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Seedling Emergence of *Blighia sapida* (K. D. Konig) Seeds Subjected to Different Lengths of Storage and Pretreatments

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Authors' contributions

This work was carried out in collaboration between the both authors. Author OOO designed the study and supervised author TNO in carrying out the experiment. Author TNO wrote the first draft while author OOO restructured the manuscript and edited it appropriately. Both authors managed the literature searches and approved the final manuscript.

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ABSTRACT

Aims: The study investigated the influence of different lengths of storage at room temperature and pretreatments on seedling emergence of *Blighia sapida* seeds.
Study Design: Randomized complete block design with four replicates was used.
Place and Duration of Study: The experiment was conducted at the nursery of Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti, Nigeria for about three months.
Methods: Seeds of *Blighia sapida* under five lengths of storage at room temperature being Fresh, 7 days, 14 days, 21 days and 28 days were sown in germination boxes filled with topsoil after subjection to four pretreatments thus: Control, Hot water, Soaking in water at room temperature and Scarification. Cumulative germination count was done daily till no further germination was observed for about a week.
Results: Seeds sown Fresh and pretreated by Soaking in water at room temperature had the highest germination percentage of 96.7% followed by those under Control with germination

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percentage of 76.7%. Seeds stored for 7 days had germination percentage of 33.3% under Control and Scarification whereas the ones subjected to Soaking in water at room temperature produced 40% germination percentage. Hot water pretreatment gave 0% germination under the different lengths of storage. In seeds stored for 14 days, 3.3%, 6.7% and 13.3% germination percentages were observed for Control, Soaking in water at room temperature and Scarification respectively. However, seeds stored for 21 days and 28 days gave 0% germination for the different pretreatments.

Conclusion: Fresh seeds of *B. sapida* are the most suitable for sowing whereas they can also be stored at room temperature for 7days to obtain appreciable viable seeds beyond which viability cannot be guaranteed even with the use of different pretreatments.

Keywords: Seed storage; room temperature; germination; recalcitrant.

1. INTRODUCTION

Seed storage is an essentiality in supplying good quality seeds for a planting programme whenever needed [1,2], whereas planting programme is rather inevitable considering many factors. Such factors include high rate of deforestation in developing countries, indiscriminate logging activities and climate change. Seed is the common propagation material for most tropical tree species [3] because propagation from seed is considered inexpensive and usually effective, and is therefore a viable method for *ex-situ* conservation of plant genetic resources [4]. This may be one of the reasons why propagation of tropical tree species still relies heavily on availability of seeds. Furthermore, the proper handling of seeds through the processes of ripening or maturation, collecting, processing, storage, dormancy, stratification and germination is required for success in producing a new seedling [5]. It has also been discovered that seed maturation does not correspond many times with seedling production thereby making seed storage a very important part of seed handling.

The period of storage is often limited by the technical and physiological storage potentials, that is, the length of time seeds of a particular species will survive under the available storage conditions. Therefore, in order to maintain viability over a prolonged period, it is important that the optimal storage environment for the species is met, as much as possible. However, even under the best storage conditions, some species will only be viable for a very short time nevertheless deterioration in such species may be delayed by adopting the best possible storage conditions, but long term storage is not possible for those species. Even where fruiting is regular and abundant every year, it is rather cheaper to collect surplus seeds to cover for several years rather than collecting seeds every year [6]. The importance of seed storage has been recognized ever since human beings began to domesticate plants. The length of successful seed storage depends on both the objectives and the species concerned, but the overall objective is to help maintain viable seeds from one growing season to the next; that is for months or even years depending on when the seeds will be needed.

Many tropical fruit tree species exhibit uncertain storage behavior. Seeds have traditionally been grouped into two according to their physiological storage potentials viz. recalcitrant and orthodox. Even with these two broad groups, variations exist among each group. Likewise, viable period for seeds of some species can be prolonged by certain storage media. [7] said other storage media such as cold storage rooms and cryopreservation could prolong viability of seeds but some of these are not readily available or are limited by one factor or another in a developing country like Nigeria. Also appropriate seed pretreatments are required for nursery establishment and production of maximum number of quality seedlings with minimum cost, time and labour [8].

Blighia sapida (K.D. Konig) has been classified as a neglected and underutilized species which signify that its potentials both as an income generating species as well as its contribution to human well being have been grossly underexploited. This neglect places it in danger of continued genetic erosion and disappearance. *Blighia sapida* commonly called ackee apple is a tropical fruit belonging to the Sapindaceae family. It is a woody perennial multipurpose fruit tree species native to the Guinean forests of West Africa but has traversed the Atlantic Ocean making the Caribbean its home. *Blighia sapida* occurs naturally from Senegal to Cameroon and Equatorial Guinea, and possibly also in Gabon. It is commonly planted in its natural area of distribution, as fruit tree and ornamental shade tree. It has been introduced in many other tropical countries and in some subtropical regions such as Florida (United States) and is widely cultivated as fruit and ornamental tree in India and tropical America. It had already been introduced in tropical America by the end of the 18th century, and has since become locally naturalized [9]. Although so far overlooked by researchers in Nigeria, the tree is highly valued by farmers and is an important component of traditional agroforestry systems in some other countries. Furthermore, the seed storage behavior of *B. sapida* is said to be uncertain [10] whereas the benefits derivable from *B. sapida* are quite enormous. It is said to be a nice ornamental tree, especially when decorated with the brightly coloured fruits. It is also considered useful for planting to improve soil fertility and reduce erosion through its large rooting system [11]. Boiled arils are an ingredient of a popular traditional dish in Jamaica, together with salt fish. In West Africa, arils are sometimes eaten raw, fried or roasted while canned ackee is exported primarily to the United kingdom and Canada [12]. Considering the diverse benefits derivable from *B. sapida*, this study on its length of storage at room temperature and pretreatments to aid its seed germination becomes important. The use of storage at room temperature is to give the knowledge of when *B. sapida* seed will still retain its viability without access to any sophisticated means of storage. Also, the pretreatments adopted in this study are those that are socially acceptable and extra precaution may not be needed to apply them. Thus, this study aimed at evaluating the viability of *B. sapida* seeds subjected to different lengths of storage at room temperature and assessment of its early growth.

2. MATERIALS AND METHODS

2.1 Fruit Collection, Depulping and Seed Weight Determination

Mature fruits of *Blighia sapida* were collected from Oye-Ekiti, Ekiti State, Nigeria (latitude 7°8'N and longitude 5°3'E). These were carefully depulped using hand to avoid seed damage. Six hundred (600) seeds of *Blighia sapida* were then selected based on their good morphological characteristics. These were divided into five parts with each part containing 120 seeds. The five parts stand for the different lengths of storage for *Blighia sapida* seeds being Fresh, 7 days storage, 14 days storage, 21 days storage and 28 days storage represented as LS1, LS2, LS3, LS4, and LS5 respectively. Storage was done at room temperature by spreading the seeds on trays in the laboratory. Forty seeds were randomly selected under each length of storage and weighed on sensitive weighing balance in four replicates.

2.2 Effect of Pretreatments on the Germination of *Blighia sapida* Seeds

The pretreatments used for aiding germination in *B. sapida* seeds were Control, Hot water, Water at room temperature and Scarification represented as PT1, PT2, PT3 and PT4 respectively. In Control, no form of pretreatment was applied while for hot water, water was allowed to boil before soaking the seeds in it and the water was allowed to cool with the seeds for 24 hours. In the case of water at room temperature, the seeds were soaked in water for 24 hours before sowing. Scarification was done using sand paper to inflict scar on the seeds thereby forcing the seed coat open to encourage imbibition of water and gas.

Seeds under each length of storage were further sub-divided into four parts with each part subjected to the different pretreatments and each had thirty seeds which were sown in germination trays previously filled with topsoil in three replicates. Watering was done daily in the morning using finely-meshed watering can. Germination count was done daily until no further germination was observed for about a week. Germination was taken to have occurred when the plumule emerged on the soil surface.

2.3 Statistical Analysis

The data collected were subjected to two-way Analysis of Variance (ANOVA) and the various means were separated using Duncan's Multiple Range Test (DMRT).

3. RESULTS

3.1 Seed Weight

Mean seed weight values showed significance among the different lengths of storage of *Blighia sapida* when subjected to Analysis of Variance (ANOVA) at $\alpha = 0.05$. However, when Duncan's Multiple Range Test (DMRT) was used to separate the means, it revealed that LS1 was significantly different ($p<0.05$) from LS2, LS3, LS4 and LS5. Contrariwise, LS2 and LS3 were not significantly different ($p>0.05$) from each other but different from those of LS4 and LS5.

Furthermore, LS4 and LS5 did not differ from each other at p>0.05 (Table 1).

Table 1. Differences in mean seed weight of *B. sapida* from the different lengths of storage

Length of storage (days)	Seed weight (g)
0	36.40[a]
7	27.50[b]
14	26.03[b]
21	22.73[c]
28	22.47[c]

Note: Means with the same letter are not significantly different

3.2 Germination of Ackee Apple Seeds

3.2.1 Germination of seeds sown fresh using different pretreatments

Germination began on the 11th Day after Sowing (DAS) for seeds subjected to PT3 and was completed on the 19th DAS whereas it began on the 12th DAS for seeds subjected to PT1 and PT4, and was completed on the 20th DAS. Germination percentages of 76.7%, 0%, 96.7% and 73.3% were recorded for PT1, PT2, PT3 and PT4 respectively (Table 3). Fig. 1 shows the cumulative number of seedlings for *B. sapida* seeds sown fresh.

3.2.2 Germination of seeds sown after 7 days of storage subjected to different pretreatments

Germination started on the 12th DAS for seeds subjected to PT3 and was completed on the 20th DAS whereas at the 13th DAS, germination began for seeds subjected to PT1 and PT4 and was brought to an end on the 20th and 19th DAS respectively. Percentage germination of 33.3%, 0%, 40.0% and 33.3% were recorded for PT1, PT2, PT3 and PT4 respectively (Table 3). Fig. 2 reveals the cumulative number of seedlings for *B. sapida* seeds sown after 7 days storage.

3.2.3 Germination of seeds sown after 14 days storage under different pretreatments

Germination began on the 13th DAS for seeds under PT4 and was completed on the 18th DAS. Whereas germination which began on the 14th DAS for seeds under PT1 and PT3 came to an end on the 14th and 17th DAS respectively. Germination percentages of 3.3%, 0%, 6.7% and 13.3% were recorded for PT1, PT2, PT3 and PT4 respectively (Table 2). Fig. 3 indicates the cumulative number of seedlings for *B. sapida* seeds sown after 14 days storage.

3.2.4 Germination of seeds sown after 21 days and 28 days storage subjected to different pretreatments

None of the seeds germinated all through the period of the experiment even under the different pretreatments. Therefore, germination percentage was 0% for the two lengths of storage under the various pretreatments (Table 2).

3.2.5 Analysis of Variance (ANOVA) results for germination rate of *Blighia sapida* across lengths of storage and pretreatments

ANOVA result showed no significance at $\alpha = 0.05$ for lengths of storage, pretreatments and; interaction between storage and pretreatments at 11 DAS. Although at 12 DAS, ANOVA result indicated significance for lengths of storage, whereas, it revealed no significance for pretreatments and; interaction between storage and pretreatments. Furthermore, at 13 DAS, 14 DAS up to 21 DAS there was significant difference for lengths of storage, pretreatments and; interaction between storage and pretreatments.

Table 2. Mean germination percentage of *Blighia sapida* seeds subjected to different lengths of storage and pretreatments

Lengths of storage	Germination percentage (%)				Germination begins (DAS)				Germination ends (DAS)			
	PT1	PT2	PT3	PT4	PT1	PT2	PT3	PT4	PT1	PT2	PT3	PT4
LS1	76.7	0	96.7	73.3	12	0	11	12	19	0	19	20
LS2	33.3	0	40.0	33.3	13	0	12	13	20	0	20	19
LS3	3.3	0	6.7	13.3	14	0	14	13	14	0	17	18
LS4	0	0	0	0	0	0	0	0	0	0	0	0
LS5	0	0	0	0	0	0	0	0	0	0	0	0

**DAS means days after sowing*

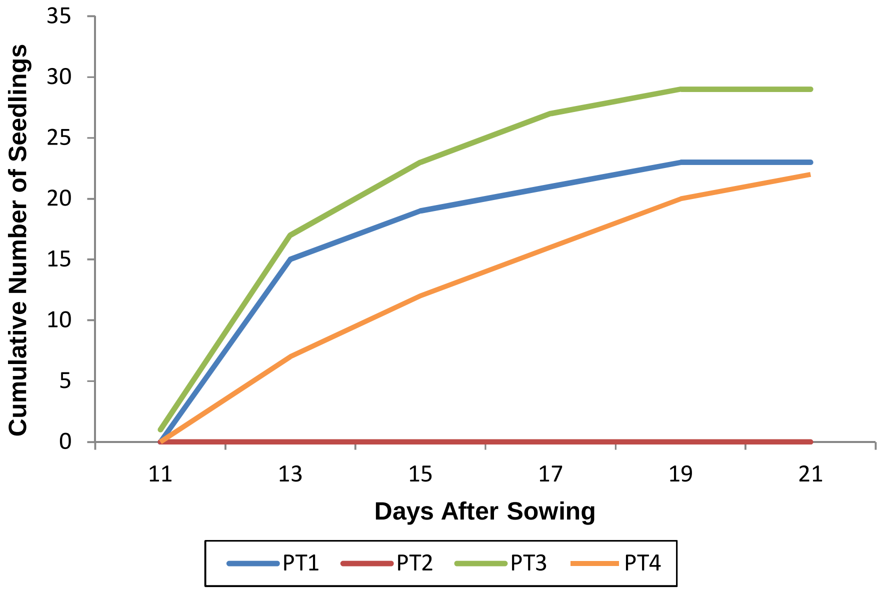

Fig. 1. Cumulative germination of *Blighia sapida* seeds sown Fresh under different pretreatments

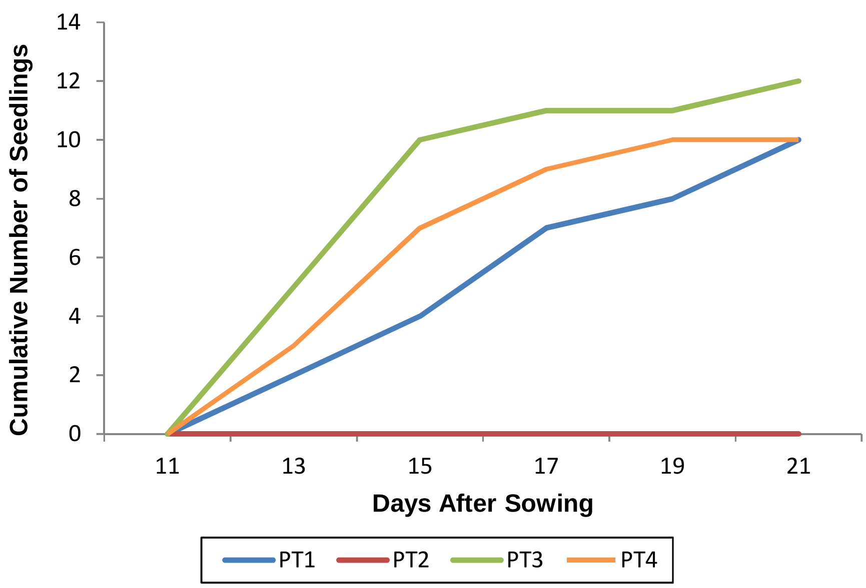

Fig. 2. Cumulative germination of *Blighia sapida* seeds sown after 7 days storage under different pretreatments

When Duncan's Multiple Range Test (DMRT) was used to separate the mean germination for the different lengths of storage, no significance ($p>0.05$) was shown at the 11th DAS for Fresh (LS1), 7 days of storage (LS2) and 14 days of storage (LS3). However at the 12th DAS, LS2 and LS3 were different ($p<0.05$) from LS1 but they were not different ($p>0.05$) from each other. Furthermore, DMRT showed significant difference ($p<0.05$) among LS1, LS2 and LS3 from 13th DAS till the experiment was concluded on the 21st DAS (Table 3).

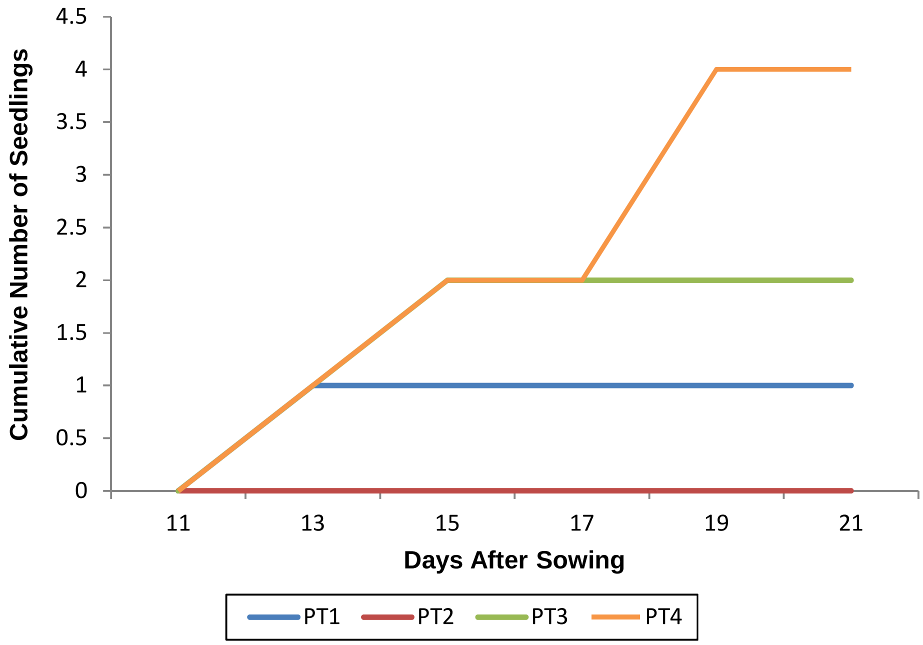

Fig. 3. Cumulative germination of *Blighia sapida* seeds sown after 14 days storage under different pretreatments

Table 3. Mean germination rate of *Blighia sapida* seeds across the different lengths of storage

DAS	Lengths of storage		
	LS1	**LS2**	**LS3**
11	0.08^{a}	0.00^{a}	0.00^{a}
12	1.67^{a}	0.17^{b}	0.00^{b}
13	3.42^{a}	0.83^{b}	0.08^{c}
14	4.00^{a}	1.25^{b}	0.25^{c}
15	4.50^{a}	1.75^{b}	0.33^{c}
16	4.83^{a}	2.00^{b}	0.33^{c}
17	5.33^{a}	2.25^{b}	0.42^{c}
18	5.67^{a}	2.33^{b}	0.80^{c}
19	6.00^{a}	2.42^{b}	0.58^{c}
20	6.17^{a}	2.67^{b}	0.58^{c}
21	6.17^{a}	2.67^{b}	0.58^{c}

Note: Means with the same letter across rows are not significantly different
**DAS means days after sowing*

3.2.6 Mean germination rate of *Blighia sapida* seeds across the various pretreatments

The mean values for PT1, PT2, PT3 and PT4 were not significantly different (p>0.05) from one another when they were subjected to DMRT at 11 DAS. However, at 12 DAS, the mean value for PT3 differed significantly (p<0.05) from PT2 whereas, it did not differ (p>0.05) from PT1 and PT4, likewise PT2 did not differ (p>0.05) from PT1 and PT4. Also, at the 13th DAS, 15th DAS and 16th DAS, DMRT showed that PT1 was significantly different (p<0.05) from PT3 and PT4, all of which differed from PT2. Furthermore, at the 14th DAS there was no significant difference (p>0.05) among the pretreatments while at the 20th and 21st DAS, only the mean value of PT2 was significantly different from those of other pretreatments (Table 4).

Table 4. Mean germination rate of *Blighia sapida* seeds across the various pretreatments

DAS	Pretreatments			
	PT1	PT2	PT3	PT4
11	0.00[a]	0.00[a]	0.11[a]	0.00[a]
12	0.67[ab]	0.00[b]	1.00[a]	0.78[ab]
13	1.89[ab]	0.00[c]	2.44[a]	1.44[b]
14	2.44[b]	0.00[d]	3.33[a]	1.56[c]
15	2.67[ab]	0.00[c]	3.78[a]	2.33[b]
16	3.11[ab]	0.00[c]	3.89[a]	2.56[b]
17	3.22[b]	0.00[c]	4.44[a]	3.00[b]
18	3.44[b]	0.00[c]	4.56[a]	3.44[b]
19	3.56[a]	0.00[b]	4.67[a]	3.78[a]
20	3.78[a]	0.00[b]	4.78[a]	4.00[a]
21	3.78[a]	0.00[b]	4.78[a]	4.00[a]

Note: Means with the same letter across rows are not significantly different
**DAS means days after sowing*

4. DISCUSSION

There was reduction in seed weight as lengths of storage increased which indicated reduction in moisture content of the seeds. Also, it was realized that Fresh seeds which had the highest weight were the first to germinate in comparison with other lengths of storage. This result agrees with that of [13] who observed that the progeny of *Dialium guineense* Willd with the highest seed weight were the first to germinate.

Considering the different lengths of storage, seeds sown fresh had the highest germination rate above the other lengths of storage while onset of germination increased with increasing length of storage. The result of this study agrees with that of [7] who reported prompt germination in seeds of *Dacryodes edulis* G. Don sown fresh in comparison with seeds stored for different lengths of time. Similarly, seeds sown fresh had the highest germination percentage while this reduced with increasing lengths of storage till no germination was observed in seeds stored for longer duration. This result still agrees with that of [7] who observed that *D. edulis* seeds sown fresh produced the highest germination percentage while this reduced till no germination was observed with increasing lengths of storage at room temperature. [14] observed that seeds are affected by desiccation and are considered short-lived. However, not all tree seeds are affected by desiccation but only those that fall under the category of being recalcitrant. It is generally known that tropical recalcitrant seeds cannot be dried without causing damage to the endospermic unit of the seed. Thus, germination of seeds in this group dried prior to sowing should not be expected [2]. Likewise, [15] reported that fresh seeds with a certain level of moisture content (15-90%) normally germinate with a germination percentage of 80-90% but that even a small decrease in moisture content will lead to a significant decrease in seed germination. They further observed fresh seeds as having the highest germination percentage than those stored for different lengths of time.

Furthermore, under the various pretreatments, the highest germination percentage was observed for seeds soaked in water at room temperature in both seeds sown Fresh and the ones stored for 7 days prior to sowing followed by Control. Although under 14 days length of storage, Scarification produced the highest germination percentage (13.3%) that was not appreciable followed by water at room temperature (6.7%). However, this result is not in agreement with the work of [16] who reported that soaking Prosopis seed in water at room temperature is generally ineffective in breaking dormancy. In the case of *B. sapida* used in this study, soaking in water at room temperature for 24 hours produced the highest germination percentage. It can then be inferred that seeds of different tree species differ in their response to various pretreatments. Furthermore, hot water gave 0% germination percentage across the assessment period and this could be as a result of the fact that the embryo of *B. sapida* seeds cannot withstand hot water pretreatment. Whereas, in the case of Prosopis seed [16] reported that hot water pretreatment is suitable in breaking its seed dormancy. Moreover, the various pretreatments used in this study produced very low germination percentage in seeds of *B. sapida* stored for 14 days while they did not aid germination at all in seeds stored for

21 days and 28 days. The loss of moisture earlier observed in *B. sapida* seeds as lengths of storage increased must have negatively affected their viability. This result disagrees with that of [17] who observed that viability of *Mimosa foliolosa* Benth seeds can be maintained for at least three years under artificial storage conditions. Their study suggested alleviation of seed dormancy with storage time which they attributed to probable seed coat softening under artificial condition.

5. CONCLUSION

The difference observed in germination percentages of *B. sapida* from the five different lengths of storage used in this experiment showed that Fresh seeds of *B. sapida* are the most suitable for sowing whereas they can also be stored at room temperature for 7 days to obtain appreciable viable seeds beyond which viability cannot be guaranteed even with the use of different pretreatments. Also, soaking seeds of *B. sapida* in water at room temperature is the best pretreatment to aid its germination. It is therefore recommended that seeds of *Blighia sapida* be sown as soon as collection is made but not stored beyond 7 days. Where this does not correspond with planting time, they can be raised in the nursery first before eventual planting out. Moreover, considering the fact that *B. sapida* seeds are desiccation sensitive, it can be concluded that they are recalcitrant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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