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Vegetative Propagation of *Vitex doniana* Sweet from Root Segments Cuttings: Effects of Substrate and Length of Cuttings on the Rooting Ability

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ABSTRACT

In the Guinea savannah highlands of Cameroon, Vitex doniana (Verbenaceae) belongs to the category of tree species which in the wild provide important non-timber forest products to populations. The present study is designed to assess the effects of substrate and length of cuttings on the rooting ability. The root system of 15 genotypes was partially excavated to a depth of 20 cm. Root Segments Cuttings (RSC) of 10, 15 and 20 cm long were carefully cut and arranged vertically in a non-mist propagator, in three different substrates (sand, sawdust and black soil-sawdust). The experimental design used was a split-plot with three replications. The main treatment consisted of three substrates. The sub-treatment was represented by three lengths of RSC. The experimental unit was constituted of 10 cuttings. The best rate of budding (51.52 ± 18.37%) was obtained in the mixture black soil-sawdust while for the rate of rooting (55.55 ± 16.66%), it was in sand. However, there was no significant difference between substrates for this parameter. Concerning the length of cuttings, the rate of budding ranged significantly from 33.19 ± 18.37% in RSC of 15 cm to 66.94 ± 15.38% for those of 20 cm long (0.01<0.05). For the rooting, the rate of rooting oscillated between 31.10 ± 13.98% in RSC of 10 cm and 54.44 ± 13.89% in those of 15 cm. The maximum number of leafy shoots (2.66 ± 0.69 cm) and of roots (5.35 ± 1.37 cm) was developed on RSC of 20 cm long and 15 cm respectively. The longest roots were registered in sawdust (6.63 ± 1.01 cm). This information is important in developing appropriate strategies toward domestication of the species.

Keywords: Cameroon, Vitex doniana, Vegetative Propagation, Domestication, Rooting ability, Root segments cuttings

INTRODUCTION

In the tropics, non-timber forest products provided by species present socio-economic stakes and the life of numerous populations are directly linked [1]. The great utility of the species can become harmful for it. Although, it can be an asset in favor of its promotion and the acquaintances of the establishment modality of germplasm development can help in the choice of the appropriate regeneration method and management of the species. *Vitex doniana* Sweet is among the most socio-economic important tree species of the Guinean Savannah Highlands, common in tropical Africa [2,3]. Vegetative propagation of trees remains understudied in Africa in general and in northern Cameroon in particular. Such methods however provide potential for producing trees and shrubs with high social and economic value. *V. doniana* is a species that emits suckers [4-7] and, as many suckering species, it seems easy to obtain clones of selected *V. doniana* [5] through Root Segments Cuttings (RSC). To promote a sustainable domestication of species in developing countries, this extremely cheap and easy method can allows rural people to maintain in their field or near their home a copy of an exceptional tree that fits with their standards [8]. Despite this importance and benefits provided by *V. doniana*, its domestication has long been poorly studied in the area, comparing with other species [9-11].The current work is designed to characterize the rooting media and the length of cuttings. Therefore, the

purpose of the present study was to assess the effects of rooting media and length of cutting which are among the key factors which influence the rooting ability.

MATERIALS AND METHODS

Study area

Root segments used in the study were sampled in Guinean Savannah Highlands of Adamawa. This region is characterized by a Guinean climate, with two seasons: a rainy season from April to October and a dry season from November to March. For the period 2000-2012, the average annual rainfall was 1447 mm, the average annual temperature 22.3°C, the average annual relative humidity 67% and the average annual evaporation 1645 mm. The region is delimited by two boundaries: The Sudanese savannas in the north and the semi-deciduous Guinean vegetation in the south. The area is covered with shrubland and/or woody savannah dominated by *Daniellia oliveri* and *Lophira lanceolata* [12]. The evolution of the vegetation is severely hampered by human impacts [13]. The soil of the area is rich in ferruginous compounds from granites and gneiss [14].

Preparation and cultivation of root segments cuttings

The experiment was conducted at the nursery of the University of Ngaoundere during 25 weeks, from January to November 2015. Plant material used in the experiment was collected from superficial roots of 15 adult *V. doniana* trees of dbh>50 cm. These genotypes were selected in the Bini-Dang upper Guinean savannahs (altitude 1081 m, 13°33'130" East, 7°25'127" North). Preferred characteristics of local populations were considered in the selection of the parent trees: good sanitary condition, large fruits, regular production and low branching. After partial excavation to a depth of 20 cm, the root segments of 1.1-2.5 cm diameter were collected using a pruner, then wrapped in a moistened newsprint paper and transported in a cooler to the nursery. Upon arrival at the nursery on the same day, the root segments were cut into three size classes 10, 15 and 20 cm long Root Segments Cuttings (RSC); then inserted in rooting media vertically. A one cm notch was made at the distal end of each RSC [15].

A total of 270 RSC were obtained and arranged vertically in substrate in a non-mist propagator [16]. A non-mist propagator is a wooden frame enclosed in a single sheet of polythene such that the base is completely water tight. The frame was covered tightly with single piece polythene and a closely-fitting lid. It was 3 m long versus 1 m wide and 1m height at the back and 0.5 m in the main façade. The polypropagator was divided in three equal compartments thus corresponding to three replications. The polythene base of the propagator was covered with a thin layer of sand to protect the polythene and large stones were placed on top of the sand to a depth of 10-15 cm. This was then covered by successive layers of small stones and gravel to a total depth of 20 cm. The spaces between stones and gravel were filled with water. The saturated layers of stones and gravel were covered by three rooting media: Saw Dust (Sd), Sand (Sa) and 50/50% homogeneous mixture of black soil-sawdust (Bs-Sd). The rooting media remained moist by capillarity and could be dampened from the above as necessary. This resulted in a permanently humid environment throughout the propagation period. The RSC were watered twice a day (morning and evening) using a hand sprayer. An open cylinder made of PVC pipe was inserted vertically into the medium and stones. This pipe was used as the filling point for the water and allowed a regular check of the water table. Temperature within the polypropagator was 28-30°C while the humidity was around 85-90% after watering. Starting from the end of the eighth week, corresponding to the first appearance of leafy shoots, a systematic inventory was conducted weekly to assess the number of RSC that emit one or more leafy shoots, the number of leafy shoots and the number of leaves per shoot. Regarding rooting, the RSC with one or more leafy shoots were meticulously excavated each week and the presence of new roots noted. Un-rooted cuttings were reintroduced into the substrate. A root segment cutting with at least one leaf is considered budded. A cutting was considered as rooted if the length of the root was greater than 1 cm [9]. The standard RSC diameter was chosen based on previous study on the same species [10]. Rooted cuttings were inserted vertically in large perforated polyethylene bags (27 cm diameter × 40 cm height) containing the black soil-sawdust which was found performant. These bags were introduced in acclimatization propagators and watered mornings and evenings. During the acclimatization phase, the propagators were left open each night during a month. Watering was then reduced to once a day. After this acclimatization phase, the plants were transferred to the field.

Experimental design and data analysis

The experimental design was a split-plot design with three replications. The substrate was the main treatment with three modalities, while the length with three classes represented the secondary treatment. The experimental unit consisted of 10 cuttings and the 15 genotypes were not tested separately. The following parameters were determined:

a) The rate of budding, the number of leafy shoots per RSC, the height and number of leaves of each leafy shoot. Budding corresponds to bud burst of latent buds that emit one or more leafy shoots on the RSC.

b) The rate of rooting (equated to the success rate of the RSC), the pole on which the leafy shoots have developed (distal or proximal pole), the number and length of the roots. The distal pole corresponds to the extremity of the RSC which was originally located farthest from the base of the parent tree. For this part, these data were collected at the end of the experiment (25 weeks).

Quantitative data were subjected to an analysis of variance (ANOVA) and Post-hoc comparisons were done with the Duncan's Multiple Range Test (DMRT) when significant main effects were detected. All the analyses have been performed using the Statgraphics 5.0 software.

RESULTS

Effect of the substrate and the length of RSC on the leafy shoot formation

Rate of budding: The first leafy shoots were observed in all RSC length and substrate, 6 weeks after planting. By the end of the experiment (25 weeks), the rate of budding varies from 45.56 ± 12.89 both in sand and sawdust to 55.21 ± 18.37 % in black soil-sawdust mixture (Figure 1). Nevertheless, there was no significant difference between substrates (0.90>0.05).

Concerning the length of the RSC, the rate of budding ranged from 33.19 ± 18.37% in RSC of 15 cm to 66.94 ± 15.38% for those of 20 cm long (Figure 2). In contrast to substrate, there was a significant difference between RSC length (0.01<0.05).

For the substrate *RSC length interaction, the rate of budding oscillated between 29.16 ± 20.38% in RSC of 15 cm cultivated in sand and 80.33 ± 20.38% in those of 20 cm planted in Black soil-sawdust, however this interaction was not significant (0.79>0.05).

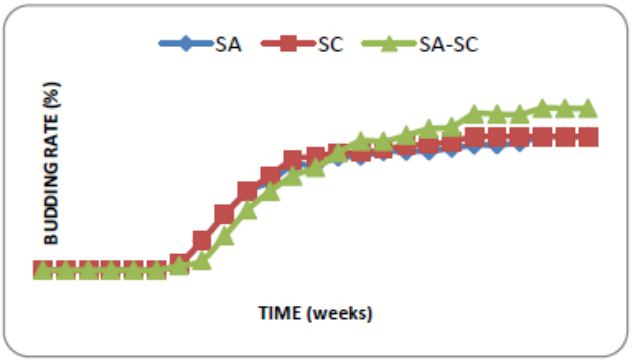

Figure 1: Rate of budding of *Vitex doniana* according the substrate.

Average number of leafy shoots per RSC: The average number of leafy shoot at the end of the experiment varied from 1.94 ± 0.88 in black soil-sawdust to 2.64 ± 0.66 in sawdust. Meanwhile there was no significant difference among substrates (0.17>0.05).

For the RSC length, the average number of leafy shoot oscillated between 1.94 ± 0.64 in RSC of 10 cm 2.66 ± 0.69 for those of 20 cm. However, there was no significant difference between RSC length (0.11>0.05).

The substrate by RSC length interaction was equally not significant (0.07>0.05). The average number of leafy shoots ranged from 1.34 ± 1.15 in RSC of 15 cm planted in Black soil-sawdust mixture to 3.64 ± 0.72 in RSC of the same length planted in sawdust.

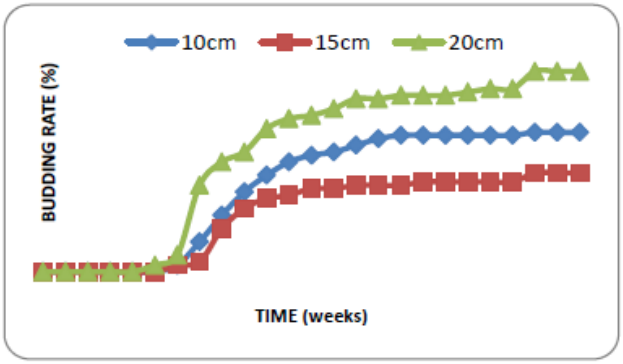

Figure 2: Length of root segments cuttings.

Height of the leafy shoots and average number of leaves per leafy shoot: by the week 25, the average height of the leafy shoots ranged from10.00 ± 2.66 cm in black soil-sawdust to 11.75 ± 1.99cm in sawdust (Table 1). There was a significant difference between substrates (0.03<0.05).

For the RSC length, the height of the leafy shoots varied from 7.92 ± 1.9 cm in RSC of 10 cm to 16.03 ± 2.10 cm in those of 20 cm long (Table 1). The RSC length affected significantly the height of the leafy shoots (0.0001< 0.001).

Concerning the substrate by RSC length interaction, the average height of the leafy shoots oscillated between 5.93 ± 1.89cm in RSC of 10 cm long cultivated in black soil-sawdust and 17.05 ± 1.86 cm in those of 20 cm in sawdust (Table 1). This interaction affected significantly the height of the leafy shoots (0.01<0.001).

The average number of leaves per leafy shoot varied significantly (0.014<0.05) from 10.00 ± 4.00 in sand to 20.00 ± 6.00 in sawdust (Table 1).

In contrast to substrate, the average number of leaves ranged from 11.00 ± 5.00 in RSC of 10 cm to 17.00 ± 6.00 in those of 20 cm. However there was no significant difference between the RSC length (0.20>0.05).

The interaction substrate-RSC length was significant on the number of leafy shoots (0.002<0.01). The average number of leaves varied from 3.00 ± 2.00 in RSC of 15 cm in black soil –sawdust mixture to 30.00 ± 6.00 in RSC of the same length in sawdust (Table 1).

Table 1: Growth Characteristics of RSC plants in the nursery after 25 weeks.

Treatments	Number of leafy shoots				Height of the leafy shoots (cm)				Number of leaves per leafy shoot			
	10 cm	15 cm	20 cm	Mean	10 cm	15 cm	20 cm	Mean	10 cm	15 cm	20 cm	Mean
Sand	2.42 ± 0.61	1.,83 ± 1.06	2.62 ± 0.60	2.3 ± 0.75	11.47 ± 1.86ab	8.29 ± 3.19c	14.70 ± 1.82a	11.48 ± 2.29	17.00 ± 5.00a	6.00 ± 2.00b	13.00 ± 5.00ab	10.00 ± 4.00
Sawdust	1.77 ± 0.65	3.64 ± 0.72	2.52 ± 0.61	2.64 ± 0.66	6.36 ± 1.95b	11.85 ± 2.16ab	17.05 ± 1.86a	11.75 ± 1.99	8.00 ± 6.00c	30.00 ± 6.00a	20.00 ± 5.00a	20.00 ± 6.00
Soil/ Sawdust	1.65 ± 0.62	1.34 ± 1.15	2.84 ± 0.87	1.94 ± 0.88	5.93 ± 1.89b	7.72 ± 3.47c	16.36 ± 2.64a	10.00 ± 2.66	9.00 ± 5.00c	3.00 ± 2.00b	20.00 ± 8.00a	11.00 ± 5.00
Mean	1.94 ± 0.62	2.27 ± 0.97	2.66 ± 0.69	2.29 ± 0.76	7.92 ± 1.9	9.28 ± 2.94	16.03 ± 2.10	11.07 ± 2.31	11.00 ± 5.00	13.00 ± 4.00	17.00 ± 6.00	13.00 ± 5.00

Both the maximum average height (17.05 ± 1.86 cm) of the leafy shoots and the maximum number of leaves per leafy shoot (20.00 ± 6.00) were reached in RSC of 20 cm long in the sawdust.

Location of the leafy shoots formation: The leafy shoots were developed at two locations on the RSC inserted vertically: 85.5% on the proximal pole and 14.5% in the middle.

Effect of the substrate and the length of RSC on root formation

Rate of rooting: The rooting rate of the RSC varied from 35.55 ± 17.13% in black soil-sawdust to 55.55 ± 16.66 % in sawdust by the end of the experiment (25 weeks). However there was no significant difference between substrates (0.46>0.05).

For the RSC length, the rooting rate ranged from 31.10 ± 13.98% in RSC of 10 cm to 54.44 ± 13.89% in those of 15 cm. There was no significant difference among the cutting lengths (0.08>0.05).

Table 2: Growth characteristics of roots in the nursery after 25 weeks.

Treatments	Rate of rooting (%)				Number of roots				Length of the roots (cm)			
	10 cm	15 cm	20 cm	Mean	10 cm	15 cm	20 cm	Mean	10 cm	15 cm	20 cm	Mean
Sand	46.66 ± 13.98	56.66 ± 13.98	63.33 ± 22.11	55.55 ± 16.69	3.86 ± 0.84	5.63 ± 1.57	2.75 ± 0.83	4.08 ± 1.08	6.01 ± 0.90	6.73 ± 1,67	4.33 ± 0.88	5.69 ± 1.15
Sawdust	26.66 ± 13.98	46.66 ± 13.98	48.33 ± 23.71	40.55 ± 17.22	4.14 ± 0.94	5.55 ± 1.04	4.48 ± 0.88	4.72 ± 0.95	6.39 ± 1.00	7.28 ± 1.10	6,22 ± 0.93	6.63 ± 1.01
Soil/ Sawdust	20.0 ± 13.98	60.0 ± 13.71	26.66 ± 23.71	35.55 ± 17.13	2.67 ± 1.03	4.88 ± 1,50	3.80 ± 0.80	3.78 ± 1.11	4.85 ± 1.10	5.92 ± 1.59	5.24 ± 0.86	5.33 ± 1.18
Mean	31.10 ± 13.98	54.44 ± 13.89	46.10 ± 23.17	43.88 ± 17.01	3.56 ± 0.93	5.35 ± 1.37	3.67 ± 0.83	4.19 ± 1.04	5.75 ± 1.00	6.64 ± 1.45	5.26 ± 0.89	5.88 ± 1.11

The substrate by RSC length interaction showed that the rooting rate oscillated between 20.0 ± 13.98% in RSC of 10 cm long cultivated in the mixture black soil-sawdust and 63.33 ± 22.11% in 20 cm in sawdust (Table 2). Despite the variation, the interaction was not significant (0.73>0.05).

Average number and length of RSC roots: The average number of roots varied from 3.78 ± 1.11 in black soil-sawdust to 4.72 ± 0.95 in sawdust. Despite the variation observed, there was no significant difference between substrates (0.26>0.05). For the length of the cuttings, the average number of roots ranged from 3.56 ± 0.93 in RSC of 10 cm to 5.35 ± 1.37 for those of 15 cm.

Concerning the length of RSC, the average number of roots oscillated between 3.56 ± 0.93 in RSC of 10 cm and 5.35 ± 1.37 for those of 15 cm.

For the interaction substrate-RSC length, the average number of roots ranged from 2.75 ± 0.83 for RSC of 20 cm long inserted in sand to 5.55 ± 1.04 in RSC of 15 cm cultivated in sawdust (Table 2). The effect of substrate was not significant on the number of roots (0.26>0.05), nor the one of RSC length (0.65>0.05) and their interaction (0.50>0.05).

Roots of shorter length (5.33 ± 1.18 cm) were observed in the black soil-sawdust while the longest roots developed in the sawdust (6.63 ± 1.01 cm). There was a significant difference between substrates (0.007<0.01).

On the other hand, the length of the RSC had no significant effect (0.58>0.05) on root length. The average length of the roots ranged from 5.26 ± 0.89 cm in RSC of 20 cm to 6.64 ± 1.45 cm for those of 15 cm.

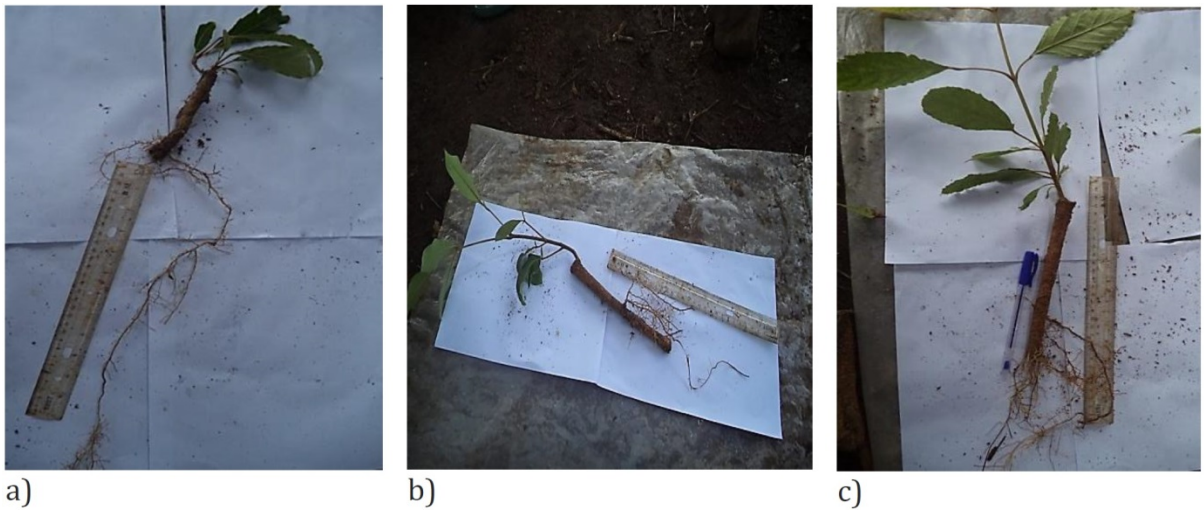

Figure 3: RSC of different lengths rooted: 10 cm (a), 15 cm (b) and 20 cm (c).

The substrate by RSC length interaction did not affect the length of the roots (0.63>0.05). Nevertheless, the average length of the roots varied from 4.85 ± 1.10 cm in RSC of 10 cm cultivated in black soil-sawdust mixture to 7.28 ± 1.10 cm for RSC of 15 cm planted in sawdust. Roots appeared in distal pole (95.75%) and middle section (4.05%) of the RSC (Figure 3).

DISCUSSION

Recently, there has been increased realization of the need to domesticate indigenous tree species, around the tropics, and to develop the appropriate methods and techniques to capture and select genetic variation in these species [17]. In facing woody species' domestication and conservation challenges one should consider the potential of vegetative propagation, and particularly the root segments cuttings method, which is very cheap to carry out, easy to handle and quite successful [18,19]. Such methods however provide potential for producing trees and shrubs with high social and economic value. *Vitex doniana* belongs to this category of species in Africa [9]. However vegetative propagation of these trees remains understudied in Africa.

In this experiment, leafy shoots were developed after six weeks compared to eight in previous works [10]. The precocity observed may be attributed to the genotype properties of parent trees from which RSC were harvested. Like in previous works, budding and rooting of the RSC are separated in time. The separation of these two phenomena allows leafy shoots to supply adventitious roots with carbohydrates. This separation imposes to cuttings the use in priority their resources for the development of the leafy stem. This explains why long RSC were rich in resources and produced more buds, more leafy shoots and consequently more roots. After development, young leaves will produce by photosynthesis, auxins and carbohydrates which will be transferred via elaborate sap in the basal part of the RSC to induce rhizogenesis. The height of leafy shoots and length of roots were significantly improved in sawdust. This substrate by its porosity and lightness allowed the growth of shoots and roots. It permitted good circulation of water and oxygen in the medium. This capacity has been reported in others species [9]. RSC length affected significantly the rate of budding. Similar results were reported in Senegal in *Lawsonia inermis* [20], in Benin in *Daniellia oliveri* [21] and Burkina Faso in *Detarium microcarpum* [15]. These authors mentioned that RSC of 15 and 20 cm long were more efficient on the growth of cuttings (budding rate, height) than those of 10 cm which were unsuitable due to poor sprouting ability. This is in agreement with a number of previous studies, in which a similar range of lengths has resulted in successful sprouting. For *F. albida* and *Spathodea campanulata*, *Detarium microcarpum* [15,22], and successfully used 15-20 cm long segments of RSC while for *M. crassifolia*, 10 cm long root long root segments exhibited the best sprouting efficiency [23]. In this experiment, the substrate influenced significantly the height of leafy shoots as well as length of roots. In line with our experiment, it was reported that sand-ferruginous soil affected the growth of RSC of *Daniellia oliveri* [21]. Master the regeneration of a species by vegetative propagation is an important step toward its domestication. Vegetative propagation permits to select individuals for which genetic characters are known, and multiply the material in a great scale and conserve their characters [24,25].

CONCLUSION

This study demonstrated that *V. doniana* could be very easily propagated from RSC harvested in January (i.e., near the middle of the dry season). The length of the RSC is an important factor that influences the formation of leafy shoots and adventitious roots.

For future work, it is envisaged to evaluate the effects of (i) the harvest season of the RSC (beginning of the dry season *vs.* end of the dry season), (ii) the effect of mycorhiza, (iii) the effect of the horizontal vs. vertical or slant positioning of RSC in the substrate, on the ability to form new leafy shoots and a dense root system, and (iv) determine the phytochemical substances involved in the process of caulogenesis and rhizogenesis of RSC.

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