Vegetative propagation of *Vitex doniana* Sweet (*Verbenaceae*) by root segments cuttings: effects of mother tree diameter and sampling distance of cuttings

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Abstract: The Guinea Savannah Highlands harbor numerous plant species of great socio-economic importance exploited by farmers in the wild. Sweet belongs to these multifunctional tree species. Despite its importance in rural back yard, little scientific research is devoted to it. The main purpose of this work is to contribute to its domestication through root segments cuttings. The assessment of the effect of tree diameter as well as the sampling distance of root segments cuttings (RSC) from them was undertaken in the nursery. The root system of 45 trees was partially excavated to a depth of 20 cm. RSC of 20 cm long were carefully cut and arranged vertically in a non-mist propagator, in black soil-sawdust substrate. The experimental design was a split-plot with three replications. The tree diameter used was the main treatment while the sampling distance was the sub-treatment. The experimental unit was 10 cuttings. The results showed that the high rate of budding of RSC class ($84.44 \pm 14.69\%$), was obtained from trees of 5-10 cm diameter. The diameter class significantly influenced the budding rate (0.004 < 0.01) contrary to the sampling distance (0.99 > 0.05). The diameter class of trees used significantly influenced rooting (0.02 < 0.05). For the interaction dbh*sampling distance of the tree, the rooting rate varied from $53.33 \pm 5.97\%$ in RSC collected at 25 cm from trees of 10.1-20 cm dbh to 96.66±5.97% in those harvested at 100 cm from trees with 5-10cm dbh.

Keywords: domestication, Vitex doniana, *vegetative propagation, root segments cuttings, tree diameter, sampling distance*

Introduction

The Guinean Savannah Highlands (GSH) harbor numerous tree species that have traditionally provided people's needs for the full-range of non-timber forest products (NTFP). Sweet. belongs to these categories of tree species (Mapongmetsem *et al.*, 2008). Known as African savannahs' black plum, it is widespread in tropical Africa, commonly found from Senegal to Cameroon and Somalia as well as in Central Africa. It is of great socio-economic interest (Wala *et al.*, 2009; Oladele, 2011) mainly for its

extensively documented food, medicinal and handicraft properties (Mapongmetsem *et al.*, 2012a).

The high utilization pressure on the species combined with the low regeneration ability cause a steady depletion of natural populations while no clear and straight forward conservation strategies have been developed neither at community level nor at national level. Yet, farmers attempted regeneration of by seeds but faced constraints related to seed dormancy and long growing period, which resulted overall in very poor results (Thies, 1995; Sanoussi et al., 2012). Other methods of regeneration must be explored in order to propose appropriate solutions for the conservation of the tree species, highly demanded by local populations, and to allow its reintroduction into farmers' production systems (Mapongmetsem et al., 2012a; Meunier et al., 2006). Despite the importance of the species in Africa, scientific studies on its regeneration are scarce, except those of Fawa (2015), Mapongmetsem et al. (2012b), Mapongmetsem et al. (2016) and Sanoussi et al. (2012) related to effects of substrates and RSC diameter. is a species that emits suckers (Thies, 1995; Bellefontaine, 2005; Oumorou et al., 2010; Fawa, 2015) and, as many suckering species, it seems easy to obtain clones of selected (Bellefontaine, 2005) through root segments cuttings (RSC). To promote a sustainable domestication of the species in developing countries, this extremely cheap and easy method allows rural people to maintain in their field or near their home a copy of an exceptional tree that fits with their standards (Meunier et al., 2006). This technique is currently the most suitable tool to propagate elite trees with advantageous phenotypic and genotypic characters (Nsibi et al., 2003; Stenvall et al., 2005 and 2006).

The main purpose of this study was to determine factors which affect the success of clonal propagation of the Black Plum by root segments cuttings. The major problems in operational and research uses of vegetative propagules relate to the effect of the age and location of the propagule from the mother tree and its ability to grow as a tree. The lack of data on these factors is a major handicap for the promotion of this technique. Here it is hypothesized that mother tree diameter and sampling distance of RSC from it are among the key factors that affect the regeneration capacity of the material and the overall ability to grow.

Materials and methods

Study area

Root segments used in the study were sampled in Guinean Savannah Highlands of Adamawa region. 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 2000-2015, the average annual rainfall was 1447.75 mm, the average annual temperature 22.3 °C, the average annual relative humidity 67.5 % and the average annual evaporation 1645 mm. The region is delimited by two boundaries: the Sudanese savannahs in the north and the semi-deciduous Guinean vegetation in the south. The area is covered with shrubland and/or woody tree savannah dominated by and (Letouzey, 1968). The evolution of the vegetation is severely hampered by *Daniellia oliveri* and *Lophira lanceolata* human impacts (Ibrahima *et al.*, 2006).

Preparation and cultivation of root segments cuttings

The experiment was conducted at the nursery of the University of Ngaoundere (Cameroon) during 20 weeks, from July 7 to December 15, 2015. Plant material used in the experiment comes from superficial roots of 45 adult trees of dbh> 5 cm. These trees were selected in the Bini-Dang Guinean Savannah Highlands (Alt.: 1081 m, LE: $13^{\circ}33'130$ ° E, LN: $7^{\circ}25'127$ ° N). Preferred characteristics of local populations were considered in the selection of the trees used: good sanitary condition, large fruits, regular production and low branching. After partial excavation of the root system to a depth of 20 cm, the root segments were collected using a pruner from trees of different diameters at breast height (dbh) at three different distances from them. The root segments cuttings (RSC) of 20 cm long and 1.1-2.5 cm diameter were 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 cuttings were inserted vertically in rooting medium (mixture black soil/sawdust 50/50%). A 1 cm notch was made at the distal end of each RSC (Ky-Dembélé *et al.*, 2010).

A total of 270 RSC were obtained and arranged within 3 cm of substrate in a non-mist propagator (Leakey et al., 1990). 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 a single piece polythene and a closely-fitting lid. It was 3 m long versus 1m 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 a rooting medium composed with 50/50 % homogeneous mixtures of black soil/sawdust (Bs-Sd). The rooting medium 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. Hygrothermometer was used by the way. Starting from the seventh 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 leafy shoots or not were meticulously excavated at the end of the experiment (20th week) and the presence of new roots noted. Unrooted cuttings were reintroduced into the substrate. A cutting was considered as rooted if the length of the root was greater than 1 cm (Mapongmetsem et al., 2012b). 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 during preliminary investigations. 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 open field.

Experimental design and data analysis

The experimental design was a split-plot design with three replicates. The mother tree dbh was the main treatment with three classes (5-10 cm, 10.1-20 cm and > 20 cm), while the sampling distance with three modalities (25 cm, 50 cm and 100 cm) represented the sub-treatment. The experimental unit consisted of 10 cuttings and the 45 trees were not tested separately and distributed randomly among treatments. The standard rooting medium, RSC diameter and its length were chosen based on previous work done on the same species (Mapongmetsem *et al.*, 2016). 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, proximal, median, etc.), the number and length of the roots. The distal pole contrary to proximal corresponds to the extremity of the RSC which was originally located farthest from the base of the mother tree. These data have been collected at the end of the experiment (20th week).

Quantitative data were subjected to an analysis of variance (ANOVA) and Posthoc 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.



Figure 1 - Rate of budding of Vitex doniana according the mother tree diameter (a) and sampling distance of Root segments cuttings (b)

Results

Effect of the mother tree diameter and the sampling distance of RSC on leafy shoots formation

Rate of budding

The first leafy shoots were observed at the beginning of the 7th week for cuttings of the entire diameter at breast height (dbh) class and sampling distance modalities of RSC collected. The rate of budding at the end of the essay (20th week), varied from 51.11 ± 14.69 % in cuttings from trees of dbh equal to 10.1-20 cm and 95.11 ± 14.69 %

in those belonging to 5-10 cm dbh (Fig. 1a). A significant difference was registered (0.004 < 0.01).

In contrast to dbh of the plants, the sampling distance of the cuttings from the trees had no significant effect on the budding rate (0.99>0.05). At the end of the experiment (20^{th} week), the budding rate was 68.89 ± 14.69 % for the RSC harvested at the distance of 25 cm from the foot of trees and 83.33 ± 14.69 % for those harvested at 100 cm of them (Fig. 1b).

Concerning the interaction dbh*sampling distance of RSC, no significant difference was detected (0.72>0.05). The budding rate oscillated between 50.0 ± 14.69 % from RSC harvested from trees of 5-10 cm dbh for the entire class distance and 86.66 ± 14.69 % for those collected at 100 cm distance of the trees of 10.1-20 cm dbh.

Average number of leafy shoots per RSC

By the end of the experiment (after 20 weeks), the average number of leafy shoots ranged from 2.22 ± 0.34 for RSC from trees of 5-10 cm dbh to 3.59 ± 0.38 for those from trees of dbh more than 20 cm (Table 1). The number of leafy shoots/RSC was significantly different between RSC (0.0004<0.001). Trees with large diameter induced important number of leafy shoots on RSC.

For the sampling distance of RSC, the number of leafy shoots varied from 2.26 \pm 0.41 for cuttings harvested at 50 cm from the trees to 3.78 \pm 0.51 for those collected at 25 cm of trees (Table 1). There was a significant difference between the sampling distance of RSC (0.0031 < 0.01).

The interaction dbh*sampling distance of collection of RSC showed that at the end of experiment, the number of leafy shoots varied from 1.45 ± 0.41 in RSC collected at 50 cm from trees with 10.1-20 cm dbh to 5.80 ± 0.84 in those harvested at 25 cm of those with the same diameter as previously (Table 1). The sapling distance by diameter interaction affected significantly the number of leafy shoots (0.0005 < 0.001).

Height of the leafy shoots and average number of leaves per leafy shoot

At the end of the experiment 20^{th} week, the average height of leafy shoots ranged from (5.74±0.73 cm) in RSC from trees with 5-10 cm dbh to 7.42±1.23 cm in cuttings from trees having 10.1-20 cm dbh (Table 1). Despite the observed variability, the dbh of trees had no significant effect on the height of leafy shoots (0.16>0.05).

The average height of leafy shoots ranged from 4.94 ± 0.89 cm in cuttings collected at 50 cm from the trees to 7.58 ± 1.11 cm in those harvested at 25 cm from it (Table 1). However, there is no significant difference between RSC concerning their sampling distance from tree (0.30>0.05).

For the sampling distance by dbh interaction, the height of the leafy shoots ranged from 3.01 ± 1.05 cm in RSC harvested at 50 cm from the trees with dbh more than

TREATMENTS -	NUMBER OF LEAFY SHOOTS				HEIGHT OF THE LEAFY SHOOTS (cm)				NUMBER OF LEAVES PER LEAFY SHOOT				
	25 cm	50 cm	100 cm	Mean	25 cm	50 cm	100 cm	Mean	25 cm	50 cm	100 cm	Mean	
5-10 cm	1.84±0.35c	2.14±0.34c	2.68±3.34b	2.22±0.34b	6.82±0.76c	5.77±0.73ab	4.75±0.73b	5.74±0.73a	7.74±1.34b	9.87±1.29bc	11.28±1.29b	9.63±1.30a	
10.1-20 cm	5.89±0.84a	1.45±0.41b	3.05±0.45ab	3.46±0.56ab	9.42±1.82a	6.06±0.90ab	6.78±0.98c	7.42±1.23a	27.02±3.19a	6.38±1.58c	11.44±1.73b	14.94±2.16a	
>20 cm	3.63±0.34b	3.19±0.48ab	3.98±0.33a	3.59±0.38a	6.51±0.75c	3.01±1.05b	8.76±0.72a	6.09±0.81a	12.40±1.31b	8.63±1.85bc	11.68±1.27b	10.90±1.43a	
Mean	3.78±0.51a	2.26±0.41c	3.23±0.37ab	3.09±0.42	7.58±1.11a	4.94±0.89a	6.76±0.81a	6.41±0.92	15.72±1.94a	8.29±1.57c	11.46±1.43b	11.82±1.63	

Table 1 - Growth Characteristics of RSC plants in the nursery after 20 weeks

Means followed by the same letter are not statistically different

20 cm to 9.42 ± 1.82 cm in those collected at 25 cm from the one with dbh 10.1-20 cm (Table 1). The interaction affected significantly the height of the leafy shoots (0.0007<0.001).

The average number of leaves per leafy shoot varied from 9.63 ± 1.30 in RSC collected from trees of 5-10 cm dbh to 14.94 ± 2.16 in those harvested from trees with 10.1-20 cm dbh (Table 1). However, there is no significant difference between RSC concerning the number of leaves/picked up shoot (0.20 >0.05).

In contrast to the diameter at the breast height of the trees, the sampling distance of RSC had a significant effect (0.01 < 0.05) on leaves formation. The average number of leaves/picked up varied from 8.29 ± 1.57 in leafy shoots of RSC harvested at 50 cm from the trees to 15.72 ± 2.16 in those collected at 25 cm (Table 1). The sampling distance impacted the average number of leaves/picked shoot.

For the sampling distance*dbh interaction, the average number of leaves per leafy shoot varied from 6.38 ± 1.58 in cuttings harvested at 50 cm from trees of 10.1-20 cm dbh to 27.02 ± 3.19 in those collected at 25cm from the one with the same diameter as previously (Table 1). This interaction was significant (0.0000<0.001).

Effect of the mother tree diameter and the sampling distance of RSC on roots formation

Rate of rooting

The rooting rate of RSC varied significantly (0.02<0.05) from 69.41 ± 6.89 % for RSC harvested from trees of 10.1-20 cm dbh to 86.66 ± 5.76 % in those from trees of 5-10 cm dbh (Table 2). The RSC have often developed root system with dense and long roots. Generally, roots appeared in different poles of the cuttings (distal, middle and middle-distal) with a preference for distal (Fig. 2a). Among the rooted cuttings, 1.10 % of them rooted without producing leafy shoots.

For the sampling distance of RSC, the rooting rate oscillated between 69.78 ± 6.69 % in cuttings harvested at 25 cm from trees and 90.52 ± 6.89 % in those collected at 100 cm from the trees (Table 2). Despite disparity observed between the rate of rooting, there was no significant difference between RSC (0.15 > 0.05).

The rooting rate varied from 53.33 ± 5.97 in RSC collected at 25 cm from trees of 10.1-20 cm dbh to 96.66 ± 5.97 in those harvested at 100 cm from trees with 5-10cm dbh (Table 2). Nevertheless, the interaction dbh*sampling distance was not significant (0.06 > 0.05).



Figure 2 - Formation of roots in distal (a), median (b) and median-distal (c) poles of the RSC with leafy shoot or without (d) in V. doniana

Average number and length of RSC roots

At the end of the experiment (20^{th} week) , the average number of roots per RSC varied from 6.33 ± 1.41 in RSC collected on trees with dbh more than 20 cm to 8.78 ± 1.16 in those from the one having 5-10 cm dbh (Table 2).

However there was no significant difference between RSC as far as the dbh of trees was concerned (0.096 > 0.05).

Concerning the sampling distance, the average root number ranged from 6.37 ± 1.41 in RSC harvested at 100 cm from trees to 8.58 ± 1.39 in cuttings collected at 50 cm from it (Table 2). Neither the diameter of the plant (0.096 > 0.05), nor the sampling distance of RSC (0.22 > 0.05) collected from trees significantly influenced the number of roots per root segments cuttings.

On the other hand, for the interaction dbh*sampling distance of RSC, the average root number varied significantly(0.0003<0.001) from 3.34±1.55 in cuttings collected

TREATMENTS	RATE OF ROOTING (%)					LENGTH OF THE ROOTS (cm)						
	25 cm	50 cm	100 cm	Mean	25 cm	50 cm	100 cm	Mean	25 cm	50 cm	100 cm	Mean
5-10 cm	86.66±5.36b	86.66±5.97b	86.66±5.97b	86.66±5.76a	5.08±1.18b	11.33±1.17a	9.93±1.15a	8.78±1.16a	5.66±1.22e	16.69±1.21a	8.96±1.19c	10.36±1.20a
10.1-20 cm	53.33±5.97c	60.0±5.97c	94.92±8.75a	69.41±6.89b	10.42±1.74a	5.42±1.54c	5.86±1.54b	7.23±1.41a	16.73±1.80a	6.32±1.60d	6.96±1.67d	10.00±1.69a
>20 cm	69.36±8.75b	96.66±5.97a	90.00±5.97a	85.34±6.89a	6.66±1.51c	9.00±1.48a	3.34±1.55c	6.33±1.51a	10.51±1.56b	8.08±1.53c	5.40±1.60e	7.99±1.56b
Mean	69.78±6.69a	81.10±5.97a	90.52±6.89a	80.47±6.51	7.38±1.47	8.58±1.39	6.37±1.41	7.44±1.36	10.96±1.52a	10.36±1.44a	7.10±1.48a	9.45±1.48

Table 2 - Growth characteristics of roots in the nursery after 20 weeks

Means followed by the same letter are not statistically different

at 100 cm from trees with dbh more than 20 cm to 11.33 ± 1.17 in cuttings harvested at 50 cm from the one of 5-10 cm dbh (Table 2).

Roots of shorter length $(7.99\pm1.56 \text{ cm})$ were observed in RSC harvested on trees with dbh more than 20 cm, while the longest $(10.36\pm1.20 \text{ cm})$ were developed on RSC collected from the one of 5-10 cm dbh (Table 2). However, there was no significant difference between RSC (0.35>0.05).

The sampling distance of the RSC had a significant effect (0.01 < 0.05) on root length. The average root length was 7.10 ± 1.48 cm for RSC collected at 100 cm from trees and 10.96 ± 1.52 cm for those harvested at 25 cm from that (Table 2).

For the dbh*sampling distance of RSC, the average length of the roots ranged from 5.40 ± 1.60 cm in cuttings harvested at 100cm from trees of dbh>20 cm to 16.73 ± 1.80 in those collected at 25 cm from the one with 10.1-20 cm dbh (Table 2). The dbh*sampling distance interaction had a significant effect on the length of root (0.001<0.01).

Discussion

Previous studies on the effects of rooting media, root length and diameter of showed that it can be propagated vegetatively (Mapongmetsem et al., 2012b; Sanoussi et al., 2012; Mapongmetsem et al., 2016). Budding and Rooting in cuttings of tropical trees is regulated by a combination of physiological processes. Each of these processes is in turn influenced by many morphological and anatomical factors which result from interactions with the pre-severance stockplant environment, that affects intershoot competition and topophysis (different parts of the plant showing phase variation), as well as the conditions in the propagator and a set of post-severance treatments (Tchoundjeu et al., 2002). Rooting is maximized when the optimal combinations of factors is achieved. Dick and Dewar (1992) modeled these factors and indicated that optimal conditions are met by maximizing the availability of carbohydrates at the cutting base. In this experiment, the diameter of the tree used by the sampling distance of RSC influenced significantly the formation of leafy shoots as well as roots in terms of their number, height and length. The importance of these factors (tree diameter at breast height, sampling distance of RSC from tree used) on the rooting ability of root segments cuttings of tropical species

is not well known for many species. A few number of comparative studies between different sampling distance of RSC and tree diameter from which RSC have been collected have indicated that under similar conditions different species have different preferences for the low distance from trees used (Nsibi et al., 2003; Bellefontaine et al., 2010) and young trees (Nsibi et al., 2003; Diaminatou et al., 2008; Ky-Dembélé et al., 2010). This is in agreement with a number of previous studies in which a similar range of distances or diameters have resulted in successful sprouting and rooting. In the present experiment, sprouting efficiency, the height of leafy shoots and the number of leaves per leafy shoot did not vary significantly between the class diameter and distances from the root collar, even though cuttings taken away (100 cm) from the root collar of the mother tree exhibited the best rooting efficiency $(90.52\pm6.89\%)$ compared to the low (25 cm) distance (69.78±6.69%). This was likely due to a combination of factors such as availability of stored carbohydrate reserves, potential sites for new root or sucker primordia, or even availability of residual water reserves to tide over the segment and developing suckers while new fine roots are developing. These results are in accordance with those of Ky-Dembele (2010) related to effects of propagation environment (high versus low humidity), cutting length and diameter, alignment of root segments (horizontal versus vertical) and distance from the root collar of mother tree on the regeneration ability of root segments of . Root segments of showed strong polarity, with most of the shoots developing towards the proximal end while roots at the distal or median-distal poles. This was expected because of the vertical position of RSC in rooting media, and hormonal control, a mechanism which interacts with carbohydrate supply for bud initiation and subsequent growth from root segments of woody plant species. According to Robinson and Schwabe (1977) and Ede et al. (1997), the polarity is due to the transport of auxin, a shoot suppression hormone that is acropetal in roots, away from the proximal end towards the root tip. In attached roots, auxin from the aerial part of the tree would normally prevent bud initiation, but when this supply ceases upon detachment of the root, depletion of auxin will allow preferential bud initiation to occur at the proximal end, a phenomenon regarded as an extension of apical dominance, the regeneration of new roots was a slower process compared to shoot regeneration (Ky-Dembele, 2011). This is in accordance with all previous studies consulted, in which rooting time is often longer than sprouting time (Hartmann et al., 2002, Stenvall et al., 2005). As suggested by these authors, this feature may indicate that the sprouting process promotes initiation of adventitious rooting because the carbohydrate supply from the leaves may support root elongation. In this experiment, the position of roots appearance on RSC varied considerably (middle, distal, middle-distal). Moreover, some of them rooted without sprouting. These results are consistent with many previous studies, which have shown that cuttings derived from juvenile stockplants are easier to root than those derived from mature stockplants (Ky-Dembele et

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al., 2010, Mapongmetsem, 1994; Mapongmetsem *et al.*, 2012b) and that shoots originating from juvenile zones of a mature tree exhibit juvenile characteristic. The fact that RSC rooted without sprouting suggested that budding is not prior to rooting may be for some species. RSC which rooted without sprouting formed few roots. This suggested that leaves play however an important role by synthesizing carbohydrate used by the cutting to increase and produce new roots.

The best diameter at breast height of tree used which exhibited the high budding rate ($84.44 \pm 14.69\%$) and rooting rate ($86.66 \pm 5.76\%$) was 5-10 cm. The high budding rate ($63.3 \pm 14.69\%$) as well as rooting rate ($90.52\pm6.89\%$) was recorded at 100 cm from the tree while the maximum number of roots per RSC was registered in cuttings harvested at 50 cm from the mother tree. The highest rooting rate ($96.66\pm5.97\%$) was recorded in RSC collected at 50 cm of tree with more than 20 cm dbh. These results were significantly improved compared to those obtained (83.33%) previously on the same species (Mapongmetsem *et al.*, 2016).

Conclusion

This study opens opportunity for domestication and the development of cultivars of desirable traits of *V. doniana* for fruits collection to supply the market. The diameter at breast height (dbh) of the mother tree (5-10 cm) and the sampling distance of RSC (100cm) from it are important factors to consider in vegetative propagation from RSC. For future work, it is planned to evaluate the effects of (i) the mycohiza, (ii) the horizontal vs. vertical or slant positioning of RSC in the substrate, (iii) the harvest season of the RSC (beginning of the dry season vs. end of the dry season), (iv) and the effect of genotypes and origin (provenance) of RSC, on the ability to form new leafy shoots and a dense root system.

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