IMPROVING PROPAGATION SUCCESS OF *D. MELANOXYLON* (AFRICAN BLACKWOOD) IN TANZANIA (II): ROOTING ABILITY OF STEM AND ROOT CUTTINGS OF *DALBERGIA MELANOXYLON* (AFRICAN BLACKWOOD) IN RESPONSE TO ROOTING MEDIA STERILIZATION IN TANZANIA

[1]WBA Washa, AMS Nyomora[2] and HVM Lyaruu[2]
[1]Mkwawa University College of Education, P.O. Box. Private Bag Iringa, Tanzania
[2]University of Dar es Salaam, P.O. Box 35060 DSM, Tanzania
Corresponding author: E-mail: nyomora@amu.udsm.ac.tz

ABSTRACT

Dalbergia melanoxylon *is a plant with valuable wood in the world and therefore is over harvested for timber while its regeneration is very low. The propagation efforts by techniques such as tissue culture or mycorrhiza have not been investigated which instigated conduction of this study. Soil and cuttings were collected from Kilwa, Kilosa and Babati for rooting test, potting media and soil characterization. The results showed that fresh soil improved rooting characteristics while sterilized soil did not due to the presence of mycorrhiza in fresh soil compared to none in sterilized media. The overall rooting in fresh soil in non-mist propagator was higher, 100% for softwood and 37% for root cuttings while none rooted in the open nursery. The results revealed that there was no significant correlation between rooting and collection sites while cutting type and the soil type used significantly influenced rooting. Softwood cuttings that weighed less than 15g significantly increased rooting at ($P < 0.05$) compared to cuttings that weighed above 15g. It was recommended to propagate* Dalbergia melanoxylon *using softwood cuttings placed in a non-mist propagator and using fresh soil infected with mycorrhiza.*

Key words: *Dalbergia melanoxylon*, non-mist propagator, rooting.

INTRODUCTION

Dalbergia *melanoxylon* Guill. & Perr, also known as African Blackwood, African ebony, Zebrawood or Mpingo, belongs to Family Fabaceae, sub-family Papilionoideae. It is a flowering plant, native to the seasonally dry regions of Africa (IUCN 2008). *Dalbergia melanoxylon* heartwood is fine grained, resistant to insect attack and is one of the most valuable timbers in Africa and therefore valued for use in wood wind instruments, clarinets, oboes, and pipes (Arbonnier 2004). The foliage and pods are used as animal forage. Various parts of the tree including barks, roots and leaves have various local medicinal uses including sacred rituals. Infusion made from its roots has been reported to treat abdominal pain, hernia, gonorrhea and as abortifacient or treating complications from abortions (Bryce 1967). The bark from roots and stems is anti diarrhoea and the smoke from its burning roots is inhaled to treat headache and bronchitis (Bryce 1967). Decoction from the bark is also used for cleaning wounds (Bryce 1967).

Due to its high demand for local and commercial purposes, *Dalbergia melanoxylon* wood is currently being over harvested. The IUCN listed *Dalbergia melanoxylon* in red listing meaning that it is neither endangered nor of least concern but nearly threatened if propagation efforts on the species are not taken immediately. This status of *D. melanoxylon* is due to over harvest of the species, low regeneration

ability while the advanced propagation techniques such as use of tissue culture and inoculating with mycorrhiza have not been investigated (Readhead and Temu 1981). *Dalbergia melanoxylon* have very low seed germination, rooting ability, seed viability and seedling growth rate and very long rotation time of 70-100 years (Amri 2008, Washa 2008). Mycorrhiza, *mykós*, "fungus" and *rriza*, "roots", is a symbiotic association between a fungus and the roots of a vascular plant (generally mutualistic, but occasionally weakly pathogenic). In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in arbuscular mycorrhizal fungi, or extracellularly as in ectomycorrhizal fungi. They are an important component of soil life and soil chemistry (Harley and Smith 1983; Jackson and Mason 1984; Kanyagha 2008). The relationships are envisaged to effect bi-directional movement of moisture and nutrients whereby plants donate their carbon assimilates to the fungus and inorganic nutrients absorbed by fungus are made available to the plant. Nutrients delivered by the mycorrhizal fungi to the plant roots are metabolized by the plant leading to improved plant growth and reproduction (Borowics 2001). Established types of mycorrhiza include Ectomycorrhizae (ECM) or sheathing mycorrhizae of the classes Basidiomycetes and Ascomycetes, Vesicular arbuscular mycorrhizae (VAM) belonging to class Zygomycetes, Orchid mycorrhizae belonging to class Basidiomycetes, Ericoid mycorrhizae belonging to order Ericales and Arbutoid mycorrhizae (Ectondomycorrhizae) which are similar to ectomycorrhizae by forming sheath, but they belong to the genera Arbutus (Brundrett *et al*. 1996). Redhead and Temu 1981 reported general research on mycorrhizal association and other plant species in Tanzania but lack of specific studies on mycorrhizal association in *Dalbergia melanoxylon*.

The current research endeavored to delineate the effect of the mycorrhizal association on rooting ability of *Dalbergia melanoxylon* root and stem cuttings aiming at improving the propagation success of this species for the purpose of cultivating it and therefore contribute to rescuing it from extinction.

MATERIAL AND METHODS

Sample collection

Ten (10) *Dalbergia melanoxylon* trees approximately 1 to 2 years old and at least 20 meters apart were selected in each of the three sites for sample collection. A total of 420 different cuttings types namely 100 softwood, 100 semi-hardwood and 100 hardwood cuttings measuring 20cm long each, as well as 120 root cuttings measuring 10cm long each were collected. Root cuttings were taken from the top soil within 15 cm of the tree root-soil zone. Roots were traced from their attachment to the stem before severing them from their stems using a machete. One hundred (100) roots were used for rooting tests while 20 roots were reserved for fungi isolation for the other experiment. A total of 120 kg of soil sample from soil rhizospheres were collected within 5mm from their roots (Table 1). One hundred (100) kg of this soil was used for potting medium in non-mist propagator and the open nursery while 20 kg of this soil was reserved for fungi isolation and sterilization. The same number of cuttings and as much soil were sampled for use in the open pots for comparison. All samples were secured in cool boxes maintained at 5^{o}C and transported to Botany Department of the University of Dar Es Salaam for rooting tests.

Table 1: Sampling distribution

	Provenances		
Parameter	**Kilwa**	**Kilosa**	**Babati**
#Softwood	34	33	33
#Semi-hardwood	33	34	33
#Hardwood	33	33	34
#Root cuttings	40	40	40
Soil rhizosphere	40kg	40kg	40kg

Experimental set-up and parameters assessed

The experiment was set in a split-split plot design whereby provenances from the 3 sites were assigned to main plots, the potting media status (non- inoculated versus inoculated) was assigned to sub plots while cutting types were designated to the sub-sub plots. Each main plot used soil from its respective site. Fresh soil mined from the experimental sites and used without sterilization was considered as inoculated media while similar soil but heated in an oven at 120 ^{0}C for 24 hours was used as non- inoculated. Sub samples (20kg) of soil were reserved for isolation and identification of mycorrhizae. Two non-mist propagators were used; one for the stem cuttings and the other for the root cuttings. Non-mist propagator provides optimum conditions of the rooting media and protects the rooting media from rain, wind and pests.

Treatment of cuttings was done according to Magingo *et al*. (2001) and that of the soil was done according to (Allen 1989). The stem cuttings were inserted in the medium at a depth of 20mm while root cuttings were inserted in the medium at a depth of 15mm as described by (Leakey 1990). Records on performance were taken once a week and at every moment of opening the propagator, a thin spray of water was applied to maintain high and constant humidity. Parameters observed included sprouting and callus percentage, percentage rooting, temperature and humidity measured by hygrothermometer, seedling height, number of branches measured by counting, root length, fresh and dry weight, number of roots as well as percentage of dead cuttings. To protect cuttings from fungal attack, a fungicide was occasionally sprayed inside the propagators. Rooting of cuttings was monitored for 3 months. Cuttings in the open space were raised in similar rooting media and watered using 2000ml/20L pot/day for the same period of 3 months.

Data analysis

The data was analyzed according to standard procedures as described by (Zar 1988) using SPSS and Graph Padprism4 software. Analysis of variance ANOVA was computed to test the significance of treatments while the significant differences between treatment means were assessed using Duncan's multiple ranges Test and Dunnett's Multiple Comparison Test.

Soil analysis

Soil samples from the three sites were prepared and analyzed according to (Allen 1989) whereby the physical characteristics were determined using sieve and pipette method; exchangeable cations were determined using 1M Ammonium acetate (pH 7) extractions followed by determination by flame photometer and AAS. Calorimetric determination was used to analyze Total Nitrogen (TN) and PO_4^-. While TN was determined using Kjeldhal digestion followed by calorimetric indophenol blue titration method, PO_4^- was determined using ascorbic acid method. Organic matter (OM) was determined using Rapid dichrometry oxidation technique. A pH meter electrode was used to measure pH while an electro conductivity meter was used to measure EC.

RESULTS

Characteristics of potting media

The results show that physical and chemical characteristics of fresh and sterilized soils from Kilwa, Kilosa and Babati Districts

were similar (Table 2). With assistance of the soil triangle chart, the soil texture was assessed as sandy clay loam. It was found that the soil characteristics namely soil pH, N, $P0_4$, OM, CEC, EC, Na^+, K^+, Ca^{2+}, Mg^{2+}, soil texture, aeration porosity, water holding capacity and total porosity were all the same in the two soil treatments. On contrary the amount of organic matter differed with Kilwa and Kilosa having 5% each while Babati registered only 2.6% organic matter. Organic matter content between fresh and sterilized soil from respective sites did not differ significantly. This can be supported by (Fernandez *et al*. 1997) findings who observed that below 150^{o}C there was no effect on forest soil organic matter but at a temperature of 220^{o}C at least 37% of the forest organic matter was lost. The pH of soil from the three sites was around 8 implying that it was alkaline. In a related study (Washa *et al*. 2012a) micro organisms, namely mycorrhiza were isolated in fresh soil samples but not in sterilized soil sample.

Table 2: Characteristics of the potting media

	Parameter	**Sites**		
		Kilwa	**Kilosa**	**Babati**
Chemical characteristics	Soil pH	7.80	7.90	8.08
	N^{-1} (%)	0.42	0.41	0.23
	$P0^{3-}_4$ (%)	0.011	0.013	0.035
	OM (%)	5.52	5.16	2.58
	CEC (meq./100g)	53.14	38.42	63.76
	EC($S{\cdot}m^{-1}$)	101	186.1	118
	Na^+ (meq./100g)	1.32	1.16	1.44
	K^+ (meq./100g)	4.26	3.19	4.17
	Ca^{2+} (meq./100g)	9.14	6.75	11.26
	Mg^2 (meq./100g)	6.4	4.26	8.12
Physical characteristics	Sand (%)	45	75	53
	Silt (%)	17.7	10.0	17.0
	Clay (%)	37.3	15.4	30
	Texture	Sandy clay loam	Sandy clay loam	Sandy clay Loam
	Aeration porosity	5.0	5.02	5.01
	Water holding capacity	50.0	80	58.0
	Total porosity	32	33.3	33.3

[z] There was no difference in Physical-chemical properties of fresh and sterilized soil media except that microorganisms were found in the fresh and not the sterilized soil media. **NB:** (meq. /100g) = milliequivalent per 100 g, ($S{\cdot}m^{-1}$) = siemens per metre.

Proportion of rooting, callusing and sprouting under different treatments

The overall rooting ability was higher in the non-mist propagator than in the open nursery. At least 34% of the 400 cuttings set in the non mist propagator rooted while none rooted in the open nursery. This includes 100% of the softwood cuttings, 37% of the

root cuttings and none of the semi-hardwood and hardwood cuttings set in the non-mist propagator rooted. About 54% of the 400 cuttings in the non mist propagator developed callus whereas only 38% of the 400 cuttings set in the open nursery did so. Likewise, 74% of cuttings in the non-mist propagator sprouted as opposed to only 38% in the open nursery (Table 3). On the other hand, significant differences in rooting percentage was found between cutting types and between media treatments, that is, 100% for softwood and 37% root cuttings in the non-mist propagator rooted while none of the hardwood and semi-hardwood in the non mist propagator and the open nursery rooted (Table 3). No significant differences in rooting percentage was found between different populations which averaged 1.75%, 1.60% and 1.50% for Kilosa, Babati and Kilwa provenances respectively (Tables 4).

Table 3: Rooting characteristics recorded 3 months after beginning of the experiment (P=non-mist propagator, O = in the open pots)

	Rooting parameters in %					
Cutting type	**Rooting in P**	**Rooting in O**	**Callus in P**	**Callus in O**	**Sprouting in P**	**Sprouting in O**
Softwood	100	00	50 ±4	40 ±3	50 ±9	40 ±3
Semi-hardwood	00	00	50 ±4	40 ±3	90 ±9	40 ±3
hardwood	00	00	50 ±4	40 ±3	85 ±9	40 ±3
root	37	00	67 ±4	30 ±3	70 ±9	30 ±3

Table 4: The proportion of rooting parameters in response to different treatments

Media	**Rooting parameter**	**Kilosa**	**Babati**	**Kilwa**	**Mean**
Inoculated	Rooting status (%)	1.73	1.60	1.50	1.30 ±0.42
	No. roots/cutting	0.95	0.91	2.01	1.30 ±0.50
	Root lenght (cm)	0.94	1.02	8.22	3.37 ±0.52
	No. branches/cutting	1.30	1.17	2.30	1.59 ±0.32
Non-inoculated	Rooting status (%)	1.62	1.75	1.64	1.67 ±0.40
	No. roots/cutting	0.97	0.95	1.05	0.99 ±0.32
	Root length (cm)	1.02	1.06	2.50	1.52 ±0.34
	No. branchs/cutting	1.47	0.98	1.72	1.39 ±0.41

There was a significant difference at $P<0.05$ in sprouting percentage between cutting types and media treatments where 50% of softwood cuttings, 90% of semi-hardwood cuttings, 85% of hardwood cuttings as well as 70% of root cuttings in the non-mist propagator sprouted while only 40% softwood, semi-hardwood and hardwood cuttings and 30% of root cuttings in the open nursery sprouted (Table 3). Callusing did not differ significantly since 50% of softwood cuttings, semi-hardwood and hardwood cuttings in the non-mist propagator and 40% of all cutting types in the open nursery callused. About 67% of root cuttings in the non-mist propagator callused and only 30% roots cuttings in the open nursery developed callus. Rooting, callusing, and sprouting percentage were relatively higher in cuttings placed in non-mist propagator compared to those placed in the open nursery (Table 3)

Number of roots per cutting

There was a significant difference at P<0.05 in the number of roots that developed between cutting types treatments. Cuttings in the open nursery did not root at all. Cuttings that rooted in the non-mist propagator portrayed an average number of 3.84 of in softwood stem cuttings compared to only 1.20 roots in root cuttings. None of semi-hardwood and hardwood stem cuttings rooted (Table 5). The number of roots per cuttings sampled from different populations differed from an average of 2 roots in Kilwa provenance compared to an average of 0.9 roots from Kilosa and Babati populations (Table 4).

Seedling biomass

There was a difference in seedling weight between different cutting types in inoculated and non-inoculated media but not statistically where softwood cuttings weighed 25.22gm in inoculated and 26.95gm in non-inoculated soils respectively (Tables 6). For semi-hardwood and hardwood cuttings, the differences were also not statistically significant in the non-inoculated media. There were no significant differences in seedling biomass between provenances from the 3 sites studied.

Table 5: Effect of cutting type on rooting characteristics in the non-mist propagator

Cutting type	Cutting initial wt(gm)	Seedling fresh wt(gm)	Seedling dry wt (gm)	#roots/ cutting	Root length (cm)	Rooting status (%)	Seedling height (cm)
Soft wood	7.5	15.2	7.7	3.8	4.3	1.0	32.1
Semi hardwood	33.3	34.9	17.6	0	0	2.0	23.1
Hard wood	34.1	33.5	16.7	0	0	2.0	23.1
Root cuttings	19.7	20.8	10.3	1.2	9.1	1.6	18.1
Std error	0.4	0.4	0.2	0.0	0.2	0.0	0.3

Table 6: Pair wise comparisons between rooting parameters as affected by the potting media in the non-mist propagator recorded during rooting test and after rooting test

Parameter	Fresh soil	Sterilized soil
Initial cutting wt(gm)	22.1	25.2
Seedling final fresh wt(gm)	25.2	26.9
Seedling dry weight (gm)	12.8	13.4
Rooting percentage	1.6	1.7
No. of roots/cutting	1.6	0.9
Seedling root length (cm)	5.8	0.9
Seedling height (cm)	25.8	22.4

Seedling height and Root length

There was a significant difference at P<0.05 in seedling height between different media treatments and between cutting types (Tables 6 and 7). Fresh soil resulted in growth of 25.8cm height and only 22.4cm in sterilized soil. Softwood grew to a height of 32.11cm, none in semi-hardwood, and hardwood while root cuttings resulted in an average of 18 cm tall seedlings. Plant height was measured from the pot level to the top of the plant and not just the new growth. Root length from different treatments showed significant difference at P<0.05 (Tables 6 and 7). For example, roots in fresh soil media were 5.76cm long while those

from sterilized soil were only 0.90cm long. Softwood and root cuttings from fresh soil were 4.35cm and 9.13cm tall respectively. Root length also varied with population at $P<0.05$ whereby seedlings from Kilwa had the longest roots of 8.22cm compared to Babati and Kilosa provenances that had only 1.00cm and 0.9cm long roots respectively (Table 6).

Table 7: Comparison test analysis results
Dunnett's Multiple Comparison Test between fresh and sterilized soil rooting parameters

	Dunnett's Multiple Comparison Test	**Mean Diff.**	**q**	**P value**	**95% CI of diff**
Fresh soil	treatment (inoculated) vs status	0.25	0.7139	P > 0.05 **NS**	-0.6289 to 1.129
	treatment (inoculated) vs # root	1.116	3.182	P < 0.01 *	0.2356 to 1.996
	treatment (inoculated) vs root length	1.02	2.91	P < 0.05 *	0.1401 to 1.900
	treatment (inoculated) vs #branch	0.73	2.085	P > 0.05 **NS**	-0.1489 to 1.609
	treatment (inoculated) vs final ht	-20.43	58.33	P < 0.01 *	-21.30 to -19.55
Sterilized soil	treatment(non inoculum) vs status	-0.565	1.127	P > 0.05 **NS**	-1.823 to 0.6934
	treatment(non inoculum) vs # root	-0.6131	1.221	P > 0.05 **NS**	-1.873 to 0.6469
	treatment(non inoculum) vs root length	-4.765	9.504	P > 0.05 **NS**	-6.023 to -3.507
	treatment(non inoculum) vs #branch	-0.7	1.396	P > 0.05 **NS**	-1.958 to 0.5584
	treatment(non inoculum) vs final ht	-24.72	49.31	P > 0.05 **NS**	-25.98 to -23.47

DISCUSSION

The fresh soils which had mycorrhiza fungi greatly influenced rooting in terms of rooting percentage, root numbers, root length and seedling biomass when used as rooting media. Soil which had been heated at 120^{o}C for 24 hours did not influence rooting performance because the sterilization process killed soil microorganisms including mycorrhizae fungi and their spores although the physical chemical characteristics of the fresh and sterilized soil used seemed to be the same. There were no differences in the chemical contents of the two soils with exceptions of organic matter in soils from Babati which was slightly low compared to those from Kilwa and Kilosa. This could have accounted for the slight difference in the rooting percentage, root numbers per cutting and number of seedling branches between the 3 provenances. All provenances grew in alkaline soil of pH 8 implying that they could have had the same ability of growing plants. Plant success in the environment depends on their ability to produce roots in large quantities, greater root length and general seedling vigour. A good rooting medium characteristic includes ability to allow good aeration, retain high moisture, does not get water logged and encourage good root development.

Large root number and mass of a plant species strongly supports it in mining through the soil resulting in high plant tolerance to drought, floods, bush fire and other environmental disturbance to an extent that a plant can inhabit a diverse of habitats. This is true for *Dalbergia melanoxylon* which is wide spread in tropical Africa from Senegal and Cote d'Ivore in the West, to Kenya and Ethiopia in the East, and extending South to South Africa. It is found in at least 26 sub-Saharan countries (Nshubemuki 1993). In Tanzania, *Dalbergia melanoxylon* is distributed in T_4, T_5, T_6, T_8 floristic regions respectively covering Shinyanga and Mwanaza (T_4), Itigi and Dodoma (T_5), Muheza and Matombo (T_6) and Mtwara (T_8).

The effect of mycorrhizal fungi on increased root length of *Dalbergia melanoxylon* which was observed in this study supports the most common method of propagation this species by root suckers regeneration from root. It is very common to see *Dalbergia melanoxylon* plants radiating almost in a single file from the mother plant that extends over 30 to 50 meters giving off a number of plantlets along their length (Munyanziza *et al*. 1994, Washa 2008). Fresh soil and root fragments from sampled *Dalbergia melanoxylon* provenances had micro organisms including mycorrhizal fungi. Mycorrhizal associations interact with the anatomical and physiological activities of their host plants by altering efficiency in nutrients acquisition, soil structure, plant-water relations, drought resistance, resistance to pathogens, and reduction in environmental pollutants thereby increasing energy production which can increase metabolic activities including meristematic cell division of the plants or cuttings as also reported by (Borowics 2001).

Active cellular activities are more concentrated near shoots and roots tips that bear meristematic cell (Borowics 2001). This could have accounted for the higher rooting percentage, root length, seedling height, and number of roots produced in softwood cuttings and root cuttings which mostly contain the meristematic cells while semi hardwood and hardwood cutting did not root. Semi hardwood and hardwood cuttings contain cells that are differentiated which have thus to de-differentiate into meristematic tissue before they can differentiate roots and this was evidenced by the high callusing. *Dalbergia melanoxylon* cuttings rooted easily and formed large root numbers, taller seedling height and root length in non-mist propagator and did not

root as easily in the open. This is because a non-mist propagator reserves high humidity which reduces evapotranspiration losses that are responsible for drying out of cuttings in open nurseries. The study also showed that softwood cuttings in the open dried out easily through loss of water because they are tender unlike the semihard, hardwood and root cuttings which could retain more water and survive by stored food in their lignified tissues.

Recommended optimum physical factors for root initiation in plant cuttings includes: 1-4 nodes, 50 -60mm long (5-6cm), leaf area of $50cm^2$, large diameter (1cm≥) and position of nodes depends on types of plants. In this research they were maintained within the recommended specifications both in non-mist propagator and the open. Leakey 1990 recommended that propagating conditions for root initiation should include moderate temperature ($30\text{-}40^0C$), moderate low light intensity, very high humidity (above 90%), suitable rooting medium and protected from rain drops, winds, pests and pathogens. To maintain these conditions, a non-mist propagator was used in this study where recommended temperature and humidity were attained as opposed to the open nursery where the mean temperature was 30^oC and humidity was 66%. Use of a non-mist propagator provides a very practical and successful system for vegetative propagation of trees.

Factors which influenced rooting

Rooting ability of cuttings in this study was affected by rooting media containing mycorrhiza, rooting environment and the cutting type. Other factors which might have affected rooting although their effects were not statistically significant included age, initial cutting weight, diameter and collecting time of the cutting. Mycorrhiza inoculated media (fresh soil) had higher ability of producing root number, increased root length and seedling height at $P < 0.01$, $P < 0.05$ and $P < 0.01$ respectively compared to non-inoculated media which was not statistically significant ($P < 0.05$). This method of inoculation has also been successfully used by Magingo *et al*. 2001 and Feng *et al*. 2002). This study has proven the importance of using a non-mist propagator of which the cutting had significant higher percentage in rooting, callusing and sprouting which were 100%, 50% and 50% respectively compared to open nursery which had 0%, 40% and 40% respectively. This is due to the ability of a non-mist propagator to maintain optimum rooting conditions and protection of the cuttings and rooting media from harsh conditions out side. Non-mist propagator has been successful used to propagate tropical trees such as *Albizia quachepele, Bombacopsis quinata, Brachystegia sp* and *Pterocarpus sp* (Leakey *et al*. 1990 and Magingo *et al*. 2001).

In this study, softwood cuttings which were leafy were the only stem cuttings that rooted in the non-mist propagator and were the first to dry and die in the open nursery. Cellular activities in a plant are more concentrated on the tips areas in which there are apical meristematic cells which are responsible for elongation of shoot and root tips. Softwood cuttings in this study also had relatively less number of nodes, diameter as well as cutting initial weight. This increased their ability to loose water in the open nursery. Highly lignified tissues in the semi-hardwood and hardwood cuttings were the reason for not rooting in the non-mist propagator since they had little cellular activities compared to root and shot tips. Open nursery was not able to meet the optimum rooting conditions that is why nothing rooted in the open pots. These findings are in agreement with findings by (Magingo *et al*. 2001). In their study they found that leafy cuttings rooted easily in a non-mist propagator and died easily in the open pots by loosing water. Semihardwood and hardwood cuttings

sprouted but did not root due to high lignifications and less meristematic cell that de-differentiate as callus prior to rooting. Callus formation is normally the common route to root formation but this final stage was not observed in this study in the open nursery and lignified stem cuttings (semi hardwood and hardwood).

Also shoot formation prior to root formation in semi-hardwood and hardwood cuttings hindered root emergence because shoots out-compete roots for available photoassimilates as well as increasing evapo-transpiration losses to the extent that the cuttings dry out and die. An appropriate season for collecting cuttings as indicated in this study is during the dry season compared to the rainy season although their effects were not statistically significant. During this study dry season samples were collected from June to August while rainy season samples were collected from January to April. The results are in agreement with findings by (Jonsen 1986) who reported that optimal seasons for rooting of cuttings were during the dormant period. This time is associated with the lowest metabolic activity such that reserved photoassimilates in the tissue can be preferentially utilized by the rooting processes unlike the rainy season during which there are other active metabolic processes.

Effect of soil nutrients and characteristics on rooting of the cutting

The agreed optimal soil characteristics value for root development include loam soils, sandy loam and clay loam soil texture, soil pH between 5.5-7.1, soil EC between 0-400µscm^{-1}, soil organic matter > 50%, soil CEC > 20 meq./100g, soil Na^{+} between 0-13%, soil phosphorous > 4 mg/100g and soil total nitrogen > 1.5% (Jaenicke 1999). In this study, pH was higher (7.8-8.08) while CEC and EC were low (38-64 meq/100g for CEC and 101-186µscm^{-1} for EC). For example Kilwa was a site with the highest level of soil total nitrogen of 0.42% which is a poor soil compared to the optimal nitrogen value in the soil. Babati site had the highest soil phosphorus level of 0.04 mg/100g which is very low compared to the optimally value; likewise, soil organic matter that ranged from 2.58-5.52%. Such poor soil cannot sustain rooting without amendments and this explains why fresh soil which had mycorrhiza fungi gave relatively higher performance than sterilized soil.

CONCLUSION AND RECOMMENDATIONS

It is possible to propagate *Dalbergia melanoxylon* by seedlings produced from stem and root cuttings in the nursery in order to initiate small and large scale plantations of the species in Tanzania and rescue it from extinction. This study recommends propagating *Dalbergia melanoxylon* using mycorrhizal fungi incorporated in the soil in order to maximize rooting ability. In order to enhance natural regeneration of *D. melanoxylon*, there is a need to restore mycorrhizal fungi by re-inoculating the sites so as to facilitate better growth and rehabilitation. Induction of mycorrhizae using other types of inoculum rather than use of soil containing infected roots could also be tried to quantify the effects of mycorrhizae on the *D. melanoxylon* cuttings for the purpose of replanting and regeneration. Use of softwood cuttings weighing between 2g to 15g which rooted easily in the non-mist propagator should be adopted. As the growth rate of this species is slow, it is also recommended to embark on research on agronomic manipulation aimed at speeding up the growth rate of the species in order to attain a harvestable size within the shortest possible time.

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