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Effects of scarification and darkness on nuts germination and plants growth of two local varieties of *Irvingia gabonensis (Aubry-Lecomte)*

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ABSTRACT
Objective: Improving the conditions of seedling production of wild mango trees may increase its growth. Here, we evaluate the effect of scarification on the germination capacity of nuts from two local varieties (*Wossro* and *Sissro*) of wild mango tree *Irvingia gabonensis* grown in Benin.
Methodology and Results: The ripe fruits were collected from two plantations and dried in the greenhouse for two days. One set of the nuts was scarified on the zygotic embryo pole and the second was maintained intact. A total of 300 nuts were sown in pots containing soil and cultivate in darkness and light conditions separately. Randomized complete block design with four replications was used. Nut scarification and darkness significantly influenced (p=0.0001) the germination rate related to the first two leaves' appearance duration. Cotyledon emergency, the growth in height, and the number of leaves of the plantlets were influenced by the genotype.
Conclusion and application of findings: The findings of this study showed that the cultivation on the dark of the scarified nuts inhibited the germination rate. The appearance time of the first two true leaves is shorter in the variety Sissro exposed to darkness and longer in the variety Wossro. It is also found that the scarification of nuts improved the seedlings height, internode length, vegetative tract development, and leaves length. These findings will be used to accelerate the germination rate of wild mango tree and facilitate rapid seeding production. The next step will be to form the seedlings producers for the use of scarification technic in their field.
Keywords: *Irvingia gabonensis*, scarification, germination improvement, growth, Benin

INTRODUCTION

Non-timber forest products (NTFPs) provide nutrients that are normally in low amounts in diets, such as certain proteins, vitamins, fibre, and minerals to prevent malnutrition (Caspa *et al.,* 2020). Approximately 1.6 billion of people depend on forest resources for their livelihoods, and 1.28 billion of these, or 80%, live in developing countries (FAO, 2008). However, NTFP producers are subjected to several types of threats such as slash and burn agriculture, population explosion (Akpaca *et al.,* 2017). This leaded to the forest ecosystems degradation and advanced deforestation that explains the changes in agricultural practices and the low living standards of rural populations who depend on the natural resources with high socio-economic value (Ernst *et al.,* 2010). In Benin republic, about 175 forest plant species are consumed for their leaves, fruits, seeds, roots, tubers and flowers. Among these natural species, the most exploited are *Adansonia digitata* L., *Vitellaria paradoxa* (Gaertn), *Parkia africana* (Jacq.) Benth, *Irvingia gabonensis* (Aubry-Lecomte), *Chrysophylum albidum* (G.Don) (Codjia *et al.,* 2003). For *Irvingia gabonensis*, different studies were carried out, among others, ethnobotanical aspects, socioeconomic and ecological importance (Dansi *et al.,* 2012; Akpaca *et al.,* 2017). *Irvingia gabonensis* is an underutilized specie in Benin, classified as a neglected crop (Dansi *et al.,* 2012). Its cropping cycle is undermined by many constraints among producers, who maintain the plantation trees out of preference or secondary interest in the resources provided by the species. It provides wood and energy and contributes to the coverage of nutritional needs, especially of the most vulnerable social groups (Gbesso *et al.,* 2015). It has been estimated that the market of *Irvingia gabonensis* represents about 50 million USD in turnover (Vihotogbé, 2012) and that there is very significant trade to markets in Nigeria, Gabon and Cameroon. According to Vihotogbé *et al*. (2014), "in Central and East Africa wild apple trees are a very important source of requirement in traditional uses". The best-known propagation technique is the plant transplanting after the germination of nuts in the forest. Traditional seedling and vegetative propagation techniques did not favour large-scale production after selection of fruits by variety according to the quality and high variability within the species. Although there are methods of vegetative propagation of *Irvingia gabonensis* (Aubry-Lecomte) with low success rates (Akpaca *et al.,* 2017; Vihotogbé *et al.,* 2014), the exploitation of this agroforestry resource continues to increase that affecting the continuity of production and plantation densities. Many studies have been conducted on the factors influencing the multiplication of the species by seedlings, notably germination under various temperatures, light intensity and physiological maturity of the fruits. According to Gnahoua, (2003) drying under particular conditions would allow the reduction of the germination time by the half from six to nine days. Vihotogbé et al. (2014) showed that darkness positively influenced the germination, and that drying time negatively influenced germination rate and the time of germination. Kouame et al. (2016) showed that nut extraction accelerated the germination to 5 days. However, extracting the kernel without altering the embryo is a tedious and constraining strategy, which can lead to losses of germination capacity of the embryo. Several other factors such as the variety, the presence of pests harming the seed could influence germination. It is within this framework that, this study was conducted on the effect of scarification and darkness on *in vivo* nut germination and seedling growth of local varieties of wild mango trees.

MATERIAL AND METHODS

Plant material: The plant material was essentially constituted of fruits of *Irvingia gabonensis*. The fruits were collected from two plantations of *Irvingia gabonensis* located in the village of Hounhomey in the township of Djakotomey in the district of Couffo. The selected plantations were geo-referenced between the geographic coordinates "6°50'17.04" North; "1°41'42.42" East and 6°50'36.34' North; 1°41'48.20" East (Fig.1). Fallen fruit were daily collected and the injured fruit were removed. With the help of producers, the distinction between the two local varieties *Wossro* and *Sissro* (Fig. 2B) was made by the water content of the fruit pulp, as assessed by the thumb pressure test on the exocarp. *Wossro* local variety was identified by the lower water rate.

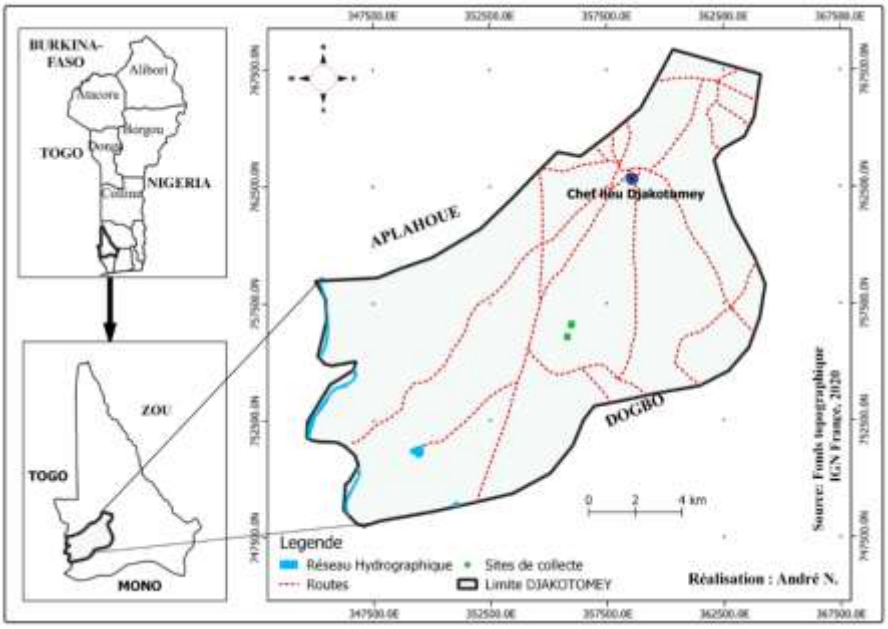


Figure 1: Collection sites of wild apple fruits (*Irvingia gabonensis* Aubry-Lecomte).

METHODS

Pre-germination treatments: After removing pulp from fruits, 304 nuts of both varieties were isolated. A half was scarified by using sandpaper and the second half was maintained unscarified. The scarified and unscarified seeds were sown in pots containing potting soil substrate at a depth of 3 cm. A part of the sowing seeds was exposed to dark and the second part to a light. After sowing, the pots were regularly watered once every 48 hours. The experimental design adopted was a complete randomized block with four factors where the block factor is considered as a random factor. Treatments were identified by the combination of the factors Variety*Scarification*Light* Block were randomized into units.

Data collection: Germination counts and measurement of growing parameters were performed every day until 48 days. A seed was considered germinated when cotyledons appeared as described by (Puy and Labat, 2012). To measure germination success according to the pre-germination treatments, the parameters evaluated were: the duration of appearance of the first two true leaves after the germination was observed, the final germination rate (TG) proposed by

Dominique, (2012); Puy and Labat, (2012) and the mean germination time (MGT) proposed by Ranal and Santana, (2006) by basing on the formula:

$$TG\ (\%) = (G*100)/N$$

with G, the total number of germinated seeds and N, the total number of sown seeds.

The mean germination time was estimated by the formula of Ranal and Santana, (2006) which stated that:

$$MGT\ (days) = \sum (ni \times ti)/N$$

where ni is the number of newly germinated seeds at time ti, ti is the number of days since the sowing date, and N is the total number of germinated seeds.

For growth, the data collected were: stem length (TL), number of leaves (NF), leaf length, and mean internode length (DB). The parameters calculated from these data were essentially averages. They were average stem length (LTMoy), average number of leaves (NFMoy), and average internode length (DBMoy).

Statistical analysis: The influence of the four factors variety, scarification, dark exposure and block on the kinetics of germination rate and germination speed was tested with the four-factor analysis of variance test for a generalized linear model. Concerning growth, the influence of nut scarification on the growth of seedlings of both varieties was tested with a three-factor analysis of variance, namely variety, scarification and block. The ANOVA test was performed in the R software version (RX643.0.1).

RESULTS

Germination kinetics: The pre-germination treatments had significantly influenced ($p< 0.0001$) the germination of *Irvingia gabonensis* nuts. Indeed, early germination was found on both varieties started from fifteen days (Fig. 3). The highest germination rate was obtained with the *Sissro* variety (51%) against (46%) for the *Wossro* variety (Fig. 2a). The germination rate evolved rapidly on the 20th day before showing a plate on the 33rd day. The highest germination peak was reached on day 46 for the *Wossro* variety against day 48 for the *Sissro* variety. Scarification had a significant influence ($p = 0.0001$) on the germination of both varieties. 55% of scarified nuts exposed to darkness were germinated against 36.66% of scarified nuts exposed to light (Fig. 2c). The unscarred nuts had a lower germination rate of 29% and 19.5% respectively for dark-exposed and light-exposed nuts respectively (Fig.2b). The dark exposition significantly improved ($p < 0.0001$) the germination rate of scarified nuts in the both varieties.

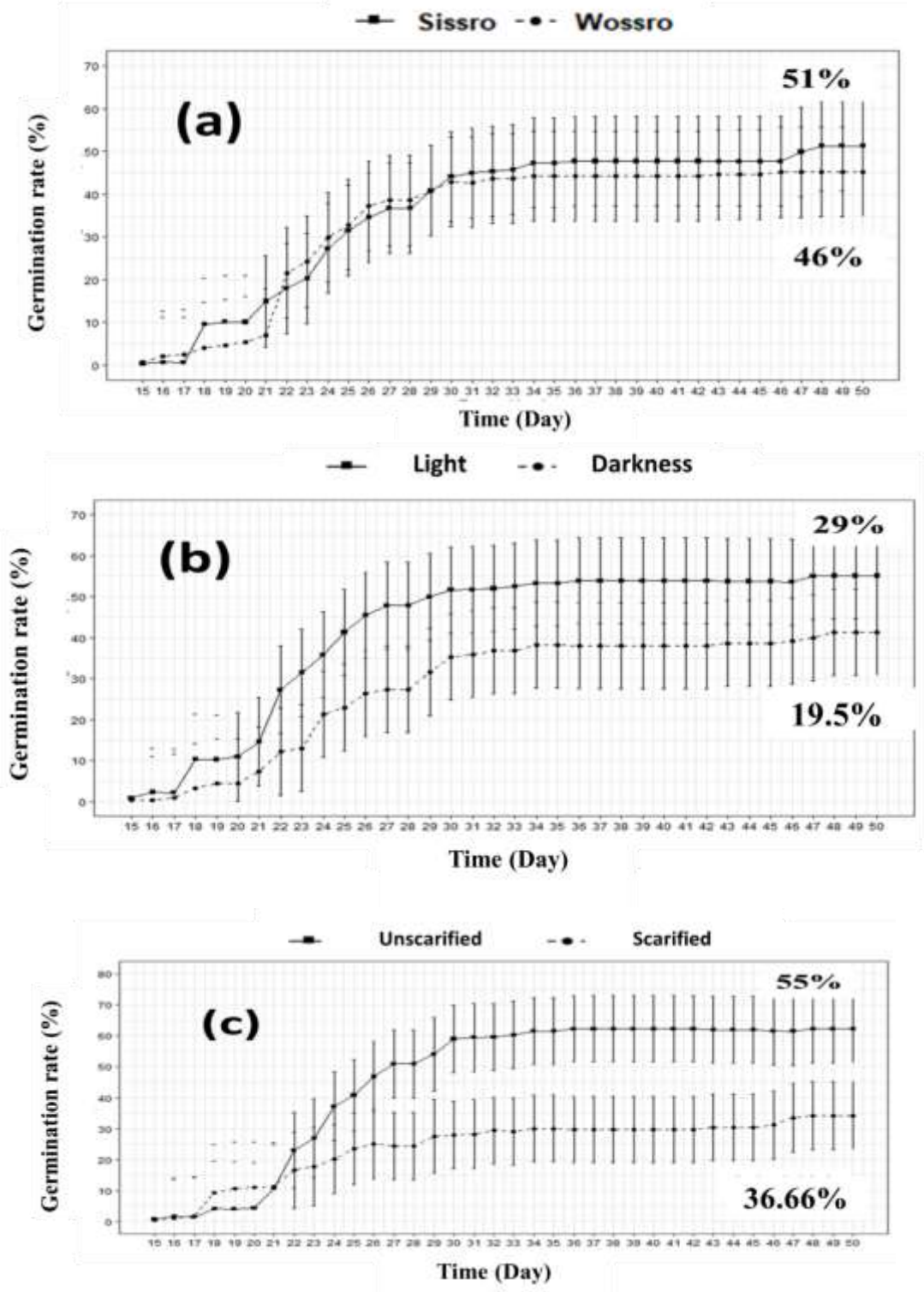


Figure 2: Kinetics of germination related to the variety (a), the light/darkness conditions (b), and the scarification treatment (c).

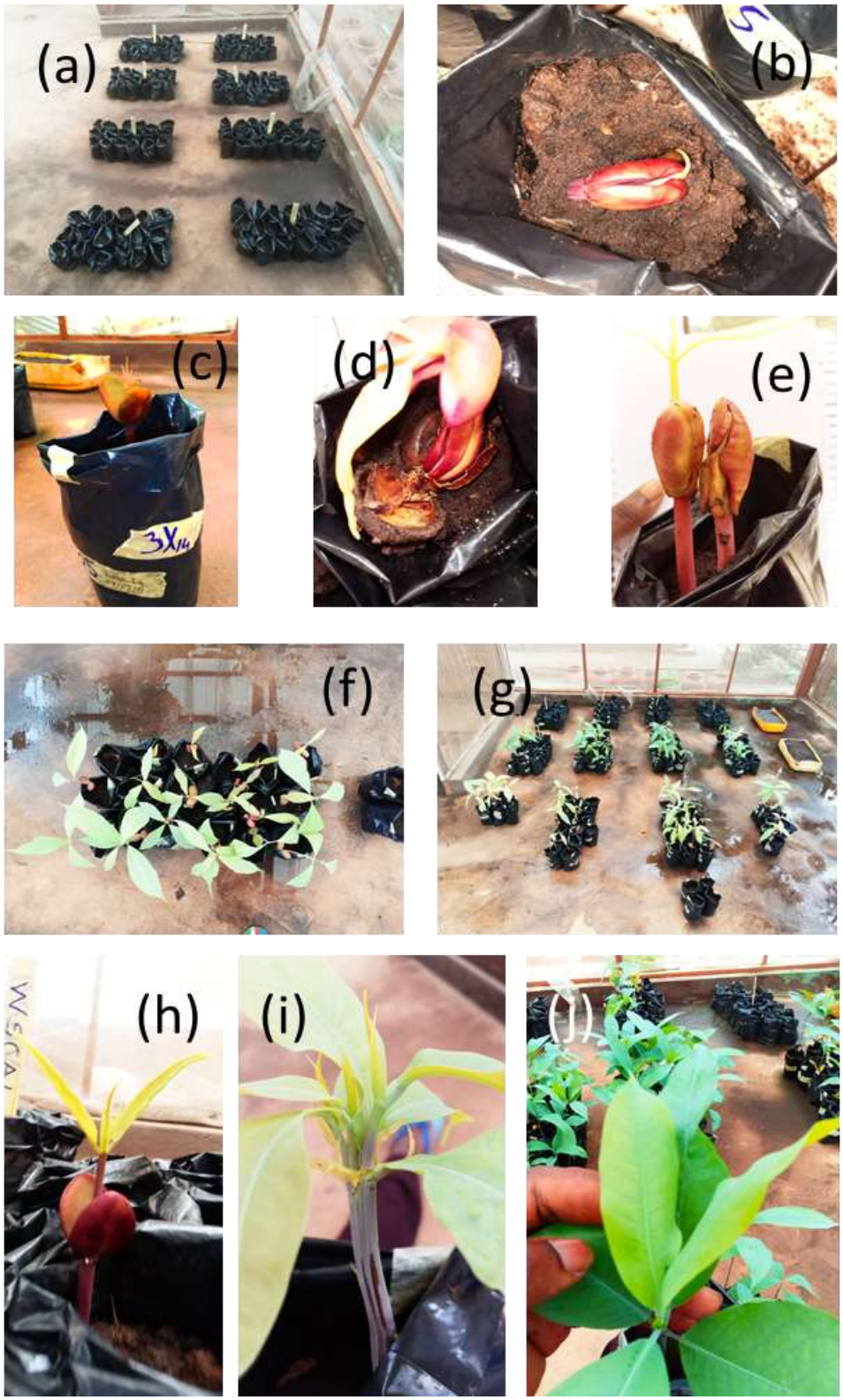
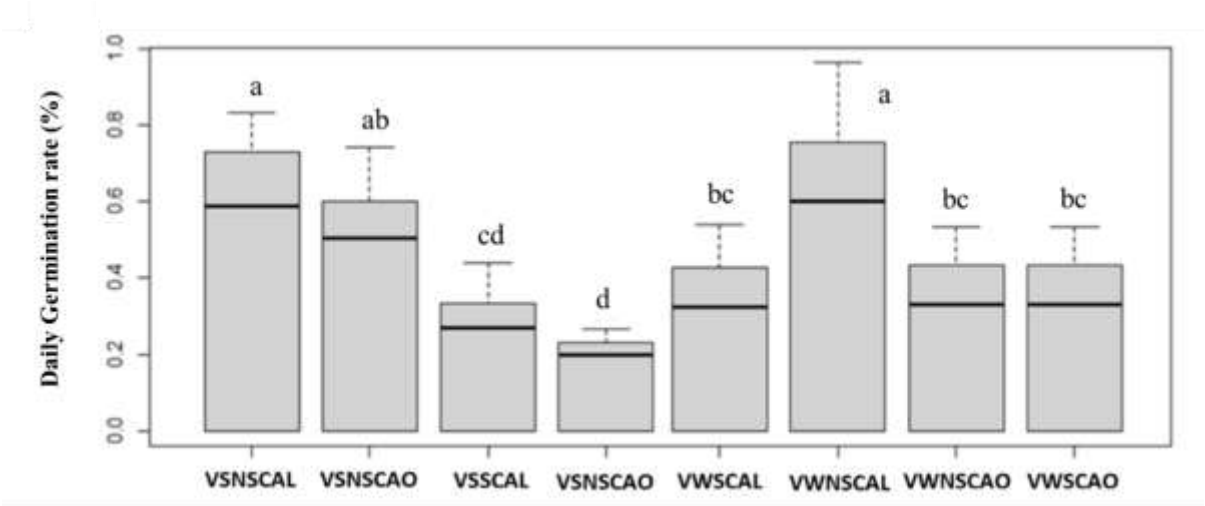


Figure 3: Steps of germination: the seeded pots (a), the evolution of germination under light (b), cotyledon emergence (c), plumule emergence (d), leafed out seedlings (e), (f), (g) and (h); leafing out development (i) and (j).

Effect of treatments on daily germination rate of nuts: The average rate of germination of nuts exposed to dark was 0.55 nuts/day for the variety *Sissro* whereas the nuts of the variety *Wossro* exposed to dark had an average time of germination of 0.36 nuts/day. When the nuts were scarified, the average rate of germination is low. For the local variety *Wossro*, the average rate of germination was 0.36 nuts/day for both the nuts exposed to light and those kept in the dark, whereas for the local variety *Sissro* the average rate varied from 0.22 nuts/day for the nuts kept in the dark to 0.36 nuts/day for the nuts exposed to light (Fig.4).

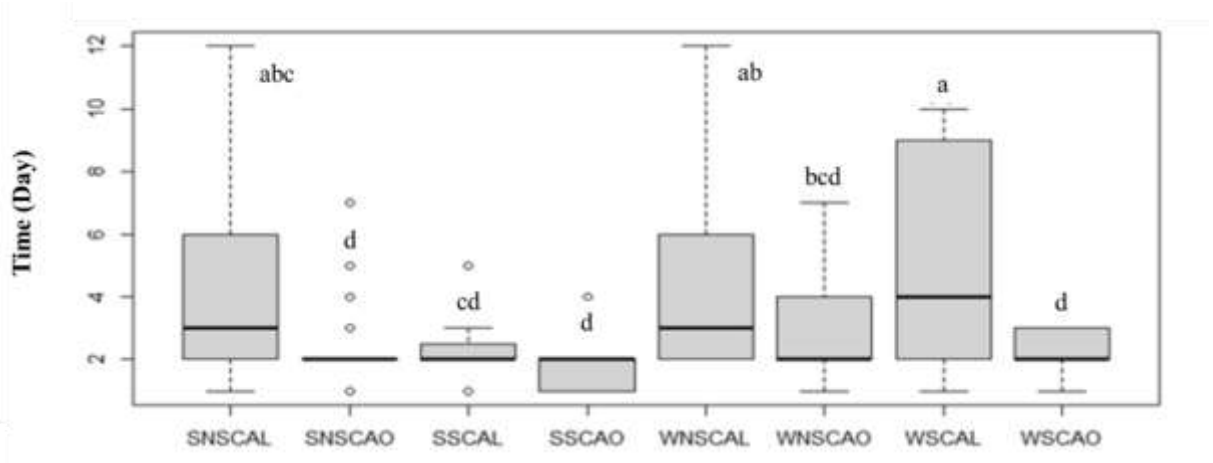


[VSNSCAL= Unscarred Sissro nuts exposed to light; VSNSCAO= Unscarred Sissro nuts exposed to darkness; VSSCAL= Scarified Sissro nuts exposed to light; VSSCAO= Scarified Sissro nuts exposed to darkness; VWSCAL= Scarified Wossro nuts exposed to light; VWNSCAL= Unscarified Wossro nuts exposed to light; VWNSCAO= Unscarified Wossro nuts exposed to dark; VSSCAO= Scarified Wossro nuts exposed to dark.]

Figure 4: Germination rates of type of nuts under different conditions.

Effect of treatments on plant growth

- **Growth at the two-leaves stage :** The time of formation to the first two true leaves was longer for *Wossro* seedlings, and shorter for *Sissro*. The seedlings that developed their leaves earlier were those of the *Sissro* variety from scarified nuts exposed to darkness. Scarification had a significant influence (p= 0.0008) on the time of formation of the first two leaves. In the scarified nuts exposed to darkness, the time of formation of the first two true leaves was shorter (2.27 days) in the variety *Sissro* compared to 1.87 days for those exposed to light (Fig. 5). In the variety *Wossro*, the time of appearance of the first two true leaves was 2.11days for the scarified nuts exposed to darkness against 5.14days those exposed to light. For seedlings from unscarred nuts exposed to both light and dark in both varieties, the time of formation to the first two leaves emergence were longer.



SNSCAL= Unscarified Sissro nuts exposed to light; SNSCAO= Unscarified Sissro nuts exposed to dark; SSCAL= Scarified Sissro nuts exposed to light; SSCAO= Scarified Sissro nuts exposed to dark; WNSCAL= Unscarified Wossro nuts exposed to light; WNSCAO= Unscarified Wossro nuts exposed to darkness; WSCAL= Scarified Wossro nuts exposed to light; WSCAO= Scarified Wossro nuts exposed to darkness.

Figure 5: Average time of appearance of the first two true leaves by treatment.

- **Growth after the two-leaves stage**

Stem length: The comparison of growth parameters for the scarification and non-scarification treatments for the two local varieties *Sissro* and *Wossro* showed that the scarification treatment had a highly significant effect ($p < 0.0001$) on seedling height growth (Fig. 6a). The length of seedlings from scarified nuts is higher (1.77 cm) than those from unscarified nuts (0.41 cm) a week after sowing (Fig. 6b). Until eight weeks, the length mean of seedlings is 21.03 cm in scarified nuts and 20.38 cm in unscarified nuts whatever the variety. It is found that although the differences between the seedlings of two varieties, the values of stem length mean remained close until eight weeks; 21.66 cm for *Sissro* and 20.25 cm for *Wossro*.

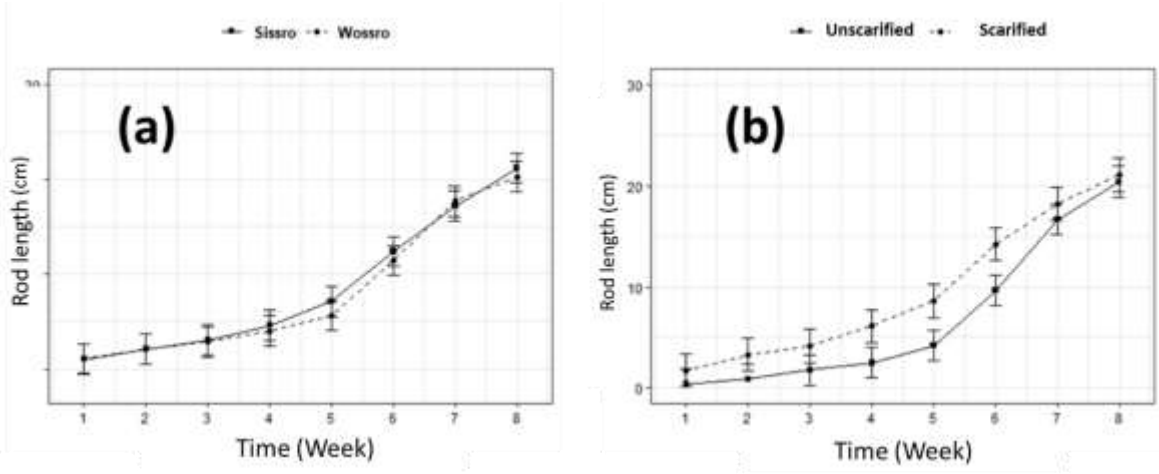


Figure 6: The height growth of seedlings after the two-leaf stage over time by (a) variety and by (b) scarification treatment.

Internode length: From five weeks, internode length mean of seedlings in *Wossro* was significantly different ($p \leq 0.0001$) from that of the *Sissro* variety (Fig.7a). Scarification had significantly increased ($p \leq 0.0001$) the internode length mean for seedlings from week 5 onwards (7b). Indeed, seedlings from scarified nuts had a mean internode length (2.16 cm) higher than those from unscarified nuts (1.67cm) whatever the variety (Fig. 7b).

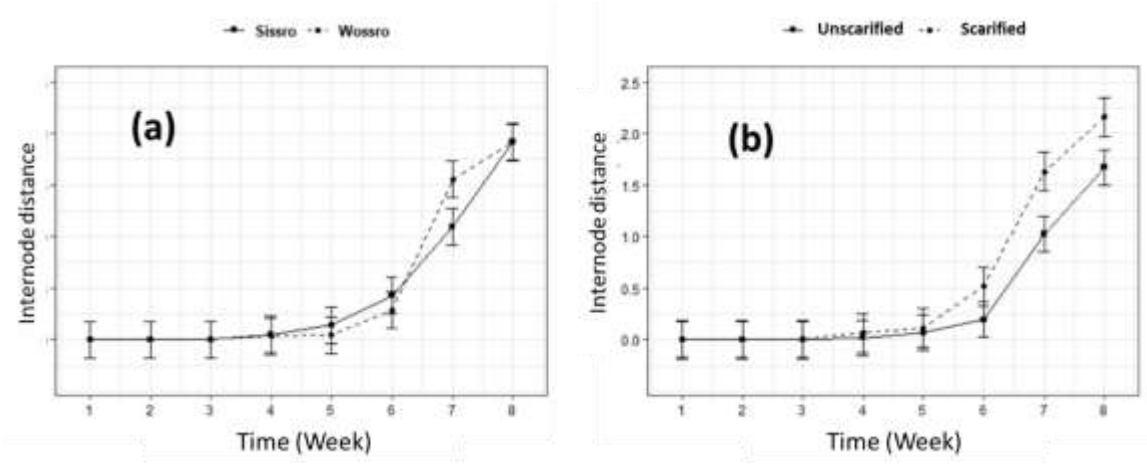


Figure 7: The evolution of growth from the internode length mean by (a) variety and by (b) scarification treatment.

Number of leaves: Observation of the evolution curves (Fig. 8a) showed that during the first five weeks, the plants of both varieties had one to two leaves on average. After the fifth week, the mean number of leaves increased very rapidly in *Wossro* variety, with mean a number of leaves reached 4.52 compared to 4.28 in *Sissro* variety. Scarification had positive and significant difference ($p \leq 0.0001$) on the development of the juvenile vegetative apparatus over time in germinated nuts of both local varieties of *Irvingia gabonensis*. Indeed, the curve (Fig. 8b) showed that the mean number of leaves from week 1 to week 8 is higher in seedlings from scarified nuts (4.59 leaves) compared to 4.21 leaves for unscarified nuts.

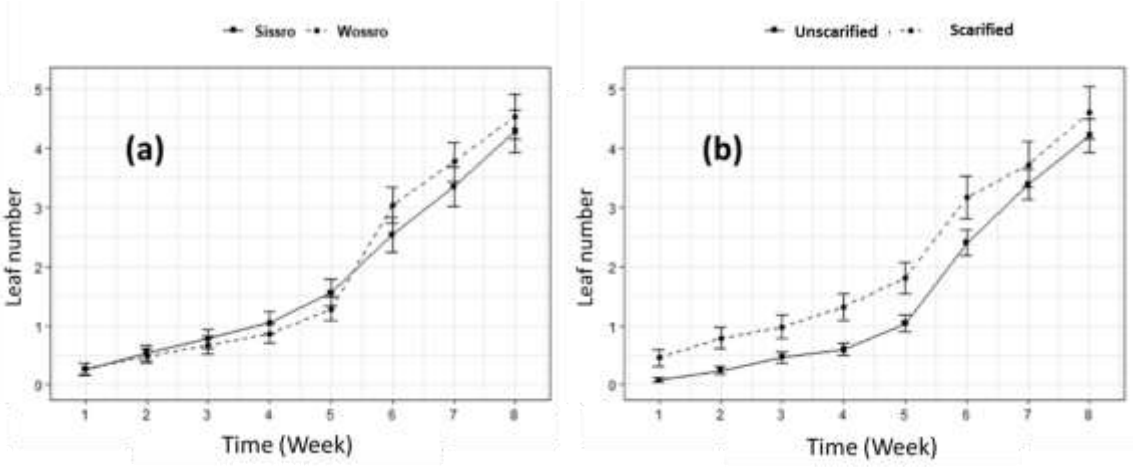


Figure 8: Evolution of the number of leaves according: (a) variety, (b): scarification treatment.

Average of leaf length: The average leaf length was significantly influenced ($p < 0.0001$) by the scarification. The average leaf lengths of seedlings from scarified nuts were longer (12.77 cm) than those from unscarified nuts (12.22 cm) (Fig. 9b). In contrast, leaf length did not vary in the two varieties *Sissro* and *Wossro* during the first five weeks (Fig. 9a). However, by the eighth week, the leaves of *Wossro* seedlings were moderately longer (12.79 cm) than those of *Sissro* variety (12.20 cm).

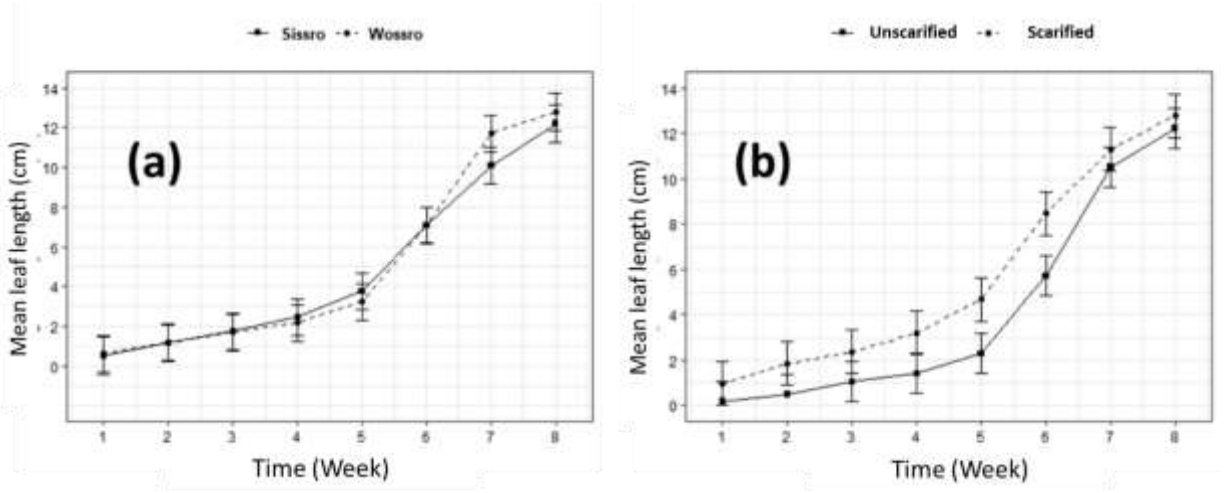


Figure 9: Evolution of the leaves length according to the (a) variety, according to the (b) scarification treatment.

DISCUSSION

For seedling propagation, epigeal scarification of dark-exposed nuts for both varieties showed better results. Indeed, the pre-germination scarification treatments had a significant influence on the germination rate of *Irvingia gabonensis* nuts. These results corroborate to those of Vihotogbé *et al.* (2014) who stated that germination rate depends mainly on drying time and germination conditions. Indeed, the fastest germinations were observed in nuts sown in the dark. However, scarification had a significant effect on the germination of nuts of both varieties with a better rate in the dark for scarified nuts. These results are contradictory to those of Vihotogbé *et al.* (2014) who stated that no pre-treatment (drying plus rehydration or scarification or special chemical treatment) is required to optimize the germination rate or time of germination of wild apples, considering the appearance of the first two true leaves as a sign of germination. Germination being defined as a process that is spread over time and whose signs and indices are quite variable, and are not limited to the appearance of the first two leaves but, involving a range of biological and physiological processes and their interactions with the environment (Bewleyl, 1997; Sanogo, 2015). According to Turner, (2010) during the germination process, the hypocotyl corresponding to the part of the seedling axis that connects the plumule to the radicle elongates in many species during germination until the vegetative apex of the plumule emerges above the soil. The appearance of the cotyledons could therefore be considered as one of the first visible signs of germination, followed by the appearance of the first two true leaves, which could be defined as the physiological response of the cotyledons' sensitivity to the presence of light (Turner, 2010). Indeed, the scarification treatment reduced the duration of first true leaf emergence for *Sissro* variety for both dark and

light treatments and in the *Wossro* variety only for the dark-exposed nuts. The variability within the results obtained could be explained by genetic and hormonal phenomena within the seed at the specific and varietal levels. The effect of nuts scarification on germination was confirmed for *Dialium guineense* willd (Fabaceae) (Assongba *et al.*, 2013), who claimed that scarification improved the germination rate of some species, and significantly reduced germination time by considering the appearance of the first two true leaves as a sign of germination, which was significantly reduced in scarified nuts of *Irvingia gabonensis*. Regarding the growth of seedlings after germination, the combination of light exposure after epigeal scarification had a significant effect (p= 0.0008) on the duration of the appearance of the first two leaves depending on the variety. Indeed, the minimum average duration of appearance of the first two leaves was two days regardless of the two varieties and treatments. However, the longest durations were obtained for the scarified nuts in *Wossro* variety exposed to light, and the shortest durations were obtained in *Sissro* variety exposed to darkness. For germination, considering the appearance of the first two leaves as the counting index, the treatment of scarified nuts in *Sissro* variety exposed to darkness gave the best results. Those results can be explained by the effect of scarification on the nuts. Indeed, scarification reduces the protective pressure of the nuts compared to the kernels, and facilitates the entry of water into the kernel and the awakening of the enzymes containing the latter for the embryos for the development of the first organs. Vihotogbé *et al.* (2014) found that high temperature and light conditions were ineffective in breaking the dormancy of recalcitrant wild apple nuts. Thus, the presence of high humidity of varying duration in the absence of light would reduce nut strength. On the other hand, the results of Kouame *et al*. (2016) showed that the extraction of the kernel from its endocarp not only allowed a gain of five days but also, would allow accelerating the growth of seedlings in plantation in wild apple trees. This is important because it could facilitate the evaluation of the effectiveness of pre-treatments on recalcitrant seeds. Several other hypotheses could be formulated in relation to the genotype effect because of the differences in the responses of the two varieties to the pre-treatments applied. Those responses could be attributable to the photosynthetic activity of the cotyledons (Turner, 2010) or to the nutrient composition of the kernels of the latter (Bewleyl, 1997). Regarding growth after the first two leaves appearance, scarification had a positive effect on height growth at the stem, internodes and leaves of both varieties. Indeed, observations of height growth from stem lengths showed that scarified nuts had much faster and distinctly different stem height growth from unscarified nuts through internode elongation. Kouame et al, (2016) made the same observations on the growth of plants from germinated kernels after kernel extraction. These results can be explained by the effect of initiating faster physiological development at the embryo level initiated by the scarification treatment on the nut. Although the genetic factor did not show great significance ($p \leq 0.3$), the phenotypic expression between variables with leaf lengths were much more expressed with the nut's scarification treatment. Gbesso et al. (2015) drew the same conclusions in the phenotypic differentiation process of these two local varieties.

CONCLUSION AND APPLICATIONS

The scarification pre-treatment influenced the average germination rate by reducing the germination rate of nuts exposed to darkness. In the light, the rate of germination in both varieties was longer. Thus, the comparison between the varieties showed that the time of

appearance of the first two true leaves was longer for seedlings in *Wossro* variety, and shorter in *Sissro* variety. The seedlings that developed their leaves earlier were those of *Sissro* variety from scarified nuts exposed to darkness. In addition, scarification reduced the duration of the first two leaves. For growth, comparison of the responses of seedlings from scarified and unscarified nuts of both varieties showed a highly significant effect of scarification on seedling height growth, increase in mean internode length, vegetative tract development and mean leaf length by increasing them independently of variety. This study results may help design strategies to optimize germination time and increase seedlings growth before plantation transplanting. They will be used to accelerate the germination rate on wild mango tree and facilitate rapid seeding production.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Akpaca, I., Yabi, I., Wokou, C.G., Tente, B.A.H., 2017. Caractérisation ds systèmes de culture de Irvingia gabonensis sur le plateau adja au sud-ouest du Bénin. Rev.Ivoir.Sci.Technol 29, 213–232.

Assongba, F.Y., Djègo, J.G., Sinsin, B., 2013. Capacité de germination de Dialium guineense willd (Fabaceae) une espèce Agroforestière. J. Appl. Biosci. 4566–4581.

Bewleyl, J.D., 1997. Seed Germination and Dormancy. Am. Soc. Plant Physiol. 9, 1055–1066.

Caspa, R.G., Nyambi, G.N., Amang, M.J., Mabe, M.N., Nwegueh, A.B., Foahom, B., 2020. Socio-economic Benefits of Non-timber Forest Products to the AFCOE2M Communities of Southern Cameroon. Sustain. Agric. Res. 9, 9.

Codjia, J.T.C., Assogbadjo, A.E., Mensah Ekué, M.R., 2003. Diversité et valorisation au niveau local des ressources végétales forestières alimentaires du Bénin Matériel et méthode. Cah. d'études 12, 19p.

Dansi, A., Vodouh, R., Azokpota, P., Yedomonhan, H., Assogba, P., Adjatin, A., 2012. Diversity of the Neglected and Underutilized Crop Species of Importance in Benin. Sci. World J. 2012, 19p.

Dominique, H., 2012. Ecologie de la germination des espèces indigènes de La Réunion. CIRAD-Université de la Réunion.

Ernst, C., Verheggben, A., Mayaux, P., Hansen, M., Defourny, P., 2010. Etat des forêts de l'Afrique Centrale: cartographie du couvert forestier et des changements du couvert forestier en Afrique centrale. Rapport d'évaluation. COMIFAC, 20.

Eyog, M.O., Gaoué, O.G., Dossou, B., 2002. Programme de ressources génétiques forestières en Afrique au sud du Sahara Programme de ressources génétiques forestières en Afrique au sud du Sahara1 241p.

F.A.O, 2008. Analyse des aspects socio-économiques des produits forestiers non-ligneux (PNFL) en Afrique Centrale.

Gbesso, F.G.H., Lougbegnon, T., Sossou Agbo, P., 2015. Critères de reconnaissance paysanne et variabilité phénotypique de deux variétés de Irvingia Gabonensis Aubry-Le Comte dans le Sud-Ouest du Bénin. Rev.Ivoir.Sci.Technol 26, 99–114.

Gnahoua, G.M., 2003. Irvingia gabonensis un fruitier sauvage d ' avenir. In: Boborou.

Kouame, N.M.-T., Mangara, A., Soro, K., N'Guessan, K., 2016. Étude de la germination des graines de Irvingia gabonensis, Centre-Ouest de la Cote d'ivoire dans la région du GÔH. Passage de paris 13, 545–555.

Puy, D., Labat, 2012. Traitement de scarification pré-germinatif des graines pour une restauration écologique des zones dégradées Cas de Millettia taolanaroensis (Fabaceae). Biodiversité des écosystèmes intertropicaux 43, 637–647.

Ranal, M.A., Santana, D.G.D.E., 2006. How and why to measure the germination process ? Rev. Bras. Bot. 2, 1–11.

Sanogo, I.S., 2015. Essais de germination et conservation de fruits et graines de quelques espèces ligneuses à usages multiples au Mali. University of Gand, Belgique.

Turner, M., 2010. Les semences, 1st ed. Center for Technical Agriculture.

Vihotogbé, R.., Houessou, L.G., Assogbadjo, A.E., Sinsin, B., 2014. Germination of seed from earlier fruits of bitter and sweet African Bush Mango Trees. African Crop Sci. J. 22, 291–301.

Vihotogbé, R., 2012. Characterization of African Bush Mango trees with emphasis on the differences between sweet and bitter trees in the Dahomey Gap (West Africa) Romaric Vihotogbe. Wageningen University.