Industrial Crops and Products 91 (2016) 332–339

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Storage, oil quality and cryopreservation of babassu palm seeds

Rodrigo Aparecido Domingues de Oliveira[a], Silma da Conceição Neves[a], Leonardo Monteiro Ribeiro[a,*], Paulo Sérgio Nascimento Lopes[b], Flaviano Oliveira Silvério[b]

[a] Departamento de Biologia Geral, Universidade Estadual de Montes Claros, 39401-089 Montes Claros, MG, Brazil
[b] Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, 39404-547 Montes Claros, MG, Brazil

ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form 20 July 2016
Accepted 25 July 2016
Available online 1 August 2016

Keywords:
Attalea vitrivir
Embryos
Germination
Fatty acids
Seed quality
Palms

ABSTRACT

Attalea vitrivir is a neotropical palm with exceptional potential for biofuel production. We evaluated the effects of different storage methods on seed and oil quality and the viability of using cryopreservation for conserving its genetic resources. Fruits were stored for 365 days in the open air (OA); under shade (SH); in the shade in polyethylene bags (PO); and in a cold chamber at 10 °C (CC); seeds were stored for 180 days in SH, PO and CC. We evaluated embryo viability as well as the acid value and fatty acid profile of the oil. Isolated embryos and seeds were stored for 90 days at room temperature (average 25 °C); −20 °C; and at −20 °C following freezing at −196 °C. Embryos were also stored at −196 °C. The storage methods used preserved embryo viability, which remained above 90%, with the exception of seed storage in PO. The oil had an initial acid number of 0.9% and a predominance of lauric acid, and a final acid number of less than 1.6% in all treatments. Saturated fatty acid and lauric acid contents increased with storage. The freezing methods preserved embryo viability and seed germinability. *A. vitrivir* seeds demonstrate orthodox behavior and are highly storage-tolerant, which is favored by the high stability of their oils. Cryopreservation of embryos shows potential usefulness for the conservation of the genetic resources of this species.

1. Introduction

Palm trees of the babassu complex (*Attalea* sp.) (Fig. 1a) are widely distributed in tropical America, with *Attalea vitrivir* being native to the Cerrado biome (neotropical savanna) in central Brazil (Dransfield et al., 2008; Lorenzi et al., 2010; Neves et al., 2013). Babassu palms produce large numbers of fruits and seeds (Fig. 1b) (Neves et al., 2013) and have been used by traditional populations as a source of raw materials for rural buildings, crafts, and as food (Lorenzi et al., 2010; May et al., 1985), constituting one of the major non-timber extractivist resources in the country (Ferrari and Soler, 2015; Teixeira, 2008). *Attalea vitrivir* fruits have a woody endocarp that can be used to make charcoal for the steel industry, and its seeds have high oil contents with high potential for biofuel production (Fig. 1c–d) (CETEC, 1983). Preliminary estimates indicated that the species has been potential to produce more than 30,000 kg of fruits and 1000 kg of oil per hectare annually under cultivation (Guedes et al., 2015).

Commercial cultivation and the conservation of natural populations of *A. vitrivir* are hampered by a lack of information concerning their biology (Neves et al., 2013), including seed behavior during storage. These studies are important for propagation management and raw material storage for agro-industrial purposes (Besbes et al., 2004; Hong and Ellis, 1996; Ribeiro et al., 2012).

Hong and Ellis (1996) proposed a widely used protocol for classifying seed behavior during storage, although the application of this methodology is difficult with palm trees due to the anatomical characteristics of their diaspores – especially the presence of a hard endocarp and their pronounced dormancy (Ribeiro et al., 2012). The use of *in vitro* embryo cultures to estimate seed viability has proven useful in studies of some palm species (Dias et al., 2015; Ribeiro et al., 2012) and may contribute to evaluating seed quality in *A. vitrivir*.

The definition of appropriate conditions for oilseed storage through biochemical assessments is important because triglycerides commonly undergo alterations that reduce their quality (Edem, 2002; Evaristo et al., 2016; Siles et al., 2013). Fatty acid profiles are useful for estimating the susceptibility of seeds to

* Corresponding author at: Departamento de Biologia Geral, Universidade Estadual de Montes Claros, Campus Prof. Darcy Ribeiro, 39401-089 Montes Claros, MG, Brazil.
E-mail address: leonardo.ribeiro@unimontes.br (L.M. Ribeiro).

http://dx.doi.org/10.1016/j.indcrop.2016.07.039
0926-6690/

Fig. 1. A natural population of *Attalea vitrivir* (A) with bunches of fruits (B). Ripe fruit and seed (C). Transversal section of the fruit showing the epicarp (ep), mesocarp (me), endocarp (en), and seeds (se) (D).

degradation (Besbes et al., 2004) and for evaluating the suitability of their oils for industrial applications (del Río et al., 2016). The proportions of free fatty acids indicate the states of oil deterioration that occurs through processes such as hydrolysis, oxidation, and fermentation and can significantly limit its use (Miraliakbari and Shahidi, 2008).

Cryopreservation of orthodox seeds and isolated fragments of recalcitrant seeds, such as embryos (Sisunandar et al., 2010), embryonic axes (Corredoira et al., 2004) or plumules (Chmielarz et al., 2011) is important in the ex situ conservation as a backup for orthodox seeds storage and more efficient and low cost germplasm storage for recalcitrant seeds (Engelmann, 2004; Hong and Ellis, 1996; Wen and Wang, 2010), although effective protocols are available for only a few palm species (Dias et al., 2015; Engelmann et al., 1995; Ngobese et al., 2010). Tolerance to cryopreservation is associated with seed behavior during storage (Ngobese et al., 2010; Wen and Wang, 2010), and studies related to this subject with *A. vitrivir* could contribute to seed management for propagation and the conservation of its genetic resources.

The present study evaluated the quality of *A. vitrivir* seeds during storage and cryopreservation in order to: (i) classify seed behavior during storage; (ii) evaluate the effect of storage methods on embryo viability and oil quality; and, (iii) estimate the potential of cryopreservation for the conservation of the genetic resources of this species.

2. Materials and methods

2.1. Collection and storage of fruits and seeds

Attalea vitrivir fruits were collected after natural abscission in wild populations (Fig. 1a) located in a protected area of Rio Pandeiros (APA-Pandeiros) in the municipality of Januária in northern Minas Gerais State, Brazil (15°26′10″S; 44°40′44″W). The presence of a yellow abscission scar on the fruits was considered indicative of recent abscission.

The fruits (Fig. 1B–C) were evaluated in the field, and those showing signs of predation or microbial attacks were discarded. Some of the fruits were stored for 365 days under the following conditions: in the open air under nursery conditions, with the fruits lying directly on the ground (open air); in the shade in an open shed, with fruit held in raphe bags (shade); in the shade, with the fruits held in polyethylene (500 μm thick) bags (shade/polyethylene); and in a cold chamber at 10 °C (cold chamber). The average annual temperature at the experiment site was 24.2 °C (average maximum 30.1 °C; average minimum 18.4 °C), the annual rainfall was 1134 mm, and the average relative humidity of the air was 66%.

Seeds were harvested from other fruits by cracking the endocarp with an ax; the seeds were then selected (Fig. 1C–D) and subsequently stored for 180 days under the following conditions: in the shade in paper bags under laboratory conditions at 25 °C (shade); in the shade hand-held in polyethylene bags under laboratory conditions (shade/polyethylene); and in a cold chamber at 10 °C (cold chamber).

2.2. Evaluations of seed water contents

Before storage, and after 15, 40, 90, 180 and 365 days, the seeds were removed from the fruits (using a saw and a vise). After determining their fresh weights, four replicates of 10 seeds were placed in a drying oven for 24 h at 105 °C and their dry weights subsequently measured. Water content was calculated considering the differences between their fresh and dry masses, and expressed based on fresh mass (Brasil et al., 2009). Isolated seeds had their water contents determined after 15, 60 and 180 days of storage.

2.3. Evaluations of embryo viability and vigor

Embryo viability was assessed by *in vitro* germination, and vigor estimated considering seedling root emission capacity (Ribeiro et al., 2012). The seeds were removed from the fruits prior to storage and after 90, 180 and 365 days of storage, disinfected using 6% sodium hypochlorite, rinsed three times with distilled water, and transferred to a laminar flow chamber. The embryos were removed, disinfected in a 0.5% Cl solution for 10 min, and then rinsed three times with sterile distilled water. The embryos were then inoculated into test tubes (12 × 1 cm) containing 2 mL of: MS medium (Murashige and Skoog, 1962) at 75% of its original concentration; 0.4 mgL^{-1} thiamine; 1 mgL^{-1} pyridoxine; 0.5 mgL^{-1} nicotinic acid; 100 mgL^{-1} *myo*-inositol; 0.5 gL^{-1} hydrolyzed casein; 3 gL^{-1} activated charcoal; 30 gL^{-1} sucrose; 6 gL^{-1} agar; and the pH was adjusted to 5.7 (Ribeiro et al., 2012).

The experiment was conducted in a factorial manner (4 storage methods × 3 storage times); the initial condition was considered an additional treatment. We used a completely randomized design with five replicates of 10 test tubes with one embryo in each. The test tubes were kept in a germinator in the absence of light at 30 °C for 30 days. Successful germination was considered to be a greater than 50% elongation of the initial embryo length, as well as the number of seedlings emitting roots (Ribeiro et al., 2012). Isolated seeds were evaluated by the same procedure after 60 and 180 days of storage. In this case, the experiment was conducted in a factorial scheme (3 storage methods × 2 storage times), considering the initial condition as an additional treatment. In both experiments, the numbers of germinated embryos and seedlings showing root emission were converted to percentages and arcsine transformed for analysis of variance; the means were compared using the Tukey test, at a 5% probability level.

2.4. Evaluations of the free fatty acid content of the oil by its acid value

Seeds extracted from fruits before storage, and after 15, 40, 90, 180 and 365 days, were placed in polyethylene bags and frozen at −20 °C until the oil was extracted. Oil was extracted by mechanically pressing the seeds in an adapted bench vise. Samples of 0.1 g oil were titrated using 0.01 N NaOH, with phenolphthalein as the indicator. The free fatty acid contents were calculated based on the molecular weight of oleic acid (Instituto Adolfo Lutz, 1985). Four replicates were used per treatment, and the titrations were performed in triplicate.

The experiment was conducted in a factorial scheme (4 storage methods × 5 storage times). For isolated seeds, the evaluations were performed at 15, 60 and 180 days in a factorial scheme (3 storage methods × 3 storage times). In both evaluations the initial condition was considered as an additional treatment. The data were submitted to analysis of variance, and Tukey's test (at 5% probability) was used to compare the means.

2.5. Evaluations of fatty acid profiles

Oils were obtained from seeds prior to storage, and after fruit storage for 15, 40 and 365, as described above; the lipids extracted using a chloroform-methanol mixture (Folch et al., 1957). The lipid fraction was suspended in a methanolic solution of 10% potassium hydroxide (KOH) and saponified for 10 min at 90 °C. The saponifiable organic phase was then separated and filtered, the extract dried under liquid nitrogen, and the fatty acids esterified using a methanolic solution of 14% BF3 for 3 min at 90 °C. The fatty acid profiles were obtained using a gas chromatograph (7890A GC, Agilent Tecnhologies, Santa Clara, USA) equipped with an electron-impact ionization detector (GC–MS) and a DB-5MS capillary column (Agilent Technologies, 30 m length × 0,25 mm internal diameter × 0.25 μm film thickness), He was used as the carrier gas. The samples were analyzed in triplicate, with four replicates for each treatment.

The effects of storage methods and times on saturated fatty acid contents and the percentages of the three predominant fatty acids were evaluated. For isolated seeds, the evaluations were carried out after 15 and 180 days. The experimental design and statistical analyses were similar to those described for evaluating the percentages of free fatty acids.

2.6. Cryopreservation of seeds and embryos

Seeds were obtained from freshly harvested fruits, sterilized as previously described, and stored in the shade; their water contents were monitored daily until reaching 5% (Hong and Ellis, 1996). Part of the seed lot was then wrapped in aluminum foil and stored for 90 days: at −20 °C, with prior freezing in liquid nitrogen for 5 minutes (fast freezing); at −20 °C (slow freezing); or at room temperature (average 25 °C) for 90 days. The same conditions were used for storing isolated embryos held in eppendorf tubes; additional samples were also stored at −196 °C in liquid nitrogen. After storage, the seeds and embryos were thawed at 25 °C, for 24 h. In the case of the embryos frozen in liquid nitrogen, thawing was conducted by maintaining them first at −20 °C for two hours and then at 25 °C for 24 h.

After 90 days, the seeds of each treatment were sterilized as described above and placed in polyethylene containers to germinate (with sterilized vermiculite moistened to 80% retention capacity with distilled water). The seeds were then kept in germination chamber at 30 °C, in the dark, for 60 days. Evaluations of germination (considering the protrusion of cotyledonary petiole, the indicative of the completion of germination in palm seeds) were made weekly (Ribeiro et al., 2012); the seeds that did not germinate were subjected to the tetrazolium test (Ribeiro et al., 2010). The experiment was established in a randomized design, with four treatments (storage conditions and the initial condition) with five replicates of twenty seeds each.

The embryos were subjected to *in vitro* culture after storage as previously described. The experiment was established in a randomized block design, with five treatments (storage conditions and the initial condition) with five replicates of 10 embryos each. The incubation conditions, the criteria for the evaluations of germination and root emission, and statistical analyzes were the same as those described previously for the fruit storage experiment.

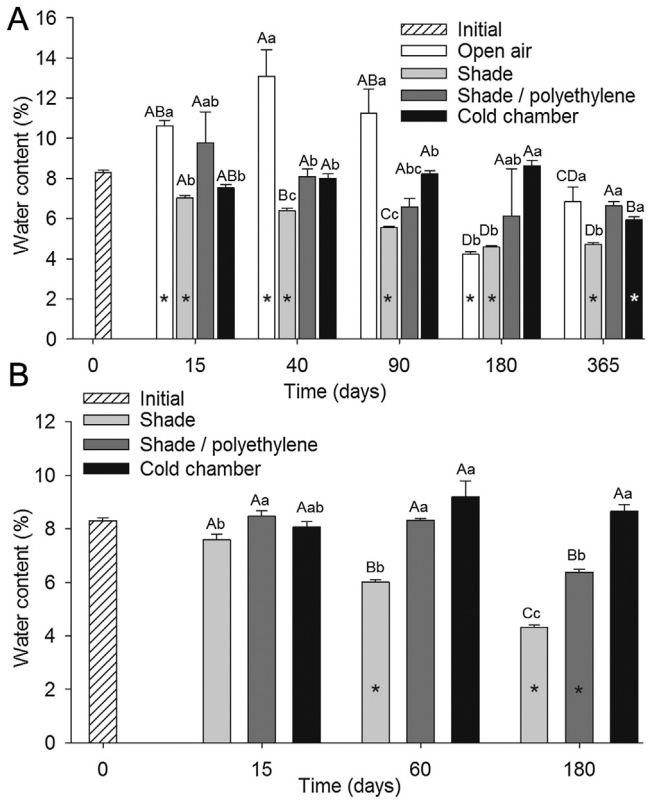

Fig. 2. Water content of *Attalea vitrivir* seeds obtained from stored fruits (A). Water contents of isolated stored seeds (B). The same letters indicate no significant differences between the storage times, using the Tukey test, at 5% probability. Capital letters indicate the effects of time within each storage condition, and lowercase letters indicate the effects of the storage condition within each time period. Asterisks indicate significant differences as compared to initial conditions. Vertical bars indicate standard errors of the means.

3. Results

3.1. Water content

The seeds showed an 8.3% water content before storage. There were decreases in seed water contents after 15 days when the fruits were stored in the shade, and after 365 days in the cold chamber; the water contents of seeds stored in the shade/polyethylene remained constant (Fig. 2A). Storage in the open air resulted in greater variations in water contents due to natural rainfall events. At the end of the experiment, the fruits stored in the shade showed lower water contents when compared to the other treatments. The water contents of the isolated seeds stored in the cold chamber remain constant during the evaluation period. Seed water contents decreased during storage in the shade after 60 days, and in the shade/polyethylene after 180 days (Fig. 2B).

3.2. Embryo viability and vigor

There were no effects of fruit storage time on the germinability of their embryos, which averaged 96.6%. Fruit storage in the shade/polyethylene and in the cold chamber provided higher embryo germination rates that storage in the shade (Fig. 3A). Root emission capacity by seedling attained higher values when the fruits were stored for 180 days (Fig. 3B), and root emissions were not influenced by the storage method (Fig. 3C).

Storage of isolated seeds in the shade/polyethylene decreased embryo germination, which was initially 100% after 180 days (Fig. 3D). The root emission capacity of seedlings increased with storage time (Fig. 3E), with storage in the shade giving better results than storage in a cold chamber (Fig. 3F).

3.3. Oil free fatty acid contents

All of the fruit storage methods resulted in increased oil acidity relative to the initial condition (0.87%), with the highest values resulting after storage in the open air (Fig. 4A), although the maximum acid value was still low (<1.6%). Storage in a cold chamber better preserved oil quality in relation to storage in the open air, but did not differ from storage in the shade/polyethylene after 40 days. No differential effects of storage methods on acid values were observed with isolated seeds, with those values increasing over time and reached a maximum value of 1.2% after 180 days (Fig. 4B).

3.4. Fatty acid profiles

The oils extracted from *A. vitrivir* seeds had high saturated fatty acid confidence (average 82.2%) (Fig. 5). The predominant fatty acids were lauric $C_{12:0}$ (50.8%), oleic $C_{18:1}$ (18.8%), myristic $C_{14:0}$ (14.3%), palmitic $C_{16:0}$ (8.7%), capric $C_{10:0}$ (4.4%), and stearic $C_{18:0}$ (2.8%). Total levels of saturated fatty acid and lauric acid in the oil increased during fruit storage, with slight decreases in the levels of myristic and oleic acid (which were not influenced by storage methods).

Isolated seeds stored in the shade and in a cold chamber (for 15 and 180 days respectively) showed increased levels of saturated fatty acids in relation to their initial conditions (Fig. 6). In the case of storage in the shade/polyethylene, the oil showed an increase in saturated fatty acid content after 15 days, but decreasing by the end of the study period. Lauric acid levels increased during storage in the shade after 15 days, during storage in the shade/polyethylene after 15 days, and during storage in a cold chamber after 180 days (Fig. 7A). The myristic acid content decreased with storage in the shade during the entire study period, and during storage in a cold chamber after 180 days (Fig. 7B), but the oleic acid content (which decreased over time) was not affected by the storage method (Fig. 7C).

3.5. Cryopreservation of seeds and embryos

No effects of storage methods on seed germination were observed, averaging 67%. The tetrazolium test indicated that the seeds that did not germinate had nonviable embryos. The different storage conditions used for holding isolated embryos did not show marked differences between them, nor compared to initial *in vitro* germination (mean 84.4%). Embryo storage at room temperature as well as fast and slow freezing did cause decreased root emission capacity, however, as compared to initial conditions (Fig. 8).

4. Discussion

4.1. Seed behavior during storage

A. vitrivir seeds demonstrated orthodox behavior in storage. Its seeds are dispersed with low water contents (below 10%), which is typical of the orthodox condition. According to criteria established by Hong and Ellis (1996), *A. vitrivir* seeds maintain their viability when dried to below 5% water content or when stored at −20 °C for 90 days. Even fruit storage in the open air for 365 days, with the seeds being subjected to large temperature and humidity variations, did not affect embryo viability. The ability to withstand dehydration is important for seed survival during storage and under stress conditions, and is not observed in seeds showing recalcitrant behavior that are also intolerant of low temperatures (Angelovici et al., 2010; Hong and Ellis 1996; Tweddle et al., 2003).

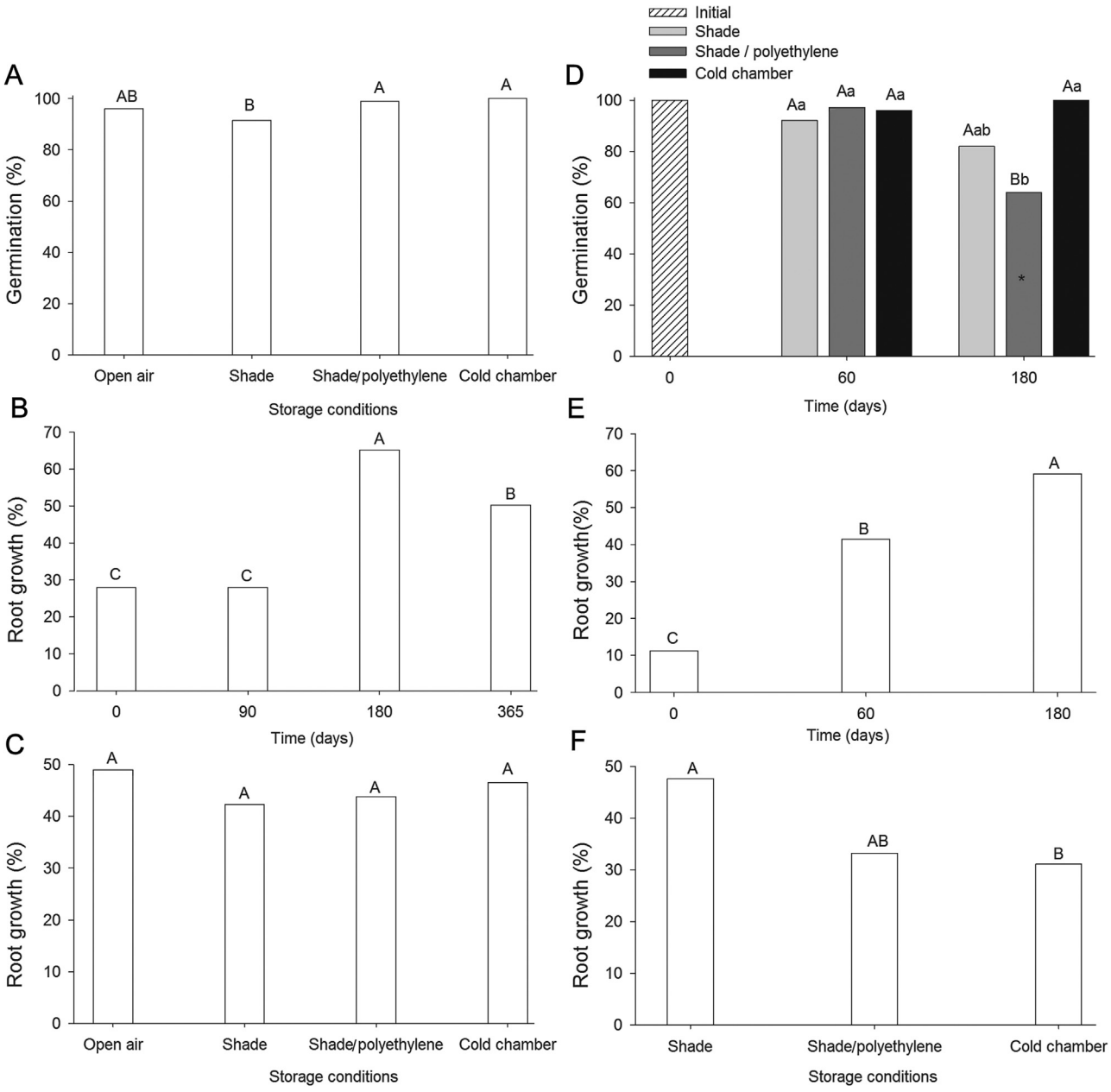

Fig. 3. In vitro germination of *Attalea vitrivir* embryos (A, D) and seedling root emission (B–C and E–F) as a function of different methods of fruit (A–C) and isolated seed storage (D–F). The same letters indicate no significant differences between the storage times, using the Tukey test, at 5% probability. In E, the capital letters indicate the effects of time within each storage condition, and the lowercase letters indicate the effects of the storage conditions within each time period. Asterisks indicate significant differences as compared to initial conditions.

As palms have wide geographic distributions (Dransfield et al., 2008), their seeds exhibit a great diversity of behaviors during storage, reflecting their adaptations to many different environments (Orozco-Segovia et al., 2003). Seeds of species from savanna regions or other arid environments, such as *Acrocomia aculeata* (Jacq.) Lodd. ex. Mart. and *Washingtonia filifera* (Linden ex André) H. Wendl. are usually classified as orthodox (Dickie et al., 1992; Ribeiro et al., 2012), while seeds of species adapted to moist environments, such as *Euterpe edulis* Mart. and *Mauritia flexuosa* L. F., are commonly recalcitrant (Panza et al., 2009; Silva et al., 2014). There are, however, species adapted to a wide variety of environments, such as *Phoenix reclinata* Jacq. and *Butia capitata* (Mart.) Becc., whose seeds are classified as intermediate because they are tolerant of dehydration but sensitive to low temperatures (Dias et al., 2015; Fintel et al., 2004). The results obtained in the present study indicated that the seeds of *A. vitrivir* follow the most common pattern due to their orthodox behavior and adaptations to the Cerrado biome (neotropical savanna) (Neves et al., 2013). It is possible that desiccation tolerance has an important role in the interaction of this species with the highly seasonal climate of the Cerrado and contributes to the formation of persistent soil seed banks (which will be discussed in future works). Their high tolerance to storage should facilitate seed handling for artificial propagation.

The observed increases in seedlings root emission capacity with storage may be associated with alterations of the hormonal balance of the embryos. The seeds of many species are sensitive to post-ripening, which implies physiological changes resulting from the long periods of time (months) that the seeds remain dehydrated after dispersal (Angelovici et al., 2010; Finch-Savage and Leubner-Metzger, 2006). Post-ripening is commonly related to overcoming

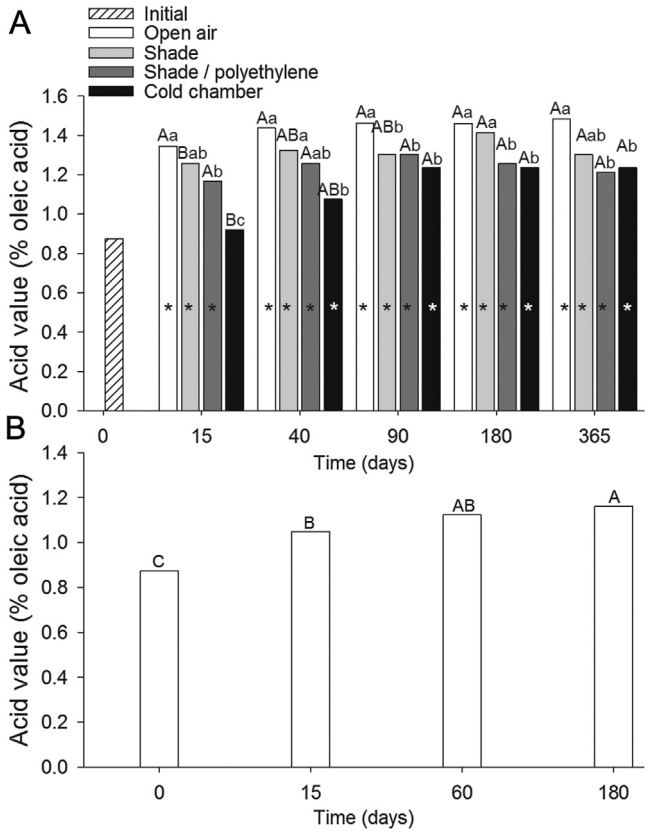

Fig. 4. Acid values of *Attalea vitrivir* seed oils under different methods of fruit (A) and isolated seed storage (B). The same letters indicate no significant differences between the storage conditions. In A, the uppercase letters indicate the effects of time within each storage condition, and the lowercase letters indicate the effects of the storage conditions within each time period. Asterisks indicate significant differences in relation to initial conditions.

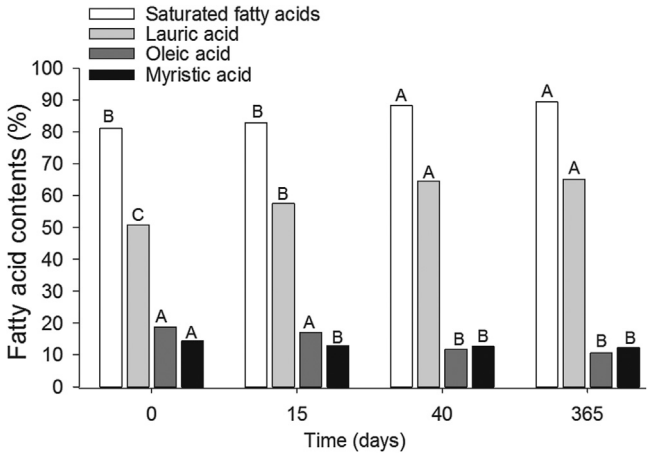

Fig. 5. Levels of saturated fatty acids, as well as lauric, myristic and oleic acids in the seed oils obtained from the *Attalea vitrivir* fruits exposed to different storage conditions. For each storage condition, the same letters indicate no significant differences by the Tukey test, at 5% probability.

dormancy and promoting germination. Although *A. vitrivir* seeds show only low levels of dormancy (Neves et al., 2013), it is possible that hormonal changes occur during storage that facilitate germination and seedling development, as has been reported for several other species (Bewley et al., 2013).

The pericarp has an important role in the conservation of the *A. vitrivir* seeds, as noted by Neves et al. (2013). We observed that isolated seeds demonstrated more pronounced effects of storage

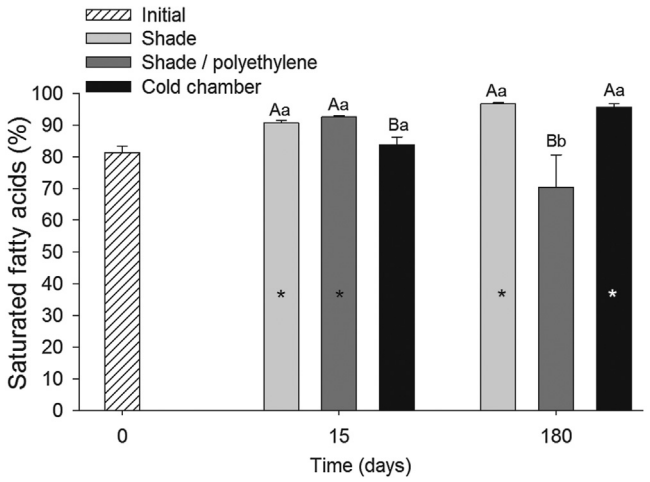

Fig. 6. Levels of saturated fatty acids in the oils obtained from *Attalea vitrivir* seeds exposed to different storage conditions. The same letters indicate no significant differences between the storage times by the Tukey test, at 5% probability. The capital letters indicate the effects of time within each storage condition, and the lowercase letters indicate the effects of the storage condition within each time period. Asterisks indicate significant differences compared to initial conditions. Vertical bars indicate the standard errors of the means.

conditions on their germination and on seedling root emission. It is therefore recommended that seeds should be stored intact within the fruit at ambient temperatures, as their germination will remain undiminished for several months – representing a low cost method that favors seedlings production for commercial plantations, or for the conservation of natural populations.

4.2. Storage effects on oil quality

The seed oil of *A. vitrivir* demonstrated substantial stability and remains well-conserved under different fruit and seed storage conditions. Free fatty acid percentages can be used to evaluate oil conservation status as measures of the cleavage of the triglyceride carbon chains (Besbes et al., 2004; Evaristo et al., 2016). We observed little change in the acid numbers during storage, regardless of the method used, with maximum values below 1.6%. These results indicate that *A. vitrivir* oil will retain the main features necessary for good quality biodiesel production under storage (Evaristo et al., 2016; Ferrari and Soler, 2015; Martínez et al., 2014).

A. vitrivir oil has a high saturated fatty acid content, mainly due to the presence of high percentages of lauric acid (which provides oxidative stability) (Besbes et al., 2004; del Río et al., 2016). The fatty acid profile of this oil is very similar to other babassu species that have been studied (Ferrari and Soler 2015; Santos et al., 2013) and to the oil of the economically important palm species *Elaeis guineensis* Jacq. (Eden, 2002) and *A. aculeata* (CETEC, 1983). Its high lauric acid content makes this oil interesting for various industrial applications such as thermal energy storage (Yuan et al., 2014) as well as the production of nanoparticles (Mamani et al., 2013) and construction materials (Cellat et al., 2015).

The increases in the percentages of saturated fatty acids encountered under most storage conditions are related to the reduction of oleic acid, which is unsaturated and therefore most vulnerable to degradation (Miraliakbari and Shahidi, 2008). Interestingly, seed storage under shade/polyethylene conditions gave different results, which were probably due to oxygen flow restriction and the consequent reduction in the levels of lipid peroxidation that maintained the percentages of saturated fatty acids (Besbes et al., 2004; Siles et al., 2013). It is worth emphasizing that fruit storage in the shade provided results similar to cold storage at the end of the 365-day test period in relation to the acid indices and fatty acid

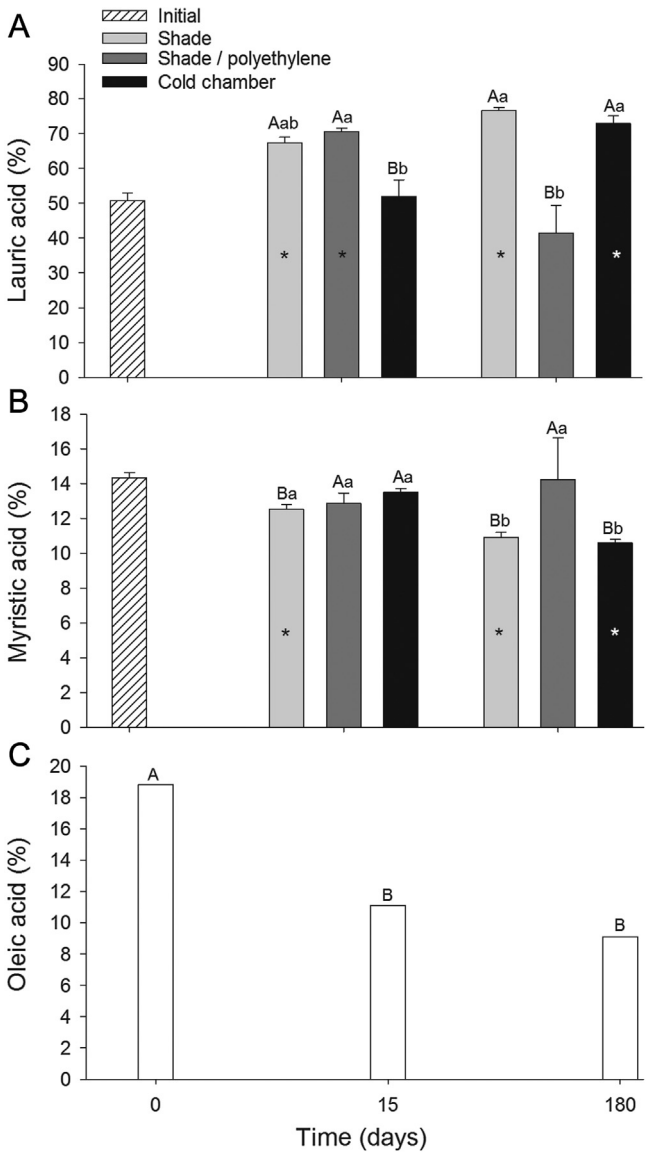

Fig. 7. Levels of lauric (A), myristic (B) and oleic (C) acids in *Attalea vitrivir* oils obtained from seeds exposed to different storage conditions. The same letters indicate no significant differences between the storage times by the Tukey test, at 5% probability. The capital letters in A and B indicate the effects of time within each storage condition, while the lowercase letters indicate the effects of the storage conditions within each time period. Asterisks indicate significant differences as compared to initial conditions. Vertical bars indicate the standard errors of the means.

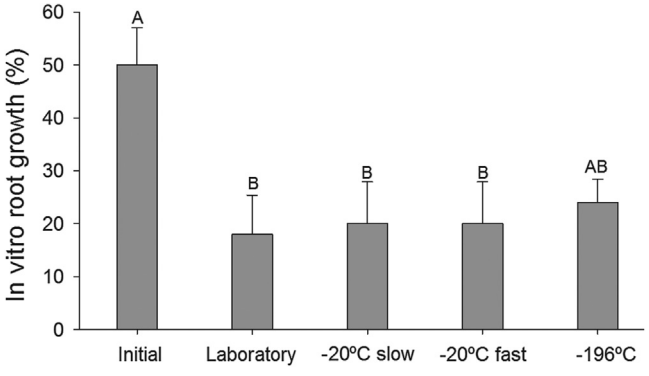

Fig. 8. Emission of roots by *Attalea vitrivir* seedlings during *in vitro* culture of embryos exposed to different storage conditions. The same letters indicate no significant differences between the storage times by the Tukey test, at 5% probability. Vertical bars indicate the standard errors of the means.

profiles – thus indicating a simple and inexpensive method for raw material storage for agro-industrial use.

4.3. Cryopreservation for the conservation of genetic resources

Both cryopreservation of isolated embryos and seed freezing show potential for genetic resource conservation in *A. vitrivir*. Cryopreservation has been shown to be an effective technique for the long-term preservation of the seeds and embryos of palm species such as *E. guineensis* and *B. capitata* (Dias et al., 2015; Ellis et al., 1991; Grout et al., 1983). The success of this technique depends on the existence of low tissue water contents that help prevent cell damage caused by the formation of ice crystals during freezing (Engelmann, 2004; Sisunandar et al., 2010). The seeds of many palm species that are dehydration-tolerant are sensitive to low temperatures (Dickie et al., 1992; Orozco-Segovia et al., 2003). *A. vitrivir* seeds are dehydration-tolerant until reaching water contents below 5%, as demonstrated in the storage experiment. Additionally, the isolated embryos and seeds retained their viability when stored at low temperatures, although there were decreases in seedling root emission capacity when the seeds were stored in a cold chamber and when isolated embryos were stored at −20 °C. The storage of isolated embryos at −196 °C and seeds at −20 °C represents a simple and inexpensive method for *A. vitrivir* germplasm conservation.

5. Conclusion

A. vitrivir seeds demonstrate orthodox behavior and high storage tolerance. Its oil has low acidity, a predominance of lauric acid, is very stable, and retains its quality under different fruit and seed storage conditions. Cryopreservation of isolated embryos as well as freezing seeds have potential for use in the conservation of the genetic resources of this species.

Acknowledgments

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity grants awarded to F.O. Silverio, P.S.N. Lopes and L. M. Ribeiro.

References

Angelovici, R., Galili, G., Fernie, A.R., Fait, A., 2010. Seed desiccation: a bridge between maturation and germination. Trends Plant Sci. 4, 211–218.
Besbes, S., Blecker, C., Deroanne, C., Lognay, G., Drira, N.-E., Attia, H., 2004. Quality characteristics and oxidative stability of date seed oil during storage. Food Sci. Technol. Int. 10, 333–338.
Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., 2013. Seeds: Physiology of Development, Germination and Dormancy. Springer, London.
Brasil, Ministério da Agricultura, Pecuária e Abastecimento, 2009. Regras para análise de sementes, Mapa/ACS, Brasília.
CETEC, Centro Tecnológico de Minas Gerais, 1983. Producão de combustíveis líquidos a partir de óleos vegetais: Estudo das oleaginosas nativas de Minas Gerais (Biofuels production from vegetable oils: study of native oleaginous plants from Minas Gerais), MG, Belo Horizonte, pp. 152.
Cellat, K., Beyhan, B., Güngör, C., Konuklu, Y., Karahand, O., Dündar, C., Paksoy, H., 2015. Thermal enhancement of concrete by adding bio-based fatty acids as phase change materials. Energy Build. 106, 156–163.
Chmielarz, P., Michalak, M., Pałucka, M., Wasileńczyk, U., 2011. Successful cryopreservation of *Quercus robur* plumules. Plant Cell Rep. 30, 1405–1414.
Corredoira, E., San-Jose, M.C., Ballester, A., Vieitez, A.M., 2004. Cryopreservation of zygotic embryo axes and somatic embryos of European chestnut. Cryo Lett. 25, 33–42.
Dias, D.S., Lopes, P.S.N., Ribeiro, L.M., Oliveira, L.A.A., Mendes, E.V., Carvalho, V.S., 2015. Tolerance of desiccation and cryopreservation of *Butia capitata* palm seeds. Seed Sci. Technol. 43, 90–100.
Dickie, J.B., Balick, M.J., Linington, I.M., 1992. Experimental investigations into the feasibility of *ex situ* preservation of palm seeds; an alternative strategy for biological conservation of this economically important plant family. Biodivers. Conserv. 1, 112–119.

Dransfield, J., W. Uhl, N., Asmussen, C. B., Baker, W. J., Harley, M. M., Lewis, C. E., 2008. *Genera Palmarum* –The Evolution and Classification of Palms.
Edem, D.O., 2002. Palm oil: biochemical, physiological, nutritional, hematological, and toxicological aspects: a review. Plant Food Hum. Nutr. 57, 319–341.
Ellis, R.H., Hong, T.D., Roberts, E.H., Soetisna, U., 1991. Seed storage behaviour in *Elaesis guineensis*. Seed Sci. Res. 1, 99–104.
Engelmann, F., Dumet, D., Chabrillange, N., Abdelnour-Esquivel, A., Assy-Bah, B., Dereuddre, J., Duval, Y., 1995. Factors affecting the cryopreservation of coffee, coconut and oil palm embryos. Plant Genet. Resour. Newsl. 103, 27–31.
Engelmann, F., 2004. Plant cryopreservation: progress and prospects. In Vitro Cell. Dev. Biol. Plant 40, 427–433.
Evaristo, A.B., Grossi, J.A.S., Pimentel, L.D., Goulart, S.M., Martins, A.D., Santos, V.L., Motoik, S., 2016. Harvest and post-harvest conditions influencing macauba (*Acrocomia aculeata*) oil quality attributes. Ind. Crops Prod. 85, 63–73.
Ferrari, R.A., Soler, M.P., 2015. Obtention and characterization of coconut babassu derivatives. Sci. Agric. 72, 291–296.
Finch-Savage, W.E., Leubner-Metzger, G., 2006. Seed dormancy and the control of germination. New Phytol. 171, 501–523.
Fintel, G.T., Berjak, P., Pammenter, N.W., 2004. Seed behaviour in *Phoenix reclinata* Jacquin, the wild date palm. Seed Sci. Res. 14, 197–204.
Folch, J., Less, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Grout, B.W.W., Shelton, K., Pritchard, H.W., 1983. Orthodox behavior of oil palm seed and cryopreservation of the excised embryo for genetic conservation. Ann. Bot. 52, 381–384.
Guedes, M.L., Ferreira, P.H.G., Santana, K.N.O., Pimenta, M.A.S., Ribeiro, L.M., 2015. Fruit morphology and productivity babassu palms in northern Minas Gerais state. Brazil. Rev. Árvore 39, 883–892.
Hong, T.D., Ellis, R.H., 1996. A Protocol to Determine Deed Storage Behaviour. International Plant Genetic Resources Institute, Rome.
Instituto Adolfo Lutz, 1985. Normas Analíticas, Métodos Químicos E físicos Para Análises De Alimentos. Instituto Adolfo Lutz, São Paulo.
Lorenzi, H., Noblick, L.R., Kahn, F., Ferreira, E., 2010. Brazilian Flora: Arecaceae (Palms). Instituto Plantarum, Nova Odessa.
Mamani, J.B., Costa-Filho, A.J., Cornejo, D.R., Vieira, E.D., Gamarra, L.F., 2013. Synthesis and characterization of magnetite nanoparticles coated with lauric acid. Mater. Charact. 81, 28–36.
Martínez, G., Sánchez, N., Encinar, J.M., González, J.F., 2014. Fuel properties of biodiesel from vegetable oils and oil mixtures. Influence of methyl esters distribution. Biomass Bioenergy 63, 22–32.
May, P.H., Anderson, A.B., Balik, M.J., Frazão, J.M.F., 1985. Subsistence benefits from the babassu palm (*Orbignya martiana*). Econ. Bot. 39, 113–129.
Miraliakbari, H., Shahidi, F., 2008. Oxidative stability of tree nut oils. J. Agric. Food Chem. 56, 4751–4759.
Murashige, T.M., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.
Neves, S.D.C., Ribeiro, L.M., Cunha, I.R.G., Pimenta, M.A.S., Mercadante-simões, M.O., Lopes, P.S.N., 2013. Diaspore structure and germination ecophysiology of the babassu palm. Flora 208, 68–78.
Ngobese, N., Sershen, Z., Pammenter, N.W., Berjak, P., 2010. Cryopreservation of the embryonic axes of *Phoenix reclinata*, a representative of the intermediate seeds category. Seed Sci. and Technol. 38, 704–716.
Orozco-Segovia, A., Batis, A.I., Rojas-Aréchiga, M., Mendoza, A., 2003. Seed biology of palms: a review. Palms 47, 79–94.
Panza, V., Pighin, D., Láinez, V., Pollero, R.J., Maldonado, S., 2009. Storage lipids and proteins of *Euterpe edulis* seeds. Biocell 33, 99–106.
Ribeiro, L.M., Garcia, Q.S., Oliveira, D.M.T., Neves, S.C., 2010. Critérios para o teste de tetrazólio na estimativa do potencial germinativo em macaúba. Pesqu. Agropec. Brasil 45, 361–368.
Ribeiro, L.M., Oliveira, T.G.S., Carvalho, V.S., Silva, P.O., Neves, S.C., Garcia, Q.S., 2012. The behaviour of macaw palm (*Acrocomia aculeata*) seeds during storage. Seed Sci. Technol. 40, 344–353.
Santos, D.S., Silva, I.G., Araújo, B.Q., Lopes Júnior, C.A., Monção, N.B.N., Citó, A.G.L., Souza, M.H.S.L., Nascimento, M.D.S.B., Costa, M.C.P., 2013. Extraction and evaluation of fatty acid composition of *Orbignya phalerata* Martius oils (Arecaceae) from Maranhão State, Brazil. J. Braz. Chem. Soc. 24, 355–362.
Siles, L., Cela, J., Munné-Bosch, S., 2013. Vitamin E analyses in seeds reveal a dominant presence of tocotrienols over tocopherols in the Arecaceae family. Phytochemistry 95, 207–214.
Silva, R.S., Ribeiro, L.M., Mercadante-Simões, M.O., NunesY.R.F. Lopes, P.S.N., 2014. Seed structure and germination in buriti (*Mauritia flexuosa*)—the swamp palm. Flora 209, 674–685.
Sisunandar, P.A.S., Samosir, Y., Rival, A., Adkins, S.W., 2010. Dehydration improves cryopreservation of coconut (*Cocos nucufera* L.). Cryobiology 61, 289–296.
Teixeira, M.A., 2008. Babassu—a new approach for an ancient Brazilian biomass. Biomass Bioenergy 32, 857–864.
Tweddle, J.C., Dickie, J.B., Baskin, C.C., Baskin, J.M., 2003. Ecological aspects of seed desiccation sensitivity. J. Ecol. 91, 294–304.
Wen, B., Wang, R., 2010. Pretreatment incubation for culture and cryopreservation of Sabal embryos. Plant Cell Tiss. Org. 102, 237–243.
Yuan, Y., Zhang, N., Tao Wenquan Cao, X., He, Y., 2014. Fatty acids as phase change materials: a review. Renew. Sust. Energ. Rev. 29, 482–498.
del Río, J.C., Evaristo, A.B., Marquesa, G., Martín-Ramos, P., Martín-Gild, J., Gutiérrez, A., 2016. Chemical composition and thermal behavior of the pulp and kernel oils from macauba palm (*Acrocomia aculeata*) fruit. Ind. Crops Prod. 84, 294–304.