Growth Response of *Pterocarpus mildbraedii* (Harms) Seedlings Inoculated with Arbuscular Mycorrhizal Fungi (AMF) under Salt Stress in Calabar, Nigeria

ABSTRACT

Aim: To determine the influence of four AMF strains on dry biomass of root, stem and leaf of *P. mildbraedii* under salt stress.

Place and Duration of Study: Calabar in the south-south zone of Nigeria. A study was conducted between February and May, 2021.

Methodology: The experiment was a 9 × 3 randomised complete block design comprising of nine treatments with three replicates, totalling 27 pots. The *P. mildbraedii* seedlings were inoculated with four AMF strains (*Glomus intraradices*, *Glomus etunicatum*, *Glomus mossae,* and *Glomus occultum*) and were subjected to a 75 mM level of sodium chloride (NaCl) salt stress. AMF colonization and dry biomass were determined at harvest (12 weeks after emergence).

Results: The results clearly indicated that all the salt stressed seedlings had higher AMF root colonization than their unstressed counterparts. Seedlings treated with *G. intraradices* (*Gi*) in combination with NaCl (*Gi*NaCl) recorded the highest root colonization of AMF of 96.24% and 66.77% as the lowest in seedlings inoculated with *G. intraradices.* The total dry weight of the inoculated seedlings were significantly ($P \leq 0.05$) higher than the control even under salt stress. However, inoculation with *G. intraradices* recorded the highest total dry weight of 16.03g/plant followed by *G. mossae* with 14.00 g/plant.

Conclusion: AMF-inoculated plants can successfully cope with different ecological extremes (salinity) and subsequently help improve afforestation in coastal areas of Nigeria. Improvement in dry biomass accumulation is an essential mechanism of salinity tolerance in mycorrhizal plants.

Keywords: Arbuscular mycorrhizal fungi; Pterocarpus mildbraedii; salt stress; seedlings inoculated; growth response.

1. INTRODUCTION

Coastal areas are important land reserve resources, gaining global interest for their vegetation, conservation, and rehabilitation [1]. The Calabar coastal area lies in the tropical high rainforest belt of Nigeria with an annual rainfall of 2500 – 3500 mm [2]. However, vegetation restoration of local coastal areas has not been effectively accomplished due to shallow water table, regular stormy weather, and high salt accumulation. Salt stress in this area is the underlying and severe limitation that affects the conservation and restoration of vegetation [3]. High salt concentration in plants above certain threshold levels could lead to oxidative damage and ion injury to plants causing metabolic disorder, lessen activities of biocatalysts, suppression of the photosynthetic activity of leaves and disintegration of cell membrane integrity, consequently, resulting in limited plant growth and development [4].

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that institute an endosymbiotic association with the roots of vascular plants [5]. AMF have favorable effects on photosynthetic activity, decrease disease invasion, improve mineral nutrient uptake in the host plant and confer tolerance to abiotic stresses caused by salinity stress, heavy metals and abrupt temperature changes [6]. The favorable effects of

AMF on host plants are the production of polyamines and antioxidants, osmotic adjustment, help with water transport in plants exposed to salinity stress, and maintenance of an ionic balance in the Na^+/K^+ ratio through the external mycelium to improve plant growth, survival, and development under salt stress [7,8]. However, there is paucity of information on how AMF affect growth and the salt tolerance of indigenous trees species in south-south coastal areas of Nigeria.

There are thirty-five (35) species of Pterocarpus that are presently accepted [9]. The species are widely distributed, extending across the African continent, but *Pterocarpus mildbraedii* (Harms) is a distinct sub-species confined to Eastern Tanzania [10]. It is an evergreen or semi-deciduous tree belonging to the family *Fabaceae* and found in the lowland, dry evergreen and riverine forest. As an important economic indigenous tree species in Cross River State, Nigeria, *P. mildbraedii* yield valuable timber traded as paduak [11]. The paduak reddish wood is valued for its toughness, stability in use and decorativeness. *P. mildbraedii* is well adapted to grow in local, moderately saline soil and is a likely candidate for coastal areas afforestation project [12]. However, despite its prolific seed production and wide range of uses, *P. mildbraedii seedlings* have low survival rates in the soil of local coastal areas due to high soil salinity, which limits their use for the afforestation of this area [11]. Therefore, the present study was conducted based on the hypothesis that AMF could promote the growth, biomass and salt tolerance of *P. mildbraedii* seedlings. A pot experiment was performed in a field to determine the influence of four AMF strains (*Glomus intraradices*, *Glomus etunicatum*, *Glomus mossae* and *Glomus occultum*) on dry biomass of root, stem and leaf of *P. mildbraedii* under salt stress (75 mM NaCl).

2. MATERIALS AND METHODS

2.1 Study Area

The field work was conducted from February 2021 to May 2021 and lasted for 12 weeks in the University of Calabar, Cross River state, Nigeria. Calabar was in the tropical high rainforest agro-ecology of the equatorial climatic belt of Nigeria (Latitude $5^0$00' and $5^0$40'N, Longitude $8^0$40' and 8º62'E) and is about 70m above sea level [2]. It has a bimodal annual rainfall distribution that ranges from 2500-3500 mm with a mean annual temperature range of 22.2ºC to 38.2ºC and a relatively high humidity range of 75-90%.

2.2 Plant Seedlings, AMF Inocula and Soil

The seeds of *P. mildbraedii* where obtained from ministry of Agriculture Department of Forestry, Calabar in January 2019 and were subjected to pre-treatment according to the methods adopted by ILDIS [13]. Four AMF strains (*Glomus intraradices*, *Glomus etunicatum*, *Glomus mossae* and *Glomus occultum*) of inocula consisting of spores, mycelium and infected root fragments were provided by the International Institute for Tropical Agriculture, Ibadan. Soil sample was collected at 0-20 cm depth and was sterilized by heating using the hot air oven at 160ºC for 2 hours and used as the growth medium.

2.3 Inoculation, Treatments and Experimental Design

The pots (39 cm diameter and 49 cm deep) were each filled with 9 kg sterilized soil and placed on racks under natural field conditions. These were watered to field capacity and left to drain overnight. Fifty grams (50 g) of crude inoculum was placed 3 cm below the surface of the soil in AMF designated pots before sowing to produce mycorrhizal plants [14]. Seedlings of uniform height were selected after thinning at one week after emergence (WAE). The treatments consisted of un-inoculated and without NaCl (control), *G. etunicatum* (*Ge*), *G. etunicatum* with NaCl (*Ge*NaCl), *G. intraradices* (*Gi*), *G. intraradices* with NaCl (*Gi*NaCl), *G. mossae* (*Gm*), *G. mossae* with NaCl (*Gm*NaCl), *G. occultum* (*Go*), and *G. occultum* with NaCl (*Go*NaCl). Differential salinization commenced at two weeks after emergence of seedlings to avoid subjecting plants to osmotic shock [15]. The salt seedlings were watered with 50 ml of 75 mM NaCl solution per pot, once a week. The experiment was a 9x3 randomized complete block design which comprised of nine (9) treatments with three (3) replicates totaling 27 pots.

2.4 Growth Measurements

Twelve weeks after emergence (12WAE), the seedlings were carefully uprooted after thoroughly watering the soil to loosen it. Each seedling was separated into roots, stems, and leaves and were placed in a separately labeled paper envelope and then oven dried at 70 ^{0}C to constant dry weight [16]. The total plant dry weight was 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 seedling was calculated as a ratio of root dry weight to shoot dry weight;

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

2.5 AMF Colonization

Fresh feeder root subsamples were taken at the time of harvest to determine the percentage of AMF colonization of the roots. The feeder roots were thoroughly washed in distilled water and fixed in 50% ethanol. Clearing and staining were carried out following the method of Koske and Gemma [17]. The stained roots were assessed for AMF colonization using the gridline intersect method of Giovanneti and Mosse [18]. Stained roots were spread on a gridline plate and viewed under a dissecting microscope at x45 magnification. The percentage root colonization was computed using the following equation;

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

2.6 Statistical Analysis

The mean values obtained from the replicate readings (n) were used to calculate the standard error. Differences between means were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test was used to separate the means at P <0.05. All data were analyzed using the statistical package for social sciences (SPSS) (version 20.0 for windows) [19].

3. RESULTS

3.1 Soil and AMF Colonization

The physicochemical analysis of the experimental soil used revealed that physically it was composed of 78.7% sand; 9.0% silt; 12.3% clay; 1.86% organic matter with a sandy loam texture and a pH of 5.69. Chemically, it was made up of 0.08 mg/kg total nitrogen; 85.0 mg/kg available P; 1.40 cmol/kg Ca; 0.8 cmol/kg and 0.11 cmol/kg K.

Mycorrhizal colonization was not found in the roots of *P. mildbraedii* control seedlings. Salt stress in most cases generally increased the percentage root AMF colonization (Table 1). At 12WAE all the salt stressed seedlings had higher percentage root AMF colonization than their unstressed counterparts. Meanwhile, seedlings inoculated with *G. intraradices* under NaCl stress (*Gi*NaCl) recorded the highest significant (P ≤ 0.05) AMF root colonization of 96.24% and the lowest was 66.77% in seedlings inoculated with *G. intraradices* (Table 1).

Table 1. Effect of mycorrhizal inoculation on the AMF colonization of *Pterocarpus mildbraedii* seedlings under salt stress

Treatment	% AMF at harvest
Control	0.00
Ge	86.14 ± 2.14^{bc}
*Ge*NaCl	89.86 ± 0.78^{bc}
Gi	66.77 ± 2.30^{a}
*Gi*NaCl	96.24 ± 0.96^{c}
Gm	69.49 ± 7.77^{a}
*Gm*NaCl	83.95 ± 0.03^{b}
Go	80.83 ± 3.60^{b}
*Go*NaCl	91.31 ± 3.33^{bc}

** Means of three replicates ± standard errors of mean (S.E.M). Means within each column followed by different letters are significantly different at P <0.05 according to Duncan's multiple range test*

3.2 Plant Growth

Generally, AMF inoculated seedlings had higher significant (P ≤ 0.05) dry matter yields than un-inoculated (control) plants regardless of the 75mM NaCl treatment (Table 2). However, salt stress significantly (P ≤ 0.05) decreased the root, stem, leaf, and total dry weight of the *P. mildbraedii* seedlings compared to the inoculated non-stressed seedlings.

Furthermore, the highest values of root, stem, leaf and total dry weight (P ≤ 0.05) values of 5.83, 3.73, 6.47 and 16.03g / plant were recorded, respectively, by seedlings inoculated with *G. intraradices*.

The root: shoot ratio did not follow any consistent pattern and un-inoculated control seedlings gave the highest significant (P ≤ 0.05) mean value of 0.64g/g and the lowest value of 0.48g/g was observed in *G. intraradices* inoculated stressed

(*Gi*NaCl) seedlings (Table 2). Meanwhile, the difference in root: shoot ratio between G. intraradices inoculated seedlings (Gi) and G. intraradices stressed seedlings (GiNaCl) was significant (P ≤ 0.05).

4. DISCUSSION

Plant productivity and growth are negatively affected by various biotic and abiotic stresses. Salinity stress is detrimental to trees and has greatly affected the structure of coastal forests and afforestation projects [16] in Nigeria [11]. However, a possible approach to alleviate salinity includes inoculating transplants by AMF [20]. AMF improves salinity tolerance in plants by altering biochemical and physiological processes, such as improving nutrient availability, water uptake, ionic homeostasis and increasing photosynthetic efficiency [21].

Table 2. Effect of mycorrhizal inoculation on the dry biomass of *Pterocarpus mildbraedii* seedlings under salt stress at harvest (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	2.81±0.06a	1.25±0.09a	3.17±0.13a	7.07±0.19a	0.64±0.02c
Ge	3.93±0.14bc	2.20±0.20abc	5.13±0.18c	11.27±0.52c	0.56±0.02b
*Ge*NaCl	3.02±0.09ab	1.70±0.17ab	3.70±0.35ab	8.42±0.48ab	0.57±0.03b
Gi	5.83±0.29e	3.37±0.40e	6.47±0.26e	16.03±0.96e	0.57±0.01b
*Gi*NaCl	3.30±0.18ab	2.55±0.20cd	4.33±0.17b	10.18±0.54abc	0.48±0.00a
Gm	5.00±0.45d	3.70±0.40e	5.63±0.19cd	14.00±1.15de	0.54±0.02ab
*Gm*NaCl	3.20±0.20ab	2.15±0.09bc	4.53±0.40b	9.45±0.63abc	0.51±0.01ab
Go	4.51±0.44cd	3.13±0.09de	6.03±0.39de	13.68±0.93d	0.49±0.03a
*Go*NaCl	3.10±0.20ab	2.00±0.06abc	4.03±0.07b	9.13±0.33abc	0.51±0.02ab

** Means of three replicates ± standard errors of mean (S.E.M). Means within each column followed by different letters are significantly different at P <0.05 according to Duncan's multiple range test*

There was no AMF colonization in *P. mildbraedii* control seedlings due to non-mycorrhization. AMF colonization depends on the host specificity, plant genotypes, nutrient availability and alteration in nutrient uptake under diverse environmental conditions [22]. In the present study the result indicates that inoculated seedlings of *P. mildbraedii* under NaCl stress present higher AMF colonization percentages than their unstressed counterparts. Although colonization percentages differed among AMF strains. These findings are consistent with the reports by Osim [3] and Osim [11] that the percentage colonization of AMF in *Terminalia ivorensis* and *P. mildbraedii* seedlings increases significantly with 75mM NaCl stress, suggesting that sporulation could be stimulated under salt stress that will increase the length of the hyphal and branched absorbing structures (BAS) of Fungi [23]. This is in contrast to the findings of Wang et al. [16], who reported a reduction in AMF colonization percentages of *Zelkova serrata* seedlings under salt stress, due to suppression of AMF hyphal growth, sporulation and spore germination.

Also, the highest significant (P ≤ 0.05) percentage root AMF colonization recorded by *P. mildbraedii* seedlings inoculated with *G. intraradices* under NaCl stress (*Gi*NaCl) is similar to the work of Osim [3] who reported that *Terminalia ivorensis* seedlings inoculated with *G. intraradices* under NaCl stress gave the highest percentage root AMF colonization which could be due to the colonization capacity of *G. intraradices* and the species of trees [16].

The results of the present study clearly demonstrate that inoculation with AMF can lead to a great stimulation of dry matter yield of *P. mildbraedii* seedlings, the magnitude of this improvement varying between mycorrhizal treatments, with or without NaCl stress. Inoculation resulted in significant (P ≤ 0.05) increases in dry matter content over the non-inoculated control seedlings, and there were also significant (P ≤ 0.05) differences between the different mycorrhizal fungi in their ability to enhance dry matter content of *P. mildbraedii*. This observation is consistent with the results of previous studies on the accumulation of dry biomass of *Terminalia ivorensis* [3]. It is also consistent with the reports of Auge et al. [24], Baltazar-Bernat et al. [6] and Chandrasekaran [22], that varying responses of different fungi 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. This could favor some physiological processes for the plant like water, nutrient uptake and photosynthetic efficiency, keeping higher osmoprotectants and enzyme activities and a high K^+/Na^+ ratio.

Plant growth and biomass suffered a lot under salinity stress. Salt stress inhibits the photosynthetic ability of plants leading to decrease in plant biomass accumulation and growth [16]. However, the significant ($P \leq 0.05$) decrease in dry matter content under 75 mM NaCl stress in this work may be due to the fact that plants exposed to salinity are subject to three primary physiological stresses; the toxicity of sodium (Na^+) and chloride (Cl^-) ions, the danger of physiological drought, and nutrient imbalance [25]. In this present work, the effects of NaCl stress on dry biomass may have been alleviated by AMF colonization (Table 2). These findings are similar to the observations of Al-Karaki [26]; Giri and Mukerji [27]; Evelin et al. [28] who reported that AMF can alleviate the salt stress on plants by inhibiting the high uptake of Na^+ and Cl^- and their transfer to the shoot. The effects of mycorrhizae on development depend on the type and dose of AMF in relation to the host plant species [6].

The biomass allocation in this work was high in the leaf tissues compared to that of stem or root tissues signifying that leaf is the primary sink in *P. mildbraedii* seedlings (Table 2). Root growth in *P. mildbraedii* appears to be less affected in spite of the negative effects of NaCl on roots, while the stem was drastically affected, so the dry weight ratio was higher in seedlings grown under NaCl stress than in the control environment (Table 2). These observations are similar to the results of Osim [3] on dry biomass accumulation of *Terminalia ivorensis* seedlings and also the reports of Singh et al. [29] and Giri et al. [30] on tomato. This could be attributed to the uptake of carbohydrates from the plant, AMF enhancement of the sink capacity of the root system and this in turn increase the photosynthetic performance of plant leading to improved plant growth [31]. The enhanced mycorrhizal tolerance to salinity stress was closely associated to a higher accumulation of soluble sugars in plant roots (Feng et al. 2022).

The highest significant root: shoot ratio ($P < 0.05$) recorded by noninoculated control seedlings in this present research may have been due to the significant decrease ($P \leq 0.05$) decrease in shoot (stem) dry weight due to nonarbuscular mycorrhizal colonization (absence of colonies in the roots) [32,26]. This is in contrast with the results of previous researchers [3,30] who observed a higher root: shoot ratio in *Terminalia ivorensis*, tomato and *Acacia nilotica* inoculated with AMF than non-mycorrhizal seedlings. This could be due to the availability of more nutrients to plant roots in direct contact with the soil as a result of the synergistic effect of the symbionts that results in an increase in root dry weight [33].

Physiological differences exist between mycorrhizal and non-mycorrhizal plants, and between mycorrhizal fungi in their effects on host species [6]. The highest significant ($P \leq 0.05$) dry biomass (root, stem, leaf and total dry weight) of *P. mildbraedii* seedlings inoculated with *G. intraradices* found in this present research is in harmony with the results of previous work on *Terminalia ivorensis* seedlings [3]. He reported the highest root, stem, leaf and total dry weight in *Terminalia ivorensis* seedlings inoculated with *G. occultum*. An increase in the developmental and biomass variables of plants inoculated with mycorrhizae has been observed. The effects of the mycorrhizae on development depend on the type and dose of AMF in relation to the host plant species [6]. Also, AMF ability to absorb nutrient is found to vary with the fungal and plant species and concentration of salt in soil [16,33].

5. CONCLUSION

AMF acts as a growth regulator in most terrestrial environments and has been used as a bioameliorator of saline soil. The result of this study has shown that inoculation with appropriate AMF can enhance biomass production and accumulation of *P. mildbraedii* seedlings in saline (75mM level) sandy loam soil of Cross River Basin in Calabar Nigeria. Therefore, the use of *G. intraradices* as the most promising candidate in the rehabilitation of afforestation and vegetation in coastal areas of Nigeria is recommended.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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