Journal of Agriculture and Environment for International Development - JAEID 2014, 108 (1): 43 - 62
DOI: 10.12895/jaeid.20141.195

Sustainable use and conservation of *Vitex doniana* Sweet: unlocking the propagation ability using stem cuttings

Enoch Gbenato Achigan Dako[1*], Sognigbe N'Danikou[1,2], Dèdéou Apocalypse Tchokponhoue[1], Françoise Assogba Komlan[3], Mahamane Larwanou[4], Raymond Sognon Vodouhe[2], Adam Ahanchede[1]

[1] *Horticulture and Genetic Unit, Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi, Cotonou, Republic of Benin.*
[2] *Bioversity International, West and Central Africa Office, Cotonou, Republic of Benin.*
[3] *National Agricultural Research Institute of Benin (INRAB), Cotonou, Republic of Benin.*
[4] *African Forest Forum, Nairobi, Kenya.*

Corresponding author: enoch.achigandako@gmail.com

Submitted on 2013, 28 September; accepted on 2014, 19 February. Section: Research Paper

Abstract: One of the major constraints for domesticating widely used wild tree resources by local communities is the lack of adequate propagation techniques. In the case of *Vitex doniana*, seed propagation has usually been reported difficult and vegetative regeneration is rarely explored. To understand how stem cutting size or hormone application affect the sprouting and early growth ability in that species we used two categories of cutting diameter (1cm<D1<2cm and 2cm<D2<4 cm) and three categories of cutting length (L1 = 30 cm, L2 = 50 cm, L3 = 70 cm) and hormone application as fixed factors in a split-split-plot design. A cohort of ten cuttings were used as experimental unit to measure cutting sprouting rates, the sprouting time, the number of new buds, the twig length and the number of leaves. Generalized models with poisson or binomial error distribution were used to test for deviance. To account for pseudoreplication due to repeated measures, we used generalized mixed effect models fit by maximum likelihood. Allometric analyses were performed to understand how the main factors affect the relationships between the twig length and the number of leaves. The results reveal that hormone effect on cutting sprouting was not obvious. However, cutting diameter and length significantly affect sprouting rates, the sprouting time, the number of new buds, the twig length, and the number of leaves produced. Stem cutting of 30 cm height and 2 to 4 cm diameter performed well (100% sprouting) compared to others. The relationships between the twig length and the number of leaves also changes with different cutting diameter and length. These findings offer new insights into the agroforestry promotion of *Vitex doniana* to reduce the pressure on wild tree population in Benin.

Keyword: Domestication, Human pressure, Vegetative propagation, Vitex doniana.

Introduction

To keep off pressure on useful natural resources, domestication has obviously been practiced by local communities as an immediate and affordable option in developing countries. Domestication is a process that takes wild species into cultivation through the management of its regeneration and propagation, the development of adequate husbandry, and techniques for adaptation in agricultural systems to meet specific and well-defined objectives that serve human needs (Simons and Leakey, 2004; Zeder, 2006; Vodouhè *et al.,* 2011). Domestication and promotion of agroforestry species have a lot of potential to improve livelihoods in rural areas. Several tree species offer this potential and were involved in incipient domestication strategies at programme level or in community-based practices. These include for instance *Adansonia digitata* (Assogbadjo *et al.,* 2008), *Ziziphus mauritiana* (Danthu *et al*., 2004), *Vitellaria paradoxa* and *Parkia biglobosa* (Teklehaimanot, 2004), *Uapaca kirkiana*, *Strychnos cocculoides*, *Parinari curatellifolia*, and *Sclerocarya birrea* (Akinnifesi *et al*., 2006), *Dacryodes edulis* and *Irvingia gabonensis* (Leakey *et al*., 2004), and *Vitex doniana* (Achigan-Dako *et al.,* 2011). In Benin, domestication of *Vitex doniana* has regularly been raised as a way out of the current threat on the plant (Oumorou *et al*., 2010; Achigan-Dako *et al*., 2011; N'Danikou *et al.,* 2011). It was also suggested in Cameroon (Mapongmetsem *et al.,* 2012) where the resource plays a vital role in sustaining livelihoods of local communities.

Vitex doniana (Verbenaceae) has numerous utilisations with promising economic potential for poverty alleviation in rural and peri-urban areas in Africa. The species contributes to the improvement of soil fertility by litter production and is a good candidate in agroforestry systems (Mapongmetsem, 2005). The wood is variously used in house construction, furniture, tools and agricultural implements making; it is suitable as firewood and for charcoal production. The blackish pulp of the matured fruits is sweet and edible, and is eaten fresh. This pulp also serves in jam preparation. A beverage is made from the fruit juice, whereas boiled fruits are the basis for an alcoholic liquor and wine (Ky, 2008). In traditional medicine *Vitex doniana* has several applications (Egbekun *et al.,* 1996; Ladeji *et al.,* 2005; Ky, 2008; Padmalatha *et al.,* 2009). For instance the leaf, the bark, dried and fresh fruit serve as ingredients in many preparations to treat or heal diseases including conjunctivitis, headache, stiffness, measles, rash, fever, chickenpox, hemiplegia, respiratory diseases, ankylostomiasis, rachitis, gastro-intestinal disorders, jaundice, kidney troubles, leprosy, liver diseases, bleeding after childbirth and diarrhoea. *Vitex doniana* is an important indigenous fruit or leafy vegetable in Africa (Burkill, 2000; Maundu *et al.,* 2009). In Benin *V. doniana* is one of the most consumed leafy vegetables collected from the wild (Achigan-Dako *et al.,* 2011). Specifically, the fon community in the neighbourhood of the Dan forest in southern Benin listed *V. doniana* as one of the top three wildharvested food in their area (N'Danikou *et al.,* 2011). The species belongs to a sophisticated market chain whereby fruits and parboiled leaves are traded in urban and peri-urban markets (Achigan-Dako *et al.,* 2010) or timbers traded between Gabon and Europe (Ky, 2008). This local, regional and sometimes international trade in *V. doniana* has been for many years based on wild populations from which leaves, fruits or timbers are collected by local collectors. Because of its multiple benefits to the community, *V. doniana* faces, on the one hand, an unprecedented harvest pressure. On the other hand, the regeneration of *V. doniana* has always been challenging particularly when it comes to seed (Yarou, 2007; Ky, 2008; Oumorou *et al.,* 2010; Ahoton *et al.,* 2011; Mapongmetsem *et al.,* 2012; Sanoussi *et al.,* 2012). The species has proved difficult to germinate from fresh seed while existing trees are aging. Germination trials usually produce low success rates (Ky, 2008). Various pre-treatments reported by Yarou (2007) lead to only 20% germination when seeds are involved. Ahoton *et al.* (2011) or Chamshama *et al.* (2001) have respectively reached 58% and 61% (the highest rates reported so far) when stones are broken with physical shock prior to sowing or when seeds were sown at 4 mm depth. Moreover, the germination of lots was far from homogeneous and can last two to six months before reaching those percentages. The low germination rate and the long duration before germination are altogether challenging for any horticultural programme involving *V. doniana* while no study has established the seed storage behavior for this species.

The high utilisation pressure on the species combined with the low regeneration ability cause a steady depletion of natural populations and no clear and straightforward conservation strategies have been developed neither at community level nor at national level apart from the fact that the species might be protected in national reserves where it occurs (Oumorou *et al.,* 2010; N'Danikou *et al.,* 2011). At the community level, although people are aware of the threat on *V. doniana*, there are limited conservation actions taken to sustain the utilisation of the species. Conservation actions usually referred to include the sparing of the tree during land clearing (Oumorou *et al.,* 2010; N'Danikou *et al.,* 2011; Vodouhè *et al.,* 2011).

To promote a sustainable domestication of the species the central question has recurrently been how to improve seed germination or develop alternative regeneration methods. In the context of rapid depletion of natural population of the species, alternative propagation methods such as cuttings regeneration which showed some positive responses in *Vitex madiensis* (Mapongmetsem, 2006) might be a viable answer. The steady interest in vegetative propagation has arisen not only from the desire to conserve the species in its agroecological systems but more importantly to promote large-scale cultivation, higher yields, early fruiting and better quality products for agroforestry development (Leakey and Akinnifesi, 2008). However, standardization of propagation techniques has been one of the greatest challenges of tree domestication (Kumar, 2008). For instance, previous experience of stem cuttings multiplication in *V. doniana* is rarely documented although regeneration from suckers has been observed in nature. Against this background the following questions are raised: 1) how prone are stem cuttings (juvenile or hardwood) of *V. doniana* to vegetative multiplication? 2) What should be the characteristics (diameter and length) of those cuttings? 3) What is the effect of hormonal application on the sprouting of cuttings? How can farmers be provided with high quality propagules in a timely manner for sustainable production of *V. doniana* leaves in urban and/or peri-urban areas? Here we hypothesize that cutting length and diameter affect the sprouting capacity of the material and the overall ability to grow. We test the sprouting and growth ability of cuttings of *V. doniana* of various diameter and length classes. We also investigate the hormonal effect on the sprouting and how fast this happens under such circumstances.

Materials and methods

The species: **Vitex doniana**

Vitex doniana is largely widespread in Africa and can grow in a diversity of habitats (Ky, 2008). It is a deciduous small to medium-sized tree up to 25 m tall with branchless bole for up to 11 m. Bole diameter can grow up to 90 cm, often slightly fluted at base. The bark surface is greyish white to pale greyish brown, fissured and scaly; the inner bark is yellowish white, darkening to brown. Leaves are opposite, digitately compound with in general 5 leaflets. The inflorescence is an axillary cyme, orange-brown hairy. Flowers are bisexual, zygomorphic. The fruit is an obovoid to oblong-ellipsoid drupe, purplish black, fleshy, with woody 4-celled stone, up to 4-seeded. The seed has no endosperm.

Sprouting experiment

Cuttings were collected from an 11-year old tree in Kanzounkpa (Abomey-Calavi Commune, South Benin). Vernier calliper was used to select stem cuttings of different diameter and length. These cuttings were placed into germination polyethylene bag which were filled with sterilized soils. After planting cuttings were watered twice a day around 9 am and 4 pm.

We used a split-split-plot experimental design with the application of a growth hormone (25% Indole-3-butyric acid) as the main factor. This was combined with two diameter categories (secondary factor) for cuttings. The first class includes cuttings with diameter (D1) ranging from 1 to 2 cm and the second category includes cuttings with diameter (D2) between 2 and 4 cm. For each diameter class three length categories were used for cuttings: 30 cm, 50 cm and 70 cm (tertiary factor). The experimental design was made up of 12 different treatments (Table 1) with 120

Table 1 - Experimental design for stem cuttings propagation in Vitex doniana.

Hormone application	Diameter category	Length category
No hormone application (H0)	1 > D1 > 2 cm	L1 = 30 cm L2 = 50 cm L3 = 70 cm
	2 > D2 > 4 cm	L1 = 30 cm L2 = 50 cm L3 = 70 cm
With hormone application (H1)	1 > D1 > 2 cm	L1 = 30 cm L2 = 50 cm L3 = 70 cm
	2 >D2 > 4 cm	L1 = 30 cm L2 = 50 cm L3 = 70 cm

experimental units using ten individuals per treatment.

Data collection started eight day after planting when the first budding appeared. These include: the budding time of each cutting category, the number of buds per cuttings, the twig length, the twig basal diameter, the number of leaves per branch. For each individual cutting, growth data were collected on three twigs every five days for 55 days.

Data analysis

For each variable, descriptive statistics were computed to determine the average values, the median, the interquartiles, and the standard deviation per treatment. Summary data are illustrated by various graphs.

To test the effects of hormone application and the cutting size on the ability to bud (i.e. if cutting length and diameter are involved in budding ability) we used the generalized linear model (glm) with binomial distribution (Achigan-Dako *et al.*, 2009). Model simplification was done by removing non-significant terms. Models were tested with Chi test to select the simplest.

We tested the effects of the same factors on the time to budding. As these are count data, the generalized linear model was applied using poisson distribution. In case of over dispersion (extra unexplained variation in the response) we used quasi-poisson or quasi-binomial given the type of data in presence.

The effects of the hormone application or the cutting length and diameter on the number of buds and the number of leaves per twigs were analyzed using the generalized linear mixed models with poisson distribution.

The effects of the same factors through 60 days on the twig length and the twig basal diameter were tested using mixed effects models with the maximum likelihood method. Mixed models were compared using Akaike Information Criterion (AIC), chi-square, and anova depending on the error distribution used. Allometric relationships were tested between the number of leaves and the twig length to understand how these relationships evolves among cuttings of different size. We tested whether slopes are similar and if so if lines elevation is the same or if they are shifted significantly. All analyses and graphs were performed in R version 2.15.2 (R Developement Core Team, 2013) with packages including lme4, nlme, smatr, gregmisc.

Results

Effects of hormone, cutting diameter and length on sprouting ability

Sprouting rate

On average 53% of the cuttings sprouted during the experiment regardless of size or hormone application. The sprouting rates varies from 20% in cuttings with big length (70 cm) and small diameter (1cm < d1 < 2 cm) to 100% in cuttings with small

Figure 1 - Cohort of ten stem cutting of Vitex doniana *(size: 30 cm height with diameter between 2 and 4 cm) showing 100% sprouting.*

length (30 cm) and big diameter (2 cm < d2 < 4 cm) (Fig. 1). The sprouting rates decrease as the cutting length increases (Fig. 2) and cuttings with big diameter exhibit the highest sprouting rates (100% for L1, 70% for L2 and 40% for L3). Analysis of deviance using the generalized linear models with quasi-binomial error distribution indicates that there is no three of two levels interaction effects between factors such as hormone application, cutting diameter and cutting length ($p > 0.05$ in all cases). However, the effects of cutting length and cutting diameter on the sprouting rates were highly significant ($p < 0,001$). The sprouting rates are not similar when cuttings have different length and diameter. The hormone application did not reveal any significant effects on the sprouting ability ($p = 0.208$). Models comparison using *F*-test indicates that the simplest model with no interaction effects was the best ($p < 0.01$).

Sprouting time

On average 18 days was required to reach 53% sprouting rates for cutting of various length and diameter. The sprouting time varies from 8 to 49 days. The shortest sprouting time was recorder for cuttings with small length and big diameter while the lengthiest sprouting time was recorded for cuttings with medium length. The sprouting time seems to increase with cutting length for cuttings with small diameter (Fig. 3) while for cutting with big diameter this trend was not observed. Likewise, the sprouting time seems to be shorter with hormone application. The analysis of deviance using the generalized linear models with quasi-poisson distribution indicate a highly significant three-level interactions between factors ($p = 0.004$). The sprouting time increases as cutting length increases with a highly significant interaction effect from cutting diameter and the application of hormone.

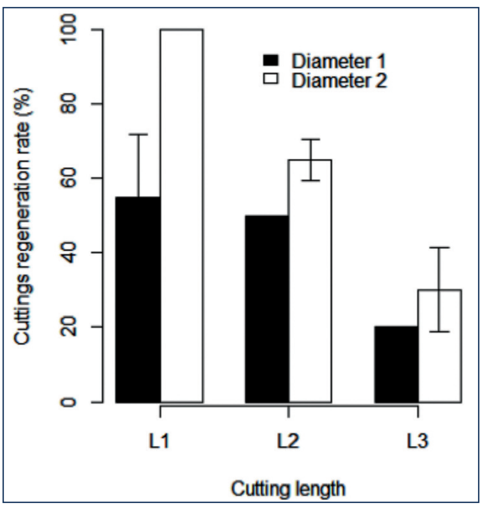

Figure 2 - Sprouting rates in Vitex doniana *given cutting lengths and diameters. L1 = 30 cm, L2 = 50 cm, L3 = 70 cm; diameter category 1 (1cm<D1<2cm), diameter category 2 (2cm<D2<4cm).*

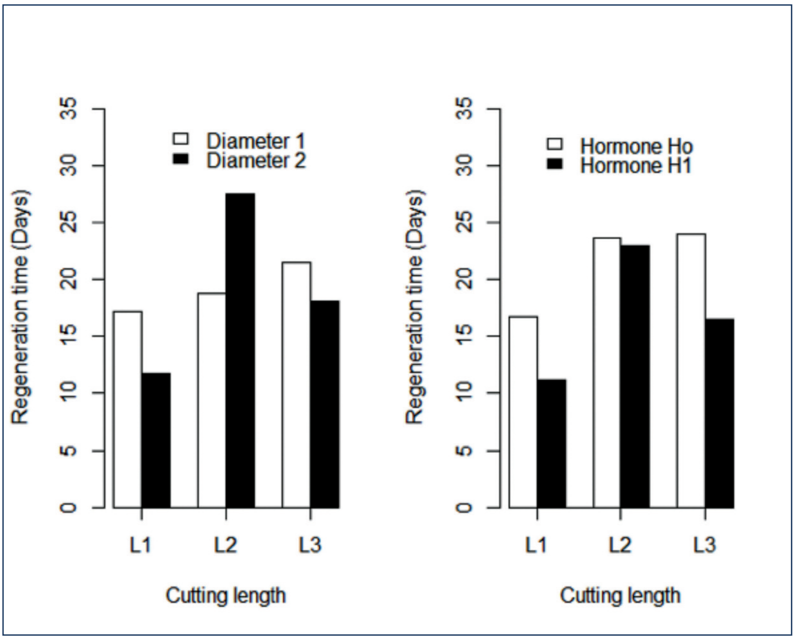

Figure 3 - Sprouting time (number of day after planting) at which cuttings produce the higher number of buds as presented in Figure 1. L1 = 30 cm, L2 = 50 cm, L3 = 70 cm. Diameter category 1 (1cm<D1<2cm), diameter category 2 (2cm<D2<4cm). Hormone Ho corresponds to no application of hormone and Hormone H1 corresponds to application of hormone.

Number of buds produced

The variation of the number of buds produced within two-month growth indicate different situation for each cutting length, cutting diameter and hormone application combination (Fig. 4). The number of buds varies between 0 and 16. For the whole growth period this number was on average higher for cutting with small length and big diameter without hormone application (7.2 ± 3.1). Cutting with small diameter did not perform well particularly when hormone was applied. The number of buds in this case varies on average between 1.06 ± 2.32 and 2.72 ± 2.66 for the whole growth period. In most cases the highest number of buds was obtained before 35 growth days. Analysis of deviance performed with the generalized linear mixed models using growth period as random factor support a significant three-level interaction between hormone application, cutting length, and cutting diameter ($p < 0.001$). The number of buds produced within 55 days period significantly varies with cutting length, cutting diameter and hormone application.

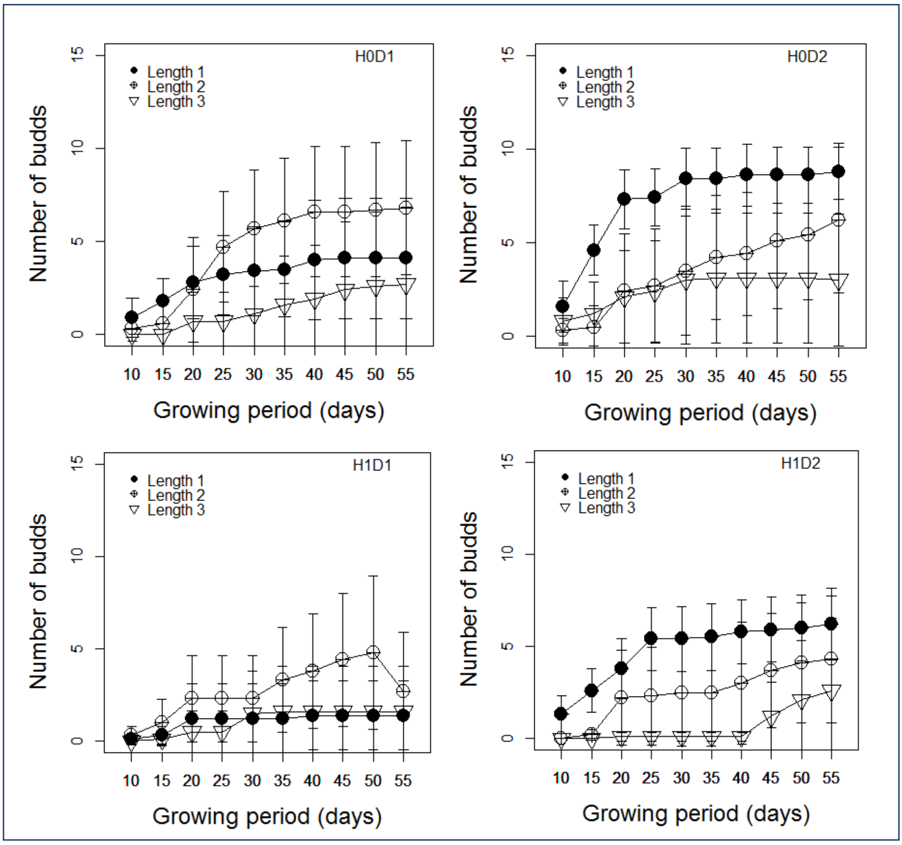

Figure 4 - Evolution of the average number of buds per cutting size and hormonal application according to growing period. H0D1: No hormone application for diameter category 1 (1 cm <D1< 2 cm); H0D2: No hormone application for diameter category 2 (2 cm < D2 < 4 cm); H1D1: With hormone application for diameter category 1 (1 cm < D1 < 2 cm); H1D2: With hormone application for diameter category 2 (2 cm < D2 < 4 cm).

Effects of hormone, cutting diameter and length on plant growth

Twig length

Twig length varies between 0 and 13.2 cm for all treatment combinations. The best performances were recorded for cuttings with small length, big diameter without or with hormone application (Fig. 5). However, twig lengths are slightly higher when hormone is applied. Analysis of deviance indicated several two-level interactions between hormone application and cutting length and cutting diameter ($p < 0.001$). Twig length grows more rapidly in cuttings with small length and big diameter, and that have received a hormone dose. The same trend was observed for twig diameter (data not shown).

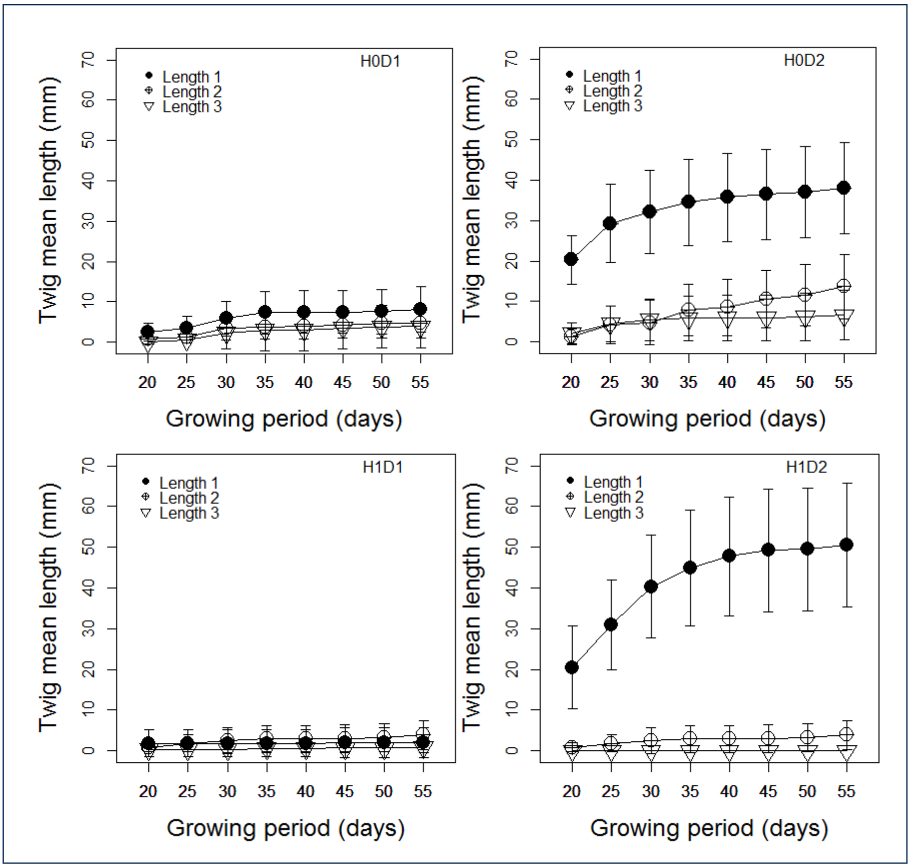

Figure 5 - Evolution of the twig mean length per cutting size and hormonal application according to growing period. H0D1: No hormone application for diameter category 1 (1 cm < D1 < 2cm); H0D2: No hormone application for diameter category 2 (2 cm < D2 < 4cm); H1D1: With hormone application for diameter category 1 (1 cm < D1 < 2cm); H1D2: With hormone application for diameter category 2 (2 cm < D2 < 4cm).

Number of leaves produced

A very limited number of leaves (maximum 11 per cutting) were produced during the first two-month growth of the cuttings. The best performance was noticed for cutting with small length, big diameter and that have received a dose of hormone (Fig. 6). On average 4 to 5 leaves were produced for this cutting category. Analysis of deviance indicated a three-level interaction between hormone application, cutting length and cutting diameter ($p < 0.001$). The number of leaves increases in cuttings with small length and big diameter, particularly when the cuttings have received a dose of hormone. It is also important to report that all leaves produced with stem cuttings were digitately compound with five leaflets.

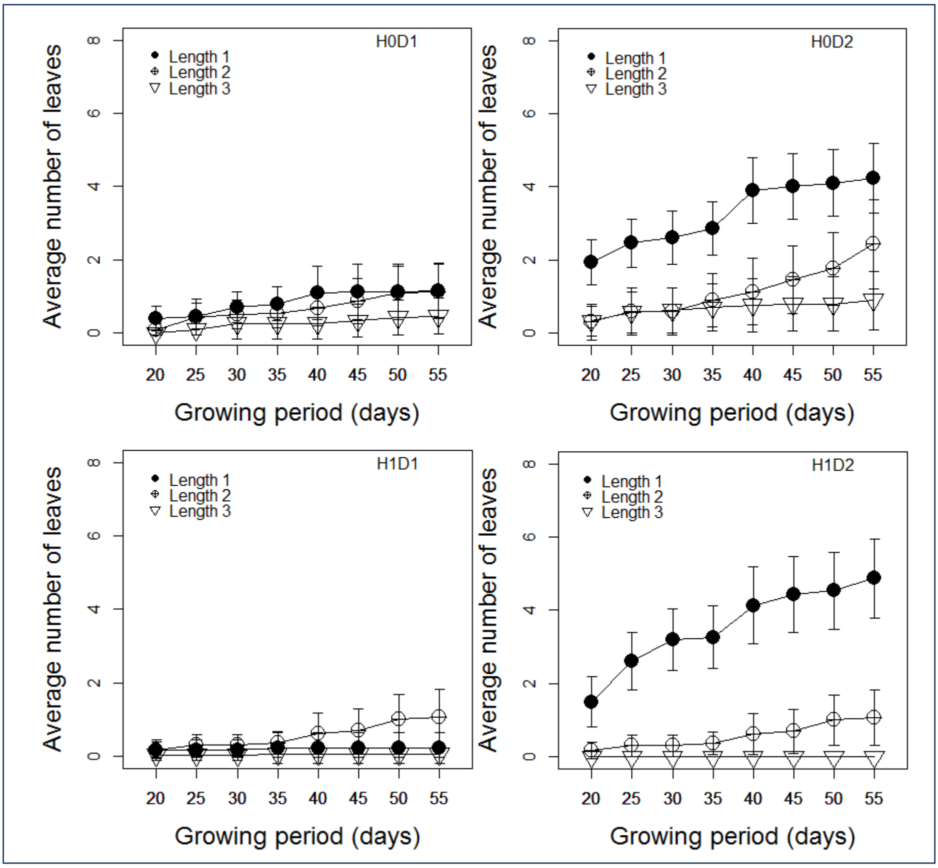

Figure 6 - Evolution of the average number of leaves per cutting size and hormonal application according to growing period. H0D1: No hormone application for diameter category 1 (1 cm < D1 < 2 cm); H0D2: No hormone application for diameter category 2 (2 cm < D2 < 4 cm); H1D1: With hormone application for diameter category 1 (1 cm < D1 < 2 cm); H1D2: With hormone application for diameter category 2 (2 cm < D2 < 4cm).

Allometric relationships between twig length and number of leaves

Allometric relationships between the twig length and the number of new leaves for the three cutting length categories (Figure 7) indicates that lines among categories have equal slope ($p = 0.362$), significant difference in lines elevation ($p = 0.016$) and significant shift along common axis ($p = 0.025$) when cuttings with small size diameter are used with no hormone application.

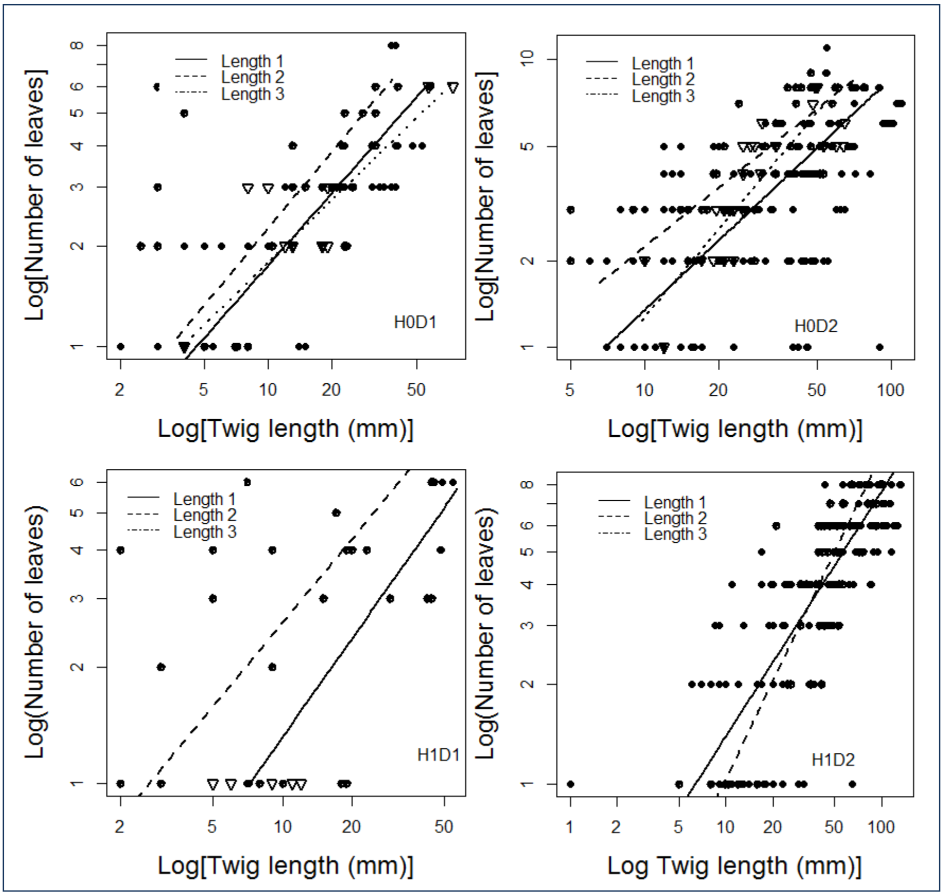

Figure 7 - Allometric relationships between the number of leaves and twig length. H0D1: No hormone application for diameter category 1 (1 cm < D1 < 2cm); H0D2: No hormone application for diameter category 2 (2 cm < D2 < 4cm); H1D1: With hormone application for diameter category 1 (1 cm < D1 < 2cm); H1D2: With hormone application for diameter category 2 (2 cm < D2 < 4cm).

Regression line coefficients are presented in Table 2. Cuttings with big diameter without hormone application revealed, however, significantly different slopes among length categories ($p = 0.016$). With hormone application (Fig. 6, H1D1 and H1D2) the allometric relations between twig length and leave number were reduced to cutting length category 1 and 2 because there were many null values for category 3. Here, the lines slopes were significantly different for cuttings with small diameter and for cuttings with big diameter as well ($p < 0.001$). The relationship between twig length and the number of leaves produced in *Vitex doniana* when vegetative propagation is used, depends on the cutting's diameter at least in the early growth stage as described here.

Table 2 - Linear relationships (y = ax+ b), coefficients of determination (r^2) and corresponding P-values of two growth parameter [Twig length (mm) and Number of leaves)] for Vitex doniana *using three cutting length and two diameter size categories with or without hormone application.*

Cutting category	Cutting Length	Slope	Elevation	R^2	P-value
No hormone application, small diameter					
	L1 = 30 cm	0.719	-0.475	0.55	<0.000
	L2 = 50 cm	0.763	-0.407	0.23	<0.000
	L3 = 70 cm	0.618	-0.365	0.82	<0.000
No hormone application, big diameter					
	L1 = 30 cm	0.805	-0.677	0.32	<0.000
	L2 = 50 cm	0.684	-0.335	0.50	<0.000
	L3 = 70 cm	1.015	-0.910	0.68	<0.000
Hormone application, small diameter					
	L1 = 30 cm	0.848	-0.728	0.97	<0.000
	L2 = 50 cm	0.721	-0.306	0.15	<0.006
Hormone application, big diameter					
	L1 = 30 cm	0.737	-0.593	0.59	<0.000
	L2 = 50 cm	0.97	-0.963	0.78	<0.000

Discussion

Sprouting and growth of stem cuttings

Vegetative propagation offers the opportunity to rapidly overcome the continuing depletion and erosion of natural genepool of a species. It is a valuable means of propagation (Leakey *et al.,* 1982; Teklehaimanot, 2004). However, propagation by mature stem cuttings is notoriously difficult (Leakey and Akinnifesi, 2008) and not many species of the Verbenaceae family have been investigated for vegetative propagation (Mapongmetsem, 2006). Particularly for *Vitex doniana*, a very limited number of studies has reported on the use of stem cutting as propagation material and includes Sanoussi *et al.* (2012) who investigated the use of stems and roots cutting on different substrate, Mapongmetsem *et al.* (2012) who studied the potential of young cuttings to regenerate in various soil types with hormone stimulation. Unlike our study, the plant material used by Sanoussi *et al.* (2012) was from various young plants and was not systematically grouped in size categories. The highest regeneration rate obtained by Sanoussi *et al.* (2012) was 50.7% and this was attributed to samples origin and environmental conditions among other factors. Results presented by

Mapongmetsem *et al.* (2012), were based on cuttings of very small size (3-5 cm) of 1.5 month-old and revealed 40% regeneration in non-thinned cuttings and 100% loss in thinned cuttings. Our results which focus on sprouting suggest that when size is adequately selected the regeneration can be improved. This was observed for cuttings with 30 cm length and 2 to 4 cm diameter big. Cutting length and diameter concomitantly contribute to good sprouting. This is in agreement with findings of Mapongmetsem (2006) who indicated that *Vitex madiensis* propagates well with stock plant heights between 0 and 25 cm. We conclude that the regeneration rates in *V. doniana* might differ when cuttings have different length and diameter. For vegetative propagation in that species, not only the type of cutting material plays a role, but also the size of the planting material particularly when it comes to stem cuttings.

The effects of hormone application were not clear. In many occasions cuttings without hormone application performed better. Our results suggest that hormone application may not be necessary for cutting sprouting in *Vitex doniana* or maybe the application method (powdering the basal area of cuttings) does not produce the desired effects. This situation was observed as well in *Vitex doniana* juvenile cuttings tested on different substrate (Mapongmetsem *et al.,* 2012), in *Lovoa trichilioides* (Tchoundjeu and Leakey, 2001) and in *Dacryodes edulis* (Avana *et al.,* 2002). Also in *Uapaca kirkiana* marcots no root-initiation hormone was required (Akinnifesi *et al.,* 2006). The effects of hormone application on regeneration and root formation vary in plant family, species and even varieties (Leakey *et al.,* 1990) and root-initiation hormone is not required for *Vitex doniana*.

The sprouting time increases with cutting length. Cutting with big length and small diameter sprouted slower than those with small length and big diameter or those with big length and big diameter. This reinforces the idea that there is a high interaction effect between cutting length and diameter toward successful vegetative regeneration.

The number of buds produced within 55 days period significantly varies with cutting length, cutting diameter and hormone application. Cuttings with big length do not necessary produce more buds. Our findings reveal that cutting diameter is more crucial for budding and the number of buds largely exceeds 8 when cuttings have small length and big diameter. The number of buds produced is higher than indicated by Sanoussi *et al.* (2012). Moreover twig length grows more rapidly in cuttings with small length and big diameter. The same trend was observed for twig diameter. The number of leaves increases in cuttings with small length and big diameter, particularly when the cuttings have received a dose of hormone. Hormonal effect was not perceptible on sprouting rate and time but on twig growth where an interaction effect among the main factors was noticed. The fact that cuttings produce directly multi-lobed leaves suggests that vegetative propagation methods can shorten the juvenile phase in *Vitex doniana* as observed in *Uapaca kirkiana* (Akinnifesi *et al.,* 2006). However, this hypothesis needs to be tested.

We also observed that the lengthier the twig, the higher the number of leaves. This positive relationship between twig length and the number of leaves was supported by allometric analysis which revealed that the nature of the relationship between twig length and the number of leaves is determined by the initial size of the cuttings particularly by diameter. We conclude that the relationship between twig length and the number of leaves produced in *Vitex doniana* when vegetative propagation is used depend on the cutting's diameter at least in the early growth stage.

Research and Development Implications for conservation and use

Knowledge on how to domesticate *Vitex doniana* is scarce. A limited number of studies have attempted to fill the gap (Mapongmetsem *et al.,* 2012). Our study shed light onto the characteristic of planting material that can be used to ensure proper vegetative propagation in *Vitex doniana*. It highlights the importance of length and diameter of stem cuttings that can be used to increase success rates. Several research and development opportunities arise from these findings and include a) clonal selection and genetic improvement of the production cycle (improvement of precocity), and the leave or fruit yields, b) micro-propagation of juvenile shoots, (see Ahmad and Anis (2007) for *Vitex negundo*), the analysis of local perception on how to best integrate *Vtex doniana* as cultivated tree into horticultural production system to increase parboiled leaves market.

Other research avenues can be investigated to increase knowledge on *Vitex doniana* and support agroforestry application. These include the characterization and evaluation of wild populations, the analysis of seed storage behaviour, and the analysis of the socio-economic determinant affecting the consent of local communities to conserve *in situ* wild populations.

To the best of our knowledge little information is available on the genetic structure of wild populations of *Vitex doniana*. This can be achieved through a thorough analysis of the morphological variation in population which can be fine-tuned with molecular check between morphological variation and genetic structure.

Although vegetative propagation might be a quick option to grasp, the propagation by seed should not be overlooked. Vegetative propagation should be complementary to seed multiplication. Massive propagation of unique genotype is another risk that should be monitored as occasional disturbances might cause a total loss of germplasm. In this regards, one should closely look into the seed storage behaviour of *Vitex doniana* and investigate the factors that determine seed viability in the short, medium and long term.

Conclusion

Diameter and length of stem cuttings affect the sprouting ability in *Vitex doniana*. Yield components such as twig length and number of leaves are also affected by the initial size of the stem cutting. The number of buds and leaves produced is improved when cuttings have small length (30 cm) and diameter comprised between 2 and 4 cm. Further investigation into the initial size of stem cuttings will offer opportunity to develop adequate mass propagation techniques for *Vitex doniana*.

Acknowledgements

This research was jointly funded by the African Forest Forum (AFF) and New Alliance Trzust (NAT).

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