

## Storage Potential of Local Brazilian Pine Seed Varieties

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### ABSTRACT

Brazilian pine seeds (*Araucaria angustifolia*) are recalcitrant, and there are no studies evaluating the longevity of their different varieties. Our objective was to evaluate the capacity of different varieties of Brazilian pine seeds to maintain their physiological quality during storage. Seeds of the varieties: *sancti josephi* (I), *angustifolia* (II), *caiova* (III) and *indehiscens* (IV) were collected from two populations located in Santa Catarina, and stored under laboratory conditions and in a cold room for 90 days. On average, freshly harvested seeds showed 88% viability, and varieties II and III maintained the greatest viability (with the greatest vigor for variety II) after 90 days in storage. Varieties I and II maintained their pre-germinative metabolism for a longer period than the other varieties during storage. Therefore, seeds from the *angustifolia* variety (II) have higher storage potential than the other varieties, maintaining approximately 61% viability at 90 days of storage.

**Keywords:** viability, vigor, seed conservation.

## 1. INTRODUCTION

Brazilian pine (*Araucaria angustifolia* (Bert) O. Ktze.) is the only species from the *Araucariaceae* family which naturally occurs in Brazil (Schlögl et al., 2012). The natural populations of the species are highly degraded and fragmented (Vibrans et al., 2011), hindering gene flow and favoring the occurrence of genetic erosion. It is estimated that between 2% to 12% of the original area with araucaria populations remains (in Brazil and Argentina) (Ribeiro et al., 2009), and approximately 7% in Santa Catarina state (Vibrans et al., 2011). Therefore, Brazilian Pine is listed on the main Endangered species lists (Brasil, 2008; IUCN, 2013), and many efforts have been made to propagate and conserve the species (Balbuena et al., 2011).

Brazilian pine seeds are recalcitrant and have short longevity under natural conditions, reaching a maximum of six months (Fowler et al., 1998; Garcia et al., 2014; Pieruzzi et al., 2011), which makes it difficult to implement measures that seek to restore degraded populations. Several efforts have been made to investigate methods that extend the storage life of Brazilian pine seeds (Amarante et al., 2007; Caçola et al., 2006; Fowler et al., 1998; Piriz Carrillo et al., 2003). It is believed that seeking varieties whose seeds have superior longevity is an alternative for developing seed conservation technologies.

The first reports regarding the identification of Brazilian pine botanical varieties were made by Reitz & Klein (1966), who reported the existence of nine varieties: *elegans*, *sancti josephi*, *angustifolia*, *caiova*, *indehiscens*, *nigra*, *striata*, *semi-alba* and *alba*. According to Mattos (2011), however, the species presents five varieties: *angustifolia*, *indehiscens*, *caiova*, *dependens* and *vinacea*, in addition to the *catharinensis* variety. With the help of farmers from Três Barras in Santa Catarina, Zechini et al. (2012) recognized four local varieties: *sancti josephi*, *angustifolia*, *caiova* and *indehiscens*. Recently, local extractors from Urubici and Paineel identified a total of 11 local ethnovarieties (Adan et al., 2017).

These varieties mainly differ depending on the coloration and maturation season of the pine nuts. The *sancti josephi* variety has an early maturation season (February-March), popularly known (in Brazil) as “São José” (Reitz & Klein, 1966; Zechini et al.,

2012) or “25 de Março”. The *angustifolia* variety is known as “comum” or “meia estação”. Its seeds ripen between the months of April and May, and they are most commonly found by farmers in Santa Catarina (Zechini et al., 2012). *Caiova* or “kayuvá” variety as it is known, generally has larger pine nuts (pinhão), which mature between June and August (Mattos, 2011; Zechini et al., 2012). The *indehiscens* or “macaco” variety start their maturation season in August (and may occur until January), however its main characteristic is the fact that the seeds do not release from the female strobili/cone when ripe, and they remain attached to the parent tree (Reitz & Klein, 1966; Mattos, 2011; Zechini et al., 2012).

Despite few reports in the literature that identify the different varieties of Brazilian pine, some studies have been recently developed aiming to characterize (Zechini et al., 2012) and evaluate the initial growth of the varieties (Coutinho & Dillenburg, 2010). However, there are no reports of studies evaluating the physiological quality and storage potential of the different varieties. Thus, the objective of this study was to evaluate the seed behavior of different Brazilian pine varieties in terms of maintaining physiological quality during storage, aiming to prolong the seed availability period for the purposes of utilization and conservation.

## 2. MATERIAL AND METHODS

Local varieties of *Araucaria angustifolia* were identified based on farmer observation and according to the maturation season of the pine cones (pinhas), corresponding to the varieties: I – “*sancti josephi*”, known as “25 de março”; II – “*angustifolia*”, or “comum” or “meia estação”; III – “*caiova*”, or “kayuvá”; IV – “*indehiscens*”, or “macaco”. Mature seeds from the four varieties were collected from two natural populations located in the municipalities of Paineel and Urubici - Santa Catarina, corresponding to Lots 1 and 2, respectively, from  $15 \pm 3$  matrices/population. Collection was performed between the months of March and August, where cones (pinhas) with isolated spots or those grouped forming brown spots were considered mature (Mattos, 2011).

Seed samples from both lots and from the four varieties were individually homogenized and separated into four replications, and then submitted to storage

conditions: natural laboratory environment and cold room (temperature of  $10 \pm 3$  °C and relative air humidity of  $45 \pm 5\%$ ), in sealed plastic packages and in packages with a porosity of  $0.015 \mu\text{m}$ . The laboratory environment condition was performed in the city of Lages (SC), as its climatological characteristics resemble those of the seed collection sites. As a reference, data from EPAGRI (2014) indicate temperature and relative humidity variation for the storage period (Table 1). Physiological quality evaluations were performed on freshly harvested seeds and after 30, 60 and 90 days of storage.

The degree of moisture was determined using four replications of three seeds cut transversely, according to the greenhouse method at  $105 \text{ °C} \pm 3 \text{ °C}$  for 24 hours (Brasil, 2009). Seed germination was evaluated visually, considering emerged seeds as those that had a primary root visibly protruding (minimum of 5 mm) still inside the plastic packaging during storage.

Seed viability was evaluated by pH exudate and tetrazolium tests, using 25 embryos per replication. The pH of the exudate was performed by individually immersing the embryos in 5 mL of water for 30 minutes at  $25 \pm 1$  °C, followed by the addition of two drops of a sodium carbonate (8 g/L) and phenolphthalein (5%) solution (Araldi & Coelho, 2015b). The tetrazolium test was performed by immersing the embryos in 0.1% tetrazolium solution at  $25 \pm 1$  °C for 1 hour (Oliveira et al., 2014). Viable embryo analysis was carried out by staining the soaking solution (pH of the exudate) and tissue staining (tetrazolium), associating them with the extent and location of the damage.

Electrical conductivity was determined using 10 embryos per replication, soaked in 75 mL of distilled water and maintained at  $25 \pm 1$  °C for 12 hours (Medeiros & Abreu, 2007). The electrical conductivity of the solution was measured using a portable Quimis

QQ95 conductivity meter, and the results expressed as  $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$  of embryos.

The experiment was carried out according to a completely randomized design. The data were submitted to the normality test and transformed in arcsine  $\sqrt{\%}$ , followed by analysis of variance to detect differences between the lots; and then grouped in the storage period as sub-divided parcels. Means separation was performed using the SNK test at 5% probability, and regression analysis using the SAS statistical program (SAS, 2009).

### 3. RESULTS AND DISCUSSION

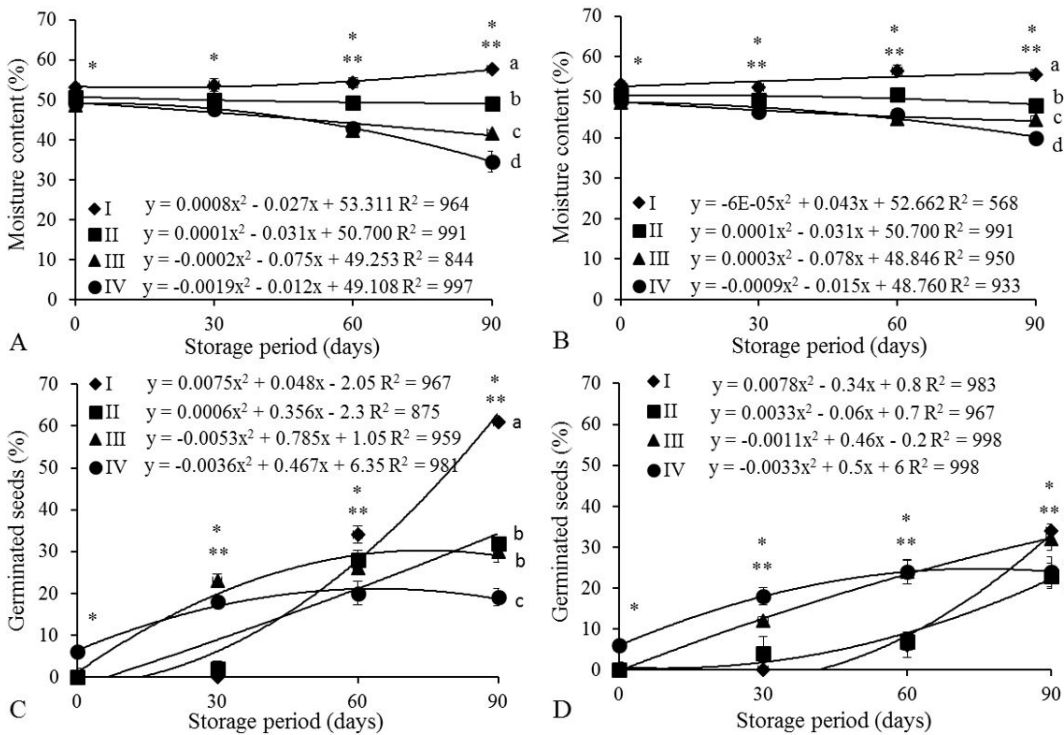
In freshly harvested seeds, it was possible to observe differences regarding the degree of moisture ( $P \leq 0.05$ ) between the varieties (Figure 1A, B), ranging from 53.1% (variety I) to 48.7% (variety III). Moisture reduction during storage only occurred for varieties III and IV for both storage conditions. No significant interaction ( $P \leq 0.05$ ) between the varieties and the storage conditions were observed for seed moisture.

Some researchers indicate that the lethal level of moisture for Brazilian pine seeds is between 25 and 38%, meaning that there would be a total loss of viability below this threshold (Eira et al., 1994; Espindola et al., 1994). Only seeds from variety IV stored for 90 days under laboratory conditions had their moisture content reduced to values within this threshold, at 34.6%.

Brazilian pine seeds remain metabolically active during storage, and present changes associated with the germination process (Amarante et al., 2007; Araldi & Coelho, 2015a); a characteristic that constitutes the basis of its recalcitrant behavior (Pammenter & Berjak, 2013). In fact, seed germination

**Table 1.** Reference temperature and relative humidity for the storage period of *Araucaria angustifolia* seeds from varieties I (*sancti Josephi*), II (*angustifolia*), III (*caiova*) and IV (*indehiscens*), according to EPAGRI (2014).

Variety	Temperature (°C)			Relative humidity (%)		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum
I	11.7	24.8	-1.8	85	98	35
II	12.1	25.6	-1.8	85	99	44
III	13.0	29.2	-1.5	82	99	39
IV	15.1	29.2	-1.5	81	99	39



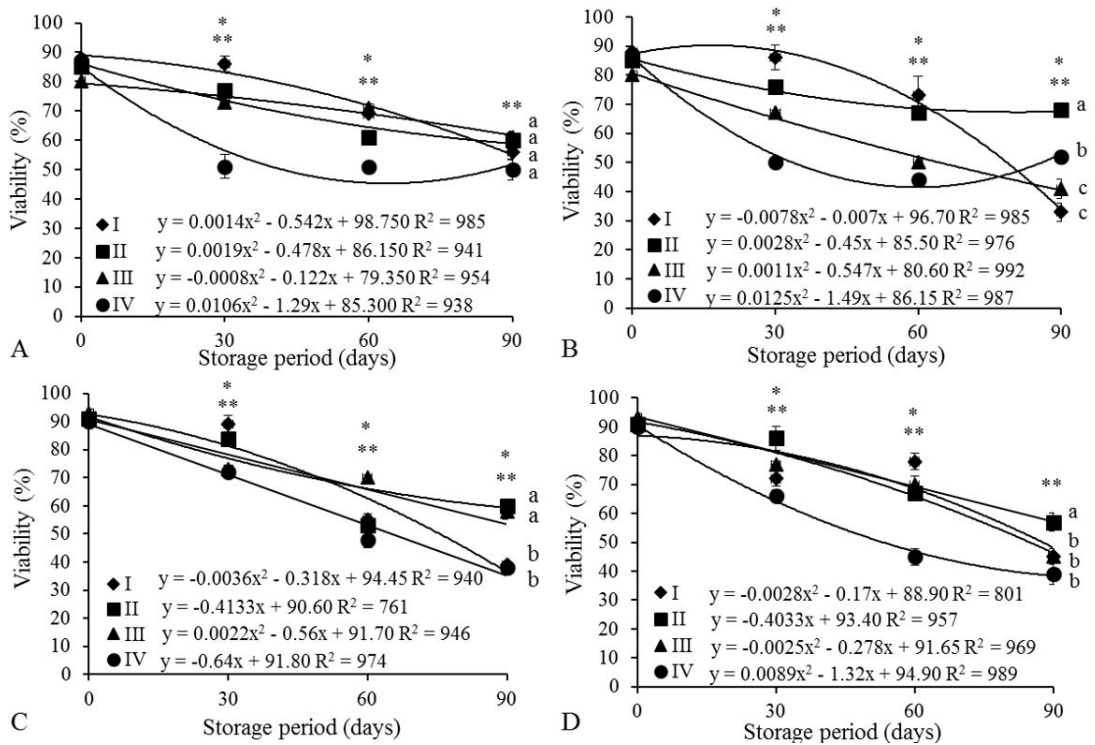
**Figure 1.** Moisture content of *Araucaria angustifolia* seeds from varieties I (*sancti Josephi*), II (*angustifolia*), III (*caiova*) and IV (*indehiscens*) during storage under laboratory environment conditions (A) and cold chamber (B), and germinated seeds upon storage under laboratory environment conditions (C) and cold chamber (D). The letters refer to the means separation by SNK test ( $P \leq 0.05$ ) and compare the varieties. \*Indicates significant differences ( $P \leq 0.05$ ) for at least one variety in relation to others; \*\*Indicates significant differences ( $P \leq 0.05$ ) between the storage period in relation to the previous period, for at least one variety.

under storage was observed inside the plastic package. For freshly harvested seeds, only variety IV presented germinated seeds (6%) (Figure 1C, D). At 30 days of storage, only varieties III and IV presented germinated seeds for both storage conditions, while varieties I and II maintained their pre-germinative metabolism. Although variety I seed germination occurred at later stages of storage (at 60 days) in relation to the others, this variety showed the highest percentages of germinated seeds at 90 days of storage for both conditions. This result can be correlated to the higher moisture content presented by this variety at 90 days of storage.

Regarding storage conditions, it was possible to observe significant differences ( $P \leq 0.05$ ) at 60 and 90 days; however, these discrepancies are due to a higher percentage of germinated seeds in the laboratory environment, mainly observed in variety I. Some authors observed a positive correlation between Brazilian pine germination and temperature during storage, up to a limit of 20 °C

(Amarante et al., 2007). Under cold room conditions (10 °C), 23 to 34% of germinated seeds were observed at 90 days, varying between the varieties. At temperatures of 10 °C, other authors observed 21% germination at 90 days of storage (Amarante et al., 2007), and 24% germination at 120 days at a temperature of 5 °C (Garcia et al., 2014).

The mean viability of the freshly harvested seeds was 85% (pH exudate) and 91% (tetrazolium), without significant differences ( $P \leq 0.05$ ) between the varieties (Figure 2). A reduction in viability was observed for all varieties within the 90-day period, regardless of the storage condition. Only varieties II and III (for laboratory storage) and variety II (for cold room storage) presented significantly higher viability than the others ( $P \leq 0.05$ ) at 90 days for both viability tests (pH of exudate and tetrazolium). By calculating an average of the viability tests, it could be observed that the viability of Brazilian pine seeds can be maintained



**Figure 2.** Viability of *Araucaria angustifolia* seeds from varieties I (*sancti Josephi*), II (*angustifolia*), III (*caiiova*) and IV (*indehiscens*) evaluated by pH exudate test during storage under laboratory environment conditions (A) and cold chamber (B), and by tetrazolium test under laboratory environment conditions (C) and cold chamber (D) conditions. The letters refer to the means separation by SNK test ( $P \leq 0.05$ ) and compare the varieties. \*Indicates significant differences ( $P \leq 0.05$ ) for at least one variety in relation to the others; \*\*Indicates significant differences ( $P \leq 0.05$ ) between the storage period in relation to the previous period, for at least one variety.

around 60% (variety II) and 59% (variety III) in a laboratory environment at 90 days of storage, and 62% (variety II) in cold room conditions, meaning that these varieties present greater storage potential under the temperature and humidity conditions tested.

In fact, seeds from variety IV stored for 90 days in ambient conditions (34.6% humidity) presented the lowest percentage of viability (on average 44%, pH of exudate and tetrazolium). However, this value was not significantly different ( $P \leq 0.05$ ) from the viability of varieties I, II and III (pH of the exudate), and variety I (tetrazolium) at 90 days of storage, nor did it differ from the viability presented by this variety (IV) at 90 days of storage in a cold room (on average 45%, with 39.8% humidity). Therefore, these results indicate that changes in viability resulting from desiccation would be more pronounced in more advanced stages of water loss.

Electrical conductivity provided indications of vigor between the varieties and during storage. Lower electrical

conductivity values occurred for varieties I, II and III (Lot 1, conductivity of 51.44; 56.48 and 61.34  $\mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$ , respectively), and II (Lot 2, conductivity of 45.79  $\mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$ ) in freshly harvested seeds (Table 2), indicating that regardless of origin, the variety II seeds potentially have a high vigor. In general, the electrical conductivity of the samples increased during storage, especially for variety IV (conductivity greater than 191.20  $\mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$ ), regardless of lot and storage condition, indicating that this variety shows less vigor in relation to the others.

By reaching physiological maturity, the seeds usually reach their maximum vigor, and its decrease during storage reflects the hydrolysis of the accumulated reserves in the seed, resulting from the respiratory and consumption activities in the pre-germination process (Guedes et al., 2013). In fact, an increase in respiratory activity of Brazilian pine seeds during storage was observed at 90 days under the temperature of 10 °C (Amarante et al., 2007).

**Table 2.** Electrical conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ ) of *Araucaria angustifolia* embryos from varieties I (*sancti Josephi*), II (*angustifolia*), III (*caiova*) and IV (*indehiscens*), from lots 1 and 2 during storage.

Variety	S.C.	Storage period (days)							
		0		30		60		90	
----- Lot 1 -----									
I	LE			56.64		62.51		32.45	
	CF	51.44	Aa	69.38	Aa	75.52	Aa	60.42	Aa
II	LE			81.28		81.26		85.76	
	CF	56.48	Aa	92.33	Ab	88.19	Ab	92.19	Bb
III	LE			80.30		82.79		86.79	
	CF	61.34	Aa	77.50	Aa	81.81	Aa	86.06	Ba
IV	LE			70.58		155.08		243.70	
	CF	87.35	Ba	91.93	Aa	167.92	Bb	257.60	Cc
CV (%)				12.89					
----- Lot 2 -----									
II	LE			88.04		71.21		65.89	
	CC	45.79	Aa	89.90	Ab	79.84	Ab	88.12	Ab
III	LE			82.19		84.54		87.79	
	CC	70.28	Ba	79.92	Aa	82.06	Aa	89.31	Aa
IV	LE			78.08		121.33		191.20	
	CC	83.75	Ba	92.68	Aa	135.42	Bb	215.10	Bc*
CV (%)				9.96					

The letters refer to the means separation by SNK test ( $P\leq 0.05$ ) and compare: varieties in the column (uppercase); storage periods in the line (lowercase); \*Indicates significant differences between storage conditions (SNK test;  $P\leq 0.05$ ). S.C. – storage condition; LE – laboratory environment; CC – cold chamber.

With the exception of variety IV from Lot 2, no differences between storage conditions were found for conductivity results. In comparing the lots, it was observed that on average Lot 2 presented lower solute leaching ( $P\leq 0.05$ ), and therefore higher vigor.

Viability and vigor results were generally higher for variety II. Such results seem to influence the growth of seedlings, since the *angustifolia* variety (variety II) presented superior growth in comparison to the *caiova* and *indehiscens* varieties (Coutinho & Dillenburg, 2010). Additionally, variety II seeds had a slower germination process, with the occurrence of germination under storage only after 30 days, indicating that this variety could be kept longer than the others. This is because once the germination begins during storage of recalcitrant seeds, there is a gradual increase in the metabolic activity and additional water for the tissues will be required. If the conditions are not ideal for completing the process (as in the case of the seeds that remain stored), deterioration will commence (Pammenter & Berjak, 2013; Walters, 2015). Therefore, Brazilian pine seeds that maintain

pre-germinative metabolism for longer can be stored for a longer period (Araldi & Coelho, 2015a).

The total time elapsed during seed development seems to contribute to the discrepancies observed between the varieties, since pollination occurs from September to October for all varieties, while seed maturation generally occurs between March and August (Mattos, 2011; Zechini et al., 2012). This fact may have contributed to the variety I seeds, which mature in March, presenting higher humidity due to early maturation. In seeds from variety IV on the other hand, in which maturity occurs in July/August, the seed development period is longer, and consequently the seeds are more exposed to climatic variability and possible loss of vigor.

With a view towards species conservation and maintaining genetic diversity, we emphasize the importance of using other varieties, either for food purposes or for seedling production. Further experiments should be conducted to improve management and promote the use and conservation of the species.

#### 4. CONCLUSION

Mature Brazilian pine seeds present viability above 80% when freshly harvested, regardless of the variety; however, *angustifolia* (II) seeds presented the greatest vigor. *Angustifolia* (II) seeds had the greatest storage potential in relation to the other varieties (*sancti josephi*, *caiova* and *indehiscens*), maintaining 60% viability at 90 days storage under ambient conditions, and 62% under cold room conditions.

#### ACKNOWLEDGEMENTS

C. G. Araldi wishes to thank the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) for providing a PhD scholarship. C. M. M. Coelho would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity fellowships granted.

#### SUBMISSION STATUS

Received: 6 nov., 2015

Accepted: 18 apr., 2017

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