Research Journal of Agriculture and Biological Sciences, 5(4): 524-532, 2009

Evaluation of Provenances and Rooting Media for Rooting Ability of African Blackwood (*Dalbergia melanoxylon* Guill. & Perr.) Stem Cuttings.

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Abstract: Vegetative propagation of *Dalbergia melanoxylon* Guill. & Perr. collected from six provenances was investigated in three rooting media (river sand, forest topsoil and mixture 1: 1 (v/v) of river sand and forest topsoil). Tthe effect of provenance and rooting media were highly Significant ($p<0.001$) for the percentage rooted stem cuttings. Interactive effect on provenance and rooting media was not significant in all rooting parameters evaluated. The highest and lowest rooting percentages with respect to provenance were 22.7% and 12.4% recorded from Madale and Mikumi respectively. River sand media revealed significant highest rooting ability of cuttings in all rooting parameters among the three media tested ($p< 0.05$). Significant positive correlation ($p< 0.01$) was revealed between rooting parameters while rooting and sprouting parameters were negatively correlated. Vegetative propagation of *D. melanoxylon* is a means for promotion of this species for domestication, conservation and as an asset for clonal forestry.

Key words: *Dalbergia melanoxylon,* Domestication, Rooting media, Stem cuttings, Non- mist propagator, Provenance.

INTRODUCTION

African Blackwood (*Dalbergia melanoxylon* Guill. & Perr.) is an economically important tree with high-quality wood and one of the most expensive timbers in the world used for the manufacturing of musical instruments and decorative objects such as carvings [23]. As a result of the valuable products derived from *D. melanoxylon,* high exploitation pressure has been exerted to the extent of threatening its genetic diversity and future existence in its natural habitats [18]. Natural regeneration of *D. melanoxylon* is limited as the species is usually raised from the seeds with poor germination [32]. Moreover, seeds of *D. melanoxylon* do not retain their viability for more than one year [30]. Vegetative propagation for domestication of *D. melanoxylon* is considered important [27].

Successful propagation of tropical trees by stem cuttings depends on many factors which could have direct effect on the cutting or through their interactions, such as rooting media and provenance type [33, 20, 39]**.** Stem cuttings collected from different provenances is important for identifying best rooting genetic material to overcome the known limitations associated with their propagation. Variation in rooting ability for cuttings collected from different provenances has been demonstrated for tree species by several researchers [12, 35]. Provenance variation in rooting ability of stem cuttings could be due to variation in physiological status of cuttings or natural genetic variation [35].

Rooting media has been found to be one of the most the most important factors which affect rooting success of cuttings [42]. Rooting media physical properties can also have a profound effect on the supply of water and air to the growing plant [6]. Physical properties of rooting media include total porosity, bulk density, air space, water holding capacity and available water content. Of these, aeration and moisture content appear to be the two properties of major concern in a rooting media which affect the emergence and vigor of roots with consequent effect on quality of rooted cuttings [25,]. [20] reported that an ideal rooting media is known to provide sufficient porosity to allow good aeration and this ensures adequate oxygen availability for the developing rooting system.

To optimize the technology of propagating of this economically important tree, this study was therefore undertaken with objectives to determine the effect of provenance, rooting media and their interactive effects on the success vegetative propagation of *Dalbergia melanoxylon.*

MATERIALS AND METHODS

Plant Material Sources for Stem Cuttings: Stem cuttings were collected from six natural populations of *Dalbergia melanoxylon* located in six different sites in eastern part of Tanzania; namely Mikumi National Park

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and Mkundi in Morogoro region, Ubena and Kibaha in Coast region, Madale and Mbezi in Dar es Salaam region. Selected stem cuttings sources ranged from $06^{0}4I'$ 43.5" S to 07^{0} 06' 16.1"S latitude and $039^{0}08'$ 41.6"E to $037^{0}14'$ 10.3.5"E longitude and altitude from 104 to 522 m a.s.l. Stem cuttings were taken from mature shoots of root suckers, then enclosed in polythene bags and were transported to the propagation site at the Department of Botany, University of Dar es Salaam.

Experimental Design: Spilt plot experimental design with three block replicates was used whereby the main the main plots were six provenances, subplots were three rooting media namely river sand (SA), forest topsoil (FS) and mixture 1: 1 (v/v) of river sand and forest topsoil (SA + FS). River sand media was the sand collected from the river. Forest topsoil was collected at a depth of 1- 10 cm from the fallow forest near the botanical garden at the Department of Botany, University of Dar es Salaam. Water holding capacity and porosity of the rooting media were determined by methods as recommended by [22]. The rooting media were air dried and then sterilized by moistening them with sodium hypochlorite (3.5%) diluted in water at a ratio of 1:9 of sodium hypochlorite: water (volume by volume) as recommended by [15]. For stem cuttings preparation, only basal cutting positions were used in this study and these were taken from node 13 from the apex of the shoot of root suckers (number 1 = apex). Cuttings had a length of 15 cm and diameter range of 0.6 to 1.4 cm.

Ten cuttings were used per replicate such that with the three replications a total of 540 cuttings were used for the whole experiment (ie 10 cuttings x 3 replications x 6 provenances x 3 rooting media). The basal ends of each stem cutting of *D. melanoxylon* were treated with auxin, Indole -3 Butyric Acid (IBA) concentration of 300 ppm (part per million). Treated basal ends of each stem cutting of *D. melanoxylon* were air dried briefly for 5 minutes before insertion to a depth of 15 - 25 mm in different rooting media in a non mist propagator as described by Leakey *et al.,* (1990). Humidity in the propagators was maintained at 85 ± 2 % and maximum and minimum day-night temperature at 30± 1 ^{0}C to 25 ± 1 ^{0}C respectively. Whenever the propagator was opened for inspection, mist spraying was applied to raise the relative humidity inside the propagator.

Observations and Recording for Rooting Experiments: Observations on rooting experiments were made twice a week and whenever the propagator was opened cuttings were sprayed with fine jet of water to maintain humidity. A cutting was considered rooted if it has at least one primary root $\geq$ 1 mm long. Data collections were in terms rooting and sprouting parameters. Rooting parameters evaluated were rooting percentage, root number, root lengths, root dry weight and callus formation for per cutting. Sprouting parameters were percentage cuttings sprouts, percentage cuttings survived, shoot number and shoot height per cutting. Evaluation of both rooting and sprouting parameters were made after 50 days from planting in the non-mist plant propagator. For measurements of root dry weight, roots were washed, removed sand and oven dried at 70 ^{0}C for 72 hours and weighed.

Data Analysis: Data analysis was done using Genstat 5 Release 7.22 DE computer software package. Analysis of variance (ANOVA) procedures were used to test for significant effect of treatments, followed by Duncan's Multiple Range Test (DMRT) for comparisons of means of different treatments. Correlation coefficients (Pearson) were also determined in order to know the strength of linear relationship among dependent variables. Before analysis in order to improve assumptions of normality, data in terms of percentages were converted by arc-sine transformation, whereas in terms of numbers were converted by square root transformation

RESULTS AND DISCUSSION

Effect of Provenance in Rooting Ability: Analysis of variance on the effect of provenance in rooting ability of *Dalbergia melanoxylon* stem cuttings was evidently significant ($p < 0.001$) only for percentage rooted cuttings among the rooting parameters evaluated (Table 1). The effect of provenance on other rooting parameters for of stem cuttings was not significant different. The percentages of rooted cuttings were significantly different ($p < 0.05$) among provenances (Fig 1). The highest and lowest rooting percentage was 22.7% and 12.4% from Madale and Mikumi provenances respectively. Rooting percentages for cuttings from Mbezi, Kibaha, Ubena and Mkundi provenances were not significantly different as revealed by DMRT (Fig 1).

Significant provenance variation in rooting ability of *D. melanoxylon* as revealed from the present investigation may be directly or indirectly controlled by stock plant genotype or may be caused by stock plant environmental factors [29]. Climate and microclimate of the provenance stands influences the accumulation of carbohydrates within stem tissue, and these factors may vary from year to year [12, 35]. It has been argued that provenance variation in rooting success can be attributed to the interaction between the physiological and morphological characteristics of the stalk plant so

Table 1: Analysis of variance values (mean squares) for the effect of provenance, rooting media and their interactions on rooting parameters of *Dalbergia melanoxylon.*

Source of variation	df	Rooting parameters				
		Rooted cuttings(%)	Root number per cutting	Root length per cutting (cm)	Root dry weight per cutting (g) x 10^{-5}	Callused cuttings (%)
Rep	2	396.91 ^{ns}	16.46 ^{ns}	20.90 ^{ns}	2.90 ^{ns}	282.10 ^{ns}
PRV	5	184.32*	2.30 ^{ns}	4.77 ^{ns}	0.65 ^{ns}	98.77 ^{ns}
Error (a)	10	8.02	2.42	2.50	0.63	128.02
RME	2	1656.17*	167.86*	164.70*	32.54*	3587.65*
RME x PRV	10	29.51 ^{ns}	1.60 ^{ns}	2.25 ^{ns}	0.66 ^{ns}	40.25 ^{ns}
Error (b)	24	84.92	2.32	3.23	0.26	94.41

ns = not significant; * significant at p< 0.001; df = degree of freedom; Rep = replicate blocks; PRV = provenance; RME = Rooting media.

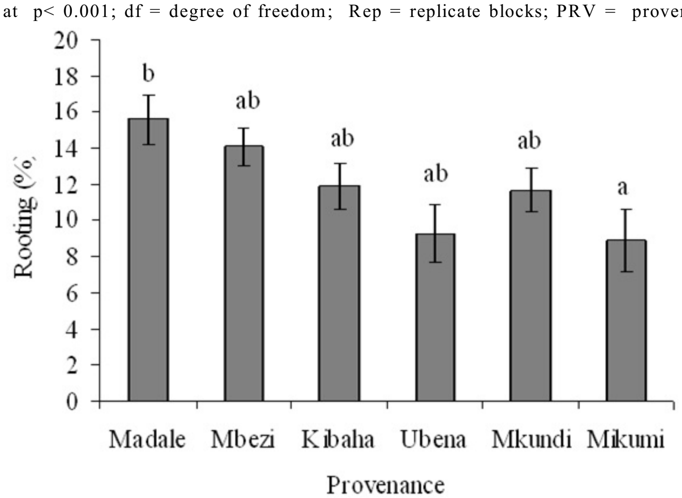

Fig. 1: Effect of provenance on rooting percentage of *D.melanoxylon* stem cuttings. The same letter indicate no significant difference at p<0.05 (DMRT).

called the 'C-effects' [24]. The C-effects have been identified as non- random parental or environmental effects (eg. age and physiological condition of the donor plant) that cause adventitious rooting ability to resemble each other more strongly than would be expected according to genetic relationship only [42, 17].

Variation in rooting ability between provenances has also been reported in several hardwoods, such as *Eucalyptus camaldulensis* [38] or *Calliandra calothyrsus* [12]. Besides environmental conditions and physiological status of the original of plant material causing variation in rooting among provenances, other attention has also been paid to the to the tree- to- tree genetic variation among provenances as a cause of variation in rooting ability of stem cuttings [21].

Effect of Rooting Media: Analysis of variance showed that different soil media had significant effect (p< 0.001) in all rooting parameters in terms of rooting percentage, root number, root length, root dry weight and callus percentage (Table 1). The interactive effect of rooting media and provenance were not significant different in all rooting parameters. Means separation by DMRT showed that the highest and significant (p< 0.05) rooting percentage and number of roots was measured in sand media (SA) followed by mixture of river sand and forest topsoil (SA+FS) media while forest top soil (FS) had the lowest rooting percentage and number of roots (Fig 2). Mean root length and root dry weight per cutting were also highest and significant (p< 0.05) in sand media followed by mixture of sand and forest top soil media and least in forest top soil media (Fig 3). Percentage of callus formation was significant different (p< 0.05) among rooting media. The highest percentage of callus formation was observed in (SA) media followed by mixture (SA + FS) media, and lowest in (FS) media (Fig 4). Physical properties of all rooting media used indicated that there were some variations in percentage of aeration porosity as well as water holding capacity (Table 2). The percent of aeration porosity was highest for the river sand media at 30.3% and lowest for the forest top soil media at 9.1%. Water holding capacity ranged between 28.3% for river sand (SA) to 45.4% for forest topsoil media (FS).

Table 2: Physical properties of rooting media

Physical properties	Rooting media		
	Sand (SA)	Mixture (SA+FS)	Forest topsoil (FS)
Total porosity (%)	58.6	49.2	54.5
Water holding capacity (%)	28.3	34.0	45.4
Aeration porosity (%)	30.3	15.2	9.1

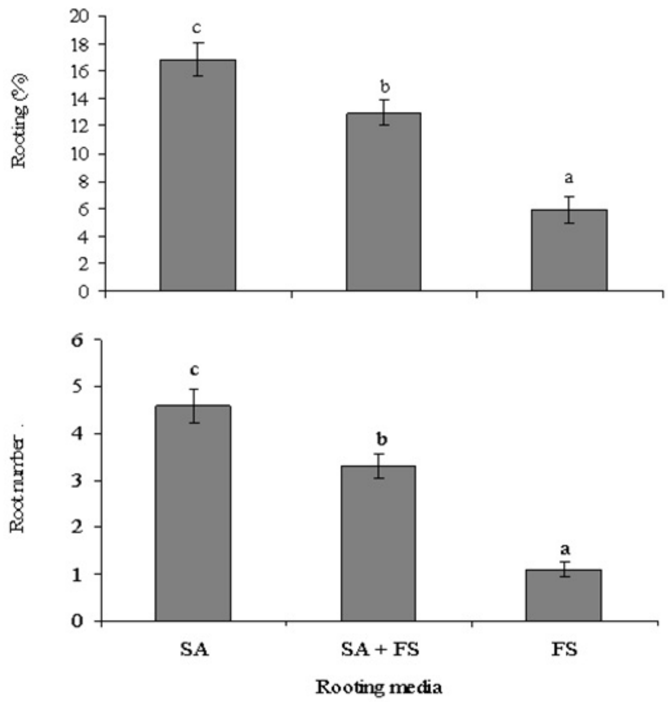

Fig. 2: Effect of rooting media on percentage rooting and root number of *D.melanoxylon* stem cuttings. The same letter indicate no significant difference at $p<0.05$ (DMRT). SA=River sand; SA+FS=Mixture of river sand and forest topsoil FS=Forest top soil.

The importance of rooting medium for rooting of cuttings is widely recognized [19, 3, 39]. Evidence from this study indicates that river sand is the best rooting media of *D. melanoxylon* stem cuttings. Stem cuttings rooted in sand as propagation media revealed high performance in rooting ability for all rooting parameters compared to mixture of river sand and forest topsoil or sole forest topsoil media used in this study. Similar results for higher rooting percentages in sand media were identified in *Cordia alliodora* [25]. Sand was also identified as best rooting medium for *Gongronema latifolia* stem cuttings [2]**.** The variation in response to different media has also been reported for other tree species. For example, in southwest Cameroon and Ghana, highest rooting percentages of *Irvingia gabonensis* [40] and *Milicia excelsa* [33] were recorded in sawdust. However, in similar studies conducted in Costa Rica with *Cordia alliodora* and *Vochysia hondurensis*, higher rooting percentages were reported for fine sand and fine gravel respectively [25]. Between species difference in rooting ability in different rooting media is explained by their xeromorphic or hydromorphic status [26], and the effects of this on the water relations of the cuttings [28].

The responses to different rooting media suggest the existence of significant variation in their physical characteristics particularly difference in total porosity, water holding capacity and aeration porosity of the media used**.** In this study, although both river sand and forest top soil had high total porosity compared to the mixture river sand and forest top soil media, only river sand media had highest aeration porosity which was 31.3 %, while the lowest aeration porosity was 9.1% for the sole forest top soil media. For optimal rooting and growth conditions, [41] stated that aeration porosity of media should range from 25 to 35% with 20 to 30% easily available water which is within the range found in river sand media used in this study. An appropriate rooting medium generally has an optimal volume of gas filled pore space and oxygen diffusion rate

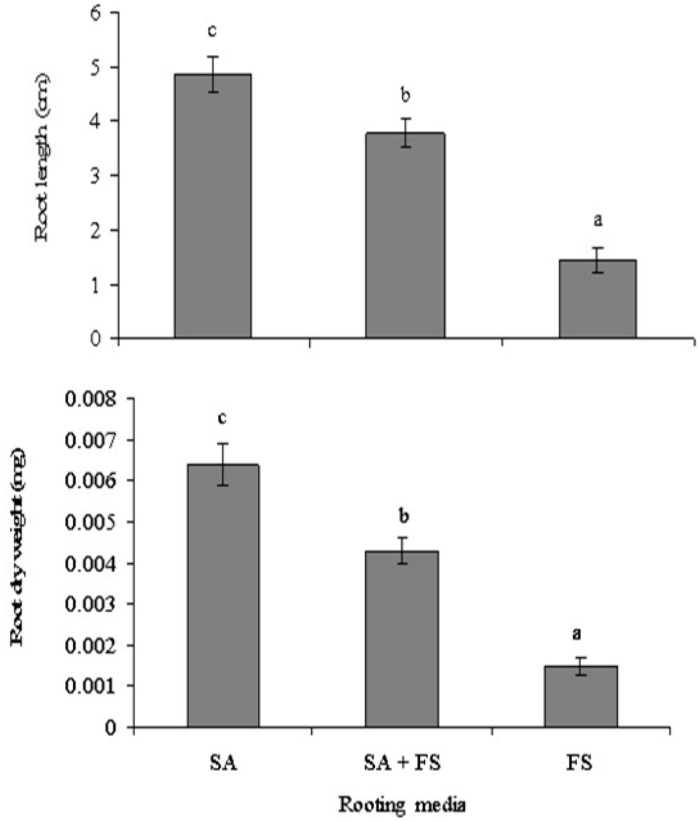

Fig. 3: Effect of rooting media on root length and root dry weight of *D.melanoxylon* stem cuttings. The same letter indicate no significant difference at $p<0.05$ (DMRT). SA=River sand; SA+FS=Mixture of river sand and forest topsoi;l FS=Forest top soil.

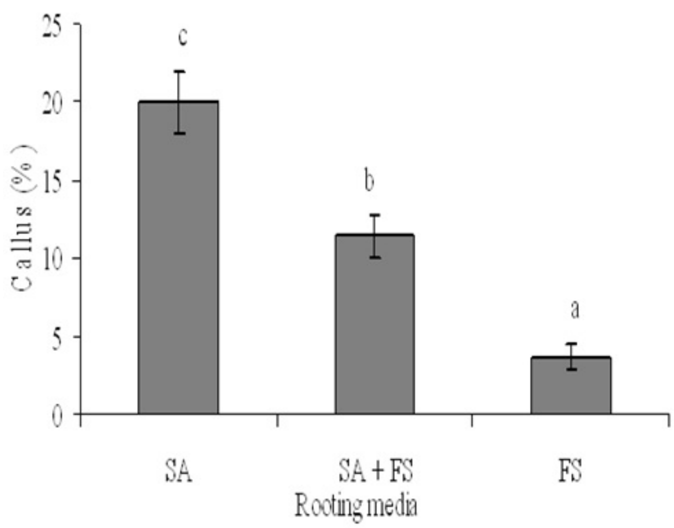

Fig. 4: Effect of rooting media on percentage of callus formation of *D.melanoxylon* stem cuttings. The same letter indicate no significant difference at $p<0.05$ (DMRT). SA=River sand; SA+FS=Mixture of river sand and forest topsoil FS=Forest top soil.

adequate for the needs of respiration [16]. [9] stated that media physical properties should not be constrained to just measurements of air-filled porosity, water-holding capacity, and bulk density, but included gas exchange characteristics.

Significant rooting in sand may be attributed to better aeration and water drainage because of higher aeration porosity, which have been reported to promote root development [34, 36]. The well aerated and a loose texture of sand allows room for higher numbers of roots and vigorous root growth. The quality of roots in terms of number and length is very important for the successful establishment of the cuttings after detachment from the propagator. Cuttings rooted in sand medium also had well differentiated callus and higher percentage of callus formation than mixture media or forest top soil only. Rooting medium can influence the type of callus produced, which in turn can affect emergence of newly-formed adventitious roots [20].

Poor rooting recorded in a forest topsoil media for all rooting parameters could also be attributed to resistance to penetration which [8] argued that is dependent on water content, bulk density, structure and strength of the soil. The forest topsoil also do not have the required aeration porosity for sufficient gas exchange, which can lead to rotting of the cuttings as it has been for *D. melanoxylon* cuttings in this study where mortality of cuttings was highest in forest topsoil media compared to other rooting media used. Other findings reported by [19] for use of forest topsoil as rooting media has recorded poor rooting percentage for *Chrysanthenum sp* cuttings and suggested that the poor rooting may reflect anoxia associated with high water content, the case which also could be applied for *D. melanoxylon* stem cuttings rooted in forest topsoil media used in this study.

The combination of river sand with forest topsoil in 1: 1 (v/v) yielded improved rooting successes and this is an indication of the importance of good aeration for root initiation and development in rooted stem cuttings. Even though, mixture of river sand and forest topsoil media performed better than sole forest topsoil in most of the attributes, sand media will be preferred to other media because it is readily available and affordable. Also, continuous collection of forest topsoil for rooting is not advisable because it is bulky, heterogeneous in mineral composition and could cause environmental hazard such as soil erosion [14] .

Correlation Analysis of Rooting and Sprouting Parameters: All rooting parameters; rooting percentage, number of roots, root length, root dry weight and callus percentage were positively correlated ($p<0.01$). Low significant positive correlation ($p<0.05$) was observed between rooting parameters and shoot height (Table 3). Low significant positive correlation was also observed between rooting parameters and percentage of cuttings that survived (rooted and health unrooted cuttings) at the end of experiment. Negative correlations were observed between rooting parameters and number of sprouted shoots per cutting. The number of shoots depended on the number of nodes per cutting. Shoot height was negatively correlated with number of shoots (Table 3).

In this study, rooting parameters in terms of the number of roots, root length, root dry weight, and rooting percentage were all positively correlated. High root numbers and root weights per rooted cuttings suggest well-developed root system which is a good indicator of field performance [11]. Planting stock with good root system would therefore confer better adaptation in the field and thus ultimately resulting in better survival and growth. The beneficial effect of long root should allow the uptake of nutrients outside the initial depletion zone [10]. The relationship between rooting parameters and shoot number sprouts of *D. melanoxylon* was found to be negatively correlated agreeing with other studies which have shown that shoot production on cuttings was negatively associated with root emergence [28].

A negative correlation between the sprouting of cuttings and their rooting is known to exist and is a common observation when propagating trees by stem cuttings [26]. For example [28] found a strong correlation relationship in *Cordia alliodora* between the sprouting parameters of cuttings and the rooting parameters. They attributed the lack of rooting to the shoot acting as a strong competing sink for assimilates. [33] working with *Milicia excelsa* also reported a negative correlation between shoot production and root emergence which in their study found that *Milicia excelsa* cuttings had a higher probability of producing a shoot and a lower probability of rooting. They hypothesized that absence of a leaf promoted shoot growth which was detrimental to rooting because it competed for photo assimilates. Shoot production therefore may be considered an indicator of metabolic activity within the cutting rather than causally related to root emergence.

Shoot sprouting and growth appeared in *D. melanoxylon* stem cuttings before root formation at the base of the cuttings. Several other studies have shown that shoot growth/flushing of buds is not necessarily a good indicator of root development [37, 36]. During vegetative propagation, early differentiation and growth of leaf buds is dependent on food reserves available in the cuttings [37, 13]. Moreover during early establishment of rooted cuttings energy allocation might be strongly biased towards roots, and therefore shoot growth may reduce root formation because of competition between

Table 3: Correlation coefficients of rooting and sprouting parameters measured for *D. melanoxylon* stem cuttings

	Rooted (%)	Root number	Root length (cm)	Root weight (mg)	Callused (%)	Sprouted (%)	Shoot number	Shoots height (cm)	Survived (%)
Rooted (%)	-								
Root number	.77(**)	-							
Root length	.76(**)	.90(**)	-						
Root weight (mg)	.79(**)	.86(**)	.84(**)	-					
Callused (%)	.46(**)	.54(**)	.44(**)	.49(**)	-				
Sprouted (%)	.06	.07	.08	.02	.01	-			
Shoot number	-.08	-.01	-.01	-.04	.01	.02	-		
Shoots height (cm)	.35(*)	.40(*)	.39(*)	.42(*)	.38(*)	.10	-.02	-	
Survived (%)	.29(*)	.18(*)	.18(*)	.18(*)	.17(*)	.28(*)	.05	.03	-

* Indicate significant at p< 0.05.
** Indicate significant at p< 0.01.

roots and shoots for nutrient reserve [11]. [7] also reported that where root formation lags behind shoot formation, survival of cuttings becomes low. Shoot production therefore may be considered an indicator of metabolic activity within the cutting rather than causally related to root emergence in stem cuttings during vegetative propagation.

Conclusion: On the basis of findings of this investigation, it is concluded that stem cutting should be collected from superior provenances for vegetative propagation of *D. melanoxylon* and the superior individuals should be identified from the superior provenance then, clonal propagation methods can be used to multiply the desired genotypes. River sand should be used as rooting media. Stem cuttings rooted in river sand will result in higher rooting percentage, root number, root lengths and root dry weights which are all essential rooting parameters of cuttings for better adaptation in the field when transplanted. Evidently, the method of vegetative propagation of *D. melanoxylon* needs to be taken into account in the promotion of this species for domestication, conservation and as an asset for clonal forestry.

ACKNOWLEDGMENTS

One of the authors, Amri, E. is grateful to the International Foundation for Science (IFS) for financial support to conduct the research project.

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