Effects of phytohormones and alternative sources on the propagation of *Ternstroemia cameroonensis* Cheek. by marcotting in the Lebialem Highlands, Cameroon.

ABSTRACT

Marcotting is a method of vegetative propagation which still finds its relevance in the present day forest management and routine practice to produce viable seedlings for the regeneration of *Ternstroemia cameroonensis*. There is dearth of information regarding the propagation of *T. cameroonensis* by marcotting. Hence effects of phytohormone application (Indole-3-acetc acid, Indole-3-butyric acid) and alternative sources (coconut water) on the propagation of *T. cameroonensis* by marcotting were investigated in the Lebialem Highlands. Mortality rates, number of roots, root length as well as marcotts position in the crown was evaluated. The propagation trials were carried under natural environmental conditions. Marcotts had an overall survival percentage of 35.41%. Those established at the middle of the crown had the highest survival percentage (15.27%) followed by those at the lower (11.80 %). Also marcotts established on branches with larger diameter (4-6 cm) and shorter length (50 cm) had the highest survival percentage (20.13 and 19.44 respectively). Again marcotts treated with IBA and CW had the best performance compared to other pre-treatments. According to the result, *T. cameroonensis* can be amenable through marcotts.

Keywords: *Ternstroemia cameroonensis*, phytohormone and alternative sources, rooting of marcotts, branch diameter and length, marcottst position.

1. INTRODUCTION

Ternstroemia cameroonensis is a highly endangered medicinal plant species found only in the Lebialem highlands [1; 2]. It growing 15-18m tall with inflorescence of four to five single flowers on the end of a stem, oval fruits and leathery leaves 6-7 x 22.5 [3]. This plant is of pharmaceutical importance to the indigenous people as decoction of bark is used to treat anaemia, stomach ache, vomiting, urinary tract infections, infertility and epilepsy [4]. A poster depicting the species, for use in Cameroon and promoting its conservation was

produced and distributed by Kew [5]. Tacham *et al*. [6], report that the species is overexploited for sale locally. No regeneration has been detected possibly because any seedlings that develop might be swept away by run-off down the steep slopes on which the surviving trees grow [2]. The work of Nkemnkeng *et al*. [7] indicate that the species is highly vulnerable in the Lebialem highland due method of exploitation with few mature individuals in the wild. The species has not been cultivated nor seed-banked. Seeds of some species of *Ternstroemia* have been shown to be dormant, particularly *Ternstroemia washikiatii* germinating after six to seven months [8]. Recent study of Nkemnkeng *et al.* [9] indicates that propagation of *T. cameroonensis* through seed and stem cuttings were inappropriate. However, a great deal of work is required to fully develop the propagation potential of this species.

Asexual propagation describes the regeneration and multiplication of plant from vegetative parts, like buds, leaves, single cells/tissues as well as cuttings of roots and stems [10]. It offers a wide array of applications in indigenous fruit tree domestication efforts and general plant genetic resources conservation programmes. With these applications, experts are able to select desired characters available in the wild tree population by fixating the genetic variation of such trees in the natural stands. Ultimately, the objective is to multiply large quantity of improved seedlings for interested farmers and other users [11, 12]. 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 marcotts 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 [13, 14].

Diverse factors (genetic, environmental and physiological) may influence the rooting and shooting abilities of marcotts. Studies had shown that rooting ability of marcotts in some species may vary with marcott length, branch diameter, bark thickness, rooting substrate, hormone type and concentration [13, 15]. 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 purpose of this study was to determine the effect of phytohormones as well as alternative sources, branch diameter, branch length and the marcott's position in the crown on rooting and survival of weaned marcotts.

2. METHODOLOGY

2.1. Study site

This study was carried out in two phases. The first phase in the Lebialem Highlands located in the North Eastern part of the Southwest Region of Cameroon between latitude 5º38 N and 5º43 N and longitude 9º58 E and 10º27 E [16].

The climate of this region is that of the Cameroon mountain range characterized by high winds and low sunshine. The average daily temperature varies very much with season and ranges from 17^{o}C to 32^{o}C [17]. The area is between altitudes 1000 m to 2700 m above sea level and annual rainfall is 2222 mm.

2.2. Marcotts preparation and setting

Trees were selected in the wild considering areas which are less prone to anthropogenic activities. On each selected tree marcotts were set at each level that is lower, middle and upper section of the crown. These levels consisted the blocks within which were treatment and repetitions on different tree. The marcotts were set up is a 2x2x4 factorial design with 3 levels and 3 repetitions on different trees that is a total of 144 marcotts were setup for this trial. Marcotts were set up in August on orthopropic and oblique oriented branches in the morning to reduce stress that may arise with extreme environmental conditions during the day. 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 and coconut water on the stripped area, it was covered with moist decomposed sawdust wrapped in a transparent plastic sheet and secured at each end with a rubber band [18]. Three experiments were setup with details presented below (Figure 1).

- Experiment 1: Effect of IAA, IBA and Coconut water on marcotts' rooting abilities

In this experiment, three trees were used for this experiment. On each tree marcotts setting were taken at random in the crown. After stripping, rooting reagents were applied and the branch was covered with moist decomposed sawdust. A total of 48 marcotts were established per tree with IAA applied to 12 of them, IBA to another 12, CW applied to 12 and 12 marcotts left untreated (control).

- Experiment 2: Effect of marcotts's position on rooting ability

Position of marcotts on the tree was also evaluated. On this note, the tree crown was divided three portions namely: lower, middle and upper sections. In each section, four marcotts were set with respect to treatment:, IAA, IBA, CW untreated and (control).

❖ Experiment 3: The effect of branch length and diameter on marcotts' survival

Marcotts were established on branches with different branch length and diameter. Branch length and diameter were within different ranges that is 40-60 cm (L_{50}), 80-120 cm (L_{100}) and 2-4 cm (D_3), 4-6 cm (D_6) respectively [18]. Equally after stripping, rooting reagent were applied and covered with decomposed sawdust using polythene paper and tied at both ends. The marcottsts were watered with about 20 ml of water every 2 weeks using a 10 ml syringe. The experimental design was randomized complete block. Assessment of dependent variables that is rooted marcotts, unrooted marcotts and mortality were evaluated every after 14 weeks for a period of 10 months.

2.3. Data collection.

This consisted of survival percentage, number of rooted marcotts as well as root length. All these were evaluated every after 12 weeks for a period of 8 months. Number of roots were counted on each rooted marcotts while the root length was measured using a meter rule. The survival percentages were evaluated based on the formula below.

$$Survival\ percentage = \frac{number\ of\ marcotts\ survived}{total\ number\ of\ marcotts\ established}$$

2.4. **Data analysis**.

Data collected were entered into excel and subjected into various analysis.

Survival percentages of rooted marcotts, number of roots as well as root length were subjected to analysis of variance (ANOVA) using the statistical programme XLSTAT, 2016 where the least significant differences (LSD) between the mean were detected and separated at $p \leq 0.05$.

3. RESULT

3.1 Effects of coconut water (CW) and hormones (IAA and IBA) marcotts rooting of *T. cameroonensis*.

Out of the 144 marcotts established, 51 survived after a period of 8 months given a survival percentage of 35.41%. Those established at the middle of the crown had the highest survival percentage (15.27%) followed by those at the base (11.80 %) and lastly by those at the top (8.33%). Also marcotts established on branches with larger diameter (4-6 cm) and shorter length (50cm) had the highest survival percentage that is 20.13% and 19.44 respectively

while those with smaller diameter and longer branch length had the least. Generally, marcotts treated with IBA and CW had the best performance compared to other treatments.

3.2 Effect of coconut water, and hormones on rooting ability, and marcotts performance

There was a significant difference in marcotts survival percentage, number of roots and roots length with respect to pre-treatment. IBA had the best survival percentage (47.22%), number of roots (2.33) and root length (2.76 cm) followed by CW with survival percentage (41.57%), root number of roots (0.83) and root length (1.33 cm) while the control treatment had the least (table 1).

3.3 Effect of branch diameter and branch length on rooting ability and marcotts performance

Branch length and diameter had no significant effect on the survival percentages of marcotts. This is also observed in branch length and diameter of 80-120 cm and 2-3 cm respectively. There was a significant difference in the rooting of marcotts within branch length of 40 – 60 cm and diameter of 4-6 cm. Marcotts of branch length within 40-60 cm had the highest number of roots (1.44), root length (1.64 cm) while those of branch length within 80-120 cm had the least number of roots (0.167), root length (0.083 cm) (table 2). In addition, marcotts of branch diameter within 4-6 cm had the highest number of roots and root length (1.72 and 1.972 cm respectively) while those of branch length within 2-3 cm had the least number of roots and root length (0.056) (Table 3).

3.4 Effect of marcotts position on rooting ability and marcotts performance

Marcotts position did not significantly affected survival percentage, as well as number of roots and root length. Despite this, marcotts established at middle of the tree crown had better performance in terms of survival percentages (66.67%), number of root (4.67) and root length (5.67 cm) than those in lower and upper positions (table 4, 5 and 6).

3.5 Survival of marcotts of *T. cameroonensis*

Rooted marcotts of *T. cameroonensis* were weaned into polythene bags at the nursery (Figure 26). Out of 51 rooted marcotts weaned, only seven survived giving a percentage of 13.72% after a period of two months.

4. DISCUSSION

Ascertaining the suitability of marcotting or any other vegetative propagation technique for that matter for the propagation of any particular species before recommendation for large scale use is very important to avoid the loss of tree stands to mortality due to incompatibility

The results are however in agreement with the findings of Steward, [19] who reported that variation in the rooting of marcotts is a factor that can be attributed to environmental conditions that increases the chances of marcotts or mother tree being susceptible to varying degrees of stress and therefore different levels of response to the marcotting procedure. Marcotts within the diameter class of 4-6 cm performed better maybe due to high carbohydrate and auxin reserves. This corroborates with the results of Assah, [20]; Okonkwo *et al*. [21] on vegetative propagation of *Dacryodes edulis*, *Garcinia kola* respectively through marcotting. This result is supported by the work of Menzel, [22] who reported variations in root growth in marcotted trees of *Artocarpus altilis*, and the findings of Kengue, [14] and Mialoundama *et al*., [15] who found differences in root volume in marcotted trees of *Dacryodes edulis* and *Ricinodendron heudelotii* respectively and associated the variations to differences in size of marcotted branches. This is in agreement with Brian and Nina, [23] who postulated that large diameter tree branches tend to have higher carbohydrate and auxin reserves than smaller ones and are therefore more likely to root better than their smaller counterparts. The benefit of the present study is that it reveals that the high carbohydrate reserves in larger diameter branches that promotes rooting also promotes and supports the survival of marcotts while still attached to the mother plant.

In addition, marcotts position equally had an influence on rooting ability, number of roots and root length. Those at the middle of the crown rooted better maybe due the high humid content compared to lower part as well as upper parts of the crown. This is apparent with the findings of Ogbu *et al*. [24] that adventitious root formation by marcotted branches of *P. macrophylla* required high humid condition which is naturally guaranteed during the peak of wet season weather, and vice versa. Obviously, this behaviour may be attributed to the prevailing high moisture and cool temperature associated with the period of peak rainfall. It has been long established that root formation is influenced by auxins produced by the plant itself [10; 25; 26]. The physiology of tree growth phases show that as plant undergoes active vegetative growth cycle; buds release endogenous auxin that moves basipetally to stimulate root production [25; 26]. So by implication and in response to the deliberate wound caused by girdling in marcotting operation, branches of the species began formation of adventitious roots. This is an affirmation of similar reports by Awodoyin and Olaniyan [25], Leakey [27] and Hartmann *et al*. [10] that infliction of wounds on stem brings about auxin induced stimulation of vascular meristematic tissues to form adventitious roots in cuttings and layering techniques.

5. **CONCLUSION**.

Rooting ability of *T. cameroonensis* marcotts proved successful in phytohormones (IAA and IBA) as well as alternative sources (coconut water). IBA had the best performance followed by coconut water while IAA was the least. Thus, rooting of marcotts of *Ternstroemia cameroonensis* with plant growth regulator (IBA, CW) presents a viable propagation technique to be used in enrichment planting programmed of this important highly endangered medicinal plant, though weaned marcotts had a very low survival percentage.

COMPETING INTERESTS DISCLAIMER:

The authors declare that they have no competing interests. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AUTHORS CONTRIBUTION.

Francoline Jong Nkemnkeng, Walter Ndam Tacham and Christiana Ngyete Nyikop Mbogue carried out the field exercise and produced the first draft of manuscript while Mendi Grace Anjah and Victor François Nguetsop edited and fine-tuned the first draft manuscript. Mendi Grace Anjah and Victor François Nguetsop supervised the work. All authors read and approved the final manuscript.

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Tables

Table 1: Effect of coconut water and hormones on rooting ability and marcotts performance.

Treatment	Sur%	NR	RL

Control	27.78[a]	0.389[a]	0.305[a]
CW	41.67[ab]	0.83[a]	1.33[ab]
IBA	47.22[b]	2.33[b]	2.76[b]
IAA	25.0[a]	0.5[a]	0.56[a]

Means followed by the same letters in the same column are not significantly different at p≤0.05

Sur%: survival percentage, NR: number of roots, RL: root length

Table 2: Effect of branch length on rooting ability and marcotts performance

L	SUR%50	SUR%100	NR 50	NR100	RL.50	RL 100
Control	33.33[a]	22.22[a]	0.22[a]	0.167[a]	0.22[a]	0.083[a]
CW	44.44[a]	38.89[a]	0.389[ab]	0.44[a]	0.44[ab]	0.89[a]
IBA	50.0[a]	44.44[a]	1.44[b]	0.89[a]	1.64[b]	1.125[a]
IAA	27.78[a]	22.22[a]	0.278[a]	0.22[a]	0.22[a]	0.33[a]

Means followed by the same letters in the same column are not significantly different at p≤0.05

50, 100: range of branch length (40-60 cm and 80-120 cm respectively)

Table 3: Effect of branch diameter on rooting ability and marcotts performance

Diameter	SUR.3	Sur.6	NR.3	NR.6	RL.3	RL.6

Control	22.22[a]	33.33[a]	0.056[a]	0.33[a]	0.11[a]	0.194[a]
CW	33.33[a]	50.0[a]	0.167[a]	0.67[ab]	0.25[a]	1.083[ab]
IBA	44.44[a]	50.0[a]	0.61[a]	1.72[b]	0.79[a]	1.972[b]
IAA	22.22[a]	27.78[a]	0.056[a]	0.44[a]	0.056[a]	0.5[ab]

Means followed by the same letters in the same column are not significantly different at p≤0.05

3, 6: range of branch diameter (2-4 cm and 4-6 cm respectively)

Table 4: Effects of position on survival percentage of marcotts

	D.6, L.50			**D.3, L.50**			**D.6, L.100**			**D.3, L.100**		
Treatment	L	M	U	L	M	U	L	M	U	L	M	U
Control	33.33[a]	33.33[a]	33.33[a]	33.33[a]	33.33[a]	33.33[a]	50.0[a]	33.33[a]	33.33[a]	0[a]	33.33[a]	0[a]
CW	66.67[a]	33.33[a]	33.33[a]	33.33[a]	33.33[a]	33.33[a]	33.33[a]	66.67[a]	33.33[a]	33.33[a]	33.33[a]	33.33[a]
IBA	66.67[a]	66.67[a]	33.33[a]	33.33[a]	66.67[a]	33.33[a]	33.33[a]	66.67[a]	33.33[a]	33.33[a]	66.67[a]	33.33[a]
IAA	33.33[a]	33.33[a]	33.33[a]	33.33[a]	33.33[a]	0[a]	33.33[a]	33.33[a]	0[a]	33.33[a]	33.33[a]	0[a]

Means followed by the same letters in the same column are not significantly different at p≤0.05

L: Lower position, **M**: Middle position, **U**: Upper position

Table 5: Effects of position on number of roots of rooted marcotts.

	D.6, L.50			**D.3, L.50**			**D.6, L.100**			**D.3, L.100**		
Treatment	L	M	U	L	M	U	L	M	U	L	M	U
Control	0.33[a]	0.67[a]	0[a]	0[a]	0.33[a]	0[a]	0.67[a]	0[a]	0.33[a]	0[a]	0[a]	0[a]
CW	0.67[a]	0.67[a]	0[a]	0.33[a]	0.67[a]	0[a]	1.0[a]	1.0[a]	0.67[a]	0[a]	0[a]	0[a]
IBA	0.67[a]	4.67[a]	0.67[a]	0.67[a]	1.67[a]	0.33[a]	1.33[a]	2.67[a]	0.33[a]	0.67[a]	0.33[a]	0[a]
IAA	0.33[a]	1.0[a]	0[a]	0.33[a]	0[a]	0[a]	0[a]	1.33[a]	0[a]	0[a]	0[a]	0[a]

Means followed by the same letters in the same column are not significantly different at p≤0.05

Table 6: Effects of position on root length of rooted marcotts.

	D.6, L.50	**D.3, L.50**	**D.6, L.100**	**D.3, L.100**

Treatment	L	M	U	L	M	U	L	M	U	L	M	U
Control	0.167[a]	0.5[a]	0[a]	0[a]	0.67[a]	0[a]	0.33[a]	0[a]	0.167[a]	0[a]	0[a]	0[a]
CW	0.33[a]	0.83[a]	0[a]	0.167[a]	1.33[a]	0[a]	0.67[a]	4.33[a]	0.33[a]	0[a]	0[a]	0[a]
IBA	0.5[a]	5.67[a]	0.5[a]	0.5[a]	2.33[a]	0.33[a]	0.83[a]	4.0[a]	0.33[a]	1.0[a]	0.583[a]	0[a]
IAA	0.33[a]	0.67[a]	0[a]	0.33[a]	0[a]	0[a]	0[a]	2.0[a]	0[a]	0[a]	0[a]	0[a]

Means followed by the same letters in the same column are not significantly different at p≤0.05

Figures

Figure 1: Marcotts establishment and monitoring in the field; A: stripped branch; B and C: stripped branch covered with decomposed sawdust and tied with polythene paper; D: irrigation of marcotts in the dry season.

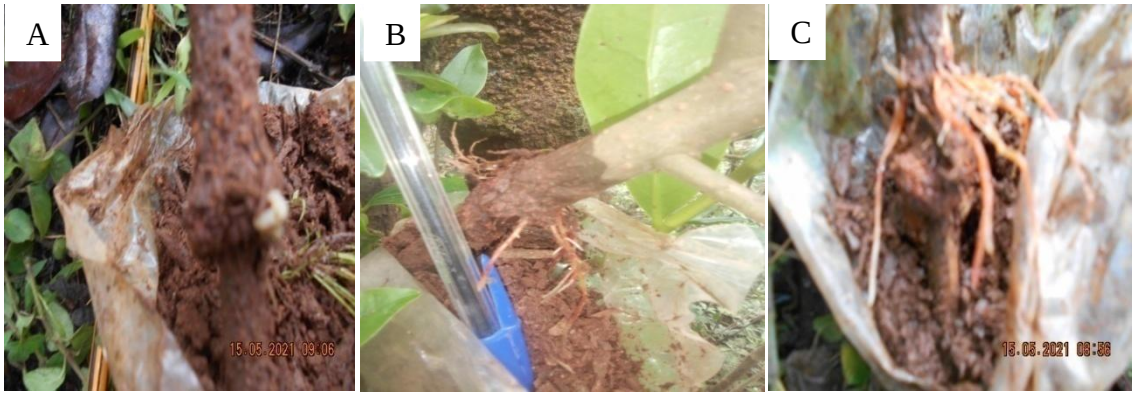

Figure 2: Characteristics of root emergence from marcotts of *T. cameroonensis*; A: most marcotts treated IAA and at upper position, B and C: marcotts treated with IBA, CW and at middle and lower position.

C

Figure 3: Transplant of weaned marcotts of *T. cameroonensis***; A:** rooted marcotts transplanted into polythene bags; B and C: sprouted transplanted marcotts.