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Effect of Salinity Stress on Mycorrhizal Association and Growth Response of *Telfairia occidentalis* Hook F. infected by *Glomus geosporum*

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ABSTRACT

The effect of arbuscular mycorrhizal fungi (*Glomus geosporum*) inoculation on *Telfairia occidentalis* grown in saline soil was investigated in a pot experiment. The experiment was laid out in a completely randomized design, with treatments replicated thrice. Standard recommended methods were used to determine photosynthetic pigments, minerals contents, biomass yield, AMF colonization and dependency. Our results show that saline soil treatment significantly ($p=0.05$) reduced total photosynthetic pigments contents - from 39.73 to 21.30 mg/kg, percentage AMF root colonization - from 53.97 to 22.41%, mineral contents; N - from 5.84 to 3.13%; P - 424.11 to 212.31 mg/kg; K - 3215.00 to 1220.00 mg/kg; Mg - 326.00 to 107.04 mg/kg; and Ca - 1640.00 to 813.00 mg/kg. Biomass yield of *T. occidentalis* was also significantly ($p=0.05$) reduced. In contrast, mycorrhizal dependency was significantly ($p=0.05$) increased in saline soil plants - from 15.13% to 100.00%. Herein, inoculation with *G. geosporum* significantly ($p=0.05$) increased total photosynthetic pigments - from 39.73 to 45.53 mg/kg; N - from 5.84 to 6.07%; P - 424.11 to 463.00 mg/kg; K - 3215.00 to 3470.12 mg/kg; Mg - 326.00 to 345.00 mg/kg and Ca - 1640 to 1658.12 mg/kg; leaf dry weight - from 0.13 to 0.17g; vine dry weight - 5.21 to 5.81g; roots dry weight - 0.57 to 1.03 and total dry weight - 5.91 to 7.01g. Biomass yield was also significantly increased. *R. irregularis* colonization (from 22.41 to 53.97%) and mycorrhizal dependency in *C. maxima* was evident in both saline and non-saline soil treatments. The results of this work have shown that *G. geosporum* can enhance the ability of *T. occidentalis* to resist salt stress (possibly through several morphological/physiological changes and through improved vigour) via the extensive network of the mycorrhizal roots (which increases nutrient and water uptake). Inoculation with appropriate AMF can, therefore, be used to increase the productivity of *T. occidentalis* in saline soils.

Keywords: Arbuscular, *Glomus geosporum*, Mycorrhiza, Salinity, Stress, *Telfairia occidentalis*

1. INTRODUCTION

Salinity affects nearly every aspect of the physiology and biochemistry of plants and significantly diminishes its growth and yield. At present, about 20% of the world's cultivated land and approximately half of all irrigated land are affected by salinity [1]. Therefore, salinity is one of the most significant abiotic factors limiting crop productivity [2]. The most important process that is affected in plants growing under saline conditions is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO₂ concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophyll and carotenoids.

Plant growth and biomass production is an integrative measurement of plant response to the salt stress conditions; therefore, the symbiotic efficiency of AM fungi has been measured in terms of plant growth or biomass accumulation [3, 4]. Several researchers demonstrated that under salt stress plants colonized by mycorrhizal fungi grow better and produce more biomass than non-mycorrhizal plants [4-7]. The improved growth of mycorrhizal plants under salt stress has been suggested to be attributed to better nutrient uptake by mycorrhizal plants [6, 8]. Besides, mycorrhizal fungi modify morphogenetic characters of roots under salt stress. They alter meristematic activity of root apices and promote formation of lateral roots [9]. These modifications in root morphology could influence acquisition of mineral nutrients and water use efficiency of host plant growing in a saline soil. Moreover, AM symbiosis influences various morphological parameters such as plant height, leaf area, root density, and fresh and dry plant weight under saline conditions [10].

T. occidentalis is a member of the Cucurbitaceae family and is indigenous to Southern Nigeria [11]. The fluted pumpkin grows in many nations of West Africa, but is mainly cultivated in Nigeria, used primarily in soups and herbal medicines [12]. Although the fruit is inedible, the seeds produced by the gourd are high in protein; fat and can therefore, contribute to a well-balanced diet. *T. occidentalis* is traditionally used by an estimated 30 - 35 million indigenous people in Nigeria, including the Efik, Ibibio and Urhobo [11]. Therefore, the objective of this study was to study the effect of *Glomus geosporum* on the growth of *T. occidentalis* under salinity conditions.

2. MATERIALS AND METHODS

2. 1. Study Area

Saline soil and salt water were collected from the saline ecosystem of Iwuochang, Ibeno Local Government Area (Latitude 4.56°N and Longitude 7.57°E), Akwa Ibom State, Nigeria, with an annual rainfall of about 4021 mm and mean temperature variation of 22 – 31 °C. The experiment was set up in a safe and secured environment at Mbioto 1, Etinan Local Government Area (Latitude 4.51°N and Longitude 7.50°E), Akwa Ibom State, Nigeria, with an annual rainfall of about 4000 mm and mean temperature variation of 26 – 36 °C. Non-saline soil for the control and non-saline treatments was obtained from a farmland in Mbioto 1, Etinan Local Government Area; fresh water was used for watering the non-saline and control treatments.

2. 2. Experimental Materials

The experimental soils were sterilized in bits for two hours in the oven at 100 °C to kill weed seeds and soil microorganisms and sieved through a 2 mm mesh to remove pebbles. Matured seeds of *T. occidentalis* were collected from Akwa Ibom State Agricultural Development Project (AKADEP). AM Fungi *G. geosporum* (60 – 65 spores per 5 g) was purchased from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

2. 3. Planting

About Five (5) seeds of *T. occidentalis* were sown in their respective earthen pots filled with about 10 kg of sterilized soils. The plants treated with species of AM fungi were inoculated with about 25 g of *G. geosporum* (60 – 65 spores per 5 g) was placed in the bucket at 15 cm depth, before planting, the plants inoculated were allowed to establish for up to 2 weeks before being treated with the first dose of salt (30 ml every 3 days). This is to ensure the establishment of AM colonization and avoid sudden plant death due to salinity shock.

Table 1. Experimental Design.

Treatments	Meaning
S- M-	- Salinity, - Mycorrhiza
S+ M-	+ Salinity, - Mycorrhiza
S+ M+ (<i>Gg</i>)	+ Salinity, + Mycorrhiza (<i>G. geosporum</i>)
S- M+ (<i>Gg</i>)	- Salinity, + Mycorrhiza (<i>G. geosporum</i>)

2. 4. Estimation of Photosynthetic Pigments

At Leaf chlorophyll meter was used for non-destructive estimation of the total photosynthetic pigments of *T. occidentalis*.

2. 5. Determination of Mineral Content

The plant samples were transferred to Ministry of Science and Technology, Akwa Ibom State for mineral analysis. Mineral contents: Nitrogen (N) was determined using the Macro-Kjeldahl method while calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P) of plant samples were determined by atomic absorption spectrophotometer (AAS), flame photometry and spectrophotometry according to the methods of AOAC [13] and Khan *et al.* [14].

2. 6. Determination of Moisture Content

The moisture content of the plant samples was determined using the formula:

$$\text{Moisture Content} = \frac{\text{Difference Between Fresh and Dry Weight}}{\text{Fresh Weight}} \times 100$$

2. 7. Determination of Leaf, Shoot and Root Dry Weight

Leaf dry weight was determined by drying the plant leaves, shoots and roots to constant weight in oven at 70 °C.

2. 8. Determination of Root Length

The plate was prepared as follows: a transparent plastic plate/tray (296 × 210 × 1 mm) was placed on a paper where the appropriate size of the grids was drawn. The grids were masked with 1 mm wide adhesive tape. The grid size was determined depending on the sample size [15]. The number of intersections between grid lines and roots, which appeared as black dots or short lines were counted. To obtain the best estimation, we avoided counting the dots which did not reach the center of the grid width according to the counting rules proposed by Tennant size [15].

2. 9. Determination of Root/Shoot Ratio

The plant sample was dried in an oven at 100 °C, the aerial part was cut from the root section and each section was weighed separately. Root/shoot ratio was calculated using the formula:

$$\text{Root/Shoot ratio} = \frac{\text{Dry Weight of Root}}{\text{Dry Weight of Shoot}}$$

2. 10. Quantification of Arbuscular Mycorrhizal Colonization in Plant Roots

Feeder roots of about 2 – 4 cm of *T. occidentalis* were separately collected, fixed in 50% ethanol and stored for colonization assessment. The fixed roots were rinsed in tap water before clearing in 10% KOH w/v and autoclaved for about 15 minutes at 121 °C autoclave-resistant glass containers that are less than one-third full to avoid overflow in the autoclave. Cleared roots were collected on a fine sieve and rinsed with water several times before being transferred into the staining solution. Staining of the plants roots was carried out using 5% ink diluted in vinegar (5% acetic acid). The roots segments were soaked in the ink and left in staining solution at room temperature for one day. Stained roots were later destained in 50% glycerol for 1 hour [16].

Stained roots were randomly dispersed in a 9 cm diameter Petri plate with grid lines. Vertical and horizontal gridlines were scanned at ×40 magnification with a dissecting microscope. The proportion of root length that is mycorrhizal and total root length can then be calculated from a conversion factor derived from the total length of grid lines and the area of the dish. A minimum of 100 intersections was used to assess the stained root samples; the samples were re-randomized and counted several times. Mycorrhizal root colonization was thus determined by estimation of percentage of root segments containing hyphae, arbuscules and vesicles [17].

$$\text{MC} = \frac{\text{Total number of roots infected intersecting gridlines}}{\text{Total number of roots intersecting gridlines}} \times 100$$

2. 11. Determination of Mycorrhizal Dependency (MD)

Mycorrhizal dependency (MD) was calculated according to the following formula:

$$MD = \frac{DW \text{ inoculated Plants} - DW \text{ non-inoculated Plants}}{DW \text{ inoculated Plants}} \times 100$$

2. 12. Statistical Analysis

The study was conducted using complete randomized design with six (4) treatments with three (3) replicates. 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 (\pm 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 $p=0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Photosynthetic pigments contents of *T. occidentalis* such as chlorophyll a, chlorophyll b and carotenoids grown in saline soil was significantly ($p=0.05$) reduced when compared to the control (Table 2). Inoculation with AMF significantly ($p=0.05$) increased these pigments in the test plant both in saline and non-saline soil treatments (Table 2). This observation agrees with the work of Jing *et al.* [18] who demonstrated that the total Chlorophyll concentration significantly decreased after exposing *Suaeda aralocaspica* to higher salinity, which they attributed to the destruction of the chloroplast structure. Amira and Abdul [19] also recorded decrease in chlorophyll 'a', 'b', and total chlorophyll in *V. faba* exposed to saline stress. This also agrees with Tort and Turkyilmaz [20] report that the exposure of barley (*Hordeum vulgare* L.) to zero, 120, and 240 mM of sodium chloride led to the decrease in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content. This could possibly have been as a result of the suppression of precursors of chlorophyll biosynthesis [21, 22], and lowering of magnesium uptake [23, 24].

Table 2. The Effect of AMF Inoculation on the Photosynthetic Pigments Content of *T. occidentalis* Grown in Saline Soil

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoids (mg/g)	Total Photosynthetic Pigments (mg/g)
S- M-	*29.44 \pm 1.12 ^a	8.15 \pm 0.42 ^a	2.14 \pm 0.27 ^a	39.73 \pm 2.54 ^b
S+ M-	14.42 \pm 0.42 ^c	4.89 \pm 0.08 ^b	1.99 \pm 0.21 ^b	21.30 \pm 1.57 ^d
S+ M+ (Gg)	18.06 \pm 0.72 ^b	7.43 \pm 0.31 ^a	2.07 \pm 0.11 ^a	27.56 \pm 2.04 ^c
S- M+ (Gg)	33.32 \pm 0.42 ^a	9.44 \pm 0.75 ^a	2.77 \pm 0.47 ^a	45.53 \pm 2.11 ^a

*Mean of three replicates \pm SEM. ^aMeans within of each column followed by different letters are significantly different at $p=0.05$ according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (Gg) – *Glomus geosporum*, (Ri)

Inoculation with AMF significantly ($p=0.05$) increased the Biomass Yield of *T. occidentalis* in saline and non-saline soil treatments. The growth enhancement was highest when AMF inoculation was done to plant grown in non-saline soil treatments (Table 3). Plant growth and biomass production is an integrative measurement of plant response to the stress conditions; therefore, the symbiotic efficiency of AM fungi has been measured in terms of plant growth or biomass accumulation [3, 4, 25]. Inoculation of *T. occidentalis* with arbuscular mycorrhizal fungi (AMF) (*G. geosporum*) in non-saline soil treatments significantly ($p=0.05$) increased their biomass accumulation, root/shoot ratio and root length above the control in both saline and non-saline soil treatments for both croppings. Abdel-Latef and Chaoxing [7] reported higher accumulation of biomass in shoot tissues than root tissues and suggested that it could have been due to a greater allocation of photosynthate to the shoot than root tissues in mycorrhizal plants.

The mineral composition of *T. occidentalis* (N, P, K, Mg and Ca) were significantly ($p=0.05$) reduced in saline soil treatments in this study (Table 4). The reduction of N, P, K, Mg and Ca composition in *T. occidentalis* in saline soil treatment observed in this study is in agreement with the findings of Evelin *et al.* [6, 25] who reported reduction in the uptake and concentration of P in plant tissues of fenugreek plants under NaCl-induced salinity in the soil. AMF application improved N, P, K, Mg and Ca uptake of *T. occidentalis* in this study. This agrees with the findings of Evelin *et al.* [6] that inoculation with *G. intaradices* (*R. irregularis*) improved the total N concentration in shoot and root of fenugreek plants over non-inoculated plants. Application of AM fungi can result in a more efficient assimilation of N, P, K, Mg and Ca in the host plants, due to the nitrate assimilation in the extra radical mycelia through the activity of nitrate reductase located in the arbuscular containing cells leading to the formation of arginine, which catabolizes and produces other substances of ammonia; increased production of enzymes controlling the primary nitrogen fixation in the extra-radical mycelia, whereas enzymes controlling arginine catabolism are up regulated in the intra-radical mycelia; decreasing the toxic effects of Na ions by reducing its uptake and this may indirectly help in maintaining the chlorophyll content of the plant [25, 26, 27].

Table 3. Effect of AMF Inoculation on the Biomass Yield of *T. occidentalis* Grown in Saline Soil

Treatments	Leaf Dwt (g·plant ⁻¹)	Vine Dwt (g·plant ⁻¹)	Roots Dwt (g·plant ⁻¹)	Total Dry Weight (g·plant ⁻¹)	Root/Shoot ratio
S- M-	0.13 ^b	5.21 ^a	0.57 ^b	5.91 ^a	0.11 ^b
S+ M-	0.06 ^c	0.69 ^c	0.10 ^d	0.85 ^c	0.14 ^a
S+ M+ (Gg)	0.11 ^b	1.42 ^b	0.23 ^c	1.76 ^b	0.16 ^a
S- M+ (Gg)	0.17 ^a	5.81 ^a	1.03 ^a	7.01 ^a	0.18 ^a

^aMeans within of each column followed by different letters are significantly different at $p=0.05$ according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (Gg) – *Glomus geosporum*

Table 4. Effect of Arbuscular Mycorrhizal Fungi (AMF) Inoculation on the Mineral Nutrient Contents of *T. occidentalis* Grown in Saline Soil

Treatments	N (%)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)
S- M-	*5.84 ^a	424.11 ^b	3215.00 ^b	326.00 ^b	1640.00 ^b
S+ M-	3.13 ^b	212.31 ^d	1220.00 ^d	107.04 ^d	873.00 ^d
S+ M+ (<i>Gg</i>)	4.07 ^a	246.77 ^c	1570.00 ^c	294.06 ^c	1080.00 ^c
S- M+ (<i>Gg</i>)	6.07 ^a	463.00 ^a	3470.12 ^a	345.00 ^a	1658.12 ^a

*Mean of three replicates. ^aMeans within of each column followed by different letters are significantly different at p=0.05 according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (*Gg*) – *Glomus geosporum*



Figure 1. *T. occidentalis* under salt stress showing chlorosis as a result of nutrient deficiency due to sodium toxicity

AMF root colonization (MC) of *T. occidentalis* was significantly (p=0.05) reduced in saline soil treatment when compared to non-saline treatments (Table 5). This agrees with the work of Beltrano *et al.* [28] who reported roots of pepper plants were highly colonized by *G. intaradices* and were higher than other reports by Kaya *et al.* [29] with *G. clarum*, Ruscitti *et al.* [30]; Cekic *et al.* [31] with *G. mosseae* and *G. intaradices*. The ability of *G. intaradices* to colonize the roots of pepper plants declined with increasing NaCl levels [28] as also observed in this study.

Table 5. Arbuscular Mycorrhizal Fungi (AMF) Colonization of *T. occidentalis* Grown in Saline Soil.

Non-inoculated treatment	Root colonization (%)	Mycorrhizal Dependency (%)	Inoculated treatments	Root colonization (%)	Mycorrhizal Dependency (%)
S-M-	0.00	0.00	S+M+ (<i>Gg</i>)	*22.41 ^c	100.00 ^a
S+M-	0.00	0.00			
			S-M+ (<i>Gg</i>)	53.97 ^a	15.13 ^b

*Mean of three replicates. ^aMeans within of each column followed by different letters are significantly different at p=0.05 according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (*Gg*) – *Glomus geosporum*

4. CONCLUSION

Soil salinity is one of the most severe abiotic stresses affecting plant establishment, growth and production worldwide as observed in this study. Results of this study revealed that salt stress negatively affected photosynthetic pigments contents, biomass yield, minerals contents, mycorrhizal colonization and dependency of *T. occidentalis*. The effects of mycorrhizal symbiotic association on *T. occidentalis* showed improvements on the photosynthetic pigments contents, biomass yield, minerals contents, mycorrhizal colonization and dependency of the test plants. Using different mechanisms *T. occidentalis* in association with arbuscular mycorrhizal fungi can tolerate or survive soil salinity. However, in the presence of the fungi, plant ability to resist the stress increases as a result of morphological and physiological changes and improved vigour, extensive network of the mycorrhizal plant roots and enhanced nutrient uptake are all among the processes that made the mycorrhizal inoculated plants to survive under salt stress. Inoculation with appropriate AMF can therefore be used to increase the productivity of *T. occidentalis* in saline soils.

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