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Endangered macro propagation of medicinal plant, *Strychnos henningsii* (gilg), (loganiaceae) for sustainable conservation

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Strychnos henningsii **(Gilg), (Logoniaceae), is an important medicinal plant that is facing threat of extinction in Kenya owing to indiscriminate and unsustainable harvesting in the wild. The wide scale use of this species resulted to its over-exploitation by the herbalists, hotels, restaurants owners and the local people. Effects of growth hormones, rooting substrates, leaf area and nodal position on stem cuttings of *S. henningsii* were investigated. Six hundred and sixty nodal cuttings were obtained from Kabiruini forest in Central Kenya. Uniform cuttings from two different nodal positions and with different lamina area were planted in three different media and treated with different auxins. Cuttings were assessed for percentage survival, percentage rooting, number of roots per cutting and the length of the longest root per cutting. ANOVA was carried out on the data collected and LSD at 5% probability level used to compare significantly different means. Mean percentage survival of 67.31% was recorded with whole leaf cuttings from the apical node. The highest mean value of 5.86 in the number of roots per rooted cutting was recorded with Seradix 2 powder. Leaf size had a highly significant effect on the rooting of cuttings as whole leaf rooted better than half leaves.**

Key words: Auxins, threatened, medicinal plant, *S. henningsii*, over-exploitation.

INTRODUCTION

Strychnos henningsii (Gilg), local names: Koffiehardepper (Afrikaanas), Muteta (Kikuyu/ Kamba), Maset (Kipsigis), Entuyesi (Maasai), Muchimbi (Meru), Kapkamkam (Pokot), Nchipilikwa (Samburu), Turukukwa (Tugen) and Yapolis (Turkana) (Maundu and Tengas, 2005), is a member of the family *Logoniaceae.* The common names are Red bitter berry (English) (Gachathi, 2007), Henning's Strychnos (Maundu and Tengas, 2005).

The species is an indigenous and threatened plant species in Kenya. It is an erect, much brunched forest tree with dark green flossy leaves. It is a native of Angola, Kenya, Mozambique, South Africa, Swaziland, Tanzania and Uganda.

In East Africa, *S. henningsii* is used in the preparation of fatty-meat and milk soups (Chapman et al., 1997). Roots, stem and bark are boiled in soup for fitness, painful joints and the general body pains among the Kikuyu, Maasai and Kamba communities (Palgrave, 1988; Beentje, 1994; Maundu et al., (1999); Gachathi, 2007). The soup is claimed to be an aphrodisiac and is used as a remedy for colic, to relieve nausea and treat syphilis (Palgrave, 1988).

In the African traditional medicine, it is used for the treatment of various ailments including rheumatism, gastrointestinal complications and possibly of value in dy-

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smenorrhoea (Hutchings, 1989; Watt and Breyer, 1962; Pujol, 1993; Hutchings, 1996). The roots bark and green fruits of *Strychnos* species are used as a remedy for snakebites (Tits et al., 1991; Ben-Erik Van wyk et al., 1997). The bark decoction is employed as a remedy for rheumatism and arthritis (Palgrave, 1988; Beentje, 1994). The ground bark is a mouth antiseptic and applied onto wounds in cattle and horses to hasten healing (Gachathi, 2007). The fruits and the bark contain a poisonous bitter alkaloids; the bark is used in traditional medicine as a purgative (Palgrave, 1988; Noad and Birnie, 1989). Some alkaloids have been used in anesthesiology due to their muscle relaxing effects (Bruneton, 1995). Mbeere people use the fruits to flavor their beer (Maundu et al., 1999; Gachathi, 2007). *S. henningsii* has a potential in the development of new antinociceptive and antispasmodic drugs (Tits et al., 1991).

Its valued timber is dark gray, heavy, hard, durable and termite resistant. Wood is used for fencing and making hut poles and tool handles by Maasai community (Maundu and Tengäs, 2005; Gachathi, 2007). The species is important in protecting soils from water erosion in highland areas. Its physical attributes, shiny foliage, pleasant shade and fragrant flowers make it a suitable choice for gardening (ICRAF, 1992).

With the alarming increase in over-exploitation and destruction of natural forest in search of these traditional herbs, the future of trees is on-farms. A classic example of such species is *S. henningsii* and in fact it has been report as disappearing in Mwingi areas of Eastern Kenya (Musila et al., 2004; Schmeltzer, 2008).

Due to the scarceness of populations of *S. henningsii,* the development of a vegetative method of propagation using cuttings is a priority. In this context, a true-to-type copies of relict individuals will be achieved, and therefore avoid the extinction of small local populations and loss of their genetic diversity.

As demand for medicinal plants continue to accelerate, species preservation is perceived to depend on sustainable methods and cultivation (Njoroge, 2010). Cultivation of medicinal plants species may be the only solution for their rapid conservation (Lange, 1998). In Asia, more and more medicinal plants are being depleted some becoming endangered hence cultivation is being viewed as a viable alternative source of these resources despite the challenges in *ex-situ* management strategies (Bisht et al., 2006; Sher et al., 2010).

There is, therefore, the need to domesticate and introduce these useful forest species to agro-ecosystems in order to prevent their extinction. Leaky and Simons (1998) noted that the development of vegetative propagation techniques represents the first step in the process of domestication of a tree species. Attempts made to establish large plantations of some forest tree species have showed little or no success due to inadequate silvicultural knowledge about the mode of planting, soil type, suitable nutrients requirements, nursery techniques and early growth behaviour that will guarantee effective seed germination. Delay or failure of seed to germinate in the nursery is a serious constraint on the efficiency of nursery management. Vegetative propagation offers a unique opportunity of avoiding the problems of recalcitrant seed predominant in tropical tree species and also facilitates the transfer of the genetic potentials as well as the non additive variance of the parent to the new plant (Puri and Khara, 1992). Greater variation between species occurs in the ease with which shoots can be rooted (Kuria et al., 2010). However; the rooting media, the type of auxin and its concentration, and leaf area of cuttings are known to influence the rooting ability of stem cuttings (Mudge and Brenna, 1999). This research aimed at investigating the effects of growth hormones, rooting media, lamina area and nodal position of *S. henningsii* stem cuttings with a view of developing an efficient method for vegetative propagation in view of the urgency to develop measures for ex situ conservations of the depleted natural populations and the growing demand for its medicinal properties.

MATERIALS AND METHODS

Plant materials

The cuttings of *S. henningsii* used in this research were taken from Kabiruini forest in Central Kenya. The cuttings were harvested and transported to Jomo Kenyatta University of Agriculture and Technology (JKUAT) botanical garden where the study was conducted.

Stem cuttings

At the green house, a total of 660 stem cuttings each with two lateral leaves left intact and termed as full leaved cuttings and or both trimmed each by half and termed as half leaved cuttings were prepared and divided equally among the three rooting media. All the cuttings were uniformly trimmed to 5 cm in length. In every medium, 110 stem cuttings were half leaved while the remaining one hundred and ten stem cuttings were full leaved cuttings. For the nodal position, 110 stem cuttings were excised from the apical position of the mother plant while the remaining one hundred and ten stem cuttings were excised from the basal position of the mother plant. The cuttings were treated with indole butyric acid (IBA), naphthalene acetic acid (NAA), indole acetic acid (IAA) and Seradix 2 powder at different concentrations of 0, 50,

Table 1. Effect of lamina area, nodal position and an average concentration across the hormones on the mean number of roots produced per rooted cutting, mean root length per cutting, mean percentage survival and mean percentage rooting.

Treatments	Mean number of roots ± SE	Mean root length (cm) ± SE	Mean percentage survival ± SE	Mean percentage rooting ± SE
Leaf size x Nodal position				
Whole leaf x apical node	4.90±0.23^{a}	5.14±0.51^{a}	67.31±2.90^{a}	49.26±3.25^{a}
Whole leaf x basal node	3.24±0.61^{b}	3.50±0.22^{b}	63.67±3.84^{a}	32.89±1.27^{b}
Half leaf x apical node	4.05±0.12^{a}	4.41±0.24^{a}	65.54±1.72^{a}	34.03±1.10^{b}
Half leaf x basal node	2.93±0.46^{b}	3.42±0.37^{b}	56.90±2.81^{b}	24.40±3.83^{c}
Concentration of hormones				
Control	3.00±0.31^{b}	2.43±0.49^{c}	66.11±3.32^{a}	34.02±2.50^{b}
50 mg/l	3.11±0.23^{b}	2.80±0.12^{c}	64.83±3.95^{a}	28.19±1.38^{c}
100 mg/l	4.16±0.14^{a}	3.72±0.29^{b}	68.22±1.09^{a}	46.64±3.57^{a}
150 mg/l	4.80±0.34^{a}	4.78±0.37^{a}	74.10±0.16^{c}	52.11±1.03^{a}

Data presented as the mean value ± standard error after 16 weeks of rooting from three independent experiments each with four replicates.

100, and 150 mg/l using quick dip method (Oni, 1987) except for Seradix 2 powder where the cuttings were moistened at their bases and dipped into the rooting powder before inserting in to the rooting medium. The cuttings were basally dipped up to 2 cm in different solutions of IBA, IAA and NAA at 0, 50, 100 and 150 mg/L for about 5 seconds and immediately transferred in to the rooting media. Sixty cuttings were treated with each type of auxin except for Seradix rooting powder; thus 5 cuttings were allocated to each treatment level, leaf size and nodal position; while 20 cuttings each were under Seradix 2 powder and control treatment.

The cuttings were planted in polybags filled with three (3) different rooting media namely: forest top soil, sandy soil and red sub-soil and arranged in a 3 x 4 x 2 x 4 x 2 factorial design in the green house under a high humidity, tightly sealed polythene paper. Watering was done every morning and evening with a knapsack sprayer.

Statistical analysis

The cuttings were assessed for the following parameters after 16 weeks; mean percentage of surviving cuttings which was determined as the number of living plants per total cuttings planted per treatment, mean number of roots formed per cutting, mean root length per rooted cutting and mean percentage of rooted cuttings. Analysis of variance (ANOVA) was carried out on the data collected for the different parameters and least significant difference (LSD) at 5% probability level was used to compare the significantly different means using SAS statistical package version 9.1.3.

RESULTS

Percentage survival of cuttings

The percentage survival of cuttings showed substantial variation among the leaf size and hormone concentration; and rooting media and hormone concentration ($p< 0.05$). From ANOVA, interactions between leaf size and concentration of hormones were observed to have significant effects on cuttings survival percentages. The highest survival percentage with a mean value of 67.31% was obtained among whole leaf cuttings from the apical node while the half leaf size cuttings from the apical node had 65.54%. Basal nodes with whole leaf size and half leaf size had 63.67% and 56.90% survival percentages respectively (Table 1).

Table 2. Effect of rooting media and average concentration across hormones on the mean number of roots per rooted cutting, mean root length per rooted cutting and the percentage survival of the cuttings.

Rooting medium/ hormone concentration	Mean no. of roots	Mean root length (cm)	Mean percentage survival (%)	Mean percentage rooting (%)
Forest soil x Control	3.32±0.28^{a}	3.33±0.18^{a}	63.32±2.21^{a}	32.21±2.89^{a}
Forest soil x 50 mg/l	2.79±0.22^{b}	3.23±0.33^{a}	70.10±1.44^{b}	34.04±1.23^{a}
Forest soil x 100 mg/l	3.90±0.27^{c}	4.11±0.29^{b}	79.42±2.39^{c}	45.90±0.36^{b}
Forest soil x 150 mg/l	4.31±0.04^{c}	5.44±0.03^{c}	81.19±1.08^{c}	56.13±2.70^{c}
Red soil x Control	3.36±0.33^{a}	3.16±0.42^{a}	61.87±3.52^{a}	29.40±0.37^{a}
Red soil x 50 mg/l	2.91±0.00^{b}	3.41±0.19^{a}	65.31±1.04^{a}	32.92±2.43^{a}
Red soil x 100 mg/l	2.84±0.14^{b}	4.40±0.11^{b}	76.24±4.09^{c}	41.07±4.49^{b}
Red soil x 150 mg/l	4.08±0.29^{c}	5.23±0.26^{c}	78.21±3.13^{c}	51.72±3.11^{c}
Sandy soil x Control	2.80±0.16^{b}	3.42±0.19^{a}	62.44±2.86^{a}	27.81±2.52^{a}
Sandy soil x 50 mg/l	2.53±0.08^{a}	3.15±0.44^{a}	64.78±2.08^{a}	33.59±0.14^{a}
Sandy soil x 100 mg/l	3.41±0.24^{a}	3.50±0.07^{a}	71.26±0.03^{b}	42.65±2.98^{b}
Sandy soil x 150 mg/l	3.67±0.17^{a}	4.37±0.14^{b}	71.00±0.13^{b}	43.38±2.19^{b}

Data presented as the mean value ± standard error after 16 weeks of rooting from three independent experiments each with four replicates.

Table 3. Effect of rooting media on the mean number of roots produced per rooted cutting, mean root length per rooted cutting, mean percentage survival and mean percentage rooting of the cuttings.

Rooting medium	Mean number of roots ± SE	Mean root length (cm) ± SE	Mean percentage survival ± SE	Mean percentage rooting ± SE
Forest top soil	4.80±0.13^{a}	5.08±0.37^{a}	77.51±0.21^{a}	66.27±1.06^{a}
Red sub-soil	4.47±0.59^{a}	4.65±0.52^{a}	62.39±0.60^{b}	51.31±2.26^{b}
Sandy soil	2.64±0.33^{b}	2.83±0.28^{b}	49.31±1.53^{c}	27.81±1.59^{c}

Data presented as the mean value ± standard error after 16 weeks of rooting from three independent experiments each with four replicates.

As shown in Table 1, the highest mean percentage survival was 74.10% for the cuttings treated with 150 mg/l concentration, 68.22% for cuttings treated with 100 mg/l concentration while cuttings subjected to 50 mg/l concentration and control had 64.83% and 66.11% respectively. In the interaction of the rooting media and hormone concentration, 81.19% survival percentage of cuttings were recorded in cuttings planted in forest soil and hormones at 150 mg/l concentration, sandy soil with no hormones (control) had the least mean of 62.44% percentage survival (Table 2). Rooting media also had significant effect on the cuttings survival. Forest top soil had the highest mean percentage survival of 77.51%; Red sub-soil had 62.39% while sandy soil had the least percentage survival of 49.31% (Table 3).

Percentage of rooted cuttings

The interaction between rooting media and hormone concentration was statistically significant for mean percentage rooting (Table 2). 56.13% percentage rooting of cuttings was recorded in cuttings planted in forest top soil and hormones at 150 mg/l concentration. Red sub-soil at 150 mg/l concentration and forest top soil at 100 mg/l concentration had 51.72 and 45.90% percentage rooting respectively.

There was an increase in the mean percentage rooting with the increase in hormone concentration (Table 2). As shown on Table 1, the interaction between the leaf size, nodal position and hormone concentration had significant effect on the rooting of cuttings. Full-leaf cuttings from apical

Table 4. Effects of rooting hormones on the mean number of roots, mean root length and mean percentage rooting of stem cuttings of *S. henningsii.*

Rooting Hormone (auxins)	Mean number of roots ± SE	Mean root length (cm) ± SE	Mean percentage rooting ± SE
Seradix 2 powder	5.86 ± 0.42[a]	6.20 ± 0.14[a]	59.72 ± 2.34[a]
IBA	4.41 ± 0.13[b]	5.01 ± 0.03[b]	55.86 ± 3.91[a]
NAA	3.16 ± 0.05[c]	3.65 ± 0.35[c]	32.54 ± 2.02[c]
IAA	4.27 ± 0.25[b]	4.98 ± 0.27[b]	41.30 ± 2.62[b]

Data presented as the mean value ± standard error after 16 weeks of rooting from three independent experiments each with four replicates.

Plate 1. Seedlings of *S. henningsii* grown in forest top soil medium.

node produced the highest percentage of rooted cuttings with 49.26%. Rooting was poor for half-leaf cuttings from basal node. Rooting media also presented significant effects on the rooting of *S. henningsii* cuttings. Forest top soil showed the highest mean value of 66.27% for rooting percentage while the least mean of 27.81% was recorded in cuttings planted on sandy soil (Table 3). All the cuttings treated with Seradix 2 powder and IBA rooted better than those cuttings treated with IAA and NAA. However, both Seradix 2 powder and IBA were statistically similar in their effects but had significant effects (p<0.05) compared to IAA and NAA (Table 4).

Number of roots per cutting

The mean number of roots per rooted cutting was significantly affected by the leaf size and concentration of hormones (p<0.05). Whole-leaf size cuttings from the apical node had higher mean value of 4.90 for mean number of roots produced per cutting while half-leaf cuttings from basal node had 2.93 indicating a highly significant difference in the number of roots produced by both leaf sizes and nodal positions (Table 1). Forest top soil medium resulted in higher mean number of roots produced per cutting as compared to red sub-soil and sandy

soil. However, forest top soil medium remained statistically at bar with red sub-soil but both were significantly different from sandy soil (Table 3). Auxins produced a significant effect on the mean number of roots produced per rooted cutting. Seradix 2 powder recorded the highest mean number of roots per cutting with 5.86. IBA had 4.41 while NAA and IAA had 3.16 and 4.27 respectively (Table 4)

Lengths of roots per cutting (cm)

The effect of auxin type, auxin concentration, rooting media, as well as the interaction between the lamina area and the nodal position, and the rooting media and auxin concentration remained statistically significant for the length of roots per cutting of *S. henningsii* (p<0.05).Forest top soil had the highest value of 5.08 cm for root length followed by red sub-soil with 4.65 cm while sandy soil had the least, 2.83 cm for root length (Table 3). Whole leaf size cuttings from the apical node had the highest mean length of 5.14 cm for root length while half leaf size cuttings from the apical node had 4.41 cm. Auxins at 150 mg/l concentration had the highest mean value of 4.78 cm for root length. 100 mg/l concentration of hormone had 3.72 cm while the control experiment and 50 mg/l concentration of hormone had values of 2.43 cm and 2.80 cm respectively for mean root length (Table 1).

Cuttings treated with Seradix 2 powder produced longer roots. Both IBA and IAA were statistically similar in their effect (Table 4).

DISCUSSION

The development of propagation method is the first step in any domestication effort (Leakey and Simons, 1998). The results of the present study indicated that *S. henningsii* stem cuttings can be successfully propagated (Plate 1). The possibility of rooting stem cuttings of *S. henningsii* is crucial to its domestication strategy. The results of this study indicate the important role of determining the optimal rooting conditions in the process of vegetative propagation. The ability of cuttings to survive and produce long and massive roots is very important. Forest top soil followed by red sub-soil was able to serve this purpose in the studied species. Sandy soil was too porous and could not keep enough humidity required by the cuttings. The results across the three media conforms to those reported by Hartmann et al, (1997) who reported that an ideal propagation medium is known to provide sufficient porosity to allow good aeration and this ensures adequate oxygen availability for the developing roots system. It is important to select medium with the collect characteristic as there can be marked differences between root formations by the same species on different media (Kuria et al*.,* 2010). According to Leakey et al, (1990) and Mesen, (1993), different media have differing air: water ratios. Usually to enhance the rooting potential of nodal cuttings of plant species, the cuttings are treated with rooting hormones (auxins). Nanda, (1975) noted that auxins are associated with the division and elongation of meristematic cells and the differentiation of reserved food materials, because auxins increase the activity of hydrolyzing enzymes. The use of external hormones in stimulating root growth or length was necessary in the propagation of this species. Cuttings without hormones (control) and those with 150 mg/l concentration of hormones were statistically different from each other. The ability of auxins to promote adventitious root development in stem cuttings is well known, and has been attributed to enhanced transport of carbohydrates to the base of the cuttings (Hartmann et al., 1990). The type of auxin used also affected the performance of cuttings. The rooting percentage of cuttings was increased by auxins treatment, especially treatment by Seradix 2 hormone and IBA. These findings are partially in line with Kuria *et al.,* 2010 who found that 0.3 mg/l IBA and 0.4 mg/l NAA induced root formation in *Warbugia ugandensis* cuttings after 28 days in the rooting media. As presented in Table 4, Seradix 2 powder together with IBA had the highest values for all the parameters that were tested. The superior performance of Seradix 2 powder could be attributed to its 3 key components which included: 0.3% IBA which is an auxin, NAD which acts as a carrier and Thiram which is a fungicide. Cuttings with whole leaf size actually produced better results than those with half leaf area. The importance of leaf area on the rooting ability of cuttings of tropical species has been documented (Leaky and Couts, 1989). These findings are in line with those of Badji *et al.,* (1991), who noted that the presence of leaves promoted rooting and significantly improved survival of *Acacia senegal* cuttings. Apical cuttings rooted better than basal cuttings in this study. Different species exhibit varying rooting success for cuttings taken from the apical, sub-apical, mid-position and basal region of the parent stem (Wilson, 1993). Similar results have been reported for

Triplochiton scleroxylon (Leakey, 1983) and *Nauclea diderrichii* (Matin, 1989). These trends could be ascribed to increasing lignification and secondary thickening from top to base or higher concentrations of auxins present in the terminal section of the shoot (Hartmann et al., 1990).

CONCLUSION

This study demonstrates that *S. henningsii* can be reproduced through macropropagation using stem cuttings.

The cuttings require a medium that is not too open but still allows for good drainage and sufficient spaces to prevent water logging and subsequent rooting of the cuttings. The retention of some active leaves on the cuttings is also important for this species. The best result was actually obtained in cuttings with high concentration of hormones. This result can tell that this species is hard to root. With the increasing demand for the traditional herbs, *ex situ* conservation programmes and true to type mass propagation of *S. henningsii* could benefit from the findings of our study.

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