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Relative Growth Rate and Net Assimilation Rate of Black Afara (*Terminalia ivorensis*) Seedlings Grown with Arbuscular Mycorrhizal Fungi (AMF) under NaCl Stress in Calabar, Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aim: To ascertain how four distinct arbuscular mycorrhizal fungi (AMF) species affect the relative growth rate (RGR) and net assimilation rate (NAR) of *Terminalia ivorensis* under NaCl stress.
Study Location and Length: Calabar is situated in southern Nigeria. The study was carried out from February to May, 2022
Methodology: The field procedure was a 9x3x6 completely randomized design with nine treatments and three duplicates, which were further replicated six (6) times, totaling 162 pots. The *Terminalia ivorensis* young plants were induced using four different AM fungi species (*Glomus mossae, Glomus intraradices, Glomus occultum* and *Glomus etunicatum*) and predisposed to

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75mM of NaCl stress. At six sequential harvests of 2, 4, 8, 10 and 12 weeks after emergence (WAE), dry biomass and AMF colonization were determined. relative growth rate and net assimilation rate were calculated using the dry weight data obtained at harvest (4,6,8,10 and 12 WAE).

Results: All the NaCl stressed young plants indisputably showed higher colonization of AMF in their roots than their unstressed replica. Young plants inoculated using *Glomus intraradices* (*Gi*) in fusion with NaCl (*Gi*NaCl) had the maximum AMF root colonization of 98.35%, while 31.78% was the least in plants treated with *G.occultum*(*Go*) at 12 and 8 WAE, respectively. Plants treated with *Glomus occultum* revealed 0.46$gg^{-1}wk^{-1}$ as the highest RGR value and 0.22$gg^{-1}wk^{-1}$ was recorded in *Gm, Gm*NaCl and *Go*NaCl as the least value at 12WAE. Furthermore, *Glomus etunicatum* treated plants recorded the maximum significant ($p \leq 0.05$) NAR of 0.55$gg^{-1}wk^{-1}$ and the least was 0.31$gg^{-1}wk^{-1}$ in seedlings inoculated with *G.occultum* under salt stress at 12 WAE.

Conclusion: The regeneration of afforestation and vegetation in Nigeria's coastal areas can be facilitated by inoculating with the right AMF, which can also increase the biomass production and accumulation of *Terminalia ivorensis* seedlings.

Keywords: Arbuscular mycorrhizal fungi; black afara (Terminalia ivorensis); relative growth rate; net assimilation rate; NaCl stress.

1. INTRODUCTION

Coastal regions, which span the globe from tropical regions to the Arctic and Antarctic, which symbolize distinct ecosystems that can be found between land and the ocean [1]. With a 2500–3500 mm yearly rainfall, the Calabar coastline region is situated in Nigeria's high tropical rainforest belt [2]. Nevertheless, the effective completion of vegetation regeneration of nearby coastal areas has been hampered by the excessive salt accumulation, shallow water table, and frequent severe weather. The primary and severe constraint on the preservation and restoration of vegetation in this area is the salinity of the soil [3]. Growing salt concentrations in the soil limit plant growth and development by decreasing the plant's ability to absorb water and negatively affecting osmotic balance, net photosynthetic rate, nutrient absorbance, stomatal conductance, metabolic processes, and hydraulic conductivity [4].

Obligate biotrophs known as arbuscular mycorrhizal fungus (AMF) form mutualistic symbiotic relationships with the roots of vascular plants [5]. By selectively absorbing K rather than Na, AMFs are hypothesized to have a cushioning effect in salt-affected soils, hence lowering the salt load of plant cells [6]. The buildup of betaines, sugars, and prolines (osmoprotectants), which also create a helpful water gradient in roots even at greater Na concentrations in soil solutions, facilitated osmotic adaptations in AMF-infected plants. Additionally, AMF improves food availability, ionic balance, water the impact of AMF [6] on the growth and salt tolerance of native tree species in Nigeria's southern coastal regions is, nevertheless, poorly understood.

Within the Combretaceae family, *Terminalia ivorensis* (A.chev) is a huge forest tree with deciduous leaves that grows widely throughout the world's tropical and subtropical areas. It is natively found in Eastern, Western, and Southern Africa in about fifty species [7]. Timber from *T.ivorensis*, an economically significant native tree species is utilized in Cross River State, Nigeria, in construction, especially for plywood production, joinery, flooring, fine woodwork, building etc [8]. Because *T. ivorensis* can grow well in local, somewhat salinized soil, it is a viable choice for the coastal afforestation project [8]. Despite their many applications, *T.ivorensis* seedlings are not very likely to survive in the high salinity soil of the nearby coastal areas, which limits their use for afforestation in this location [3].

As a result, the current investigation was carried out with the assumption that AMF may increase *T. ivorensis* seedlings' growth biomass and salt tolerance. The impact of four distinct AMF species; *Glomus occultum, Glomus mossae, Glomus intraradices,* and *Glomus etunicatum* on the net assimilation rate (NAR) and relative growth rate (RGR) of *T. ivorensis* under NaCl stress were investigated in a pot experiment carried out in a field. There is no previous report of any research work on AMF and *Terminalia ivorensis* in Calabar.

2. MATERIALS AND METHODS

2.1 Research Area

The 12-week field study took place at the University of Calabar in Cross River State, Nigeria, between February and May of 2022. At roughly 70 meters above sea level, Calabar is located in the agro-ecology of the tropical high rainforest of Nigeria's climate in the equatorial area (Latitudes $5^0$00' and $5^0$40'N, Longitudes $8^0$04' and $8^0$62'E) [2]. With an average yearly temperature range of 22.2-38.2^{0}C and a comparatively high range of humidity of 75 to 90%, it has a bimodal distribution of rainfall, with a range of 2500–3500 mm of rainfall per year.

2.2 Soil, AMF Inocula, and Plant Seedlings

The *T. ivorensis* seeds were acquired in January 2022 from the Calabar, Department of Forestry, Ministry of Agriculture, and pre-treated using Wardsworth's recommended procedures [7]. The International Institute for Tropical Agriculture, Ibadan, gave four different AMF species; *Glomus etunicatum*, *Glomus intraradices*, *Glomus mossae,* and *Glomus occultum* of inocula made up of infected root fragments, mycelium, and spores. The soil sample was taken between 0 to 20 cm below the surface, and it was employed as a growth medium after being sterilized for two hours at 160^{0}C [9,3].

2.3 Treatments, Inoculation, and Experimental Planning

Nine kilograms of sterilized soil were placed into each of the 39 cm in diameter and 49 cm deep pot, before being set up on racks in an actual field. These were irrigated until completely soaked, then allowed to drain overnight. To create mycorrhizal plants, fifty grams (50g) of coarse inoculum was placed in pots, 3 cm below the soil's surface, intended for AM fungi assigned pots (9). After thinning, seedlings with uniform heights were chosen one week following emergence (WAE). The treatments are made up of; control (without AMF and NaCl), *Glomus etunicatum* (*Ge*), stressed *Glomus etunicatum* (*Ge*NaCl), *Glomus intraradices (Gi),* stressed *Glomus intraradices* (*Gi*NaCl), *Glomus mossae* (*Gm*), stressed *Glomus mossae* (*Gm*NaCl), *Glomus occultum* (*Go*) and stressed *Glomus occultum* (*Go* NaCl). Two weeks following seedlings emergence, differential salinization was started in order to prevent plants from experiencing osmotic shock [10]. Once a week, 50 milliliters of a 75 mM NaCl solution per pot was used to irrigate the salt seedlings. A total of 162 pots were involved in the 9x3x6 completely randomized design experiment, which included nine (9) treatments with three (3) replicates, each of which was further reproduced six (6) times (according to the six harvesting periods).

2.4 Destructive Harvest and Biomass Determination

Total plant biomass was destructively determined on six successive harvest dates (2, 4, 6, 8, 10 and 12 weeks after emergence) After deeply soaking the soil to soften it, the seedlings were gently removed at each harvest. Each plant was divided into its stems, leaves and root. It was then put in a paper package with a distinct label, and it was oven dried at 70 degree Celsius until it reached a constant dry weight [1]. The total dry weight of the plant was calculated by adding the dry weight of the stem, leaf, and root. The net assimilation rate (NAR) and relative growth rate (RGR) were then computed using the dry weight data that were acquired. At harvest and at 4, 6, 8, 10, and 12 WAE, RGR and NAR were noted as;

$$RGR = \frac{InW_2 - InW_1}{t_2 - t_1}\ gg^{-1}wk^{-1}$$

Where, In= Natural logarithm, W_2 and W_1= total dry weight at successive harvest stages, t_2 and t_1 = time interval respectively and is expressed as g/g/week [11].

NAR; It was calculated as the change in total plant biomass per leaf weight and time [12]

$$NAR = \frac{2(W_2 - W_1)}{(t_2 - t_1)(LDW_2 + LDW_1)}\ gg^{-1}wk^{-1}$$

where LDW_2 and LDW_1= leaf dry weight at successive harvest stages

2.5 AMF Colonization

At the time of each harvest, fresh feeder root subsamples were collected in order to calculate the proportion of AMF root colonization. After being properly cleaned in distilled water, the feeder roots were fixed in 50% ethanol. Staining and clearing were done using Koske and Gemma's approach [13]. We assessed the

stained roots for AMF colonization using Giovannetti and Mosse's gridline intersect technique [14]. On a gridline plate, stained roots were spread out and examined at x45 magnification using a dissecting microscope. The following formula was used to get the percentage of root colonization:

$$Root\ colonization(\%) = \frac{Number\ of\ arbuscular\ mycorrhizal-positive\ segments}{Total\ number\ of\ segments\ examined} X\ 100$$

2.6 Statistical Analysis

The standard error was computed using mean values derived based on the repeated measurements (n). One-way analysis of variance (ANOVA) was utilized to ascertain mean differences, and Duncan's multiple range test was employed to separate the means at p≤0.05. The statistical program for social sciences (SPSS) (version 20.0 for Windows) was used to analyze all of the data [15].

3. RESULTS

3.1 Soil and Colonization by AMF

Physically, the soil used for the experiment constituted of 9.0% silt, 78.7% sand, 1.86% organic matter and 12.3% clay. It had a pH of 5.69 and a sandy loam texture according to the physicochemical study. Chemical composition: 0.8 cmol/kg Mg, 1.40 cmol/kg Ca, 85.0 mg/kg accessible P, 0.11 cmol/kg K, and 0.08 mg/kg total nitrogen.

The roots of *T. ivorensis* control seedlings did not exhibit mycorrhizal colonization. With the exception of two weeks after emergence (2WAE), salt stress continuously and significantly (P≤0.05) enhanced the percentage root AMF colonization (Table 1). Inoculating seedlings with *Glomus mossae* (*Gm*) produced the least mean value of 54.18%, while plants inoculated with *Glomus intraradices* (*Gi*) under NaCl stress, which produced the maximum percentage root colonization of 98.35% (Table 1).

3.2 Destructive Harvest and Biomass Determination

Generally, relative growth rate progressively decreases with time in all treatments across Table 2 (from 4-12 WAE). For all treatments, the relative growth rate (RGR) of seedlings at 6 and 12 WAE did not differ significantly (P≥0.05). Furthermore, at 12WAE, seedlings injected with G. occultum showed the greatest RGR mean value of 0.46gg-^{1}wk-^{1} and the lowest value of 0.22gg-^{1}wk-^{1} in *Gm*, *Gm*NaCl and *Go*NaCl (Table 2).

The net assimilation rate was consistently higher in seedlings inoculated with AMF than their stress counterparts at 4,6,8,10 and 12WAE, except in seedlings inoculated with *G. intraradices* and stressed at 4 and 12WAE (Table 3). Seedlings stressed with NaCl recorded a reduction in NAR in most cases at 6,8,10 and 12WAE, except in seedlings inoculated with *G. intraradices* at 12WAE (Table 3). Seedlings infected with *G. etunicatum* also showed the highest significant (p≤0.05) results at 12WAE. In seedlings injected with G. occultum under salinity stress, the lowest NAR was 0.31g/g/wk, and the highest was 0.55g/g/wk (Table 3).

Table 1. Impact of mycorrhizal inoculation on *T. ivorensis* seedlings' root AMF colonization percentage under salt stress (%)

Treatment	2WAE	4WAE	6WAE	8WAE	10WAE	12WAE
Ge	64.49±2.07^{c}	72.98±1.76^{d}	63.95±2.54^{c}	63.86±2.12d^{e}	67.86±1.96^{c}	71.26±2.01^{c}
*Ge*NaCl	68.08±1.51^{c}	91.09±1.02^{f}	96.59±0.89^{e}	96.14±0.59^{g}	93.03±0.56^{e}	94.16±1.03^{f}
Gi	52.68±3.61^{b}	53.29±3.48^{b}	41.41±3.32^{a}	37.77±3.62^{b}	53.94±3.82^{b}	54.67±3.69^{a}
*Gi*NaCl	55.23±3.43^{b}	97.74±0.37^{g}	91.81±0.67^{e}	89.58±0.59^{f}	96.44±1.18^{e}	98.35±0.54^{f}
Gm	57.14±1.03^{b}	47.21±0.89^{a}	57.19±0.82^{b}	52.05±0.98^{c}	50.40±1.94^{ab}	54.18±0.89^{a}
*Gm*NaCl	58.02±1.03^{b}	79.11±0.69^{e}	73.95±0.79^{d}	64.96±0.93^{e}	81.04±1.07^{d}	77.12±1.14^{d}
Go	42.86±0.00^{a}	43.87±0.77^{a}	39.06±0.62^{a}	31.78±0.50^{a}	45.69±0.74^{a}	54.24±2.45^{a}
*Go*NaCl	43.26±1.39^{a}	63.52±0.77^{c}	72.24±0.97^{d}	59.58±1.14^{d}	70.50±0.96^{c}	65.48±0.79^{b}

** Means of three replicates ± "standard errors of mean (SEM) Duncan's multiple range test indicates that means within each column followed by different letters are substantially different at P ≤ 0.05*

Table 2. Impact of mycorrhizal inoculation on *Terminalia ivorensis* relative growth rate (gg^{-1} wk^{-1}) under salt stress

Treatment	4WAE	6WAE	8WAE	10WAE	12WAE
Control	1.30±0.00^{b}	0.29±0.06^{a}	0.16±0.01^{a}	0.25±0.01^{b}	0.25±0.02^{a}
Ge	1.49±0.08^{bc}	0.43±0.06^{a}	0.48±0.02^{f}	0.37±0.01^{c}	0.24±0.00^{a}
*Ge*NaCl	1.39±0.09^{bc}	0.36±0.07^{a}	0.30±0.00^{cd}	0.32±0.00^{c}	0.27±0.02^{a}
Gi	1.63±0.04^{c}	0.37±0.00^{a}	0.38±0.03^{e}	0.31±0.01^{c}	0.29±0.08^{a}
*Gi*NaCl	1.32±0.04^{c}	0.28±0.02^{a}	0.21±0.01^{ab}	0.20±0.04^{ab}	0.33±0.06^{a}
Gm	0.67±0.09^{a}	0.38±0.04^{a}	0.30±0.03^{cd}	0.32±0.04^{c}	0.22±0.00^{a}
*Gm*NaCl	0.59±0.06^{a}	0.32±0.04^{a}	0.24±0.02^{bc}	0.25±0.02^{b}	0.22±02^{a}
Go	0.77±0.15^{a}	0.63±0.25^{a}	0.35±0.02^{de}	0.32±0.01^{c}	0.46±0.00^{a}
*Go*NaCl	0.67±0.17^{a}	0.28±0.03^{a}	0.24±.04^{bc}	0.17±0.01^{a}	0.22±0.01^{a}

** Means of three replicates ± "standard errors of mean (SEM) Duncan's multiple range test indicates that means within each column followed by different letters are substantially different at P ≤ 0.05*

Table 3. Impact of mycorrhizal inoculation on *Terminalia ivorensis* net assimilation rate (gg^{-1} wk^{-1}) under salt stress

Treatment	4WAE	6WAE	8WAE	10WAE	12WAE
Control	1.76±0.00^{c}	0.64±0.10^{ab}	0.19±0.08^{a}	0.40±0.00^{ab}	0.46±0.02^{bc}
Ge	1.64±0.12^{bc}	0.88±0.05^{b}	0.73±0.03^{e}	0.69±0.00^{ab}	0.55±0.01^{d}
*Ge*NaCl	1.89±0.13^{c}	0.62±0.11^{ab}	0.45±0.01^{cd}	0.98±0.51^{b}	0.44±0.03^{b}
Gi	1.34±0.06^{b}	0.60±0.14^{ab}	0.56±0.02^{d}	0.59±0.02^{ab}	0.50±0.03^{cd}
*Gi*NaCl	1.35±0.05^{b}	0.37±0.20^{a}	0.28±0.02^{ab}	0.25±0.09^{a}	0.51±0.06^{cd}
Gm	0.82±0.08^{a}	0.52±0.05^{ab}	0.44±0.05^{cd}	0.76±0.05^{ab}	0.51±0.00^{cd}
*Gm*NaCl	0.75±0.04^{a}	0.45±0.03^{a}	0.33±0.02^{bc}	0.53±0.10^{ab}	0.41±0.04^{b}
Go	0.79±0.10^{a}	0.48±0.03^{ab}	0.49±0.02^{d}	0.58±0.05^{ab}	0.46±0.01^{bc}
*Go*NaCl	0.69±0.15^{a}	0.33±0.04^{a}	0.29±0.09^{ab}	0.19±0.04^{a}	0.31±0.00^{a}

** Means of three replicates ± "standard errors of mean (SEM) Duncan's multiple range test indicates that means within each column followed by different letters are substantially different at P ≤ 0.05.*

4. DISCUSSION

Numerous abiotic stressors negatively impact plant growth, which lessens the encouragement of plant growth and explains the decline in agricultural productivity [16]. Extreme salinity damages trees and has a negative impact on the composition of coastal forests and vegetation restoration initiatives [1] in Nigeria [3]. On the other hand, AMF inoculation improves a plant's ability to withstand salt by altering physiological and biochemical processes, including enhanced growth, development, and metabolism [6].

Because *T. ivorensis* control seedlings were not mycorrhized, there was no AMF colonization. The potential of the AMF to colonize is influenced by plant genotypes, host specialization, nutrient availability, and fluctuations in nutrient uptake under different environmental conditions [17]. The findings of Chandrasekaram's [17] report, which indicate that the strains of a fungus influence symbiotic efficiency in addition to the host species and environmental factors, align with the differences observed in the current study's colonization patterns and the AMF between the various strains' reactions to NaCl stress. Additionally, the current study's findings show that *T. ivorensis* seedlings that were inoculated under NaCl stress had larger percentages of AM fungi colonization than plants that were not affected. These results are consistent to reports by [18,3] that show a significant increase in the percentage of AM fungi colonization in *Pterocarpus mildbraedii* and *Terminalia ivorensis* seedlings under 75mM NaCl stress. This implies that sporulation, which lengthens the hyphal and branching absorbing structures (BAS) of fungus, may be stimulated by salt stress [19]. This contradicts the results of Wang *et al*. [1], who found that AMF hyphal development, sporulation, and spore germination were suppressed, leading to a decrease in the percentage of AM fungi colonization of *Zelkova serrata* plants subjected to salt stress.

Plant growth rate is measured using a metric called relative growth rate, or RGR. It calculates the rise in dry matter at a specific time with a specific amount of assimilatory material [20]. A

plant's RGR is influenced by a variety of plant characteristics, such as biomass allocation, germination time, and life history [21]. Relative growth rate (RGR) is a common metric used to describe growth. However, the steady decline in the relative growth rate over time (4 to 12WAE) observed in this study is consistent with the findings of Rees et al., [22]. He discovered that as size increased, RGR decreased and that physiology, morphology, and allocation underwent systematic modifications. The RGR may decrease over time due to accelarating resource constraints (e.g., self-shading or soil resource depletion). Additionally, RGR frequently decreases over time as plant biomass rises [23, 24]. In the meantime, Osim [3] found that the *T. ivorensis* seedlings' leaf tissues had a higher biomass allocation than their root or stem tissues, suggesting that, the leaf serves as the main sink. However, because of the high dry mass of the leaves, there is a significant carbon loss through leaf respiration, which lowers the RGR significantly.

A helpful metric for assessing a plant's photosynthetic efficiency is the net assimilation rate, or NAR. It has to do with how quickly the dry weight of the entire plant increases per unit of leaf area and unit of time, which is related to the photosynthetic activities of the leaf. Expressed per unit leaf area, it is primarily the net consequence of carbon gains (photosynthesis) and losses (exudation, respiration, and volatilization). NAR was determined in this study by calculating the variation in total plant biomass per leaf weight and time [12]. The significant variation in NAR found in this work is consistent with Chango and Velly's [25], which found that the rate of photosynthesis in siliquae increases and in leaves decreases as crops mature (i.e., at the siliquae production stage). This could be explained by the varying pace at which photoassimilate is supplied to the plant depending on its stage of vegetative growth as a result of siliquae production [25]. Concurrently, the current study of RGR and NAR reductions of *T. ivorensis* seedlings under salt stress are comparable to Downton's findings [26]. He discovered that at 75 mM NaCl, the concentration of carbohydrates in grapevine leaves decreased by 20–40%. This indicated that the decreased growth was caused by a decrease in photosynthesis, which was the consequence of a decrease in chlorophyll content and CO_2 assimilation (27). Moreover, Juniper and Abbott [28] documented the direct impact of NaCl on the fungal plant species, which led to a drop in NAR as a result of a rise in respiration rate (carbon loss) and energy expenditure. This is in contrast to the results of several other studies [29-31], who found that AMF-inoculated plants under salt stress had higher NAR. These data show that salt lessens the interference that salt causes with the production of chlorophyll in mycorrhizal plants, which promotes photosynthesis [29]. AMF play a crucial in biofortification of afforestation, and vegetation restoration of coastal regions. Application of AMF is a rather economical technique.

5. CONCLUSION

The coordinated operation of interdependent stems, leaves, and (mycorrhizal) roots determines how well a tree performs. The method accurately determines that net assimilation rate (NAR) is closely connected with relative growth rate (RGR) since RGR decreases with time due to increased resource constraint (as in the loss of soil resources or self-shading). During the course of the growth cycle, individuals are destructively sampled regularly. Applying a traditional growth study to successive harvests will yield the same results. Since age and size are interchangeable, all strategies are therefore equally legitimate. AMF has been applied to saline soil as a bioameliorator. In Calabar, Nigeria's salty sandy loam soil, inoculating seedlings of *T.ivorensis* with the right AMF can increase biomass production and seedling accumulation. As a result, it is advised that *G.mossae*, the most promising contender, be used to restore afforestation and vegetation in Nigeria's coastal areas.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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