The effect of auxins (IBA, NAA) on vegetative propagation of medicinal plant

*Bobgunnia madagascariensis* (Desv.) J.H.Kirkbr & Wiersema.

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ABSTRACT: *Bobgunnia madagascariensis*, an important medicinal plant is difficult to propagate through seed germination. The study was conducted to develop a procedure for its regeneration through vegetative propagation by stem cuttings. The effects of auxin type [IBA (indole-3-butyric acid) or NAA (*α*–naphthalene acetic acid)] and their different levels of concentration 0, 100, 300 and 500 ppm were investigated to determine their ability to promote adventitious rooting in *B. madagascariensis* stem cuttings. The effect of auxin type was significant (*p* < 0.01) only for percentage rooting and root number per cuttings while the effect of auxin concentration was evidently significant (*p* < 0.01) in all rooting parameters evaluated in terms of percentage rooted cuttings, root number per cuttings, root length and root dry weight. The effect of auxin types and concentration showed that the highest and significant (*p*<0.05) rooting percentage was observed under IBA treatment at 300 ppm (39.53%). IBA revealed significant higher performance in rooting ability of *B. madagascariensis* than NAA in all rooting parameters evaluated and the effect being more pronounced at 300 ppm. Vegetative propagation will ensure sustainable exploitation this economically important medicinal plant.

Key words: Auxin, *Bobgunnia madagascariensis*, IBA, NAA, Stem cuttings.

INTRODUCTION

*Bobgunnia madagascariensis* (Desv.) J.H.Kirkbr & Wiersema formally known as *Swartzia madagascariensis* is a wild leguminous tree widely distributed in savannah and dry forest regions of Africa (Hostettmann *et al*., 2000; Schaller *et al*., 2001; Smith and Allen, 2004). It is widespread throughout the Miombo woodland areas of Tanzania. Different parts of the tree are used in traditional medicine to treat various diseases. The tree bears large fruits which have been reported to be toxic to snails related to the causative agent of human schistosomiasis (Lwambo and Moyo, 1991). The fruit pulp also exhibit high trypanocidal activity (Atawodi, 2005). Extracted portions of the leaves, stem have shown antifeedant activity against flour beetle of maize, Tribolium casteneum (Adeyemi *et al*., 2010). Antifungal products have also been isolated from the root bark (Hostettmann *et al*., 2000; Schaller *et al*., 2001; Hostettmann and Marston, 2002). Apart from its medicinal and insecticidal properties, this tree has a very strong, durable and termite resistant wood used in building and making of local musical instruments (Kirkbride and Wiersema, 1997).

The commercial harvesting and sale of roots of this species is widespread in Africa (Hostettmann *et al*., 2000). Unsustainable harvesting through uprooting of the whole plant or removal of the roots reduces regeneration and thus, threatens local populations. Unfortunately, *B. madagascariensis* is a difficult tree to propagate because it has low seed viability, poor germination and slow growth of its seedlings (Mbuya *et al*., 1994).

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Although some efforts to improve seed germination through pretreatments have been reported (Amri, 2010; Blackson et al., 2011), methods for vegetative propagation of mature trees by stem cuttings have not yet reported. Vegetative propagation through stem cuttings is highly needed to optimize its growth and establishment for sustainable availability of harvestable products in the future.

Vegetative propagation through stem cutting is important for mass production of improved materials within a short time, and for perpetuating the characteristics of the parent plant (Hartmann et al., 2002). Many tropical tree species have been successfully propagated vegetatively by stem cuttings (Leakey et al., 2006; Tchoundjeu et al., 2006, Amri et al., 2010). Vegetative propagation is considered the ideal not only for maintaining certain desirable traits of a threatened species but also desirable alternative to gathering seeds thus eliminating reliance on seasonally available seeds. (Hartmann et al., 2002; Tchoundjeu et al., 2004). Exogenous application of commercially available auxins like indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) promotes rooting in stem cuttings (Tchoundjeu et al., 2002; Gateable´ and Pastor 2006; Husen and Pal, 2007) and the specific action of these growth hormones are not known in this species. Therefore, the present study was conducted to investigate the effect of auxin type IBA or NAA and auxin concentrations on rooting ability of B. madagascariensis stem cuttings.

MATERIALS AND METHODS
Fresh stem cuttings of B. madagascariensis were collected from Nyanganje provenance (latitude S 07°56’S, longitude 036°39’ E), Ifakara. Cuttings were taken from shoots of mature plants then enclosed in polythene bags, held on ice, and transported to the propagation site at the Department of Botany, University of Dar es Salaam. Two types auxins namely indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) were used in treating the cuttings at concentration levels 0, 100, 300, and 500 ppm. Untreated cuttings serving as control treatment (0 ppm) were dipped into a 50% isopropyl alcohol-deionized water solution.

The experiments were conducted in a split-split-plot design with a factorial arrangement using 3 replications. Auxin treatments were main-plots, while auxin concentrations were sub-plots. Each replication consisted of 10 cuttings such that with the three replications a total of 240 cuttings were used for the whole experiment (ie 10 cuttings x 3 replications x 2 Auxin x 4 concentrations). Treated basal ends of each stem cutting of B. madagascariensis were air dried briefly for 5 minutes before insertion to a depth of 3 cm in rooting media which was a mixture of fine gravel and sand in a non-mist plant propagator as described by Leakey et al., (1990). Humidity in the propagators was maintained at 86 ± 2 % and maximum and minimum day-night temperature at 31± 1 0C to 26 ± 1 0C 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
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. Data collections were in terms rooting and sprouting parameters. Rooting parameters evaluated were rooting percentage, root number, length of the longest root, root dry weight and callus formation for per cutting. A cutting was considered rooted if it has at least one primary root > 1 mm long. 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 60 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 °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 rooting and sprouting parameters as 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
Propagated stem cuttings of *B. madagascariensis* were evaluated after 60 days. Analysis of variance (ANOVA) showed significant difference (p < 0.01) for auxin type only for percentage rooting and root number per cuttings among the rooting parameters evaluated. The effect of auxin concentration in rooting ability of *B. madagascariensis* stem cuttings was evidently significant (p< 0.01) in all rooting parameters evaluated in terms of percentage rooted cuttings, root number per cuttings, root length and root dry weight. There was no significant interaction between treatments for all rooting parameters. (Table 1).

Means separation by DMRT for the effect of auxin types and concentration, showed that the highest and significant (p<0.05) rooting percentage was observed under IBA treatment at 300 ppm (39.53± 2.82%) followed by IBA 500 ppm (24.83± 2.06 %) and IBA 100 ppm (23.50± 1.22%). Significant lowest rooting percentage (16.20± 2.2 %) was observed under NAA treatment at 100 ppm (Table 2). The number of roots per cutting was significant high under the treatment at IBA 300 ppm (7.60 ± 0.86) followed by treatments IBA 500 ppm (4.43± 0.73), Other auxins treatments were not significantly different from each other and highest average number of roots for the NAA treatment was 2.60± 0.43 at a concentration of 300 ppm (Table 2).

Significantly maximum length of longest primary root was noticed in the treatment IBA at 3000 ppm (6.83± 0.34 cm) followed by IBA 500 ppm (4.43± 0.73 cm) and NAA 00 ppm (3.63± 0.14 cm). Whereas the minimum length of longest primary root was noticed in NAA 1000 ppm (1.57 ± 0.6cm) which was not significant different from the rest of auxins treatments (Table 2). Highest and significant root dry weight was noticed in the treatment IBA 300 ppm (17.67± 0.92g x 10^{-3}) followed by IBA 500 ppm (8.0± 0.26g x 10^{-3}) other treatments were not significantly different from each other (Table 2).

### Table 1. Analysis of variance for the effect of auxin type and concentration on rooting parameters of *B. madagascariensis*

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Source of variation</th>
<th>df</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rooted cuttings (%)</td>
<td></td>
<td>Root number per cutting</td>
</tr>
<tr>
<td>Rep</td>
<td>9</td>
<td>32.67ns</td>
<td>0.88ns</td>
<td>0.77ns</td>
</tr>
<tr>
<td>Auxin</td>
<td>1</td>
<td>316.83*</td>
<td>24.60*</td>
<td>17.17</td>
</tr>
<tr>
<td>Concentration</td>
<td>3</td>
<td>1024.43*</td>
<td>28.11*</td>
<td>25.37*</td>
</tr>
<tr>
<td>Auxin x Concentration</td>
<td>3</td>
<td>83.71ns</td>
<td>7.01ns</td>
<td>4.36ns</td>
</tr>
</tbody>
</table>

ns = not significant; * significant at p< 0.001; df = degree of freedom; Rep = replicate blocks.


**Table 2.** The effect of auxin type and concentration on rooting parameters of *B. madagascariensis*

<table>
<thead>
<tr>
<th>Auxin Type</th>
<th>Auxin Conc.(ppm)</th>
<th>Rooting parameters</th>
<th></th>
<th></th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rooted cuttings (%)</td>
<td>Roots number per cutting</td>
<td>Root length (cm)</td>
<td>Root dry weight(g) x 10^-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>23.50± 1.22^{ab}</td>
<td>2.17± 0.13^{b}</td>
<td>2.27± 0.14^{a}</td>
<td>3.67± 0.36^{a}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>39.53± 2.82^{c}</td>
<td>7.60 ± 0.73^{c}</td>
<td>6.83± 0.34^{c}</td>
<td>17.67± 0.92^{d}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>24.83± 2.06^{ab}</td>
<td>4.43± 0.86^{ab}</td>
<td>4.07± 0.47^{ab}</td>
<td>8.0± 0.26^{c}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16.20± 2.2^{a}</td>
<td>1.43± 0.84^{a}</td>
<td>1.57 ± 0.6^{a}</td>
<td>3.0± 0.18^{a}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>22.00± 1.36^{ab}</td>
<td>2.60± 0.43^{a}</td>
<td>3.03± 0.14^{ab}</td>
<td>5.67± 0.82^{ab}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>20.60± 1.57^{ab}</td>
<td>2.07± 0.64^{a}</td>
<td>1.80± 0.04^{a}</td>
<td>3.0± 0.71^{a}</td>
<td></td>
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</tr>
</tbody>
</table>

Means in column bearing the same letter indicate no significant difference at p<0.05.

It was observed that at higher concentration the efficiency of both IBA and NAA decreased as it was marked by low performance in all rooting parameters evaluated. In general, the effect of IBA in rooting ability of *B. madagascariensis* was more pronounced than NAA in terms of rooting percentage, number of primary roots, root lengths and root dry weight. No rooting was recorded on untreated stem cuttings. Means in column bearing the same letter indicate no significant difference at p<0.05.

When correlation analysis of rooting and sprouting parameters was done it showed that 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).
Table 3. Correlation coefficients of rooting and sprouting parameters for *B. madagascariensis* stem cuttings

<table>
<thead>
<tr>
<th></th>
<th>Rooted (%)</th>
<th>Root number</th>
<th>Root length (cm)</th>
<th>Root weight (g)</th>
<th>Callused (%)</th>
<th>Sprouted (%)</th>
<th>Shoot number</th>
<th>Shoots length (cm)</th>
<th>Survived (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rooted (%)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root number</td>
<td>.78 **</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root length</td>
<td>.75**</td>
<td>.88**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root weight (g)</td>
<td>.76**</td>
<td>.83**</td>
<td>.81**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callused (%)</td>
<td>.48**</td>
<td>.56**</td>
<td>.47**</td>
<td>.51**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprouted (%)</td>
<td>.08 ns</td>
<td>.07 ns</td>
<td>.09 ns</td>
<td>.03 ns</td>
<td>.02 ns</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot number</td>
<td>-.07 ns</td>
<td>-.01 ns</td>
<td>-.02 ns</td>
<td>-.05 ns</td>
<td>.02 ns</td>
<td>.03 ns</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoots length (cm)</td>
<td>.36*</td>
<td>.39*</td>
<td>.40*</td>
<td>.43*</td>
<td>.38*</td>
<td>.10 ns</td>
<td>-.03 ns</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Survived (%)</td>
<td>.31*</td>
<td>.19*</td>
<td>.18*</td>
<td>.20*</td>
<td>.18*</td>
<td>.29*</td>
<td>.06 ns</td>
<td>.04 ns</td>
<td>-</td>
</tr>
</tbody>
</table>

Indicate significant at p< 0.05.
** Indicate significant at p< 0.01.
ns = not significant

**DISCUSSION**

This study has shown that auxin type and concentration proved important factors in vegetative propagation of *B. madagascariensis* while untreated cuttings produced virtually no roots. Auxins have shown promising results at various concentrations for rooting cuttings of several other tropical plants species (Teklehaimanot et al., 2004; Atangana et al., 2006). Cuttings from some species root readily without auxin treatment, while cuttings from others do not root easily (Hartmann et al., 2002; Blythe et al., 2004). Auxins in addition to enhancing the rate of adventitious root development also increased the number of roots initiated per cutting in *B. madagascariensis*. Similar findings have also been reported in a variety of species (Tchoundjeu et al., 2004; Atangana et al., 2006). Auxin is thought to enhance enzyme activity, thus increasing starch hydrolysis and facilitating mobilization. The degree of rooting is considered to be determined by the relative production of endogenous auxins and inhibitors in the branches.(Nandi et al., 2002). Auxin has a relationship with mobilization of starch which can trigger energy for root initiation (Nanda and Anand, 2006). It seems that application of IBA and NAA elevated the level of...
endogenous auxins resulting in the full expression of rooting ability.

IBA was more effective than NAA in enhancing rooting ability in *B. madagascariensis* stem cuttings. The result complies with that of other tropical tree species like *Prunus africana* (Tchoundjeu et al., 2002) and *Juniperus procera* (Negash, 2002) reported IBA significantly enhanced rooting ability compared to NAA. Further it has been evident that IBA is more effective in increasing the number of sprouts per cutting as the case in *J. curcas* and *J. glandulifera* (Kochhar et al., 2005) and in *Pongamia pinnata* (Kesari et al., 2009) compared to NAA. IBA is more effective in inducing plants rooting than NAA, because the former is more stable than the latter, and more important, IBA can be changed into IAA by the mechanism of parallel to fatty acid oxidation (Berleth et al., 2007).

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 (Davis and Jacobs, 2005). 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 (Clark and Schlarbaum, 2000).

Shoot sprouting and growth appeared in *B. madagascariensis* stem cuttings before root formation at the base of the cuttings. Other studies by Puri and Thompson (2003) have shown that shoot growth/flushing of buds is not necessarily a good indicator of root development because during vegetative propagation, early differentiation and growth of leaf buds is dependent on food reserves available in the cuttings. 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 roots and shoots for nutrient reserve (Davis and Jacobs, 2005). 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
The results presented in this work have revealed that exogenous application of auxin can stimulate root production in *B. madagascariensis*. IBA should be used for vegetative propagation by stem cuttings since it is more effective in this regard than NAA in promoting higher root number per cuttings. Vegetative propagation by stem cuttings in *B. madagascariensis* may solve not only the problem of inadequate seed supply due to infrequent flowering, low seed viability, poor germination and slow growth of its seedlings rapid, but also will reduce time taken by the plant to reach maturity age. Propagation through stem cuttings will ensure conservation, availability and sustainable exploitation this economically important medicinal plant.

REFERENCES


