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Effects of indole butyric acid and coconut liquid endosperm on rooting of *Crateva adansonii* stem

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Abstract
Indole butyric acid (IBA) and coconut liquid endosperm (CLE), two rooting agents, were tested in three concentrations to see how they affected the rooting of *Crateva adansonii* DC stem cuttings. 300 mg/L, 200 mg/L, and 100 mg/L (IBA) of rooting material and 100%, 80%, and 60% of CLE were the concentrations that were assessed. This was done in three replications using completely randomized design (CRD). The setup, which lasted six months, was on exhibit in the Botanic Garden at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. While control stem cuttings took the longest to start and finish bud break on the stem cuttings, higher concentrations of the rooting chemical substances, 300 mg/L IBA and 100 percent CLE respectively, influenced the shortest times (initial and final). The results showed that the control had the fewest buds that sprouted into leaves, whereas 300 mg/L IBA and 100 percent CLE had the highest percentage of buds that turned into leaves on the stem cuttings. Regardless of the treatment concentrations utilized, a thin callus formed on the stem cuttings. Observations on rooting revealed that not all callused stem cuttings subsequently took root. IBA and CLE were applied to stem cuttings in various concentrations, and the results were seedlings with lateral and feeding roots. The maximum percentage of rooting response was seen in stem cuttings treated with 300 mg/L IBA and 100 percent CLE, respectively, while the control did not root. The findings of measuring the aerial and sub aerial growth characteristics of seedlings revealed that 300 mg/L IBA and 100 percent CLE had a greater impact on the development of higher values of the growth parameters examined than cuttings treated with lower concentrations of the two rooting agents. Based on the findings of this study, it is possible to draw the conclusion that *C. adansonii* can be multiplied vegetatively by using rooting agents to help stem cuttings take root. It is recommended that more research be done using rooting medium with a higher concentration and rooting agents like auxins at different concentrations.

Keywords: *Crateva adansonii*, Indole buteric acid (IBA), Coconut liquid endosperm (CLE), Rooting and callus

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INTRODUCTION

There are roughly 70 species in the genus *Crataeva* (*Capparaceae*), most of which are found in the tropics and subtropics (Kumar *et al*., 2020). One of these, *Crateva adonsinii*, exhibits remarkable health advantages. It is a medium-sized deciduous tree that bears the name of the Greek botanist *Cratevas* (Kumar *et al*., 2020). Due to poor seed germination and poor seedling establishment, this tree is rarely widely planted (Igoli *et al*., 2014). In nature, the tree only generates a small number of root suckers, which limits its range (Kher *et al*., 2016). In the savanna and forest regions, it grows as a little tree and is extensively dispersed. They are frequently observed along riverbanks throughout Africa and resemble the Asian *Crateva religoasa* (Igoli *et al*., 2014).

Although *Crateva adansonii* is consumed as food and is significant in medicine and commerce, very little is known about its nutritional worth. According to Agbankpe *et al*. (2015), who established their nutritional benefits, they are high in calcium, zinc, and proteins. When it comes to treating ear infections, the leaves are highly sought after in Eastern Nigeria, whilst the Yoruba ethnic group in Western Nigeria uses the same leaves as a mild headache counter irritant (Agbankpe *et al*., 2015). Rubefacient substances used on cysts include the ground leaves and bark (Kabore *et al*., 2015). The bark of the plant has been used to cure ailments like syphilis, jaundice, yellow fever, mycotic infections, and to hasten the healing of wounds, according to Ajali *et al*. (2010), Adjabga *et al*. (2017), and Kumar *et al*. (2020). The long list of successes recorded from the use of the extracts of the various tissues of the species in treatment of different ailments and hence, the need to make available large quantity of the plant materials, which can sustain biotechnological exploitation excited interest in selecting rooting of the stem cuttings which is an aspect of vegetative propagation for this study.

Among all seedling multiplication approaches, rooting of stem cuttings has become the fastest method used to produce large quantity of uniform seedlings. It contributes significantly to the conservation of the species both for present as well as future biotechnological exploitation of its medicinal properties and sufficiently ensures their availability for research activities (Yeshiwas *et al*., 2015). Above all, the present study will provide baseline information on the methods for the vegetative propagation of the species, and suggest which can be adopted by local nursery men and farmers for best result.

Although, some researchers have attempted studying the impact of IBA on the propagation of plants, such as *Bambusa arundinacea* (Venkatachalam *et al*., 2015), *Rosa rubiginosa* (Yeshiwas *et al*., 2015), *Duranta erecta* (Shiri *et al*., 2019), *Ocimum gratissimum* (scent leaf) and *Pterocarpus mildebraedii* (Okafor *et al*., 2020), and *Cornus sericea* (Inoue *et al*., 2022), there is paucity of information on the possible application of IBA and CLE in propagating *Crateva adansonii*. Several reports have shown that seedlings produced by seeds take longer time to yield, probably because of the effect of prolonged seed dormancy, hence, delaying biotechnological exploitation of their resources (Nzekwe, 2006; Igoli *et al*., 2014; Adjagba *et al*., 2017). Based on insufficient knowledge on how to solve the problem of delayed seed germination of *Crateva adansonii*, coupled with numerous untapped health benefits derivable from its seeds, leaves, wood, root and bark which of course has skyrocketed its demands, it has become necessary to develop other methods of propagating this important plant species so as to enable full exploitation of its medicinal attributes. The efficiency of two rooting agents on the rooting and seedling development of mature stem cuttings of *Crateva adansonii* DC (*Capparaceae*) was thus compared.

MATERIALS AND METHODS

Site description for the experiment

The research was done in the botanic garden of the University of Nigeria, Nsukka Department of Plant Science and Biotechnology, during the rainy season under cover of shade. Nsukka is located on latitude 6^{o}51’56” N, longitude 7^{o}24’22” E and 1,410ft above sea level. The approach involved planting of the stem cuttings in poly pot and mechanically irrigating the setup, avoiding the use of mist drip irrigation or bottom heat technique (Griffith, 1940). The Centre for Basic Space Science at the University of Nigeria, Nsukka, provided meteorological information such as average monthly rainfall, temperature, and humidity during the course of the study.

Materials description

Mature coconut fruits (Ghana variety, because of its high liquid endosperm content) bought from local market (Nsukka main market) were extracted of the liquid endosperm content.

Indole-3-butyric acid and branches of *Crateva adansonii* trees were obtained from the departmental laboratory and botanic garden, respectively. Uniform stem cuttings were derived from the *Crateva adansonii* branches.

Top soil and poultry droppings were collected from uncultivated land from the departmental botanic garden and poultry farm of the Faculty of Agriculture, respectively.

Plate 1: the image of a typical *Crateva adansonii* plant.

Preparation of media and rooting substances

The growing substrate was prepared from a 2:1 mixture of top soil and poultry manure by thoroughly mixing 40kg of the former with 20kg of the latter. Polypots measuring (12 x 25 cm) in size were used for the experiment. The polypots which contained 0.25kg of the media were perforated at the base and sides to allow for adequate drainage of excess moisture. The choice of the growing medium which is otherwise referred to as the amended media was based on the reported qualities of mixed/amended media over single medium. Nzekwe (2006) projected such media as the best being well-aerated and could retain enough moisture for plant growth over a given period.

The concentrations of IBA (SimSon Chemtech, India) used were prepared by dissolving it into three levels *viz;*300mg, 200mg and 100mg separately in one litre of distilled water. Coconut liquid endosperm 100ml (100%) was the undiluted stock, 80 ml was diluted with 20ml water to get 80%, this was followed by dilution of 60 ml with 40ml water to get 60%.

Preparation of stem cuttings

Defoliated *C. adansonii* branches, each of which carried 4-6 buds, were trimmed to length (30 cm) from trees in the Department's botanic garden. 42 stem cuttings of *C.adansonii* in total, divided into 3 replicates, were utilized for the experiment's 7 treatments (3 levels of IBA, 3 levels of coconut liquid endosperm, and control) (2 cuttings per treatment x 7 treatments x 3 replicates).

Before planting, each cutting lot was tied with twine and submerged for an hour in the appropriate rooting chemical substance concentration (300mg/L, 200mg/L, 100mg/L IBA, and 100%, 80%, and 60% CLE). According to Hartmann and Kerster's (2002) advice, the upper cut end of the stem cuttings was sealed with paraffin wax before planting to reduce water loss, particularly from the pith. Cuttings that had not been bathed in rooting agent served as the control for the experiment.

Stem cuttings were planted in a slanting orientation, one cutting per polypot.

Experimental design

Complete randomization design (CRD) was used in the experimental design. The setup was on exhibit in a shaded area of the Department's botanic garden, which received daily mechanical irrigation and manual weed removal. Observations were made on sign of bud breaking which represented growth activities on the cuttings.

Data collection

Daily checks were done to see if the stem cuttings' metabolic processes started up again during each watering cycle. The parameters tracked included initial and final bud sprouting times, the quantity of sprouted buds produced by each stem cutting, and the times when buds developed into photosynthesis-producing leaves. After the development of the leaves, stem cuttings were randomly selected at four-week intervals to examine callus and root development. Cuttings that had developed calluses and roots were labeled and replanted following each sampling.

Aerial growth and rooting parameters

The following aerial growth parameters such as: number of sprouted buds on stem cutting with emphasis on the times of the first and last bud sprouting as well as the quantity of buds that developed into leaves were recorded likewise, the following rooting parameters such as: number of callused and rooted stem cuttings, type of callus developed, types and length of five longest roots in each treatment were also observed and recorded. For each parameter, mean of ten stem cuttings were determined. Leaf area was estimated by adding a constant to the product of the leaf's length and width, whereas using a meter rule, the root length was calculated and recorded in centimeters, the rest parameters were counted.

Data analysis

Using SPSS, the data were subjected to an analysis of variance (ANOVA), and Duncan's New Multiple Range Test (DNMRT) was used to differentiate the means at $P \leq 0.05$. Plates, figures, and tables are used to show pertinent data and observations.

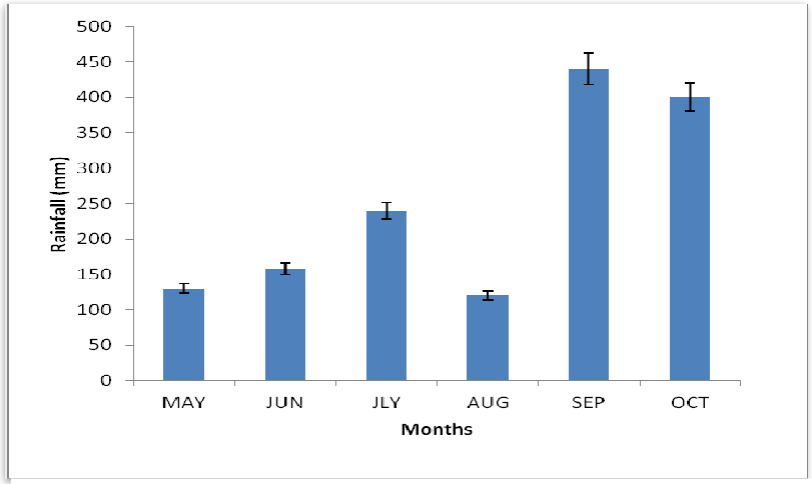
RESULTS

Meteorological results comprising of rainfall, relative humidity and temperature

The result for the mean monthly rainfall throughout the duration of the trial is presented in Figure 1. The results showed that mean monthly rainfall ranged from 120 to 440±12.73mm, with September (440±12.73mm) as the wettest month. This was followed by October (400±12.73mm) while the least was recorded in August (120±12.73mm). The mean monthly relative humidity as presented in Figure 2 showed that relative humidity ranged from 48 to 75±9.64% and that the most humid month was recorded in September (75±9.64%) also. This was followed by October (70±9.64%) and July (60±9.64%), while August (48±9.64%) was the least. The results of the determination of mean monthly temperature are summarized in Figure 3. There were variations in the mean monthly temperature. The results showed that the mean monthly temperature had a range of 28.4±0.29^{o}C to 29.8±0.29^{o}C and the hottest month was August (29.8±0.29^{o}C). This was followed by May (29.6±0.29^{o}C) and October (29.6±0.29^{o}C). The months of July and September (28.4±0.29^{o}C) each had the coolest temperature comparatively.

Periods of bud breakage (initial and final)

The results to determine the initial and final period of bud breakage on the stem cuttings are presented in Table 1. The outcomes revealed variation among the varying levels of each treatment in both bud breakage periods. Irrespective of rooting substance and their concentrations all the stem cuttings including the control (untreated stem cuttings) had bud flush as indicated in Plates 2a and b. The initial period of bud break ranged from 25.50±0.65 to 37.50±0.65 days after planting (DAP). Stem cuttings that have been treated with 300mg/L of IBA had the earliest period of bud break (25.50±0.65) DAP. It was followed by cuttings of stems that have been treated with 100% coconut liquid endosperm (26.50±0.65) DAP while the control took the longest period (37.50±0.65) DAP to experience sprouting. This make stem cuttings treated with IBA to have taken shorter period (25.50± 0.65) DAP to sprout comparatively. Sprouting observed in stem cuttings treated with CLE varied from 26.50±0.65 to 37.50±0.65 DAP.



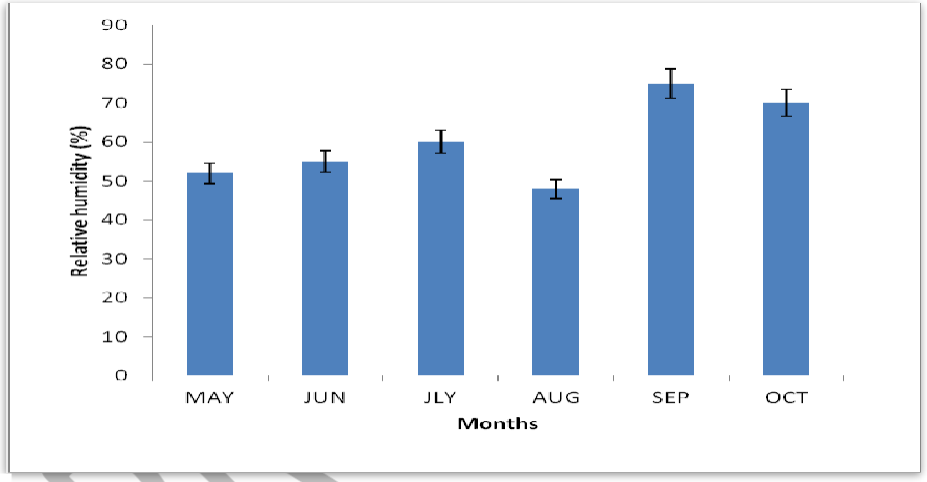


Figure 2: The average monthly change in relative humidity

Figure 3: Mean monthly temperature variation

The period of finial bud break (termination of bud sprouting) ranged from 33.75±0.48 to 48.75±0.85 DAP. The stem cuttings treated with 300mg/L IBA were also the earliest to terminate bud sprouting which was recorded from 33.75±0.48 to 42.00±0.91days after planting whereas the controls took the longest periods to terminate their bud sprouting (47.50±1.04 to 48.75±0.85) DAP.
Comparatively, the initial period DAP to bud sprout termination at stem cuttings treated with 300mg/L of IBA was the shortest at 25.50±0.65 DAP. In CLE, 100%-treated stem cuttings had the shortest beginning time (26.50±0.65 DAP), whereas 60%-treated stem cuttings had the longest (37.50±0.65 DAP).

Percentage bud breakage

The effect of rooting substance on percentage of bud breakage at the end of bud sprouting is presented in Table 2. The results showed variation among the treatment means and the values ranged from 67.25±0.85% to 88.50±0.65%. Stem cuttings immersed in 200 mg/L of IBA produced the maximum bud sprout (88.50±0.65%), followed by those treated with 100 percent CLE (88.00±0.71%). Stem cuttings that had been bathed in 300mg/L of IBA (83.50±0.65%) came next. The results also demonstrated that the control (67.25± 0.85 %) and stem cuttings soaked in 60 percent CLE had the least. As seen in Plates 2a and b, several cuttings treated with Indole Butyric Acid (IBA) and Coconut Liquid Endosperm (CLE) had multiple buds flush. Comparatively, bud break percentage was higher in stem cuttings soaked in three different concentrations of IBA than it was in three different concentrations of CLE.

Percentage of sprouting buds that grew into leaves

The percentage of buds that eventually turned into photosynthesis leaves is shown in Table 3. According to the findings, not all of the buds that emerged on the stem cuttings survived and turned into leaves (Plate 3). Between 59.62±1.07% to 84.25±2.17% of sprouting buds turned into leaves. The buds that developed on stem cuttings treated with 100 percent CLE had the maximum leaf development, measuring 84.25±2.17%. Buds that emerged on stem cuttings treated with 300mg/L and 200mg/L IBA had values of (82.75±1.11%) and (79.75±0.85%), respectively. The next to appear on stem cuttings was from the control, which had a bud development into leaves percentage of 59.62±1.07%., this was the lowest.

Comparatively, the findings demonstrated that stem cuttings treated with CLE had lower percentages of buds developing into leaves than those that sprouted on stem cuttings treated with the three dosages of IBA. Additionally, regardless of the rooting chemical concentration, the data revealed that roughly 25% of the stem cuttings had numerous buds flush that turned into leaves (Plate 4).

Development of Callus on the stem cuttings of *Crateva adansonii* as influence by IBA and CLE

The observations made on the stem cuttings' basal region one month after the growth of the leaves revealed that not all of the randomly picked stem cuttings had calluses. Additionally, the stem cuttings generated light calluses, as seen in plates 5a and b, regardless of the type of rooting material and its concentration. Additionally, stem cuttings of the control that survived did not exhibit callus development, regardless of the growth of leaves. Further investigation revealed that some callused stem cuttings had sprouted among the cuttings treated with coconut liquid endosperm and indole butyric acid (IBA) (CLE).

Callus percentage affected by Indole Butyric Acid (IBA) and Coconut Liquid Endosperm (CLE)

The percentage callus production ranged from 28.00±2.16% to 81.75±1.18%, according to the results shown in Table 4. On the stem cuttings treated with 300mg/L of IBA, the highest percentage callus production, 81.75±1.18 % was noted. Cuttings treated with 100% CLE came in second (81.25±1.49%), while those treated with 60% of a similar chemical had the lowest value (28.00±2.16%). Comparatively, the results showed that CLE-treated stem cuttings exhibited lower percentage callus formation than those treated with the three levels of IBA concentrations. The outcomes also demonstrated that greater concentrations of CLE (100%) and IBA (300 mg/L) significantly influenced very high percentage callus formation on the stem cuttings, with respective values of 81.25±1.49% and 81.75±1.18%.

Table 1: Rooting agents Indole Butyric Acid (IBA) and Coconut Liquid Endosperm (CLE) effects on the times of bud breakage on *C. adansonii* stem cuttings

	Periods of bud breakage (Days)	
Treatments	Initial (DAP)	Final (DAP)
IBA (mg/L)		
100	34.50 ± 1.04^{ab}	42.00 ± 0.91^{b}
200	33.50 ± 1.04^{b}	38.50 ± 0.65^{c}
300	25.50 ± 0.65^{c}	33.75 ± 0.48^{d}
Control	37.00 ± 0.91^{a}	48.75 ± 0.85^{a}
LSD($P < 0.05$)	2.85	2.29
CLE (%)		
60	37.50 ± 0.65^{a}	46.00 ± 1.08^{a}
80	30.50 ± 0.65^{b}	38.50 ± 0.65^{b}
100	26.50 ± 0.65^{c}	35.75 ± 0.85^{b}
Control	37.50 ± 0.65^{a}	47.50 ± 1.04^{a}
LSD($P < 0.05$)	1.99	2.84

In the vertical column, values with the same alphabet(s) do not significantly differ from one another ($P < 0.05$).

Table 2: Coconut liquid endosperm (CLE) and indole butyric acid (IBA) effects on the percentage of bud breaking on *Crateva adansonii* stem cuttings

Treatments	Bud breakage (%)
IBA (mg/L)	
100	83.50 ± 0.65^{b}
200	88.50 ± 0.65^{a}
300	83.50 ± 0.65^{b}
Control	78.50 ± 0.65^{c}
LSD (P<0.05)	1.98
CLE (%)	
60	67.25 ± 0.85^{c}
80	76.50 ± 0.65^{b}
100	88.00 ± 0.71^{a}
Control	67.25 ± 0.85^{c}
LSD (P<0.05)	2.35

Values in the vertical column that share the same alphabet(s) do not significantly differ from one another (P 0.05).

Plates 2a: Indole butyric acid influences the bud sprouts on *C. adansonii* stem cuttings (IBA)

Plates 2b: *C. adansonii* stem cuttings' bud sprouts are impacted by coconut liquid endosperm (CLE)

Plate 3: On *Crateva adansonii* stem cuttings treated with 100% Coconut Liquid Endosperm, leaves have developed (CLE)

Plate 4: A 300mg/L (IBA)-treated stem cutting of the *Crateva adansonii* plant displaying several buds that eventually turned into leaves.

Table 3: Effects of coconut liquid endosperm (CLE) and indole butyric acid (IBA) on the percentage of sprouting buds that grow into leaves on *Crateva adansonii* stem cuttings

Treatments	Percentage development of sprouted bud into leaves (%)
IBA (mg/L)	
100	73.25 ± 1.36[b]
200	79.75 ± 0.85[a]
300	82.75 ± 1.11[a]
Control	59.75 ± 1.55[c]
LSD (P<0.05)	3.84
CLE (%)	
60	65.50 ± 0.65[c]
80	72.50 ± 1.04[b]
100	84.25 ± 2.17[a]
Control	59.62 ± 1.07[d]
LSD (P<0.05)	4.18

Values in the vertical column that share the same alphabet (or alphabets) do not substantially differ from one another (P ≤0.05).

Table 4: *Crateva adansonii* stem cuttings' percentage callus formation in response to indole butyric acid (IBA) and coconut liquid endosperm (CLE)

Treatments	Percentage callus (%)
IBA (mg/L)	
100	46.50 ± 1.71[c]
200	65.25 ± 1.25[b]
300	81.75 ± 1.18[a]
Control	0
LSD (P<0.05)	3.73
CLE (%)	
60	28.00 ± 2.16[c]
80	65.00 ± 1.22[b]
100	81.25 ± 1.49[a]
Control	0
LSD (P<0.05)	4.47

Values in the vertical column that share the same alphabet(s) do not significantly differ from one another (P 0.05).

Indole Butyric Acid (IBA) and Coconut Liquid Endosperm (CLE) impact on root growth of *Crateva adansonii*

Following studies on how the stem cuttings formed their roots, it was discovered that the stem cuttings developed two different kinds of roots: lateral roots and feeding roots. The results also showed that the stem cuttings' sides had few lateral roots and only a few light clustered feeding roots at the cut-end. Irrespective of the rooting material and its concentrations, the observations were the same on all rooted stem cuttings. Plate 6 displays the outcome of the rooted stem cutting of *Crateva adansonii* treated with 300mg/L IBA and displaying the main, lateral, and feeder roots.

Counting and measuring the length of roots on stem cuttings as influenced by different concentrations of IBA and CLE

The number of roots, including lateral and feeder roots, per stem cutting and the average length of the five longest roots per cutting are determined. The results are provided (Plate 6 and Table 5). In general, the results revealed diversity between the various treatments, including the different IBA and CLE concentration levels. Per stem cutting, there were anywhere between 15.25±0.48 to 25.50±1.32 roots. The stem cuttings soaked in 300 mg/L IBA produced the most roots (25.50±1.32), followed by those soaked in 200 mg/L IBA and 100 percent CLE (19.25±0.48), and the stem cuttings soaked in 60 percent CLE (15.25±0.48) produced the fewest roots. The longest five roots on each stem cutting ranged in length from 8.13±0.05 cm to 8.60±0.04cm on average. The results also showed that stem cuttings soaked in 200mg/L IBA and 80% CLE produced the longest roots (8.60±0.04 cm). Stem cuttings soaked in 300 mg/L IBA 8.53±0.03cm followed, while stem cuttings soaked in 100 mg/L IBA 8.13±0.05cm had shortest roots.

Comparatively, the findings showed that stem cuttings treated with different concentrations of IBA developed more roots on the stem cuttings than stem cuttings treated with different concentrations of CLE. Further analysis of the results revealed that CLE concentrations of 100%, 80%, and 60%, which produced 19.25±0.48, 16.00±0.41 and 15.25±0.48 correspondingly, had a greater influence on the development of roots than higher IBA concentrations (300mg/L and 200mg/L) did. According to the findings, stem cuttings treated with IBA concentrations produced longer roots than stem cuttings soaked with CLE.

Aerial growth parameters of rooted stem cuttings

Table 6 shows the results of calculating the mean values of some aerial growth metrics of the rooted stem cuttings, including branch count, branch length, and mean leaf area.

On the stem cuttings, there appeared somewhere between 5.25±0.48 to 12.75±0.85 branches. The stem cuttings that had been treated in 300mg/L of IBA had the most branches on them, which was followed by stem cuttings soaked in 100 percent CLE (8.75±0.48). Stem cuttings bathed in 100mg/L of IBA had the least. The results also demonstrated that the branches' lengths varied from 3.75±0.48 cm to 8.75±0.48cm. The longest branches were formed by stem cuttings that had been immersed in 100 mg/L (8±0.75cm) of IBA. Stem cuttings bathed in 200mg/L IBA and 100% CLE followed after that.

The seedlings' mean leaf area was calculated, and the results showed that it ranged from 23.50±0.65 cm^2 to 26.75±0.48 cm^2. The broadest leaves, measuring 26.75±0.48, were produced by stem cuttings immersed in 100 mg/L IBA and 60 percent CLE. Stem cuttings immersed in 100 percent CLE generated the narrowest leaves (23.50±0.65cm^2), followed by cuttings soaked in 300 mg/L IBA, 200 mg/L IBA, and 80 percent CLE, which all had leaves with an area of 24.25±0.48cm^2.

DISCUSSION

The results from the meteorological data within the period of the trial which included: mean monthly rainfall, humidity, temperature from May to October showed high rainfall and relative humidity incidence alongside low temperature. The results agree with the earlier reports of Araya *et al*. (2007) and Sani *et al*. (2016) who reported May to October as the period with best result in their various trials. Studies on rooting of stem cuttings by (Sani *et al*.,2016) were favoured by the wet season, characterized by high rainfall, high humidity and low temperature.

The first indication of life renewal that might lead to further development was seen in the bud breaking. Buds sprouted on all stem cuttings, including the control, regardless of the rooting chemicals used or their concentrations (untreated stem cuttings). On stem cuttings, the time between the first and final bud sprouting varied. This supports past studies that described the steps involved in the production of roots on stem cuttings treated with rooting agents, including bud break, leaf formation, callus formation, and rooting (Gilani *et al*., 2019). The development of adventitious roots is the primary process in asexual propagation. Adventitious root growth is influenced by both internal and external influences (Cibele *et al*., 2013). The most significant impact on internal factors is attributed to phyto-hormones, with preference to auxins but IBA has been reported to have greater ability to induce adventitious roots formation than indole acetic acid-IAA (Ludwig-Muller, 2000; Hakan and Kerim, 2013) and this may be due to increased stability of IBA. Similar report was also upheld by Ludwig-Muller (2000) and Fattorini *et al*. (2017). Therefore, indole-butyric acid (IBA) has been advocated as a better hormone to encourage the development of roots in plant stem cuttings.

According to the current study, the great susceptibility of the species to bud sprouting under any degree of concentration of any known rooting medium, maybe related to the species' genetic makeup, could be the cause of the bud breakage as observed on all of the stem cuttings, including the control.

a **b**

Plates 5: The stem cuttings treated with coconut liquid endosperm (CLE) in (a) and indole butyric acid (IBA) in (b) developed calluses and roots.

Plate 6: Rooted stem cutting of *Crateva adansonii* with main, lateral, and feeder roots after treatment with 300mg/L indole butyric acid IBA.

Table 5: Effect of coconut liquid endosperm (CLE) and indole butyric acid (IBA) on the quantity of roots and average length per stem cutting of *Crateva adansonii*

Treatments	Number of roots per cutting	Mean length of five longest roots per stem cutting (cm)
IBA (mg/L)		
100	16.50 ± 0.65^{c}	8.13 ± 0.05^{b}
200	19.25 ± 0.48^{b}	8.60 ± 0.04^{a}
300	25.50 ± 1.32^{a}	8.53 ± 0.03^{a}
Control	0.00 ± 0.00^{d}	0
LSD (P<0.05)	2.38	0.01
CLE (%)		
60	15.25 ± 0.48^{b}	8.43 ± 0.05^{b}
80	16.00 ± 0.41^{b}	8.60 ± 0.04^{a}
100	19.25 ± 0.48^{a}	8.40 ± 0.06^{b}
Control	0.00 ± 0.00^{c}	0
LSD (P<0.05)	1.24	0.131

Values in the vertical column that share the same alphabet (or alphabets) do not substantially differ from one another (P ≤0.05).

Table 6: Some *Crateva adansonii* aerial growth metrics after treatment with coconut liquid endosperm (CLE) and indole butyric acid (IBA)

Treatments	Number of branches	Length of branches (cm)	Mean leaf area (cm)
IBA (Mg/L)			
100	4.75 ± 0.48^{b}	8.75 ± 0.48^{a}	26.75 ± 0.48^{a}
200	7.00 ± 0.71^{b}	8.00 ± 1.29^{a}	24.25 ± 0.48^{b}
300	12.75 ± 0.85^{a}	5.25 ± 0.75^{b}	23.50 ± 0.65^{b}
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0
LSD	1.85	2.42	1.28
CLE (%)			
60	5.25 ± 0.48^{b}	4.00 ± 0.41^{b}	26.75 ± 0.48^{a}
80	5.75 ± 0.63^{b}	3.75 ± 0.48^{b}	24.25 ± 0.48^{b}
100	8.75 ± 0.48^{a}	6.25 ± 0.75^{a}	23.50 ± 0.65^{b}
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0
LSD	1.42	1.50	1.44

Values in the vertical column that share the same alphabet (or alphabets) do not substantially differ from one another (P ≤0.05).

Since plant meristematic tissues produce hormones including cytokinin and IAA, Azizi and Sahebi (2015) suggested that these hormones may be to blame for the early bud sprouting observed on all of the stem cuttings. The discrepancy in the times that the stem cuttings' buds sprouted could be explained by the interaction between the hormones that the plant naturally produces and the rooting agents (IBA and CLE) that were administered. A lack of intrinsic hormone concentration in the cutting may have contributed to the lengthier time it took the control group to start early bud sprouting. This may be the real cause of the high rate of single and multiple bud sprouting seen in stem cuttings treated with additional and variable concentrations of CLE and IBA.

There aren't many comprehensive studies on the growth of buds that emerged from cuttings to become photosynthesizing leaves. In the current investigation, it was found that the untreated stem cuttings had a higher rate of none sprouting into genuine leaves than the stem cuttings treated with low quantities of IBA. This may be because the hormones supplied in lesser concentrations were insufficient to encourage the production of leaves. According to Nzekwe (2002) and Sani *et al*. (2016), not all buds that emerged on stem cuttings lasted through leaf development.
The findings demonstrated that higher concentrations of IBA (300 mg/L) and Coconut liquid endosperm (CLE) had a greater influence on the proportion of buds that developed into leaves, up to 83 mg/L for IBA and 84 mg/L for CLE, respectively. The higher concentrations of rooting components that provided the stem cuttings with additional rooting substance for subsequent developmental activities appear to have had an impact on the higher percentages of bud development into leaves. The 83 percent successful bud development into leaves on 300mg/L IBA as opposed to 84 percent impacted by 100 percent CLE reveals that CLE performs better than the former and points to the need for future studies to use IBA concentrations above 300mg/L for better results. Low percentage of untreated stem cuttings' buds developing into leaves could be a result of depletion of the naturally produced auxin in the stem cuttings, thus the use of untreated stem cuttings of *Crateva adansonii* in the vegetative propagation of the species may not be hoped upon for the routine generation of the species seedlings. The transformation of buds into leaves suggested that additional nutrients were being produced by photosynthesis in the leaves. According to Nzekwe (2006), a plant's capacity to absorb nutrients through photosynthesis increases in direct proportion to the number of healthy leaves it produces. This rise is mostly attributable to auxins, which promote photosynthesis, participate in the light reaction, and activate the enzyme rubulose-bisphosphate carboxylase, increasing leaf nutrition (Al-hasnawi , 2012). This is consistent with research done by Amrut and Rajput (2013) on fenugreek and Ullah *et al*. (2013) on marigold (*Tagetes erecta L*.).

The observations made on the stem cuttings demonstrated that callus production was not implied by the appearance of bud rupture and eventual leaf development seen in all of the stem cuttings. These results agree with those of Puffy *et al* (2008). The development of two morphologically different types of calluses on the stem cutting of the same plant species has been documented by a number of authors (Nzekwe, 2006; Puffy *et al*.,2008; Sani *et al*., 2016). On stem cuttings treated with rooting agents prior to rooting, they also observed the development of two physiologically distinct types of calluses. They classified the first callus type as "heavy calluses," characterized by heavy swelling of the stem cuttings and basal region, associated with vertical swelling. According to Norhayati *et al*. (2013), strong callus proliferations were a reliable indicator of how quickly stem cuttings will root. The second callus type was also referred to as "light calluses" by the authors and was capable of producing seedlings (rooted stem cuttings) with just lateral and feeder roots and no tap root system.

The findings of the current investigation demonstrated that stem cuttings associated with seedling formation with just lateral and feeder roots, without a tap root system, were only mildly callused, regardless of the concentration of rooting agent. This is also consistent with what Puffy *et al*.(2008) observed. Despite light calluses noticed on stem cuttings, irrespective of the concentrations of the rooting substance, cuttings treated with higher concentrations of IBA (300mg/L, 200mg/L) and CLE (100%, 80%) had higher percentages of callusing than those treated with lower concentrations of IBA (100mg/L) and CLE (60%). The production of light calluses observed in this study could be an indication that further investigation involving the use of higher concentrations of IBA or other rooting substance should be encouraged to find out if there could be changes in aerial and basal growth parameters. The higher concentrations of IBA (300 mg/L) and CLE (100%) is believed to be responsible for the highest percentage rooting observed on the stem cuttings up to 80% out of all the already callused stem cuttings leaving the control with no root development.

The inability of the control to develop root could possibly have been because of the insufficiency of the available hormone to have initiated root formation. The breaking of hydrogen bonds between cellulose microfibrils by proteins from IBA is what causes the trend in root length, encouraging cell wall thinning and eventual cell enlargement (Kumar *et al*., 2015; Qu Yang *et al*., 2015). The rate of cambium dedifferentiation is raised, hydrolytic activity is accelerated, and callus production is boosted at optimal exogenous IBA, all of which contribute to better root length (Gilani *et al*., 2019). The action of the cytokinins and auxin in coconut water may be to blame for this. Thus, it is clear that the presence of cytokinins and IBA in the coconut water encouraged the growth of large numbers of undifferentiated cells (callus). After being exposed to certain hormones, such as auxins (found in coconut water), which formed roots, the callus cells are then encouraged to grow roots. The influence of growth regulators IBA on the translocation of metabolites and metabolism of carbohydrates may also be responsible for the increase in root length. When compared to the other auxines, Hakan and Kerim (2013) saw that IBA produced a larger output of roots in *Melissa officinalis.* The outcome is in line with Okunlola (2016) investigations, which found that rooting hormones applied to *Bougainvillea spectabilis* wood cuttings had a substantial impact on the length of roots compared to the control. These findings concur with those of Oluwagbenga (2016) who discovered that coconut water treated *Parkia biglobosa* plant had more roots per plant than the control. Given the reduced time needed for callus formation and the increased cambium dedifferentiation, Singh *et al*. (2014) noted that there are more roots at the optimal IBA concentration. These numerous cells will differentiate into root cells. The pace of disappearance of amyloplasts is accelerated by optimal IBA. During rooting, amyloplast levels naturally decrease (Singh *et al*., 2014). Our results show that at optimal indole-3-butyric acid, amyloplast decline can be improved and cambium activities are stimulated, which will mobilize stored food supplies to the root initiation site and encourage the production of many roots (Gilani *et al*., 2019).

From the study's findings which revealed that rooted stem cuttings did not produce taproot system implied that either the concentrations of the chemical substances were not sufficient to have an impact on seedling production that resembles the parent stock, or the seedlings require more growth in the nursery. Despite producing seedlings that had no taproot system, the understudy species can be multiplied using stem cuttings and the vegetative propagation of the species can immensely contribute to the production of large quantity of seedlings which can help in the future exploitation of the species medicinal potentials. Such seedlings would be uniform and from that resources can be exploited from the species as noted by Lodama and Robbertse (2016). Based on the high percentage callusing of the cuttings treated with 300mg/L IBA, which had no taproots, there is need to evaluate the effects of other rooting substances at higher concentrations on production of species seedlings that resembles the parent stock. The finding is in conformation with the report of Sani *et al*. (2016) on the investigation on *Moringa oliefera* that showed that IBA and IAA respectively influenced the development of aerial and sub aerial parameters of the plant.The same concentrations of the treatments in the current study produced larger values of plant aerial growth characteristics like number of leaves, length of branches, mean leaf area, and sub-aerial growth parameters like number of roots and root length than their lower concentrations did. The development of higher values of the aerial and sub-aerial growth parameters appears related to the early periods of bud breakage and leaf development.

CONCLUSION AND RECOMMENDATIONS

This study's findings allow us to draw the conclusion that *Crateva adansonii* can be multiplied by treating the stem cuttings with the appropriate concentration of IBA preferably above 300 mg/L. The high percentage of rooting response of cuttings treated with 300mg/L IBA and 100% CLE concentrations can be dependable for a high volume of seedling production required for the species genetic resource conservation in large proportion needed for future biotechnological exploitation for its medicinal bioactive potentials. However, further studies could be encouraged especially with the use of other rooting substances. Low percentage development of buds on low concentration treated stem cuttings of the respective rooting substances implied that the concentrations of the rooting chemicals were not adequate for obtaining good responses by the stem cuttings, hence higher concentrations are recommended for further studies. Based on the high percentage callusing of the cuttings treated with 300 mg/L IBA, which had no tap roots, there is need to evaluate other rooting substances at higher concentrations on production of species seedlings that resemble the parent stock. The production of light calluses observed in this study implied that further studies involving the use of higher concentrations of IBA or other rooting substance can be investigated to find out if there could be changes in aerial and basal growth parameters as well as heavy callus formation.

Conflict of interest

The authors have no conflict of interest to declare

Author Contribution

Study design – NMC, ON, NNO, OVO, OAA, EOC, data collection – NMC, ON, NNO, data analysis – NMC, OVO, manuscript writing – NMC, ON, EOC, OVO, OAA, critical revision – NMC, ON, NNO, EOC, study supervision – NMC, ON, NNO, OVO

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