

## Physiological Quality of Stored *Dipteryx alata* Vogel Seeds: Tetrazolium Test and Radiographic Imaging Standardization

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### Abstract

The *baru* (*Dipteryx alata* Vogel), is fruit tree displaying economic, environmental, industrial and food importance. The objective of this study was to adapt to the tetrazolium test methodology to *baru* seeds and evaluate the physiological quality of seeds stored for 12 months at 10 and 20 °C, using X-ray images as a toll for detection damage throughout the storage period while also carrying out germination, emergence and electrical conductivity tests. *Baru* seeds remained viable for 12 months of storage, albeit with a small decrease in vigor after nine months. The applied tetrazolium test methodology was efficient in determining internal seed damage. The pre-soak time for the tetrazolium test and solution concentration was 24 hours and 0.05%. No changes in seed morphology and radiographic images were observed during the storage periods, regardless of the assessed temperature. The X-ray test enabled a precise visualization of internal *baru* seed structures.

**Keywords:** Storage, *baru*, Viability, X-ray, vigor.

### 1. INTRODUCTION AND OBJECTIVES

The *baru* (*Dipteryx alata* Vogel) is a native species from the *Cerrado* domain, a fruit displaying high potential, used as a food item and in the medicinal and industrial areas. Its seeds present high nutritional value, with high protein and essential amino acid content, as well as considerable amounts of calcium, zinc, iron and fiber, thus considered an excellent dietary ingredient (Fernandes et al., 2010). Another important factor contributing to *baru* valuation is the fact that it is an excellent soil nitrogen fixator (Mota et al., 2012). This species belongs to the Fabaceae family and has been applied in recovery and planting areas aiming at pasture enrichment. It has wide distribution, especially in the drier regions (Oliveira et al., 2006; Soares et al., 2008). However, due to agro-industrial activity advances and human occupation of the *Cerrado* domain, the *Cerrado* territory has become fragmented, leading to vegetation restriction and habitat loss for various species (Klink & Machado, 2005; Brannstrom et al., 2008; Carvalho et al., 2009), including the *baru*.

Seed physiological quality, longevity and vigor are determining factors to ensure germination and seedling formation success, as well as plant species preservation. In this sense, orthodox seeds have mechanisms that favor their survival in different conditions. Fact, which contributes to understanding the behavior of seeds during storage (Bragante et al., 2018). The seeds of species of the Fabaceae Family, give most of them, an orthodox character. Tolerating prolonged periods of storage with a water content of approximately 5% (Freitas et al., 2019). However, there are few works with *Dipteryx alata* Vogel that describe the characteristics of seeds in this regard.

Therefore, it is important to create strategies to assess the viability of seeds. Maximizing the storage process to ensure minimal deterioration and loss of characteristics (Toledo et al., 2009). These strategies include factors that can be controlled in storage environments, such as in a temperature and humidity control chamber (type BOD).

It is widely recognized that storage is a viable means of maintaining seed physiological quality and guiding their

behavior over time, becoming especially important in contributing to the conservation of genetic information, leading to species preservation (Walters et al., 2005). Several techniques have been tested during seed storage targeting at metabolism conservation and reduction and aiming at high vigor maintenance, such as low water content and reduced temperature conditions (Kohama et al., 2006).

Different tests can be performed to evaluate seed vigor (Abreu, 2011; Alves, 2012), although all require specific species standardization. The tetrazolium test, based on tissue viability via respiratory activity (França Neto, 1998; Azerêdo et al., 2011), is noteworthy in this regard. X-ray imaging is also efficient in evaluating seed vigor, as it is an accurate, fast and non-destructive method that allows for the detection of internal seed damage.

X-ray imaging allows for the visualization of damage potentially caused by malformations due to harvesting, drying or insect attacks (Cícero & Júnior, 2003; ISTA, 2004; Pinto et al., 2007). It is important to note that seeds without full internal structure development increase the chances of low vigor seedling development or no development at all (Burg et al., 1994; Carvalho & Nakagawa, 2012). This technique is promising and aids in the selection of high-quality seeds which will, in turn, lead to best seedling development (Pinto et al., 2012; Lima et al., 2013). However, efficient, X-ray testing, as well as tetrazolium testing, also require standardization, taking into account X-ray intensity and exposure times. Thus, the adaptation and definition of these methodologies for the study of native Brazilian seeds is vital for the success of preservation programs (Ataide et al., 2012).

Given the above, the aim of the present study was to adapt to the tetrazolium test methodology for the assessment of *baru* seeds and evaluate the physiological quality of seeds stored during 12 months at 10 and 20 °C using X-ray images as a tool for damage detection during the storage period, in addition to germination, emergence and electrical conductivity tests.

## 2. MATERIALS AND METHODS

### 2.1. Fruit sampling

*Baru* fruits were collected at the *Bem Posta* Farm, located in the municipality of Portelândia, GO, Brazil, at 17° 15' S - 52° 40' N and 549 m altitude. Two fruit batches were purchased, harvested in August 2014 and 2015, with ripe and whole fruits scattered on the ground. After sampling, the fruits were taken to the Federal Goiano Institute Seed Laboratory, located in the Rio Verde municipality, where the fruit processing and experiments were carried out. Seeds were obtained after opening the fruits with the aid of a PIT BULL® hand press.

### 2.2. Water content

The water content of the seeds was determined by oven method at 105 ± 3 °C for 24 hours, adapted according to Brasil (2009), using four replications consisting of five seeds, corresponding to 10 grams. The seeds were weighed on an analytical balance with 0.001g precision and calculations were performed according to the following formula 1:

$$\% \text{ de Umidade } (U) = \frac{100(P - p)}{P - t}$$

Where P: initial weight, weight of the container more weight of the moist seed (g); p: final weight, weight of the container more the weight of the dry seed (g); t: tare weight, container. (% wet weight; w.b.).

### 2.3. Assay 1. Definition of the tetrazolium test methodology for *baru* seeds

The purpose of this test was to standardize the use of the tetrazolium test for *baru* seeds, using seeds from the two fruit batches collected in August 2014 and 2015. *Baru* fruits harvested in 2014 were stored in unsealed Styrofoam boxes and maintained at room temperature of 25 ± 3 °C for 12 months. The fruits harvested in 2015 remained under the same condition for 60 days. Seed extraction was performed via fruit opening with the aid of a PIT BULL® hand press. After seed removal, they were stored in a BOD incubator at 20 °C. Three tetrazolium salt solution concentrations, equivalent to 0.05, 0.075 and 0.10%, and two pre-soaking times were tested. Initially, three repetitions consisting of 10 seeds were distributed on germitest sheets, moistened with three times their dry weight and maintained in the BOD incubator for 24 and 36 hours. The established pre-imbibition period was intended to facilitate seed tegument removal and activate the cellular seed metabolism. Tegument removal was performed with the aid of a scalpel blade from the superficial and longitudinal section towards the raphe. After tegument removal, the pre-soaked seeds were immersed in 30 mL of a tetrazolium salt solution at the aforementioned concentrations above for two and a half hours in the BOD incubator at 35 °C.

Seed vigor evaluation was performed through individual determinations using a magnifying glass, classified into four classes, as follows: very vigorous (VV), viable (V), deteriorated (D) and unviable (UNV).

### 2.4. Assay 2. Seed Storage

*Baru* fruits harvested in 2014 were stored in unsealed Styrofoam boxes and maintained at room temperature of

25 ± 3 °C for 12 months and extracted from the fruits as mentioned previously.

The first lot was randomly divided into five samples, each representing a storage period, as follows: zero (control), three, six, nine and twelve months. The initial water content of the seeds was obtained from the control sample, equal to 7,49% (w.b.). Then, the seeds were packed in hermetically sealed glass jars and the voids were filled with cotton to reduce oxygen exchanges with the investigated material. The seeds from the five samples were then maintained in a BOD incubator at 10 and 20 °C. At each time point, subsamples of storage at 10 and 20 °C were removed and submitted to water content determination and vigor evaluations were conducted through electrical conductivity tests, x-ray imaging, germination and emergence assessments. The length and dry mass of the plants were determined to quantify *baru* seedling growth.

### 2.5. Electrical Conductivity Test (EC)

The electrical conductivity of the seed imbibition solution was determined according to a methodology adapted from Vieira and Krzyzanowski (1999). Four replicates consisting of five previously weighed seeds immersed in 75 mL of distilled water for 24 hours in a BOD incubator at 30 °C were used. The soaking solution was assessed using a TECNAL model TEC-4MP benchtop conductivity meter.

### 2.6. X-ray test and radiographic image analyses

X-ray images were obtained at the Seed Analysis Laboratory belonging to the Lavras Federal University Department of Agriculture, using a Faxitron X-Ray Corporation X-ray scanner MX-20.

A total of 100 seeds were used to standardize the test reading. The seeds were separated into ten repetitions comprising ten units, fixed with double-sided tape on transparent acrylic plates and sequentially numbered. Exposure time and radiation intensity were set at 30 kv for 45 seconds, respectively. Radiographic images were then saved and analyzed for internal seed morphology assessments.

### 2.7. Germination test

The seeds were placed on germitest sheets moistened with distilled water 2.5 times their dry weight and maintained in a Mangesldorf germinator at 30 °C. Germination was evaluated for eight days, with each seed individually evaluated according to the numerical X-ray image sequence. During this period, almost all seeds presented a root length of 0.5 cm or more. After germination, seedlings were maintained

in the germinator for up to 15 days to even out their growth. Subsequently, seedling lengths (root and shoot) were evaluated with the aid of a digital caliper, while their dry weight was also determined using a 0.001 g precision analytical balance after drying in a forced-air drying oven at 65 °C for 48 hours.

### 2.8. Seedling emergence test

Seedling emergence was conducted in a greenhouse with automated sprinkle irrigation (28.4 °C ± 4 °C and relative humidity of 75 ± 5%) four times a day for eight minutes over beds containing washed sand as a substrate. Seed distribution on the substrate consisted of four repetitions with 25 seeds arranged horizontally with the hilum facing down up to a depth of 2.5 cm. The criterion used to detect seedling emergence was set as when the two cotyledons were positioned above the substrate. The seedlings were maintained in the beds for 30 days, when seedling length and dry weight were determined.

### 2.9. Statistical design

The experimental design was completely randomized, consisting of four replications. The vigor classes of the tetrazolium test were subjected to analysis of variance by Tukey test at 5% probability. And the analyzed variables of seed storage were submitted to regression analysis by the SISVAR statistical program. Regression models were adjusted, and the graphs were plotted using the Sigma Plot, software version 12.0.

## 3. RESULTS AND DISCUSSION

### 3.1. Assay 1. Tetrazolium Test

The tetrazolium solution concentrations used in this study were equally efficient in identifying damage present in *baru* seeds. As all, concentrations allowed for salt reaction endosperm and embryo reserve tissues, regardless of the seed lot (Figure 1). Thus, aiming at lower costs, a 0.05% concentration and pre-imbibition time of 24 hours are recommended, as this condition is sufficient to activate initial seed metabolism and promote the salt reaction with seed tissues (Figure 1 AD).

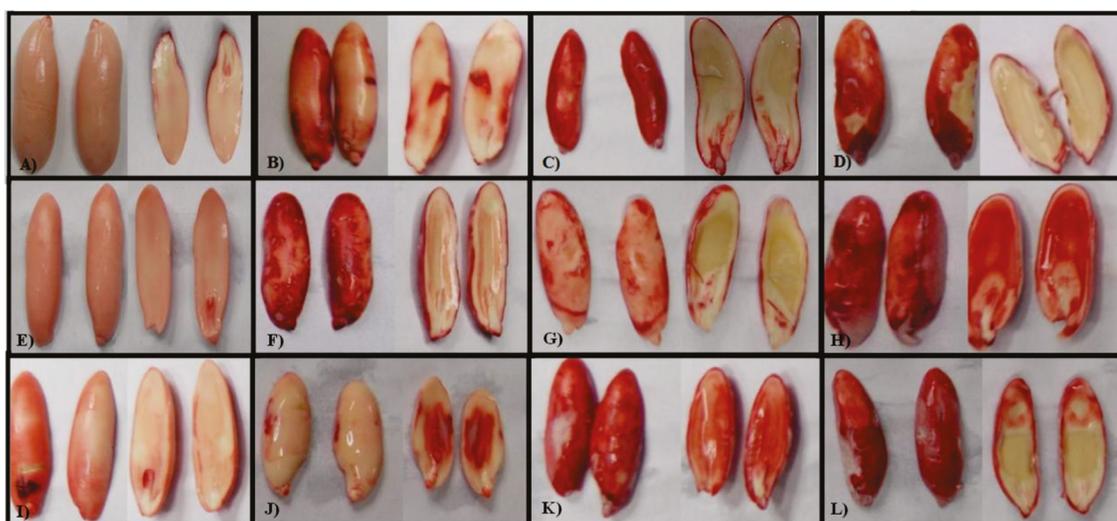
In a study by Brito et al. (2020), in order to evaluate the vigor of *Tabebuia aurea* seeds under conditions similar to those described for *baru* seeds, the authors showed that the best concentration was 0.05% of the tetrazolium salt for four hours of soaking. For these authors, the tetrazolium test has become increasingly efficient in the methodologies for assessing the vigor of forest species. Although the concentration of the tetrazolium salt solution used is efficient for both, *baru*

seeds need a longer exposure time. Since, the seeds have a very thick endosperm. Which probably influences the absorption time of the solution. Affirming the relevance and importance of vigor test adequacy for seeds, Medeiros et al. (2020) observed in seeds of *Amburana cearenses* (Allemão) A.C. Smith, significant correlations in the quantification of vigor of normal seedlings when compared to vigor using the tetrazolium test.

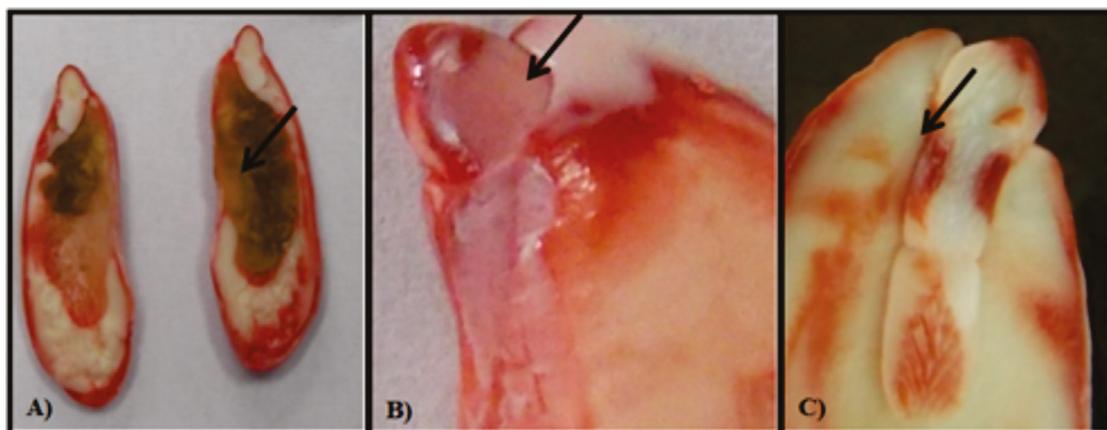
In Figure 1 it is possible to verify that the most vigorous tissues of *baru* seeds have pink color in its entirety, viable seeds have about 40% of the most intense color, the seeds deteriorated and unviable, more than 40% of the tissues with carmin red color. These color patterns were also observed by Carvalho et al. (2017) in *Libidibia ferrea* seeds that the

ideal concentration for the test was 0.05% for six hours of soaking. And in *Jatropha molissima* seeds that 0.25% of the tetrazolium concentration was necessary to react with seed tissues for four hours (Walter et al., 2020).

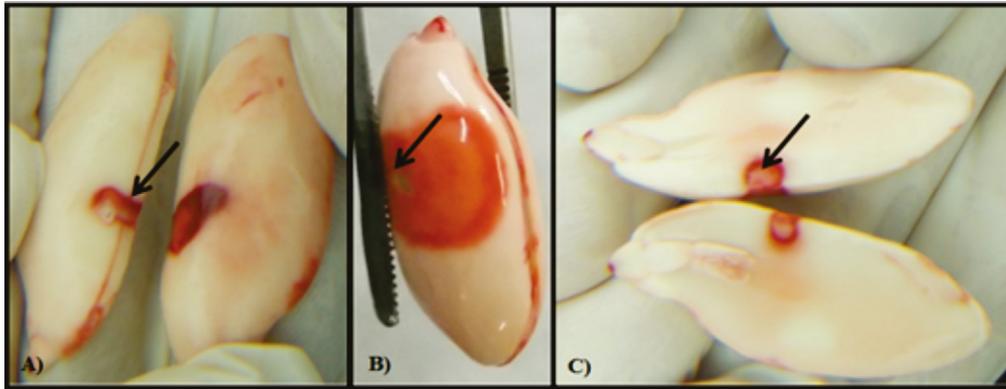
The exposure time (24 hours) for *baru* seeds was higher compared to other studied seeds, probably can be related to a large amount of endosperm reserve material. Other tissue damage at the different concentrations evaluated is evidenced in Figures 2 and 3 presents the types of damage caused by moisture, insect attacks and mechanical damage in detail. However, it is noteworthy that these damages were found in a very small fraction of the assessed seeds considering the number of seeds used for this test, regardless of the fruit collection year.



**Figure 1.** Tetrazolium test on *baru* (*Dipteryx alata* Vogel) seeds subjected to different concentrations for 24 hours. Where concentrations are indicated by the lines, 0.05% (A, B, C and D); 0.075% (E, F, G and H) and 0.10% (I, J, K and L). And the vigor classes in the columns, (A, E and I) very vigorous seeds (VV); (B, F and J) viable seeds (V); (C, G and K) deteriorated seeds (D); (D, H and L) unviable seeds (UNV).



**Figure 2.** Tetrazolium test performed on *baru* seeds (*Dipteryx alata* Vogel). (A) seed presenting endosperm tissue necrosis, indicated by the arrow. (B and C) Moisture damage in the embryo region, indicated by the arrow.

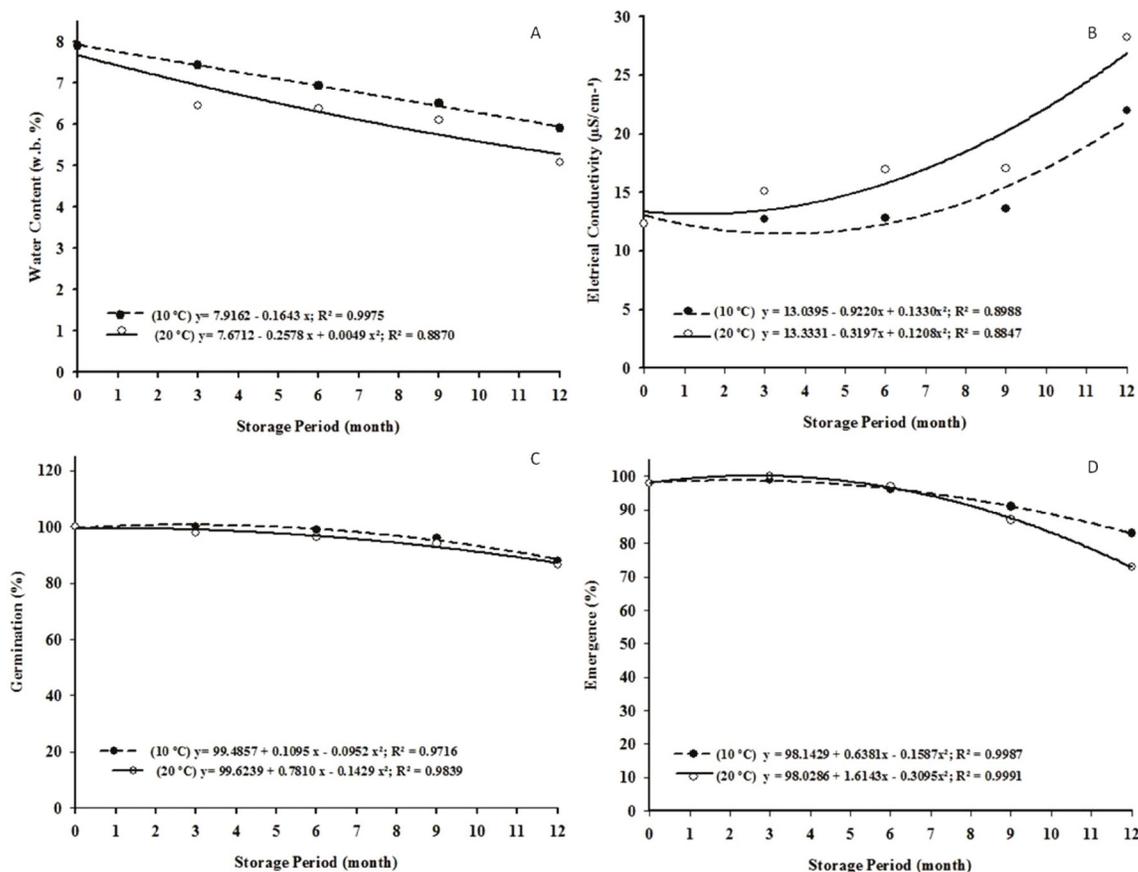


**Figure 3.** Details evidenced by the black arrow indicate mechanical damage (A) and insect damage (B and C) in *baru* seeds (*Dipteryx alata* Vogel) assessed by the tetrazolium test.

### 3.2. Assay 2. Seed Storage

One of the most important aspects to be evaluated during seed storage is seed water content. This factor is determinant to conservation conditions, since it exerts direct influence on seed metabolism (Carvalho & Nakagawa, 2000; Nagel & Börner, 2010). In the present study, the water content of *baru* seeds decreased throughout the 12 months of storage. An interaction between time and temperature was noted, and

according to the obtained values, the seeds initially stored at 7.49 water content decreased to 6.12 and 5.39% (w.b.) at 10 and 20 °C, respectively (Figure 4A). Similar behavior has been observed for soybean seeds stored at 20 °C for six months, exhibiting decreasing water content during storage (Smانيotto et al., 2014). It is noteworthy that, although *baru* seeds displayed loss of water content, they still maintained high germination potential of above 85% at 12 months of storage (Figure 4 C).



**Figure 4.** Water content, electrical conductivity, germination percentage and emergence percentage of *baru* seeds (*Dipteryx alata* Vogel) throughout 12 months of storage at 10 and 20 °C. w.b. = wet base.

In this study, electrical conductivity, indicative of *baru* seed membrane permeability, increased during 12 months of storage (Figure 4B). This increase was not, however, directly linked to their deterioration, as germination and emergence tests detected high physiological quality seeds (Figure 4 C and D), of above 85% and 70%, respectively. Seed deterioration can occur during storage under unfavorable environmental conditions. One of the first signs of such damage is increased membrane permeability. This, however, will not always indicate a high degree of deterioration, as cell structures may be rewarded by repair mechanisms (Panobianco et al., 2007), which may still result in high seed vigor, corroborating the results of this study.

Studies conducted with soybean seeds by Fessel et al. (2010) corroborate the results reported herein, as those authors verified that 10 and 20 °C for up to 15 months of storage led to an increase in solutes present in the electrical conductivity soaking solution, but no seed deterioration. In contrast, Pontes et al. (2006) reported that increased electrical conductivity in *Caesalpinia peltophoroides* (Benth.) seeds is associated to decreased germination. This species, also classified as orthodox, reached rates below 70% at 8 months of storage at 20 °C. Sequentially to these results, Silva et al. (2018) checked an increase in the values of electrical conductivity in *Jatropha curcas* seeds stored for 18 months. In which, with the increase in conductivity, there was a marked reduction in vigor, reaching 15% of germination. Afzal et al. (2019) also emphasize the importance of storing seeds in hermetically packaged and low water content. Studies carried by these authors with *Moringa oleifera* seeds, detected lower values for electrical conductivity when stored at 10 °C with 8% moisture and hermetically sealed. While seeds with high water content are subject to deterioration.

These results demonstrate the importance of studies to define storage time and the temperature for seeds in general, as the results vary even among orthodox species, and vigor and, consequently, longevity may be affected by intrinsic inter-specific factors over time (Murthy et al., 2003).

*Baru* seeds maintained high germination rates in the present study (Figure 4 C). In accordance with the germination data obtained herein, seedling emergence evaluations indicate that *baru* seeds remained viable during storage (Figure 4 D). What was also verified in seeds of *Melanoxylon brauna* Schott, by Freire et al. (2021) who observed high percentages (85%) of germination, when it is were maintained in a refrigerator at  $\pm 4$  °C for up to 12 months with a water content close to 5%. Once in studies by Ribeiro et al. (2018) was noted that seeds of *Erythrina velutina* Willd., stored at 10 °C for up to 14 months registered germination above 90%, however, showed oscillation in the rate of seed moisture after six months of storage.

According to data obtained in the present study, *baru* seeds initially presented 99% emergence, decreased prominently after nine months. No interaction between time and temperature was observed, however, lower emergence percentages were observed at 20 °C (73%) (Figure 4 D). In the studies carried out by Araújo et al. (2017) with seeds of *Amburana cearensis* (Allemão) A.C. Sm., there was reduction in the percentage of emergence when maintained at 25 °C, counterpart, the seeds of *Amburana cearensis* stored in a refrigerated and hermetically sealed environment remained viable for up to 24 months, corroborating the results of the present study.

Regardless of temperature, no effect of storage time on *baru* seedling length and dry mass was observed, even with a slight reduction in vigor, as evidenced by decreased germination and emergence over time.

X-ray tests were performed to support the germination and emergence test results. No temperature effect was noted regarding internal and external seed morphology. Thus, only images referring to the 10 °C storage temperature and its respective seedlings are presented (Figure 5 A-E). All internal and external structures generally remained intact throughout the storage periods.

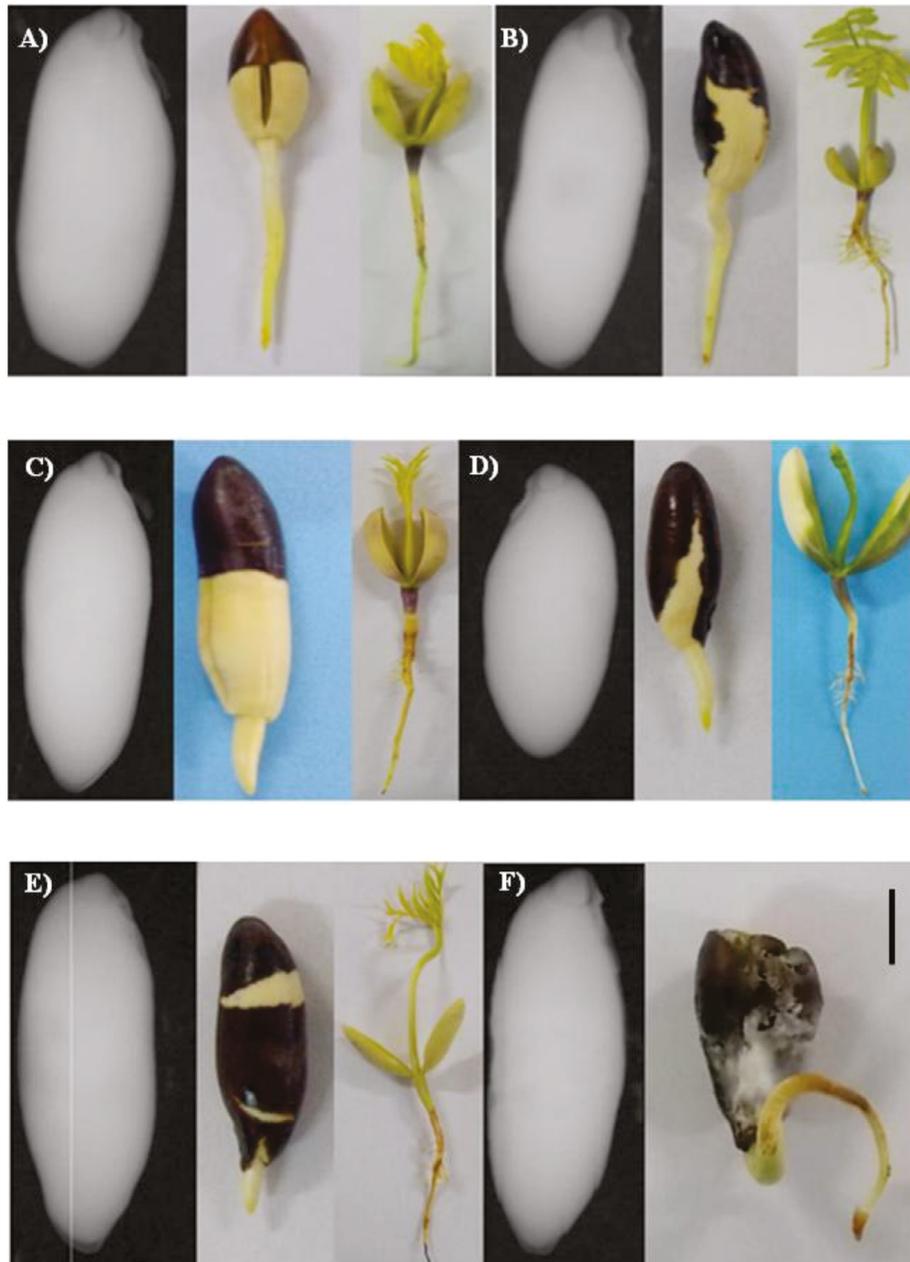
It is well known that decreased reserve areas in seed compromises seed quality (Oliveira et al., 2003). Contrary to what was expected with the conditions of storage and reduction of water content (7.49, 6.12 and 5.39%), there was no change or reduction in the area between the endosperm and the tegument (Fig. 5 A-E), regardless of the evaluated temperature. Even in analyzes carried out immediately after collection, or even, up to 12 months of storage. Corroborating the high germination and emergence percentages. Otherwise, Machado & Cicero (2003) report that seeds from forest species, due to uneven maturity and/or development, usually present internal damage. They also indicate that seeds with 75 to 50% filled embryonic cavities generate abnormal seedlings or do not germinate when evaluating white pepper seeds.

Seedling mortality from *baru* seeds in this trial was below 15% and occurred after nine months of storage, more accentuated at the 12<sup>th</sup> month (Figure 5 F). In addition, the low mortality percentage observed in this study is due to microorganism contamination, and not morphological changes. Effect similar to this fact, was reported by Pupim et al. (2008) in which fully formed *embaúba* seeds do not generate seedlings due to contamination and physiological deterioration. Still, Gomes et al. (2016) when evaluating radiographic images of *Platypodium elegans* Vog. verified that “full” seeds, that is, with all the internal structures necessary for seedling development, accounted for between 60 and 72% germination. Parallel to these studies, Medeiros et al. (2018) evaluated the internal morphology of *Leucaena leucocephala*

seeds with moisture of about 8%, and stated that lower the water content of the seeds, better radiation absorption. In these conditions, the authors recorded a germination percentage above 65% for *L. leucocephala* seed lots that showed integrity of the internal morphology.

Expressive values were also denoted in *Crotalaria juncea* L. seeds with water content between 8 and 9%, where 77% of the seeds generated normal seedlings. These authors evidenced that each species has peculiarities, in terms of

density and chemical composition and are directly related to the visualization of seed structures (Arruda et al., 2016). Because of that, it is considered for *baru* seeds that the water content contributed positively to the analysis of radiographic images. And that, the large amount of reserve material present in *baru* seeds and its orthodox character seem to confer longevity during storage for the seeds. These data, referring to the composition of *baru* seeds were analyzed in previous studies by Silva et al. (2020).



**Figure 5.** X-ray images of *baru* seeds (*Dipteryx alata* Vogel) during different storage periods at 10 °C. (A) Control, (B) Stored at 10 °C for 3 months. (C) Stored at 10 °C for 6 months. (D) Stored at 10 °C for 9 months. (E) Stored at 10 °C for 12 months. (F) dead seeds by microorganism contamination.

## 4. CONCLUSIONS

The tetrazolium test applied to *baru* seeds was efficient at all analyzed concentrations, although the recommended pre-imbibition time and concentration are, respectively, 0,05% and 24 hours for *Dipteryx alata* Vogel.

The X-ray test is proven to be a promising and accurate technique for visualizing internal baru seed structures. In addition, there was no effect of damage from storage on the morphological characteristics of the seeds analyzed by the X-ray test.

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