Enhancing germination and seedling growth in *Vitex doniana* Sweet for horticultural prospects and conservation of genetic resources

Sognigbé N'Danikou[1,2]*, Enoch G. Achigan-Dako[1], Dèdéou A. Tchokponhoué[1], Françoise Assogba Komlan[3], Jens Gebauer[4], Raymond S. Vodouhè[2], Adam Ahanchédé[1]

[1] Hortic. Genet. Unit, Fac. Agron. Sci, Univ. Abomey-Calavi, 01 BP 526, Tripostal Cotonou, Rep. Benin
[2] Bioversity Int., West Central Afr. office, 08 BP 0932, Cotonou, Rep. Benin, ndanikou@gmail.com
[3] Ntl. Agric. Res. Inst. Benin (INRAB), BP 884 Cotonou, Repub Benin
[4] Fac. Life Sci., Hochschule Rhein-Waal, Marie-Curie-Straße 1, D-47533 Kleve, Germany

* *Correspondence and reprints*

Received 17 September 2013
Accepted 3 December 2013

Fruits, 2014, vol. 69, p. 279–291

DOI: 10.1051/fruits/2014017
www.fruits-journal.org

***Resumen Español**, p. 291*

Enhancing germination and seedling growth in *Vitex doniana* Sweet for horticultural prospects and conservation of genetic resources.

Abstract – Introduction. Many fruit trees with a hard seed coat exhibit seed dormancy, posing problems for their regeneration. *Vitex doniana*, an indigenous multipurpose but threatened fruit and vegetable tree that supports the livelihoods of many households in West Africa, is a typical example. **Materials and methods**. In our research, we evaluated five dormancy-breaking treatments. We tested the effects of sulphuric acid at 95% concentration (T1); 3 d sun-drying + 48 h soaking in tap water (T2); 3 d alternation of 8 h sun-drying + 1 h soaking in tap water (T3); 2 weeks sun-drying with regular watering in the daytime (T4); and physical shock (T5). These treatments were compared with two controls (T0 and Tc), with seeds from two different sources. The germination percentage, mean germination time, time to first germination and time to threshold germination (20%) were compared; the seedling height, diameter and biomass produced were monitored for 15 weeks. We used generalised linear models and correlation tests to compare the effects of the various treatments on germination and seedling growth. **Results and discussion**. T3 significantly enhanced seed germination in *V. doniana* (72% after 12 months). T4 best promoted homogeneity in germination ($p < 0.01$), followed by T3. The best seedling growth was obtained with T4 and T3. Alternation of sun-drying followed by soaking of seeds, a technique with almost no cost, improved seed germination in *V. doniana* and, in 33 d, just over 1 month, 20% germination can be achieved. *Vitex doniana* is a fast-growing species (at the nursery stage), in contrast to the common opinion. **Conclusion**. Our method should be further investigated to assess the adequate soaking and drying length so as to speed up germination and reach homogenous cohorts.

Benin / *Vitex doniana* / plant domestication / sexual reproduction / seed dormancy / seed germination / seedlings / growth rate

Amélioration de la germination et de la croissance de *Vitex doniana* Sweet pour l'horticulture et la conservation des ressources génétiques.

Résumé – Introduction. De nombreuses espèces d'arbres fruitiers ayant des graines à téguments durs présentent une dormance problématique pour leur régénération. *Vitex doniana*, une plante légumière et fruitière autochtone à usage multiple, mais menacée, qui permet la subsistance de nombreux ménages en Afrique de l'Ouest, est un exemple typique. **Matériel et méthodes**. Dans notre étude, nous avons évalué cinq traitements de levée de la dormance des semences. Nous avons testé l'effet de l'acide sulfurique à une concentration de 95 % (T1) ; 3 j de séchage au soleil + 48 h de trempage dans l'eau courante (T2) ; 3 j d'alternance de 8 h de séchage au soleil + 1 h de trempage dans l'eau courante (T3) ; 2 semaines de séchage au soleil avec un arrosage régulier pendant la journée (T4) ; et choc physique (T5). Ces traitements ont été comparés à deux témoins (T0 et Tc), constitués respectivement de semences provenant de deux sources différentes. Le taux de germination du lot (ou traitement), le temps moyen pour la germination des semences du lot, le temps pour la première germination dans le lot et le temps pour atteindre le seuil de 20 % de germination ont été comparés ; puis la hauteur, le diamètre au collet des plantules et la production de feuilles ont été suivis pendant 15 semaines. Nous avons utilisé les modèles linéaires généralisés, les modèles mixtes et les tests de corrélation pour comparer les effets des différents traitements sur la germination et la croissance des plantules. **Résultats et discussion**. T3 a considérablement amélioré la germination de *V. doniana* (72 % après 12 mois). T4 a donné la meilleure homogénéité de la germination ($p < 0{,}01$), suivi de T3. La meilleure croissance des plantules a été obtenue avec les traitements T4 et T3. L'alternance du séchage au soleil suivie par un trempage des semences dans l'eau courante, une technique presque sans coût, a amélioré la germination des graines de *V. doniana* et 20 % de taux de germination ont pu être obtenus en 33 j, soit en un peu plus d'un mois. Contrairement à l'opinion habituelle, *V. doniana* est une espèce à croissance rapide (en pépinière). **Conclusion**. Des recherches devraient être poursuivies afin de déterminer la durée optimale de l'alternance séchage-trempage permettant d'accélérer la germination des graines et d'obtenir des populations homogènes.

Bénin / *Vitex doniana* / domestication des plantes / reproduction sexuée / dormance des semences / germination des graines / plantule / taux de croissance

1. Introduction

Seed dormancy is an innate adaptive strategy of some plant species to tide over environmental conditions. Although dormancy is a natural process that allows the long-term conservation of most orthodox seeds, this can become a constraint in plant production. Dormant seeds are often difficult to regenerate, with the risk of losing genetic diversity with each regeneration cycle. Dormancy occurs in 69.6% of all major taxonomic groups [1], and this situation is harmful to the world's food supply [2]. Different dormancy types have been described [3], and physiological and physical classes are known to be the most common [4]. During the last decades, several studies have focused on physical dormancy in forest species and many seed treatments have been applied to break dormancy and improve germination. These treatments showed positive effects on some species but cannot be standardised. In addition to low germination rates there is the erratic pattern of germination in most forest seeds with a hard coat.

Our study focused on black plum (*Vitex doniana* Sweet, Verbenaceae) producing orthodox seeds with a hard coat, often called "stones". The species was chosen for being multi-purpose with important socio-economic [5–8] and agronomic potential [9, 10] in West Africa. These functions make the species very coveted in the region and this has resulted in high pressure on natural populations [7, 10, 11]. The threat level reported from parklands in northern Benin reached about 95.65% [10]. For Eyog-Matig [12], the main external factor threatening the species is the intensive exploitation of plant parts for several purposes. Meanwhile, seed germination remains a bottleneck due to dormancy, which hampers natural regeneration of the species [7, 13].

Despite its high value for local populations, there is no evidence of any conservation initiatives from harvesters [7]. Also, very few attempts have been made to break seed dormancy in the species [9, 14]. To the best of our knowledge, no standardised dormancy-breaking treatment is available for *V. doniana*. Moreover, how to obtain homogenous seedling cohorts has remained an important bottleneck for proper propagation of the species in Benin and elsewhere in Africa. For other Verbenaceae such as *Tectona grandis* L.f. (teak), better germination rates (50% to 79%) were obtained when using particular seed extraction techniques [15], dry heating pre-treatments [16] or alternate wetting and drying procedures [17]. However, we still do not know whether the treatments used for teak are transferable to *V. doniana* as species of the same family may not necessarily respond in the same way to seed dormancy-breaking treatments. In our study, we aimed to i) investigate the effects of alternative dormancy-breaking treatments on *V. doniana* seed germination, and ii) evaluate the effect of these treatments on seedling vigour and growth. We hypothesise that the dormancy-breaking treatment applied to *T. grandis* seeds should improve seed germination in *V. doniana*, as both species belong to the same plant family. The experiment was carried out at the Agonkanmey research station of the National Agricultural Research Institute of Benin (INRAB), located in the Abomey-Calavi district.

2. Materials and methods

2.1. Plant material and experimental design

Prior to applying dormancy-breaking treatments we carried out the seed coat imbibition test following methods by Baskin *et al.* [18] and Phartyal *et al.* [19] on non-scarified seeds of *Vitex doniana* to ascertain whether seeds presented a physical dormancy or not. Three lots of 100 non-scarified seeds each were randomly selected from the seed population. Seed down was carefully removed with a razor blade, but not to be confused with scarification as the seed coat was not injured. We took this measure because down on *V. doniana* seeds takes up a considerable amount of water during seed imbibition and introduces a bias into the results. Seeds were wrapped with an imbibed cloth (100% cotton) and stored for 24 h. The imbibed seeds were weighed to the nearest 0.001 g, before and after the imbibition test. Water intake by non-scarified seeds was

Table I.
Definition of dormancy-breaking treatments for seeds of *Vitex doniana* (Benin).

Treatment abbreviation	Treatments definitions
T0	Control: seeds from market (trial 1) or collected under a tree (trial 2)
Tc	Control: seeds harvested green at physiological maturity from tree but ripened with ash
T1	One hour soaking in sulphuric acid at 95% concentration and washing with tap water
T2	Three days sun drying + 48 h soaking in tap water
T3	Three days alternation of eight hours sun-drying + one hour soaking in tap water
T4	Two weeks sun-drying with regular watering at day time (“teak treatment”)
T5	Physical shock (seed coat fissuring)

expressed as: $\{W_{intake} = [(W_i - W_d) / Wd] \times 100\}$, where W_d = mass of dry seeds; and W_i = mass of imbibed seeds.

We used *V. doniana* seeds collected from various sources but used separately in a trial repeated twice (two cycles). Each trial was made up of a series of experiments. The first trial (A1) was undertaken from September 2011 to September 2012. The second trial (A2) was carried out between December 2011 and October 2012. Seeds used in trial A1 were bought from Akassato market (Abomey-Calavi district). Seeds used in trial A2 were from fallen fruits collected under a mother tree located in the same district (6°40’ N, 2°34’ E). An extra seed lot harvested at physiological maturity from a tree in the same area was used as a second control (Tc). These fruits were ripened (turning mature green fruit to dark black) by adding ash, as is traditionally done in the region. Therefore, five treatments were applied to seed lots in both trials and two control treatments were used (*table I*). The experimental design was a complete randomised block comprising five blocks of 10 seeds (total 10 seeds) per treatment, and seeds were sown in a black polystyrene nursery bag of 29,673 cm^3 ($\pi \times 15$ cm × 15 cm × 42 cm), with the hilum of the seed above the soil, and watered daily. A seed was registered as germinated when the embryo plant broke through the seed and soil and was visible outside. Seedlings were raised under ambient conditions on the initial soil, under shelter and were watered every day. No fertiliser was provided.

2.2. Data collection

Germination was recorded daily, from the day of sowing through to the end of the experiments, while seedling growth (height, number of leaves, basal stem diameter) was measured weekly. Measurement of growth parameters started two weeks after germination through to the end of the study.

2.3. Data analysis

To estimate seed lots’ germination ability, we calculated the germination percentage as: [$Germ_p = n_{gp}/N$], with n_{gp} being the number of seeds that germinated (n_g) in a seed lot or treatment p and N, the total number of seeds sown in the seed lot (50 for all lots). The germination speed was assessed by calculating:

a) the time to first germination (TFG),

b) the mean germination time (MGT) as:

$MGT_p = \sum_{i=1}^{k} ngpi \times ti / \sum_{i=1}^{k} ngpi$, where p is the seed lot, t_i is the time taken since the germination experiment started to date i, n_{gpi} is the total number of seeds in lot p that germinated at date i, and k is the time elapsed from the start to the end of the experiment.

c) the time necessary to reach 20% germination (t_{20}).

The generalised linear model with binomial and quasi-binomial (to account for over-dispersion) error structures was used

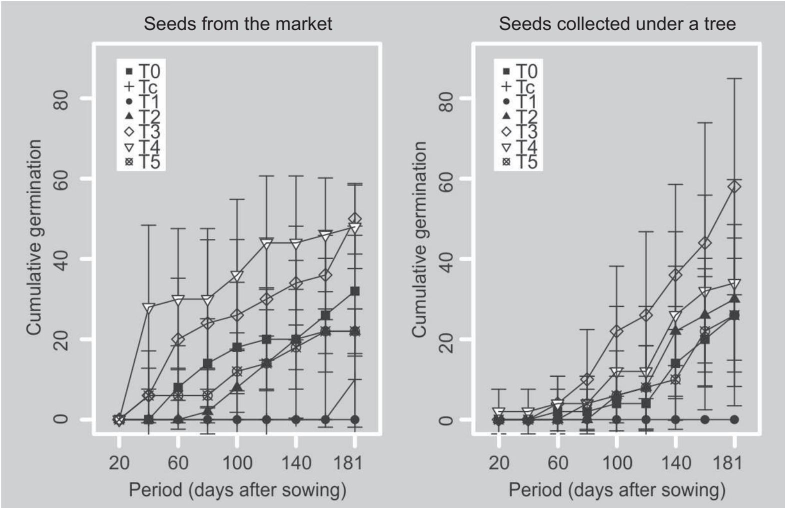

Figure 1.
Germination of *Vitex doniana* seeds. T0-Control: seeds from the market (trial 1) or collected under a tree (trial 2); Tc-Control: seeds harvested green at physiological maturity from a tree but ripened with ash; T1: 1 h soaking in sulphuric acid at 95% concentration and washing with tap water; T2: 3 d sun-drying + 48 h soaking in tap water; T3: 3 d alternation of 8 h sun-drying + 1 h soaking in tap water; T4: 2 weeks sun-drying with regular watering in the daytime ("teak treatment"); T5: physical shock (seed coat fissuring).

to test the effects of the different dormancy-breaking treatments on germination. Effects of the treatments on the time to first germination, the mean germination time and the duration to reach 20% germination were compared using the generalised linear model with a quasi-Poisson error structure. A mixed-effect model with the Akaike Information Criterion (AIC) was used to analyse the effects of fixed factors on seedling growth (diameter, length and number of leaves). Correlation tests and simple regression were used to analyse relationships among growth parameters. Statistical analyses were performed in R statistical software (version R.2.15.2, 2012), with nlme and gregmisc packages added to the basic ones.

3. Results

3.1. Effects of treatments on germination of *V. doniana* seeds

The results of the imbibition test on non-scarified seeds indicated a low water intake (W_{intake} = 19.9% of mass increase). After 6 months of the experiment, the germination percentage varied from 0% to 50% in trial A1, and from 0% to 58% in trial A2 (*figure 1*). In both trials, it clearly appeared that there was a significant difference in the treatment effects on germination ($p < 0.01$). Alternation of soaking and drying for 3 d (T3) is the best treatment for breaking dormancy in the species (50% and 58% germination rates for A1 and A2, respectively), followed by treatment T4 (2 weeks sun-drying with regular watering in the daytime) with 48% and 34% germination rates for A1 and A2, respectively. No germination was recorded for treatment T1 where seeds were soaked in sulphuric acid at 95% concentration for 1 h, removed and washed immediately with tap water. We also found that seeds harvested green at physiological maturity (Tc) did not germinate at all. The same trend in germination remained 12 months after sowing ($p < 0.05$), with seed lots T3 and T4 of trial A1 reaching 54% germination each. As for the trial A2, after 12 months of the experiment, there was a very significant difference in germination rates of seed lots which received treatments T3 and T4 (p = 0.01), with treatment T3 reaching a 72% germination rate.

3.2. Effects of treatments on germination speed

The germination speed was assessed using three parameters: the mean germination time (MGT), the time to first germination (TFG) and the time to 20% germination (t_{20}). In trial A1, the trends in these parameters indicated that treatment T4 showed the best performance, followed by T3 (*figure 2*). In trial A2 the trends in the same parameters indicated the best performance for T3, followed by T4 (*figure 3*). In general, seeds in trial A1 showed a higher germination speed than in trial A2 (*table II*). On average, the MGT of most seed lots was below 100 days in trial A1 (*figure 2*); in trial A2, it was higher (*figure 3*). On average, the shortest time before the first germination was recorded 29 days after seeds were sown in treatment T4 of trial A1 (*figure 2*). That treatment also showed the lowest t_{20} in trial A1, followed by T3 (*table II*). A similar trend was observed in trial A2, with the best performance obtained in treatments T3 and T4 (*figure 3*).

The generalised linear model analysis with a quasi-Poisson error structure revealed a significant difference in the effects of the treatments on germination speed of seed lots ($p < 0.001$). In trial A1, the best (lowest) and most significant MGT

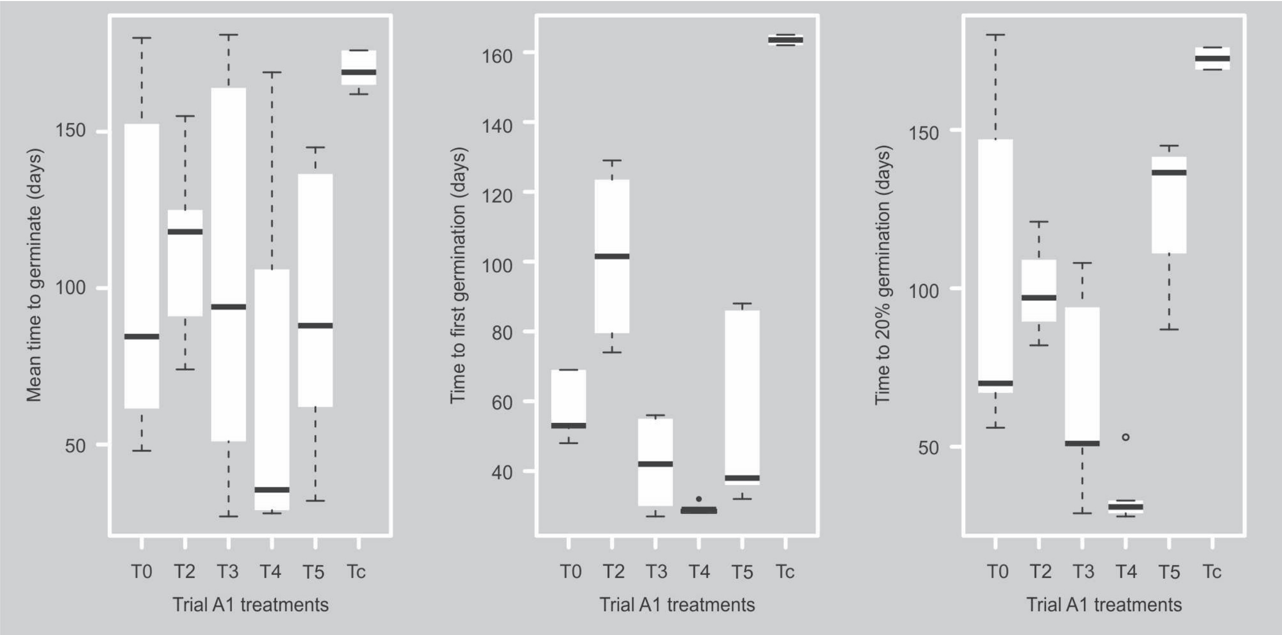

Figure 2.
Germination speed of *Vitex doniana* seeds in trial A1 (seeds from the market). T0-Control: seeds from the market; Tc-Control: seeds harvested green at physiological maturity from a tree but ripened with ash; T2: 3 d sun-drying + 48 h soaking in tap water; T3: 3 d alternation of 8 h sun-drying + 1 h soaking in tap water; T4: 2 weeks sun-drying with regular watering in the daytime ("teak treatment"); T5: physical shock (seed coat fissuring).

(65 d on average, $p = 0.007$) was observed in seed lot T4 and the highest value (169.6 d on average) in Tc (*table II*). The results of the GLM analysis with a quasi-Poisson error structure also indicated that the treatments applied significantly affected the time to first germination ($p < 0.01$), with quicker germination in T4 (29.2 d on average, with the earliest occurring at 28 d after sowing) and T3 (42 d on average, with the earliest occurring at 27 d). The highest TFG was observed in the Tc seed lot (after 160 d). As for the threshold set at 20% germination, there was a very significant difference among treatments ($p < 0.01$). Indeed, 20% of seeds germinated in the T4 and T3 seed lots before 40 d and 60 d after sowing, respectively. In contrast, even at 70 d, 20% germination was not yet achieved in the other seed lots (*figure 2*).

In trial A2, the average mean time for germination varied from 119 d (T4) to 136.53 d (T0) (*table II*). However, the statistical test indicated that the treatments applied did not seem to affect this parameter ($p = 0.202$). Meanwhile, the time for first germination and time to 20% germination were not significantly different among treatments ($p > 0.05$). Notwithstanding this, the highest germination speed was recorded for seeds in the T3 treatment followed by those in the T4 treatment.

3.3. Seedling growth

3.3.1. Main seedling stem diameter at ground level

The mean seedling stem diameter at ground level ranged between 0.378 mm and 2.27 mm in the first series of experiments, and between 0.68 mm and 2.92 mm in the second cycle. Our results revealed that, throughout the experiment, the best seedling diameter growth in the season of the trial A1 was noted in T4 and T3 seed lots (*figure 4*). The statistical test using mixed effect models indicated that the dormancy-breaking treatments applied very significantly affected seedling growth in diameter ($p < 0.001$). The T4 and T3 treatments showed a greater effect on seedling growth and constitute a homogeneous statistical group, followed by T0, T2 and T5. The slowest growth was recorded in the T5 and T2 treatments. In trial A2, treatment T3 had by far the greatest effect on seedling diameter growth ($p < 0.001$), followed by the T4 and T2 treatments (*figure 4*).

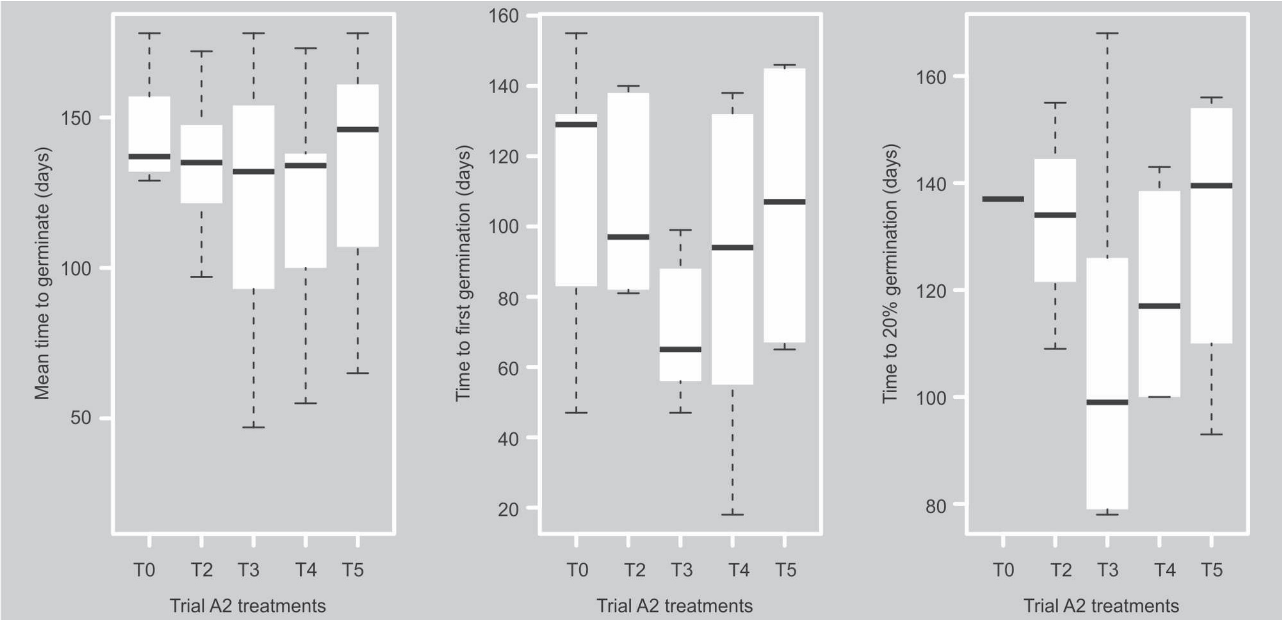

Figure 3.
Germination speed of *Vitex doniana* seeds in trial A2 (seeds collected under a tree). T0-Control: seeds under a tree; T2: 3 d sun-drying + 48 h soaking in tap water; T3: 3 d alternation of 8 h sun-drying + 1 h soaking in tap water; T4: 2 weeks sun-drying with regular watering in the day-time ("teak treatment"); T5: physical shock (seed coat fissuring).

3.3.2. Seedling height

The best seedling growth in height was recorded in the T4 and T3 seed lots (*figure 5*), in both trials. It is nonetheless worth mentioning that seedlings from seed lot T3 showed a remarkable height growth in the second series of experiments (*figure 5*). The smallest seedling height at the end of the experiment (1.14 cm) was noted in seed lot T2. Statistical tests revealed that the treatments applied greatly affected seedling growth in height, with a marked effect of T4 and T3 ($p < 0.001$). After 14 weeks of nursery monitoring in trial A2, the mean height of seedlings in seed lot T3 was more than two times higher compared with seedlings in other treatments. This is supported by the mixed effect model analysis, which indicated that the effect of treatments on seedling height was highly significant ($p < 0.001$).

3.3.3. Biomass production: growth of compound leaves

At 2 weeks' age, seedlings produced simple leaves, with two pairs in most cases. The generalised linear model analysis of leaf production revealed that there were highly significant differences in the effect of treatments ($p < 0.001$) in both trial series. In trial A1, there was no significant difference between seedlings of the T4 and T3 treatments, which produced more leaves at the nursery stage (4.7 leaves on average after 112 d) compared with those of the T0, T2 and T5 treatments (*figure 6*). In the second series of experiments, the treatment T3 showed the best effect on leaf production (*figure 6*) compared with the others ($p < 0.001$).

3.3.4. Correlation among growth parameters

Correlations among height, diameter and number of leaves produced by *V. doniana* seedlings were studied in the best-performing treatments (*table III*). The values of r ($r \geq 0.91$) and p ($p < 0.001$) observed indicated that there were robust and significant correlations among all the growth parameters that were investigated. Analysis of the regression revealed that seedling diameter and height explained production of leaves better.

4. Discussion and conclusion

4.1. Seed germination

The emerging studies dealing with seed dormancy in non-wood forest tree species have

Table II.
Effect of dormancy-breaking treatments on germination speed for seeds of *Vitex doniana* (Benin). Trial 1: seeds from the market; trial 2: seeds collected under a tree.

Treatments[1]	Mean germination time (days)		Time to first germination (days)		Time necessary to reach 20% germination (days)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
T0	104.75 ± 49.93 b	136.53 ± 35.65 a	66.8 ± 25.94 c	109.2 ± 43.48 a	104 ± 55.79 c	137 ± 0 a
Tc	169.6 ± 6.34 c	–	163.5 ± 2.12 e	–	172.5 ± 4.94 d	–
T2	111 ± 24.65 b	131.4 ± 27.5 a	101.5 ± 26.18 d	107.6 ± 29.36 a	100 ± 19.67 c	133 ± 18.81 a
T3	100.68 ± 55.15 b	123.72 ± 39.69 a	42 ± 13.54 b	71 ± 21.8 a	66.4 ± 33.15 b	110 ± 37.83 a
T4	65 ± 45.86 a	119.05 ± 37.92 a	29.2 ± 1.64 a	87.4 ± 51.13 a	34.8 ± 10.33 a	119.25 ± 22.52 a
T5	95.18 ± 44.86 b	133.84 ± 39.09 a	56 ± 28.39 c	106 ± 39.76 a	126.25 ± 26 c	132 ± 28.99 a
P value	0.007**	0.202 ns	0.0002***	0.123 ns	0.002**	0.266 ns

[1] For the treatment characteristics, see table I.
** Significant at α = 0.01; *** significant at α = 0.001; ns = not significant.
Means followed by the same letter within a column are not statistically different.

used a diversity of techniques to treat seeds. Most often these techniques are complementary but sometimes face the major problem of reproducibility. In our paper, the results were confirmed by duplicating the experiment in two trials. This study is the first of its kind whereby alternation of soaking in water and drying under sunshine was used to improve seed germination in *Vitex doniana,* although the principle was recently reported to be most successful on *Cassia leptophylla* Vogel and *Senna macranthera* (Collad.) H.S.Irwin & Barneby seeds by de Paula *et al.* [20].

Vitex doniana is known to have a hard seed coat, which induces very poor germination rates, and seedlings regenerated from seeds are scarce in natural habitats [21]. Many seed treatments have been suggested to break dormancy and improve germination without any conclusive recommendations. In 2005, the highest germination rate reported by Mapongmetsem *et al.* [9] was 11.25%. A few years later, Ky [13] reported in his review that 34% germination was reached in Côte d'Ivoire with stones dipped in sulphuric acid at 95% concentration, a fact not corroborated by our results. Here, no germination was recorded for sulphuric acid treatment, neither in the first trial, nor in the second one. Certainly, the seed embryo was damaged during soaking in the sulphuric acid. The situation raises two questions:

1) In which conditions is the use of sulphuric acid successful for *V. doniana* and how long should the seed be soaked in the acid to expect germination at a high rate?

2) How successfully can such a technique be taught to farmers while promoting the propagation of the species, knowing the risks associated with the misuse of sulphuric acid?

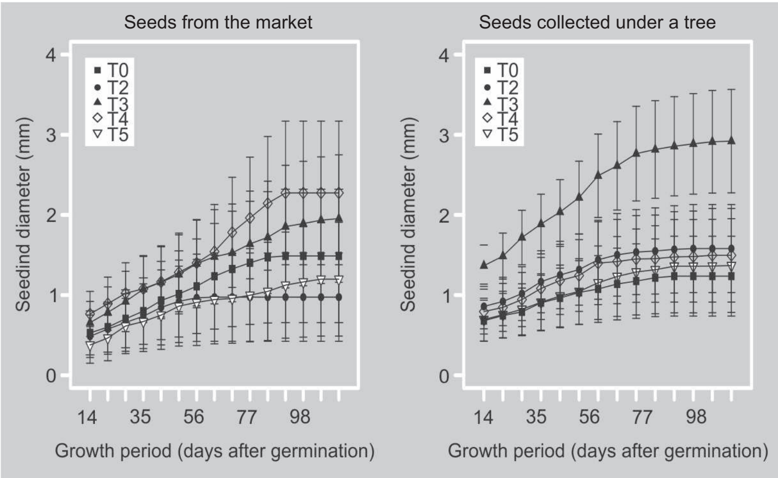

Figure 4.
Average diameter growth of *Vitex doniana* seedlings. T0-Control: seeds from the market (trial 1) or collected under a tree (trial 2); T2: 3 d sun-drying + 48 h soaking in tap water; T3: 3 d alternation of 8 h sun-drying + 1 h soaking in tap water; T4: 2 weeks sun-drying with regular watering in the daytime ("teak treatment"); T5: physical shock (seed coat fissuring).

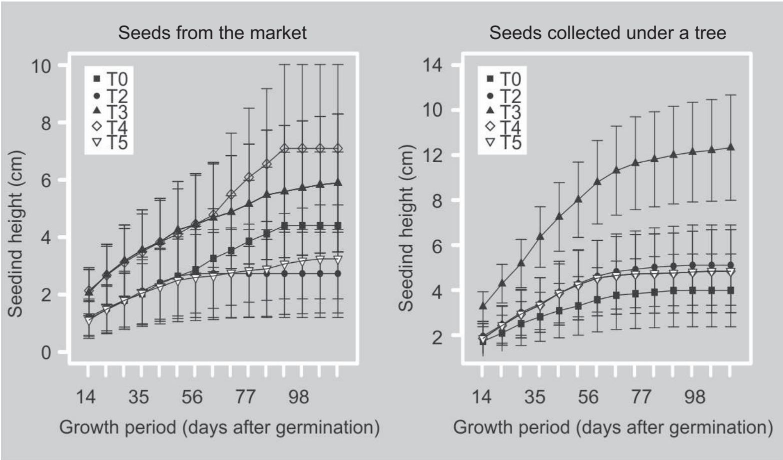

Figure 5.
Average growth in height of *Vitex doniana* seedlings. T0-Control : seeds from the market (trial 1) or collected under a tree (trial 2); T2: 3 d sun-drying + 48 h soaking in tap water; T3: 3 d alternation of 8 h sun-drying + 1 h soaking in tap water; T4: 2 weeks sun-drying with regular watering in the daytime ("teak treatment"); T5: physical shock (seed coat fissuring).

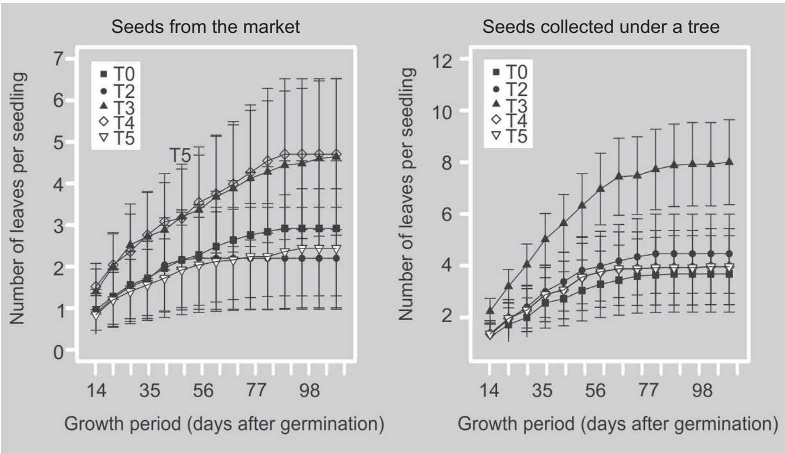

Figure 6.
Average leaf production by *Vitex doniana* seedlings. T0-Control : seeds from the market (trial 1) or collected under a tree (trial 2); T2: 3 d sun-drying + 48 h soaking in tap water; T3: 3 d alternation of 8 h sun-drying + 1 h soaking in tap water; T4: 2 weeks sun-drying with regular watering in the daytime ("teak treatment"); T5: physical shock (seed coat fissuring).

Based on the current knowledge the answers to these questions are not straightforward. This explains why the potential offered by our results offers ways toward effective domestication and horticultural production of a vegetable tree such as *V. doniana*.

At six months, in both trials, the best dormancy-breaking treatment in our study was T3 (three days' alternation of 8 h sun-drying + 1 h soaking in tap water), with 50% and 58% germination rates for each trial. A similar germination rate was reported with physical shock by Ahoton *et al.* [14]. The physical shock, in our investigation, exhibits 24% and 30% germination rates in trials A1 and A2, respectively. The difference in results might be explained by the absence of a standardised method for physical shock. The shock pressure may vary from one person to the other. After 12 months of the experiment, the highest germination rates remained in treatment T3 (54% and 72%, respectively, in A1 and A2). The highest germination exhibited by consecutive alternation of drying and soaking (T3) could be explained by suitable water uptake by water-impermeable layers of palisade dislocation, which is important for seed germination [22]. Furthermore, alternating drying and soaking induced temperature fluctuation within the seed coat and such fluctuation is reported to promote germination [23]. The ability of such hydration-dehydration treatments to improve seed germination had also been observed on *Ailanthus excelsa* Roxb. by Ponnuswamy *et al.* [24]. This is demonstrated by the lower germination rate in T2, a variant of drying and soaking with no alternation. Therefore, this confirms our hypothesis that the best dormancy treatment in *Tectona grandis* also works for *V. doniana*, which belongs to the same family. In trial A1, the second control (Tc) did not germinate before 6 months, in contrast to T0. Seeds used in Tc were harvested green at physiological maturity directly from the mother tree, while seeds in T0 were bought ripe in a market. Here, we suspect that the seed maturity might have an important role to play [25], and germination might be strongly associated with the maturity level. This is under the control of the ratio of germination inhibitors and promoters, which is responsible for physiological dormancy and decreases with seed maturity. The germination rates obtained in our study are significantly higher than the ones reported in Cameroon on another species of the same genus, *Vitex madiensis* Oliv., exhibiting 5.68% as the maximal germination percentage with manual scarification [26].

Regardless of the treatments that were applied, it was observed that the seeds bought from the market germinated better, compared with those seeds that were

Table III.
Correlations among growth parameters for seedlings of *Vitex doniana* (Benin). For all the treatments, the *P* value is < 0.001.

Treatments	Parameters tested	*r*-value	Regression
T3-Trial A1	Diameter-Height	0.97***	Number of leaves = 0.118 + 0.960 Diameter + 0.447 Height; (r^2 = 0.93, *p* = 0.000)
	Diameter-Number of leaves	0.95***	
	Height-Number of leaves	0.96***	
T4-Trial A1	Diameter-Height	0.97***	Number of leaves = 0.234 + 1.62 Diameter + 0.148 Height; (r^2 = 0.94, *p* = 0.000)
	Diameter-Number of leaves	0.97***	
	Height-Number of leaves	0.95***	
T3-Trial A2	Diameter-Height	0.95***	Number of leaves = 0.537 + 1.13 Diameter + 0.381 Height; (r^2 = 0.86, *p* = 0.000)
	Diameter-Number of leaves	0.91***	
	Height-Number of leaves	0.92***	

*** Correlation highly significant.

collected ripe under the mother tree. Most often, vendors collect most *V. doniana* fruits at physiological maturity and use a traditional process by adding ash to turn them ripe. The process takes some days before fruits are sent to market for sale, but the duration of storage varies from 3 to 7 d. The main issue here is that storage must be favourable for seed germination, but the duration and conditions are important issues that need to be further investigated.

Based on our results and previous dormancy investigations in the species, it appears that *V. doniana* exhibits a higher rate of physical dormancy (class D), according to Baskin and Baskin's [27] classification. Considering the results of seed coat imbibition and the fact that pre-treated seeds continue to germinate even after 12 months, we suspect a double dormancy (physical and physiological), a situation that will be investigated further.

4.2. Germination speed

Germination in *V. doniana* is generally known to be spread over a long period, but this can be shortened. In our study, the first germination was recorded 18 d after sowing in trial A2. The earliest germinations were recorded in T4 and T3, which used drying and soaking alternation. This effect was also observed on teak [28] and *Eryngium foetidum* L. [22] seeds. The time to first germination obtained, though slightly higher than the ones reported by Ahoton *et al.* [14], was in line with most forest species with a hard seed coat. For instance, in Saudi Arabia, *Juniperus procera* seeds were reported to start germination 21 d after sowing [29], whereas *Strychnos cocculoides* Baker seeds often germinated 28 d after sowing in Zambia [30]. Treated seeds of other species such as *Sorbus mougeotii* Godr. & Soy.-Will. took until 105 d to 119 d for the first germination [31].

Although earliness of germination is desired, the mean germination time (MGT) is also a useful parameter for measuring germination range and speed in time and expresses seed vigour. The MGT showed acceptable values with regards to the available literature. Faster germination was recorded in seeds submitted to moisture and heat stress; this was observed in our study, where the T4 and T3 treatments offered the lowest MGT. Another parameter used to measure germination speed in this research is the time to reach the threshold germination rate (the highest common germination rate reached in all treatments of a trial), which is 20% (t_{20}). This parameter, together with the MGT, is important and will probably determine the acceptance of any introduction of wild species into active domestication by farmers and horticulture professionals. In fact, the more homogenous the germination, the more economically profitable the nursery production. Our results revealed that, in 33 d, just over 1 month, 20% germination can be achieved with *V. doniana* seeds treated by alternating moistening and drying (T4 and T3). The lowest germination speed in T2 revealed that just soaking followed by drying is not sufficient, and this should be alternated to break the hard seed coat to allow layers' water intake.

4.3. Effect of treatments on seedling growth

The vigour of seedlings is known to be affected by seed and soil quality, water supply and other environmental conditions. In our paper, we highlight that, even if a quality seed is sown on a fertile soil with regular water supply, the vigour of the seedling will be affected by the time taken to germinate. In fact, the seed embryo is weakened over time if it takes longer than normal to germinate. Also, soil and other environmental conditions became poorer over time, as no fertiliser was supplied in this work. Seed dormancy-breaking treatments involving alternation of moistening and drying (T3 and T4) were the most effective. These treatments significantly affected *V. doniana* seedling growth. However, it is worth mentioning that in studies of this type authors often considered only the success event (germination) in their analysis of growth data, eliminating cases of failure (no germination). Doing this inevitably introduces a bias as failure could also be a result of the treatment. Instead, in the current research, we included all experimental units (all the 50 seeds that were initially sown) of each treatment. This way of analysing data eliminates the bias introduced by an unequal number of seedlings on the overall performance of a seed lot or treatment. However, the individual performance (growth) of seedlings was expressed based on the effective number of seedlings that germinated in each treatment, and it is shown that *V. doniana* is a fast-growing species (at least at the nursery stage), as reported by another study [14], in contrast to the common opinion. At 15 weeks' age, the height and diameter of seedlings on average were 18.67 cm and 5.98 mm, respectively; whereas they produced seven pairs of leaves on average. Nonetheless, the growth of the species should be monitored over years in horticulture or agroforestry systems.

The analysis of the growth data revealed two important phases in *V. doniana* development in the nursery. The first phase started from 14 d to 63 d after germination and the second phase from 63 d through to the end of the nursery period. The first phase tallied with the active seedling growth period, where leaf production started with two pairs in the best conditions and increased by an additional pair every week. As for the second phase, it could reflect two important phenomena corresponding either to vegetative rest or nutrient deficiency. In the case of the first phenomenon, it means that a vegetative rest can occur even at 2 months, and thus shorter than the 5 months reported in a previous study [14]. In this case, we would suggest taking *V. doniana* seedlings out of the nursery after 2 months. The results of the correlations among growth parameters at 6 months indicated that seedling height and diameter are strongly and positively correlated and both with the number of leaves produced. However, under some unfavourable environmental conditions, such as poor soil, poor light and pest damage by cutting the top of plants, these relationships might not be detectable.

4.4. Implications for horticulture prospects and genetic resource conservation

The trade of the parboiled leaves of *V. doniana* has for a long time sustained livelihoods in Benin and elsewhere in West Africa [6, 32]. The need for sustainable management of the current threatened wild populations calls for innovative practices to domesticate the species. Our results offer great potential for the effective propagation of black plum.

Seeds have the advantage of concentrating the highest genetic information of any other plant part. Thus, sexual propagation is preferable if there is a cost-effective means to use it to propagate a species. As multiplication by sexual reproduction is well handled, a strategic action might be to organise an *ex situ* conservation approach, studying the possibilities for cultivation in orchards or in intensively managed agroforestry systems. In fact, consumption of *V. doniana* leaves is significant among urban and peri-urban populations of Benin. A strategic way to continue providing such a commodity to consumers remains the integration of *V. doniana* trees in horticultural systems in urban and peri-urban settings. Producing *V. doniana* in horticultural systems will enlarge the resource base and keep pressure off natural stands. These stands are heavily harvested with consequences such as reduction of population size and structure, and the absence of juveniles for succession [21, 33].

If regenerating *V. doniana* using seed is possible, enhancing and homogenising seed germination is desired. Thus, reaching and reducing the time to 100% germination remain important challenges. Our investigation revealed that alternation of soaking and drying is the best seed treatment to achieve better germination and seedling growth. In view of the current findings the next steps towards domestication of *V. doniana* in Benin and West Africa should elucidate the effects of seed storage duration on seed viability and germination, length of soaking and drying alternation, the part played by physiological dormancy in the species, and the function-structure relationships of *V. doniana* trees in cultivation.

Acknowledgements

This research was supported by the New Alliance Trust and the African Forest Forum. We are also grateful to Mr Carlos A. Houdegbe and Mrs Chaldia O.A. Agossou for their help in the field during the experiment.

References

[1] Baskin J.M., Baskin C.C., Classification, biogeography, and phylogenetic relationships of seed dormancy, in: Smith R.D., Dickie J.B., Linington S.H., Pritchard H.W., Probert R.J. (Eds.), Seed conservation: turning science into practice, R. Bot. Gardens, London, U.K., 2003, 517–544.

[2] Cohn M.A., Hilhorst H.W.M., Alcohols that break seed dormancy: the anesthetic hypothesis, dead or alive?, in: Viémont J.D., Crabbé J. (Eds.), Dormancy in plants: From whole plant behaviour to cellular control, CABI Publ., Wallingford, U.K., 2000.

[3] Hilhorst H.W.M., Standardizing seed dormancy research, Methods Mol. Biol. 773 (2011) 43–52.

[4] Baskin J.M., Baskin C.C., New approaches to the study of the evolution of physical and physiological dormancy, the two most common classes of seed dormancy on earth, in: Nicolás G.K.J.B., Côme D., Pritchard H.W. (Eds.), The biology of seeds: recent research advances, Proc. 7th Int. Workshop on Seeds, CAB Int., Salamanca, Spain, 2003.

[5] Codjia J.C., Assogbadjo A.E., Ekué M.R.M., Diversité et valorisation au niveau local de ressources végétales forestières alimentaires du Bénin, Cah. Agric. 12 (2003) 322–331.

[6] Achigan-Dako E.G., N'Danikou S., Assogba-Komlan F., Ambrose-Oji B., Ahanchede A., Pasquini M.W., Diversity, geographical, and consumption patterns of traditional vegetables in sociolinguistic communities in Benin: implications for domestication and utilization, Econ. Bot. 65 (2011) 129–145.

[7] N'Danikou S., Achigan-Dako G.E., Wong J.L.G., Eliciting local values of wild edible plants in southern Benin to identify priority species for conservation, Econ. Bot. 65 (2011) 381–395.

[8] Dadjo C., Assogbadjo A.E., Fandohan B., Glèlè–Kakaï R., Chakeredza S., Houehanou T.D., Van-Damme P., Sinsin B., Uses and management of black plum (*Vitex doniana* Sweet) in Southern Benin, Fruits 67 (2012) 239–248.

[9] Mapongmetsem P.M., Benguellah M.B., Nkongmeneck B.A., Ngassoum M.B., Gübbük H., Baye–Niwah C., Longmou J., Litterfall, decomposition and nutrients release in *Vitex doniana* Sweet and *Vitex madiensis* Oliv. in the Sudano–Guinea savannah, Akdeniz Üniv. Ziraat Fak. Dergisi 18 (2005) 63–75.

[10] Oumorou M., Sinadouwirou T., Kiki M., Glèlè-Kakai R., Mensah G.A., Sinsin B., Disturbance and population structure of *Vitex doniana* Sweet in northern Benin, West Africa, Int. J. Biol. Chem. Sci. 4 (2010) 624–632.

[11] Ajenifujah-Solebo S.O., Aina J.O., Physicochemical properties and sensory evaluation of jam made from black-plum fruit (*Vitex doniana*), Afr. J. Food Agric. Nutr. Dev. 11 (2011) 4772–4784.

[12] Eyog-Matig O., Networking on forest genetic resources in sub-Saharan Africa with special attention to Bioversity-SAFORGEN Programmes, in: Vodouhe R., Atta-Krah K., Achigan-Dako G.E., Eyog-Matig O., Avohou H. (Eds.), Plant genetic resources and food security in West and Central Africa, Reg. Conf., Bioversity Int., Roma, Italy, 2007.

[13] Ky K.J.M., *Vitex doniana* Sweet, in: Louppe D., Oteng-Amoako, A.A., Brink M. (Eds.), Prota 7 (1): Timbers/Bois d'oeuvre 1, PROTA, Wageningen, Pays-Bas, 2008.

[14] Ahoton L.E., Adjakpa J.B., Gouda M., Daînou O., Akpo E., Effet des traitements de semences du prunier des savanes (*Vitex doniana* Sweet) sur la régénération et la croissance des plantules, Ann. Sci. Agron. 15 (2011) 21–35.

[15] Dabral S.L., Extraction of teak seeds from fruits, their storage and germination, Indian For. 102 (1976) 650–658.

[16] Suangtho V., Factors controlling teak (*Tectona grandis* Linn. f.) seed germination and their importance to Thailand, Austr. Ntl. Univ., Australia, 1980.

[17] Chacko K., John S., Asokan A., Evaluation of some pre-sowing treatments for germination of teak (*Tectona grandis* Linn. f.) fruits, Ann. For. 5 (1997) 55–56.

[18] Baskin J.M., Davis B.H., Baskin C.C., Gleason S.M., Cordell S., Physical dormancy in seeds of *Dodonaea viscosa* (Sapindales, Sapindaceae) from Hawaii, Seed Sci. Res. 14 (2004) 81–90.

[19] Phartyal S.S., Baskin J.M., Baskin C.C., Thapliyal R.C., Physical dormancy in seeds of *Dodonaea viscosa* (Sapindaceae) from India, Seed Sci. Res. 15 (2005) 59.

[20] de Paula A.S., Delgado C.M.L., Paulilo M.T.S., Santos M., Breaking physical dormancy of *Cassia leptophylla* and *Senna macranthera* (Fabaceae: Caesalpinioideae) seeds: water absorption and alternating temperatures, Seed Sci. Res. 22 (2012) 259–267.

[21] N'Danikou S., Diversity, management and conservation of wild edible plants in the Fon community of Agbohoutogon, South Benin, Bangor Univ., Thesis, Gwynedd, Wales, U.K., 2009, 65 p.

[22] Mozumder S.N., Hossain M.M., Effect of seed treatment and soaking duration on germination of *Eryngium foetidum* L. seeds, Int. J. Hortic. 3 (2013) 1046–1051.

[23] Hansen O.B., Leivsson T.G., Germination and seedling growth in *Abies lasiocarpa* (Hook.) Nutt. as affected by provenance, seed pretreatment, and temperature regime, Scand. J. For. Res. 5 (1990) 337–345.

[24] Ponnuswamy A.S., Chellapilla K.L., Vihaya-Ray R.S., Surendran C., Effect of collection date and hydration – dehydration treatment on seed viability and vigor of *Ailanthus excelsa* Roxb, Seed Sci. Technol. 19 (1991) 591–595.

[25] Loha A., Tigabu M., Teketay D., Lundkvist K., Fries A., Provenance variation in seed morphometric traits, germination, and seedling growth of *Cordia africana* Lam., New For. 32 (2006) 71–86.

[26] Mapongmetsem P.M., Domestication of *Vitex madiensis* in the Adamawa highlands of Cameroon: phenology and propagation, Akdeniz Üniv. Ziraat Fak. Dergisi 19 (2006) 269–278.

[27] Baskin J.M., Baskin C.C., A classification system for seed dormancy, Seed Sci. Res. 14 (2004) 1–16.

[28] Yadav J.P., Pre-treatment of teak seed to enhance germination, Indian For. 118 (1992) 260–264.

[29] El-Juhany I.E., Arel M.I., Al-Ghamdi M.A., Effects of different germination and early establishment of the seedlings of pre-treatments on seed of *Juniperus procera* trees, World Appl. Sci. J. 7 (2009) 616–624.

[30] Mkonda A., Lungu S., Maghembe J.A., Mafongoy P.L., Fruit and seed germination characteristics of *Strychnos cocculoides* an indigenous fruit tree from natural populations in Zambia, Agrofor. Syst. 58 (2003) 25–31.

[31] Jensen M., Effects of seed maturity and pretreatment on dormancy and germination of *Sorbus mougeotii* seeds, Scand. J. For. Res. 186 (2003) 479–486.

[32] Maundu P., Achigan-Dako E.G., Morimoto Y., Biodiversity of African vegetables, in: Shackleton C.M., Pasquini M.W., Drescher A.W. (Eds.), African indigenous vegetables in urban agriculture, Earthscan, London, U.K., 2009.

[33] Agossou O.A.C., Pressions anthropiques et stratégies locales de domestication de *Vitex doniana* Sweet dans la commune de Djidja au sud Benin, Fac. Sci. Agron., Univ. Abomey-Calavi, Mémoire, Bénin, 2011, 34 p.

Mejora de la germinación y del crecimiento de *Vitex doniana* Sweet para la horticultura y la conservación de los recursos genéticos.

Resumen – Introducción. Muchas especies de árboles frutales con semillas de tegumentos duros presentan una latencia problemática para su regeneración. Un ejemplo típico es *Vitex doniana*, una planta leguminosa y frutal autóctona de uso múltiple, aunque amenazada, que permite la subsistencia de numerosos hogares en África del Oeste. **Material y métodos**. En nuestro estudio, evaluamos cinco tratamientos de levantamiento de la latencia de las semillas. Testeamos el efecto del ácido sulfúrico en una concentración del 95% (T1); 3 d de secado al sol + 48 h de inmersión en agua corriente (T2); 3 d de alternancia de 8 h de secado al sol + 1 h de inmersión en el agua corriente (T3); 2 semanas de secado al sol con un regado regular durante el día (T4); y choque físico (T5). Estos tratamientos se compararon con dos testigos (T0 y Tc), constituidos respectivamente de semillas procedentes de dos fuentes diferentes. Se compararon el índice de germinación del lote (o tratamiento), el tiempo medio para la germinación de las semillas del lote, el tiempo para la primera germinación en el lote y el tiempo para alcanzar el tope del 20% de germinación, a continuación durante 15 semanas se hizo el seguimiento de la altura, el diámetro en el cuello de las plántulas y la producción de hojas. Utilizamos los modelos lineares generalizados, los modelos mixtos y los tests de correlación para comparar los efectos de los diferentes tratamientos en la germinación y el crecimiento de las plántulas. **Resultados y discusión**. T3 mejoró considerablemente la germinación de *V. doniana* (el 72% después de 12 meses). T4 dio una mejor homogeneidad de la germinación ($p < 0,01$), seguido de T3. El mejor crecimiento de las plántulas se obtuvo con los tratamientos T4 y T3. La alternancia del secado al sol seguido por una inmersión de las semillas en agua corriente, una técnica casi sin coste, mejoró la germinación de las semillas de *V. doniana* y el 20% de índice de germinación pudo obtenerse en 33 d, es decir casi 1 mes. Contrariamente a la opinión habitual, *V. doniana* es una especie de crecimiento rápido (en vivero). **Conclusión**. Deberían realizarse más investigaciones con el fin de determinar la duración óptima de la alternancia secado-inmersión que favorezca la germinación de las semillas, y obtener las poblaciones homogéneas.

Benin / *Vitex doniana* / domesticación de plantas / reproducción sexual / dormancia de semillas / germinación de las semillas / plántulas / índice de crecimiento