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Chemical composition, embryo anatomy and viability by tetrazolium test of pyrenes of *Euterpe edulis* Mart.

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ABSTRACT

The advancement of knowledge and technologies for production, quality assessment and conservation of seeds of *E. edulis* is a need imposed by the high demand for this plants. Information on biometry, chemical composition and anatomy of seeds and seedlings give basis for the marking of future studies. The objectives of this study were to describe the biometrics and chemical composition of fruits and seeds, the anatomy of embryos; assess the viability of seeds by tetrazolium and classify the seeds of *Euterpe edulis* Mart. as for desiccation and storage tolerance. Seeds were analyzed for biometrics, water content, chemical composition, germination and seedling emergence. For the tetrazolium test, embryonic axes were immersed in a solution at 40°C for one, two, four and six hours. As for desiccation tolerance, seeds with water content of 45.7, 12 and 5% were evaluated for germination. *E. edulis* seeds have 83.1% carbohydrates and recalcitrant behavior, and the tetrazolium test conducted at 40°C in solution at 0.2% concentration for two hours proved to be adequate to estimate seed viability.

Key words: anatomy, Arecaceae, biometry, germination, viability

Composição química, anatomia do embrião e viabilidade pelo teste de tetrazólio em pirênios de Euterpe edulis Mart.

RESUMO

A demanda por sementes de *E. edulis* torna necessário o desenvolvimento de conhecimentos e tecnologias favoráveis à sua produção, avaliação da qualidade e conservação. Informações sobre biometria, composição química e descrição anatômica da semente e plântula possibilitam o balizamento de estudos futuros. Os objetivos deste trabalho foram: descrever a biometria e composição química de frutos e sementes, a anatomia dos embriões; avaliar a viabilidade pelo tetrazólio e classificar as sementes de *Euterpe edulis* Mart. quanto a tolerância à dessecação e ao armazenamento. As sementes foram submetidas à determinação biométrica, teor de água, composição química, avaliação da germinação e emergência de plântulas. Para o tetrazólio, eixos embrionários foram imersos na solução, a 40 °C por uma, duas, quatro e seis horas. Quanto a tolerância à dessecação, sementes com teores de água de 45,7; 12 e 5% foram avaliadas pelo teste de germinação. As sementes de *E. edulis* possuem 83,1% de carboidratos, são de comportamento recalcitrante e o teste de tetrazólio conduzido a 40 °C, em solução com concentração 0,2%, durante duas horas é adequado para estimar a viabilidade das sementes.

Palavras-chave: anatomia, Arecaceae, biometria, germinação, viabilidade

Introduction

The highest number of genera and species of palm trees is found in the tropics, most notably in Brazil. Palm trees are used in numerous ways, such as production of handicrafts, coal, dyes, fresh consumption and industries: food, cosmetics, timber, medicine, culinary, ornamental, among others. Juçara palm tree (*Euterpe edulis* Mart. - Arecaceae) is a species that stands out because its potential for commercial exploitation of palm hearts. In natural areas, populations of *E. edulis* have been destroyed due to the extraction.

Thus, because of the need for new repopulations, whether by replacement through replanting of disturbed areas, or by commercial plantations, the use of pyrenes, henceforth treated as seeds, of good physiological quality is essential for the success of the activity, since propagation of juçara palm hearts occurs exclusively through sexual means. Thus, it is imperative to know the physical and morphological characteristics of this species, as well as its behavior regarding desiccation and storage tolerance in order to develop appropriate techniques for processing and conservation.

Some studies have suggested that *E. edulis* seeds have recalcitrant behavior because they are sensitive to desiccation and storage (Martins et al., 2009; Roberto & Habermann, 2010), and drying can cause changes that accelerate deterioration (Nascimento et al., 2007). Desiccation reduces the viability of recalcitrant seeds by causing the formation of superoxide and hydrogen peroxide that destroy membrane lipids and lead to loss of cellular compartmentalization (Pukacka & Ratajczak, 2006).

Considering the process of deterioration of recalcitrant seeds, the evaluation of their viability with fast and effective methods such as the tetrazolium test is essential in production programs. Tetrazolium is a seed viability assessment based on the activity of dehydrogenase enzymes involved in respiration. Therefore, reduction reactions of the tetrazolium salt solution occur only in living cells and cause changes in the color of embryonic tissues, whereas dead tissues retain their original color (Silva et al., 2012).

In order to obtain accurate and reliable data using the tetrazolium test, besides the knowledge of the embryonic structure, standardization and improvement of specific techniques for each species is necessary, such as preparation procedures of seeds for dying, as well as the correct definition and interpretation of the resulting color (Flores et al., 2011; Silva et al., 2012).

Given the importance of the species and the absence of information, this study had the objective of investigating the biometrics and chemical composition of fruits and seeds; describing the anatomy of the embryo; assessing the viability of seeds by tetrazolium and classifying them as for desiccation and storage tolerance.

Materials and Methods

Ripe fruits newly harvested in the region of Ribeirão Preto from 20 arrays placed, at least, 100 m distant from each other, were packed in Styrofoam boxes and sent to the Seed Analysis Laboratory of the Department of Crop Production of the UNESP, Jaboticabal Campus.

Initially, fruits were washed in tap water over the sieve and then immersed in 0.05% sodium hypochlorite (NaOCl) for five minutes, discarding those that floated. Subsequently, they were washed in running water and visually sorted. Malformed and irregular fruits were excluded, and the selected fruits were dried in the shade, on paper towel, until losing surface moisture.

The extraction of the pulp (mesocarp + epicarp) for the analysis of chemical composition was performed manually by rubbing the fruits against the sieve to obtain the required amount of pulp. Pyrenes, consisting of endocarp and seed, were subjected to the same procedure but, in this case, under running water. After that, they were immersed in sodium hypochlorite solution, as aforementioned, rinsed under running water and dry in the shade, on a tray with paper towels, for as long as necessary to reducing the surface wetness.

Biometric data of 100 fruits and 100 seeds were obtained using a caliper in millimeters, with average and the standard deviation. The weight of thousand seeds (Brasil, 2009) and their water content was also obtained.

For determination of the chemical composition of fruit pulp and seeds, samples were oven-dried with forced air circulation at 55°C for 72 hours. Then, they were cooled in glass desiccators and sent for grinding. After this, the material was sent to be analyzed in the laboratory of the Food Technology Institute – ITAL, Campinas, SP.

The composition consisted of moisture and ash (AOAC, 2005; IAL, 2005), total lipids (IAL, 2005), and protein and calories (IAL, 2005). The total amount of carbohydrates was calculated by the difference: 100 - (moisture + ash + protein + total amount of lipids) (IAL, 2005). Starch was determined according to the Ewers method modified by Hardorn & Doewelaar (Diemair, 1963).

Whole and intact embryonic axes were used in the tetrazolium test. These were obtained by positioning the germ pore of the seed slightly ahead the pincer grippers, one of which went on the raphe and the other in the opposite position. After dividing the seed into two parts, the part containing the embryo was placed on small wooden board, and the endosperm was cut with a knife slightly ahead of the embryonic axis and in its direction, leaving it loose to be removed with the stylus. During the extraction process, the embryonic axes were kept on moistened filter paper with distilled water in petri dishes until completing the number needed for each treatment, for then apply the tetrazolium test.

The excision of 50 whole and intact embryonic axes for each treatment (five repetitions of 10 embryonic axis) took one hour and a half, with aid of two people.

To perform the tetrazolium test, embryonic axes were dipped in plastic cups containing solution with 2,3,5-triphenyl tetrazolium chloride (0.2%), kept in the dark at 40°C by 1, 2, 4 and 6 hours. Five replicates of ten embryonic axes for each treatment were used, in addition to the control treatment for each period where only buffer solution was used. After deadlines expired, these were washed three times and stored in distilled water for viability assessment under 10 and 15 fold magnifying lens.

The germination test was conducted in germination chambers with four replications of 25 seeds lying in *Sphagnum* sp. moistened with distilled water up to 60% of its holding capacity, in plastic boxes (28.5 × 18.5 x 10 cm), at constant 30 °C, under fluorescent white light and photoperiod of eight hours. Seeds were considered germinated when the primary root reached length equal or superior to the largest lenght of seed (Figure 1).

Seedling emergence was held under uncontrolled conditions in a laboratory environment. There were four replications of 25 seeds positioned at 1cm deep in washed sand, moistened with distilled water up to 60% of its holding capacity in boxes similar to those used in the germination test. The maximum and minimum temperatures were recorded on a daily basis, and the averages for the period studied were 28 and 24.4°C, and the criterion adopted was the formation of normal seedlings (Figure 1).

For the anatomical description of the embryo, permanent slides of longitudinal and cross sections of embryos were prepared following the methodology described by Johansen (1940), using the triarch quadruple color technique (Hagquist, 1974).

Following the protocol proposed by Hong & Ellis (1996) for the study of desiccation and storage tolerance, seeds of *E. edulis* were placed in 30 cm - diameter glass desiccators, kept at 20°C, and containing 20% of the own volume of silica gel (one kg per dissector). The silica gel was replaced when its color changed from blue to pink. The mass of the seeds was periodically measured until reaching the water content desired according to the formula: % WL = (100 × (MCi - MCf)/(100- MCf)), where: % WL: percentage of weight loss; MCi = initial moisture content (% wet basis) and MCf = final moisture content (% wet basis).

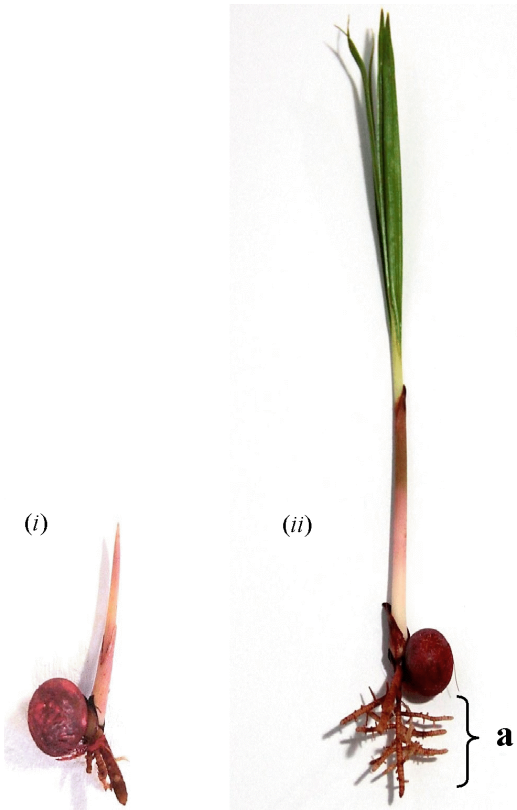

Figure 1. Seedling germination and emergence criteria of *Euterpe edulis*: (i) primary root length equal or superior to the largest length of seed; (ii) normal seedling; a) primary root

Water content and germination tests were determined: a) immediately after the processing (control), b) after reaching estimated water content between 10 and 12%, c) after reaching estimated water content of 5%, d) after storage under moisture contents of 5%, packed under vacuum, at -20°C for three months.

The water content, wet basis, of seeds was determined at 105 ± 3°C for 24 hours with two replicates of 10 to 30 units each (Brasil, 2009).

Before the germination test, seeds subjected to storage at -20°C were defrosted at 20°C for 24 hours.

Before germination and seedling emergence, seeds were chemically treated with the fungicide fludioxonil (25 g L^{-1}) by diluting 20 mL of the commercial product in 50 mL of water.

For statistical analysis, the experimental design was completely randomized, and data were previously transformed into arcsen $(x/100)^{1/2}$ and subjected to analysis of variance, and means were compared by Tukey test ($p = 0.05$).

Results and Discussion

Ripe fruits of *E. edulis* have spherical shape, purple-black epicarp, with the stigma remnants and pedicel scar (Figure 2). The mesocarp is thin and the endocarp has long fibers. Values of the smallest and largest diameter of the fruits were 12.79 and 14.41 mm (standard deviation of 0.54 and 0.61 mm, respectively, and CV = 4.2), while the minor and major diameters of seeds were 12.02 and 13.33 mm (standard deviation of 0.57 and 0.77 mm, respectively, CV = 5.2%). The mass of 1000 seeds was 716 g (38% water content) and 1 kg contains 1,397 units, differing from information in the literature that one kilogram of *E. edulis* seeds contains about 750 units (Lorenzi et al., 2004).

Variations in the dimensions of fruits and seeds are subject to the influence of factors such as genetic variability of parenting, weather conditions, availability of water and nutrients during the process of maturation and water content. Thus, the distinction of seeds by weight and size can improve the lots in relation to uniformity of emergence and seedling vigor (Moura et al., 2010).

In addition, *E. edulis* individuals producing large seeds have an ecological advantage because they may have greater germination success and vigorous seedlings (Pizo et al., 2006). Seed size can be an important factor to determine which individuals are able to highlight the numerous seedlings that can be found in the understory (Pizo et al., 2006).

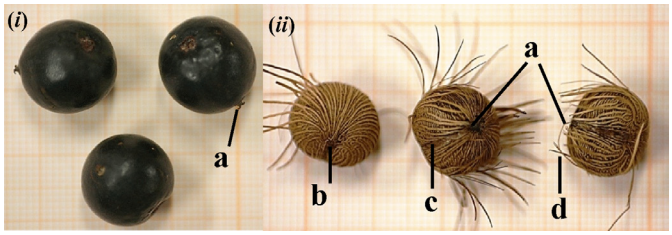

Figure 2. (i) Ripe fruits of *Euterpe edulis*. a) Remnants of stigma; (ii) Seeds of *Euterpe edulis*. a) Germ pores; b) Hilo; c) Raphe; d) Endocarp fibers

The classification of seeds by density is also a strategy used for the uniform emergence of seedlings, since this allows obtaining seedlings of similar size and/or vigor. Thus, higher density of seeds may be potentially stronger, resulting in more vigorous seedlings.

Regarding chemical composition, the fruit pulp and seed of *E. edulis* have a low percentage of ash, total lipids and proteins (below 6.3%), and both have a percentage of total carbohydrates above 80% (Table 1), while the amount of starch in the seeds was considered undetectable (< 0.10 g 100 g^{-1}).

In the proximate determination of *E. edulis* seeds, 83.1% of total carbohydrates, 3.9% of crude protein and 5.9% of lipids. These values agree partially with those obtained by Borges et al. (2011) for fruits and seeds of *E. edulis.* However, these authors reported that the place of production can influence the chemical composition of fruits and seeds.

When assessing the viability of *E. edulis* embryos through the tetrazolium test (Brasil, 1992), those with at least the embryonic axis colored are considered viable, the region located in the proximal region of the embryonic axis. However, in this study, embryos were colored only in the distal region, contradicting Brasil (1992), which in fact should have led the authors to withdrew the recommendation of the new edition, in Brazil (2009).

In longitudinal section of *E. edulis* embryos (Figure 3), the embryonic axis is located in its proximal region (h), and most of the cotyledon is in the distal region (i) (Figure 3).

The proximal region (h) corresponds to the cotyledon petiole and involves the embryonic axis and the distal region (i) refers

Table 1. Chemical composition (%) of the fruit and seed pulp of *Euterpe edulis*

Structure	Humidity	Ashes	Total lipids	Protein (N×5.75)	Total carbohydrates[1]	Calories[2]
	(g.100 g^{-1})					(kcal 100 g^{-1})
Fruit pulp	3.4	4.3	5.8	6.3	80.2	398.2
Seed	5.2	1.9	5.9	3.9	83.1	401.1

[1]Calculated by the difference: 100 - (g 100 g^{-1} humidity + g 100 g^{-1} ash + g 100 g^{-1} protein + g 100 g^{-1} total lipids).[2] The calorific value of the sample was calculated as the sum of the percentages of protein and carbohydrates multiplied by factor 4 (kcal g^{-1}) plus the total lipid content multiplied by the factor 9 (kcal g^{-1}).

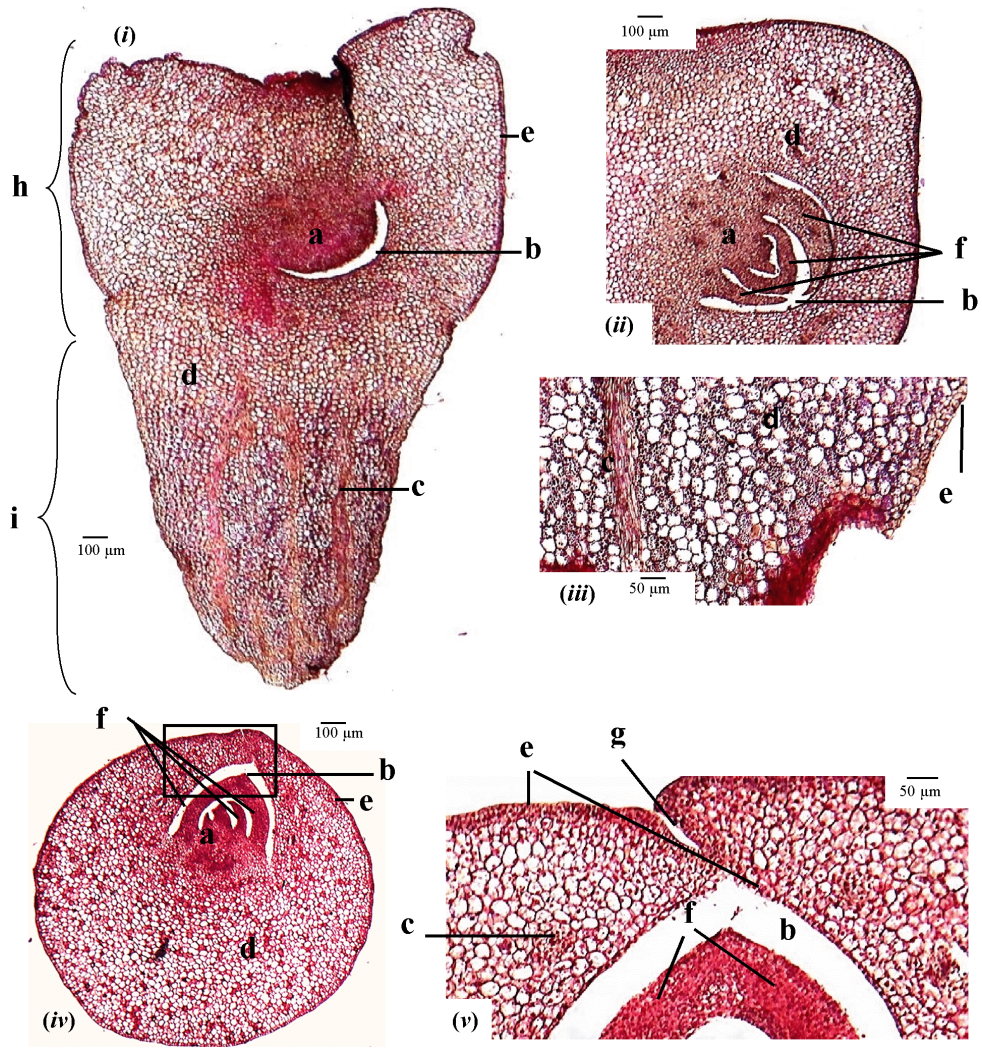

Figure 3. *Euterpe edulis* embryo: (i) Longitudinal section; (ii) Detail of the embryonic axis with greater tissue differentiation; (iii) Detail of the cotyledon distal region; (iv) Cross section of the embryo in the embryonic axis region; (v) Detail of cotyledonary slit. a) embryonary axis; b) cotyledon cavity; c) procambium; d) cotyledon; e) protoderm; f) leaf primordia of the embryo; g) cotyledonary slitt; h) proximal region; i) distal region of the embryo

to the cotyledon limbus, which becomes the haustorium in the germination process. Likewise, in juçara, two distinct regions occur in the embryo, which were separated by difference in color and/or light constriction (Aguiar & Mendonça, 2003). The plumule is differentiated in three leaf primordia (f), located within the cavity of the cotyledon petiole (b). The cotyledonary slit (g) is conspicuous, similarly to that observed in the bacabinha palm seedlings (Oliveira et al., 2010).

In the petiole and in the cotyledon limbo, the protoderm (e) is uniseriate and formed by flat cells (Figure 3e), similarly to embryos of other palms, such as bacabinha (Oliveira et al., 2010) and *Phoenix roebelenii* (Iossi et al., 2006).

In cotyledons the parenchyma is composed of cells with thin walls, large intercellular spaces and abundant granular content (Figure 3), similar to the seed haustorium of peach palm, which consists of parenchymatous cells with intercellular large spaces with abundant starch (Silva et al., 2006). In the proximal region (h), the meristem is formed by cells with more thickened walls and less intercellular spaces, bordered by raphides mainly around the plumule, while in the distal region of the embryo (i), early differentiation of vascular bundles (c) is observed, as data in Figure 3.

The need to remove only the embryo as preparation method for staining in tetrazolium test was also reported for seeds of other palm species such as peach palm (Ferreira & Sader, 1987) and macaúba (Ribeiro et al., 2010;. Rúbio-Neto et al., 2012). Removal of this structure allows the tetrazolium solution to diffuse up through the membranes, uniformly coloring vital tissues.

When vigorous seeds are slowly colored, they get a bright pink tone in the surface, what indicates that the solution had difficulty to penetrate into tissues due to increased integrity of the cell membrane system. Older seeds, deteriorated or damaged are usually colored faster and more deeply and show reddish color (Silva et al., 2012). However, the intense red color may indicate excessive time of contact with the tetrazolium or inadequate solution concentration.

The results obtained with seeds whose water content was 35.6%, coming from fruits harvested and pulped one week before processing and packaged in trays at room temperature at constant 20°C, were not satisfactory. Embryos were slightly shriveled and yellow when sectioned, and became brown, as initial tests, after having been subjected to immersion tests in distilled water (controls) or tetrazolium solution, at 40°C for 2 hours (Figure 4).

Then, new tests were proceeded, fruits were harvested, pulped and embryos were extracted on the same day (seeds with 49.6% of moisture), which showed satisfactory results on the color pattern, which made it possible to assess the lot as for viability (Figure 5).

In order to make the tetrazolium test for *E. edulis* seeds, preliminary tests were performed (data not shown) with variation in the concentration of salt solution (0.05, 0.075 and 0.1%), but these methods have not been consolidated, because when embryos underwent the procedure, this resulted in oxidation of tissues, giving them a brown color, and when they were processed after one week (Figure 4).

In the tetrazolium test, embryos stained on the whole surface of the distal region were considered viable, and with the results, 58% were considered viable in the period of one hour, 66% in two hours, 67% in four hours and 72% in six hours of soaking. Thus, the same pattern of quality was seen when seeds were subjected to controlled conditions as well as environment variables, because they reached 64% and 65% of germination and seedling emergence, respectively.

For the estimation of viability by the tetrazolium test to be valid, a tolerance of up to five percent points of difference up or down of the tetrazolium in relation to the germination percentage (original data) is accepted, and major differences indicate problems in the implementation or evaluation of tests.

For *E. edulis* seeds, periods of two and four hours were adequate to estimate viability. However, in order that this interpretation be correct, due to excessive coloration which may indicate high metabolic rate and thus low vigor or seed number, the staining period can be reduced to two hours, according to the proper staining (Figure 5) and less than 5% of difference in the results of germination and seedling emergence.

In the case of the study of the desiccation of tolerance and storage, germination happened only in control seeds with 45.7% water content at the time of assessment (Table 2), while for other treatments, reducing the water content of the seeds

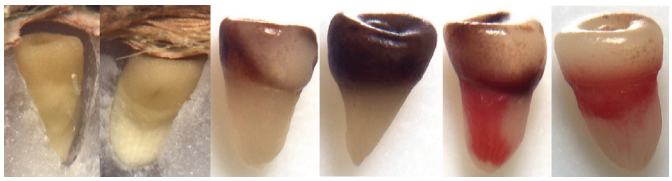

Figure 4. *Euterpe edulis* embryos of seeds with 35.6% water content. From left to right: two embryonic axis of freshly cut seed; two subjected to control treatment (distilled water, at 40°C for 2 h); and other two subjected to TZ treatment under concentration of 0.2% at 40°C for 2 h)

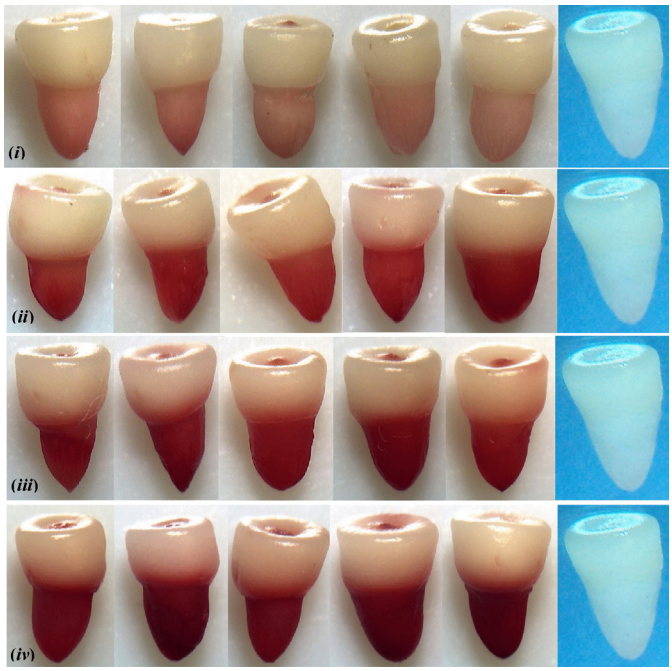

Figure 5. *Euterpe edulis* embryos submitted to the tetrazolium test at a concentration of 0.2% at 40°C for one (i), two (ii), four (iii) and six hours (iv) compared with embryonic control on the right axis

Table 2. *Euterpe edulis* seed germination with different water contents

Seed water content (%)	Period in desiccators and storage (days)	Germination (%)	
		Original data	Transformed data [1]
Witness (45.7)	0	72	58 a[2]
12	21	0	0 b
5	26	0	0 b
5 (3 months a -20 °C)	116	0	0 b
Teste F			212.78**
DMS (5%)			8.42
CV (%)			27.42

[1]Arcsine transformation $(x/100)^{1/2}$.

[2]Numbers followed by the same letter, vertically, do not differ according to Tukey test at 5% probability.

** Significant at 1% probability.

caused death thereof and, therefore, did not germinate. Thus, according to the methodology proposed by Hong & Ellis (1996), seeds of *E. edulis* have recalcitrant behavior as for desiccation and storage tolerance.

As in this study, germination and vigor were hampered by partial drying and increased storage time in the case of seeds of *E. edulis*, due to variation depending on the production environment, leading to different recalcitrant levels (Martins et al., 2009). For other species of the genus *Euterpe*, recalcitrant behavior of seeds was also observed, as in *E. espiritosantensis* (Martin et al., 2007) *E. oleracea* (Nascimento et al., 2007). Moreover, it is important to consider that the environment in which the seeds develop determine the pattern of desiccation tolerance because tropical species seeds develop under high humidity and they virtually do not lose water during maturation, suffering injuries when submitted to drying (Nascimento et al., 2007).

Conclusions

Euterpe edulis Mart. seeds are rich in total carbohydrates in composition and contain no starch;

They have recalcitrant behavior and desiccation and storage tolerance;

The tetrazolium test conducted at 40°C under 0.2% solution for two hours is adequate to estimate seed viability;

Germination is of the type adjacent ligular.

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