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Rapid results of peach palm seed viability: a methodological proposition for the tetrazolium test

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ABSTRACT: Prior to commercialization, seeds of peach palm (*Bactris gasipaes* Kunth) have to undergo the germination test, whose well-established methodology takes 120 days. Due to their recalcitrant behavior, the seeds have short longevity when stored (around 30-45 days), which makes it challenging to select the most viable ones for marketing. This study aimed to determine a methodology for the tetrazolium test to be carried out in peach palm seeds, in order to fast deliver results that can be correlated to the germination test. Different forms of pre-conditioning, preparation, and staining were investigated via moisture content, germination, and tetrazolium tests, so as to define the vital parts of the seed and sort out the viability classes. For the seed lot under study, the tetrazolium test delivered results supported by the germination test when the following procedures were adopted: pre-conditioning by water submersion (20 °C for 24 h), longitudinal cut adjacent to the embryo, and half-seed immersion (embryo + endosperm) in a 1.0% tetrazolium solution for 4 h at 30 °C. Having fulfilled these criteria, it became possible to separate the peach palm seeds into two classes (viable or non-viable).

Index terms: Arecaceae, *Bactris gasipaes*, germination, physiological quality, biochemical test.

Resultado rápido da viabilidade de sementes de pupunha: proposta de metodologia para o teste de tetrazólio

RESUMO: Para comercialização das sementes de *Bactris gasipaes* Kunth, é necessária a realização do teste de germinação, cuja metodologia já se encontra estabelecida e tem duração de 120 dias. Em função do comportamento recalcitrante, a semente possui curta longevidade no armazenamento, em geral 30 a 45 dias, o que dificulta a seleção de sementes viáveis para a comercialização. O trabalho teve por objetivo estabelecer uma metodologia para condução do teste de tetrazólio em sementes de pupunha para obter resultados rápidos e que possam ser relacionados com o teste de germinação. Foram realizadas avaliações do teor de água, teste de germinação e teste de tetrazólio, considerando-se diferentes formas de pré-condicionamento, preparo e coloração, as áreas vitais da semente e estabelecendo as classes de viabilidade. O teste de tetrazólio, em função dos resultados obtidos para o lote estudado, forneceu resultados correlacionados ao teste de germinação mediante sua condução com pré-condicionamento por imersão em água (20 °C por 24 h), corte longitudinal adjacente ao embrião e imersão de metade da semente (embrião + endosperma) em solução a 1,0%, por 4 h a 30 °C. Assim, foi possível separar as sementes de pupunha em duas classes (viáveis e não viáveis).

Termos para indexação: Arecaceae, *Bactris gasipaes*, germinação, qualidade fisiológica, teste bioquímico.

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INTRODUCTION

Brazil is the biggest world producer and consumer and places second in exportation of hearts of palms. After the prohibition of the extractive exploitation of *juçara* palm trees (*Euterpe edulis* Martius), native to the Brazilian Atlantic Forest, peach palm (*Bactris gasipaes* Kunth) became a sustainable alternative for supplying the demand. The species has been cultivated mostly by small farmers (Graefe et al., 2013), which contributes to the generation of income and job opportunities locally, thus helping to contain the rural exodus.

The sustainability of the peach palm culture encompasses the development of research for the reuse of residues generated by its processing. Currently, agro-industrial by-products have been employed as a substrate for mushrooms (Sales-Campos et al., 2011) and seedlings (Sá et al., 2020), for manufacturing wood biocomposites (Haro et al., 2018) and wooden panels (Quinaya et al., 2016; Pinheiro et al., 2017), for fabricating biodegradable packaging (Silva et al., 2017; Melo-Neto et al., 2018), as a complement in animal nutrition (Schmidt et al., 2010; Santos-Cabral et al., 2015), and as a soil conditioner (Bellettini et al., 2017). The fruit has been used for flour production (Martínez-Girón et al., 2017), and the oil has applications in cosmetology (Mujica et al., 2017).

The potential of the species has raised interest in its cultivation, which relies heavily on the seed (diaspore) as the main alternative for multiplication (Belniaki et al., 2020). It is worth remarking that the vegetative propagation still has some obstacles, such as the difficulty of rooting, the low survival rate of the offshoot cutting technique (Tracz et al., 2009), and the lack of consolidated micropropagation protocols (Graner et al., 2015).

Performing the germination test is a prerequisite for marketing forest seeds (Brasil, 2016). The methodology for peach palm has already been established and recommends to finish the test at 120 days after sowing (Brasil, 2013), as the seeds exhibit dormancy (Nazário et al., 2017). Notwithstanding, due to their recalcitrant behavior (Bovi et al., 2004), stored peach palm seeds have short longevity (30-45 days) (Ramalho et al., 2005), which makes it unfeasible to obtain the germination result in time for the commercialization of the lots.

For that reason, the tetrazolium test might be an option to speed up the viability assessment of peach palm seeds. However, the Instructions for Forest Species Seed Analysis (*Instruções para Análise de Sementes de Espécies Florestais*) contains no specific methodology recommendation (Brasil, 2013). A preliminary study adapting the tetrazolium test for peach palm can be found in the literature, though (Ferreira and Sader, 1987). It requires making an excision in the embryo, an unpractical and time-consuming procedure, which expends a large number of seeds and depends on the skill of the analyst to prevent the loss of material due to mechanical injuries. Moreover, disregarding the evaluation of the seed endosperm makes the test interpretation incomplete, as the reserve tissue is responsible for nourishing the embryo for months, until the complete seedling formation. This reinforces the need for more detailed testing for this plant.

The present study aimed to adapt the methodology of the tetrazolium test for *B. gasipaes* seeds, so as to obtain rapid results that can be related to germination.

MATERIAL AND METHODS

Peach palm seeds were provided by the Project for Dense-spacing Consortium Reforestation (*Projeto de Reflorestamento Econômico Consorciado Adensado* – RECA), in *Nova Califórnia*, Brazilian state of *Rondônia*. After being harvested, they were treated with the systemic fungicide carbendazim (benzimidazole) at 100 mL.100 kg^{-1} of seeds. The material was packed in a plastic bag, which was stowed in a cardboard box and ultimately shipped by air to the laboratory of seed analysis, in *Curitiba*, state of *Paraná*.

In the laboratory, the sample was manually homogenized and divided into four subsamples of similar mass (statistical replications). First, the moisture content was determined in two replications of five whole seeds, via the oven method at 105 ± 3 °C for 24 h (Brasil, 2009). The germination test was conducted in eight replications of 25 seeds, which were sown at a depth equivalent to their diameter. In this process, the fertile germination pore (with the fibre plug) was put in contact with the substrate bed, in a fashion that the other two sterile germination pores faced up. Sterilized finegranulometric vermiculite was used as the substrate, placed inside plastic boxes perforated at the bottom (17.5 x 13.2 x 11.5 cm). The sets were moistened with a volume of water equivalent to the substrate retention capacity. Finally, the plastic boxes were stored in a Mangelsdorf-model germinator at 25 °C (Brasil, 2013) and under a light source.

The germination was tested 120 days after the experiment was settled (Brasil, 2013), when the seedlings were removed from the substrate and evaluated for well-developed roots (both primary and adventitious) and first cataphyll emergence. The results were expressed in percentage of normal seedlings.

In turn, the investigation of the tetrazolium test followed these procedures:

Pre-conditioning: The whole seeds were submerged in water at 20 °C for 24 hours, inside a plastic box (17.5 x 13.2 x 11.5 cm). This step proved to be necessary for facilitating the cutting on the otherwise hard endocarp.

Preparation: A longitudinal cut adjacent to the embryo (Figure 1A) was performed with a bench guillotine (Figure 1B), and the half seed without the embryo was disposed. In pre-tests, the guillotine did not injure the embryo during cutting – unlike what frequently happened when a vise was used, so this tool was discarded. The guillotine cuts were verified individually in a stereomicroscope, before staining the material, to avoid releasing the embryo during the washing step, which would compromise the test interpretation. The excess of endosperm, often observed in some cuts, were carefully removed during the analysis with the aid of a fine-tipped scalpel, without damaging the embryo.

Staining: Four replications of 25 seed halves containing the embryo (embryonic axis + cotyledon) and the endosperm were stained by direct immersion in 35 mL of tetrazolium solution. The process was carried out inside 200-mL disposable plastic cups, which were placed inside a BOD chamber at 30 °C, in the dark. Two concentrations of the tetrazolium solution (0.5 and 1.0%) and two staining times (2 and 4 h) were considered. Following each staining period, the samples were double-washed inside a container, with the aid of a sieve, so as not to remove the embryo under running water.

After that, the vital areas of the seed were established with the aid of a stereoscopic microscope equipped with a camera. The intensity of the color and both integrity and texture of the tissues were analyzed in each treatment, aiming to determine the viability classes (viable or non-viable) in comparison to the equivalent results achieved from the germination test.

The statistical analysis used the general linear models (GLM) approach, in which the normal and binomial distributions were investigated to identify the model that best fitted the data. The choice of the model took into account the graphical analysis of the residues, and also used the Akaike information criterion, which penalizes excessively complex or poorly adjusted models (Cordeiro and Demétrio, 2013). Thus, the study adopted a normal distribution, represented by the equation: $f\ (y, \mu, \sigma^2)\ = \frac{1}{\sqrt{2\pi\sigma^2}}\ exp\left[-\frac{1}{2}\frac{(y\ -\ \mu)^2}{\sigma}\right]$. In the context of this study, y represents the ratio of viable seeds, μ is the average ratio of viable seeds, σ^2 is the variance, and π is approximately to 3.1416 (Olsson, 2002).

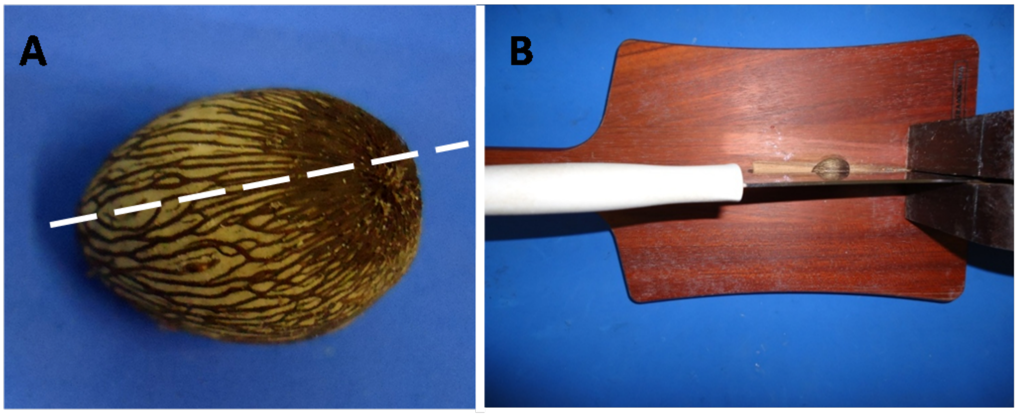

Figure 1. Preparation of a peach-palm seed (*Bactris gasipaes*) to expose the embryo for the tetrazolium test. (A) detail of the longitudinal cutting, adjacent to the embryo; (B) bench guillotine used to cut the seeds.

The experiment complied with a completely randomized design, with four replications. The means were compared by the Scott-Knott test at a 5% probability. The data on the moisture content of the seeds were not evaluated statistically.

RESULTS AND DISCUSSION

The initial moisture content of the peach palm seeds (before testing) was 44.9%, which confirms the recalcitrant behavior of the species (Bovi et al., 2004; Barbedo, 2018). This value is higher than the critical moisture content of 38.0% defined by Ferreira and Santos (1992), therefore indicating that the seeds were apt for the experiment.

The characterization of the seed parts revealed a lignified endocarp (Silva et al., 2006), surrounded by a thin integument (Nazário et al., 2013), covering the seed (Figure 2A). It also showed an endosperm rich in lipids – which implies less storage potential, as these compounds are unstable over time (Marcos-Filho, 2015) – and an embryo with approximately 3 mm.

The embryo has a conical shape and uniform color, and it can be divided into two regions (Figure 2B): one proximal (Rp) and the other distal (Rd) to the germination pore. The proximal portion contains the internal embryonic axis, whose plumule exhibits three primordial leaves and an undifferentiated root pole (Nazário et al., 2013). In turn, the distal region, which encompasses the cotyledon, is intensely vascularized and originates the haustorium (Dias et al., 2018). This structure is responsible for digesting the endosperm to nourish the development of the embryo (Silva and Clement, 2005).

The function of the embryo portions becomes more evident as the germination process begins (Figure 2C). In this stage, the region proximal to the pore (bearing the embryonic axis) forms the germinative button, which exits through the germination pore (Figure 2D) (Ferreira, 2005).

Cutting longitudinally, adjacent to the embryo (Figure 1A), is a technique similar to the one used for the tetrazolium testing in *E. edulis* (Oliveira et al., 2014) and *E. oleracea* seeds (Lima et al., 2018). This type of cut favors the evaluation

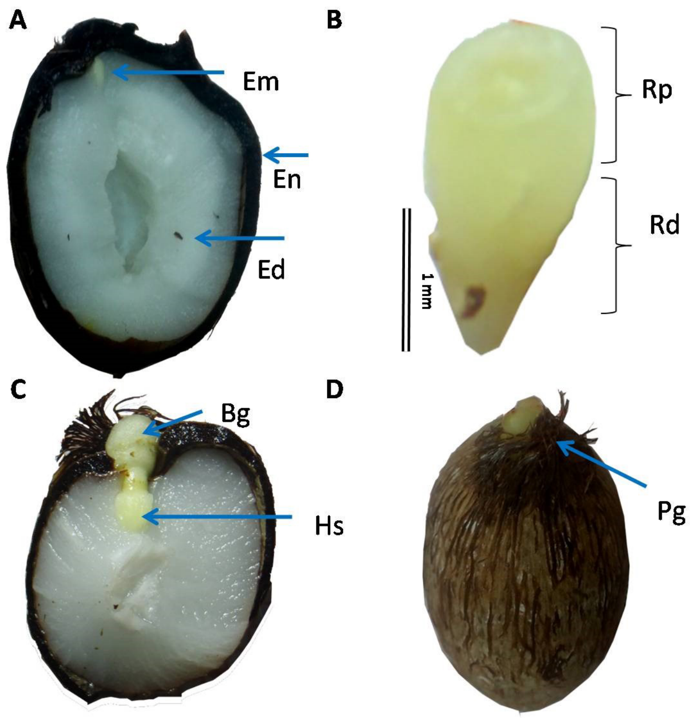

Figure 2. Parts of the peach palm seed (*Bactris gasipaes*). (A) Em – embryo, En – endocarp, Ed – endosperm. (B) structure of the embryo: Rp – region proximal to the germination pore (embryonic axis); and Rd – region distal to the germination pore (cotyledon). (C) evolution of the embryo at the beginning of germination: Bg – germinative button and Hs – haustorium. (D) Pg – germination pore.

of the endosperm, and it is internationally recommended for interpreting the tetrazolium test in most tree and shrub seeds (ISTA, 2015).

The interpretation of the results was based on the change of color of the living tissues when in contact with the tetrazolium solution. This phenomenon reflects the activity of the dehydrogenase enzymes involved in the respiratory activity, as they catalyze the reaction of the H^+ ions released, forming a non-diffusible, red-colored substance called triphenylformazan (França-Neto and Krzyzanowski, 2019).

On that account, the seeds belonged to the viable group when they displayed one of these sets of characteristic: intact endosperm and completely red-carmine embryo (Figure 3A); intact endosperm and embryo with the region proximal to the germination pore (containing the embryonic axis) stained in a less intense red and the distal part (cotyledon) in carmine red (Figure 3B); or intact endosperm with small pink central spots, germinative button colored in light red and distal region in carmine red (Figure 3C).

On the other hand, the non-viable class included seeds with one of the following set of features: intact endosperm and distinctly bicolored embryo, with an uncolored proximal region (embryonic axis) and a carmine-red distal region (cotyledon) (Figure 4A); intact endosperm and embryo with the distal region only partially stained in red (Figure 4B); intact endosperm and embryo with only part of the distal region colored in pink (Figure 4C); intact endosperm and completely unstained embryo (Figure 4D); intact endosperm and embryo with a yellowish color and some deterioration degree (Figure 4E); endosperm in an advanced stage of deterioration and pink-stained embryo (Figure 4F); absence of more than 50% of the endosperm and pink-colored embryo (Figure 4G); and, lastly, endosperm with softened consistency due to some deterioration and pink embryo with whitish spots (Figure 4H).

As for the concentration of the tetrazolium solution and the staining time, the most evident results for interpreting the peach palm seed viability were delivered by the combinations 1.0% for 2 h and 1.0% for 4 hours. These conditions produced averages statistically similar to those of the germination test. Conversely, the results of the treatments at a 0.5% concentration showed no relation with the germination test (Figure 5).

Once defined the vital areas of the seeds (Figure 2), the classes viable (Figure 3) and non-viable (Figure 4) were established. The most common examples of non-viable seeds within the studied lot included those with some pathogen-related rot (Figure 4F) and those with a bicolored embryo exhibiting a milky white proximal region (embryonic axis) and a carmine-red distal portion (cotyledons) (Figure 4A). Regarding the former case, the pathogen identification is pendent, to be addressed in further research.

The death of the embryonic axis is possibly related to the loss of water through the germination pore, as this region is more sensitive to dehydration. Problems caused by seed dehydration were also found in embryos of the palm species *Acrocomia aculeata* (Rubio-Neto et al., 2012).

Staining for 2 h at a 0.5% tetrazolium produced a very light hue in the seed tissues, leading to an underestimation of the lot viability. On the other hand, performing the process for 4 h at the same concentration overestimated the lot potential, as non-colored spots could not be easily visualized, making the result interpretation inaccurate. Nevertheless,

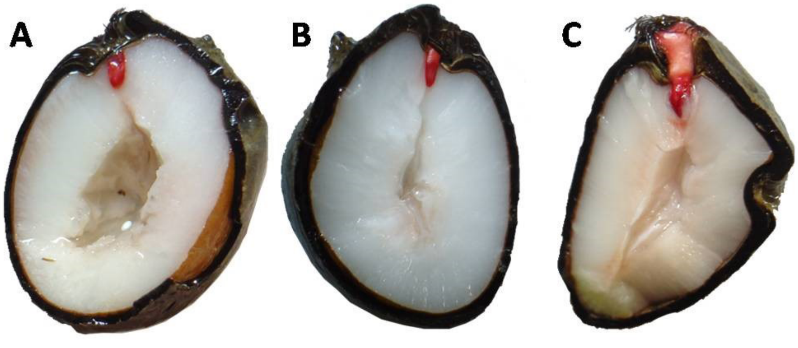

Figure 3. Types of peach palm seeds (*Bactris gasipaes*) included in the viable class by the tetrazolium test.

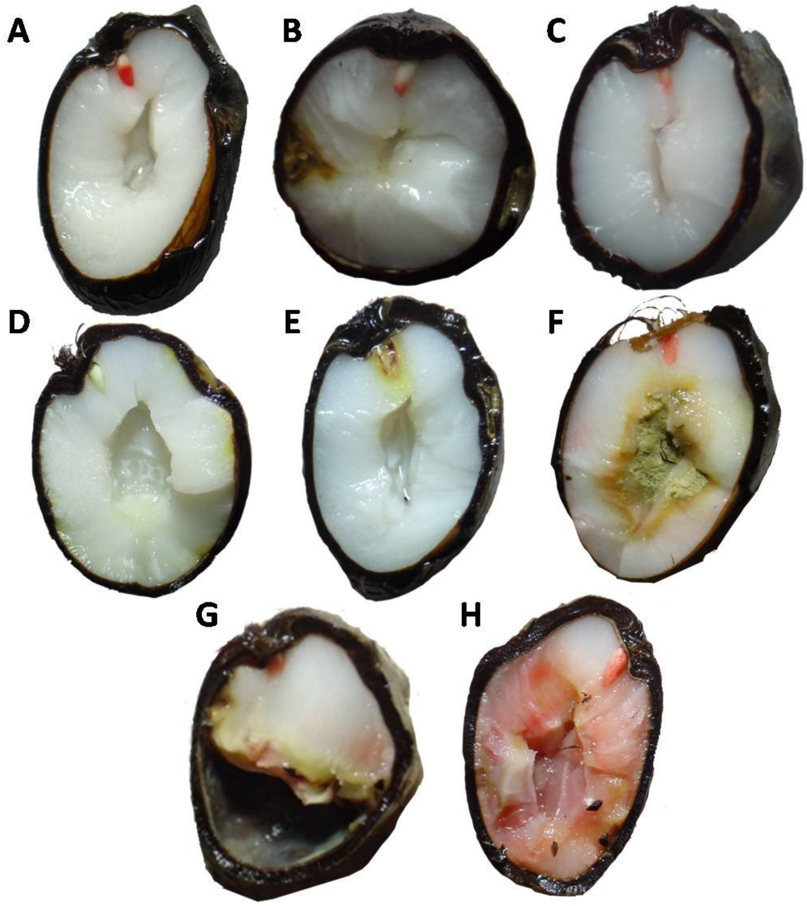

Figure 4. Types of peach-palm seeds (*Bactris gasipaes*) included in the non-viable class by the tetrazolium test.

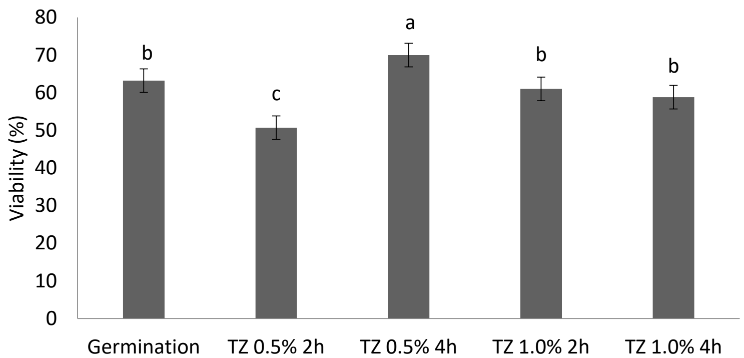

Figure 5. Viability of peach-palm seeds (*Bactris gasipaes*) assessed via germination test and tetrazolium test (using different combinations of concentration, in %, and staining time, in h). Means followed by the same letter do not differ, according to the Scott-Knott test at a 5% probability. Error bar = standard error. TZ = Tetrazolium test.

in other species of the same family, concentrations equal to or lower than the ones used in this study proved to be efficient. That was the case of 0.5% tetrazolium applied for 2 h to *A. aculeata* seeds (Ribeiro et al., 2010), and 0.2% for 4 h to *E. edulis* (Iossi et al., 2016).

Using the tetrazolium solution at 1% had the best correlation with the germination test. This concentration has also been indicated for assessing the seeds of other Arecaceae palms, such as *E. oleracea* Mart (açai) (Lima et al., 2018). Although there was no statistical difference in staining for 2 or 4 h (Figure 5), the 4-hour period delivered more consistent results. Thus, it allows the analyst to distinguish the viability classes more efficiently, assuring greater assertiveness in decision-making.

CONCLUSIONS

The tetrazolium test provides results correlated to the germination test, once the peach palm seeds are pre-conditioned by immersion in water (at 20 °C for 24 h), longitudinally cut adjacent to the embryo, and half-submerged (embryo + endosperm) in a 1.0% tetrazolium solution for 4 h at 30 °C. These conditions make it possible to separate the peach palm seeds in two viability classes: viable and non-viable.

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