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Growth Performance of Apical and Nodal Cuttings of Selected Forest Tree Species Under Different Fertilizers and Substrate Regimes in the Nursery

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ABSTRACT

The main aim of clonal propagation program is the production of high-quality timber and faster tree growth. An experiment was established from wood shoot cuttings of 3-year-old hedged Acacia and Eucalyptus stock plants grown in a hedge garden at Miro Mile 91, in northern Sierra Leone to study the effects of branch position, fertilizer application and substrate on growth of seedlings. A completely randomized design in factorial arrangement was used for experimentation. Coppice shoots collected from the apical and nodal leafy cuttings were prepared. These cuttings were planted in root trainers using soil and cocopeat as growth media and cultured in a mist chamber for rooting at 85 ± 2% relative humidity with maximum and minimum day-night temperature at 32 ± 18C and 26 ± 18C respectively. The results showed that the rooting parameters viz., percent rooting, mean number of leaves sprout, shoot length, and mean number of roots and length per cutting were significantly influenced by the branch position, substrate, and fertilizer treatments. Treatments with apical cuttings treated with 20g/L and 30g/L NPK fertilizer increased percent rooting and percent sprouting compared to those treated with 10g/L NPK and the control. Cocopeat substrate had significantly higher aeration and water holding porosity than sterilized soil. The results indicated that Apical cuttings treated with 20g/L or 30g/L NPK fertilizer using cocopeat medium could be used for successful clonal propagation of Eucalyptus and Acacia species. Apical cuttings, cocopeat substrate and NPK fertilizer are recommended for mass clonal propagation of these two species.

Keywords: Clonal propagation, apical, nodal, substrate, fertilizer, percent rooting.

INTRODUCTION

Forests play a major role in maintaining plant genetic resources, soil conservation, watershed stability, etc [1], [2] In Africa, forests occupy almost 650 million hectares and provide foreign exchange and ecosystem services that meet the needs of local populations [3]. However, anthropogenic activities of all kinds (for example, extensive itinerant and migratory agriculture, bush fires, anarchic exploitation of certain resources, animal raving) and a galloping population are exerting a strong, non-discrete and increasing pressure on forest ecosystems [4].

Forest trees are very important to our indigenous people and wildlife. Their products are present in buildings, structural and decorative materials, furniture, printing and writing paper, toilet paper, steel products, pharmaceutical products, cosmetics and they are also used for food, shelter, medicine, traditional ceremonies and creation of domestic materials like bags, fans, mats and many other necessities of life. They are also widely used in plantation forest establishment. There is increasing need for forest plants, but their population is decreasing at a rapid rate. The stock of these forest tree species has been badly depleted and natural regeneration is constrained by wild animals which rationally feed on them and also poor germination and growth rates, which might cause extinction of these species if appropriate measures are not taken. The world population has recently stretched to seven billion people and above, and daily, everyone makes use of forest products at homes, offices and schools, but often do not appreciate how important they are. Global demand for wood will increase, driven mainly by the following trends [5]. The world population is increasing, forecasts indicating that there will be nine billion people in 2050. Wood availability is reducing. Over time the global forest area and wood availability per capita has been reduced, and climate change, with increasing temperatures and water scarcity trends. Accepting and promoting them as the only way to address the wood scarcity problem and to avoid illegal logging in natural forests is a major issue, according to [6].

Clonal forestry plays a significant role in many plantation forestry operations around the world, and is now common practice in Acacias, Eucalyptus and pine plantations. The main objective of clonal propagation program is the production of high-quality timber and faster tree growth. Cloning is a propagation technology that can be very effective in making practical use, via 'clonal forestry', of proven superior individuals occurring in nature or developed by breeding. A clone comprises identical copies of an individual that have the same genetic constitution in so far as mutation has not occurred during propagation. In forestry and horticulture, a clone comprises all the descendants of a single plant, produced by vegetative propagation. A clonal plantation forestry, produced from the same parent material, consists of trees that are all expected to be genetically identical to each other. The clonal approach to plantation improvement is to identify a small number of individual trees, by replicated tests in target environments conducted over several years, that are superior in a desired combination of such traits as tree form, wood quality, speed of growth, tolerance of salinity, etc., and to reproduce them vegetatively to create a plantation of trees all identical to these superior individuals. In Sierra Leone, Miro Forestry Company is in similar direction in Eucalyptus clone production that has led to its establishment on a large scale in the field. The purpose of commercial forestry is to grow the highest value crop in an often adjustable and uncertain environment. Industrial forestry companies in Brazil have had enormous success with clonal plantations of hybrid eucalypts [7].

Clonal forestry plays a significant role in many plantation forestry operations around the world. Mini-cutting technique can enhance propagation efficiency and was developed to overcome the rooting problems of some adult plants of the genus Eucalyptus. Mini cuttings from rejuvenated propagules are used for regeneration and mass production of woody species plantlets [8]. In some clones of Eucalyptus, the mini-cuttings from rejuvenated plant material develop a better root system than is found in mini-cuttings from non-rejuvenated tissue [9]. Research results with other tree species indicate the viability of using rooted mini cuttings for commercial propagation ([10], [11]) . In the plantlet production from mini cuttings, the substrate plays a fundamental role in root initiation and growth. It keeps the mini cuttings in an upright position, provides a matrix for needed water and nutrients, and improves root differentiation and growth. Because of these specific but multifaceted requirements, the best substrate is usually composed of a variety of different materials that fulfill these physical functions and facilitate the hormonal stimulation of the desired growth responses, as well as providing availability of needed assimilates for rooting and plant health ([12], [13]).

The development of clonal propagation techniques has allowed for the full maintenance of genetic characteristics of a selected plant (donor of mini cuttings), which has provided uniform forests with rapid growth and the production of homogeneous raw materials [14], [15]. According to [16] [15] the advantages of vegetative propagation are the uniformity of forests, the adaptation of specific clones for production areas and the maximization of timber production in quantity and quality, as compared to forest plantations produced by seeds (seedlings). Moreover, clonal forests can allow for the accurate prediction of yields and, therefore, the profits.

As global demand for wood increases, planted forests will also become increasingly important. Accepting and promoting them as the only way to address the wood scarcity problem and to help suppress the demand for illegally logged timber from natural forests is a major global issue. The major problems faced by the farming community/growers are non-availability of quality planting material, costly seedling and unfledged marketing linkages. Eucalypt and Acacia clonal production will provide alternatives in this context, due to their fast growth, wood quality appropriate to many different uses, huge existing variability, and suitability for vegetative propagation. This study seeks to investigate the substrate and concentration of fertilizers which enable optimum growth performance of the clone plantlets.

The objectives of this study are to determine a suitable substrate and the effect of fertilizer and cutting techniques on sprouting and growth of Eucalyptus and Acacia tree seedlings.

MATERIALS AND METHODS

Description of Study Area

The study was conducted at the nursery site of Miro Forestry Limited (MFSL). MFSL is a sustainable and commercially focused Forestry Company operating in the Yoni chiefdom within the Tonkolili and Port Loko Districts of Sierra Leone. The company's current landholding is 25,213 Ha. It is situated about 5km Miles from Mile 91 in the Tonkolili district on Latitude 8.31^{o} and 8.51^{o} N and Longitude -12.18^{o} and -12.35^{o} W at an elevation of 80m above mean sea level.

Plate 1: Map of Sierra Leone showing area of study

Donor Plant and Coppicing

Three-year-old stock plants, which were raised using seeds of Eucalyptus and Acacia were coppiced by severing the main stem at 30.0 cm distance from the ground level. Approximately three nodes were present at each stock plant. The individual trees were first selected in Miro plantations for their morphological characteristics of growth, health, and branching patterns. The selected trees were then cut down and the coppice sprouts are used to propagate several cuttings sufficient for a field-testing program. These coppicing shoots with 5 to 6 long internodes were selected. After field collection, these were brought immediately to the nursery to be trimmed into cuttings. Final selection for inclusion in the planting program is based on field performance and nursery performance including rootability**.**

Plate3: Obtaining cuttings of Acacia and Eucalyptus from mother plants

Sampling Method

These coppicing shoots comprise 5 to 6 long internodes, which are brought immediately after field collection to the nursery are trimmed into cuttings. These cuttings consist of a 5 to 8 cm long basal internode and two opposite half leaves separated by a second internode of varying length. Half of the surface of these 2 to 4 cm-long leaves are removed to reduce evapotranspiration and water stress risks. The prepared two-node cuttings are then soaked in an aqueous fungicide solution of carbendazim. As soon as completed with the cuttings, the trays are transferred for 30 days to the rooting area, on benches at around 80cm above ground level, under 50% shade and mist water sprays maintained at 8 seconds every minute during the 6 hours following the transfer, to prevent hydrous stress. Spray regime is reduced to 15 seconds every 10 min for the next 14 days, and finally to 15 seconds every hour for the 15 last days of the rooting phase, from 9 till 16 o'clock. During this rooting period, the plantlets are subjected to different fertilizer levels of NPK (0, 10, 20 and 30g/L of N /plant). The Completely randomized block design in factorial arrangement was used.

Treatments

- 2 Tree species (Eucalyptus and Acacia).
- 2 Types of cuttings (Nodal and apical)
- 4 Fertilizer levels – 0g, 10g, 20g and 30 g/L of nitrogen per plant.
- 2 Types of substrates- Cocopeat, and sterilized soil
- Each plot contains 12 cuttings Apical and Nodal.
- Each replication contains 384 of Apical Cuttings and Nodal Cuttings.

The main treatments were (a) 2 tree species and 2 types of cuttings and sub-treatments were two different types of substrates and four rates of fertilizers treatments. As described above the shoots were collected from apical and nodal position.

Planting

The cuttings were planted into root trainers which were filled with two substrates; Cocopeat and sterilized loamy topsoil. (pH 7.0). The substrates were presoaked in tap water for 24 h before filling the trays. The cuttings were dipped in 100mg/L of IBA and planted/inserted about 1.0 cm in the substrates immediately after cutting and kept inside a mist chamber where the relative humidity was maintained at 85 ± 2% with maximum and minimum day-night temperature at 32 ± 18^{o}C and 26 ± 18^{o}C respectively.

Plate 4: Apical cutting of *E. pellita*

Plate 5: Nodal cutting of *A. mangium*

Plate 6: Acacia and Eucalyptus mini-cuttings in the Green House at Miro

Data Collection and Analysis

Calculation of Water holding Capacity and Porosity:

A container was filled with water and the volume recorded (volume of container. The sealed container was emptied and filled with dry substrate (Soil or Cocopeat). Using a measured volume of water, the substrate was irrigated in the container slowly until it is saturated with water. The saturation point is reached when water stays visible on the surface. The volume of water used was recorded. The volume of water needed to reach this point is called the total pore volume. The seal was removed from the drainage holes and the water caught as it runs out. Wait for several hours until the water has dripped out. The volume of water collected was recorded. This is the aeration pore volume. Total porosity, aeration porosity and water holding porosity were calculated using the equations.

- Total porosity = Total pore volume/container volume
- Aeration porosity= Aeration pore volume/container volume
- Water holding porosity = Total porosity - aeration porosity

Data Collection on Seedling Growth Parameters:

Data were also collected on the following parameters: percent rooting and survival, mean number of leaf sprout, shoot length, and mean number of roots and length per cutting. Data were subjected to analysis of variance (ANOVA) using Genstat Release Version 10.3DE and Least Significant Difference (LSD) test was used for mean separation.at 5 % level of probability.

RESULTS AND DISCUSSION

Physical Properties of Soil and Cocopeat Substrates

The results of the physical analysis of the substrates showed that the cocopeat had higher total porosity, aeration porosity and water retention than the soil. The total porosity of cocopeat was 75% whilst the total porosity of the soil was 50%. The total porosity of the cocopeat is within the appropriate range of total porosity values (75-85%) as proposed by [17] The Cocopeat substrate recorded higher aeration porosity (17%) and water holding (58%) than the soil substrate (Fig 1). According to [18] a total porosity of above 50%.is a good growing medium for agroforestry trees. The superior performance of cocopeat could be attributed to its rapid growth and favourable properties.

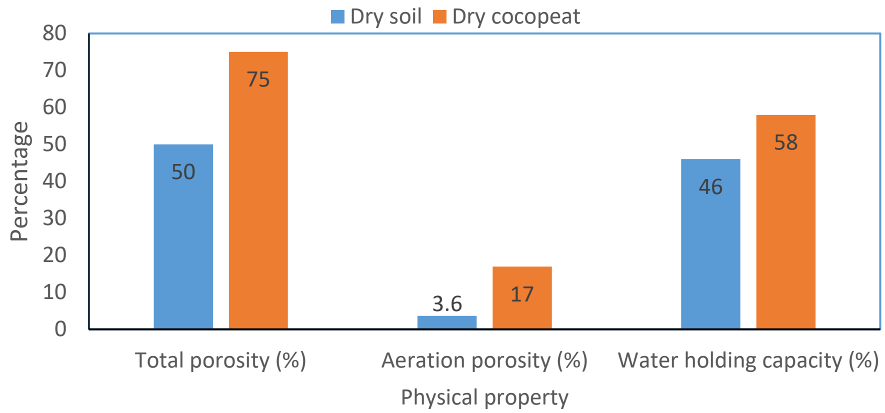

Fig 1: Physical properties of soil and cocopeat substrates.

The results of the analysis of variance also showed statistically no significant ($p = 0.47$) difference between Acacia and Eucalyptus species in mean number of leaves at 2 weeks (Table 1). Also, the type of substrate had no significant ($p = 0.25$) effect on the number of leaf sprout at 2 weeks (Table 2). However, *Acacia mangium* had higher mean number of leaf sprouting (1.48) than *Eucalyptus pellita* (1.39)

Table 1: Effect of species on mean number of leaves sprout at 2 weeks.

Species	Number of leaf sprout
Acacia mangium	1.48 a
Eucalyptus pellita	1.39 a
Mean	1.44
LSD (0.05)	0.24
CV (%)	41.9

Means with the same letters in a column are not significantly different at 5% level of probability.

Table 2: Effect of type of substrate on mean number of leaves sprout at 2 weeks.

Substrate	Number of leaf sprout
Soil	1.36a
Cocopeat	1.51 a
Mean	1.44
LSD (0.05)	0.24
CV (%)	41.9

Means with the same letters in a column are not significantly different at 5% level of probability.

Percentage Survival of Seedlings at 2 and 4 Weeks after Planting

Highly significant ($p < 0.001$) differences were observed in percentage of seedling survival at 2 and 4 weeks after planting. The mini cuttings treated with 30g/lLof NPK had the highest percent survival (61.2%) whilst those without fertilizer had the lowest percent survival (45.6%) at 2 weeks after planting. A similar trend was observed in the survival percent of seedlings except that the percentage survival declined in all the treatments. Generally, the low survival rate observed at the end of the 4 weeks could be due to lack of growth hormone and fungal infection especially in the mini cuttings grown in soil medium.

Table 3: Effect of fertilizer treatment on percent survival of cuttings at 2 and 4 weeks

Fertilizer treatment	Survival % 2 weeks	Survival % 4 weeks
0g/L	45.6b	34.6b
10g/L	64.6a	51.0a
20g/L	66.7a	52.5a
30g/L	67.7a	53.5a
Mean	61.15	47.9
LSD (0.05)	8.01	9.08
CV (%)	22.7	32.9

Means with the same letters in a column are not significantly different at 5% level of probability.

Mean Number of Roots and Rooting Percentage at 4 Weeks After Planting

The number of roots per seedling and rooting percentage are important components of seedling nursery establishment in forestry. The number roots significantly ($p < 0.05$) differed in mean number of roots between the apical and nodal cuttings of the two tree species studied. The apical cuttings significantly produced the highest mean number of roots per plant (4.56) whilst the nodal cuttings had the lowest (3.71) mean number of roots (Fig.5). Similar observations were also recorded in rooting percentage with the apical cutting recording the highest rooting percentage (42%) whilst the nodal cuttings recorded the lowest rooting percentage (31.6%). These results agree with earlier studies on *Milicia excelsa* [19] *Triplochiton. Scleroxylon* [20] and *Nauclea diderrichii* [21] who also reported that cuttings originating from the apical position of shoots displayed higher rooting percentages than those taken from the basal portions. This could be due to the high concentration of auxins in actively growing shoot tips (leaf primordia and young leaves) which are transported to the root to stimulate root formation [22]. The effect of position on rooting may be caused by variation in the physiological status of shoot/cutting tissues on stock plants resulting in occurrence of gradients along the stem axis in the cellular activity or in the level of assimilates or growth regulators or in the level of lignification etc. [23]. However, these results are contrary to those of [24] who reported that the position of cuttings within a shoot had no substantial effect on rooting percentage but influenced mean number of roots.

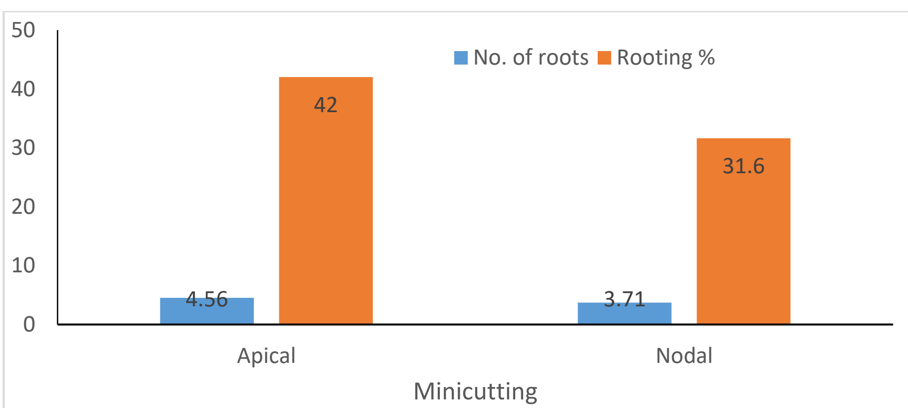

Fig 2: Mean number of roots and rooting percentage as affected by type of mini-cutting.

In other species such as *Calophyllum brasiliense* (Cam), there is no difference between apical and nodal mini-cutting for rooting [12]. However,[11] had similar results in a study with *Anandenanthera macrocarpa* (Benth) in which apical mini- cuttings were more responsive to rooting than nodal cuttings. This can be attributed to the endogenous rooting promoters by virtue of their proximity to both the sites of auxin synthesis and less differentiated tissues potentially resulting in increased differentiating cells to their meristematic condition which is essential for root initiation [25].

The mean number of roots per rooted cutting was significantly affected by the type of substrate used as growth medium. Cocopeat medium resulted in higher mean number of roots produced per cutting compared to topsoil. The same trend was also observed for rooting percentage. Cuttings grown in Cocopeat had higher rooting percentage of 42.6% than cuttings grown in soil (Fig.6)

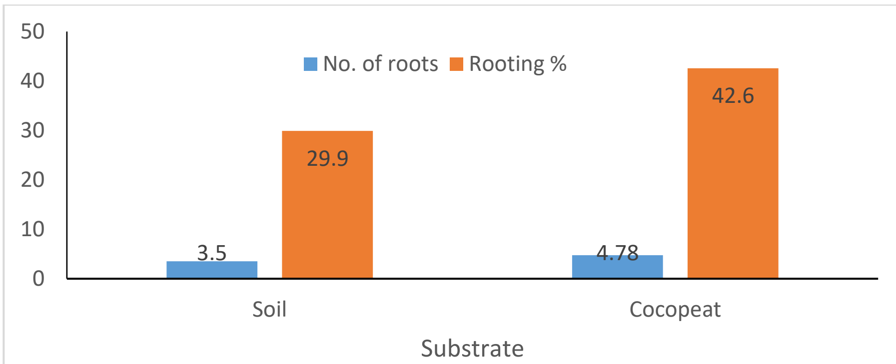

Fig 3: Mean number of roots and rooting percentage as affected by type of substrate

The results of the analysis revealed that after 4 weeks after planting both the mean number of roots/ plant and rooting increased with increase rate of fertilizer application. The mean number of roots was highest (5.25) with cuttings that received 30g/L NPK fertilizer followed by the 20g/L NPK treatment (4.67). Conversely, the least number of roots per cutting was recorded by the control with 0g/L. The same trend was observed in the rooting percentage with the 30g/L NPK treatment having the highest rooting percent (48.3%0 and the control having the lowest rooting percent (25%).

Table 4: Effect of fertilizer on mean number of roots and rooting percentage

Fertilizer treatment (g/L)	Mean number of roots	Rooting percentage
0g	3.08	25.0
10g	3.54	30.6
20g	4.67	43.1
30g	5.25	48.3
Mean	4.14	36.75
LSD (0.05)	0.87	8.99
CV (%)	36.4	42.3

Means with the same letters in a column are not significantly different at 5% level of probability.

Pearson's Correlation of Some Seedling Traits of the Tree Species

Results obtained from correlation analysis showed a significant positive correlation ($r = 0.64$, $P < 0.0001$) between leaf sprouting and number of roots. Similarly, a significant and positive relationship was observed between number of roots and survival percent ($r = 0.76$, $p = 0.0019$)

while shoot length and survival percent were negatively non-significantly correlated (Table 5). There was very weak negative correlation (r = -0.05; p =0.59) between shoot length and survival percent

Table 5: Correlation matrix of some seedling traits of the tree species

	Leaf sprout	Survival %	Shoot length	root_ Length	No. of_ roots	Root_ percent
Leaf sprout	1	0.52868	-0.10338	0.09817	0.6390	0.63532
		0.025	0.3162	0.3413	0.0001	0.021
Survival %	052868	1	-0.0554	0.04027	0.76259	0.75026
	0.025		0.5919	0.6969	0.0019	0.0311
Shoot length	-0.10338	-0.0554	1	-0.05968	-0.13618	-0.09666
	0.3162	0.5919		0.5635	0.1859	0.3488
root length	0.09817	0.04027	-0.05968	1	0.02605	0.07086
	0.3413	0.6969	0.5635		0.8011	0.4927
No of roots	0.6390	0.76259	-0.13618	0.02605	1	0.88605
	0.0001	0.0019	0.1859	0.8011		<.0001
Root percent	0.63532	0.75026	-0.09666	0.07086	0.88605	1
	0.021	0.0311	0.3488	0.4927	<.0001	

CONCLUSIONS

The results of the present study clearly revealed that

- Nursery performance of mini cuttings was generally low in terms of mean number of roots per cutting and rooting percentage which are key indications for final selection in clonal propagation.
- The low rooting success may be due to lack of growth hormone which was excluded in this experiment.
- Cocopeat substrate had higher aeration and water holding porosity than sterilized soil.
- Mini cuttings perform better in Cocopeat than soil substrate.
- The apical mini-cuttings had higher growth and produce more roots than nodal mini-cuttings.
- Mini cuttings responded to increase fertilization by increase growth performance and
- Acacia species had higher number of leaf sprout and shoot length than Eucalyptus which means it is more vigorous in growth.
- The use apical mini cuttings with fertilizer (20g/L and 30g/lL and cocopeat substrate is recommended for increase rootability in clonal propagation of Acacia and Eucalyptus trees.

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Conflict of Interest: The authors have not declared any conflict of interest.

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