Improving propagation methods of \textit{Ricinodendron heudelotii} Baill. from cuttings

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**Abstract**
\textit{Ricinodendron heudelotii} is a valuable multipurpose tree from Africa that is lacking an efficient and inexpensive vegetative propagation method. To improve multiplication, a series of nursery experiments were conducted to assess the effect of propagation media, plant growth regulators (NAA and IBA), accession source harvest timing, cutting type, and pre-treatment with honey. Maximum survival and rooting percentages of 90.2 and 93.7% respectively were achieved in all experiments. The maximum number of leaves and roots were respectively, 7.4 and 8.7; maximum root length was 15.6 cm. Fine sand was superior to sawdust or fine sand:sawdust mixture. IBA, at 100 μg/l was more effective than NAA for rooting and growth. Principal component analysis showed that, independent of the accession source, cuttings harvested from April to May were more suitable for propagation, giving the highest rates of rooting and growth. In contrast, variable results were achieved with apical cuttings (highest rooting), basal single-node leafy cuttings (highest number of leaves and length of root) and basal two-node leafy cuttings (highest number of roots). Cuttings were more successful when they were soaked in honey for 60 min. After acclimatization, maximum survival rate of plants was 67.6%. This improved protocol can be incorporated into agro forestry system in which propagation of \textit{R. heudelotii} is a component.

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1. Introduction

\textit{Ricinodendron heudelotii} Baill. (Euphorbiaceae), is a tree distributed from Guinea to Angola and in East Africa; heights range from 12 to 40 m. The species grows in secondary forest between 130 and 1030 m above sea level (Vivien and Faure, 1996). It provides food, medicines and other various products, including construction and building materials. The kernels are used as a flavoring agent, and are also a good source of oil used to make soap and varnish (Plenderleith, 2004; Tchiegang et al., 2005). The wood is used for making household implements, drums and carvings (Plenderleith, 1997); the bark is used to treat elephantiasis (Plenderleith, 2004).

Natural regeneration rates of \textit{R. heudelotii} is low and is exasperated by the negative impact of over exploitation has on natural population levels. This plant has therefore been classified as one of the top ten species to be domesticated in Central and West Africa by the International Centre of Research in Agro Forestry (Franzel et al., 1996). In the case of \textit{R. heudelotii}, the Institute of Medical Research and Medicinal Plants Studies in Cameroon (IMPM) worked with farmers to identify priorities and traits preferences for the development of vegetative propagation techniques.

Efficient propagation techniques will greatly facilitate the domestication and the improvement of the species. In previous studies on regeneration of \textit{R. heudelotii} by cuttings, Shiembo et al. (1997a) used seedlings or coppice shoots as stock material in a non-mist propagation system to study the effect of indol-3-butyric acid (IBA), leaf area and planting media on rooting. This low-technology system is designed for use in rural areas and has successfully been used for the propagation of a large number of other trees species such as \textit{Irvingia gabonensis} (Shiembo et al., 1997b), \textit{Prunus africana} (Tchoundjeu et al., 2002a, 2002b) and \textit{Vitex madiensis} (Mapongmetsem, 2006). Mapongmetsem et al. (1999) used sprouts from stump of adult trees of \textit{R. heudelotii} to investigate the effect of wood type and the cutting orientation on rooting. In both of these studies, rooting percentage was low. However, very little further research has been done on vegetative propagation of \textit{R. heudelotii}. In an attempt to further define appropriate material for use in the development of an efficient regeneration system, the effect of the physiological status and origin of the cutting material on rooting and subsequent plant regeneration of \textit{R. heudelotii} was investigated, with...
the ultimate objective of determining the most successful treatment for mass production of clonal planting stock. The effects of propagation media, auxin concentration and pre-treatment with honey were also assessed.

2. Materials and methods

2.1. Study site and stock plant production

This study was conducted from September 2008 to December 2009 in a propagation unit established in the nursery at IMPM at Yaounde in the Centre region of Cameroon, located at latitude 3° 31' N and longitude 11° 25' E, approximately 813 m above sea level. Average rainfall is 1692 mm and is bimodally distributed (Segalen, 1967).

Three sites were chosen for seed collection: Yaounde (site described above) and Mbalmayo (latitude 3° 31' N, longitude 11° 30' E; rainfall 1650 mm, altitude 600 m) in the Centre region and; Santchou (latitude 4° 37' N, longitude 9° 50' E; rainfall 1500 mm, altitude 320 m) in the West region of Cameroon. Mature seeds were collected from at least 30 randomly selected trees per site. Seeds from each site were bulked before sowing at the IMPM nursery. The resulting seedlings were planted near the nursery. These growing seedlings aged one year old were used as cutting source for the present trial.

2.2. Preparation of cuttings and propagation system

All cuttings taken for the various experiments were placed in a propagation system consisting of poly-propagators (1.5 m x 0.5 m x 1 m) divided into three compartments each. They were constructed according to the method of Leakey et al. (1990b) and placed in a shade house roofed with transparent polyethylene. This provided an irradiance level of 20–32% that was received outside the unit.

2.3. Propagation media

The propagation media were composed of fine sand (FS), sawdust (SD) or a fine sand:sawdust (1:1) mixture (FD:SD). All were treated with a systemic fungicide (Benlate) prior to use. The sawdust was obtained from male palm inflorescences. The three media were placed according to a randomized block design in the poly-propagators. Basal single-node leafy cuttings were collected from Yaounde stock plants between August to September and inserted into a rooting bed in a randomized complete block design with four replications. Each treatment contained 30 cuttings, giving a total of 360 cuttings for the whole experiment (30 cuttings x 3 treatments x 4 replications). The base of each cutting was treated with 50 μg IBA. The effect of propagation media on cuttings was assessed at three phases: root induction, growth and development (of aerial parts). Survival and rooting rate, number and length of roots and number of leaves were measured.

2.4. Growth regulators: IBA and NAA

Four concentrations of IBA and NAA were tested, (control), 50, 100, 200 and 300 μg/l. Basal single-node leafy cuttings were collected from Yaounde stock plants in August and September and then, inserted in fine sand in a rooting bed in a randomized complete block design with four replications. There were 20 cuttings per treatment, so the total for the experiment was 720 (20 cuttings x 9 treatments x 4 replications). Cuttings were assessed every 2 weeks for survival and rooting rate, number and length of roots and number of leaves.

2.5. Accession site and harvesting period

Single-node cuttings were harvested from each accession plant stock over at three time periods: April to May (CP1); August to September (CP2) and November to December (CP3). The base of each cutting was treated with the best growth regulator, as determined from the results of the previous experiment. The cuttings were inserted in a rooting bed in a randomized complete block design with four replications. There were 20 cuttings per treatment giving a total of 720 for the experiment (20 cuttings x 9 treatments x 4 replications). Cuttings were assessed after four months for survival and rooting rate, number and length of rooting and number of leaves.

2.6. Accession site and cutting type

For each accession, three types of cutting were produced to evaluate the effect on rooting success: apical cutting (C1), basal single-node leafy cutting (C2) and basal two-node leafy cutting (C3). The most appropriate harvest timing, media type and growth regulator concentration, as determined by previous experiment station were used. Cuttings of different types were inserted in a rooting bed in a randomized complete block design with four replications. Twenty cuttings were used for each treatment; thus the total for the experiment was 720 (20 cuttings x 9 treatments x 4 replications). Cuttings were assessed after four months for survival, rooting rate, number and length of root and number of leaves.

2.7. Pre-treatment with honey

Basal two-node leafy cutting from Yaounde stock plant was used to assess the influence of honey on cutting development. Cuttings were soaked in liquid honey (bought in local market) for a time of 0 (control), 30, 60, 90, 120 min. The most appropriate media and phytohormone concentration of the previous experiments were equally used. Cuttings were inserted in a rooting bed in a randomized complete block design with four replications. The experimental unit was made up of 15 cuttings with a total of 300 (15 cuttings x 5 treatments x 4 replications). Cuttings were assessed after two months for survival and rooting rate.

2.8. Substrate used for plant acclimatization

Three types of substrates were used, namely: black soil, fine sand, a mixture of black soil and fine sand (1:1). Plantlets of R. heudelotii were placed in polythene pots (10 cm diameter x 18 cm depth). After 2 months of acclimatization, survival rate and plantlet height were assessed.

2.9. Data collection and analysis

Data collected were: rate of rooting, number and length of roots per cutting, number of leaves per cutting, and survival rate. These data were subjected to tests of analysis of variance, correlation and principal component analysis. After rejecting null hypothesis of equal means using ANOVA F-test, Fisher’s LSD (Least Significant Difference) was used for comparing treatment group means at 0.05. Statistical software packages used were SPSS 17.0 and SPAD 4.01 for Windows. Values were means ± standard deviation.

3. Results

3.1. Effect of propagation media on rooting and growth

In the presence of 50 μg IBA, survival rate decreased with time and was dependent upon media type. During the root induction phase, (0 to 4 weeks), survival somewhat decreased, but the effect was not
significant \((P < 0.05)\). In contrast, survival decreased rapidly and significantly during the growth phase. After 10 weeks of culture (the development phase), the survival rate stabilized at 54.6%, 45.2% and 53.2% for fine sand, sawdust: fine sand and sawdust, respectively. By sixteen weeks, the percent rooting (53.6; 42.5; 51.2) (Fig. 1A), leaf number (3.3; 1.1; 5.2) (Fig. 1B), root number (3.5; 0.5; 3.1) (Fig. 1C) and root length (14; 5; 10) (Fig. 1D) were obtained for respectively fine sand, sawdust: fine sand and sawdust media. Significant interaction between sand and sawdust was noticed with leaf number.

3.2. Effect of IBA and NAA on rooting and growth

The effect of different IBA and NAA concentrations was assessed twelve weeks after treatment. Cutting survival rate was dependent on treatment. The control had the lowest survival (12.6%); maximum survival rates were seen with 100 \(\mu g/l\) NAA (66.5%) and 200 \(\mu g/l\) IBA (61.4%) treatments. Sixteen weeks after culture, the maximum and significant \((p < 0.05)\) rooting rate (59.6%) (Fig. 2A), number of leaves per cutting (4.1; 3.9) (Fig. 2B), number of roots (6.4; 4.4) (Fig. 2C) and length of roots (14.5; 10.9) (Fig. 2D) were obtained, respectively, with 50 or 100 \(\mu g/l\) IBA and NAA. There was a strong positive correlation between both survival and rooting rate \((R = 0.997)\) and rooting rate and number of leaves per cutting.

3.3. Effect of accessions and harvesting period on rooting and growth

For each accession tested, CP1 (the April to May harvest period) was generally more favorable for rooting and development than either CP2 (August to September) or CP3 (November to December). The survival rate ranged from 73.2% for cuttings harvested in April–May from the Yaounde accession (A1CP1) to 21.8% for cuttings harvested in November–December from the Santchou accession (A3CP3). The maximum rate of rooting (68.4%), number of roots (8.6), number of leaves (7.3) and length of roots (14.6 cm) were obtained for the A1CP1 treatment (Table 1). In general, harvesting periods were significantly different \((P < 0.05)\) from each other and there was also significant interaction between accessions and harvesting period. Principal component (Jeffer, 1967) showed that the first and second components represented 96.94% of the total variability. The first axis, which describes 89.5% of total variation, was defined by rooting rate. The second axis accounting for 7.45% of total variation was defined by leaf number, root number, root length and survival rate. Dendrogram produced using average linkage and rescaled distance methods, revealed 3 clusters (Fig. 3):

- cluster I contained cuttings harvested in August–September from the Mbalmayo (A2CP2) and Santchou (A3CP2) accessions and was characterized by the highest survival rate;
- cluster II, which comprised cuttings harvested in August–September from the Yaounde accession (A1CP2), and in April to May from the Santchou (A3CP1). Mbalmayo (A2CP1) and Yaounde (A1CP1), was characterized by the highest rate of rooting, number of leaves, root number and root length.
- cluster III contained cuttings harvested in November to December from the Mbalmayo (A2CP3), Yaounde (A1CP3) and Santchou (A3CP3) accessions; it was not influenced by any variable measured.

3.4. Effect of accession and cutting type on rooting and growth

In general, the C1 (apical, single node) cutting type had the highest survival rate while the C2 (basal, single node) cutting type gave more favorable rooting and development.

Cutting survival rate ranged from 68.9% for basal two-node leafy cuttings from the Santchou accession (A3C3) to 86% for apical, single-node leafy cuttings from the Yaounde accession (A1C1). The maximum rate of rooting (90.6%), root number (8.7), leaf number (7.4) and root length (15.6 cm) were obtained, respectively, for the basal, single-node leafy cuttings from the Mbalmayo accession (A2C2), basal, single-node leafy cuttings from the Yaounde accession (A1C2), apical single-node leafy cuttings from the Yaounde accession (A1C1) and basal, two-node leafy cuttings from the Santchou

![Fig. 1. Effect of propagation media on R. heudelotii cuttings: A) Rooting rate, B) leaf number/cutting, C) root number, D) root length. Values are means ± standard deviation. Values having the same letters are not significantly different according to Fisher’s LSD test \((P < 0.05)\).](image-url)
accession (A3C3) (Table 2). In general, accessions and cutting type and their interaction were significantly different (P < 0.05) from other treatment; principal component analysis showed that the first and second components represented 87.47% of the total variability observed. The first, which describes 69.38% of a total variation, was defined by the rooting rate, leaf number and, root length variables. The second, 18.09% of total variation comprised root number and survival rate. Average linkage and rescaled distance, techniques produced a dendrogram composed of 4 clusters (Fig. 4):

- cluster I contained apical, single-node leafy cuttings from the Mbalmayo and Yaounde accessions (A2C1 and A1C1), basal, single-node leafy cuttings from the Santchou accession (A3C2); it was characterized by the highest survival rooting rates (Fig. 5A);
- cluster II was composed of basal, single-nodes cuttings from the Yaounde and Mbalmayo accessions. These materials showed maximum leaves and longest roots (Fig. 5B);
- cluster III contained basal, two-node leafy cuttings from the Mbalmayo (A2C3), Santchou (A3C3) and Yaounde (A1C3) accessions; it was characterized by the highest root number (Fig. 5C);
- cluster IV contained apical, single-node leafy cuttings from the Santchou accession (A3C1), was not influenced by any variable measured.

3.5. Effect of honey on survival rate and rooting

In general, soaking cutting in honey improved survival and rooting and, with the exception of the longest period, the effect was time-dependent. The lowest survival (75.9%) and rooting (80%) rates were obtained in the longest time (120 min) treatment, while the maximum rate (90.2% and 93.7%, respectively) were obtained with 60 min of soaking (Fig. 6).

3.6. Effect of substrate on plant acclimatization

Plants derived from cuttings were potted on substrate containing black soil, fine sand or a 50:50 mixture of black soil: fine sand. After 6 weeks of acclimatization, the 50:50 mixture showed a success rate (67.8%) which was significantly different (P < 0.05) from that of the other substrates (49.9%). Plantlets had a mean height of 24.7, 19.4 and 16.2 cm respectively; in the 50:50 mixture, fine sand and black soil.

4. Discussion

The most effective means of improving *R. heudelotii* for on-farm use is by vegetative propagation, which can rapidly overcome the
limitations imposed by long generation times, irregular fruiting/flowering and out breeding. In this work, multiplication was achieved in non-mist propagation system, confirming results of Shiembo et al. (1997a). The fact that maximum survival and rooting percentages of 90.2 and 93.7%, respectively, were achieved in all five experiments suggests that the technique described here could be used for large-scale multiplication of the species. Previous trials of Shiembo et al. (1997a) assessed the effect of propagation medium, auxin concentration and leaf area on rooting of leafy stem cuttings; only 80% rooting was achieved, compared to over 90% in this study.

We observed that rooting is generally dependent upon pre-treatment of the cutting (with growth regulator or honey), potting media, cutting type and harvesting time. The highest rooting and survival percentage were observed in the fine sand medium. These results correspond with those of Leakey et al. (1990b), who obtained maximum rooting from cuttings of Cordia alliodora and Gymella arborea by using fine sand. Shiembo et al. (1997a) showed that for the propagation by cuttings, fine sand is superior to sawdust: fine sand, sawdust:gravel or fine sand:gravel. In contrast, experiments with Vitex macuensis (Mapongmetsem, 2006) or Vochysia hondurensis (Mesen, 1993) showed that a 50:50 mixture of sawdust:sand is favorable for propagating these species by cuttings. Different species may display contrasting rooting percentages in different rooting media which may be attributed to variation in oxygen and water content, pH or porosity of the media while survival rate of cuttings may be influenced by media humidity and inherent rooting capacity (Leakey, 1990a). In general, sand is an appropriate rooting medium, with an optimal volume of gas-filled pore-space and oxygen diffusion rate adequate for the needs of respiration (Andersen, 1986). Also, it limits development of microbial pathogens.

IBA had a stronger effect on survival and rooting rate than NAA. IBA has been used for rooting of many tropical trees such as Milicia excelsa (Ofori et al., 1996), C. alliodora (Mesen et al., 1997), Prunus sp. (Esitken et al., 2003; Tchoundjeu et al., 2002a, 2002b); Shorea parvifolia and Shorea macropera (Aminah et al., 2006) and Dalbergia melanoxylon (Amri et al., 2010). For Cordia alliodora, an IBA concentration of 1.6% produced the highest rooting percentage (Mesen et al., 1997) and cuttings without IBA failed to root (Mesen, 1993). However, in M. excelsa, a decline in rooting percentage with IBA concentrations greater than 0.2% was reported (Ofori et al., 1996). Successful rooting without applied auxin has also been reported in a number of other tropical tree species, such as Shorea macrophylla (Lo, 1985), Nauclea diderrichii (Leakey, 1990a) and Vochysia hondurensis (Leakey et al., 1990b). It is known that concentrations of auxin substantially higher than those normally found in plant tissues may cause cell death (Hartmann et al., 1990). In this study, either IBA or NAA concentrations greater than to 100 μg/l reduced rooting rates, as well as the number of leaves and roots and root-length.

Accession source had no significant effect on rooting and cutting growth but harvesting period is of importance. In contrast, Somkuwar et al. (2011) described rootstock variability in the ability for rooting based on the biochemical constituents of the Vitis sp. In this experiment, the April–May period, which corresponds to the beginning of rainy season, is generally more favorable for rooting and development of cutting than either the August–September or November–December period, which correspond to the peak rainy and end-of-rainy season, respectively. Generally, each period is characterized by specific physiological and biochemical stages. Cuttings taken in April–May and August–September were in a vegetative growth state while those harvested in November–December were dormant. In contrast, Auge (1989) demonstrated that dormant cuttings are more responsive than actively-growing cuttings. In Hevea brasiliensis, which exhibits rhythmic growth independent of seasonal cycle, cuttings harvested during the vegetative or active phase are most suitable for rooting and development (Lardet et al., 1990). Harvest period had a strong effect (68.4% of total).

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**Table 2**

<table>
<thead>
<tr>
<th>Accessions</th>
<th>Type of cutting</th>
<th>Survival rate of cutting</th>
<th>Rate of rooting</th>
<th>Number of roots/cutting</th>
<th>Number of leaves/cutting</th>
<th>Length of roots/cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yaounde (A1)</td>
<td>C1</td>
<td>86.0 ± 3.4 d</td>
<td>80.0 ± 3.0 e</td>
<td>8.3 ± 0.6 c</td>
<td>7.1 ± 0.8 b</td>
<td>12.6 ± 1.2 b</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>80.3 ± 3.2 bc</td>
<td>89.6 ± 2.8 f</td>
<td>8.7 ± 0.7 c</td>
<td>7.4 ± 0.4 c</td>
<td>12.1 ± 0.9 b</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>73.2 ± 2.8 ab</td>
<td>68.4 ± 1.5 c</td>
<td>8.0 ± 0.9 bc</td>
<td>7.3 ± 0.6 c</td>
<td>14.6 ± 1.5 c</td>
</tr>
<tr>
<td>Mbalanayo (A2)</td>
<td>C1</td>
<td>84.3 ± 3.8 d</td>
<td>80.1 ± 1.9 e</td>
<td>7.6 ± 0.7 b</td>
<td>7.0 ± 0.9 b</td>
<td>14.5 ± 0.8 c</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>75.6 ± 2.7 b</td>
<td>90.6 ± 2.9 f</td>
<td>8.1 ± 1.0 b</td>
<td>6.9 ± 0.3 b</td>
<td>11.0 ± 0.7 a</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>70.9 ± 2.6 a</td>
<td>63.5 ± 1.7 b</td>
<td>6.8 ± 0.5 a</td>
<td>6.5 ± 0.7 a</td>
<td>15.1 ± 1.3 d</td>
</tr>
<tr>
<td>Santchou (A3)</td>
<td>C1</td>
<td>82.9 ± 3.1 c</td>
<td>65.1 ± 0.9 b</td>
<td>6.0 ± 0.6 a</td>
<td>6.8 ± 0.4 b</td>
<td>10.3 ± 1.0 a</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>78.3 ± 1.9 b</td>
<td>75.6 ± 1.4 d</td>
<td>6.8 ± 0.8 a</td>
<td>6.4 ± 0.8 a</td>
<td>10.9 ± 0.8 a</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>68.9 ± 1.5 a</td>
<td>58.9 ± 0.8 a</td>
<td>7.2 ± 0.6 a</td>
<td>6.3 ± 0.3 a</td>
<td>15.6 ± 1.6 d</td>
</tr>
</tbody>
</table>

Values having the same letter(s) in the same column are not significantly different according to Fisher’s LSD Test at P < 0.05.

C1: Apical cutting; C2: Basal single-node leafy cutting; C3: Basal two-node leafy cutting.
on rooting rate. Rooting being a prerequisite for continued growth and development of plants, this factor is then very important for propagation of *R. heudelotii*.

Cutting type also had significant effects on rooting independent of the accession. It is responsible for almost 70% of observed results for three parameters measured: (rooting rate, leaf number and roots length). Undifferentiated tissues of woody plants are generally more responsive than differentiated structures (Auge, 1989). Additionally, response was enhanced when the cuttings were soaked for 60 min in honey, possibly because the honey provides protection against desiccation and rottion, which are the main causes of mortality (Shiembo et al., 1997a). Honey which is natural and non toxic compared to other substances like petroleum and silicon also used to prevent desiccation could be suitable to treat cuttings. After acclimatization, maximum survival rate of plants was 67.6%; these results are similar to those obtained by Youmbi and Benbadis (2001) for *D. edulis* and Fotso et al. (2004) for *R. heudelotii*, who used the same substrate for acclimatization as reported here. This work provides an improved protocol which can be used for propagation of *R. heudelotii* in agroforestry systems.

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