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Propagation of *Ternstroemia cameroonensis*: an approach towards the conservation of a critically endangered medicinal plant species in the Lebialem highlands, Cameroon

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Abstract

This study was carried out to investigate the regeneration potential of *Ternstroemia cameroonensis* Cheek. a critically endangered medicinal plant in the Lebialem Highlands, Cameroon by seeds and stem cuttings. Air dried seeds were subjected to abrasion with sand paper, soaked in hot water and 98% concentrated sulphuric acid at various duration. Seeds pre-treated with 98% concentrated sulphuric acid for 1 minute (T21), 3 minutes (T22) had the best latent period 43.6 and 41.25 days respectively. There was a significant difference in the germination percentage, with seed soaked in 98% concentrated sulphuric acid for 3 minutes (T22) having the highest germination percentage (20%) followed by those soaked in 98% concentrated sulphuric acid for 6 minutes (T23) with a germination percentage of 12.22%. Early growth performances of seedlings were not significant. All seeds subjected to abrasion with sand paper and those soaked in hot water at various duration failed to germinate. In addition, rooting of stem cuttings was significantly affected by the application of synthetic hormones as well as alternative sources. The best survival percentage of stem cuttings was in coconut water (CW) (28.7%) followed by IBA (22.75%). Stem cuttings with 50% leaf area had the best survival percentage (34.9%) compared to others. Low concentrations of IBA (0.5g/l and 0.2g/l) and soaking in CW for 4 h had the best performance.

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Introduction

Anthropogenic activities have caused induced pressure on plant communities resulting in the vulnerability of some species (Bell *et al.*, 2016). This has resulted in some plant species becoming rare, critically endangered or extinct. In order for continuous availability of these plant species and sustainable livelihood, there is need for conservation. This could be in-situ or ex-situ through protection or propagation. Protection could be through reinforcement of traditional ecological knowledge or government laws while regeneration could be sexually or through vegetative means. Seeds of some plant have proven to be recalcitrant thus making their propagation difficult as well as vegetative organs. Because of this, several studies have been carried out to ameliorate the effectiveness of these propagation techniques. Plant species occupy a wide variety of habitats over the other species with adaptability which determines the tendency to perpetuate into particular environment by producing their offspring's to survive (Smith *et al.*, 2001). This can happen by different reproduction methods namely, sexually by seed which is most important and asexually when reproduction by seed is limited (Toogood and Anderson, 1999). Some of these methods are inefficient due to shortcomings such as dormancy, inability of cuttings or air layering to root or runners to survive.

Several previous studies have been carried out to investigate the effects of these impediments. To ameliorate the quality of seed germination, Missanjo *et al.* (2014)., Asif *et al.* (2020) carried out various trials to break dormancy in the seeds of *Acacia polyacantha, Prosopis juliflora* and *Dalbergia sissoo respectively*. This consisted seed abrasion with sand paper, soaking in sulphuric acid at various concentrations and durations as well as in Hot water (100^{o}C) at various durations. In addition, Dunsin *et al.* (2016)., Dada *et al.* (2019)., Mbogue *et al.* (2021) evaluated the effects of various concentrations of synthetic auxins (IAA, IBA and NAA) and alternative sources (coconut water and honey) on the rooting of stem cuttings of *Annona muricata, Parkia biglobosa* and *Echinops giganteus* respectively. Alternative hormones are natural materials that possess the ability to stimulate the rooting of cuttings. They are a suitable substitute to the synthetic hormones such as auxins, cytokinins, gibberellins which are essential and popular rooting hormones. Examples of alternative hormones used are honey, coconut water, willow tea, aspirin, moringa extract and saliva (Carusetta S., 2014).

However, very little is known about the regeneration of the *Ternstoemia spp*. Information about the regeneration of this species through seed germination or vegetative propagation by stem cutting is very scarce and therefore extensive research in this aspect is inevitable. *Ternstroemia cameroonensis* was recently described by Cheek *et al.* (2017) as a new critically endangered medicinal plant endemic in the Lebialem highlands belonging to the family *Ternstroemiaceae*. 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 22.5cm (WHINCONET, 2007).

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 (Focho *et al.*, 2009). The plant is also used as a blood tonic, fuel wood, construction of hurts and rituals. Despite this importance, the plant is considered critically endangered (IUCN, 2012) and was first documented as a rare montane tree species by Letouzey (1977) restricted to these highlands.

The endangered nature of this plant may be as result of data deficient, reduction of area of occupancy, severe habitat fragmentation, extreme fluctuation or loss of habitat due to anthropogenic activities. A poster depicting the species, for use in Cameroon and promoting its conservation was produced and distributed by Kew (Cheek, 2000). Tacham *et al.* (2015), report that the species is overexploited for sale locally and in neighbouring markets. 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 (Cheek *et al*., 2017). The species has not been cultivated nor seed-banked. Equally seeds of some species of *Ternstroemia* have been shown to be dormant, particularly *Ternstroemia washikiatii* germinating after six to seven months (Xavier and Carmen, 2016). In addition, recent studies by Nkemnkeng *et al*. (2021) indicate that the species is highly vulnerable in the Lebialem highland due method of exploitation, few mature individuals with rare saplings and seedlings. Considering the role of this plant in rural livelihood, it becomes important to investigate different propagation methods for its regeneration and eminent in-situ or ex-situ conservation.

Material and methods

The study site

This study was carried out partly at Forestry and Wildlife Nursery North West Region of Cameroon. It is located between latitude 5°20 and 6°15 N and longitude 9°7 and 10°21 E between 1207-2621 m above sea level with an annual rainfall of 2400mm (Olayiwola *et al*., 2011).

Propagation by seeds

Fruit collection, seed extraction and viability test

Mature fruits were collected at the various sites in the Lebialem Highlands at Fossimondi and Agocham. Seeds were extracted from fruits at the nursery of the regional delegation of forestry and wildlife for the North West Region, Cameroon dried for two weeks under natural sunlight and preserved in polythene bags. One thousand seeds were randomly selected from lots and subjected to a viability test by floatation method. The procedure permits the estimation of percentage of viable seeds in a lot. The seeds were placed in a bucket of water at room temperature (Wamegni, 1991; Schaal, 2000). The seeds that sunk down were classified as viable seeds, while those that floated were classified as non-viable.

Effects of seed pre-treatments on germination, and early growth of T. cameroonensis

Method of seed pre-treatment was adopted from Aronu *et al*. (2010) but with some modification and a total of 390 seeds were used. These seeds were subjected to three pre-treatments that is mechanical scarification, soaked in hot water and sulphuric acid prior to sowing. This was done as follows:

Mechanical scarification

There was careful abrasion of the hard seeds coats using a sand paper. The abrasion was on various sides of the seed that is anterior (T1), posterior (T2), lateral (T3), a combination of posterior and anterior (T4) and the control (T5). The anterior end was considered as that bearing the scar and the posterior opposite to it while the lateral was the sides of the seed. Five seeds were subjected to each treatment with three repetitions thus a total of 75 seeds were involved.

Scarification in hot water

Seeds were soaked in water at 100°C at various durations that is 20s (T6), 45s (T7), 1 minute (T8), 1.5 minutes (T9), 3 minutes (T10), 5 minutes (T11), 10 minutes (T12), 15 minutes (T13), 30 minutes (T14), 60 minutes (T15), 120 minutes (T16) and the control (T17). This consisted of five seeds each with three repetitions. The seeds were immediately immersed in cold water. Thus a total of 180 seeds were used in this experiment.

Scarification in sulphuric acid.

Seeds were soaked in 98% concentrated sulphuric acid at various durations. This consisted of soaking for 5s (T18) 15s (T19), 30s (T20), 1 minute (T21), 3 minutes (T22), 6 minutes (T23), 12 minutes (T24), 24 minutes (T25), 48 minutes (T26), 90 minutes (T27) and a control (T28). A total of 165 seeds were used in this experiment. In the control, seeds were not subjected to any pre-treatments.

After pre-treatments, the seeds were sown in polythene bags with respect to pre-treatment that is one seed per polythene bag. Thus a total of 420 seeds were used for the research work. Sowing was at uniform depth estimated using a ruler and the edge of an HB pencil. The ruler was placed close to the pencil and sowing depths marked on the ruler. After sowing watering was done daily while nursery care once a month.

Germination parameters were evaluated twice a month for a period of 12 months. Early growth parameters evaluated were latent period, germination percentage, number of leaves, collar diameter and shoot height.

Germination percentage (GP): This is the percentage ratio of the total number of seeds germinated to the total number of seeds sown expressed as follows:

$GP = \frac{N}{Tn} \times 100$ (Niang *et al.*, 2010)

Where N: number of seeds germinated and
Tn: total number of seeds sown

Latent period: This is number of days taken for the first seed to germinate (Ahoton *et al.*, 2009).

Collar diameter was measured by placing the calliper 10cm above the ground along the seedling and value recorded. Height of shoot was measured by placing a measuring tape from the soil level to the tip of the shoot

While number of leaves were counted on the seedling, all these for a period of eight months.

Experimental design was split plot randomized complete block design. Sowing was done in polythene bags filled with forest top soil and placed in a shed measuring 8m X 8m. Blocks, plots and subplots were placed 30cm apart to enable free movement during watering and nursery care.

Preparation of cuttings

Cuttings for this study were harvested from the wild and conserved in a cooler containing ice blocks prior to transportation to the nursery. At the nursery, the cuttings were prepared. Using a sharp knife, the branches were cut 4–6cm in length and comprising at least two nodes.

For each cutting, all the leaves except two attached to the apical node were removed. The apical leaves were trimmed to the desired surface area (0, 50% and 100%) of their laminar area (Fig. 1). The prepared cuttings were treated with systemic insecticide and fungicide K-Optimal and Plantomil super prior to dipping in various rooting media.

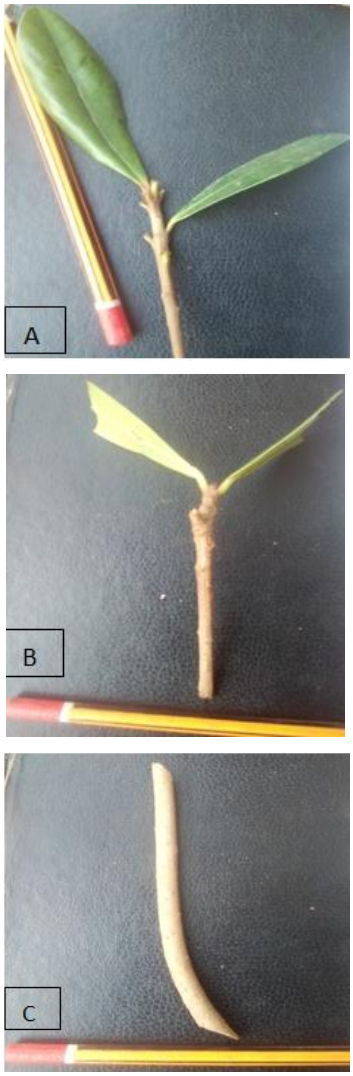


Fig. 1. Prepared cuttings of *Ternstroemia. cameroonensis* (A: cutting with 50% leave area, B: cutting with 100% leave area, C: cutting with 0% leave area.

To evaluate the efficiency of hormone treatments in promoting root initiation from stem cuttings, auxin solutions were prepared by dissolving 2g of pure auxin powder in 200mL of 90% industrial alcohol, making a concentration of 10g/l (Kanmegne *et al.*, 2015). Seven auxin treatments of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA)] respectively were tested and one control (solvent only). All auxins treatment was applied by dipping the basal end of the cutting into the solution for 5 s before inserting the cuttings in the rooting medium. The alcohol was evaporated off with a stream of cold air. Also coconut water (CW) and honey (H) were used. Cuttings were soaked in coconut water at various durations (1 hour, 4 hours, 8 hours and 16 hours) respectively.

A table spoon of honey was poured in a glass of hot water at 100 ^{o}C and allowed to cool then cuttings were soaked in it for respective durations (1 hour, 4 hours, 8 hours and 16 hours). Effects of type of cutting, hormone, coconut water and honey treatments on cutting survival were evaluated. The following formulas were used for this evaluation.

$$Rooting\ percentage = \frac{Number\ of\ cuttings\ rooted}{Total\ number\ of\ cuttings\ sown}$$

$$Mortality\ rate = \frac{Number\ of\ dead\ cuttings}{Total\ number\ of\ cuttings\ sown}$$

A total of 648 cuttings set in three blocks of a split–split plot experimental design was used. At each level, treatments were assigned at random to experimental units so as to have 7 hormone treatments × 3 leaf areas × 3 blocks × 10 cuttings.

After they were inserted in the rooting medium consisting of Fine River sand in the non-mist propagator and watered regularly to avoid desiccation. The non-mist propagators were constructed following a design based on that of Howland (1975) modified by Leakey *et al.* (1990).

Data analysis

Data collected were entered into excel and subjected into various analysis. Overall percentages were calculated using Microsoft excel while analysis of variance was done with XLSTAT, 2016 and mean separated using Duncan Multiple range test at $P < 0.05$.

Results

Propagation from seeds

Effects of seed pre-treatments on the germination and early growth of T. cameroonensis.

Seeds soaked in hot water at various durations, seeds subjected to manual scarification by abrasion with sand paper as well as those soaked in 98% concentrated H_2SO_4 from 24 minutes did not germinate. Only seeds soaked in 98% concentrated H_2SO_4 at 1 minute (T21), 3 minutes (T22), 6 minutes (T23) and 12 minutes (T24) sparingly germinated.

Effects of seed pre-treatment on latent period and germination percentage of T. cameroonensis.

Seeds soaked in 98% concentrated H_2SO_4 for 3m had the least latent period (41.25 days) followed by those soaked for 1 m (43.6 days) while the control had the highest latent period (64.3 days). Equally, Seeds soaked in 98% concentrated H_2SO_4 for 3m had the best germination percentage (20%) followed by those soaked for 6 m 12.22%) while the control had the least percentage (4.44%). There was a significant difference in the germination percentage of seeds soaked at various durations in concentrated H_2SO_4 (Table 1).

Table 1. Effects of seed soaked in 98% H_2SO_4 on latent period and germination percentage of *Ternstroemia cameroonensis*.

98% H_2SO4	Latent period/days	Germination%
T28 (Control)	64.3^{b}	4.44^{a}
T21 (1 minute)	43.6^{a}	5.56^{a}
T22 (3 minutes)	41.25^{a}	20.0^{b}
T23 (6 minutes)	46^{a}	12.22^{ab}
T24 (12 minutes)	47.66^{a}	5.56^{a}

Means followed by the same letters in the same column are not significantly different at $p \leq 0.05$.

Effects of seed pre-treatment on collar early growth of T. cameroonensis.

There was no significant difference in early growth parameters of seedlings emanating from various durations of soaking in 98% concentrated sulphuric acid. Despite this, seeds soaked for 3 minutes produced seedlings with the highest CD (0.746mm), followed by those soaked for 6 minutes (0.634mm) while the control had seedlings with the least CD (0.477mm). Also seeds soaked for 3 minutes and 6 minutes gave seedlings with the highest NL (3 leaves) while the control had seedlings with the least number of leaves (2 leaves). Furthermore, seedling from seeds soaked for 3 minutes had the highest height (2.6cm) while those from the control had the least height (1.97cm) (Table 2).

Propagation by stem cutting

Effect of leaf area, coconut water, honey and hormone treatments on the rooting and early growth of T. cameroonensis.

A total of 648 cuttings were involved in this experiment out of which 147 survived giving an overall percentage of 22.68%.

Cuttings that were soaked in coconut water at various durations had the highest survival percentage of 28.7% followed by those in IBA with 22.75% while the control had the least survival percentage of 14.81% (Table 3).

Also cuttings with 50% leaf area had the highest survival percentage (30.68%) followed by those with 100% leaf area (24.04%) while those with 0% leaf area were the least (16.8%) (Table 4).

Table 2. Effects of seed soaked in 98% concentrated H_2SO_4 on early growth parameters of *Ternstroemia cameroonensis* seedlings.

Treatment	CD (mm)	NL	H (cm)
T28 (Control)	0.477[a]	2.046[a]	1.971[a]
T21 (1 minute)	0.546[a]	2.67[a]	2.014[a]
T22 (3 minutes)	0.746[a]	3.11[a]	2.609[a]
T23 (6 minutes)	0.634[a]	3.056[a]	2.086[a]
T24 (12 minutes)	0.594[a]	2.79[a]	2.025[a]

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

Table 3. Overall effects of hormone treatment, duration of soaking in coconut water and honey on the survival of *Ternstroemia cameroonensis* cuttings.

Treatment	Survival%
IAA	17.99
IBA	22.75
CW	28.7
Honey	18.51
Control	14.81

Table 4. Overall effects of leaf area, hormone treatment, duration of soaking in coconut water and honey on the survival% of *Ternstroemia cameroonensis* cuttings.

Treatment	0% Leaf area	50% leaf area	100% leaf area
	%	%	%
Control	11	33.3	22.2
CW	25	33.3	25
Honey	22	25	27
IBA	9	34.9	23
IAA	17	26.9	23

Effects of coconut water, honey and leaf area on the mortality rate of T. cameroonensis.

There was no significant difference in mortality rate with respect to coconut water, honey, and leaf area of cuttings. Cuttings with 50% and 100% leaf area registered the least mortality rate (85%) at the control and soaking in coconut water for 8 hours respectively, Cuttings with 0% and 100% leaf area soaked in coconut water for 16 hours and 4 hours had the highest mortality rate (96.29%).

On the other had cuttings with leaf area of 50% and 100% had the least mortality for those soaked in honey for 1 hour and control (85.18%) while cuttings with 0% and 100% leaf area had the highest mortality rate (96.29%) (table 5).

Table 5. Effect of coconut water, honey and leaf area on the mortality rate of *Ternstroemia cameroonensis*.

Treatment.	MR, 0%	MR, 50%	MR, 100%
Control	92.59[a]	85.18[a]	85.1867[a]
CW 1hr	92.59[a]	88.89[a]	92.5926[a]
CW 4hr	88.89[a]	88.89[a]	96.29[a]
CW 8hr	88.89[a]	85.18[a]	88.8889[a]
CW 16hr	96.29[a]	92.59[a]	88.8889[a]
Control	92.59[a]	85.18[a]	85.1867[a]
H 1hr	92.59[a]	88.89[a]	85.18[a]
H 4hr	88.89[a]	92.59[a]	92.59[a]
H 8hr	96.29[a]	92.59[a]	96.29[a]
H 16hr	92.59[a]	92.59[a]	88.89[a]

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

MR: mortality rate, 0%, 50%, 100%: respective leaf area.

Effect of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and leaf area on the mortality rate of T. cameroonensis.

There was a significant difference in the mortality rate of cuttings immersed in IAA and IBA at various concentrations. Cuttings with 50% and 100% leaf area had the least mortality rate particularly for those immersed in 4mg/l and 1mg/l of IAA (81.48% and 85.18%) respectively. Cuttings with 0%, 100% leaf area had the highest mortality rate at 4mg/l, 10mg/l and 2mg/l (100%).

Also cuttings with different leaf area immersed in various concentrations of IBA showed no significant difference. Despite this, cuttings with 50% and 100% leaf area had the least mortality rate (77.78%, 85.18%) whereas cuttings with 0% leaf area showed the highest mortality rate (100%) at 2mg/l, 4mg/l, and 10mg/l respectively (Table 6).

Table 6. Effect of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and leaf area on the mortality rate of *T. cameroonensis*.

Treatment	MR, 0%	MR, 50%	MR, 100%
Control	96.667^{ab}	86.67^{a}	93.33^{ab}
IAA 0.5	96.29^{ab}	96.29^{a}	92.59^{ab}
IAA 1	92.59^{ab}	85.18^{a}	92.59^{ab}
IAA 2	92.59^{ab}	88.89^{a}	100.0^{b}
IAA 3	88.89^{a}	92.59^{a}	88.89^{ab}
IAA 4	100.0^{b}	88.89^{a}	81.48^{a}
IAA 5	96.29^{ab}	92.59^{a}	92.59^{ab}
IAA 10	100.0^{b}	92.59^{a}	96.29^{ab}
Control	96.667^{a}	86.67^{a}	93.33^{a}
IBA 0.5	92.59^{a}	77.78^{a}	85.18^{a}
IBA 1	96.29^{a}	85.18^{a}	96.29^{a}
IBA 2	100.0^{a}	88.89^{a}	92.59^{a}
IBA 3	92.59^{a}	88.89^{a}	92.59^{a}
IBA 4	100.0^{a}	92.59^{a}	92.59^{a}
IBA 5	96.29^{a}	88.89^{a}	92.59^{a}
IBA 10	100.0^{a}	96.29^{a}	92.59^{a}

Means followed by the same letters in the same column are not significantly different at p≤0.05.
MR: mortality rate.

Effects of soaking in coconut water, honey and leaf area on number of leaves (NL) of rooted cuttings.

There was no significant difference on the duration of soaking in coconut water, honey as well as leaf area on the number of leaves of rooted cuttings. Despite this, cuttings soaked in CW for 1 hour and 8 hours had the highest number of leaves while those soaked in honey for 8 hours had the least (0.22) followed by the control at 0% leaf area and honey at 100% leaf area. Cuttings at 50% leaf area performed better than others (Table 7).

Table 7. Effects of soaking in coconut water, honey and leaf area on number of leaves (NL) of rooted cuttings.

Treatment	NL, 0%	NL, 50%	NL, 100%
Control	0.44^{a}	0.78^{a}	0.78^{a}
CW 1hr	1.11^{a}	1.89^{a}	1.44^{a}
CW 4hr	1.0^{a}	1.0^{a}	0.33^{a}
CW 8hr	1.33^{a}	1.61^{a}	1.67^{a}
CW 16hr	0.33^{a}	1.11^{a}	1.67^{a}
Control	0.44^{a}	0.78^{a}	0.78^{a}
H 1hr	1.11^{a}	0.89^{a}	1.78^{a}
H 4hr	1.0^{a}	0.78^{a}	0.56^{a}
H 8hr	0.22^{a}	0.56^{a}	0.44^{a}
H 16hr	0.89^{a}	1.22^{a}	1.56^{a}

Means followed by the same letters in the same column are not significantly different at p≤0.05 NL: number of leaves.

Effects of soaking in coconut water, honey and leaf area on root length (RL) of cuttings.

There was a significant difference on the duration of soaking in coconut water, honey as well as leaf area on the root length of rooted cuttings. Cuttings with 50% leaf area soaked in CW for 1 hour had the highest root length (1.4cm) followed by those with 100% leaf area soaked in CW for 1 hours (0.89cm) while the control and those soaked in honey had the least root length (0.056cm, 0.0278cm) respectively. Cuttings at 50% leaf area performed better than others (Table 8) as most of them develop callus (Fig. 2).

Table 8. Effects of soaking in coconut water, honey and leaf area on root length of cuttings.

Treatment	RL 0% (cm)	RL 50% (cm)	RL 100% (cm)
Control	0.056^{a}	0.4167^{a}	0.25^{a}
CW 1hr	0.5^{a}	1.4^{a}	0.89^{ab}
CW 4hr	0.194^{a}	0.38^{a}	0.11^{a}
CW 8hr	0.61^{a}	0.59^{a}	0.5278^{ab}
CW 16hr	0.11^{a}	0.5^{a}	0.67^{ab}
Control	0.056^{a}	0.4167^{a}	0.25^{ab}
H 1hr	0.1389^{a}	0.1389^{a}	0.3056^{ab}
H 4hr	0.167^{a}	0.083^{a}	0.083^{a}
H 8hr	0.0278^{a}	0.11^{a}	0.0278^{a}
H 16hr	0.5278^{a}	0.694^{a}	0.86^{b}

Means followed by the same letters in the same column are not significantly different at p≤0.05.
RL: root length.

Fig. 2. Germination characteristics of *Ternstroemia cameroonensis* (A: seedling emergence after 50 days, B: seedling emergence after 10 months).

Effects of soaking in coconut water, honey and leaf area on shoot length (SL) of rooted cuttings

There was a significant difference on the duration of soaking in coconut water, honey as well as leaf area on the shoot length of rooted cuttings. Cuttings with 50% leaf area soaked in CW for 8 hour had the highest root length (1.67cm) followed by those with 100% leaf area soaked in CW for 1 hours (1.44cm) while cuttings with 0% leaf area soaked in honey for 8 hours had the least root length (0.056cm). Cuttings with 50% leaf area performed better than others (Table 9).

Table 9. Effects of soaking in coconut water, honey and leaf area on shoot length (SL) of rooted cuttings.

Treatment	SL, 0% (cm)	SL, 50% (cm)	SL, 100% (cm)
Control	0.136^{a}	0.83^{a}	0.33^{a}
CW 1hr	0.61^{a}	1.44^{a}	1.22^{ab}
CW 4hr	0.72^{a}	1.278^{a}	0.44^{a}
CW 8hr	0.5^{a}	1.67^{a}	1.25^{ab}
CW 16hr	0.22^{a}	0.5^{a}	1.33^{ab}
Control	0.136^{a}	0.83^{a}	0.33^{a}
H 1hr	0.33^{a}	0.67^{a}	0.6389^{a}
H 4hr	0.25^{a}	0.25^{a}	0.1389^{a}
H 8hr	0.056^{a}	0.139^{a}	0.083^{a}
H 16hr	0.61^{a}	0.67^{a}	0.861^{a}

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

SL: shoot length.

Effects of different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and leaf area on number of leaves (NL) of rooted cuttings

There was a significant difference on the effects of various concentrations of IAA and IBA as well as leaf area on the number of leaves of rooted cuttings. Cuttings with 50% leaf area in 0.5g/l, 2g/l and 5g/l had the highest number of leaves (1.89, 1.67) respectively. Cuttings with 0% leaf area and 100% leaf area dipped in IAA at 2g/l and 3g/l had the least number of leaves (0.11). Generally, cuttings with 50% leaf area dipped in IBA performed better than others (Table 10).

Effects of different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and leaf area on root development and root length (RL) of rooted cuttings

There was a significant difference on the effects of various concentrations of IAA and IBA as well as leaf area on the root length of rooted cuttings.

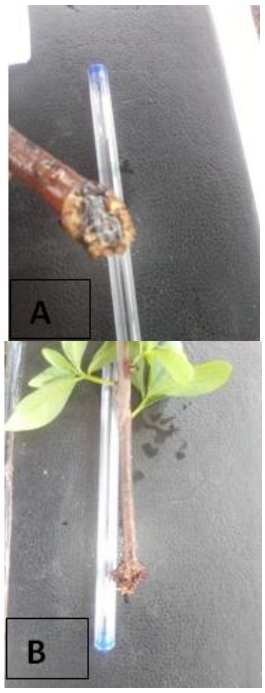
Most stem cuttings under these pre-treatment develop callus (Fig. 3). Cuttings dipped in 0.5 g/l and 2 g/l IBA had the longest root length (1.416cm, 1.56cm) respectively while those dipped in IAA at 3 g/l and 2 g/l had the least (0.056cm and 0.083cm) respectively. Generally, cuttings at 50% leaf area performed better in IBA than in IAA (Table 11)

Table 10. Effects of different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and leaf area on number of leaves (NL) of rooted cuttings.

Treatment	NL, 0%	NL, 50%	NL, 100%
Control	0.2^{a}	1.2^{ab}	0.25^{a}
IAA 0.5	0.22^{a}	-	0.56^{a}
IAA 1	0.11^{a}	0.78^{a}	0.11^{a}
IAA 2	0.33^{a}	0.61^{a}	-
IAA 3	0.11^{a}	0.33^{a}	0.278^{a}
IAA 4	-	0.33^{a}	0.278^{a}
IAA 5	0.44^{a}	0.44^{a}	-
IAA 10	-	-	-
Control	0.2^{a}	1.2^{ab}	0.25^{a}
IBA 0.5	0.56^{a}	1.89^{b}	1.33^{ab}
IBA 1	0.11^{a}	0.44^{a}	0.22^{a}
IBA 2	-	1.67^{b}	1.11^{ab}
IBA 3	0.56^{a}	0.33^{a}	0.67^{a}
IBA 4	-	0.33^{a}	0.78^{a}
IBA 5	0.44^{a}	1.67^{b}	0.78^{a}
IBA 10	-	0.22^{a}	0.44^{a}

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

NL: number of leaves.



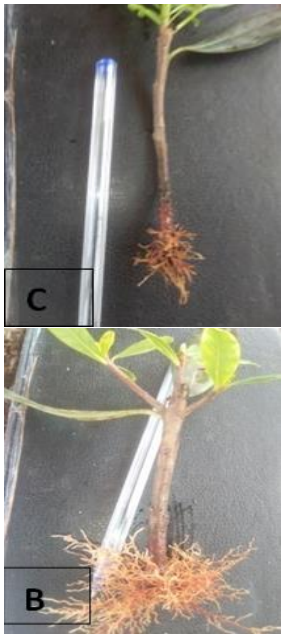


Fig. 3. Rooting characteristics of *Ternstroemia cameroonensis* (A: callus development, B: secondary root initiation, C and D: secondary root development and elongation).

Table 11. Effects of different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and leaf area on root length (RL) of rooted cuttings.

Treatment	RL, 0% (cm)	RL, 50% (cm)	RL, 100% (cm)
Control	0.175[a]	0.55[a]	0.175[a]
IAA 0.5	-	0.11[a]	-
IAA 1	-	0.11[a]	-
IAA 2	-	0.083[a]	-
IAA 3	-	0.056[a]	-
IAA 4	-	0.11[a]	-
IAA 5	0.056[a]	0.1389[a]	-
IAA 10	-	-	-
Control	0.175[a]	0.55[a]	0.175[a]
IBA 0.5	0.167[a]	1.4167[b]	0.56[a]
IBA 1	-	-	-
IBA 2	-	1.56[b]	0.78[a]
IBA 3	0.5[a]	-	0.44[a]
IBA 4	-	0.3056[a]	0.44[a]
IBA 5	0.22[a]	0.94[a]	0.61[a]
IBA 10	-	0.78[a]	0.89[a]

Means followed by the same letters in the same column are not significantly different at p≤0.05.
RL: root length.

Effects of different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and leaf area on shoot length (SL) of rooted cuttings

There was a significant difference on the effects of various concentrations of IAA and IBA as well as leaf area on shoot length of rooted cuttings. Cuttings dipped in IBA at 0.5 g/l, 2 g/l and 5 g/l had the longest shoot length (2cm, 1.78cm) respectively at 50% and 100% leaf area while cuttings with 0% leaf area had the least (0.11cm) in both media. Generally, cuttings at 50% leaf area performed better IBA than in IAA (Table 12)

Table 12. Effects of different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and leaf area on shoot length (SL) of rooted cuttings.

Treatment	SL, 0% (cm)	SL, 50% (cm)	SL, 100% (cm)
Control	0.2[a]	0.875[b]	0.45[a]
IAA 0.5	-	-	0.11[a]
IAA 1	-	0.33[ab]	0.11[a]
IAA 2	-	0.22[ab]	-
IAA 3	-	-	-
IAA 4	-	0.11[a]	0.056[a]
IAA 5	-	0.11[a]	-
IAA 10	-	-	-
Control	0.2[a]	0.875[ab]	0.45[a]
IBA 0.5	0.33[a]	2.0[b]	1.78[b]
IBA 1	0.11[a]	0.167[a]	0.167[a]
IBA 2	-	1.056[ab]	0.67[ab]
IBA 3	0.3056[a]	0.22[a]	0.278[a]
IBA 4	-	0.278[a]	0.4167[a]
IBA 5	0.167[a]	1.167[ab]	0.56[ab]
IBA 10	-	0.33[a]	0.5[ab]

Means followed by the same letters in the same column are not significantly different at p≤0.05.
SL: shoot length.

Discussion

Seed germination depends on the potential of embryo growth or the potential of growth inhibitors (Koorneef *et al.*, 2002). These potentials depend particularly on the seed structure that surrounds the embryo, i.e. endosperm, pericarp, glume and seed coat. Several dormancy types can be associated with the seed-coat, e.g. mechanical resistance, physical barrier to moisture absorption or gaseous exchange, temperature or chemical inhibition, and light sensitivity (Ellis *et al.*, 1985; Schmidt, 2000). Other factors like hormones and environmental factors also affect embryo growth (Benech *et al.*, 1998; Mares, 2005). *T. cameroonensis* seeds were subjected to different seed treatments. The best pre-treatment to enhance the germination was soaking in 98% concentrated sulphuric acid as it gave a higher germination percentage compared to the other pre-treatments.

It seemed that the seed coat is one of the inhibitor of germination in the species as seed coat hardness is an important factor that affects germination in seed (Aref *et al.*, 2011). Seed dormancy is known to occur in many tropical tree species (Amusa, 2011), most *Ternstroemia spp* have hard seed coats which maybe impervious to water and probably the cause dormancy as observed by Xavier and Carmen (2016). Uniyal *et al.* (2000) stated that seed pre-treatment are species specific and that no one type of treatment has been reported to be universally effective. Therefore breaking the seed dormancy by softening the seed test a to allow water imbibition is crucial for any afforestation programs (Aref *et al.*, 2011). The results of this study showed that all treatment did not significantly affect germination performance. Chemical scarification in 98% concentrated sulphuric acid produce some sparing germination though not appropriate while those soaked for more than 15 minute in 98% concentrated sulphuric acid fail to germinate as well as those soaked in hot water or abrasion with sand paper. This may be because the acid, hot water or abrasion burn and destroy the seeds thus preventing germination. This may also be due to the fragility of the seeds which was probably damaged in this process thus giving null percentage germination.

This observation is similar to the result of Asif *et al.* (2020) who observed that subjecting *Prosopis juliflora* and *Dalbergia sissoo to* 95% sulphuric acid and hot water treatment failed to germinate. Some seeds took a very long time to germinate maybe due to impermeable seed coat which prevented water and gases from getting into it to stimulate germination. Delayed in seed germination may also be as a result of immature embryo or seed viability. Thus, soaking in hot water or 98% concentrated H2SO4 caused serious damage and deleterious effects on the embryo. In addition, water and gas impermeability of seeds is caused by physical and biochemical obstacles of the seed coat (Bewley 1997). On the other hand, existence of inhibitory materials in the seed coat could also be considered as the reason for this kind of dormancy.

The application of a rooting hormone for the rooting of stem cuttings is widely recognized (Leakey *et al.*, 1990; Tchoundjeu and Leakey, 2001). Also substances such as *Aloe vera* gel, coconut water, *Moringa* leaf extract, honey, asprin etc contain phytohormones as chemical analysis has revealed the presence of IAA, Cytokinins, Gibberellin A1, GA3, ABA and Salicylic Acid in them (Yong *et al.* 2009; Carusetta, 2014). Synthetic hormones, alternative sources as well as leaf area significantly affected the rooting percentages and early growth parameters in our study.

In addition, IBA had the best performance at low concentration maybe due it's very important role in rooting various tropical tree species as noted by Leakey *et al.* (1982); Tchoundjeu *et al.* (2002); Amri *et al.* (2009). The different concentrations of IBA applied leading to rooting response varied for different species. We also think that the reason behind the higher efficiency of rooting under low concentrations of IBA for this species is attributed possibly to the higher level of endogenous auxin contents in the cuttings. We postulate that at higher concentration, IBA may have some negative impacts against the naturally occurring growth hormones in the cuttings. The increase in rooting percentage due to IBA treatment may be attributed to the fact that the hormone helps in mobilization of reserve food materials and differentiation of cambial initials into root primordial (Younis and Riaz, 2005).

This corroborate with the findings of Baul *et al.* (2010) who found out that rooting of wild tropical medicinal plant *Holarrhena pubescens* was effective at a low IBA concentration and also with the work of Ambebe *et al.* (2019) on the effects of synthetic hormones (IBA) and plant extracts as potential rooting enhancers in cuttings of *Vitex diversifolia* and *Cordia milleneii*. Contrary, Opuni-Frimpong *et al.* (2008) working with *Khaya anthotheca* and *Khaya ivorensis*, revealed the reverse trend where the percentage of rooting increased with increasing concentrations of IBA. Furthermore, CW produced second best performance while IAA the least. Various auxins such as IAA, IBA, NAA, and 2,4-Dichlorophenoxy Acetic Acid have been demonstrated to promote rooting in cuttings.

This is contrary to the finding of Hassanein, (2013) involving cuttings of a woody tree, *Ficus hawaii* where treatment with IAA was found to be more effective than that with either IBA or NAA. Also, the findings of Dunsin (2016) showed that CW significantly augmented rooting in *Parkia biglobosa* while honey was ineffective. Also Bhattacharya *et al.* (2010) postulated that coconut water can be used as a supplement in many laboratories to improve regeneration of plant cells. The findings also agree with Trevisan *et al.* (2005) who demonstrated the advantage of coconut water for stem elongation and plant development in fruit species. Previous study revealed that coconut water contained sugar, amino acid, myo-inositol, and micro-constituents of phenyl urea that aid tree development (Agele *et al.*, 2010). In contrast, successful rooting without applied auxin has been reported in a number of tropical tree species, such as *Nauclea diderrichii* (Leakey, 1990) and *Allanblackia floribunda* (Atangana *et al.*, 2006). Such contrasting results may be due to the variation in endogenous auxin contents at time of severance (Hartmann *et al.*, 1990).

Leaf area significantly influenced rooting of *T. cameroonensis* stem cuttings by acting on mortality rate as well as roots length and shoot length of rooted cutting. Leafless cuttings had the highest mortality rate compared to leafy cuttings. This trend has been reported in other tropical tree species such as *Lovoa trichilioides* (Tchoundjeu and Leakey 2001), *Prunus africana* (Tchoundjeu *et al.*, 2002), Cola anomala (Kanmegne *et al.*, 2015). The inability of leafless cuttings to root has been associated with the rapid depletion of carbohydrates in stem tissues; in contrast, concentrations in leafy cuttings tend to increase (Leakey *et al.*, 1982). The restriction of rooting to leafy cuttings as reported in the present study appears to confirm the hypothesis that survival and rooting of stem cuttings depend on carbohydrates formed and utilized after cuttings have been detached from the mother plant. Apart from providing a source of carbohydrates, the leaf also influence the water status of the cutting. Trimming of leaves minimizes water loss via transpiration while allowing sufficient photosynthesis to occur for root development (Mesen *et al.*, 1997).

In this study, cuttings with 50% leaf area rooted better than those with 100% leaf area while leafless cuttings were the least. The differences in root growth may also be due to the differential effects of the growth regulators on metabolites translocation and carbohydrates metabolism.

Conclusion

Ternstroemia cameroonensis seeds exhibit physical dormancy due to the hard seed coat, which affects the seed germination. The cotyledon is equally minute thus with minimal food reserves to boost germination vigour. Results obtained in this study indicate that the pre-treatment of seeds using 98% concentrated H_2SO_4 for 3 minutes could enhance seed germination as well as early growth parameters better than the control. Seed abrasion with sand paper and soaking in hot water at various durations failed to germinate indicating that they are not effective in stimulating seed germination. In addition, rooting ability of *Ternstroemia cameroonensis* stem cuttings proved successful in synthetic auxins (IAA and IBA) as well as alternative hormones (coconut water and honey). IBA (0.5 g/l) had the best performance followed soaking in coconut water (1 h and 8 h) while various concentrations of IAA and honey were the least. Thus, rooting of stem cuttings of *Ternstroemia cameroonensis* with plant growth regulator (IBA at 0.5 g/l and soaking in coconut water (1 h and 8 h) presents a viable propagation approach to be used in enrichment planting programmed of this important critically endangered medicinal plant.

Competing interest

The authors declare that they have no competing interests

Authors' contributions

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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