



DRY BIOMASS ACCUMULATION OF *TERMINALIA IVORENSIS* (A. Chev) SEEDLINGS INOCULATED WITH ARBUSCULAR MYCORRHIZAL FUNGI UNDER SALT STRESS

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ABSTRACT

Soil salinity is one of the most severe abiotic factors limiting vegetation conservation and rehabilitation of coastal areas in Nigeria. Mycorrhizal application has been proven to be a suitable biological control agent in ecological restoration of contaminated soils in terms of assisting plant survival. However, the role of arbuscular mycorrhizal fungi (AMF) in alleviating salt stress among indigenous trees species is not well known, restricting the application of AMF in the afforestation of local area. In this study, salt stress pot experiment was investigated to determine the effects of AMF on *Terminalia ivorensis* (A. Chev.) a timber species with great ability for afforestation of coastal area. The *Terminalia ivorensis* seedlings inoculated with four AMF strains (*Glomus intraradices*, *Glomus etunicatum*, *Glomus mossae* and *Glomus occultum*) were subjected to 75mM level of sodium chloride (NaCl) salt stress under field conditions. The results clearly indicated that inoculation with AMF consistently improved the root, stem and leaf dry matter of *Terminalia ivorensis* seedlings over the control plants. The total plant biomass of the inoculated seedlings were significantly ($P \leq 0.05$) higher than the control even under salt stress. However, inoculation with *G.occultum* recorded the highest total plant biomass of 15.37g/plant followed by *G.mossae* with 14.05g/plant. While, the total plant dry weight was consistently reduced in stressed seedlings. The root:shoot ratio (R/S) did not follow any consistent pattern. However, *T.ivorensis* seedlings inoculated with *G.etunicatum* gave the highest root:shoot ratio of 0.55g/g and the lowest value of 0.22g/g was recorded in *G.occultum* stressed seedlings. The enhancement in dry biomass (of root, stem and leaf) is an important mechanism of salinity stress tolerance in mycorrhizal plants. The results revealed that inoculation with AMF have the potential in ameliorating salt stress and enhancing afforestation in the coastal areas of Nigeria.

Keywords: Arbuscular mycorrhizal fungi, Salt stress, Dry biomass accumulation, Seedlings inoculated, *Terminalia ivorensis*.

1.0 INTRODUCTION

Coastal areas are globally distributed from the Arctic and Antarctic to tropical zones, describing peculiar ecosystems between ocean and land (Wang, Fu, Ren, Zhu, Lin, Zhang, Cheng, Ma and Yue, 2019). They are precious land reserve resources, attracting global attention for their vegetation conservation and rehabilitation (Rodrigues, Mascaranhas, and Jagtaup, 2011). The Calabar coastal area lies in the tropical high rainforest belt of Nigeria with an annual rainfall of 2500-3500mm (Iwena, 2008). However, vegetation rehabilitation of

local coastal area has not been efficiently accomplished because of high salt accumulation, shallow water and regular stormy weather. Soil salinity in this area is the basic and severe limitation affecting vegetation conservation and rehabilitation (Osim, 2020). High soil salinity limits nutrient and water absorption by plants, negatively affecting the ion balance in plants (Ruiz-Lozana, Porcel, Azcon and Aroca, 2012). Furthermore, high concentration of salt in plants above certain threshold levels could lead to oxidative damage and ion injury to plants causing metabolic

disorder, suppression of the photosynthetic capacity of leaves and disintegration of cell membrane integrity, finally resulting in limited plant development and growth (Kumar, Dames, Gupta, Sharma, Gilbert and Ahmad, 2015).

The association between arbuscular mycorrhizal fungi (AMF) and plant roots is a valuable ecological strategy to enable plants withstand saline environments (Lopez- Raez, 2016). AMF are microorganisms promoting plant growth and can form symbiotic association with more than 80% of plant species in terrestrial ecosystems, including saline environments (Spatafora, Chang, Benny, Lazarus, Smith, Berbee, Bomito, Corradi, Grigoriev and Gryganskyl, 2016). AMF have been known to occur naturally in saline environments (Diagne, Ngom, Djighaly, Fall, Hoher and Svistoonoff, 2020). Many researchers have reported that AMF may improve the rhizosphere soil environment and increase the soil tolerance of plants (Diagne *et al.*, 2020; Frosi, Barros, Oliveira, Santos, Ramos, Maia and Santos, 2017). AMF employ several mechanisms to improve the salt tolerance of plants to grow and develop in saline environments which include enhancing of water and nutrient absorption through extraradicular hyphae (Ruiz-Lozano *et al.*, 2012; Chandrasekaran, Boughattas., Hu., Oh and Sa, 2014), inducing the expression of stress-related genes and proteins, maintaining more favorable K^+/Na^+ ratios to alleviate toxic ions effects, improving rhizosphere soil health, increasing the production and effectiveness of some enzymes to reduce oxidative damage, and accumulating more proline and other organic solutes (osmoregulators) to reduce osmotic stress (Wang *et al.*, 2019; Diagne *et al.*, 2020; Porcel, Redondogomez, Mateosnaranjo, Aroca, Garcia and Ruiz-Lozano, 2015). However, there is paucity of information on how AMF affect growth and the salt tolerance of indigenous trees species in coastal areas, particularly in south-south Nigeria.

Terminalia ivorensis (A. Chev) is a large deciduous forest tree in the family Combretaceae and is distributed throughout

the tropical and sub-tropical regions of the world and approximately fifty species are naturally widespread in western, eastern and southern Africa (Wadsworth, 1997). As an important economic indigenous tree species in Cross River State, Nigeria, *T. ivorensis* timber is used for construction, particularly for building, joinery, fine carpentry, flooring and plywood manufacturing (Lemmens, Sderiangara and Wong, 1995). *T. ivorensis* is well adapted to grow in local, moderately saline soil and is a promising candidate for afforestation of coastal areas (Lemmens *et al.*, 1995). However, despite their wide range uses, the seedlings of *T. ivorensis* have low survival rates in the soil of local coastal areas due to high soil salinity, which limits their use for afforestation of this area. Therefore, the present study was conducted based on the hypothesis that AMF could promote the growth, biomass and salt tolerance of *T. ivorensis* seedlings. A pot experiment was performed in a field to determine the influence of four AMF strains (*Glomus etunicatum*, *Glomus intraradices*, *Glomus mossae* and *Glomus occultum*) on dry biomass of root, stem and leaf of *T. ivorensis* under salt stress (75mM NaCl).

2.0 MATERIALS AND METHODS

The field work was conducted from February 2019 to May 2019 and lasted for 12 weeks, in the University of Calabar, Nigeria. Calabar lies in the tropical high rainforest agro-ecology of the equatorial climatic belt of Nigeria (Latitude $5^{\circ}00'$ and $5^{\circ}40'N$, Longitude $8^{\circ}04'$ and $8^{\circ}62'E$) and is about 70m above sea level (Iwena, 2008). It has a bimodal annual rainfall distribution that ranges from 2500-3500mm with a mean annual temperature range of $22.2^{\circ}C$ to $38.2^{\circ}C$ and a relatively humidity that ranges from 75 – 90%.

The top soil (0-20cm depth) was collected from the study area and analyzed using standard methods for determining soil physico-chemical properties (A.O.A.C., 2003). The sieved soil was sterilized by heating using the hot air oven at $160^{\circ}C$ for 2 hours and used as the growth medium. The seeds of *T. ivorensis* were obtained from Ministry of Agriculture, Department of Forestry, Calabar, in January,

2019 and were subjected to pre-treatment according to the methods adopted by Wadsworth (1997). Four AMF strains (*G. etunicatum*, *G. intraradices*, *G. mossae* and *G. occultum*) of inocula consisting of spores, mycelium and infected root fragments were provided by the International Institute for Tropical Agriculture (IITA), Ibadan.

The experiment was a 9×3 randomized complete block design which comprised of nine treatments with 3 replicates, totaling 27 pots. The treatments consisted of un-inoculated and without NaCl (Control), *G. etunicatum* (*Ge*), *G. etunicatum* with NaCl (*GeNaCl*), *G. intraradices* (*Gi*), *G. intraradices* with NaCl (*GiNaCl*), *G. mossae* (*Gm*), *G. mossae* with NaCl (*GmNaCl*), *G. occultum* (*Go*) and *G. occultum* with NaCl (*GoNaCl*). The pots (39cm diameter and 49cm deep) were each field with 9kg sterilized soil and placed on racks under natural field conditions. These were watered to

filed capacity and left to drain overnight. 50g of crude inoculum was placed 3cm below the surface of the soil in AMF designated pots before sowing to produce mycorrhizal plants (Rabic and Almadini, 2005). Seedlings of uniform height were selected after thinning at one week after emergence (WAE). Differential salinization commenced at two weeks after emergence of seedlings to avoid subjecting plants to osmotic shock (Abbaspour, Fallahyan and Fahmni, 2005). The salt seedlings were watered with 50mls of 75mM NaCl solution per pot, once a week.

Fine terminal feeder roots were taken for AMF colonization assessment using the gridline intersect technique of Giovannetti and Mosse (1980). The presence of colonization was recorded at each point where a stained root with hyphae, vesicles or arbuscules intersected a line. The percentage root colonization was computed using the following equation:

$$\text{Root colonization (\%)} = \frac{\text{Number of arbuscular mycorrhiza-positive segments}}{\text{Total number of segments studied}} \times 100$$

Harvested seedlings were separated into roots, stems and leaves. Their biomass was determined using an electronic scale balance (model ZI: 200630014473.3), after oven drying them at 70°C until a constant weight (Wang *et al.*, 2019). The total plant dry weight was

$$\text{Root: Shoot Ratio (R:S)} = \frac{\text{Dry weight of root}}{\text{Dry weight of shoot}} \text{ g/g}$$

Mean values obtained from the replicate readings were used to calculate standard error. Differences between means were determined by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test was employed to separate the means at $p \leq 0.05$. All data were analyzed using the statistical package for social sciences (SPSS) for windows (version 20.0).

3.0 RESULTS

Table 1: Effect of mycorrhizal inoculation on the AMF colonization of *Terminalia ivorensis* under salt stress (%)

Treatment	12WAE (at harvest)
Control	0.00

computed as the sum of the dry weight of root, stem and leaf. The dry weight data obtained were then used to calculate the root : shoot ratio. The root : shoot ratio (R:S) of each plant was calculated as a ratio of root dry weight to shoot dry weight:

Mycorrhizal colonization was not found in the roots of *T. ivorensis* control seedlings. Salt stress consistently and significantly increased the percentage root AMF colonization (Table 1). At 12 WAE inoculation with *G. intraradices* under NaCl stress gave the highest percentage colonization of 98.35% while the lowest mean value of 54.18% was recorded by seedlings inoculated with *G. mossae* (Table 1).

<i>Ge</i>	71.26±2.01 ^c
<i>GeNaCl</i>	94.16±1.03 ^f
<i>Gi</i>	54.67±3.69 ^a
<i>GiNaCl</i>	98.35±0.54 ^f
<i>Gm</i>	54.18±0.89 ^a
<i>GmNaCl</i>	77.12±1.14 ^d

<i>Go</i>	54.24±2.45 ^a
<i>GoNaCl</i>	65.48± 0.79 ^b

*Means of three replicates ± standard errors of mean (S.E.M). Means within each column followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

WAE: Weeks after Emergence; *Ge*: *Glomus etunicatum*; *GeNaCl*: *Glomus etunicatum*+ Sodium Chloride; *Gi*: *Glomus intraradices*; *GiNaCl*: *Glomus intraradices* + Sodium Chloride; *Gm*: *Glomus mossae* *GmNaCl*: *Glomus mossae* + Sodium Chloride; *Go*: *Glomus occultum*; *GoNaCl*: *Glomus occultum* + Sodium Chloride

AMF inoculated seedlings generally had higher significant ($p \leq 0.05$) dry matter yields than uninoculated (control) plants regardless of the 75mM NaCl treatment (Table 2). However, salt

stress significantly ($p \leq 0.05$) decreased the root, stem, leaf and total dry weight of *T. ivorensis* seedlings compared to the inoculated non-stressed plants.

Furthermore, the highest root, leaf and total dry weight significant ($p \leq 0.05$) values of 5.36, 6.03 and 15.37g/plant respectively were recorded by seedlings inoculated with *G. occultum*, while the stem dry weight revealed the highest non-significant ($p \geq 0.05$) mean value of 3.97g/plant. Also, the root and leaf dry weight were significantly ($p \leq 0.05$) different in all treatments.

The root: shoot ratio did not follow any consistent pattern and seedlings inoculated with *G. etunicatum* gave the highest mean value of 0.55g/g and the lowest significant ($p \leq 0.05$) value of 0.22g/g was observed in *G. occultum* inoculated stressed seedlings (Table 2).

Table 2: Effect of mycorrhizal inoculation on the dry biomass of *Terminalia ivorensis* seedlings under salt stress at 12 WAE.

Treatment	Root Dry Weight (g/plant)	Stem Dry Weight (g/plant)	Leaf Dry Weight (g/plant)	Total Dry Weight (g/plant)	Root : Shoot Ratio (g/g)
Control	0.87±0.07 ^a	0.73±0.09 ^a	1.63±0.03 ^a	3.32±0.019 ^a	0.36±0.01 ^{ab}
<i>Ge</i>	4.10±0.06 ^d	2.87±0.03 ^{ab}	4.50±0.12 ^f	11.47±0.20 ^d	0.55±0.00 ^c
<i>GeNaCl</i>	1.33±0.03 ^b	1.13±0.03 ^a	3.30±0.06 ^b	5.77±0.12 ^b	0.30±0.00 ^{ab}
<i>Gi</i>	4.47±0.29 ^{de}	3.17±0.03 ^b	5.20±0.06 ^g	12.83±0.38 ^e	0.40±0.14 ^{bc}
<i>GiNaCl</i>	1.53±0.12 ^b	1.37±0.03 ^a	3.57±0.07 ^c	6.47±0.23 ^b	0.31±0.02 ^{ab}
<i>Gm</i>	4.85±0.20 ^e	3.53±0.03 ^{ab}	5.67±0.12 ^h	14.05±0.35 ^f	0.53±0.01 ^c
<i>GmNaCl</i>	2.05±0.03 ^c	1.86±0.09 ^a	3.83±0.09 ^d	7.73±0.20 ^c	0.36±0.01 ^{ab}
<i>Go</i>	5.36±0.07 ^f	3.97±0.03 ^{ab}	6.03±0.09 ⁱ	15.37±0.18 ^g	0.54±0.01 ^c
<i>GoNaCl</i>	1.17±0.07 ^{ab}	1.00±0.10 ^a	4.17±0.07 ^e	6.33±0.20 ^b	0.22±0.02 ^a

* Means of three replicates ± standard errors of mean (S.E.M). Means within each column followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

WAE: Weeks after Emergence; *Ge*: *Glomus etunicatum*; *GeNaCl*: *Glomus etunicatum* + Sodium Chloride; *Gi*: *Glomus intraradices*; *GiNaCl*: *Glomus intraradices* + Sodium Chloride; *Gm*: *Glomus mossae* *GmNaCl*: *Glomus mossae* + Sodium Chloride; *Go*:

Glomus occultum; *GoNaCl*: *Glomus occultum* + Sodium Chloride

4.0 DISCUSSION

Soil salinity is a common and serious environmental problem worldwide. Salt stress is detrimental to trees and have seriously affected the structure of coastal forests and afforestation projects (Wang *et al.*, 2019) in Nigeria. However, Mycorrhization was found to increase the fitness of the host plant by enhancing its' growth and biomass (Aggarwal,

Kadian, Neetu, Tanwar and Gupta, 2012). AMF are considered as bio-ameliorators of soil salinity tolerance of plants (Wang, Wang, Li, Wu and Huang, 2018).

There was no colonization of AMF in control *T. ivorensis* seedlings due to non-mycorrhization. The result of the present study indicates that inoculated seedlings under NaCl stress presented higher ($p \leq 0.05$) AMF colonization percentages than the unstressed seedlings. These findings are similar to the work of Aliasgharzadeh, Saleh, Towfighi and Alizadeh (2001), who found that the number of AMF spores did not significantly decrease with soil salinity, suggesting that sporulation could be stimulated under salt stress that will increase the hyphal length and branched absorbing structures (BAS) of the fungi (Jahromi, Aroca, Porcel and Ruizlo Zano, 2008). This is in contrast to the findings of Juniper and Abbott (2006), who reported a reduction in some AMF colonization of roots in the presence of NaCl, due to the direct effect of NaCl on the fungi or the timing of the observation.

The higher dry biomass (root, stem, leaf and total dry weight) of inoculated *T. ivorensis* seedlings even under NaCl stress compared with non-inoculated seedlings in this present research agrees with the findings of Aggarwal *et al.*, (2012). Who reported that mycorrhizal treated *Poncirus trifoliata* seedlings exhibited significantly higher dry biomass in saline soil as compared to non-AMF seedlings. The positive impact of AM fungi on dry matter growth under NaCl stress could partly be attributed to the enhancement of water and nutrients acquisition by external mycorrhizal hyphae, particularly Phosphorus and micronutrients (Aggarwal *et al.*, 2012), but also Nitrogen and Potassium (Wang *et al.*, 2018).

Plant growth and biomass suffered a lot under salt stress. It is well known that salt stress inhibits the photosynthetic ability of plants resulting in decrease in plant growth and biomass accumulation (Wang *et al.*, 2019). However, the significant ($p \leq 0.05$) reduction in dry matter production under 75mM NaCl

stress in this present work could be due to the fact that plants growing under salinity are subject to three primary physiological stresses (Wang *et al.*, 2018): Firstly, the toxicity of sodium and chloride ions which disrupt the normal physiological processes such as respiration, protein synthesis and photosynthesis; secondly, the danger of physiological drought due to osmotic stress, which reduced the water availability to the plant; thirdly, nutrient imbalance induced by high uptake of Na and Cl in plants. In this work, the effects of NaCl stress on dry biomass was enhanced by mycorrhizal colonization (Table 2). These findings are consistent with previous report for AMF-colonized plants under salinity stress (Aggarwal *et al.*, 2012; Wang *et al.*, 2018; Balliu, Sallaku and Rewand, 2015).

The biomass allocation in this present study was high in the leaf tissue compared to that of stem or root tissues indicating that leaf is the primary sink in *T. ivorensis* seedlings (Table 2). In spite of the negative effects of NaCl on roots, the root growth in *T. ivorensis* appears to be less affected whereas, stem was affected drastically, so that, the dry weight ratio was higher in seedlings grown under NaCl stress than in control environment (Table 2). These findings are similar to the reports of Singh, Sastry and Singh (2012) and Giri, Kapoor and Mukerji (2007) on tomato. This could be due to the uptake of carbohydrates from the plant, the AMF enhance the sink capacity of the root system and in turn, increase the photosynthetic performance of the seedlings leading to improved plant growth. The enhanced mycorrhizal tolerance to salt stress was attributed by Feng *et al.*, (2002) to a higher accumulation of soluble sugars in plant roots.

The significant ($P \leq 0.05$) higher root:shoot ratio of *T. ivorensis* seedlings inoculated with AMF in this present research is in harmony with the results of previous researchers (Giri *et al.*, 2007 and Diagne *et al.*, 2020). Who reported a higher root:shoot ratio in tomato, *Acacia nilotica* and many agricultural plants inoculated with AMF than non-mycorrhizal seedlings. This may be due to the availability of

more nutrients to the plant roots in direct contact with the soil as a result of the synergistic effect of the symbionts which resulted in an increase in root dry weight (Diagne *et al.*, 2020). Also, the lowest root:shoot ratio of *T. ivorensis* inoculated under NaCl stress recorded in this experiment is in agreement with earlier studies of Beltrano, Ruscitti, Arango and Ronco (2013). The decrease in root:shoot ratio came from increase in leaf (shoot) dry weight (Table 2). This could be due to the direct effect of NaCl on the fungi and on the plants. Also, AMF may have competed with the roots for photosynthates, thereby, limiting a higher root dry matter accumulation (Kaya, Ashraf, Sonmez, Aydemir, Tuna and Cullu, 2009).

The significant ($p \leq 0.05$) variations of root and leaf dry weight values within the treatments in this present work is similar to the findings of Aggarwal *et al.*, (2012) and Kim *et al.*, (2017). Who reported that differences in responses exist between fungal and plant species and environmental conditions. This could be due to species diversity. The varying responses to the different fungi species suggest that symbiotic efficiency is determined not only by the species and races of the fungus, but also by the host species and environmental conditions (Diagne *et al.*, 2020). Also, AMF ability to absorb nutrient is found to vary with the fungal and plant species and concentration of salt in soil (Wang *et al.*, 2019; Diagne *et al.*, 2020).

5.0 CONCLUSION

AMF improved the growth and biomass of *Terminalia ivorensis* seedlings. However, inoculation of the NaCl stressed seedlings with AMF alleviated the adverse effect (s) of salinity. This alleviation was enough for the plant to be able to efficiently resist the harmful effects of salinity at the 75mM level. Therefore, the use of *G. mossae* as the best promising candidate in afforestation and vegetation rehabilitation of coastal areas is recommendable (i.e., the present data satisfactorily support my hypothesis described in the introduction section).

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