Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 11(2): 597-608, April 2017

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Effects of substrates, different pretreatment protocols and dehydration on the induction of seeds germination of *Xylopia aethiopica* (Dunal) A. Rich.

Gabriel KANMEGNE[1*], Delphine Ache MBIBONG[1], FOTSO[2] and Dénis Ndoumou OMOKOLO[3]

[1]*Department of Plant Biology, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.*
[2]*Department of Biology, Higher Teacher's Training College of Bambili, University of Bamenda, P.O. Box 39 Bamenda, Cameroon.*
[3]*Department of Biological Sciences, Higher Teacher's Training College, University of Yaoundé I, P.O. Box 47 Yaoundé, Cameroon.*
[*] *Corresponding author, E-mail: gkanmegne@yahoo.fr , Tel: + (237) 677 49 00 69*

ABSTRACT

Despite its socio-economic importance, the cultivation of *Xylopia aethiopica* is not popular owing to the difficulty in seed germination. As a step in its domestication process, investigations were undertaken on germination requirements and desiccation tolerance of seeds. Three substrates (forest top soil, river sand and a mixture of forest top soil and river sand) and 18 pre-germination treatments including a control (untreated seeds), a mechanical scarification, six heat treatments which were done by soaking seeds in hot water (100 °C) for different lengths of time, and ten acid scarifications which were done by soaking seeds in either sulfuric acid or hydrochloric acid for different lengths of time were tested for their effect on seed germination. Results showed that mean percentage germination was higher on the mixture of forest top soil and river sand (29.4 ± 2.6%) than on other substrates tested. While untreated seeds and those soaked in hot water irrespective of the duration of treatment failed to germinate, seed dormancy was successfully broken by either mechanical or chemical scarification. Soaking seeds in either concentrated HCl or concentrated H_2SO_2 for 5 min were the most effective treatments in breaking dormancy, with 80 ± 6.3% and 70 ± 6.3% mean germination recorded respectively. The desiccation tolerance test showed that *X. aethiopica* seeds are desiccation-tolerant and their storage behaviour is orthodox. This study shows that for propagating *X. aethiopica* from seeds, it is recommended that fresh or dried seeds be soaked in either concentrated HCl or concentrated H_2SO_4 for 5 min, and that seeding be done in substrate composed of a mixture of forest top soil and river sand in a 1:1 (v/v) ratio.

Keywords: Spice tree, domestication, seed dormancy, scarification, desiccation tolerance.

INTRODUCTION

In the humid tropics of West and Central Africa, forest tree species provide an array of medicinal, nutritional and industrial produce, which are of direct relevance to the well being of the people (Gbadamosi and Oni, 2004; Kokutse et al., 2014; Alaba et al., 2015). Most of these woody perennials have multiple uses, and wherever grown, they are highly valued by the local farmers (Atangana

DOI https://dx.doi.org/10.4314/ijbcs.v11i2.6

et al., 2014). However, very little scientific knowledge is available on the growth, management and propagation needs of these species (Sinclair and Joshi, 2001). With the prevailing high demand for Agroforestry species, there is a growing need for developing simple and easy methods of growing and propagating such indigenous tree species (Leakey and Tchoundjeu, 2001).

Xylopia aethiopica (Dunal) A. Rich. is an evergreen, aromatic tree, of the Annonaceae family (APG III, 2009), that grows up to 30 m high and about 60 – 70 cm in diameter (Orwa et al., 2009). It is a native to the lowland rainforest and moist fringe forest in the savanna zones of Africa, but largely found in West, Central and Southern Africa. These trees are widely distributed in the humid forest zones especially along rivers in the drier area of the region (Orwa et al., 2009). Its common names include; African pepper, Guinea pepper, spice tree, negro pepper, West African pepper and Senegal pepper. It is one of the most high valued forest tree species of West and Central Africa where it is used extensively in African cuisine, construction and traditional medicine (Erhirhie and Moke, 2014). Indeed, the fruits serve as spice and flavouring agent in many African countries, and they used to be exported to Europe as pepper substitute (Fleischer, 2003). The stem wood on the other hand is used for house posts, scantlings, paddles, bows and fuel among the indigenes (Fleischer, 2003; Orwa et al., 2009). Almost every morphological part of the plant is used for medicinal purposes. They have been employed either as multi-component or single component remedies. The fruits form a common ingredient in many African traditional remedies in which they serve either as an excipient or active ingredient (Fleischer, 2003). Fruits and stem bark decoction are used in the treatment of bronchitis, biliousness, dysentery, asthma, arthritis, neuralgia, chronic pain, and they are suspected to enhance fertility and aid delivery (Asekun and Adeniyi, 2004). The fruits extract has been shown to be used as antimicrobial agent against gram positive and gram negative bacteria (Tatsadjieu et al., 2004). *X. aethiopica* has anti-spirochoectal properties so that it works both as a preventive measure and in treatment of primary, secondary and tertiary stages of syphilis. *X. aethiopica* has been used for treating rheumatism as well as other inflammatory conditions (Tatsadjieu et al., 2004). Other medicinal uses of *X. aethiopica* include the treatment of coughs, amenorrhea, dizziness, and headache. The pharmacological properties of different *X. aethiopica* plant parts have been attributed to numerous phytochemicals (alkaloids, saponins, tannins, flavonoids, anthraquinones, steroids and glycosides) they contain and which exhibit a wide range of biological effects as a consequence of their antioxidant properties (Fleischer, 2003; Keita et al., 2003). *X. aethiopica* is one of the many non-timber forest products that are of socio-economic importance. It commercialization contributes substantially to the socio-economic uplift of the people of its area of distribution.

Despite its socio-economic importance, the cultivation of *X. aethiopica* is not popular owing to the difficulties encountered by farmers in attempting to raise seedling in nurseries. Thus the trees are still exploited in the wild because they are not domesticated (Alaba et al., 2015). The seeds of the species are difficult to germinate hindering its domestication process. The germination difficulty in *X. aethiopica* seeds have been reported to be due to its hard coat (Udosen and Sam, 2015). Therefore, overcoming the

problem of coat-imposed seed dormancy becomes imperative, as seed germination is crucial to the regeneration of the species. A high number of previous researches reported that coat-imposed seed dormancy can be broken through scarification (mechanical and chemical) in addition to soaking the seeds in hot water (Gbadamosi and Oni, 2004; Hessou et al., 2009; Alaba et al., 2011; Ibiang et al., 2012). Nevertheless, the application and effectiveness of these treatments depend on the degree of dormancy, which varies between different species (Oliveira et al., 2003; Nascimento et al., 2009), making it difficult to prescribe a standard procedure for breaking coat-imposed seed dormancy. Different species with coat-imposed seed dormancy may require different pre-germination treatment to break dormancy and allow germination. For *X. aethiopica*, protocol for breaking seeds dormancy and enhancing germination remains unknown and, on the other hand, seed germination responses to other factors like germination substrates and seed dehydration are still to be documented. Indeed, among the different species of West and Central Africa that are of socio-economic importance, *X. aethiopica* has suffered neglect in the area of research and development.

As an important step in the process of *X. aethiopica* domestication, this study was carried out with views to 1) identifying and recommending the appropriate substrate and pretreatment protocol for breaking dormancy and enhancing the germination of seeds and 2) determining the desiccation sensitivity and storage behaviour classification of seeds.

MATERIALS AND METHODS

Seed material

Mature and disease-free pods of *X. eathiopica* were harvested from a single tree in March 2016 in Bambili, a location situated in the western highlands of Cameroon (5°58'60''N, 10°15'E, altitude 1350 m), together with local top-soil samples and immediately brought to the Laboratory of Applied Botany, Department of Plant Biology, University of Dschang (5°27'N, 10° 3'E, altitude 1,400 m). In the laboratory, mature black coloured seeds were extracted form pods 24 hours after harvest and used for the determination of initial characteristics (i.e., viability percentage and moisture content) and for further experiments.

Viability test

Seed viability was determined using 2,3,5-triphenyl-tetrazolium chloride (TZ). The TZ staining procedure is a standard test prescribed by the Association of Official Seed Analysts (AOSA) to determine the percentage of viable seeds in a batch; it was as follows: Thirty seeds from the sample were hydrated for 24 h at room temperature (24 ± 1 °C), then they were cut longitudinally and medially through the embryo and incubated in 0.1% TZ solution at 30 °C in the dark for 5 h after which they were washed several times with distilled water to remove excess solution and examined for color change (AOSA, 2000).

Moisture content determination

Moisture content determination was done by the oven dry method which consisted in weighing seed samples before and after drying them in the oven at 103 °C for 17 h. Each sample contained ten seeds. Moisture content, which was expressed as a percentage of fresh weight, was calculated using the formula MC % = [(FW – DW)/(FW)] × 100. Where FW (fresh weigh) is weigh of sample before drying and DW (dry weigh) is weigh of sample after drying (ISTA, 2004). The value of the moisture content was the mean of six

measurements at each time (six replications of ten seeds).

Germination assay

To investigate on *X. aethiopica* seeds germination, fresh seeds were extracted from pods 24 h after harvest and immediately used. A total of 18 pre-germination treatments were tested for their effects on seeds germination. These treatments involved a control (seeds sown without any treatment); a mechanical scarification (MS) consisting in peeling off the seed's coat using a sharp blade; six heat treatments consisting in soaking seeds in hot water (100 °C) for 30 sec, 1 min, 2 min, 3 min, 4 min, and 5 min, immediately followed by rapid cooling at room temperature using cool water; five sulfuric acid treatments consisting in soaking seeds in concentrated H_2SO_4 for 5, 10, 15, 30 and 60 min; and five hydrochloric acid treatments consisting in soaking seeds in concentrated HCl for 5, 10, 15, 30 and 60 min. Immediately after acid treatments, seeds were washed thoroughly with distilled water before sowing. Seeds from each pre-germination treatment were sown 2 cm depth in black plastic perforated polythene bags (20 cm high and 15 cm diameter) filled with substrate which was either forest top soil, river sand or a mixture of forest top soil and river sand in 1:1 (v/v) ratio. 25 seeds were sown in each polythene bag. The seeded polythene bags were then placed in the nursery (22 ± 3 °C) at natural photoperiod (i.e. 12 hours/day). A total of 4050 seeds were sown in three blocks of a split plot experimental design. Each main plot contained three substrates (forest top soil, river sand and 1:1 (v/v) mixture of forest top soil and river sand), whereas 18 different treatments were tested at the subplot level. Treatments were assigned at random to experimental units so as to have 3 substrates X 18 treatments X 3 replications X 25 seeds. Manually, water was applied daily to the seeded polythene bags so that the medium (substrate) was kept moist without getting waterlogged.

Seeds which emerged above the surface of the substrate as a consequence of hypocotyls elongation were recorded as having germinated. Germination was recorded daily, and the experiment was finished after 90 days, when no further germination was observed over a period of four consecutive weeks.

Desiccation tolerance test

Fresh seeds which were extracted from mature pods 24 hours after harvest were spread in a single layer on the laboratory bench top and left to dry under shade at laboratory temperature (24 ± 1 °C). Laboratory relative humidity was 55 ± 5%. At 1-week intervals, seed samples were withdrawn for moisture content measurement and germination test.

Moisture content measurement was done as described above. For the germination test, three replications of 25 seeds were also withdrawn at 1-week intervals and allow to germinate in the nursery at 22 ± 3 °C, natural photoperiod (i.e. 12 hours of light per day) and in black polythene bags filled with a mixture of forest top soil and river sand in a 1/1 (v/v) ratio as substrate. Prior to germination test, seed dormancy was broken by soaking seeds in concentrated hydrochloric acid for 5 min, followed by thorough washing with distilled water. The seeded polythene bags were then regularly watered and the record of germination ran for 90 days as described above.

Data analysis

Data analyses were performed using SPSS 12.0 software package. The dependent variable was the mean germination

percentage, whose data were transformed into arcsine square root values before statistical analysis. Analyses of variance (ANOVA) were performed to detect the level of significance of the main effects of germination substrates and pre-germination treatments, as well as the significance of their interaction effect. Means that exhibited significant differences ($P < 0.05$) were further compared using Duncan's multiple comparison test.

RESULTS

The determination of initial characteristics of seeds revealed a moisture content of 54.37 ± 0.49% fresh weight and a viability of 100%. *X. aethiopica* seed germination was spread out over the period from day-45 to day-90 after sowing. From the 90th day after sowing, there was no further germination over a period of four consecutive weeks. The germination type was epigeal, where the hypocotyls elongated and the cotyledons which were still enclosed within the seed coat were taken out of the soil (Figure 1).

Analyses of variance (Table 1) indicated significant individual influences of germination substrates ($p = 0.044$) and treatments ($p < 0.001$) on the percentage of germination, but the interaction between treatments and substrates had no significant effect ($p = 0.99$). When considering the treatments taken all together, germination percentage on the mixture of forest top soil and river sand (29.4 ± 2.6%) was significantly higher than that obtained on forest top soil alone (20.6 ± 2.6%). The percentage of germination on river sand alone (26.7 ±2.6%) was neither different from that obtained on the mixture of top soil and river sand nor different from that obtained on top soil alone (Table 2). Considering the three substrates taken all together, untreated seeds (control) and seeds soaked in hot water irrespective of the duration of treatment failed to germinate. Germination occurred only with manually scarified seeds for which 33.3 ± 6.3% was recorded as percentage of germination, and with seeds soaked in either concentrated hydrochloric acid or concentrated sulfuric acid. The effect of each individual acid strongly depended on the duration of soaking. The highest germination percentages recorded with seeds treated with concentrated HCl and concentrated H_2SO_4 were 80 ± 6.3% and 70 ± 6.3% respectively, all of which were recorded when the duration of exposure to the acid was 5 min. With both acids, above the soaking duration of 5 min, the percentage of germination gradually decreased with increasing duration of treatment. The overall highest germination percentage (90 ± 10.9%) was recorded with seeds soaked in concentrated HCl for 5 min and sown in the mixture of forest top soil and river sand as substrate (Table 2).

The desiccation tolerance test showed that fresh seeds of *X. aethiopica* had an initial moisture content of 54.37 ± 0.49%. As seeds were dried, their moisture content gradually decreased and reached the value of 7.32 ± 0.18% after four weeks of drying. At the same time, germination percentage slightly increased from 78 ± 6.8% at week-0 to 93 ± 6.8% at week-1 and then remained constant as seeds were dried till week-4 (Figure 2), indicating that there was no loss in germination percentage as result of seed dehydration.

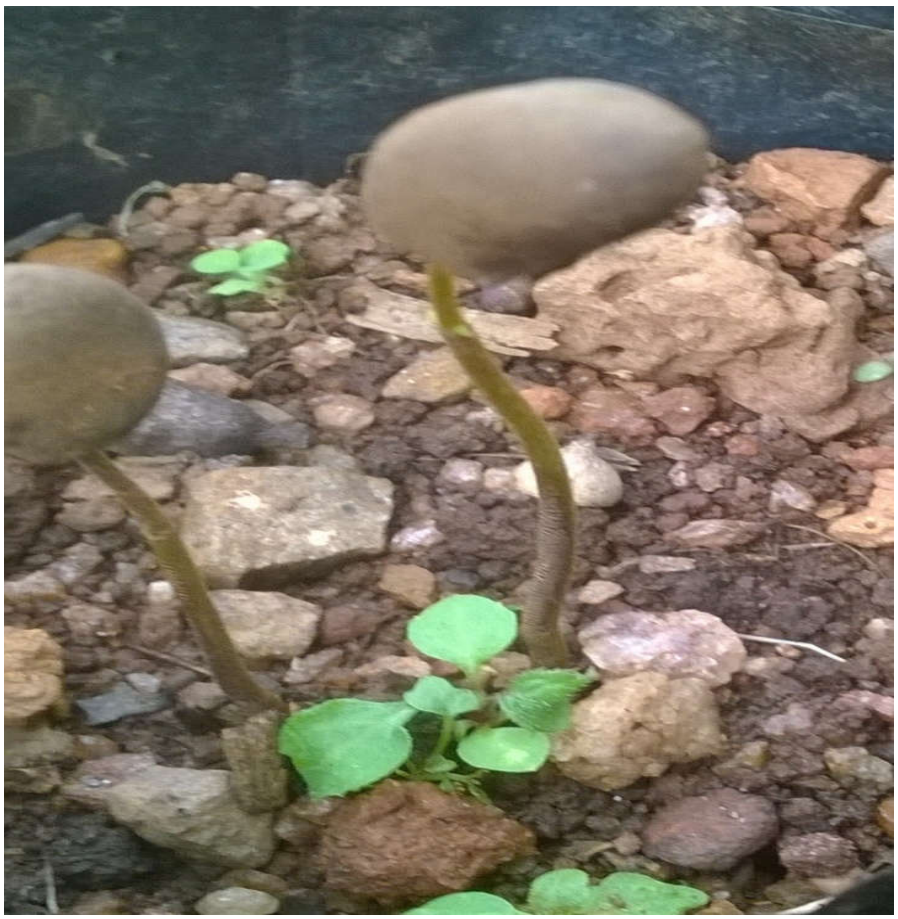

Figure 1: Germinated *Xylopia aethiopica* seeds at 50 days after sowing, showing an epigeal growth which consisted of the emergence of cotyledons above the surface of the substrate as a result of hypocotyls elongation.

Table 1: ANOVA of the effects of substrates, treatments and their interaction on the germination percentage of *X. aethiopica* seeds at three months after sowing.

Variable	**Sum of Squares**	***df***	**Mean Square**	***F***	***p***
Substrates	0.744	2	0.372	3.152	0.044
Treatments	42.667	17	2.510	21.250	< 0.001
Substrates X Treatments interaction	1.922	34	0.057	0.479	0.995
Error	57.400				
Total	138.000				

Table 2: Effects of substrates and treatments on the percentage germination of *X. aethiopica*'s seeds at three months after sowing.

Treatments	Substrates			Treatments' main effects
	Top soil	Sand	Top soil + Sand	
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{a}
Hot water 30 sec.	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{a}
Hot water 1 min.	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{a}
Hot water 2 min.	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{a}
Hot water 3 min.	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{a}
Hot water 4 min.	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{a}
Hot water 5 min.	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{a}
Manual Scarification	30 ± 10.9	30 ± 10.9	40 ± 10.9	33.3 ± 6.3^{bc}
HCl 5 min.	70 ± 10.9	80 ± 10.9	90 ± 10.9	80 ± 6.3^{e}
HCl 10 min.	70 ± 10.9	60 ± 10.9	80 ± 10.9	73.3 ± 6.3^{e}
HCl 15 min.	50 ± 10.9	60 ± 10.9	70 ± 10.9	60 ± 6.3^{de}
HCl 30 min.	20 ± 10.9	50 ± 10.9	60 ± 10.9	43.3 ± 6.3^{cd}
HCl 60 min.	10 ± 10.9	20 ± 10.9	30 ± 10.9	20 ± 6.3^{ab}
H_2SO_4 5 min.	60 ± 10.9	70 ± 10.9	80 ± 10.9	70 ± 6.3^{e}
H_2SO_4 10 min.	30 ± 10.9	50 ± 10.9	50 ± 10.9	43.3 ± 6.3^{cd}
H_2SO_4 15 min.	20 ± 10.9	40 ± 10.9	30 ± 10.9	30 ± 6.3^{bc}
H_2SO_4 30 min.	10 ± 10.9	10 ± 10.9	0 ± 10.9	6.7 ± 6.3^{a}
H_2SO_4 60 min.	0 ± 0	10 ± 10.9	0 ± 0	3.3 ± 6.3^{a}
Substrates' main effects	$20.6 \pm 2.6^{\alpha}$	$26.7 \pm 2.6^{\alpha\beta}$	$29.4 \pm 2.6^{\beta}$	

Within the same column, means ± SEs followed by the same letters are not significantly different at 5 % level; within the same row, means ± SEs followed by the same symbols are not significantly different at 5 % level.

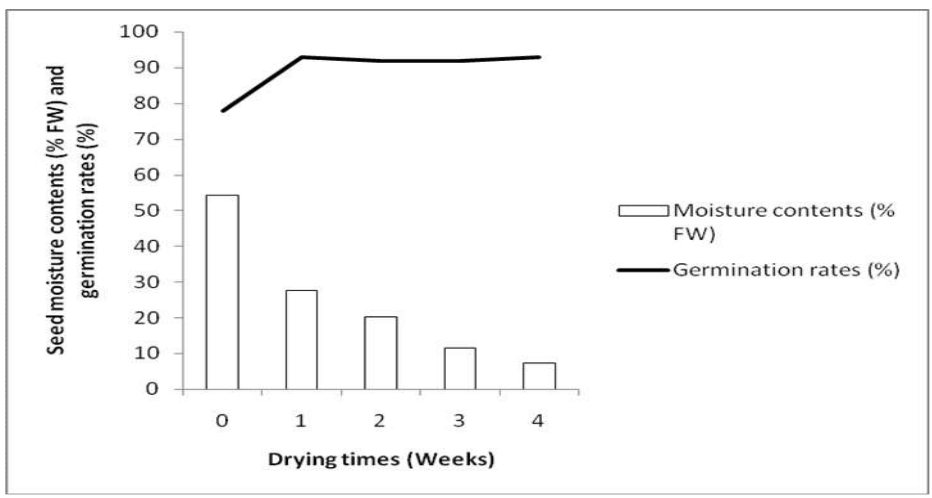

Figure 2: Moisture content and germination percentage of *Xylopia aethiopica* seeds over drying time.

DISCUSSION

The germination failure recorded with untreated control seeds in the present work is consistent with previous works which reported that *X. aethiopica* seeds are deeply dormant (Udosen and Sam, 2015; Alaba et al., 2015). The fact that seeds which were scarified either mechanically or chemically germinated without any further additional treatment is a clear indication that the seed coat is the sole barrier to germination in *X. aethiopica*. This kind of dormancy has been reported for many tropical plant species where hard seed coats make seeds impervious to water and gases thereby limiting imbibitions and germination (Baskin and Baskin, 2001; Cruz et al., 2001; Alaba et al., 2011; Ibiang et al., 2012; Udosen and Sam, 2015).

Soaking the seeds in either concentrated HCl or concentrated H_2SO_4 for 5 min resulted in highest percentage germination. Similar results have been reported for a wide range of species for which the efficiency of acid scarification in breaking coat-imposed seed dormancy has been proven (Lopez et al., 2004; Nascimento et al., 2009; Alaba et al. 2011; Musara et al., 2015). The effectiveness of HCl and H_2SO_4 in overcoming coat-imposed seed dormancy could be attributed to successful removal of several lignified layers in the testae, which are packed tightly together and contain water repelling compounds (Baskin, 2003). These layers act as a mechanical (physical) barrier to water absorption and gaseous exchange (Colling, 2009). Baskin and Baskin (2001) noted that the whole idea behind treating the seeds is to either completely remove the germination impeding seed coat or to reduce its thickness so that the seed could emerge. Removal or reduction in thickness of the seed coat allows the seed to take up water and respiratory gases thus the germination process can be initiated (Musara et al., 2015). In a previous work, Udosen and Sam (2015) reported that *X. aethiopica* seeds which were treated with concentrated H_2SO_4 for 3 min failed to germinate. This failure of germination may have resulted from an inappropriate duration of exposure to acid, since our results indicated that 5 min exposure to concentrated H_2SO_4 led to subsequent high germination percentage. Soaking seeds in concentrated acids for durations longer than 5 min resulted in lower germination percentages as compared with those exposed for 5 min. This agreed with the findings of Gbadamosi and Oni (2004) on *Enantia chlorantha* and may be due to the corrosive nature of the acid, which affected the embryo of the seeds when they were left therein for long a period of time. Seeds exposure to either concentrated HCl or concentrated H_2SO_4 for 5 min before sowing may be the vital key to the mass production of seedlings of the species for plantation establishment. Seeds treated with hot water failed to germinate irrespective of the duration of the treatment. This result is similar to that reported with *Enantia chlorantha* (Gbadamosi and Oni, 2004) and *Tamarindus indica* (Fandohan et al., 2010), but contrasts with those reported with many species including *Robinia pseudoacacia* (Mirzaei et al., 2013). This indicates that there exist some interspecies variation in the response of dormant seeds to various seed dormancy breakers, but the determinism of these variations is not well known. It seems, therefore, more appropriate not to make a generalization including all plants about the effectiveness of a specific treatment in breaking dormancy of seeds.

Substrates are not limiting factors in seed germination per se in the nursery, but rather the growth of the seedling after germination (Sounou et al., 2009). Nevertheless, a substrate needs to have adequate aeration and moisture for germinating seeds (Benvenuti, 2003). The

results of the present study showed that mixture of forest top soil and river sand showed a higher germination percentage compared to forest top soil alone. As seeds were watered on a daily basis, there was no problem of water availability. The differences among different substrates for their germination percentage as reported here may be attributed to the differences in substrate aeration. It is well known that both sand and the mixture of forest top soil and sand have textures that allow higher levels of aeration than that of forest top soil alone. Germination of *X. aethiopica* seeds may have been enhanced by the aeration condition which was more appropriate in mixture of forest top soil and sand and in sand alone than in forest top soil. Similar results have been reported for seeds of many others species (Benvenuti, 2003; Rodriguez et al., 2014).

The desiccation tolerance test revealed that moisture content drastically decreased as seeds were dried, but there was no loss in germination percentage associated to seeds dehydration. This clearly indicates that *X. aethiopica* seeds are desiccation-tolerant, and thus exhibit orthodox seed-storage behaviour. This is of great interest for conservation storage, since it is well known that contrarily to desiccation-sensitive (recalcitrant) seeds that do not tolerate dehydration and for which storage is only possible for short periods of time, desiccation-tolerant seeds can be dried and stored for a long period of time without loosing their viability (Daws et al., 2006). The slight increase in germination percentage recorded after one week drying in this study has also been observed by Prichard et al. (2004) in other tropical species including *Sterculia quinqueloba*, *Podocarpus nagi*, *Ekebergia capensis* and *Pouteria macrophylla* and by Agyili et al. (2007) in *Garcinia kola*. It has been suggested by Tompset and Pritchard (1998) that stimulation of germination in seeds due to short-term desiccation was due to the further development of the less mature seeds in the batch, with drying substituting for what may have happened naturally on the parent plant if the seeds had not been naturally, yet prematurely, abscised.

Conclusion

The results from the present study revealed that seed dormancy in *X. aethiopica* is due to the hard seed coat, which can be effectively broken by acid scarification for 5 min in either concentrated HCl or concentrated H_2SO_4. Mixture of top soil and river sand in a 1:1 (v/v) ratio performed higher germination percentage than other substrates. *X. aethiopica* seeds are desiccation-tolerant and exhibit orthodox seed-storage behaviour. This study provides useful information for domestication and large scale plantation development, and in environmental conservation efforts. It contributes to the growing interest in the domestication of the agroforestry tree species that have been traditionally important in West and Central African culture and are becoming important for sustainable rural development.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all the authors. GK designed the study, wrote the protocol, carried out the desiccation tolerance test and wrote the first draft of the manuscript. DAM collected the seeds from field, and carried out the germination trial in the nursery. F provided critical inputs towards the research, and corrected the manuscript. DNO provided laboratory facilities and supervised the

research. All the authors read and approved the final manuscript.

REFERENCES

Agyili J, Sacandé M, Koffi E. 2007. Improving the collection and germination of West African *Garcinia kola* Heckel seeds. *New For.*, **34**: 269-279. DOI: 10.1007/s11056-007-9054-7

Alaba FT, Motolani AO, Adeola AC. 2015. Efficiency of seed treatments and different growth media on the germination of seeds of *Xylopia aethiopica* (Dunal) A. Rich. *J. Biol. Chem. Res.*, **32**(2): 849-853.

Alaba FT, Oyeboade SA, Jaime ATS, Ayodele BA, Oluyemisi MA. 2011. Efficiency of sulfuric acid, mechanical scarification and heat treatments on breaking dormancy of *Tetrapleura tetraptera* seeds. *Seed Sci. Biotechnol*., **5**(1): 53-55.

AOSA (Association of Official Seeds Analysts) 2000. *Tetrazolium Testing Handbook*, Peters J (ed). Contrib. No. 29, New Mexico 88003.

APG (The Angiosperm Phylogeny Group III) 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plant: APG III. *Bot. J. Linn. Soc.,* **161**(2): 105-121. DOI: https://doi.org/10.1111/ j.1095-8339.2009.00996.x

Asekun OT, Adeniyi BA. 2004. Antimicrobial and cytoxic activities of fruit essential oil of *Xylopia aethiopica* from Nigeria. *Fitoterapia,* **75**: 368-370. DOI: http://dx.doi.org/10.1016/j.fitote.2003.12 .020

Atangana A, Khasa D, Chang S, Degrande A. 2014. *Tropical Agroforestry*. Springer. DOI: 10.1007/978-94-007-7723-1.

Baskin CC. 2003. Breaking physical dormancy in seeds – focusing on the lens. *New Phytol*., **158**: 227-238. DOI: 10.1046/j.1469-8137.2003.00751.x

Baskin CC, Baskin JM. 2001. *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*. Academic Press: San Diego, Diego, San Francisco, New York, Boston, London, Sydney, Tokyo.

Benvenuti S. 2003. Soil texture involvement in germination and emergence of buried weed seeds. *Agron. J.,* **95**: 191-198. DOI: 10.2134/agronj2003.1910

Colling J. 2009. Towards understanding the metabolism of in vitro *Sutherlandia frutescens* (L.) R. Br. Cultures. Msc Thesis, Stellenbosch University, South Africa.

Cruz ED, Martins FO, Carvalho JE. 2001. Biometria de frutos e sementes e germinação de jatobacuruba (*Hymenaea intermedia* Ducke, Leguminosae-Caesalpinioideae). *Rev. Bras. Bot.,* **24**: 161-165. DOI : http://dx.doi.org/10.1590/s0100-84042001000200005

Daws MI, Garwood NC, Pritchard HW. 2006. Prediction of desiccation sensitivity in seeds of woody species: a probabilistic model based on two seed traits and 104 species. *Ann. Bot.,* **97**: 667-674. DOI: https://doi.org/10.1093/aob/mcl022

Erhirhie EO, Moke GE. 2014. *Xylopia aethiopica*: a review of its ethnomedicinal, chemical and pharmacological properties. *Amer. J. Pharm. Tech. Res*., **4**(6): 21-37.

Fandohan B, Assogbadjo AE, Glele Kakai R, Sinsin B. 2010. Variation in seed morphometric tratits, germination and early seedling growth performances of *Tamarindus indica* L. *Int. J. Biol. Chem. Sci.*, **4**(4): 1102-1109. DOI: http://dx.doi.org/10.4314/ijbcs.v4i4.63047

Fleischer TC. 2003. *Xylopia aethiopica* (Dunal) A. Rich.: a chemical and biological perspective. *J. Univ. Sci. Technol*., **23**(2): 24-31.

Gbadamosi AE, Oni O. 2004. Effect of pretreatments on germination of seeds of the medicinal plant *Enantia chlorantha* Oliv. *Food, Agric. Environ.,* **2**(2): 288-290.

Hessou C, Glele Kakai R, Assogbadjo AE, Odjo T, Sinsin B. 2009. Test de germination des graines de *Caesalpinia bonduc* (L.) Roxb au Bénin. *Int. J. Biol. Chem. Sci.*, **3**(2): 310-317. DOI: http://dx.doi.org/10.4314/ijbcs.v3i2.44507.

Ibiang YB, Ita EE, Ekanem BE, Edu NE. 2012. Effect of different pretreatment protocols on seed germination of *Tetraptera tetrapleura* (Schum and Thonn). *IOSR J. Env. Sci. Toxicol. Food Technol.*, **2**(3): 25-29.

ISTA (International Seed Testing Association) 2004. *International Rules for Seed Testing. Rules 2004*, International Seed Testing Association: Zurich, Germany.

Keita B, Sidibe L, Figneredo G, Chalchat JC. 2003. Chemical composition of the essential oil of *Xylopia aethiopica* (Dunal) A. Rich. from Mali. *J. Essent. Oil Res*., **15**(4): 267-269. DOI: http://dx.doi.org/10.1080/10412905.2003.9712139

Kokutse AD, Adjonou K, Guelly AK, Kokou K. 2014. Bamboo resources in Togo. *Int. J. Biol. Chem. Sci*., **8**(2): 481-493. DOI: http://dx.doi.org/10.4314/ijbcs.v8i2.8

Leakey RRB, Tchoundjeu Z. 2001. Diversification of tree crops: domestication of companion crops for poverty reduction and environmental services. *Exp. Agric.*, **37**: 279-296. DOI: https://doi.org/10.1017/s0014479701003015

Lopez JC, Dais PC, Macedo CMP. 2004. Tratamentos para supercar a dormencia de sementes de *Ormosia arborea* (Vell) HarmS. *Brasil Forestal*, **80**: 25-35.

Mirzaei M, Moghadam ARL, Ardebili ZO. 2013. The induction of seed germination using sulfuric acid, gibberellic acid and hot water in *Robinia pseudoacacia* L. *Int. Res. J. Applied Basic Sci.*, **4**(1): 96-98.

Musara C, Chitamba J, Nhuvira C. 2015. Evaluation of different seed dormancy breaking techniques on okra (*Abelmoschus esculentus* L.) seed germination. *Afr. J. Agric. Res*., **10**(17): 1952-1956. DOI : 10.5897/ AJAR2014.9181

Nascimento H, Alves EU, Bruno RLA, Goncalves EP, Colares PNQ, de Medeiros MS. 2009. Superaçao da dormencia em sementes de faveira (*Parkia platycephala* Benth). *Revista Arvore*, **33**(1): 33-45. DOI : http://dx.doi.org/10.1590/s0100-67622009000100005

Oliveira LM, David AC, Carvalho MLM. 2003. Avaliação de metodos para queba da dormencia e para a desinfestação de sementes de canafistula (*Peltophorum dubium* (Sprengel) Taubert). *Revista Arvore*, **27**(5): 597-603. DOI : http://dx.doi.org/10.1590/s0100-67622003000500001

Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. 2009. Agro-forest tree database: a tree reference and selection guide. Version 4.0 (http://www.worldagroforestry.org/af/treedb/), Retrieved 2nd August 2012.

Pritchard HW, Sacandé M, Berjak P. 2004. Biological aspects of tropical tree seed desiccation and storage responses. In *Comparative Storage Biology of Tropical Tree Seeds*, Sacandé M, Joker

D, Dulloo EM, Thomsen KA (eds). IPGRI: Rome; 319-341.

Rodriguez OAV, Vazquez AP, Martinez AJ. 2014. Effects of seed weight and substrate on germination and growth of non-toxic *Jatropha curcas* L. seedlings. *Ann. Res. Rev. Biol*., **4**: 4232-4245. DOI: 10.9734/ARRB/2014/11697

Sinclair FL, Joshi L. 2001. Taking local knowledge about trees seriously. In *Forestry, Forest Users and Research: New Ways of Learning,* Lawrence A (ed). ETFRN: Wageningen; 45-61.

Sounou M, Glele Kakai R, Avakoudjo J, Assogbadjo AE, Sinsin B. 2009. Test de germination et de croissance de *Artemisia annua* L. anamed sur différents substrats au Bénin. *Int. J. Biol. Chem. Sci.*, **3**(2): 337-346. DOI: http://dx.doi.org/10.4314/ijbcs.v3i2.44505.

Tatsadjieu LN, Ngang JJE, Ngassoum MB, Etoa FX. 2004. Antibacterial and antifungal activity of *Xylopia aethiopica*, *Monodora myristica*, *Zanthoxylum zanthoxyloiles* and *Zanthoxylum leprieuril* from Cameroon. *Fitoterapia*, **74**(5): 469-472. DOI: http://dx.doi.org/10.1016/s0367-326x(03)00067-4

Tompsett PB, Pritchard HW. 1998. The effect of chilling and moisture status on the germination, desiccation tolerance and longevity of *Aesculus hippocastanum* L. seed. *Ann. Bot.*, **82**: 249-261. DOI: https://doi.org/10.1006/anbo.1998.0676

Udosen IR, Sam SM. 2015. Germination studies and early seedling growth of some local spices found in Niger Delta Nigeria. *Int. J. Sci. Res*., **4**: 818-819. DOI: 10.15373/2249555x