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Effects of root inducing agents on rooting performance of *Vitex diversifolia* stem cuttings

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Abstract

This study examined the comparative effect of potential rooting promoting substances on rooting of Vitex diversifolia semi-hardwood cuttings. Treatments were comprised of a negative (untreated) and positive (Indole-3-Butyric Acid, IBA) control, aloe vera gel (AV), coconut water (CW), and AV+CW in the ratio 1:1 (v/v). Data on root production and growth were collected four months after the application of treatments. Results indicated an absence of rooting of cuttings in the negative control. Rooting percentage was highest in AV+CW and lowest in the AV treatment which did not show a significant difference with either the controls or CW. In contrast, values of number of primary roots were highest in AV and lowest in IBA. Number of secondary roots, root system length and root fresh weight were significantly reduced by IBA. Root diameter was unresponsive to the treatments. The findings suggest that biostimulators are essential factors for rooting of Vitex diversifolia cuttings. While treatment with IBA, AV, and CW may have a comparable influence on rooting percentage, the combination of AV and CW may be a more potent promoter of the trait. In addition, the application of IBA may be less beneficial than the plant extracts for number and growth of roots.

Keywords: Cutting propagation, growth, phytohormone, rooting, semi-hardwood cuttings.

Introduction

Vitex diversifolia is a Verbanaceae of the dicot group. The small tree that may attain a height of 8 m, frequently 2 – 6 m, has a short bole and an open crown[1]. It is found at elevations of 1000 - 1800 m in which it thrives in grasslands and forests[2]. *Vitex diversifolia* has a wide ecological distribution in Africa, with occurrences in both northern and sub-Saharan countries. The tree is an important component of the mixed forest and savannah grasslands of the Bamenda Highlands where it is used for timber, carving, and medicine[3]. In addition, sale of the edible fruit in local markets constitutes an important income generating activity. An essential oil distilled from its air-dried leaves has been found to contain limonene as principal component[4]. Aside from associated pharmacological attributes, the sweet aroma imparted by the oil gives it potentials for commercialization[5]. Other commodities derived from the tree are fuel wood, charcoal[2], and chew stick for cleaning of teeth[6].

Aided by unsustainable human behavior, the Bamenda Highlands has experienced substantial degradation of its woodlands in recent years. In fact, the landscape's forest cover has declined by over 50% since the 1960s. The ecosystem is burdened by conversion of forests to farmland, cattle rearing, bushfires, abusive harvesting of plants and plant parts, and uncontrolled logging[7-10].

These have led to a decline in population of its tree species, warranting restoration. Seed is the common starting material for regeneration of *Vitex diversifolia*[5]. However, seed may not be an attractive regeneration material when particular traits are desired of the plant as it is a product of a mixture of genetic material that may be coming from unknown parents. On the other hand, a plant raised from vegetative plant parts is a clone in which the characteristics of the donor are preserved[11]. Depending on the species, vegetative propagation is easier and faster than sexual propagation[12].

Cutting propagation, and in particular stem cutting, is the most popular of vegetative propagation techniques[13]. Its wide application is due to low cost and the ease with which the procedure is performed. Although stem cuttings have the potential to expedite the mass supply of good quality regeneration material, the success rate is undermined by difficulty in rooting[14]. The rooting behavior of cuttings is influenced by ortetage, cutting type, canopy position, substrate, auxin level, rooting co-factors, and carbohydrate contents among others[15-18].

Moreover, significant interactions exist between these factors and plant species. The use of synthetic auxins in enhancing rooting in the Bamenda Highlands is timidified by unavailability and high cost. Tissue contents of phytohormones and other growth promoting factors make plant extracts potential treatments for enhancing rooting in cuttings. This study examined the effect of plant extracts on root production and initial growth of *Vitex diversifolia* semi-hardwood cuttings.

Materials and Methods

Study site: The experiment was conducted at the Reforestation Task Force (RETAFO) nursery at Bamenda III Sub-Division, Mezam Division, North West Region of Cameroon. Found between latitudes 6°15' and 6°25'N and longitudes 10°02' and 10°15' E of the Greenwich Meridian, the Bamenda III municipality is bounded by four other Sub-Divisions of Mezam namely; Bafut (North), Bamenda I (South), Bamenda II (West), and Tubah (East) Sub-Divisions (Figure-1). There are two villages in the Bamenda III council area namely Nkwen where RETAFO is located and its smaller counterpart Ndzah. The area is characterized by two seasons: the rainy season runs from mid-March to mid-November while the dry season goes from mid-November to mid-March. Under changing global climatic conditions, however, there are often modifications in the timing and duration of the seasons.

Experimental set-up: The study made use of five pre-planting treatments namely; an untreated control, 5000 mg l^{-1} Indole-3-Butyric Acid (IBA), aloe vera gel (AV) coconut water (CW) and a 1:1 AV:CW mix (AV+CW). The plant material consisted of 20 cm long non-leafy semi-hardwood cuttings taken from the lower canopy of seed-bearing trees of *Vitex diversifolia*. The trees were growing in forest fragments in neighboring the Tubah Sub-Division located between latitudes 4°50' and 5°20' N and longitudes 10°35'and 11°59' E. After dipping the lower 2 cm in the appropriate substance, the cutting was planted at a depth of 4 cm in coarse sand substrate contained in a non-mist propagator. There were two replications of each of the treatments that had 10 cuttings. A plastic enclosure over the propagator bed guaranteed a high humidity around the cuttings. Watering was done by filling water in a PVC tube that went through the coarse sand substrate to a water table consisting of successive layers of fine sand, stone, and gravel. The need for irrigation was determined from a mark created on the wall of the PVC tube. Once water was filled up to the mark, the water table was fully hydrated. Whenever the plastic sheet was slightly opened to monitor the cuttings, a light mist of water was supplied with a spray bottle before closure to maintain the humidity in the propagator. The structure was situated in a shade house roofed alternatingly with aluminum and transparent sheets. The experiment lasted four months.

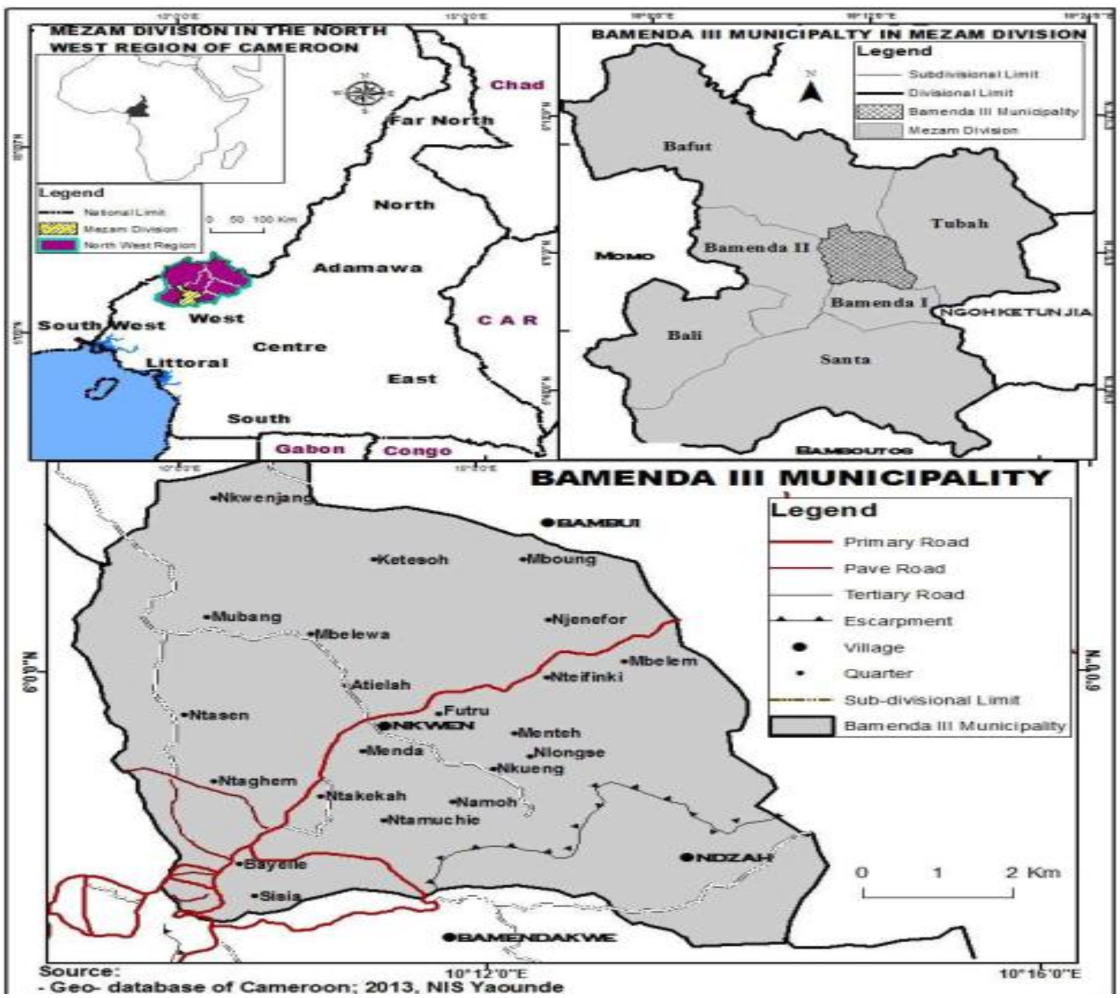

Figure-1: Location of Bamenda II Sub-Division in Mezam Division, North West Reegion, Cameroon.

Data collection: At the end of the experiment, the substrate was flooded with water and the cuttings were lifted carefully. The root system was rinsed free of substrate and the cuttings that had rooted in each treatment and replication were counted. Rooting (%) was calculated as follows:

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

Three rooted cuttings were randomly chosen per treatment × replication for further data collection. Number of primary and secondary roots per cutting was determined. The length of the root system and diameter of the main root were measured with a ruler and caliper, respectively. The diameter measurement was carried out closest to the point of attachment of the root to the cutting. The roots were detached and weighed after removing any residual water droplets with a blotting paper.

Data analysis: The data were checked for normality and homogeneity of variance and then subjected to one-way ANOVA. When the effect of pre-planting treatment was significant for a particular parameter, Fisher's LSD test was used for comparison of means. The analyses were performed in Data Desk 6.01 where the significance level was set at 5%.

Results and Discussion

All root production parameters responded to treatments. As for root growth, only root length and root fresh weight responded were affected; root diameter was not (Table-1).

Table-1: *p*-values from analysis of variance for the effect of pre-planting treatment on root production and growth of *Vitex diversifolia* semi-hardwood cuttings.

Parameter	p-value
Rooting (%)	0.0391
No. of primary roots	0.0387
No. of secondary roots	0.0003
Root length	0.0149
Root diameter	0.6499
Root fresh weight	0.0033

Root production: Rooting (%) was highest in AV+CW and lowest in the control where none of the cuttings produced root. However, the AV treatment did not differ with either the control or CW for rooting. Just as was the case with AV+CW, differences in rooting (%) between IBA and CW were not statistically significant (Table-2). The superiority of IBA in root induction has been reported previously. In rose (*Rosa* spp.), for instance, semi-hardwood cuttings treated with the hormone exhibited the shortest time lapse to root induction and the highest rooting in comparison to IAA and an untreated control[19]. Ibrahim *et al.*[20] also found significant effects of IBA alone or in interaction with branch type in favour of rooting of lemon verbena (*Lippia citriodora*) cuttings. Reason for IBA superiority. The root initiation by IBA is explained by its role in the mobilization of food reserves and differentiation of cambial initials into root primordial[21]. The finding that rooting in CW was comparable to that in the control is reflective of the absence of IBA in the plant extract as assayed by Tan and co-workers[22]. On the other hand, the potency of the AV+CW treatment in augmenting rooting is attributable to the presence of Indole-3-Acetic Acid (IAA) in the plant extracts whose combination must have led to an appreciable amount of the hormone. The IAA induces cell division in the pericycle in favor of lateral and adventitious roots formation. Other phytohormones present in the plant extracts are Gibberellic Acid (GA) and Abscisic Acid (ABA) in AV[23] and cytokinins, GA, ABA and Zeatin in CW[22,24]. Additionally, the cytokinins and auxin may stimulate the development of callus in the root formation pathway.

In contrast to IBA that kept primary root production at a minimum to a comparable extent as AW+CW, treatment with AV resulted in the highest number. The CW pre-planting treatment expressed a statistically similar number of roots as AV and AV+CW (Table-2). As for number of secondary roots, it increased from IBA to the AV, CW, and AV+CW treatments which did not differ among each other for this trait (Table-2). Such positive main effects of the plant extracts on roots count have been documented earlier by Dunsin[25] and El Sherif[23]. Number of root primordia may be elevated by exposure to exogenous auxin, leading to an increase in number of roots[26].

Root growth: Root length declined from the individual plant extracts and their combination to IBA. On the other hand, there were no significant differences between the former treatments for the parameter (Table-3). On its part, there was an incremental trend in root fresh weight from IBA to AV+CW. While AV did not differ with either IBA or CW, neither the AV nor AV+CW was significantly different from CW (Table-3). AV and CW are loaded with growth promoting factors like proteins, vitamins, sugars, and minerals that place them at an edge over IBA in driving growth[23,27,28]. The larger root size due to treatment with the plant extracts may enhance the uptake of nutrients and water for survival and growth of the cuttings in the field.

Conclusion

Treatment with AV and CW is a necessity for initiating roots in semi-hardwood cuttings of *Vitex diversifolia.* Individually, the plant extracts can increase roots per cutting while in combination they can take number of rooted cuttings to values above either untreated cuttings or those treated with IBA. Furthermore, the plant extracts are preferred substances for achieving greater initial root growth after initiation.

Table-2: Effect of rooting substances on root production on *Vitex diversifolia* semi-hardwood cuttings.

Parameter	Control	IBA	AV	CW	AV+CW	Mean ± SE
Rooting (%)	0 ± 0.00^{c}	66.67 ± 16.67^{ab}	33.33 ± 0.00bb^{c}	50 ± 0.00^{b}	83.33 ± 16.67^{a}	46.67 ± 0.67
# ofpri. roots	-	1 ± 0.00^{c}	3.5 ± 1.19^{a}	3 ± 0.91^{ab}	1.5 ± 0.29^{bc}	2.25 ± 0.60
# of sec. roots	-	19.5 ± 1.77^{b}	60.5 ± 4.95^{a}	65.5 ± 4.60^{a}	58 ± 12.73^{a}	50.88 ± 6.01
Mean ± SE	0 ± 0.00	29.06 ± 6.15	32.44 ±2.05	39.5 ± 1.84	47.61 ± 9.90	29.72 ± 4.43

IBA = Indole-3-Bytyric Acid; AV = aloe vera gel; CW = coconut water; # of pri. roots = number of primary roots; # of sec. roots = number of secondary roots. Different letters indicate statistically significant differences.

Table-3: Effects of rooting substances on root growth *Vitex diversifolia* semi-hardwood cuttings.

Parameter	Control	IBA	AV	CW	AV+CW	Mean ± SE
Root length	-	7 ± 1.41^{b}	14.45 ± 2.90^{a}	14.75 ± 2.83^{a}	16.6 ± 4.45^{a}	13.2 ± 2.90
Root diameter	-	2 ± 0.00^{a}	1.75 ± 0.25^{a}	2.25 ± 0.48^{a}	2 ± 0.00^{a}	2 ± 0.18
Root fresh weight	-	0.07 ± 0.00^{c}	0.39 ± 0.04^{bc}	0.8 ± 0.34^{ab}	1.14 ± 0.20^{a}	0.6 ± 0.15
Mean ± SE	-	3.02 ± 0.47	5.53 ± 1.06	5.93 ± 1.22	6.58 ± 1.55	5.27 ± 1.08

IBA = Indole-3-Bytyric Acid; AV = aloe vera gel; CW = coconut water; # of pri. roots = number of primary roots; # of sec. roots = number of secondary roots. Different letters indicate statistically significant differences.

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