geosporum*) in this study through several morphological and physiological processes exhibited remarkable improvement in growth morphology, total photosynthetic pigments, macronutrients composition as well as biomass yield.
Conclusion: The results of this work have shown that AMF can enhance the ability of *A. esculentus* to resist drought stress possibly through some morphological and physiological changes which improves water and nutrients uptake.

Keywords: Abelmoschus esculentus; drought; fungus; Glomus geosporum; mycorrhiza; stress.

1. INTRODUCTION

Amongst the world's abiotic stresses, drought or water deficit is regarded as one of the most serious abiotic stress that accounts for serious limitations in the growth of plants as well as total crop productivity in several agricultural regions of the world. Certain estimates have it that about one-third of agriculturally viable soils are severely affected by drought stress [1]. Many factors account for water deficit; including irregularity in rainfall dispersal, total lack of rainfall, the degree and duration of drought and the progression rate of stress [2].

Water deficit usually results in lowering the soil water potential thereby prompting cell dehydration, eventually causing inhibition in cell expansion and division, stem elongation, root proliferation, leaf size, upsetting stomatal oscillations, plant water and nutrient uptake, as well as water use efficiency [3]. As a result of drought stress, plants tend to develop sophisticated and multifarious machineries in physiological, morphological as well as biochemical physiognomies apportioning into either drought avoidance, escape or tolerance, to cope with water deficit [4].

Fig. 1. Picture of *Abelmoschus esculentus*

Beneficial microorganisms for example arbuscular mycorrhizal fungi (AMF) can colonize plants even at their natural habitat. Plant tolerance under stressed conditions as well as crop yield can be improved by inoculation of such plants with useful bacteria and fungi [5]. It is estimated that about 80% of land-dwelling plant species roots form association with arbuscular mycorrhizal fungi [6]. Many researchers have shown that arbuscular mycorrhizal fungi promote plant growth under drought stressed conditions. Several studies showed that AMF symbiosis is able to improve drought tolerance of plants [7,8].

AMF *Glomus geosporum* belongs to the family *Glomeraceae*. Spores of *G. geosporum* are formed singly in the soil; yellow (3A8) to orange (5B8); globose to subglobose; (130) 175 (260) µm diameter; sometimes ovoid; 130-150 x 220-260 µm; with a single subtending hypha. The mycorrhizae of *G. Geosporum* consisted of arbuscules, vesicles, as well as intra- and extra radical hyphae. The arbuscules and vesicles were patchily distributed along the roots examined [9].

Abelmoschus esculentus also known as okra belongs to the family Malvaceae. It is cultivated all over Nigeria where the immature fruits are cut into pieces and used as vegetables in soup (okra soup) to which they give a slimy texture. The leaves and pods are the edible portions, preferably the young pods. Therefore, the objective of this work was to assess the influences of AMF (*G. geosporum*) inoculation on the survival of *A. esculentus* under drought stress.

2. MATERIALS AND METHODS

2.1 Experimental Soil Sampling Site

The experimental soil used for this study was collected from the Botanical Garden of the Department of Biological Sciences, Ritman University (Latitude 5º11'44º N and Longitude 7º42'12º E), Akwa Ibom State, Nigeria, with an annual rainfall of about 4021 mm and mean temperature variation of 22 – 31^{o}C [10].

2.2 Experimental Setup

This experiment was set up in a completely randomized design (CRD) with all treatments replicated thrice. This gave a total of 7 treatments, 21 replicates (Table 2.1).

Table 2.1. Experimental setup design

Treatment	Explanation
Control	Watered daily, uninoculated
OAW	Watered once a week
OTW	Watered once in 2 weeks
O3W	Watered once in 3 weeks
OAW+AMF	Watered once a week + *G. geosporum*
OTW+AMF	Watered once in 2 weeks + *G. geosporum*
O3W+AMF	Watered once in 3 weeks + *G. geosporum*

2.3 Soil Sterilization and Planting

The experimental soil was steam sterilized in the oven in bits for two hours at 100^{o}C to kill weed seeds and soil microorganisms and sieved through a 2 mm mesh to remove pebbles. AMF (*G. geosporum*) was inoculated by placing about 25 g of soil/root fragments containing about 60 – 65 spores per 5 g in planting hole at 15 cm depth, before planting the *A. esculentus.*

2.4 Physicochemical Analysis of Experimental Soils

The experimental soil samples were analyzed following the standard procedures outlined by the Association of Official Analytical Chemist [11] procedure for wet acid digestions.

2.5 Growth Morphological Parameters

The shoot length, leaf area and nodes of healthy leaves from the experimental plants were taken from 2 weeks after sprouting (WAS) using standard methods.

2.6 Determination of Photosynthetic Pigments

The atLeaf handheld chlorophyll meter was used for non-destructive estimation of the total photosynthetic estimation.

2.7 Determination of Mineral Contents

The N, P, K, Ca and Mg contents of *A. esculentus* were analyzed following the standard procedures outlined by the Association of Official Analytical Chemist [11].

2.8 Determination of Biomass Yield

Values of fresh weight, leaf fresh weight, leaf turgid weight, root length, total fresh weight and

total dry weight were all determined using standard methods.

2.9 Statistical Analysis

All data in the present study were subjected to analysis of variance (ANOVA) using Statistical package for Social Sciences and data are presented as standard error of mean (± S.E.M.) of triplicate experiments. The differences between the means were separated and compared using the Duncan's multiple range tests. However, a probability level of P =.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The physicochemical analysis of the experimental soil used in this study revealed the physical and chemical properties of the soil (Table 3.1). The textural class of the soil was described as loamy sandy soil.

Results obtained from this research showed that shoot length, petiole length, internode length, number of leaves and leaf area of *A. esculentus* were all significantly (P =.05) negatively by drought stress (Fig. 3.1; Tables 3.2 – 3.5). Several researchers have corroborated the findings of this study. It has been reported that inoculation with *Glomus* species showed 1.99, 1.95, and 1.80 times higher biomass of mung bean than non-AMF treatment under 12% soil water content conditions [12]. Also, Sánchez-Díaz and Honrubia [13]; Zou et al. [14] all reported that inoculation with AMF significantly (P=.05) enhanced plant growth under drought stress than under well watered conditions.

Table 3.1. Physicochemical properties of the soil

S/N	Parameters	Values
1	Sand (%)	84.34
2	Silt (%)	4.32
3	Clay	11.34
4	Textural Class	Loamy sand soil
5	pH	6.12
6	Electrical Conductivity (ds/m)	0.06
7	Organic Matter (%)	2.90
8	Total Nitrogen (%)	0.07
9	Available Phosphorus (mg/kg)	44.62
10	Calcium (cmol/kg)	8.32
11	Magnesium (cmol/kg)	2.77
12	Na (cmol/kg)	0.06
13	K (cmol/kg)	0.14
14	EA (cmol/kg)	2.10
15	ECEC (cmol/kg)	13.37
16	Base Saturation (%)	84.29

Na = Sodium, K = Potassium, EA = Exchange Acidity, ECEC = Effective Cation Exchange Capacity, AV.P = Available Phosphorus, EC = Electrical Conductivity

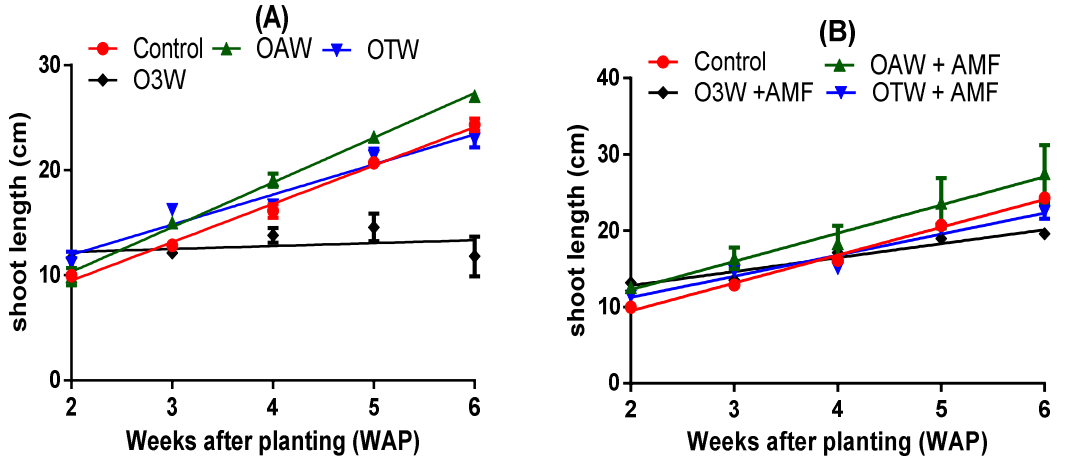

Fig. 3.1. (A) Shows shoot length responses of non-mycorrhizal *A. esculentus* under drought stress (B) shows improved growth of *A. esculentus* under drought stress as a result of AMF inoculation. Under extreme stress condition, it could be observed that AMF inoculation stimulated growth close to that of the control when compared to its uninoculated counterpart

Table 3.2. Influence of arbuscular mycorrhizal fungi inoculation on the leaf area (cm^2) of *A. esculentus* under drought stress

Treatments	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	3.84±0.98[a]	10.31±1.30[a]	12.11±2.61[a]	11.88±1.74[a]	13.24±1.80[a]
OAW	4.45±0.85[a]	13.40±2.76[a]	13.24±2.56[a]	14.43±4.42[a]	15.92±3.03[a]
OTW	4.34±0.66[a]	11.09±0.78[a]	5.50±0.52[b]	17.21±1.46[b]	10.23±3.79[b]
O3W	3.71±0.67[a]	3.13±0.46[b]	6.54±2.37[b]	7.51±0.94[c]	8.04±1.05[b]
OAW+AMF	5.14±0.28[a]	9.95±0.63[a]	8.09±0.67[b]	14.18±0.61[a]	13.51±1.16[a]
OTW+AMF	5.07±0.19[a]	13.03±0.16[a]	7.02±1.03[b]	20.85±1.41[b]	17.80±1.09[a]
O3W+AMF	5.39±0.21[a]	6.32±1.49[b]	15.44±1.61[a]	6.63±2.47[c]	2.48±0.36[c]

Means with different superscripts along the same column are significantly different (P = .05) ± Standard error

Table 3.3. Influence of arbuscular mycorrhizal fungi inoculation on the petiole length (cm) of *A. esculentus* under drought stress

Treatments	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	0.63±0.35[a]	1.53±0.27[a]	3.00±0.29[a]	3.07±0.37[a]	3.33±0.33[a]
OAW	0.97±0.28[a]	2.00±0.10[a]	3.27±0.43[a]	3.40±0.52[a]	3.40±0.67[a]
OTW	0.93±0.23[a]	1.83±0.19[a]	0.83±0.33[b]	3.20±0.06[a]	1.37±0.72[b]
O3W	0.83±0.20[a]	0.67±0.17[b]	1.33±0.42[b]	2.50±0.06[b]	1.27±0.07[b]
OAW+AMF	1.43±0.09[b]	2.23±0.19[a]	2.00±0.12[c]	2.70±0.10[b]	3.00±0.20[a]
OTW+AMF	1.33±0.07[b]	2.03±0.39[a]	0.50±0.00[b]	4.27±0.47[c]	2.93±0.35[a]
O3W+AMF	1.83±0.20[b]	1.20±0.40[b]	2.50±0.45[c]	1.77±0.43[b]	0.10±0.00[c]

Means with different superscripts along the same column are significantly different (P = .05) ± Standard error

Table 3.4. Influence of arbuscular mycorrhizal fungi inoculation on the internode length (cm) of *A. esculentus* under drought stress

Treatments	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	0.80±0.12[a]	3.40±0.10[a]	1.50±0.29[a]	2.77±0.22[a]	1.87±0.41[a]
OAW	0.87±0.12[a]	4.30±0.47[b]	2.93±0.47[b]	2.77±0.53[a]	2.03±0.26[a]
OTW	1.07±0.33[a]	4.50±0.45[b]	0.50±0.00[a]	1.47±0.47[b]	0.40±0.30[b]
O3W	1.03±0.32[a]	2.33±0.17[c]	0.77±0.27[a]	1.00±0.12[b]	0.10±0.00[b]
OAW+AMF	1.77±0.28[b]	4.73±0.77[b]	3.17±0.33[b]	3.63±0.93[a]	1.77±0.34[a]
OTW+AMF	1.40±0.20[a]	3.77±0.15[a]	0.67±0.17[a]	1.27±0.47[b]	0.97±0.09[b]
O3W+AMF	2.00±0.42[b]	2.17±0.44[c]	1.83±0.33[a]	0.10±0.00[c]	0.10±0.00[a]

Means with different superscripts along the same column are significantly different (P = .05) ± Standard error

Table 3.5. Influence of arbuscular mycorrhizal fungi inoculation on the leaf number of *A. esculentus* under drought stress

Treatments	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	4.00±0.00[a]	5.00±0.00[a]	4.00±0.00[a]	5.33±0.33[a]	5.00±0.00[a]
OAW	4.00±0.00[a]	5.00±0.00[a]	4.00±0.00[a]	4.00±0.00[b]	4.33±0.33[b]
OTW	4.00±0.00[a]	5.00±0.00[a]	2.00±0.58[b]	3.67±0.33[b]	2.33±0.33[c]
O3W	4.00±0.00[a]	1.33±0.33[b]	2.67±0.88[b]	4.00±0.00[b]	2.00±0.00[c]
OAW+AMF	4.00±0.00[a]	5.00±0.00[a]	4.00±0.00[a]	4.00±0.00[b]	4.00±0.58[b]
OTW+AMF	4.00±0.00[a]	5.00±0.00[a]	2.00±0.00[b]	3.00±0.00[c]	3.00±0.00[d]
O3W+AMF	4.00±0.00[a]	2.33±0.33[c]	3.67±0.33[a]	2.67±0.33[c]	1.00±0.00[e]

Means with different superscripts along the same column are significantly different (P = .05) ± Standard error

Total photosynthetic pigments (TPP) contents of *A. esculentus* were significantly (*P* =.05) reduced by drought stress (Table 3.6). Non-mycorrhizal *A. esculentus* treatments were more severely affected than the mycorrhizal inoculated plants. However, gradual reductions in the photosynthetic pigments were observed from the 6th week of the study as the test plants came under extreme water deficit. AMF inoculation significantly improved the TPP in the inoculated plants above the uninoculated plants.

Table 3.6. Influence of arbuscular mycorrhizal fungi inoculation on the total photosynthetic pigments (mg/kg) of *A. esculentus* under drought stress

Treatments	4 weeks	6 weeks
Control	33.53±1.82[a]	38.77±3.01[a]
OAW	35.73±1.85[a]	35.40±0.55[a]
OTW	34.17±3.03[a]	32.73±1.44[a]
O3W	35.40±4.05[a]	29.83±1.89[b]
OAW+AMF	37.40±1.39[a]	39.30±2.71[a]
OTW+AMF	44.47±0.73[b]	39.40±1.63[a]
O3W+AMF	40.93±2.35[b]	32.27±2.47[a]

Means with different superscripts along the same column are significantly different (P = .05)

Table 3.7. Influence of arbuscular mycorrhizal fungi inoculation on the biomass yield of *A. esculentus* under drought stress

Treatments	Plant fresh weight (gplant^{-1})	Leaf fresh weight (gplant^{-1})	Leaf turgid weight (gplant^{-1})	Root length (cm)	Total fresh weight (gplant^{-1})	Total dry weight (gplant^{-1})
Control	2.67±0.09[a]	1.30±0.06[a]	1.20±0.12[a]	18.50±0.12[a]	24.17±0.24[a]	6.90±0.06[a]
OAW	3.97±0.03[a]	1.60±0.12[a]	1.40±0.23[a]	16.00±1.15[b]	24.47±0.64[a]	6.70±0.06[a]
OTW	2.40±0.00[a]	1.50±0.12[a]	1.40±0.06[a]	15.00±0.58[b]	13.50±0.10[b]	3.83±0.17[b]
O3W	1.37±0.03[b]	1.20±0.12[a]	1.20±0.12[a]	10.00±1.73[b]	4.87±0.13[c]	1.27±0.09[c]
OAW+AMF	3.17±0.03[a]	1.60±0.12[a]	1.70±0.12[a]	22.70±0.12[a]	24.47±1.72[a]	9.13±0.09[a]
OTW+AMF	2.00±0.00[a]	1.70±0.17[a]	2.00±0.29[a]	19.40±0.58[a]	24.70±0.78[a]	8.20±0.06[a]
O3W+AMF	3.10±0.00[a]	1.70±0.12[a]	2.00±0.12[a]	22.00±1.15[a]	7.90±0.06[d]	7.93±0.03[a]

Means with different superscripts along the same column are significantly different (P = .05)

From this study, N, P, K, Ca and Mg composition of *A. esculentus* were significantly (*P* = .05) reduced as a result of drought stress. However, the inoculation of *A. esculentus* with *G. geosporum* significantly (*P* = .05) increased the N, P, K, Ca and Mg uptake of *A. esculentus* (Fig. 3.2). The improvement of nutrients uptake via AMF inoculation could be one of the major physiological machineries by which plants adopt in bid to tolerate drought. Many early researches have reported that there is a correlation between improved nutrients uptake with drought tolerance especially P uptake [15,16].

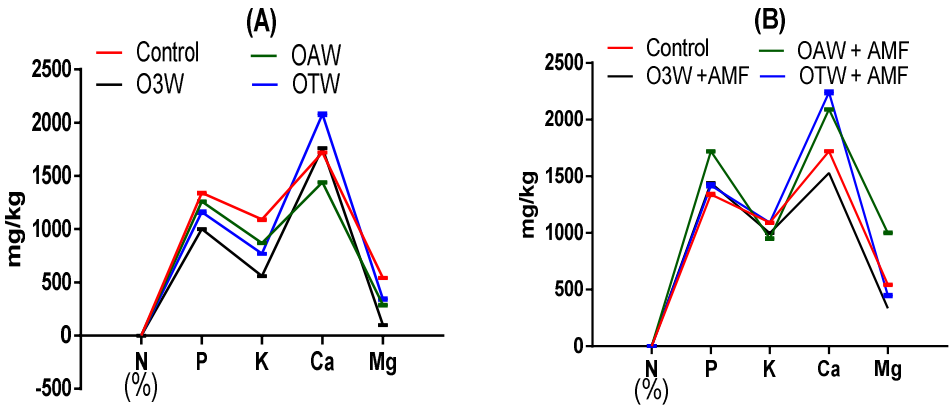

Fig. 3.2. (A) Shows macronutrients composition of non-mycorrhizal *A. esculentus* under drought stress (B) shows improved macronutrients uptake of *A. esculentus* under drought stress as a result of AMF inoculation. Under extreme stress condition, it could be observed that AMF inoculation enhanced macronutrients uptake above that of the control and that of its uninoculated counterpart

Fig. 3.3. Shows root morphology of *A. esculentus* inoculated with *G. geosporum* under drought stress

There was significant (*P* = .05) differences in plant fresh weight, leaf fresh weight, leaf turgid weight, root length, total fresh weight and total dry weight between AMF inoculated and uninoculated treatments (Table 3.7). It has been reported that as a form of adaptation AMF modifies the root morphology of inoculated plants under drought stress. Root morphology adaptation has been reported in trifoliate orange inoculated with or without *F. mosseae* by Liu et al. [17]. This kind of root adaptation resulting from AMF symbiosis provides the ability of the plant to explore more soil volume in order to access and take up nutrients and water [18].

4. CONCLUSION

From the present study, it can be concluded that drought stress inhibits growth and nutrition of *A. esculentus*. Shoot length, leaf area, leaf number, petiole and internode length were all significantly inhibited as a result of water deficit. Total photosynthetic pigments, macronutrients composition as well as biomass yield also experienced similar reductions. However, the inoculation of *A. esculentus* roots with *G. geosporum* in this study AMF through several morphological and physiological processes exhibited remarkable improvement in growth morphology, total photosynthetic pigments, macronutrients composition as well as biomass yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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