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Storage, oil quality and cryopreservation of babassu palm seeds

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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

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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%.