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Packing, moisture and environment for conservation of 'jabuticatree açu' seeds during storage

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Abstract

The objective of this study was to evaluate the viability of 'jabuticaree açu' [*Plinia cauliflora* (DC.) Berg] seeds according to water loss and packaging conservation conditions. Two experiments were carried out at UTFPR - Dois Vizinhos Campus, Parana State, Brazil. For the first experiment, the seeds were submitted to the hydro conditioning process by soaking in water during 24 hours with subsequently storage in a BOD chamber at 25°C for 0, 12, 24, 36, 48, 60, 72, 84 and 96 hours. This experiment was conducted in a completely randomized design with four replications of 50 seeds each. For the second experiment the seeds were separated into three lots: PET® bottles with lids, kraft® paper bags at room temperature and in cold storage (6°C±1°C). Each lot was stored during 0, 5, 10, 15, 20, 25, 30, 60, 90, 120 and 150 days. The experiment was carried out in a completely randomized design, in a 3x11 factorial (storage location x storage period), with four repetitions of 50 seeds each. *Plinia cauliflora* seeds present storage capacity of 96 hours at room temperature without loss of germination capacity, when previous hydro conditioning was carried out. Storage using Pet® bottles at room temperature for up to 25 days allows seeds viability.

Keywords: Plinia cauliflora, Myrtaceae, seed viability, jabuticaba

Introduction

The jabuticatree belongs to Myrtaceae family and to the genus Plinia, being a native fruit tree of Brazil, with nine well-known species, among which stands out the 'jabuticatree paulista' or 'jabuticatree-açu' [*Plinia cauliflora* (DC.) Berg], being one of the most sold species in the Brazilian South market (Danner et al., 2011a). This species presents desirable sensory characteristics that allow its 'in natura' commercialization or with processing techniques, generating jellies and fermented beverages (Sasso et al., 2010).

At the present time there are few commercial orchards of this fruit, with most of the fruit being harvest in areas of extractivism, with sales without selection criteria and in road borders. It is necessary to organize the productive chain for this fruit, selecting first the genotypes with great potential for commercial cultivation (Hössel et al., 2013) and adopt parameters for the fruit commercialization.

In this context, the use of seeds for the propagation of selected trees as matrices for the creation of commercial orchards is of great importance because, in addition to allowing the seedlings obtaining, some of them are genetically identical to the selected plant, due to the presence of the apomixia phenomenon (Wagner Júnior et al., 2011).

However, Plinia cauliflora seeds

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present limitations to storage because they are recalcitrant, ithout to be tolerant to desiccation, requiring special storage methods, which should be studied (Danner et al., 2011b). According to Ambrósio et al. (2008) after ten days of extraction, the 'jabuticatree' seeds are no more viable, due to the moisture content, below 13%. These characteristics of short viability and difficult storage are common in recalcitrant seeds, as cited by Silva et al. (2012) that when evaluating the desiccation of *Cinnamomum zeylanicum* Ness seeds verified a maximum drying time of 12 hours at room temperature without any damage to the seeds physiological quality.

Some alternatives to maintain the viability of recalcitrant seeds, are the use of low temperatures and high moisture contents. The use of low temperature has been used with satisfactory results in recalcitrant seeds, as in *Tabebuia aurea* as described by Neves et al. (2014), whose role is to reduce metabolic activity, saving more reserves for later use in germination processes, in addition to reducing the rapid water loss.

However, if the high moisture content is maintained, it is possible the germination in the storage environment and the fungi proliferation, which also compromises the seeds viability (Fior et al., 2010). In this sense, it is important to use containers that retain moisture to reduce the water exchange with the air and reduce its metabolic activity, combined with the environment conditions. Therefore, it is possible to combine the seeds pre-hydro conditioning to conditions to maintain its viability and in packages which reduces the gas exchanges, reducing the seeds metabolic activity, preventing its germination during storage.

It should be taken into consideration the minimum water tolerable values, because in native fruit the seeds viability decrease in conditions of less than 45% of water content, totally losing their germination capacity when the percentage is reached less than 15% (Delgado & Barbedo, 2007), being used as reference.

Therefore, the objective of this study was to evaluate the viability of *Plinia cauliflora* seeds, according to water loss and packaging for conservation.

Material and Methods

Two experiments were carried out at the Plant Physiology laboratory and in the Nurse Teaching and Research Unit, of the Federal Technological University of Paraná (UTFPR) - Dois Vizinhos Campus.

In both experiments, seeds of 'jabuticatree-açu' (*P. cauliflora*), obtained from fruits with complete maturation, from a single plant. For the seeds extraction the mucilage was manually removed by friction in a fine mesh sieve, adding virgin lime. Afterwards, the seeds were washed in running water and placed in paper towel, remaining for 24 hours in a shady environment to remove the excess of moisture.

In experiment 1, the seeds were weighed and submitted to the hydropriming process by imbibition in water for 24 hours and then weighed again and placed in a B.O.D. chamber at 25°C for drying, being kept in this environment for periods of 0, 12, 24, 36, 48, 60, 72, 84 and 96 hours. After each of these periods, the seeds were weighed and seeded in Tetrapak® boxes (19x7x7cm), using sand as substrate. The irrigation was performed with the aid of a spray, in two periods (in the early morning and late afternoon), always verifying the moisture of the substrate, visually.

The experiment was installed in a completely randomized design, with 4 replicates of 50 seeds per experimental unit. At 100 days the percentage of emergence, the emergency speed index (ESI) (from the twenty-fourth to the hundredth day) (Maguire, 1962) and the number of seedlings per seed were evaluated.

In the experiment 2, the seeds were separated after extraction into three lots and stored at: PET[®] bottles with lid (300 mL), kraft paper bag, closing the paper with two folds and a clamp, under cold temperature ($6^{\circ}C \pm 1^{\circ}C$) and kraft bags at roo temperature. Subsequently each lot with the respective packaging was stored for 0, 5, 10, 15, 20, 25, 30, 60, 90, 120 and 150 days. After each storage time, the seeds were seeded in sand inside a gerbox box without a lid and kept in B.O.D. at 25°C, without photoperiod (Pirola, 2013).

A completely randomized design was used in a 11x3 factorial (storage period x storage condition), with 4 replicates of 50 seeds each. One hundred days after sowing the emergence (%) and emergency speed index (from the fourteenth to the hundredth day) were evaluated (Maguire, 1962).

The data of the variables evaluated from both experiments were previously submitted to the Lilliefors normality test, with the need for transformation for emergence and ESI. The transformations were performed using $\sqrt{x} + 1$. With the data transformation, the variance analysis was performed using the Duncan's test (p \leq 0.05) for the qualitative factor, and regression (p \leq 0.05) for the quantitative factor, using the Genes software.

Results and Discussion

For the experiment 1, no significant difference between the storage periods for the variables emergence percentage, ESI and number of plantlets per seed were evaluated, with means of 88.67%; 3.20 and 1.70, respectively.

It is possible that the statistical similarity for the analyzed variables is due to the moisture content presented by the seeds at the time of sowing, since a previous hydro conditioning was realized and the drying process in the B.O.D. presented a minimum value of 76.4% (Figure 1), which does not allow the loss of viability, according to Delgado & Barbedo (2007).





According to Valio & Ferreira (1992), the seeds of 'jabuticatree-açu' (*P. cauliflora*) lost their capacity of germination with 31% of humidity. Following these parameters, with the results obtained in the present study, it was verified that it would take 11.5 days for the 'jabuticatree-açu' seeds to reach this percentage of humidity if the B.O.D. was seated at 25°C (Figure 1).

This demonstrates the beneficial effect of hydro-conditioning to maintain the high germinative capacity (88.7%) of these seeds, when stored at room temperature (25°C), up to five days, which allows other studies with this technique, analyzing it for a longer period. Differently from what was presented by Pirola (2013), in which the seeds of 'hybrid' 'jabuticatree' without pre-hydro-conditioning, with 5 days of drying at 25°C, presented no viability. This was also demonstrated by Delgado & Barbedo (2007) evaluating the desiccation tolerance of several species of the genus Eugenia (*E. brasiliensis, E. pyriformis, E. involucrata, E. uniflora, E. cerasiflora and E. umbelliflora*) during 6 days of drying, presenting about 15% of moisture content and total loss of viability.

However, it is important to observe the obtaintion of 1.7 seedlings per seed, which proves to be a polyembryonic seed species. Danner et al. (2011b) observed in 'jabuticatree' (*P. cauliflora*) the average polyembryony rate of 29.2% and 41.3% in seeds stored, being able to verify up to 5 seedlings per seed, where normally one has a zygote embryo, formed by fertilization and the rest of embryos are asexual (formed by apomixia), being these clones identical to the mother plant.

For the experiment 2 a significant interaction between the storage condition x storage time was observed for the emergence percentage (Table 1) and ESI (Table 2). Seeds maintained in a PET® bottle presented viability up to 30 days after storage, with values considered ideal (78.73% of emergence) up to the 15th day when compared to other packages (Kraft®) (Table 1).

 Table 1. Emergence percentage for 'jabuticatree' seeds according to the storage period and conditions.

	Storage condition		
Storage time	Pet bottle	Kraft paper/ room temperature	Kraft paper/ cold temperature
0	94.83 a A*	94.83 a A	94.83 a A
5	89.72 ab A	57.55 b B	66.09 b B
10	90.37 ab A	32.41 c C	67.07 b B
15	78.73 b A	14.64 d C	32.42 c B
20	39.75 c A	0.33 e C	15.21 d B
25	8.75 d A	0.0 e B	0.82 d B
30	0.18 e A	0.0 e A	0.0 d A
60	0.0 e A	0.0 e A	0.0 d A
90	0.0 e A	0.0 e A	0.0 d A
120	0.0 e A	0.0 e A	0.0 d A
150	0.0 e A	0.0 e A	0.0 d A
CV (%)		27.81	

*For each column, means followed by the same lowercase letter and, in lines, for the same uppercase letter are not statistically different, according to Duncan's test ($p \le 0.05$).

Regarding the emergence speed index (ESI), the results were similar to the obtained for emergence, with seeds from PET bottle presenting greater vigor until 25 days of storage, with a great decrease of the ESI at 5 days for seeds stored on Kraft paper (Table 2).

Seeds stored in Kraft paper, maintained on natural or cold environment presented higher emergence at time 0, but at lower temperatures they remained viable for a longer period (25 days), when compared with the natural environment (20 days). Seeds maintained in cold storage also presented satisfactory results in the storage of loquat seeds (*Eriobotrya japonica* Lindl.), according to Brasileiro et al. (2011), who also characterized them as recalcitrant.

This result in cold storage can also be associated with the lower water loss and the lower metabolic activity of the seeds, extending its viable period with higher moisture content, important for recalcitrant seeds, and greater conservation of the reserve that it are necessary for the survival of the seed in storage and subsequent vigor during germination, as observed by IVE (Table 2).

The superiority achieved with the storage in PET bottles can be associated with the lower

water vapor exchanges that this package provides during the seed storage. In addition, this packaging changes the CO₂:O₂ ratio, increasing the ratio, reducing the metabolism and without viability loss. This was also demonstrated by Pirola (2013), who used PET[®] bottles with lids and controlled temperatures (6°C) in the storage of recalcitrant seeds of *Eugenia involucrate*, increasing the period of viability of these seeds for up to 45 days.

The use of the PET® bottles to storage seeds provided less water loss, being the maximum at 140 days (13.12% - Figure 2A). When the seeds were stored on Kraft paper bags, the loss of water content was higher, at cold storage, presenting at 106 days of storage, loss of 41.75% (Figure 2B), and at room temperature and 104 days, 36.85% (Figure 2C).

The use of waterproof packaging has already shown satisfactory results in the storage of *Myrciaria dubia* and 'jabuticatree' seeds through the use of polyethylene bags (Yuyama et al., 2011; Hössel et al., 2013, respectively). This occurs because the package provides regular water loss, reducing water loss from the seed, which is needed for recalcitrant seeds.

PET® packaging is already used in

	Storage condition			
Storage time	Pet bottle	Kraft paper/ room temperature	Kraft paper/ cold temperature	
0	1.67 a A*	1.66 a A	1.67 a A	
5	1.35 bc A	0.86 b C	1.11 b B	
10	1.53 ab A	0.47 c C	1.11 b B	
15	1.26 c A	0.15 d C	0.48 c B	
20	0.47 d A	0.02 d C	0.22 d B	
25	0.18 e A	0.0 d B	0.03 e B	
30	0.0 f A	0.0 d A	0.01 e A	
60	0.0 f A	0.0 d A	0.0 e A	
90	0.0 f A	0.0 d A	0.0 e A	
120	0.0 f A	0.0 d A	0.0 e A	
150	0.0 f A	0.0 d A	0.0 e A	
CV (%)		3.74		

 Table 2. Emergence speed index (ESI) of 'jubuticatree-açu' seedling according to the storage time and storage condition.

*For each column, means followed by the same lowercase letter and, for each line, followed by the same uppercase letter are not statistically different, according to Duncan's test ($p \le 0.05$).



Figure 2. Water loss (%) of 'jabuticatree-açu' seeds according to the storage times (days) and conditions: Pet® bottles at cold temperature (A), Kraft® bags at cold temperature (B) and Kraft® bags and room conditions (C).

agriculture for corn, for example, an orthodox species. With this result, it is also promising the use for recalcitrant seeds, such as 'jabuticatree'.

Conclusion

Plinia cauliflora seeds present storage capacity of 96 hours at room temperature without loss of germination capacity, when previous hydro conditioning was carried out. Storage using Pet® bottles at room temperature for up to 25 days allows seeds viability.

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