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The influence of planting orientation and ecotype on sprouting and survival of stem cuttings of African teak (*Milicia excelsa* (Welw.) in Kenya

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Abstract

African teak (*Milicia excelsa* (Welw.) is a giant deciduous forest tree which belongs to the Moraceae family and is now threatened by extinction due to over exploitation. This study determines the influence of planting orientation and ecotype on sprouting and survival of cuttings of African teak. The cuttings were collected from the Coastal and Western regions of Kenya. A Randomized Complete Block Design was used. The cuttings were planted in three positions (horizontal, vertical, and slanting angle) in the nursery containers covered with the polyethene sheet and the planting medium was composed of river sand. Data were collected monthly on the number and height of sprouts while the cutting survival was done at the end of the experiment which was five months). Data were subjected to analysis of variance using GENSTAT 15th Edition while separation of means was performed using the Standard Error of Difference (SED). The results showed that planting orientation was significant on the sprouting of cuttings df=2, F=117.93, p-value=0.001 as well as the survival of cuttings at the end of the experiment, the cuttings which were planted vertically and slanting were higher compared with those planted in horizontal orientation. The ecotypes had a significant effect on the number of sprouts obtained by stem cuttings df=1, F=6.99, p-value=0.012 and the cuttings from the Western region were performed better than those from Coast. It was concluded that vertical or slanting orientations and Western ecotype should be considered before planting this species.

Keywords: *Milicia excelsa*; Vegetative propagation; Planting orientation; Ecotype

1. Introduction

Trees play an important role in the functioning of the savanna ecosystems by maintaining soil chemical properties and nutrient cycling and providing direct or indirect human nutrition for developing countries particularly [1,2]. Demands for land, timber, food, feed, energy, and ecosystem services are escalating and becoming more and more competitive for trees and forests [3]. Forests are frequently sacrificed when agriculture expands [4] and it is thought to be the main reason for deforestation accounting for over 80% of forest loss [2]. The most difficult and conspicuous causes of biodiversity loss and soil degradation in savanna environments are deforestation, desertification, and erosion [5]. Through programs for planting new trees, replanting existing ones, or regeneration, these restricting constraints can be addressed and ultimately reduced [6]. Only when appropriate production methods are acquired through domestication, propagation and regeneration programs will it be possible to improve the productivity and availability of this indigenous species [7].

Milicia excelsa is a commercially significant timber species native to West, Central, and East Africa. Currently, wild forests provide the majority of the timber for this species, with only a few commercial plantations established [8]. *M.*

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excelsa vulnerability by the gall-forming bug, *Phytolyma lata* makes it difficult to cultivate in plantations. The production of pest-resistant planting stock through a genetic improvement program, according to research conducted at the Forestry Research Institute of Ghana in 1996, reported initial results from seedling screening trials which indicate that pest-resistant genotypes may exist in natural population [9]. Vegetative propagation strategies are necessary required to replicate genotypes that exhibit pest. *M.excelsa* can be propagated through seedlings and wildings although their seeds are susceptible to pests [10]. Suckers are also produced, which can be used in propagation. With slow and poor germination, *M. excelsa* is not a prolific seed producer. Some authors reported that the presence of oily substances surrounding seeds delay seed germination [11]. Several researches have shown that the ease of forming adventitious roots in the stem cuttings decreases as the ortet becomes older [12]. This is a significant issue since desired phenotypic features are rarely manifested until a plant has grown to a significant size or maturity [13]. It has also been shown that in many plant species, cuttings taken from different positions within a shoot differ in their rooting ability. In addressing domestication and conservation challenges regarding sexual propagation, vegetative propagation, and particularly stem cuttings method provides potential for production of propagules with high social and economic value and provide numerous advantages [14] even though it has been proposed but little tested. This paper describes experiments which were undertaken with stem cuttings of *M. excelsa* to determine the influence of planting orientation and ecotype on sprouting and survival.

2. Material and methods

The research was conducted at the Agroforestry Tree Nursery, Egerton University, Njoro, Kenya in January to June 2022. The University is located in Njoro, a small community approximately 25 kilometers southwest of the Nakuru town. The study site lies on a latitude 0°22'11.0"S, Longitude 35°55'58.0"E and an altitude of 2,238 m above sea level. The area falls in agro ecological zone Lower Highland 3. The experimental site receives mean annual rainfall of 1200 mm, with the rain seasons being bimodal. The long rains are received from April to August and short rainfall season from October to December each year. The temperatures lie between 10.2 and 22.0 °C [15].

2.1. Experimental Procedure

The cuttings were collected from the two regions which are Taita Taveta County in Coastal province and Busia County in Western region in January 2022 from the trees which were scattered on farms. The rooting medium used in the whole experiment was composed of sand which was collected from river (approximately 2-3mm diameter) and the nursery containers were 9*16-inch length and width respectively were placed inside the polyethene sheet tunnel and positioned under a shade screen of other trees in the nursery and covered to maintain humidity. Watering was done three times per week until the end of experiment. To obtain cuttings, a cut in the base, or heading to the tip of the branches depending on the nature of each mother plant was done using sterile pruning scissors in the early morning. The cuttings were misted with water and stored in the cooler boxes with ice inside for one day from the site of collection to the planting site prior to propagation. The shoots were severed into cuttings and the foliage was removed. The stem bases of the cuttings were dipped into a rooting powder ('Seradix No.2'with 0.6% IBA, Amiran ltd, Nairobi, Kenya) prior to insertion in the nursery containers filled with rooting medium. All plant materials were of local origin. Cuttings were planted in three different orientation which are vertical, slanting angle and horizontal orientations.

A total of 270 cuttings from each region (90 cuttings per replicate) were taken, and inserted in blocks with 10 cuttings per treatment per block, the total cuttings from the two regions was 540 cuttings. Cuttings were assessed monthly for the presence of new shoots, number and length of new sprouts, and cutting mortality. Seven cuttings per treatment were randomly sampled and the cuttings were allowed to grow for five months. Data collected were subjected to analysis of variance using General Linear Model for factorial design to obtain the p value of the effect of each treatment using GENSTAT 15th edition [16]. The treatments which were found to be significant were separated using Standard Error of Difference (SED) at $p \leq 0.05$ level of significance [16]. Cuttings were defined as dead when either shrivelled or severely rotted.

3. Results

3.1. Survival of cuttings

The results of the study showed that the *M. excelsa* cuttings sprouted (Figure 1). The low survival was contributed by fungal infestation of the cuttings during the experimental period, some cuttings wilted and died (Figure 2). There was no statistical difference on percentage survival of stem cuttings from the two regions although the higher survival percentage of stem cuttings are from Coast region (41.27%) than the percentage survival from West region (39.68%) at the end of experiment.

Planting orientation of stem cuttings was statistically significant, df=2, F=40.46, p-value=0.001 on survival of stem cuttings at the end of experiment with high number of survivals in cuttings planted vertically (46.8%) followed by cuttings planted in slanting angle position (46.03%) and horizontal with 28.57% although there were no statistical differences between vertical and slanting positions (Table 1).

Figure 1 *M. excelsa* sprouted cuttings grown inside polyethene sheet tunnel

Table 1 Effects of planting orientation on cutting survival

Cutting orientation	Cutting survival (%)
Horizontal	28.57b
Slanting	46.03a
Vertical	46.83a
CV	17
SE	6.88
P Value	<.001

* Means on the same column having different superscripts are significantly different (P<0.05)

3.2. Effect of planting orientation on sprouting of *M. excelsa* cuttings

Stem cuttings were planted in three different planting orientation and showed different results after propagation. The good performance was obtained in the cuttings which were planted vertically and slanting orientation and these were higher compared with horizontal orientation (Table 2), df=2, F=117.93, p-value=0.001, this shows that there was statistically difference of planting orientation on sprouting since p<0.001. The high number of sprouts was found in cuttings from slanting position from 30 days up to 120 days of the experiment. However, the mean number of sprouts in all three positions reduced at the end of experiment.

Table 2 Effect of planting orientation on the number of sprouts

Number of sprouts					
Planting orientation	Interval of data collection				
	30 days	60 days	90 days	120 days	150 days
Horizontal	0.17b	0.45b	0.61b	0.65b	0.39b
Slanting	0.63a	0.93a	1.30a	1.34a	0.81a
Vertical	0.64a	0.93a	1.29a	1.28a	0.76a
CV	5.7	16.9	13	44.1	20.5
SE	0.06	0.13	0.14	0.48	0.13
P Value	<.001	<.001	<.001	<.001	<.001

* Means on the same column having different superscripts are significantly different (P<0.05)

The effect of planting orientation was significant (p≤0.05) on the height of sprouts produced by the stem cuttings throughout the experiment, df=2, F=82.48, p-value=0.001, this shows that there was statistically significant difference. The mean height of cuttings from the three positions increased from 30 days up to 120 days and reduced at the end of experiment (Table 3). The longest was obtained in cuttings which were planted in slanting positions.

Table 3 The effect of planting orientation on the height of sprouts

Height of sprouts (cm)					
Cutting orientation	**Interval of data collection**				
	30 days	**60 days**	**90 days**	**120 days**	**150 days**
Horizontal	0.16b	0.57b	1.16b	1.41c	1.36b
Slanting	0.53a	1.37a	2.29a	2.81a	2.49a
Vertical	0.53a	1.38a	2.21a	2.47b	2.40a
CV	5.7	5.9	12.8	15	12.9
SE	0.05	0.07	0.24	0.33	0.27
P Value	<.001	<.001	<.001	<.001	<.001

* Means on the same column having different superscripts are significantly different (P<0.05)

Figure 2 Wilted and dying *M. excelsa* cutting

3.3. Effect of ecotype on sprouting

The stem cuttings were collected from Coastal and Western region which comprises two ecotypes in the study. The ecotypes had a significant (p<0.05) effect on the number of sprouts obtained by stem cuttings at 30 days,120 and 150 days after propagation (Table 9), df=1, F=6.99, p-value=0.012. The mean number of sprouts of cuttings from Coast had increased up to 120 days and dropped on the end of the experiment while those from Western had increased up to 90 days and then reduced until the end of the experiment.

Table 4 Effect of ecotype on the number of sprouts

Number of sprouts					
Ecotype	**Interval of data collection**				
	30 days	**60 days**	**90 days**	**120 days**	**150 days**
Coast	0.44b	0.75	1.03	1.47a	0.75a
Western	0.52a	0.79	1.11	0.71b	0.56b
CV	5.7	16.9	13	44.1	20.5
SE	0.06	0.13	0.14	0.48	0.13
P Value	0.012	0.245	0.059	<.001	<.001

* Means on the same row having different superscripts are significantly different (P<0.05)

On the other hand, coast ecotype showed significantly higher number of sprouts at 120 (1.47) and 150 (0.75) days compared with the western ecotypes which showed 0.71 and 0.56 respectively. This shows the optimum period for sprouting was 120 days for the coast ecotype while for western was 90 days.

The ecotype was statistically significant df=1, F=17.38, p-value=0.001 on the height of sprouts from 30 to 60 days after propagation. There were no statistical differences on 90 days, but there were also statistical differences at 120 days of propagation (Table 5). However, at the end of experiment the mean height of cuttings from the two regions have reduced.

Table 5 Effect of ecotype on height of sprouts

Height of sprouts (cm)					
Ecotypes	**Interval of data collection**				
	30 days	**60 days**	**90 days**	**120 days**	**150 days**
Coast	0.35b	0.97b	1.87	2.38a	2.10
Western	0.47a	1.24a	1.91	2.08b	2.06
CV	5.7	5.9	12.8	15	12.9
SE	0.05	0.07	0.24	0.33	0.27
P Value	<.001	<.001	0.564	0.002	0.548

* Means on the same row having different superscripts are significantly different (P<0.05)

4. Discussion

4.1. Effect of planting orientation on sprouting and survival of *M. excelsa*

Planting orientation had a significant effect on the number of sprouts and their heights, there was no statistical differences between the vertical and slanting orientations but they had statistical difference on the horizontal position. This may be due to the fact that the cuttings planted in vertical or slanting receive more sunlight compared to that of planted horizontally, hence the photosynthesis is high and the cuttings planted horizontally would decompose if placed too deep in the rooting medium [17]. Planting methods and preplanting treatments may play a key role in improving the success of stem cutting to develop roots and shoots [18]. Since the horizontally planted cuttings took longer to emerge than those that were slanting and vertically planted, the height of the shoots of the horizontally tend to be small [19]. It has been suggested in the earlier studies that the differences between the two planting orientations may be caused by differences in the gravity orientation of the root hormones [20]. Vertical or slanting positions allows the large area of cuttings is in contact with the rooting medium for inducing roots, these positions helps to expose more area of the cambium layer, which helps in more water absorption and callus formation [21]. The study done by [22] reported that the cuttings planted with proximal and above soil had greater sprouting percentage than the cuttings planted horizontally. Since, the shoot emerges from proximal end of cutting and the roots from distal end, there was no hinderance from rooting medium in shoot emergence in vertically placed cuttings. In horizontal planting the cuttings were placed in polyethene bags and covered with sand which make the shoots difficult to emerge on the rooting media surface. The similar findings have been reported by [8] that the root cuttings displayed pronounced polarity. Shoots arose from the proximal ends of the cuttings while adventitious roots developed exclusively from the distal ends, when cuttings were inverted such that, their proximal ends were inserted into the medium, shoots always emerged from the proximal ends. Evidence of polarity in root segments has been reported in *Populus tremula* [23]. Roots cuttings in all species exhibit polarity with new shoot development occurring at the proximal end and root development at the distal end of cuttings, regardless of orientation at planting [24]. This polarity results from the polar transport of auxin, basipetally from shoots to roots, coupled with the asymmetric distribution of auxin within each cell, with higher concentration of auxin promoting root development and inhibiting shoot development. The carbohydrate and nutrient status of cuttings also influences their regenerative capacity [25].

4.2. Effect of Ecotypes on sprouting and survival of *M. excelsa*

Genetic potential, as well as propagation environment, postseverance treatment, cutting origin and environment, stockplant physiology and management have been reported to influence rooting and sprouting [26,27]. The results in present study showed that ecotype had significance effects on the sprouting of *M. excelsa.* The trends observed in this study might have influenced by environmental factors. Several studies have indicated that morphological variation is apparently the result of an adaptive response to the environment; for example, variation in growth traits and phonological traits is associated with a latitudinal and altitudinal range or by contrasting climatic conditions [28, 29]. The greater discrimination power of adaptation micro edaphic conditions compared to the geographical regions of origin of accession was found in the study done in morphological variation and ecological structure of African teak in Benin and the results clearly indicated the greater importance of environmental factors (soil texture, soil chemical characteristics, and annual rainfall) than geographical location in discriminating populations [10,30]. The differences and variations showed in the present studies between the two ecotypes might have resulted due to changes in environmental conditions and other climatic conditions. Ecotypes are genetically distinct populations of a given species, displaying phenotypic traits that maximize fitness within a particular local abiotic and biotic conditions [31, 32]. In the study done by [33] reported that termites can cause considerable damage to tree plantations, and plant susceptibility to termites, perhaps related to co-evolution between local tree ecotypes and associated termite species producing resistance to termite attack [34] but *Milicia* was reported to have lower survival when exposed to termites, in present study there was presence of termites in short time and had eaten small sprouts of cuttings which caused some of them to die, may be the termites were coming from the surrounding tree species in the nursery where the experiment was established. Some studies showed that termites feed preferentially on native species [33].

5. Conclusion

The planting orientation had a significant effect on sprouting of *M. excelsa* where vertical and slanting positions performed better compared to the horizontal planting orientation. The ecotype was also significant, the cuttings from Western region were better than those from Coast but not throughout the experiment and did not show significant difference on the cutting survival while planting orientation showed significant effect on cutting survival. It was concluded that the planting orientation particularly vertical and slanting should be considered before planting this species. Further studies should take into account the factors which can influence survival, the number of shoots produced, and adventitious root formation of cuttings as well as pest and diseases control.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest declared by the authors in regard to the findings of this research.

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