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Full Length Research Paper

Domestication of *Irvingia gabonensis* (Aubry Lecomte) by air layering

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***Irvingia gabonensis* is one of the most important indigenous fruit tree species in African humid tropics. Methods have been developed for phenotypic selection and vegetative propagation of this species; but techniques for the capture of desired traits through inexpensive technological methods are still lacking. A series of experiments were conducted to assess the effects of auxin (Seradix-2) application (treated and untreated marcotts), branch diameter (, and 4 - 5 cm), and position in the crown (lower, middle and upper) on the rooting of marcotts on one hand; and the combined effect of branch length and diameter (50 cm length, 2 - 3 cm diameter), (50 cm length, 4 - 5 cm diameter), (100 cm length, 2 - 3 cm diameter), (100 cm length, 4 - 5 cm diameter), (150 cm length, 2 - 3 cm diameter), (150 cm length, 4 - 5 cm diameter) on the survival of rooted marcotts on other hand. After 12 months, the proportion of rooted marcotts was significantly higher (P < 0.05) for untreated (50 ± 7.3%), than for treated marcotts (31 ± 6.7%). Marcotts of 3 - 5 cm diameter rooted significantly better (46.9 ± 5.1%) than those of 2 - 3 cm of diameter (32.1 ± 5.2%). Branch position in the crown did not affect rooting ability of marcotts. After 8 weeks, the highest survival rate of weaned marcotts (40%) was observed for those with 100 cm length and 2 - 3 cm diameter while the highest mortality rate was obtained for those with 150 cm length and 4 - 5 cm of diameter (90%). It can be therefore concluded from this study that *I. gabonensis* is amenable to air-layering but further studies are needed to improve the survival rate during the weaning period.**

Key words: *Irvingia gabonensis*, domestication, marcotting, rooting ability, auxin, branch diameter, length and position.

INTRODUCTION

Irvingia gabonensis (Aubry Lecomte), locally known as bush mango tree is an oleaginous fruit tree species belonging to the *Irvingiaceae* family. The edible fruit pulp contains fibre, minerals and vitamin C (Vivien and Faure, 1996; Milko, 2009). Its kernels are used as soupthick-ener, have high polysaccharide content (Ndjouenkeu et al., 1996). Between January and February 1995, it was estimated that Cameroon exported 140 tons of kernels to neighboring countries (Gabon, Nigeria and Equatorial Guinea) for an estimated income of US $ 302,000 (Ndoye et al., 1998) The oil from the kernels has a high saponification index of 233 – 250 (Okafor, 1983). In addition, the bark of bush mango is used to relieve hernia, diarrhea and yellow fever and is also used as antidote for poison and to relieve dental pain (Ayuk et al., 1999). Due to its economic, nutritional and therapeutic importance, some Cameroonian farmers protect the species, and collect its germ plasm through various tree domestication techniques ( ).

Considerable domestication research has been done on *Irvingia gabonensis.* Among others, Schiembo et al. (1996) investigated vegetative propagation of juvenile leafy stem cuttings for mass production of selected trees; Atangana et al. (2001), Leakey et al. (2002), Anegbeh et al. (2003) reported variation in fruit and kernel sizes, and weight of fruits and kernels; Omokolo et al. (2004) investigated the

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mass production of *I. gabonensis* by *in vitro* propagation; Leakey et al. (2005) investigated variation in kernel's nutritive attributes to show the great variability within trees and populations and Lowe et al. (2010) studied diversity within and among populations using molecular techniques. However, a great deal of work is required to fully develop the propagation potential of the species. One key area is understanding the factors which affect rooting and sprouting of *I. gabonensis* marcotts.

Air layering (marcotting) is a vegetative propagation technique characterized by the initiation of adventitious roots on one part of the tree branch *in situ*. After root initiation, the rooted part (marcott) is weaned from the tree and transplanted in a substrate where it grows independently of the mother tree. Like other vegetative propagation techniques, the main advantages of marcotting are cloning selected trees with desirable traits and shortening the period for fruit production (Hartmann et al., 1997; Kengue, 2001). In fact, *I. gabonensis* trees from seed- lings start bearing fruits after 7 - 10 years, while trees from marcotting can initiate fruiting after 3 - 4 years (ICRAF AHT, unpublished data).

Diverse factors (genetic, environmental and physiological) may influence the rooting and shooting abilities of marcotts. Studies had shown that rooting ability of *Dacryodes edulis* marcotts may vary with branch diameter, bark thickness, rooting substrate, hormone type and concentration and marcott length (Hartmann et al., 1997; Kengue and Tchio, 1994; Mialoundama et al., 2002). It has also been demonstrated that rooting and sprouting requirements are highly variable within and among species. Therefore, assessing factors that affect rooting and sprouting abilities of marcotts in each species is an important prerequisite for its successful propagation through air-layering.

The objectives of this study on vegetative propagation of *I. gabonensis* by marcotting were to assess the effect of Seradix -2 (auxin), branch diameter and the marcott's position in the crown on rooting and the effect of branch length and diameter on survival rate of weaned marcotts.

MATERIALS AND METHODS Non-mist giant

propagator or humidity chamber

The giant propagator modified from Leakey et al. (1990) consists of a wooden frame (2 m × 2 m × 2 m) enclosed in a single clear polyethylene. The watertight base of the propagator is covered by a layer of river sand of 20 - 30 cm depth. In this propagation system, air humidity is maintained by the provision of a water table in the sand layer, resulting in a permanently humid environment (RH = 80 - 90%). Relative humidity and temperature provide growing conditions approximately similar to field conditions in the humid lowlands of Cameroon. The giant propagator used in this study (Figure 1) was constructed under a shade house roofed with stainless zinc and translucent plastic sheets at a nursery managed by the World Agroforestry Centre (ICRAF) in Nkolbisson, Yaoundé (altitude: 700 m, latitude: 3°52' N; longitude: 11°26' E). Yaoundé is located in the central region of Cameroon within the semi-deciduous forest zone (Letouzey, 1985). The rainfall pattern is bimodal with four distinct seasons: the first rainy season (March – June) and the second raining season (August – October), the long dry season (November – February) and short dry season (July). The temperature varies between 23 and 25°C and the relative humidity normally varies between 73 and 84%, but may be as low as 60% and as high as 100%. The sides of the shade house were made of a shade cloth allowing 40% ambient light transmission to reduce air temperatures in the propagator.

Study areas for marcott preparation and setting

Marcotts were prepared and set in the Ting Melen and Biyan localities, located around Yaoundé. Trees were selected, in cocoa system, with the farmer's knowledge's contribution, using the following criteria: < 15 years old, vigor (not dry and without insect damage), and desirable fruit traits (taste, size and yield). On each selected tree, used as a block in both experiments, marcotts were set up in June on orthopropic and oblique oriented branches in the morning. The bark was completely stripped off with a knife 5 cm away from the crotch (formed by two branches) and over a length of 5 to 10 cm. After application of hormone, the debarked area was covered with moist substrate (Figure 2), wrapped in a transparent plastic sheet and secured at each end with a rubber band (Kengue, 2002). Four experiments were setup. The details of each experiment are presented below.

Experiment 1: Effect of Seradix-2 on marcotts' rooting abilities

Twelve trees were used for this experiment. On each tree, eight branches of 3 cm diameter were selected for marcott setting at random in the crown. A total of 96 marcotts were set, and Seradix-2 applied to 48 marcotts (4 per tree) while the other 48 marcotts were untreated (control). Moist decomposed sawdust was used as substrate.

Experiment 2: Effect of branch diameter on marcotts' rooting abilities

Twenty-seven trees were used. Two different ranges in branch diameter (treatments) were tested (2 - 3 and 3 - 5 cm). Diameter was measured using a caliper. The experiment was done without hormone. Moist decomposed sawdust was used as substrate. The number of marcotts per treatment was three with a total of 162 marcotts set (2 treatments × 3 marcotts / tree × 27 trees) for the whole experiment. The marcotts were set at random in the crown.

Experiment 3: Effect of marcott's position on rooting ability

Twenty-two trees were chosen. The crowns of each tree were divided into the lower, medium and upper sections. In each section, two marcotts were set without hormone; 44 marcotts were set on 22 trees for each treatment with a total of 132 marcotts set (3 treat- ments, 2 marcotts per treatment and 22 trees) for the experiment. The marcotts were set independently of branch diameter. The experimental design was randomized complete block. Statistical analysis was performed using SAS through the following model:

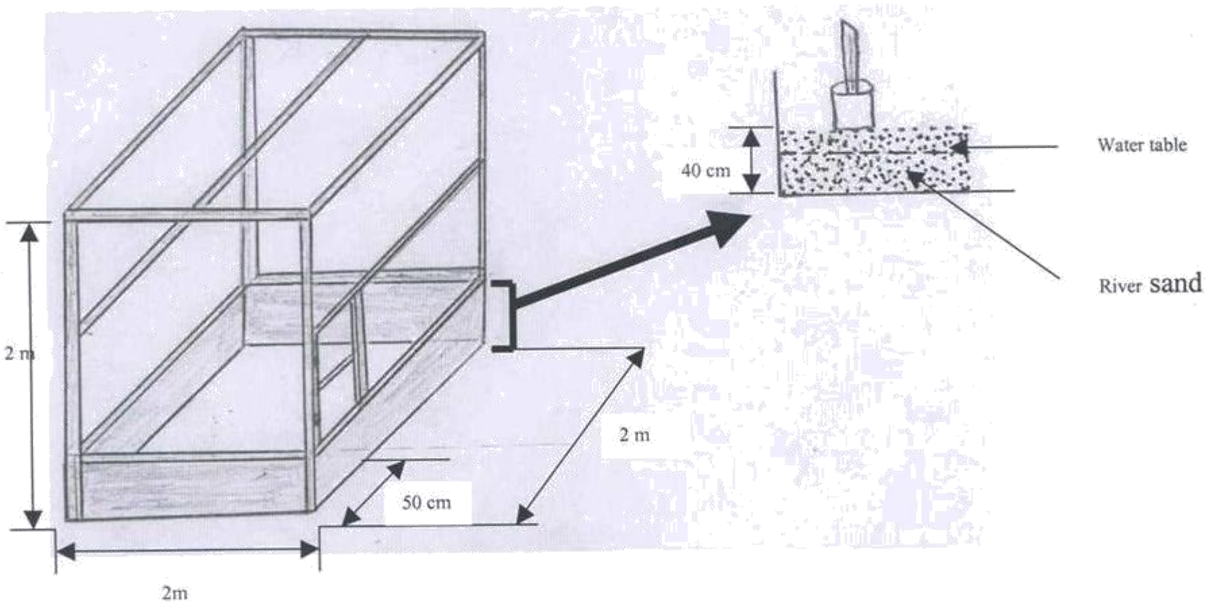

Figure 1. Humidity chamber.

Figure 2. Setting –up of *Irvingia gabonensis* marcott.

$Y_{ij} = \quad + \quad_{i} + \quad_{j} + \quad_{ij}$

Where Y is the average value of the dependant variables, effect of block, the treatment effect and the error.

Experiment 4: The effect of branch length and diameter on rooted marcotts' survival

Set marcotts were assessed at 3-months intervals for rooting. Those with more than one root of 4 cm length were considered rooted. The rooted marcotts were collected without leaves, preserved in a wet plastic bag before being taken to the nursery. Before potting, the plastic sheets stripped off and the roots carefully freed. Harvested marcotts of different diameters (2 - 3 and 3 - 5 cm) were three different lengths (50, 100 and 150 cm) and potted in 40 × 30 cm bags, filled with substrate composed of 2/3 soil and 1/3 sand. The number of marcotts per treatment was 30 and a total of 180 marcotts was used. The experimental design was completely randomized. The model was:

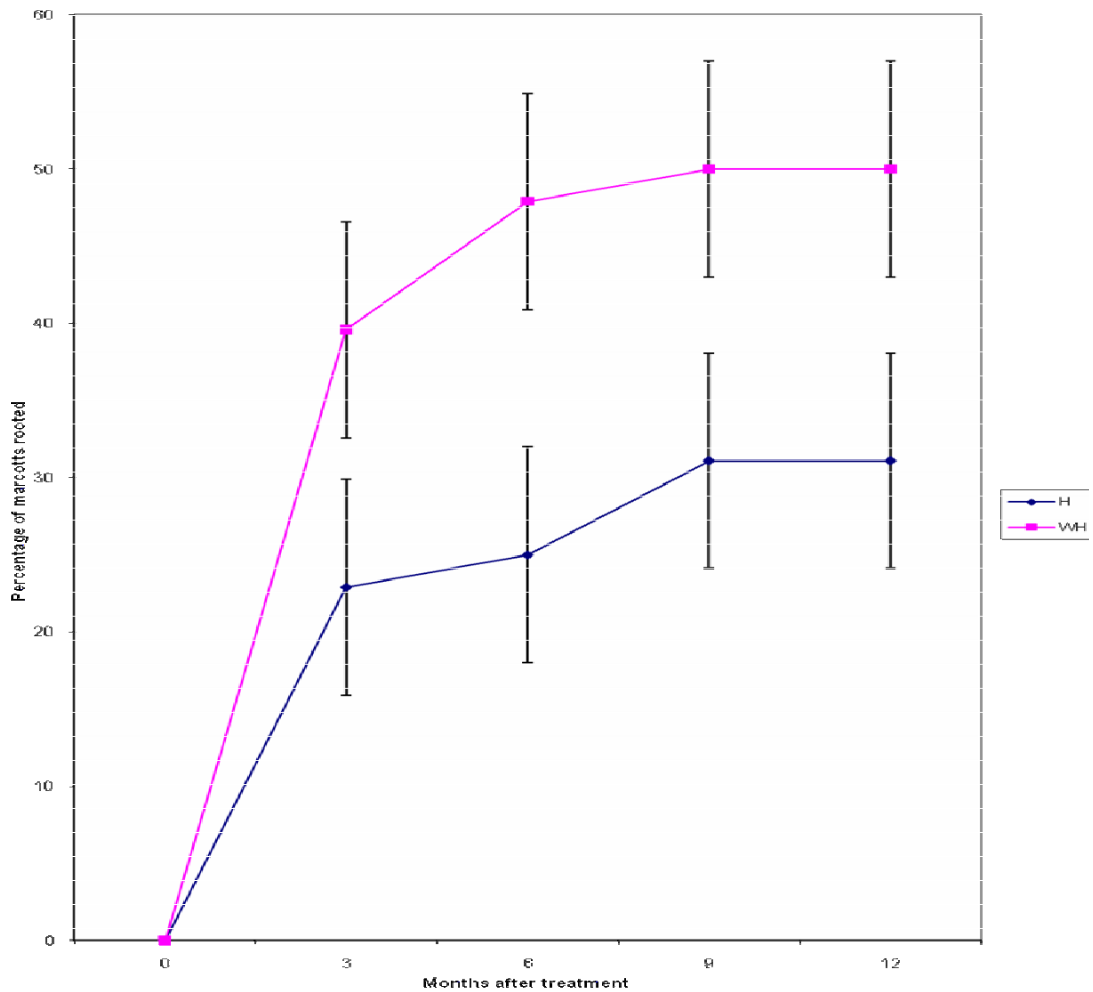

Figure 3. Effect of seradi-2 on rooting *I. gabonensis* marcotts.

$Y_i = \; + \; _i$

Where Y is the average value of the dependant variables, the overall mean and the treatment.

After potting, the wounded parts of the marcotts were protected with mastic to prevent diseases during the weaning period in the giant propagator. The marcotts were watered with about 100 ml of water every 2 to 3 days depending on the nature of the substrate.

Assessment of dependent variables

The variables assessed for rooting experiments (1, 2 and 3) were rooted marcotts, mortality and unrooted marcotts. Four assessments were done in 12 months. For weaning experiment (4), marcotts, survival rate was assessed on a weekly basis for a period of 8 weeks. In each experiment, data were collected, normality tested and transformation by arcsin applied using Excel before being subjected to analysis of variance (ANOVA) in Statistical Analysis System (SAS) using General Linear Model (GLM) procedure. The tests were done at the 5% level .

RESULTS

Effect of Seradix-2 on rooting ability of marcotts (Experiment 1)

Analysis of variance (ANOVA) indicated that Seradix-2 treatments had no significant effect on the rooting percentage of *I. gabonensis* marcotts at the third month (P = 0.196); but at the sixth, ninth and twelfth months, the hormone significantly affected the rooting percentage (P = 0.004, 0.024 and 0.024) respectively. The cumulative rooting percentage of the treated and untreated marcotts (control) at 12 months was 31 ± 6.7 and 50 ± 7.3%, respectively (Figure 3). Seradix-2 significantly increased the mortality of marcotts independent of the duration. The cumulative mortality at 3, 6, 9 and 12 months were 25 ± 6.3, 43.7 ± 7.2, 54.2± 8.9 and 58.3 ± 8.8%, respectively with seradix-2 and 4.2 ± 2.9, 27.9 ± 6.5, 33. 33 ± 6.8 and

Table 1. Mean of rooting marcotts per tree.

Tree	Rooting (%)
11	100 a
12	75.0 ab
2	62.5 ab
9	50.0 bdc
1	37.5 bdc
3	37.5 bdc
6	37.5 bdc
4	25.0 bdc
7	25.0 bdc
10	25.0 bdc
5	12.5 dc
8	0.0 d

Trees with the same letter are not significantly different (P = 5 %).

39.6 ± 7.1%, respectively for the control. Most of the marcotts were rooted or died after 3 months.

Rooting ability varied significantly among trees of *I. gabonensis* marcotts (P < 0.001) independently of the duration after the setting of the marcotts. After 9 months the rooting rate varied between 0 and 100 % (Table 1). No significant interaction was observed between tree and treatment for the rooting ability (P = 0.0765).

Effect of branch diameter on rooting ability of marcotts (Experiment 2)

Branch diameter did not have a significant effect on rooting percentage after 3 months (P = 0.19). Meanwhile, at the sixth, ninth and twelfth months, the percentage of rooted marcotts with 4 - 5 cm diameter significantly increased more rapidly than the others (P = 0.06, 0.01 and 0.01 respectively). At the end of the experiment, the overall rooting percentages of marcotts were 32.1 ± 5.2 and 46.9 ± 5.1% for 2 - 3 and 4 - 5 cm diameter, respectively. Most of the marcotts rooted were after 3 months (Figure 4).

Branch diameter significantly affected marcott mortality (P = 0.032) . Independent of the duration, mortality was significantly higher for marcotts with 2 - 3 cm diameter. The cumulative percent mortality at the twelfth month for 2 - 3 and 4 - 5 cm diameter were 67.9 ± 5.2 and 40.7 ± 5.5%, respectively. At the twelfth month unrooted marcotts with 4 - 5 cm diameter developed more callus than the others. Rooting ability also varied between trees. At the twelfth month, rooting per tree varied from 0.0 to 100%.

Effect of marcott position on rooting ability (Experiment 3)

No significant difference was observed between the three crown positions. Three months after setting the marcotts, the percent rooting of branches in the lower, middle and upper positions was 23.8 ± 3.1, 34.09 ± 7.2 and 35 ± 7.6%, respectively; while after 12 months, they were 28.2 ± 6.8, 36.4 ± 7.3 and 37.5 ± 7.7%, respectively (Figure 5). Irrespective of the treatment duration, the location of the branch in the crown had no significant influence on the mortality of marcotts. At the twelfth month assessment, the percentages of dead marcotts were 66.7 ± 7.4%, 54.5 ± 7.6 and 50.0 ± 8.0%, for lower, middle and upper positions respectively.

Effect of branch length and diameter on marcotts survival (experiment 4)

Marcotts sprouted two weeks after potting. Eight weeks after potting, the highest survival rate and the best sprouting performance was observed on marcotts of 100 cm length and 2 - 3 cm diameter, while the lowest was obtained on marcotts of 150 cm length and 4 - 5 cm diameter. Marcotts of 4 - 5 cm diameter, 100 and 150 cm length sprouted more rapidly than the others at week 2 (Table 2). Marcotts of 150 cm length and 4 - 5 cm diameter showed greatest mortality from week 3. Most of the marcotts died after sprouting (Table 3).

DISCUSSION

The importance, firstly of branch diameter, hormone, position of the marcotts in tree crown, marcotts rooting ability, and secondly of the length and the diameter of marcotts during weaning on the marcott survival is widely recognized (ICRAF-WCA, unpublished data; Kengue, 2002). The results of the experiment on Seradix – 2 showed that auxin treatment decreased the rooting percentage and increased the mortality rate of *I. gabonensis* marcotts. The same experiment were ran on *Dacryodes edulis* and *Cola nitida* and the results showed no significant effect of Seradix – 2 on the first species contrary to the second (ICRAF-WCA, unpublished data; Asaah et al. in preparation). Seradix-2 is well known as an exogenous IBA (4 – indol – 3yl butyric acid), which is an adventitious root stimulating hormone. Also, auxin concentration is known to affect the root development ability of *I. gabonensis* cuttings (Schiembo et al., 1996) with the optimal concentration of 250 µg. So, the negative effect observed here might be due to the high concentration of auxin in Seradix – 2 (0.3% of IBA). Future work should focus on IBA concentration in Seradix -2 and the evaluations

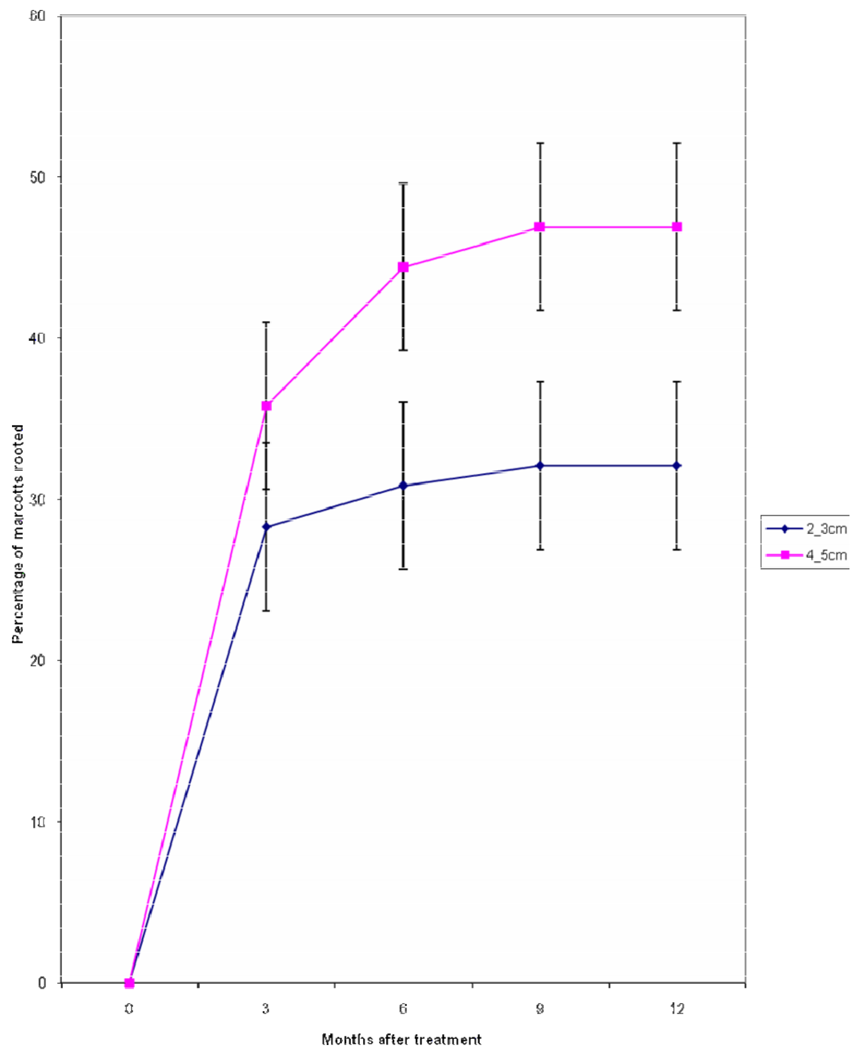

Figure 4. Effect of diameter on rooting of marcott of *I. gabonensis*.

evaluation of their influence on *I. gabonensis* marcotts. If the quantity is reasonably high, there should be positive effects as demonstrated by Mialoundama et al. (2002).

The differences in rooting in relation to marcott diameter might be due to the quantity of carbohydrate stock in the branches. Before roots initiation, the branches rely on the reserve of carbohydrates in their stem tissues. Consequently, branches with large diameters have more stored carbohydrates and auxin. This probably explained the best rooting performance of branches with 4 - 5 cm diameter. The higher mortality of marcotts with 2 - 3 cm diameter could be explained by lower reserves of carbohydrate and the tenderness of the stem tissues (Brian and Nina, 1988), compared with marcotts with 4 - 5 cm diameter. The same results were observed with *D. edulis, Ricinodendron heudelotii* and *C. nitida* (Kengue, 2002; Mialoundama et al., 2002; Asaah et al. in preparation). It is expected that branches with larger diameters should be more suitable for *I. gabonensis* air layering. However, this has not been experimentally demonstrated.

The position of the marcotts in the crown did not affect rooting ability of marcotts. These observations could be explained by two main factors of root initiation which are physiological age and carbohydrate distribution. Concerning the physiological age, the expected result was a significant rooting of upper part of the crown because of the fact that their branch tissues are younger than others. Since this was not the case, this should mean that the non significant effect should be explained by the relative equal photosynthesis distribution along the crown. In fact, Sellin and Kupper (2004) have shown high

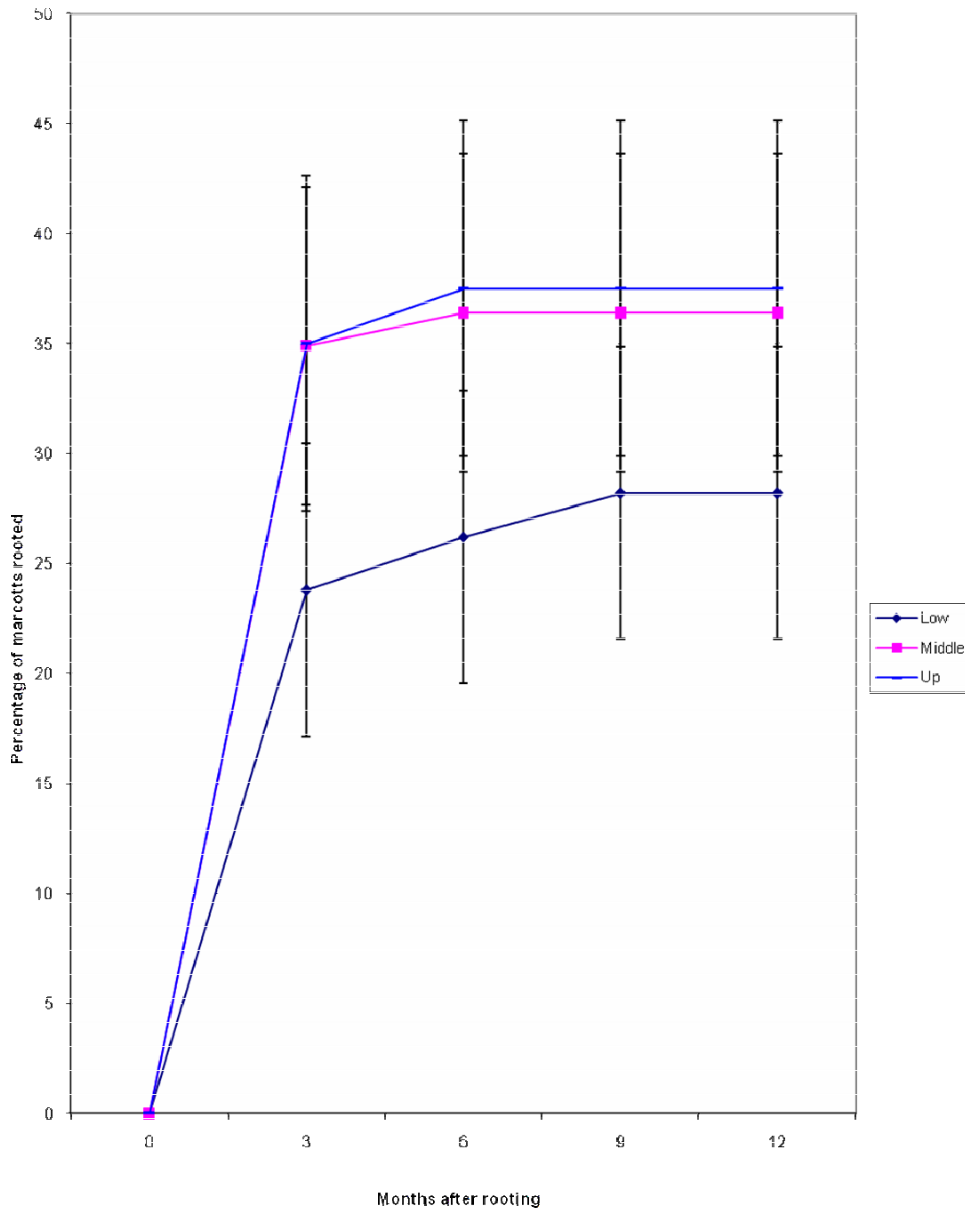

Figure 5. Effect of branch position on rooting of marcotts of *I. gabonensis*.

photosynthetic photon flux in the upper crown position, indicating that the upper crown position receives more light than the lower crown. Photosynthesis consequently, should be more active. It is worth noting that their experiment was carried out in the forest. But in the cocoa agro forest where this experiment was carried out, apparently, there was no light gradient on the crown because the integrated trees are sparser than in the forest scenario. So, there could be an equal distribution of the physiological rooting factors in the different parts of the crown. Consequently, all positions could have the same potential of rooting.

According to Tim (1988), Brian and Nina (1988), the difference in the survival rate between different lengths/diameters of marcotts could be linked to the roots quantity/marcotts volume relation-ship. In this study apparently, there was no difference between root quan-tities of different rooted marcott diameters. This means that the quantity of nutrient uptake should be the same, therefore, sufficient to nourish the marcotts with small diameters contrary to largest. In fact, marcotts with 2 - 3 cm diameter and 100 cm length could be the stem tissues with balanced gain (by photosynthesis) and loss

Table 2. Percentage of survived and sprouted marcotts at the weaning period.

Treatments*	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
(50,2-3)	0	10	10	70	40	50	20	20
(50,4-5)	0	0	50	50	60	70	20	20
(100,2-3)	0	10	40	40	50	50	40	40
(100,4-5)	0	20	40	40	60	60	50	20
(150,2-3)	0	10	20	30	40	60	60	30
(150,4-5)	0	40	60	40	50	50	50	10

* : Length and diameter of marcotts (cm).

Table 3. Percentage of dead marcotts at the weaning conditions.

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
(50,2-3)	0	0	0	0	30	70	70	70
(50,4-5)	0	0	20	20	20	30	80	80
(100,2-3)	0	0	20	20	20	20	60	60
(100,4-5)	0	0	40	40	40	40	50	80
(150,2-3)	0	0	0	10	10	30	40	70
(150,4-5)	0	0	0	20	20	50	50	90

(through transpiration) of carbohydrates. To this effect, the future work should be the quantification of roots and it effect on marcotts survival.

Regarding the effect of marcott diameter on rooting ability and the combined effects of marcott diameter and length on the survival of weaned marcotts, it is advisable to set marcotts on branches with 2 - 3 cm diameter. This branch diameter is recommended as its survival rate after weaning is higher than 4 - 5 cm diameter and its rooting rate acceptable. The rooting speed of *I. gabonensis* marcott is very high. Most marcotts rooted after 3 months in contrast to *D. edulis* in which majority of the marcotts rooted after 6 - 9 months (Kengue et al., 1998; Kengue, 2002). This could be explained by the carbohydrate reserves in relation to the diameters of the chosen branches. The *D. edulis* marcotts were set on branches with 4 - 5 cm diameter (older stem tissues) contrary to *I. gabonensis* marcotts, which were set on branches with 2 - 3 cm of diameter (young stem tissues).

Intra-specific variation is known to be a cause of variation in the rooting ability between trees. The same results (tree to tree variation) were observed on *D. edulis* marcotts (Kengue et al., 1998), *Ricinodendron heudelotii, Lovoa trichilioides* and *Khaya ivorensis* stem cuttings (Mpeck et al., 2004; Tchoundjeu, 1989). That tree to tree variation could be explained by variation in the physiological and genetic factors between trees within species. For example, Zobel (1996) showed that the initiation and the structure of the root system could be influenced by plant genotype. In all vegetative reproduction techniques (marcotting, grafting, cutting, etc), the physiological state of the mother tree is a key factor of rooting ability. Trees at the physiological stage of ascending sap are in all cases most suitable to rooting, or grafting success. The existence of genetic differences among trees within the same population is largely recognized, and this could affect the physiology of each tree, and consequently the rooting ability of marcotts (Tchoundjeu, 1989; Kengue et al., 1998; Mpeck et al., 2004).

This study has shown that *I. gabonensis* branches root, but the survival rate is still too low. Air layering will be recommended to multiply the species when the survival rate has been increased. Another option to improve survival is mycorrhizal inoculation. In fact, the soil from Cameroon rain forest zones is acid, mainly characterized by low pH, deficient on Ca, Mo, Mg, K and P and toxicity on Al, Fe and Mn (Ambassa-Kiki, 2000). According to this authors, 75 - 100% of humid forest zone of Cameroon is made up of acidic soils.. Consequently, the plants poor roots extension like *I. gabonensis* marcotts (Ingleby, 2001) should grow very slowly.

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