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Full Length Research Paper

Vegetative propagation of *Warburgia ugandensis* Sprague: An important medicinal tree species in eastern Africa

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***Warburgia ugandensis* is an important medicinal tree species whose bark is widely harvested for its valuable anti-bacterial and anti-fungal properties. Consequently, this tree species is considered threatened. Therefore, this species is ranked as one of the priority species for management and conservation. This study investigated an appropriate technique for propagation of *W. ugandensis* using stem cuttings. Three types of stem cutting (hardwood, semi-hardwood and softwood) were obtained from Mabira Forest Reserve, Uganda and propagated in a factorial experiment using non-misting tunnels. Data on callusing root and shoot formation, number and length of roots and shoots were collected over a period of 93 days. Data manipulation was done by employing general linear model analysis of variance and Chi - square tests. There was significant variation ($p < 0.05$) in callus formation, root and shoot development, number and length of roots and shoots for different stem cutting types. The highest percentage of callusing, rooting and shoot regeneration (46, 49 and 57%) was recorded in softwood cuttings which also produced the highest number and longest roots and shoots. Successful propagation of *W. ugandensis* can therefore be appropriately achieved through softwood stem cuttings rather than either hardwood or semi-hardwood cuttings.**

Key words: *Warburgia ugandensis, v*egetative propagation, Mabira forest reserve, Canellaceae.

INTRODUCTION

Warburgia ugandensis Sprague (Canellaceae) is a luxurious canopy level tree species of east Africa's semi-deciduous and moist natural forests. The tree is useful for timber for building and furniture, anti-fungal and anti-bacterial medicine (bark, roots, young twigs), mulch, green manure, shade, ornamental, resin and food seasoning (Mbuya et al., 1994; Katende et al., 1995). The leaves and seeds are used to add flavor to curries and for livestock fodder while the fruits are edible with a hot peppery taste (ICRAF, 2009). Other products from this tree include soap, toothbrushes, veterinary medicine, insecticide, firewood and charcoal (Maundu and Tengnas, 2005). The wood has high oil content and burns well with an incense-like smell while the gum/resin is used locally as glue to fix tool handles (Katende et al., 1995).

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Table 1. Distribution of the different types of stem cuttings to the three rooting media and IBA concentration.

Rooting media	IBA concentration (% w/w)	No. of softwood	No. of semi hardwood	No. of hardwood	Sub total
Milled pine bark	0.8	45	45	45	135
	0.6	45	45	45	135
	0.3	45	45	45	135
	Control	45	45	45	135
Sand	0.8	45	45	45	135
	0.6	45	45	45	135
	0.3	45	45	45	135
	Control	45	45	45	135
Soil	0.8	45	45	45	135
	0.6	45	45	45	135
	0.3	45	45	45	135
	Control	45	45	45	135
Total		540	540	540	1620

W. ugandensis is highly esteemed for its valuable pharmaceutical properties (Olila et al., 2002; Wamalwa et al., 2006). This tree species has been rated as second highest priority medicinal plant species in Kenya for detailed study (Wamalwa et al., 2006). According to FAO (1986) and Wamalwa et al. (2006), dried bark of *W. ugandensis* is commonly chewed and the juice swallowed as a remedy for stomachache, constipation, toothache, cough, fever, muscle pains, weak joints and general body pains.

The fresh roots are boiled and mixed with soup for the prevention of diarrhorea; leaf decoction baths are used as a cure for several skin diseases. The bark, roots or leaves can also be boiled in water and the decoction drunk to treat malaria (Katende et al., 1995; ICRAF, 2009). A cytoxic sesquiterpine (characterized as muzigadial) has been isolated from *W. ugandensis* and used to treat trypanosomiasis (Olila et al., 2002) and other parasitic diseases (Kioy et al., 1990) in animals.

Although, the tree is very valuable and provides many products, its availability is on the decline. It has been widely harvested and is now difficult to access (Katende et al., 1995; Amani, 1997). Thus, one way of ensuring conservation of the tree is promoting its propagation and domestication. However, although preliminary studies indicate that *W. ugandensis* has a very high seed germination rate (Mbuya et al., 1994; Katende et al., 2000), the seeds of this tree are highly recalcitrant. Apart from the foregoing, there is paucity of literature related to vegetative propagation of *W. ugandensis*. This study therefore aimed at ascertaining the most appropriate propagation *W. ugandensis* using stem cuttings.

MATERIALS AND METHODS

Experimental design

The experiments were carried out in non-misting propagation tunnels which were constructed under a shade net of 10% heat intensity penetration. The tunnels were constructed using white 0.5 mm polythene sheets, 4 cm wide wooden frames, connector aluminium wires and gravel. The experimental set-up was a 3 × 3 × 4 factorial treatment structure in a complete randomized design (Jeruto et al., 2008). Forty five stem cuttings of each type (hardwood, semi-hardwood and softwood) were treated with varying concentrations of Indole-3-butryic acid (IBA) (0, 0.3, 0.6 and 0.8%) and planted in three types of rooting substrate (milled pine bark, top soil and sand) (Scalabrella et al., 1983) (Table 1). Each set of hormone treated stem cuttings were replicated three times. In order to avoid bias, each treatment (hormone treated stem cuttings) was allocated randomly to each rooting media by use of a table of random digits (Johnson and Bhattacharyya, 2006).

Preparation of rooting substrate

Top soil and sand were pasteurized when moist for 30 min by the open air heat method and cooled (Kester et al., 1990; Larsen and Guse, 1997) while milled pine bark was fumigated with Dithane M-45® anti-fungal powder at 2 000 ppm according to manufacturer's instructions. The different rooting substrate were then laid over gravel in the non-mist propagators, watered and then covered with a 0.5 mm thick white polythene sheet (Jeruto et al., 2008).

Preparation of stem cuttings

The three types of stem cuttings were chosen according to Evans and Blanzich. (1999) and Agbo and Obi, (2007). These were: hardwood (mature leafy twigs with no obvious signs of active growth); semi-hardwood (partially mature twigs of the current

Figure 1. Shooting and rooting performance of *Warburgia ugandensis* cuttings under nursery conditions (Photos by Florence Akwatulira).

season's growth with reasonably firm wood and mature leaves) and softwood (young and succulent stems with new growth). A total of 1620 stem cuttings (540 of each type) were collected from coppices of selected *W. ugandensis* tree populations in Mabira forest, central Uganda. The bases of all the cuttings were squared by use of a sharp pair of scissors to avoid one-sided rooting. Leafy softwood and semi-hardwood stem cuttings were trimmed to 7.5 cm long while hardwood cuttings were cut to 10 cm long. Each selected cutting had two or more lateral buds and leaves (Kester et al., 1990). The cuttings were collected only in the morning hours to reduce on desiccation and kept moist/cool at all times by placing them in cool boxes (Agbo and Obi, 2007).

The cuttings were dipped in water and treated with different levels of IBA hormone to a length of 1 cm (Kester et al., 1990) and then stuck into each type of rooting media at a depth of between 2.5 and 5 cm. The rooting media were then slightly compacted around the bases of the cuttings, watered and covered with a thick polythene sheet over the wooden frames (Kester et al., 1990). The purpose of the polythene sheet was to maintain high humidity and reduce water loss from the cuttings within the chamber (Evans and Blanzich, 1999). Dithane M45 at 2 000 ppm was sprayed on all cuttings to control fungal infections (Yeboah and Amoah, 2009). Monitoring was carried out every two days in a week to remove dead leaves and cuttings. The cuttings were also watered twice a week in order to keep the rooting media moist until the cuttings had rooted (Evans and Blanzich, 1999).

Data collection and analysis

The number of cuttings alive (callused, rooted and shooted), number and length of budded shoots, number and length of roots developed per rooted cutting for each stem cutting type in the different rooting media were recorded following Yeboah and Amoah (2009). Raw data were entered in Microsoft Excel spreadsheet and analyzed for mean number and percentages of cuttings that formed callus, developed roots and sprouted shoots for the different stem cuttings (Badji et al., 1991). General Linear Model ANOVA in MINITAB 12.22 were then performed to obtain means and standard deviations for number and length of roots and shoots; and the interactions of stem cutting type and rooting media (Moreira et al., 2009). The significant effect of each factor (stem cutting type, rooting media and their interactions) on number and length of roots and shoots were separated by Tukey's pair-wise comparisons and post hoc least significant difference (LSD) test at 5% level of significance (Moreira et al., 2009). Chi – square tests were also conducted to test whether there were significant differences in callusing, rooting and shooting success of the different stem cuttings used (Badji et al., 1991).

RESULTS

Softwood stem cuttings propagated in milled pine bark recorded the highest mean number of cuttings that formed callus, developed roots and sprouted shoots (Figure 1). None of the semi-hardwood stem cuttings propagated in top and sandy soils callused, rooted or even shooted (Table 2). The mean percentage of stem cuttings that developed callus, roots and sprouted shoots was influenced by the stem cutting types. Chi-square test showed that softwood stem cuttings significantly ($p < 0.05$) recorded the greatest mean percentage of cuttings that formed callus (46%), developed roots (49%) and sprouted shoots (57%) compared to semi-hardwood and hardwood stem cuttings (Figures 2a to c).

The number of roots developed per rooted stem cutting for the different type of stem cuttings (softwood, semi-hardwood and hardwood) were significantly ($p < 0.05$) influenced by the different rooting media. The number of roots developed per rooted softwood stem cuttings propagated in milled pine bark were significantly ($p < 0.05$) higher than softwood stem cuttings propagated in both top and sandy soil media (Table 3). On the other hand, both semi-hardwood and hardwood stem cuttings

Table 2. Callusing, rooting and shooting success of stem cuttings of *W. ugandensis* in the different rooting media (N = 180).

Rooting media	Stem cutting	Number of callused, rooted and shooted cuttings			
		Minimum	Mean ± SD	Maximum	SE
Milled pine bark	Softwood	28	31.2±3.1	39	2.6
	Semi hardwood	03	04.2±3.6	09	2.8
	Hardwood	00	02.8±0.5	04	0.1
Top soil	Softwood	08	08.2±2.4	18	0.5
	Semi hardwood	00	00.0±0.0	00	0.0
	Hardwood	00	02.5±0.9	05	0.2
Sand	Softwood	00	01.8±0.5	04	0.1
	Semi hardwood	00	00.0±0.0	00	0.0
	Hardwood	00	01.8±0.5	04	0.1

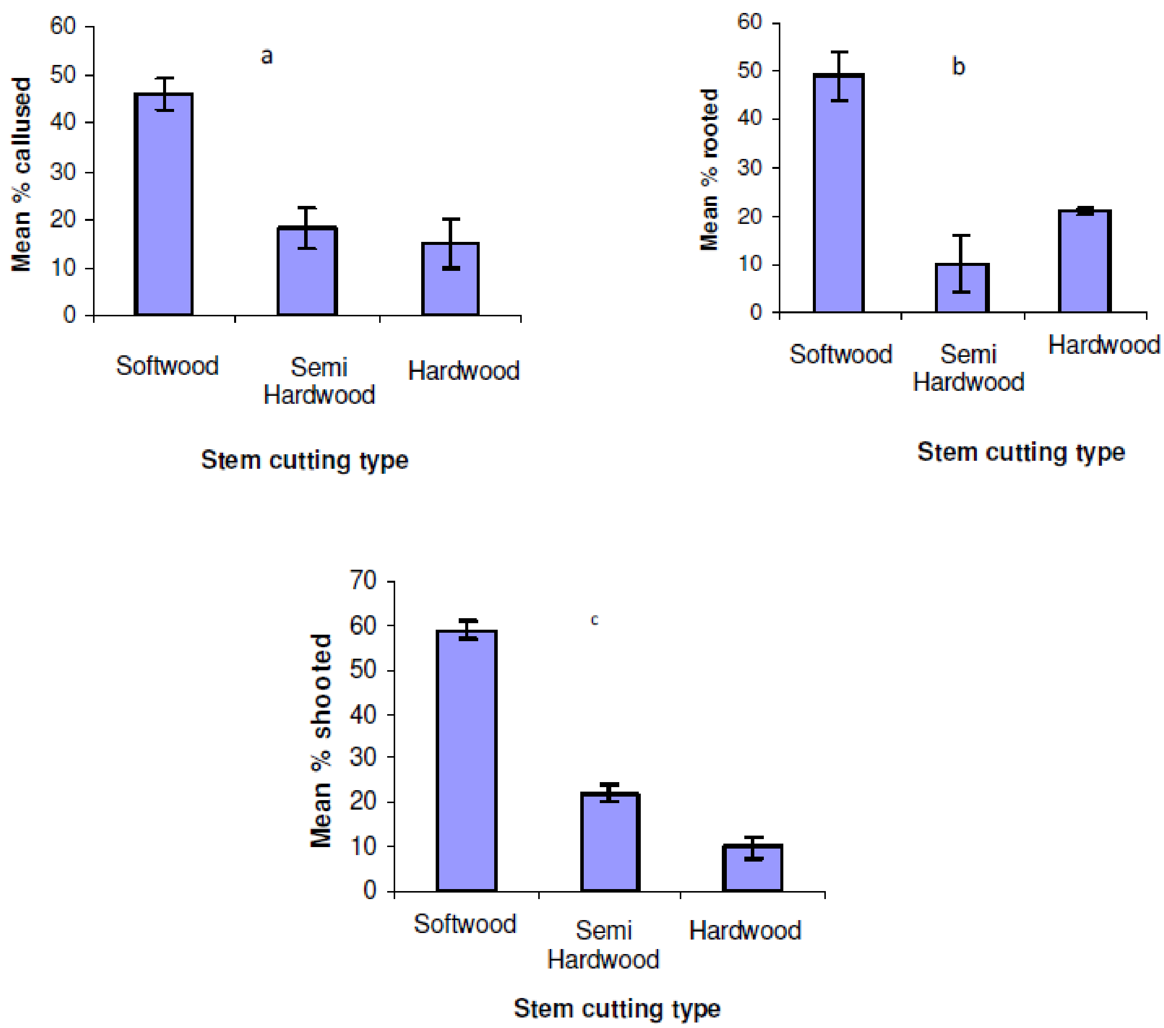

Figure 2. Mean percentage callusing, rooting and shooting of the different *W. ugandensis* stem cuttings. (a). Callusing (b). Rooting and (c). Shooting.

Table 3. Interactions between stem cutting type and rooting media on number and length of roots and shoots of stem cuttings. Numbers in each column represent mean number of stem cuttings alive after 93 days (Means of the same superscript on the same column are not significantly different at p < 0.05), using Turkey's pairwise comparison (LSD). NS = Not significant.

Rooting medium	Stem cutting	Mean±standard Error			
		Number of roots	Root length (cm)	Number of shoots	Shoot length (cm)
Milled pine bark	Softwood	3.24 ± 0.33^a	0.16	2.10 ± 0.40^a	2.12 ± 0.50^a
	Semi hardwood	0.70 ± 0.10^b	0.04	0.16 ± 0.30^b	0.25 ± 0.10^b
	Hardwood	0.06 ± 0.02^b	0.08	0.05 ± 0.10^b	0.12 ± 0.20^b
Top soil	Softwood	0.06 ± 0.02^b	0.09	0.06 ± 0.01^b	0.05 ± 0.01^b
	Semi hardwood	0.06 ± 0.01^b	0.00	0.00 ± 0.00^b	0.00 ± 0.00^b
	Hardwood	0.06 ± 0.02^b	0.00	0.00 ± 0.00^b	0.00 ± 0.00^b
Sand	Softwood	0.05 ± 0.01^b	0.12	0.04 ± 0.01^b	0.06 ± 0.02^b
	Semi hardwood	0.06 ± 0.01^b	0.03	0.09 ± 0.03^b	0.02 ± 0.01^b
	Hardwood	0.03 ± 0.01^b	0.03	0.03 ± 0.01^b	0.05 ± 0.10^b
LSD (P<0.05)		2.9	NS	1.8	2.1

Table 4. Variation in the mean number and length of roots and shoots of *W. ugandensis* stem cuttings. Numbers in each column represent mean number of stem cuttings alive after 93 days. Means of the same superscript in the same column are not significantly different at p < 0.05 using Turkey's pairwise comparison (LSD).

Treatments	Mean ± standard error			
	Number of roots	Root length (cm)	Number of shoots	Shoot length (cm)
Softwood	2.15 ± 0.28^a	1.47 ± 0.12^a	1.52 ± 0.15^a	2.4 ± 0.24^a
Semi hardwood	0.25 ± 0.13^b	0.15 ± 0.07^b	0.52 ± 0.12^b	0.8 ± 0.20^b
Hardwood	0.08 ± 0.07^b	0.02 ± 0.0^b	0.1 ± 0.06^b	0.24 ± 0.14^b
LSD (P<0.05)	2.13	1.29	0.67	0.65

propagated in all rooting media (milled pine bark, top and sandy soils) were not significantly different ($p > 0.05$). These media recorded the least number of roots which developed per rooted stem cutting. The length of roots developed per rooted stem cutting for all the stem cutting types and rooting media were also not significantly ($P> 0.05$) affected by the various rooting media used (Table 3).

The number of shoots that sprouted for the different stem cuttings were significantly ($p < 0.05$) influenced by the type of rooting media. There were more shoots developed per sprouted stem cutting for softwood stem cuttings propagated in milled pine bark compared to those propagated in top and sandy soil media. Only a few shoots sprouted for both the semi-hardwood and hardwood stem cuttings propagated in all types of rooting media. The length of shoots per sprouted stem cutting were also significantly ($p < 0.05$) influenced by the different rooting media. Softwood stem cuttings propagated in milled pine bark gave the highest mean length of shoots per sprouted stem cutting as opposed to softwood cuttings propagated in both top and sandy soils media (Table 3).

The number of roots/ shoots and length of roots/ shoots recorded per rooted and sprouted stem cutting was also influenced by the different stem cuttings (softwood, semi hardwood and hardwood). Softwood stem cuttings significantly ($p < 0.05$) developed more roots/shoots and longer roots and shoots per rooted and sprouted stem cutting respectively than semi-hardwood and hardwood cuttings. Semi-hardwood and hardwood cuttings developed almost similar number of roots/ shoots and length of roots and shoots that developed per rooted and sprouted stem cutting (Table 4).

DISCUSSION

The highest percentage of *W. ugandensis* cuttings that formed callus and developed roots were recorded by the softwood stem cuttings (Figure 1). Number of roots of *W. ugandensis* that developed from the rooting of stem cuttings was also affected by stem cutting type. Softwood stem cuttings produced higher number of roots than semi hard and hardwood stem cuttings and also gave the longest roots. These differences among soft, semi hard and hardwood stem cuttings could be due to high concentration of endogenous root promoting substances that are present in the softwood stem (apical) cuttings. According to Hartmann et al. (2002) and Hintsa (2005), endogenous substances usually arise from terminal buds which also have “more cells” capable of becoming meristematic. Thus, the production of endogenous substances may have helped the softwood stem cuttings of *W. ugandensis* to produce more and longer roots as compared to semi-hard and hardwood stem cuttings. These findings are also in agreement with the previous work of Elkhalifa (1990) and Elnour et al. (1990) on vegetative propagation of tropical trees in which softwood stem cuttings also recorded the highest rooting percentages.

The higher number and longer roots produced by softwood stem cuttings compared to the semi-hard and hardwood stems could also be due to the presence of high free auxins, sugars, and free phenols and other nutrients in the softwood stem cuttings (Agbo and Obi, 2007; Yeboah and Amoah, 2009). According to Yeboah and Amoah, (2009), plant substances such as auxins, sugars and free phenols are responsible for root initiation and deactivation of indolacetic acid (IAA) oxidases.

Differences in performance of rooting among softwood, semi-hard and hardwood stem cuttings in which softwood stem cuttings resulted into higher mean percentage of cuttings that developed roots compared to semi-hard and hardwood stem cuttings may also have been due to variations in lignifications down the stem. According to Leakey and Storeton-west, (1992), there are gradients in stem morphology such as secondary thickening and lignifications, starch, sugar content and the content of plant growth regulators and rooting co-factors that always decrease with increase in stem lignification. For instance, a study on cutting respiration rate per grain dry mass in *Prosopis juliflora* indicated that starch, sugar, plant growth regulators and rooting co-factors decreased down the stem. According to Dick et al. (1994), the decrease could be due to increased lignifications of older tissue.

It has been reported by Jacobs (1979) that softwood cuttings are rich in endogeneous auxins which decrease with increase in distal distance from the apices. According to Palanisamy and Kumar (1997), cuttings from the distal end of *Azadirachta indica* (Neem) branch rooted better than those from the middle or proximal end implying that endogenous auxin levels could be greatest at the distal end, decreasing down the proximal end. The lowest mean percentages for semi-hard and hardwood stem cuttings that rooted could have been due to low phenolic levels that have been reported to be responsible for causing a reduction in the mean percentage of rooted cuttings of the semi hard and hardwood stem cuttings (Hartmann and Kester, 2002).

Conclusions

The study provides preliminary results concerning vegetative propagation of *W. ugandensis* by stem cuttings. Softwood cuttings appear to be the most appropriate vegetative propagation candidate parts for rooting *W. ugandensis*. While mass propagation and on - farm planting of *W. ugandensis* can be promoted by using softwood stem cuttings as a source of planting materials, tissue culture using roots and leaves of *W. ugandensis* should also be tried as another means of mass production. This can be helpful in a case where the proper source of softwood stem cuttings for rooting *W. ugandensis* cannot be easily located.

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