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A comparative evaluation of Indole-3-Butyric Acid and plant extracts as potential rooting enhancers in cuttings of *Vitex diversifolia* and *Cordia milleneii*

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***Abstract*— *This study tested the effect of a synthetic phytohormone and plant extracts on rooting of cuttings. Treatments comprised of two tree species (Cordia mellineii and Vitex diversifolia) and five pre-planting rooting treatments (untreated control, Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and AV+CW in the ratio 1:1 v/v) laid out in split-plot. Data were collected four months after the application of treatments. There was neither rooting of Cordia mellineii cuttings in any of the treatments nor Vitex diversifolia cuttings in the control. Rooting percentage of Vitex diversifolia was highest in AV+CW and lowest in the AV treatment which did not show a significant difference with the control. In contrast, values of number of primary roots were highest in AV. Number of secondary roots, root system length and root fresh weight were significantly reduced by IBA. Root diameter was unresponsive to rooting enhancing treatments. The results suggest that biostimulators are essential for rooting of Vitex diversifolia cuttings. While IBA, AV, and CW may have a comparable influence on root initiation, the two plant extracts have the tendency to exhibit a stronger effect when in combination. In addition, IBA may be less beneficial for root growth than AV and CW.*

***Keywords*— *cutting propagation, growth, montane tree species, phytohormone, rooting*.**

I. INTRODUCTION

Cordia millenii Bak. (family Boraginaceae) is a multipurpose timber tree of the African tropics. Commonly known as drum tree, it attains a height of 30 (-35) m with a branchless bole and diameter of up to 10 (-15) m and 100 (-120) cm, respectively (Jiofack 2010). Although soft and fairly light in weight, the wood is durable and resistant to termites (Burkil et al. 1985-2005). It is used for construction, joinery, furniture, musical instruments, utensils, tool handles, plywood, fuel, and carving. The seeds and leaves are of medicinal value while the flowers provide nectar and pollen for bees (Jiofack 2010). In addition, trees are often preserved for shading in agroforestry systems (Fern 2019). The species has become less common in some parts of its range because of large-scale exploitation and declines in the extent of its habitat.

Vitex is the largest genus of the family Verbenaceae which is made up of 250 species that are distributed all over the world (Ganapaty and Vidyadhar 2005). Amongst them is *Vitex diversifolia* Bak., a shrub or small tree 2 - 6 (-8) m high with a short bole and an open crown (Arbonnier 2004). The species inhabits grassland, wooded grassland or dense woodlands at elevations of 1,000 - 1,800 m (Ruffo et al. 2002). In tropical Africa, its distribution spans from Senegal to Cameroon and as far as Egypt and Sudan (Arbonnier 2004). *Vitex diversifolia* is used for reforestation, carving, timber, medicine, and watershed protection (Ndenecho 2009). Moreover, the leaves yield an essential oil of such sweet and penetrating fragrance that it has been recommended for commercial development (Fern 2019).

Cordia milleneii and *Vitex diversifolia* are important components of the fast degrading forests of the western Cameroon highlands. In fact, over 50% of the forest that once covered most of the landscape has been lost since the 1960s. A high human population density due to fertile volcanic soils and adequate rainfall in the area has subjected the forest remnants to enormous pressure from agricultural encroachment, unsustainable harvest of trees

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and tree parts for medicine, fires established by farmers and graziers, and unsustainable collection of firewood and construction materials (Ndenecho 2011, Assi-Kaudjhis 2012, Kometa 2013, Sedláček et al. 2014). For instance, large pieces of *Cordia milleneii* bark continue to be stripped from the bole for hut-walls and partitions (Jiofack 2010). Thus, the forests are not only degraded but threatened. There is a need for mass production of planting materials of these and other highly important western Cameroon Highlands tree species to rehabilitate the ecosystem. Discovering a simple but efficient way of propagating the species is of particular interest in such an effort.

Vegetative propagation is a practical means for mass production of high quality regeneration stock. Unlike with sexual propagation, the new independent plant produced through vegetative means is a clone in which desirable traits of the donor are preserved (Santoso and Parwata 2014). Moreover, vegetative propagation can bypass the germination phase to reduce the rotation period of the species. Stem cutting is the most common of vegetative propagation methods for herbaceous and woody plants. Reasons for the popularity generally revolve around the low cost (Waziri et al. 2015) and ease (Dawa et al. 2017) associated with the use of the technique. The success of cutting propagation may be confounded by the age of the donor plant (Ambebe et al. 2017), growth medium (Ashiono et al. 2017), type of cutting (Washa 2014), phytohormones (Bhardwaj et al. 2017), size of the cutting and health of the donor plant (Kramer and Kozlowski 2014) among others. Furthermore, the responses of the cuttings to some of these factors are species sensitive (Hassanein 2013). Extracts from some plant species have been found to exhibit root-promoting activities due to tissue contents of phytohormones (Prades et al. 2012). This study was aimed at comparing the effects of IBA, coconut water and aloe vera gel on rooting of semi-hardwood cuttings of *Cordia milleneii* and *Vitex diversifolia*.

II. MATERIALS AND METHODS

2.1. Plant material

Semi-hardwood cuttings of *Cordia milleneii* and *Vitex diversifolia* were collected from healthy trees in disturbed forest patches in Tubah Sub-Division (4°50' – 5°20'N, 10°35' – 11°59'E; 950 – 1500 m asl) of the North West Region of Cameroon. The Sub-Division is located at 15 km from Bamenda (5°59'N, 10°11'E), the Regional headquarter. Being part of the western highland ecological zone of Cameroon, the forest and grassland vegetation of Tubah has been subjected to enormous destruction by a high population density and unsustainable human behavior (Melle et al. 2016). The cuttings were collected from the lower part of the canopy of seed bearing trees. The material from each species was sealed in a leak-proof polythene bag and transported to Bamenda III Sub-Division where cuttings of similar diameter were resized to a length of 20 cm and given a slanting cut at the distal end.

2.2. Study site

The experiment was carried out at the Reforestation Task Force (RETAFO) nursery in Bamenda III Sub-Division. The site is characterized by a rainy season that runs from April to October and a dry season from November to March. With a mean annual precipitation of 2145 mm, January is the driest month with 9 mm while September is the wettest with an average of 383 mm of precipitation. The mean annual temperature is 21.5 °C. The warmest and coldest months of the year are March and July with 23.0 °C and 20.1 °C, respectively, (Climate-Data 2019).

2.3. Treatments and experimental design

Treatments comprised of two tree species (*Cordia milleneii* and *Vitex diversifolia*) and five root promoting substances (untreated control, 5000 mg l^{-1} Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and AV+CW in the ratio 1:1 v/v). The study followed a split-plot design with tree species as the whole plot and root promoting treatment as the split-plot. There were 10 cuttings in each treatment combination replicated twice. The root enhancing treatments were applied by dipping the bases of the cuttings in the appropriate inducer for 2 minutes. They were then set to a depth of 4 cm into coarse sand in a non-mist propagator. A light overhead watering was given immediately after planting to get the cuttings settled. The propagator consisted of a large wooden box sealed with polythene sheet. A water table made up of successive layers of fine sand, stone, and gravel was created beneath the coarse sand substrate. A PVC tube installed through the substrate into the water table made it possible for the water level in the propagator to be gauged. When irrigating, water was allowed to fill the water table up to the upper limit of the uppermost (gravel) layer so that the substrate was kept moist. A wooden frame was constructed over the box and then the entire set-up was enclosed in polythene sheet to maintain a humid environment around the cuttings. Whenever the propagator was opened to inspect the cuttings and/or check the water status of the substrate, a mist of water was applied with a spray bottle to maintain the high humidity. The propagator was situated in a shade house roofed with alternating rows of transparent plastic and

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corrugated iron roofing sheets. The experiment was started on April 18 and terminated on August 26, 2018.

2.4. Data collection

The cuttings were examined regularly for rooting. At the end of the experiment, no *Cordia milleneii* cutting rooted in any treatment. The number of *Vitex diversifolia* cuttings that formed roots was noted and the rooting percentage calculated thus:

$$\text{Rooting percentage} = \frac{\text{Number of cuttings rooted}}{\text{Number of cuttings planted}} \times 100$$

Three rooted cuttings were randomly chosen from each treatment on which the primary roots were counted. In addition, the diameter of the main root, number of secondary roots on the main root, root system length, and root fresh weight were determined for each of the cuttings.

2.5. Statistical analysis

To test the effect of root promoting treatment on rooting behavior of *Vitex diversifolia*, the data were subjected to ANOVA after having ascertained that they fulfilled the test's assumptions of normality and homoscedasticity. When there was a significant effect of treatment on a given parameter, means separation was conducted with Fisher's Least Significant Difference (LSD) test. All the analyses were performed in Data Desk v. 6.01 at $p < 0.05$. *Cordia milleneii* was not included in the analysis because of the 0% rooting in the species.

III. RESULTS

3.1. Rooting percentage

There was a significant effect (p = 0.0391) of rooting treatment on rooting percentage. According to the post hoc test, the greatest proportion of rooted cuttings was obtained following treatment with AV+CW while the control was completely void of rooting (Fig. 1). However, IBA did not significantly differ with either AV+CW or any of the two plant extracts for this trait. Additionally, the difference in rooting percentage between AV and the control was not statistically significant (Fig. 1).

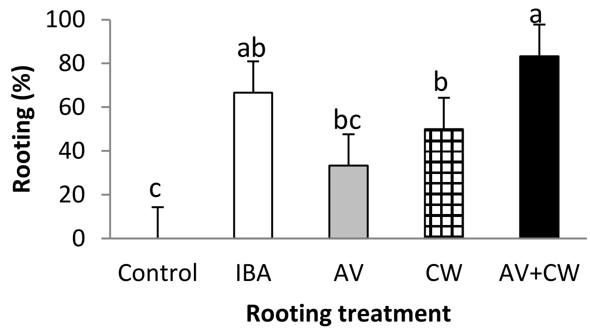

Fig. 1. Effect of Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and a combination of aloe vera gel and coconut water (AV+CW) on rooting percentage of Vitex diversifolia semi-hardwood cuttings.

3.2. Number of primary roots

The ANOVA detected a significant effect (p = 0.0387) of rooting treatment on number of primary roots. Values were highest in AV and lowest in IBA (Fig. 2). There were no significant differences in number of primary roots between CW and either AV or AV+CW. Moreover, the difference between IBA and AV+CW was not significant under the LSD test (Fig. 2).

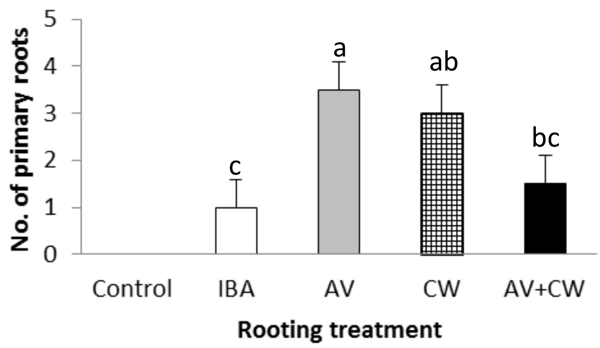

Fig. 2. Effect of Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and a combination of aloe vera gel and coconut water (AV+CW) on number of primary roots of Vitex diversifolia semi-hardwood cuttings.

3.3. Number of secondary roots

Rooting treatment had a significant effect (p = 0.0003) on number of secondary roots. It was significantly reduced by IBA (Fig. 3). Number of secondary roots did not differ between the plant extracts either individually or in combination (Fig. 3).

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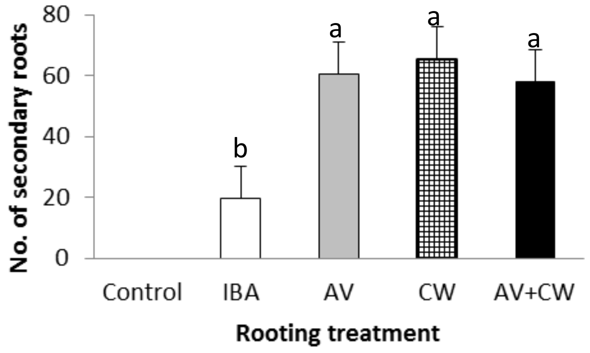

Fig. 3. Effect of Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and a combination of aloe vera gel and coconut water (AV+CW) on number of secondary roots of Vitex diversifolia semi-hardwood cuttings.

3.4. Root system length

There was a significant effect (p = 0.0149) of rooting treatment on root system length. Values of the parameter increased from the IBA to the plant extract based treatments which did not show significant differences among one another (Fig. 4).

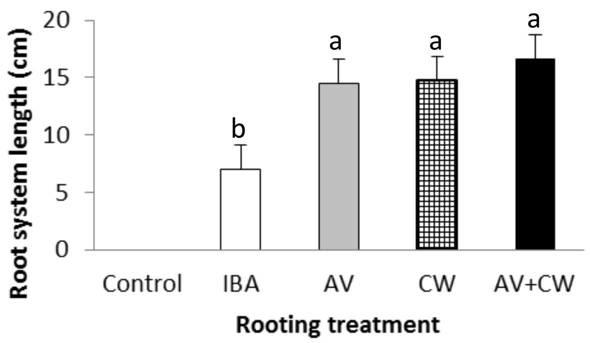

Fig. 4. Effect of Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and a combination of aloe vera gel and coconut water (AV+CW) on root system length of Vitex diversifolia semi-hardwood cuttings.

3.5. Root diameter

Diameter of the main root was not significantly (p = 0.6499) influenced by rooting treatment (Fig. 5).

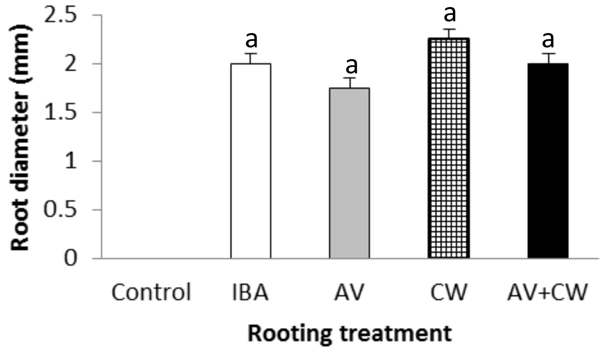

Fig. 5. Effect of Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and a combination of aloe vera gel and coconut water (AV+CW) on root diameter of Vitex diversifolia semi-hardwood cuttings.

3.6. Root fresh weight

Root fresh weight was significantly (p = 0.0033) affected by rooting treatment. Treatment of the cuttings with AV+CW resulted in the highest values of this attribute while that with IBA produced the lowest (Fig. 6). The CW treatment did not differ significantly with either AV or AV+CW for root fresh mass. Furthermore, the difference recorded between AV and IBA was statistically insignificant (Fig. 6).

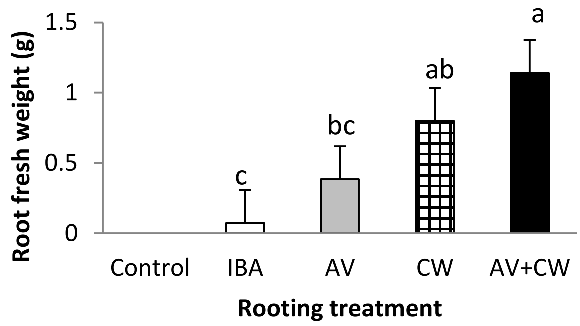

Fig. 6. Effect of Indole-3-Butyric Acid (IBA), aloe vera gel (AV), coconut water (CW), and a combination of aloe vera gel and coconut water (AV+CW) on root fresh weight of Vitex diversifolia semi-hardwood cuttings.

IV. DISCUSSION

The determining step for the success of asexual propagation is the formation of adventitious roots. Of the intrinsic factors that influence rooting, phytohormones, and in particular auxins, have been highlighted to play the most important role (Karunarathna and Harris 2016). The results obtained in the present study are in accordance with those of other investigators. As cited in Ibrahim (2015), Kelly (1985) observed that dipping the distal end of *Magnolia stellata* cuttings in IBA resulted in 30% more rooting than when untreated. The hormone has been shown to promote rooting of cuttings in other species including *Actinidia chinensis* (Morini and Isoleri 1986), *Acacia* spp (Hartmann et al. 2011), and *Rosa* spp (Dawa et al. 2017). On the other hand, Naphthalene Acetic Acid (NAA) improved number of rooted cuttings and root length of *Lippia citriodora* (Ibrahim et al 2015) while Indole Acetic Acid (IAA) influenced root formation of *Prunus* sp. cuttings (Štefančič et al. 2005). Aloe vera gel (AV) and coconut water (CW) are important sources of phytohormones. Chemical analysis has shown that AV contains Gibberellic Acid (GA3), IAA, and Abscisic Acid (ABA) (El Sherif 2017) while CW is made of IAA, Cytokinins, Gibberellin A1, GA3, ABA and Salicylic Acid (Yong et al. 2009). Various auxins such as IAA, IBA, NAA, and 2,4-Dichlorophenoxy Acetic Acid have been demonstrated to promote rooting in 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). On its part, IAA stimulates cell division in the pericycle leading to the formation of lateral and adventitious roots. In addition, the Cytokinins and auxin present in coconut water may stimulate the formation of masses of undifferentiated cells (callus) which can, in turn, be induced to form roots by exposure to the auxin. The combination of AV and CW seems to have complemented the low IAA levels in each other leading to a greater rooting percentage.

In line with the results of the present study, number of roots per cutting was significantly augmented by CW in *Parkia biglobosa* (Dunsin 2016) and AV in *Populus tremula* (El Sherif 2017). According to Rolston et al. (1996), exogenous auxin treatment can increase the number of root primordia in the basal part of cuttings with the outcome that rooting and number of roots per cutting are augmented. Furthermore, the presence of growth promoting factors (e.g. proteins, vitamins, sugars, and minerals) in AV (El Sherif 2017) and CW (Thio 1982, Jackson et al. 2004, Gopikrishna et al. 2008) gives the extracts a greater potential than IBA to drive growth. In addition to number of roots, AV and CW outperformed IBA in terms of root system length and root fresh weight. This finding concurs with the results of a previous trial involving cuttings of a woody tree, *Ficus hawaii*, and a herbaceous plant, *Chrysanthemum morifolium*, where treatment with IAA was found to be more effective than that with either IBA or NAA (Hassanein 2013). The differences in root growth may also be due to the differential effects of the growth regulators on metabolites translocation and carbohydrates metabolism.

V. CONCLUSION

The results of this study have shown that pre-planting treatment with biostimulators is essential for rooting of semi-hardwood cuttings of *Vitex diversifolia*. While synthetic IBA, AV, and CW may have a comparable influence on root initiation, AV+CW has the tendency to be a more potent rooting inducer than either of the plant extracts alone. In addition, treatment of cuttings with AV and CW may be more beneficial than IBA for root growth after initiation.

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