Four multipurpose species of the genus *Vitex* (Lamiaceae) in the Democratic Republic of the Congo show different responses to propagation techniques for nursery production

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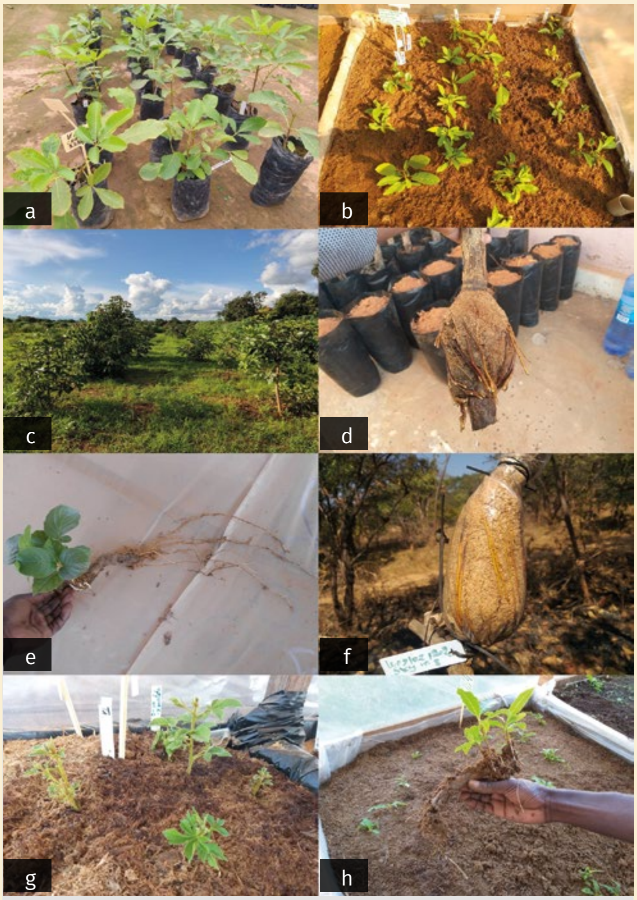

Photos 1.
a. Young *Vitex doniana* plants in the shadehouse. b. Budding of *Vitex doniana* roots in a propagation frame. c. A plantation of species of the genus *Vitex* in the experimental garden of UNILU's Faculty of Agronomic Sciences. d. Rooted marcotte of *Vitex fischeri* in Lubumbashi. e. Rooted root cutting of *Vitex madiensis* in Lubumbashi. f. Rooted marcotte of *Vitex madiensis* in Lubumbashi. g. Cutting roots of *Vitex mombassae* in a propagation chassi in Lubumbashi. Fragment of a rooted root cutting of *Vitex doniana* in a propagation frame in Lubumbashi. h. Fragment of a rooted root cutting of *Vitex doniana* in a propagation frame in Lubumbashi.
Photos D. Numbi Mujike, 2019.

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RÉSUMÉ

Quatre espèces polyvalentes du genre *Vitex* (Lamiaceae) de la République démocratique du Congo réagissent différemment aux techniques de multiplication pour la production en pépinière

Les plantes médicinales ligneuses constituent l'un des principaux produits écosystémiques fournis par la forêt claire sèche du Haut-Katanga (République démocratique du Congo). La déforestation rapide, et la demande croissante en médicaments traditionnels mettent en péril la sécurité de l'approvisionnement. Dans ce contexte, la domestication et la production en culture peuvent représenter une solution durable. Les espèces du genre *Vitex* (Lamiaceae) sont bien connues pour leur utilisation en médecine traditionnelle. Cette étude vise à optimiser les techniques de propagation de quatre espèces du genre *Vitex* (*V. doniana, V. madiensis* subsp. *milanjiensis, V. fischeri, V. mombassae*) qui coexistent dans la forêt claire sèche du Haut-Katanga. Quatre techniques, à savoir le semis, le bouturage de racines, le bouturage de tiges et le marcottage aérien, ont été testées. Les quatre espèces montrent des réponses remarquablement contrastées. *Vitex* madiensis n'est pas apte à la multiplication générative (0 % de taux de germination), mais les graines mûres scarifiées ont enregistré des taux de levée plus élevés chez *V. doniana* (90 %), tout comme les graines scarifiées récoltées à l'état vert chez *V. mombassae* (75 %). Le bouturage de racines a été plus performant chez *V. doniana* (87 %), *V. madiensis* (80 %) et *V. mombassae* (77 %), mais pas pour *V. fischeri* (0 %). Le marcottage aérien convient bien à *V. fischeri* (83 %) et *V. madiensis* (77 %), mais pas pour *V. mombassae* (0 %) dans les conditions de notre étude. Les boutures de tiges n'ont été efficaces pour aucune des quatre espèces. Des protocoles spécifiques efficaces pour la propagation représentent un premier pas vers la domestication de ces espèces en République démocratique du Congo.

Mots-clés : marcottage, bouturage, semis, multiplication végétative, domestication, *Vitex*, plante médicinale, miombo, République démocratique du Congo.

ABSTRACT

Four multipurpose species of the genus *Vitex* (Lamiaceae) in the Democratic Republic of the Congo show different responses to propagation techniques for nursery production

Woody medicinal plants are one of the main ecosystem products found in the dry woodlands of the province of Haut-Katanga (Democratic Republic of the Congo), where rapid deforestation coupled with increasing demand for traditional medicine are jeopardising supplies. In this context, domestication and cultivation may be a sustainable solution. The species of the genus *Vitex* (Lamiaceae) are well known for their uses in traditional medicine. This study aims to optimise propagation techniques for four species of the genus *Vitex* (*V. doniana, V. madiensis* subsp. *milanjiensis*, *V. fischeri, V. mombassae*) that coexist in the dry woodlands of Haut-Katanga. Four techniques, i.e., sowing, root cuttings, stem cuttings and air layering, were tested experimentally. The four species show strikingly contrasting responses. Sowing was ill-suited to *V. madiensis* (0% germination rate), but scarified ripe seeds produced high germination rates in *V. doniana* (90%) as did scarified seeds harvested from unripe (green) seeds of *V. mombassae* (75%). Root cuttings were most suitable for *V. doniana* (87%), *V. madiensis* (80%) and *V. mombassae* (77%), but not for *V. fischeri* (0%). Air layering was well suited for *V. fischeri* (83%) and *V. madiensis* (77%), but not for *V. mombassae* (0%) in the conditions of our study. Stem cuttings were not efficient for any of the four species in the conditions of our study. Effective and specific propagation protocols would be a first step towards domestication of these species in the Democratic Republic of the Congo.

Keywords: sowing, root cuttings, stem cuttings, air layering, vegetative propagation, domestication, *Vitex*, medicinal plant, miombo, Democratic Republic of the Congo.

RESUMEN

Cuatro especies polivalentes del género *Vitex* (Lamiaceae) de la República Democrática del Congo reaccionan de forma diferente a las técnicas de multiplicación para la producción en vivero

Las plantas medicinales leñosas constituyen uno de los principales productos ecosistémicos proporcionados por el bosque abierto seco del Alto Katanga (República Democrática del Congo). La deforestación rápida, y la creciente demanda de medicamentos tradicionales ponen en peligro la seguridad del aprovisionamiento. En este contexto, la domesticación y la producción en cultivo pueden representar una solución sostenible. Las especies del género *Vitex* (Lamiaceae) son bien conocidas por su uso en medicina tradicional. Este estudio pretende optimizar las técnicas de propagación de cuatro especies del género *Vitex* (*V. doniana, V. madiensis* subsp. *milanjiensis, V. fischeri, V. mombassae*), que coexisten en el bosque abierto seco del Alto Katanga. Se ensayaron cuatro técnicas, es decir, el sembrado, los esquejes de raíz, los esquejes de tallos y el acodo aéreo. Las cuatro especies muestran respuestas claramente contrastadas. *Vitex* Madiensis no es apta para la multiplicación generativa (0 % de tasa de germinación), pero las semillas maduras escarificadas registraron tasas de crecimiento más elevadas en *V. doniana* (90 %), al igual que las semillas escarificadas recogidas siendo verdes en *V. mombassae* (75 %). El esqueje de raíces fue más efectivo en *V. doniana* (87 %), *V. madiensis* (80 %) y *V. mombassae* (77 %), aunque no para *V. fischeri* (0 %). La reproducción por esquejes aéreos es conveniente para *V. fischeri* (83 %) y *V. madiensis* (77 %), pero no para *V. mombassae* (0 %) en las condiciones de nuestro estudio. Los esquejes de tallos no fueron eficaces para ninguna de las cuatro especies. Los protocolos específicos eficaces para la propagación representan un primer paso hacia la domesticación de estas especies en la República Democrática del Congo.

Palabras clave: acodo, reproducción por esqueje, siembra, multiplicación vegetativa, domesticación, *Vitex*, planta medicinal, miombo, República Democrática del Congo.

Introduction

Medicinal plants are used in the treatment of many diseases in traditional African medicine. Although traditional medicine cannot be reduced to herbal medicine alone, the vast majority of traditional recipes and treatments are plant-based. In the Democratic Republic of the Congo (D.R. Congo), more than 80% of the population uses traditional medicine to treat themselves (Okombe *et al.*, 2014; Bakari *et al.*, 2017) with, in particular, a massive use of woody medicinal plants (Lejoly *et al.*, 1992; Vwakyanakazi and Petit, 2004; Muya *et al.*, 2014; Konda *et al.*, 2012; Mbayo *et al.*, 2016; Mongeke *et al.*, 2018; Rusaati *et al.*, 2021).

The dry tropical woodlands in the south of the country, also known as miombo woodlands, are being rapidly degraded by the unsustainable harvesting of wood for charcoal making, inappropriate wildfire practices and cleared for slash-and-burn agriculture, mining activities, and urbanization (Potapov *et al.*, 2012; Useni *et al.*, 2017). This represents a serious threat to woody medicinal plants. At the same time, the demand for medicinal plants is increasing due to population growth. Supply challenges could lead to substitution with other plants, which may be inactive or even toxic, thus threatening the health and livelihoods of the people depending on them (Yamani *et al.*, 2015).

In this context, strategies for conservation and sustainable use of medicinal plants need to be developed (Maghembe *et al.*, 1998; Akinnifesi *et al.*, 2007; Meunier *et al.*, 2008; Leakey and Van Damme, 2014; Mapongmetsem and Diksia, 2014). Cultivation of overexploited plants and exclusively harvested in the wild would be a sustainable alternative as it would not only reduce pressure on wild populations, but also secure supply and generate more income for households (Simons and Leakey, 2004; Amujoyegbe *et al.*, 2012). Cultivation also has the advantage of combating adulteration by providing reliable botanical identification. However, lack of knowledge on the most appropriate propagation techniques is one of the main constraints to cultivation (Gbenato *et al.*, 2014).

Research is currently being developed in different regions of tropical Africa to assess the domestication potential of overexploited or endangered medicinal plants. This is the case of, among others, *Irvingia gabonensis* (Atangana *et al.*, 2001), *Prunus africana* and *Pausinystalia johimbe* (Tchoundjeu *et al.*, 2002, 2004), *Spathodea campanulata* (Meunier *et al.*, 2006), *Warburgia salutaris* (Hannweg *et al.*, 2016), *Ximenia americana* (Fawa *et al.*, 2015), etc.

In the Katanga region, several species of the genus *Vitex* (*V. madiensis, V. mombassae, V. doniana* and *V. fischeri*) of the Lamiaceae family are well known by local communities for their medicinal and food (fruit) value. Ethnobotanical studies in this and neighboring regions have shown that these species are widely used in traditional medicine to treat various diseases including diabetes, diarrhea, asthma, gastrointestinal parasitosis, anemia, etc. (Augustino, 2011; Bruschi *et al.*, 2014; Bakari *et al.*, 2017; Mpasiwakomu, 2021; Rusaati *et al.*, 2021).

Vitex doniana, the species whose propagation has been most extensively studied in Central and West Africa, has been shown to have a stronger ability to propagate by root cuttings compared to stem cuttings (Sanoussi *et al.*, 2012; Mapongmetsem *et al.*, 2016a). Germination is enhanced by scarification (Ahoton *et al.*, 2011; Neya *et al.*, 2017b), because seeds are surrounded by a hard endocarp (physical dormancy). Available data on the propagation of *V. madiensis* show that the species is unsuitable for seeding (Mapongmetsem, 2006). The size (diameter at chest height) of the mother plant (parent) could influence the performance of the propagation material (Mapongmetsem *et al.*, 2016b). Further investigations on the propagation in ex-situ conditions of other species of the genus *Vitex* occurring in Katanga would make available to users' protocols for cultivation, a necessary step for domestication.

In this paper, we compare propagation protocols for seeds, root and stem cuttings, and aerial layering for these four *Vitex* species (*V. doniana, V. fischeri, V. madiensis, V. mombassae*). We test i) if the protocols developed for *V. doniana* in Central and West Africa can be applied to other species of the same genus, or whether, specific protocols should be used for each of these four species; ii) whether, at the intraspecific level, the success of the propagation methods is influenced by phenotypic characteristics of the material, especially the vigour of the cuttings (stem or root).

Material and methods

Study environment

The study area, called "Plaine de Lubumbashi" (Schmitz, 1971), is located in the south of the Haut-Katanga province, in D.R. Congo. The experiments were conducted *in-situ* and in the experimental field of the Faculty of Agronomic Sciences of the University of Lubumbashi (UNILU) (E27°48'61'', S11°61'55,3''; 1,257 m a.s.l.). The climate is characterized by a 5-month rainy season (November-March) and a 5-month dry season (May-September) separated by two transition months (October and April). The average annual rainfall is ca. 1,300 mm. The average annual temperature is 20 °C with the lowest temperatures in June-July (temperatures < 10 °C can be recorded at night) and the highest temperatures in October (maximum temperatures of 39 °C have been recorded in recent years). Ferralsols are the major soil type of the region. The most common vegetation formation, is miombo woodland, i.e. dry tropical woodland dominated by three legume genera of the Caesalpinioideae subfamily, i.e. *Brachystegia*, *Julbernardia*, and *Isoberlinia* (Campbell *et al.*, 2007). Industrial and artisanal mining and slash-and-burn agriculture are the main activities of the population of the area.

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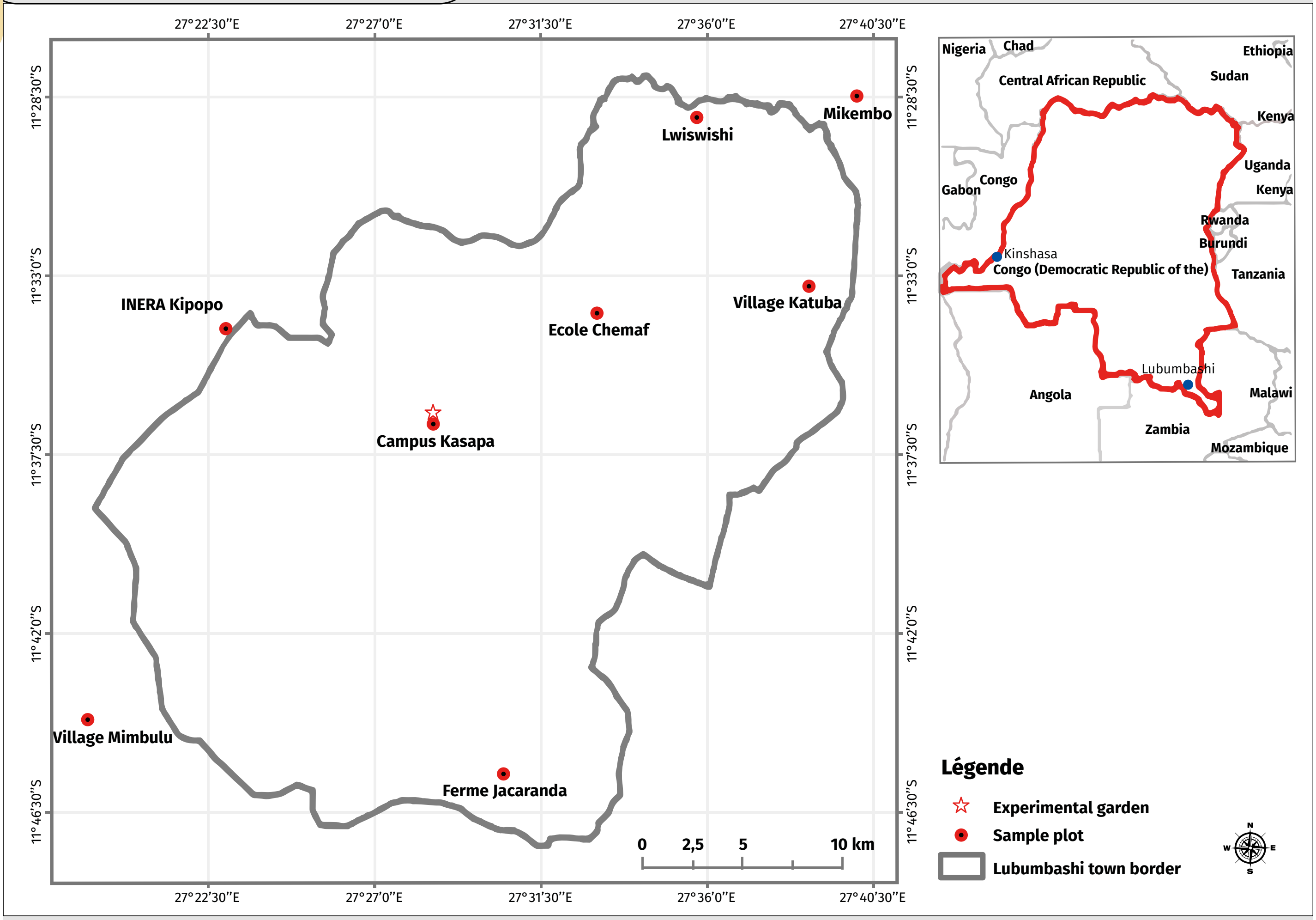

Figure 1.
Location of the sites of origin of propagation materials (Mujike, 2022).

Plant material

The genus *Vitex* comprises ca. 270 species of trees and shrubs, formerly included in the Verbenaceae family, but which phylogenetic classification now places in the Lamiaceae family (Harley *et al.*, 2004). They grow mostly in tropical regions and are easily recognized by their opposite, palmate compound leaves. The fruit is a drupe with a 4-celled pit, each cell containing a seed (Mabberley, 2017). *Vitex doniana* is widely distributed in tropical Africa; *V. fischeri* and *V. mombassae* are distributed in east and southern Africa, while *V. madiensis* subsp. *milanjiensis* is restricted to the Zambezian region. For more details on the morphological traits of the four-study species, see Meerts (2018) and Paton and Meerts (2020). Within the miombo woodland, the four species occupy slightly different niches, with *V. fischeri* occurring almost exclusively on termite mounds, on soil with high base cation contents (Nkulu *et al.*, 2022).

Germination trial

The seeds were collected from five sites: Mikembo (*V. fischeri, V. madiensis* and *V. mombassae*), Lwiswishi (*V. fischeri, V. madiensis* and *V. mombassae*), Kasapa (*V. doniana, V. fischeri, V. madiensis* and *V. mombassae*), Village Katuba (*V. doniana*) and Kipopo (*V. doniana*). The sites of origin of propagation materials are presented in figure 1 and appendix I. These sites were chosen based on the availability of the material. In each site and for each species, an average of five vigorous individuals (parent), ca. 200 m from each other and in good health were selected. As *V. madiensis* and *V. mombassae* showed variation in the leaflet number in all sites, seeds were collected from individuals with five leaflets, individuals with 3 leaflets and individuals with 3 & 5 leaflets (in the same tree). Twelve fruits were harvested from each individual. Seeds were sown in a manner that allowed identification of the parent plant in the nursery. Parent plant characteristics are presented in appendix I.

A total of 160 fruits per species were harvested and sown in March 2019 (end of the rainy season). Fruits were manually pulped with a knife. The pits were divided into four dormancy-breaking pre-treatment batches (manual scarification, soaking in boiling water until cooled, soaking in tap water at room temperature for 24 h, untreated control) and two maturity levels (ripe fruit and green fruit). Ripe fruits were identified by the blackish (*V. doniana, V. fischeri, V. madiensis*) or brownish (*V. mombassae*) color of the pericarp. The ripe fruits were collected on the ground, while the green fruits were collected from the tree. Scarification was done by breaking the pit with a hammer. The use of green fruits was chosen because *Vitex* seeds are known to be physically dormant (Belhadj *et al.*, 1998), and we thought that the endocarp of fruits harvested in the ripe state would be strongly lignified and could therefore prevent the contact of water and embryo necessary for any germination. The green fruits would have a less lignified (porous) endocarp. The black polyethylene bags of 12 cm x 20 cm containing the soil of the experimental garden (of the ferralsol type) were installed under a shade in a rectilinear way (in rows spaced of 10 cm, with a spacing of 15 cm).

The experiment was conducted in a randomized block design of eight treatments repeated twenty times with three factors: species, dormancy breaking pre-treatment and fruit maturity level. One pit per bag was sown at a depth of about 2 cm. Germination rate, lag time (period between sowing and first germination), number of leaves and plant height were observed. Approximately 22 cl of water was supplied daily to every bag for six months.

Root cuttings

This experiment was conducted in February 2019, near the end of the rainy season. Propagation materials were collected in four sites: Chemaf (*V. doniana*), Lwiswishi (*V. fischeri, V. madiensis* and *V. mombassae*), Kasapa (*V. doniana, V. fischeri, V. madiensis* and *V. mombassae*) and Kipopo (*V. doniana*). In average ten trees per species and per site were selected for collection of one root (diameter 1.1–2.5 cm) within a radius of 1.5 m around the parent, following the protocol of Sanoussi *et al.* (2012) and Mapongmetsem *et al.* (2016a). The mother plants have on average a minimum diameter of 5 cm and a maximum of 15 cm at breast height. The leaf trait characteristics were the same as those of the seedlings.

A refrigerated box was used to transport the roots to the nursery. The roots were cut into 15 cm long fragments and placed horizontally in the propagator at a depth of about 2-3 cm. The experimental design was a randomized block repeated nine times with two factors: (i) the species and (ii) the rooting substrate (decomposed sawdust and potting soil). Five root cuttings were planted for each treatment (species x substrate). The propagators are installed in a shadehouse.

A total of 360 root cuttings, i.e. 90 per species, were grown. The propagator is a wooden box of 3 m length by 1 m width and 1 m height divided into three equal compartments, following the model of Leakey *et al.* (1990). Recovery rate, lag time, number of stems, leaves, roots, root length, rooting rate (proportion of cuttings with at least one root) and plant height were observed. A cutting was considered as rooted when it had at least one root of 1 cm in length (Mapongmetsem *et al.*, 2016b). Photos 1 illustrate some images of the results obtained in the propagator (a), aerial layering (b) and plants in black polyethylene bags (c).

Stem cuttings

This experiment was conducted in April 2019. The collection sites were the same as for the root cuttings and mother plants with the same characteristics. A total of 240 stem cuttings (60 per species) of about 30 cm in length and 2.5-4 cm in diameter (Sanoussi *et al.*, 2012; Gbenato *et al.*, 2014; Mapongmetsem *et al.*, 2016ab) were harvested and cultured in black polyethylene bags of 16 cm x 30 cm in a factorial design with three blocks. Soil rich in organic matter constituted the substrate of culture. Prior to plantation, cuttings were soaken for a few seconds in a solution of auxin (Rootone). The bags were arranged in a straight line and placed under a shade (with a shade net that covers the entire nursery). A quantity of 15 cl of water was given to the cutting in the morning and evening for six months. Budding rate, rooting rate, number of new stems, number of leaves and stem height were monitored for six months.

Aerial layering

Aerial layering was carried out between December 2019 and January 2020 (in the middle of the rainy season; trees are in fruiting state), in seven sites: Lwiswishi (*V. fischeri, V. madiensis* and *V. mombassae*), INERA Kipopo (*V. fischeri, V. madiensis* and *V. mombassae*), Chemaf (*V. doniana*), Katuba (*V. doniana* and *V. mombassae*), Mimbulu 2 (*V. madiensis*) and Jacaranda farm (*V. fischeri* and *V. madiensis*). Marcottes were prepared from orthotropic branches of 3-7 cm in diameter, with a maximum of 2 marcottes per individual. The mother plants had a range dbh of 5-12 cm (*V. doniana*) and 4-10 cm (*V. fischeri, V. madiensis* and *V. mombassae*). A total of 360 marcottes were prepared, i.e. 30 marcottes per site for each species. The experimental design was a main factor design with species and sites as factors. It was not possible to evaluate the species x site interaction because the four species where not present in the same sites. Thus, the number of sites varied among the species.

Each marcotte was wrapped in translucent plastic film. The plastic sleeve was filled with decomposed sawdust as a rooting substrate. To avoid contamination, the knife was regularly washed and disinfected. The substrate was moistened every month with a syringe. Rooting rate and lag time were our observed parameters.

Influence of mother plants

The morphotype (i.e. 3 or 5 leaflets) and diameter at breast height (DBH) of all individuals or mother trees used for seed collection, stem and root cuttings and aerial layering were recorded in all sites in order to assess their influence on the seedling's performance (Momo *et al.*, 2017).

Statistical analysis

For the germination trial, ANOVA was not applied to test the effect of treatment on the germination rate as there

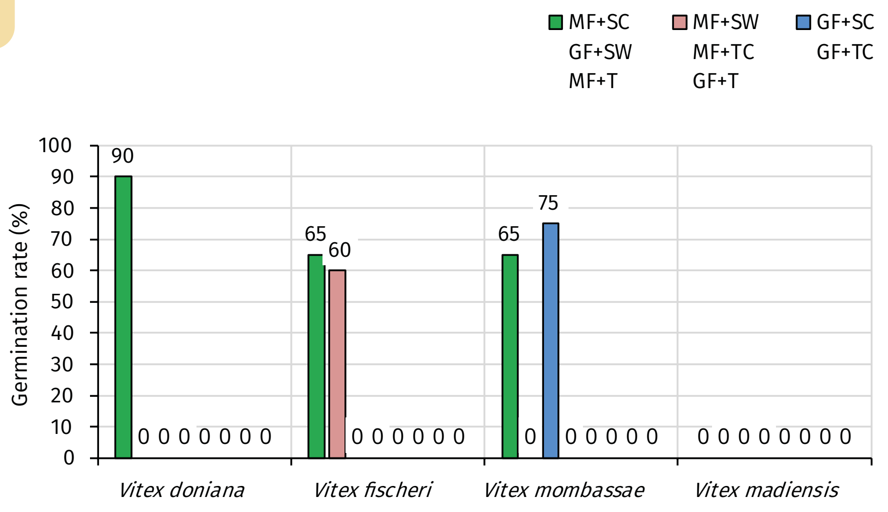

Figure 2.
Germination rate of four species of *Vitex*. 160 seeds per species. MF = mature fruit, GF = green fruit, SC = scarified fruit, SW = soaked in water at room temperature, TC = soaked in boiling water, T = control.

was only one seed per pot for each treatment. The number of germinations for each treatment was calculated based on the twenty pots in the experimental device.

Two-way ANOVA without replication was applied to the data from lag time, number of leaves and seedling height. For the lag time, species and blocks were the main factors. For *V. fischeri*, two-way ANOVA was applied to test the effect of dormancy breaking (scarification and soaking in cold water) and blocks. Fruit maturity and blocks were the main factors for *V. mombassae*. The effect of interactions (scarification x block, dormancy breaking x block and fruit maturity x block), were not calculated.

As for root cuttings, three-way ANOVA without replication was used to test the species effect, the rooting substrate effect, the block effect as well as the species x rooting substrate interactions.

For aerial layering, two one-way ANOVA was performed successively. First, one-way ANOVA was applied to test the influence of site for each species separately. As there was no significant effect of site, all the sites were pooled and a one-way ANOVA was applied to test the effect of species (all sites pooled).

A one-factor ANOVA was also performed to test the effect of the morphotype (i.e. number of leaflets) of the mother plant on the performance of the propagation material. To test the effect of mother plant diameter on the performance of the propagation material, multivariate analyses of variance (MANOVA) were performed between DBH and the different variables (emergence rate, recovery rate, number of leaves per plant, plant height, etc.).

In order to respect the normality and homogeneity of the variance of the residuals, the data of lag time, number of leaves, roots and plant height were transformed into Log 10 while those expressed in percentage were transformed into square root. The method used to compare mean values was the least significant difference at the 5% probability level (LSD) Tukey HSD test. All the statistical analyses were performed using R software 64.4.0.5.

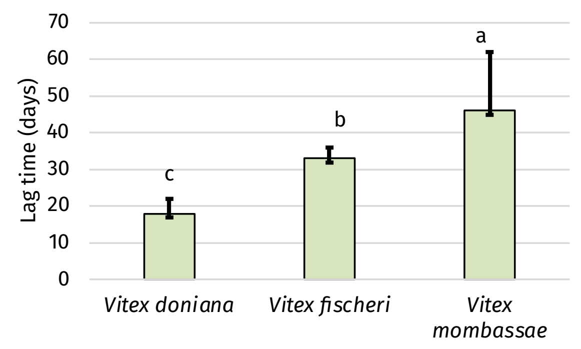

Figure 3.
Lag time of germination of mature, scarified fruits in three species of *Vitex*. 160 seeds per species. A, b, c: values sharing the same letter are not significantly different at the 5% probability threshold (Tukey's test).

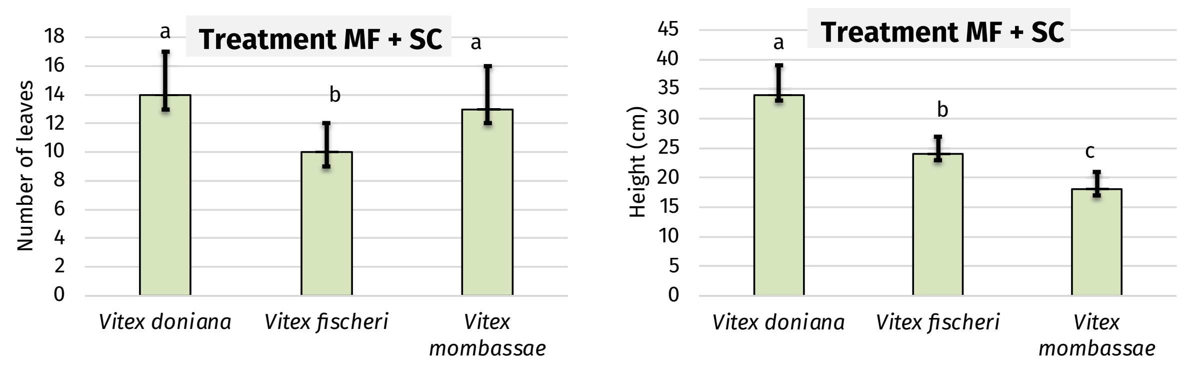

Figure 4.
Number of leaves per plant as well as plant height of *Vitex* in the nursery. Comparison of MF+SC treatment by species. 160 seeds per species. a, b, c: values sharing the same letter are not significantly different at the 5% probability threshold (Tukey test). Legend: MF = mature fruit, SC = scarified fruit.

Results

Germination

After six months, no *V. madiensis* seeds germinated regardless of fruit maturity and pretreatment to break dormancy. Similarly, there was no germination with the untreated controls, with the combination of Green Fruit x Boiling Water Dip and Green Fruit x Room Temperature Water Dip regardless of species. The other three species showed different germination patterns (figure 2). For *V. doniana*, only seeds from ripe and manually scarified fruit germinated with a rate of 90% (18/20). For *V. fischeri*, germination was recorded only for seeds from ripe fruits scarified manually (13/20, 65%) or soaked in tap water (12/20, 60%). For *V. mombassae*, germination occurred only for manually scarified seeds from green fruits (15/20, 75%) or from ripe fruits (13/20, 65%).

ANOVA (appendices II) shows that the lag time, the number of leaves as well as plant height are significantly influenced by the species ($p = 0.000$). The shortest lag time was obtained with scarified ripe seeds of *V. doniana* (18 ± 4 days), followed by scarified ripe seeds of *V. fischeri* (33 ± 3 days) while scarified ripe fruits of *V. mombassae* recorded the longest lag time (45 ± 2 days) (figure 3). The effect of block was not significant for all observed parameters.

In *V. fischeri*, lag time, number of leaves per plant and plant height were not influenced by the dormancy breaking technique (manually scarified ripe fruits and cold water soaked ripe fruits) (figure 4). In *V. mombassae*, none of the parameters were influenced by the physiological maturity of the seed. ANOVA results are presented in the appendices II.

Root cuttings

After 6 months, the recovery rate ranged from 0% (*V. fischeri*) to 79 ± 7% for *V. mombassae*, 80 ± 4% for *V. madiensis* and 87 ± 3% for *V. doniana*. The lag time was about 75 days for *V. doniana* and *V. madiensis* and about 85 days for *V. mombassae*.

No parameter was significantly influenced by the block. The rooting substrate significantly influenced the recovery rate, root emission rate and plant height ($p < 0.05$). The species effect is very highly significant on all observed parameters ($p < 0.05$). The interaction of species with rooting substrate was significant on recovery rate, lag time, number of leaves and root emission rate ($p < 0.05$). Figure 5 shows the results obtained for budding rate, recovery rate, lag time and number of leaves per plant. The other parameters were not significant. The table summarizing the results of the ANOVAs is presented in appendix III.

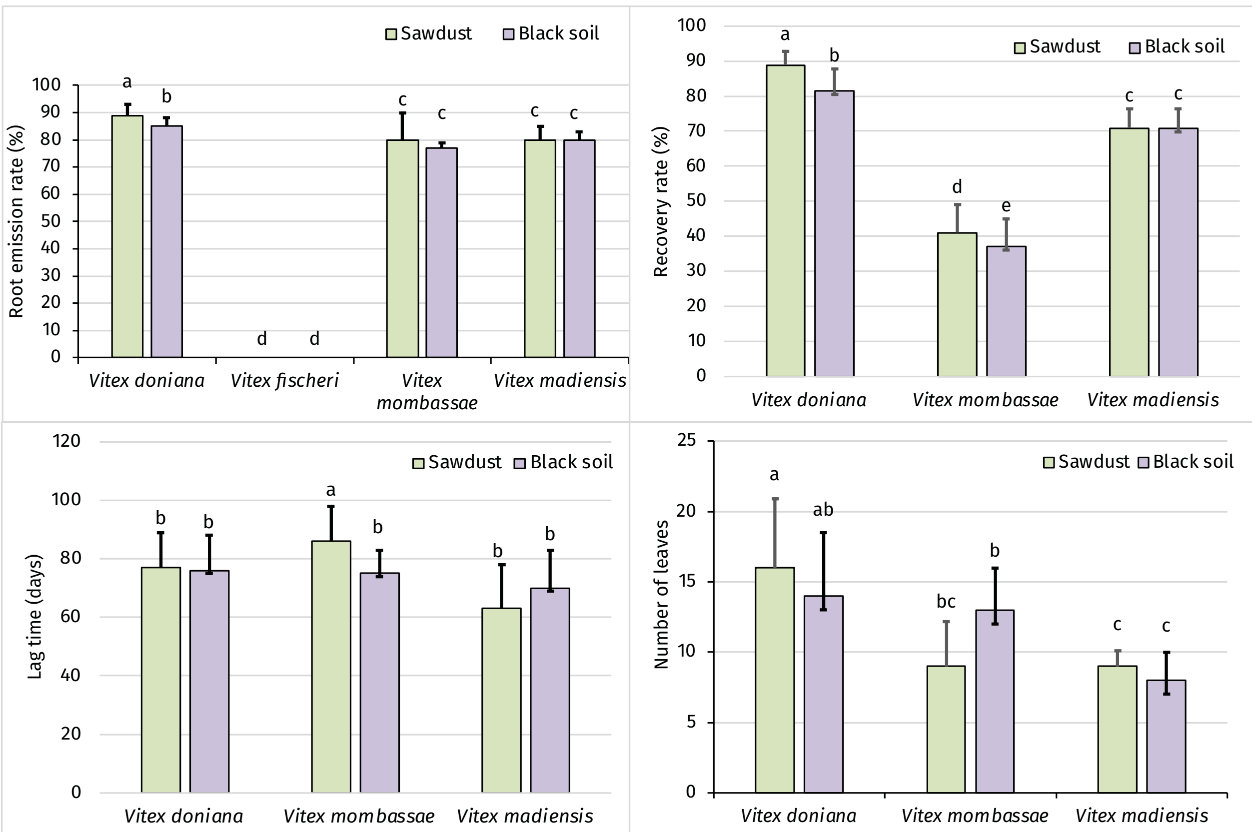

Figure 5.
Root cuttings of species of the genus *Vitex*. Budding rate, recovery rate, lag time and number of leaves per plant after six months. Mean ± Standard deviation. 360 root cuttings, 90 per species, 30 per treatment (5 x 9 replicates). a, b, c: values sharing the same letter are not significantly different at the 5% probability threshold (Tukey's test).

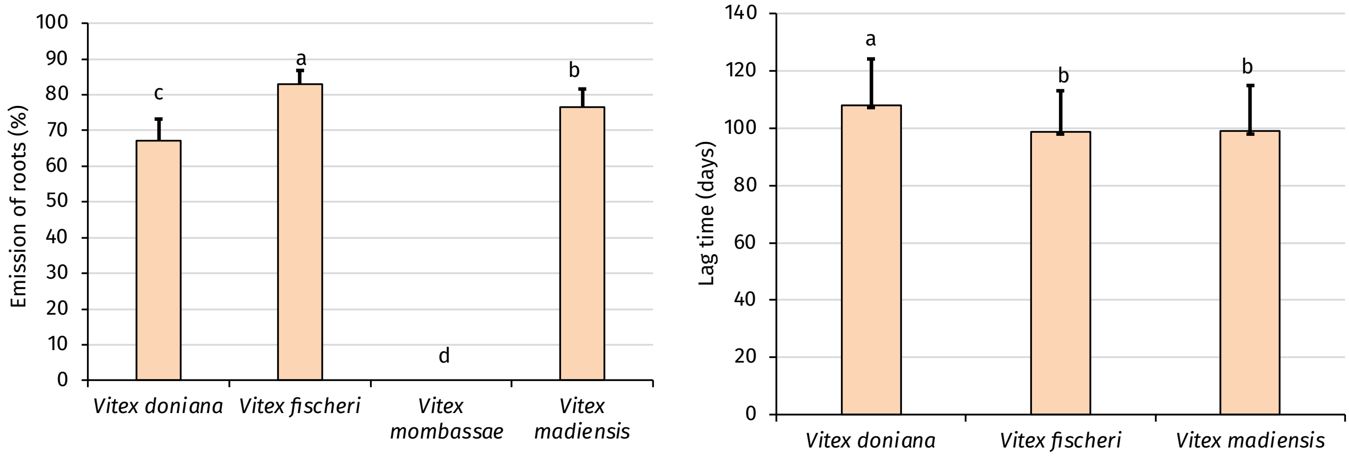

Figure 6.
Aerial potting of species of the genus *Vitex*. Root emission rate and lag time after 150 days of observations. Total number of marcottes was 360 or, 90 per species. a, b, c, d: Values sharing the same letter are not significantly different at the 5% probability threshold (Tukey's test).

Stem cuttings

The recovery rate of stem cuttings was 25 ± 9% (*V. madiensis*), 30 ± 10% (*V. fischeri*), 43 ± 10 (*V. doniana*) and 63 ± 15% (*V. mombassae*). The lag time was 22 ± 4 days (*V. mombassae*), 24 ± 4 days (*V. madiensis*), 29 ± 3 days (*V. doniana*) and 34 ± 3 days (*V. fischeri*). However, no roots were produced, regardless of species. A mortality rate of 100% was recorded for all cuttings four months after planting in pots. The figure illustrating the evolution of the survival rate of stem cuttings in the nursery is presented in appendix IV.

Aerial layering

Data were pooled after non-significant ANOVA test for site effect. The results obtained show that no rooting of marcottes could be obtained for *V. mombassae*. For the other three species, the rooting rate was high, ranging from 67 ± 5% (*V. doniana*), 77 ± 5% (*V. madiensis*) to 83 ± 4% (*V. fischeri*). The lag time was 99 ± 20 days (*V. madiensis*), 99 ± 14 days (*V. fischeri*) and 108 ± 16 days (*V. doniana*).

The influence of species was very highly significant on rooting rate ($F(3,8) = 5903.94$; $p = 0.000$) as well as on lag time ($F(2,201) = 6.55$, $p = 0.002$). Figure 6 shows schematically the results for root emission rate and the lag time. The results of the ANOVAs for the site effect are presented in appendix V.

Influence of the mother plant

The MANOVA results showed a positive significant influence of mother plant diameter on the recovery of root cuttings of *V. doniana* ($P = 0.000$) and *V. mombassae* ($p = 0.006$). The diameter of mother plants also had a positive significant influence on the number of roots of *V. doniana* ($p = 0.002$). The recovery and the number of roots increased with the diameter of the mother plants. For all the other propagation techniques and parameters there was no significant influence of mother plant diameter.

Discussion

Different responses of the four *Vitex* species tested

We have compared the suitability of different germination techniques in four congeneric species of *Vitex*, a genus of woody species occurring in tropical Africa. Apart from the negative results obtained for stem cuttings, and the lack of germination of unscarred seeds for all the species, there were striking differences in the responses of the four species to all the other techniques. The failure of stem cuttings under the conditions of our study is surprising because previous works used this technique successfully for *V. doniana* and *V. madiensis* (Mapongmetsem *et al.*, 2016a; Gbenato *et al.*, 2014; Sanoussi *et al.*, 2012). Future work should use cuttings from 1- or 2-y old shoots and/or test other growth conditions.

Concerning the germination of seeds, our results show that all the species have physical dormancy (lignified endocarp), and require dormancy breaking. This confirms previous results for *V. doniana* and *V. madiensis* (Belhadj *et al.*, 1998; Mapongmetsem, 2006; Gbenato *et al.*, 2014; N'Danikou *et al.*, 2014; Neya *et al.*, 2017ab). Interestingly, the species show contrasting response to the different dormancy breaking techniques, with *V. fischeri* seeds being able to germinate after soaking in water at room temperature while only mechanical scarification with a hammer was efficient for the other species. This is likely due to the smaller pit size and thinner endocarp of *V. fischeri*. *V. madiensis* subsp. *milanjiensis* was found to be unsuitable for propagation by seed. In Western Africa, difficult germination of the other subspecies of *V. madiensis* (subsp. *madiensis*) was reported by Mapongmetsem (2006). Seed viability should be assessed in *V. madiensis*. Nkulu (2022) found very high rates of selfing in *V. madiensis*, therefore inbreeding depression could possibly affect seed viability.

For all the other techniques, the contrasting responses of the four species are surprising, because all our species co-occur in sympatry in the dry woodlands of Haut-Katanga. Interestingly, the four species occupy somewhat different niches. In particular, *V. fischeri* occurs only on high *Macrotermes* termite mounds, which have quite different soil conditions compared to the surrounding woodland (Mujinya *et al.*, 2010; Cuma Mushagalusa *et al.*, 2020). *V. madiensis* and *V. mombassae* are never found on termite mounds, and tend to occur in different plant communities (Nkulu *et al.*, 2022). It is possible that the regeneration niches of the four species are somewhat different.

Previous studies (Sanoussi *et al.*, 2012; Mapongmetsem, 2006; Mapongmetsem and Diksia, 2014; Mapongmetsem *et al.*, 2016a,b) suggested that *V. doniana* and *V. madiensis* were more suitable to multiplication by root cuttings than stem cuttings. This is confirmed by the results of this study. Interestingly, the high recovery rate of root cuttings of *V. doniana* can be related to the frequent observation of suckers in this shallow-rooted species in nature (Mujike, 2022). The complete failure of aerial layering in *V. mombassae,* in contrast to the other species, is intriguing and deserves further investigation.

Conclusion : Practical implications for seedling production and propagation of the four *Vitex* species

The objective of this study was to identify the most suitable propagation techniques for the four species of the genus *Vitex* from miombo in Haut-Katanga through a comparison of several propagation methods in a domestication perspective. This has made it possible to provide users with the first data on the propagation of *V. fischeri* and *V. mombassae*. The results show that the same protocol does not apply to all four species. Each of them presents one or two most appropriate modes of propagation. Stem cuttings were identified as the least appropriate mode of propagation for the four species under the conditions of this study. These results lead to the following practical recommendations for the propagation of *Vitex* species in Haut-Katanga (table I).

All the results, especially those from the root cuttings, showed a more significant effect of species compared to substrate (sawdust and potting soil). This suggests that the use of sawdust or potting soil in the production of propagating material of the four *Vitex* will depend on their availability.

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Data access

The data collected in the field and used for the statistical analysis and writing of this article are freely available on the ZENODO platform. https://zenodo.org/deposit/8355009#

Table I.
Preferred propagation techniques for four *Vitex* species occurring in Haut-Katanga dry tropical woodlands.

Propagation technique	*Vitex doniana*	*Vitex fischeri*	*Vitex madiensis* subsp. *milanjiensis*	*Vitex mombassae*
Stem cuttings	-	-	-	-
Root cuttings	++	-	++	++
Aerial layering	+	++	++	-
Seed germination (soaking in water)	-	+	-	-
Seed germination (mechanical scarification)	++	+	-	++

- = poor, + = good, ++ = very good.

Appendix I.

Table II.
Parent plant characteristics. Characteristics of seedlings (mother plants) of 4 species of the genus *Vitex* used in the experiments.

Species	Vegetative propagation		Generative propagation	
	Sample (n)	DBH (cm)	Sample (n)	Fruit diameter (cm)
Vitex doniana	15	5-13	30	1.8 ± 0.1
Vitex fischeri	15	6-12	30	0.7 ± 0.06
Vitex madiensis	15	5-10	30	1.6 ± 0.1
Vitex mombassae	15	5-10	30	2.2 ± 0.2

DBH = diameter at breast height.

Appendices II: ANOVA germination.

Table III.
Effect of species for the scarified wall fruit treatment. 2-factor ANOVA without replication: ***: p < 0.01, **: p < 0.01, *: p < 0.05, NS: p > 0.05.

Observed parameters	Source of variation	F value	P value
Lag time	Block	(11,22) = 1.30	NS
	Species	(2,22) = 34.8	***
Number of leaves	Block	(11,22) = 1.243	NS
	Species	(2,22) = 13.2	***
Plant height	Block	(11,22) = 1.24	NS
	Species	(2,22) = 65.4	***

Table IV.
Effect of scarification vs. cold water soaking for *V. fischeri*, 2-factor ANOVA without replication: ***: p < 0.01, **: p < 0.01, *: p < 0.05, NS: p > 0.05.

Observed parameters	Source of variation	F value	P value
Lag time	Block	(11,11) = 2.17	NS
	Treatment	(1,11) = 2.92	NS
Number of leaves	Block	(11,11) = 0.36	NS
	Treatment	(1,11) = 12.69	**
Plant height	Block	(11,11) = 0.823	NS
	Treatment	(1,11) = 6.41	**

Table V.
Effect of fruit maturity vs. scarified seeds of *V. mombassae*. Two-factor ANOVA without replication: ***: p < 0.01, **: p < 0.01, *: p < 0.05, NS: p > 0.05.

Observed parameters	Source of variation	F value	P value
Lag time	Block	(12,12) = 0.558	NS
	Treatment	(1,12) = 2.630	NS
Number of leaves	Block	(12,12) = 1.281	NS
	Treatment	(1,12) = 0.357	NS
Plant height	Block	(12,12) = 2.910	NS
	Treatment	(1,12) = 0.178	NS

Appendix III: ANOVA root cuttings.

Table VI.
Effect of species, substrate and interaction (sub x sp) on performance of root cuttings. 3-factor ANOVA without replication: ***: p < 0.01, **: p < 0.01, *: p < 0.05, NS: p > 0.05.

Observed parameters	Source of variation	F value	P value
Budding rate	Block	(8,40) = 1.075	NS
	Substrate	(1,40) = 5.165	*
	Species	(2,40) = 41.636	***
	Sub. x sp.	(2,40) = 6.701	**
Lag time	Block	(8,40) = 1.351	NS
	Substrate	(1,40) = 1.539	NS
	Species	(2,40) = 14.043	***
	Sub. x sp.	(2,40) = 7.268	**
Number of leaves	Block	(8,40) = 1.110	NS
	Substrate	(1,40) = 0.069	NS
	Species	(2,40) = 24.626	***
	Sub. x sp.	(2,40) = 8.512	***
Recovery rate	Block	(8,40) = 2.98	NS
	Substrate	(1,40) = 41.98	***
	Species	(2,40) = 4,095.97	***
	Sub. x sp.	(2,40) = 27.60	***
Number of roots	Block	(8,40) = 1.817	NS
	Substrate	(1,40) = 0.772	NS
	Species	(2,40) = 25.714	***
	Sub. x sp.	(2,40) = 1.097	NS
Plant height	Block	(8,40) = 0.719	NS
	Substrate	(1,40) = 19.006	***
	Species	(2,40) = 19.960	***
	Sub. x sp.	(2,40) = 1.231	NS

Appendix IV: Stem cuttings.

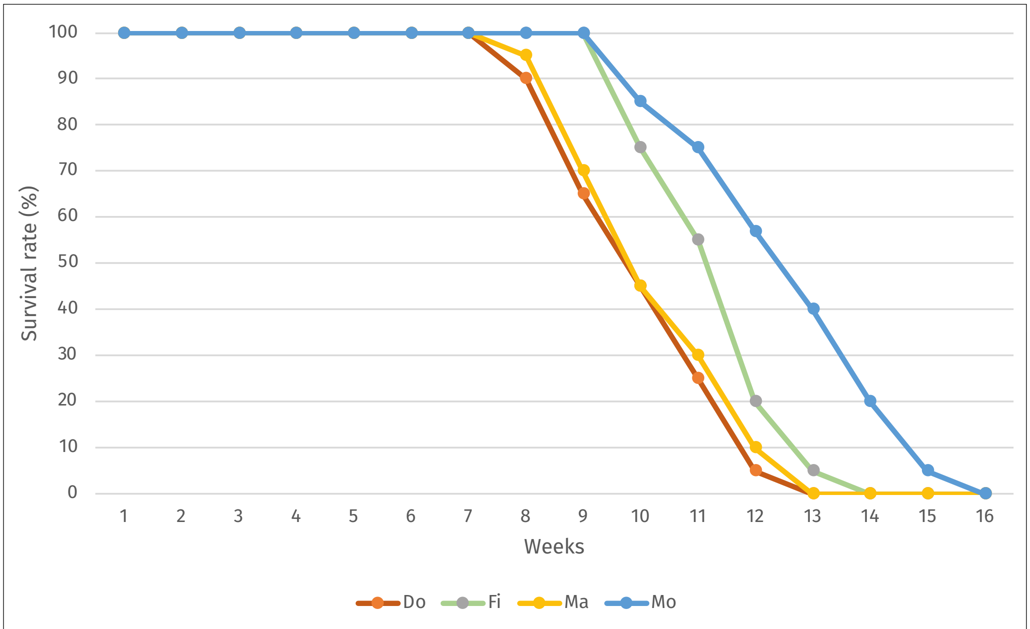

Figure 7.
Stem cuttings. Evolution of the survival rate of stem cuttings in the nursery. Do = *V. doniana*; Fi = *V. fischeri*; Ma = *V. madiensis*; Mo = *V. mombassae*.

Appendix V: ANOVA aerial layering.

Table VII.
One-factor ANOVA to test the layering site effect: ***: p < 0.01, **: p < 0.01, *: p < 0.05, NS: p > 0.05.

Species	Observed parameters	F value	P value
Vitex doniana	Lag time	(2,57) = 0.07	NS
	Recovery rate	(2,8) = 0.057	NS
Vitex fischeri	Lag time	(2,72) = 3.30	NS
	Recovery rate	(2,8) = 0.092	NS
Vitex mombassae	Lag time	(2,66) = 6.94	NS
	Recovery rate	(2,8) = 5.49	NS

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