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Early harvest increases post-harvest physiological quality of *Araucaria angustifolia* (Araucariaceae) seeds

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Abstract: *Araucaria angustifolia* is a conifer native to Brazil and is an endangered species. Since this species seeds have a short period of viability, its vulnerability is higher. Thus the aim of this study was to evaluate the physiological quality of *A. angustifolia* seeds during the development and post-storage periods. For this, cones of *A. angustifolia* were collected from a natural population in Curitibanos, Santa Catarina, Brazil, in March, April, May and June 2012. The collected seeds were classified into developmental stages of cotyledonary, I, II and III according to the month of collection; a total of 10 cones were collected for each stage. Seeds were stored in a refrigerator for 60 and 120 days, and were submitted to a chamber germination test (25 °C-photoperiod 12 h). Additionally, seeds were tested for moisture content (105 °C for 24 hours), tetrazolium (0.1 % for 1 hour) and vigor (electric conductivity [75 mL distilled water at 25 °C], germination speed index, and shoot and root length). Our results showed that during seed development, moisture content decreased from the cotyledonary stage (66.54 %) to stage III (49.69 %), and vigor increased in the last stage. During storage, moisture content at cotyledonary stage and stage I was stable. On the other hand, stored seeds exhibited a decrease in moisture content after 120 days at stages II and III. Physiological quality at the cotyledonary stage resulted in an increased germination rate of 86 % and 93 % after 60 and 120 days of storage, respectively; unlike stages II and III exhibited a decrease in seed viability and vigor after storage. Electrical conductivity was higher for fresh seeds at the cotyledonary stage, than for those stored for 60 and 120 days. However, in other stages, released leachate content after 120 days of storage, increased with the advance of the collection period. Germination speed index and shoot and root lengths after storage were highest for seeds at the cotyledonary stage and stage I; unlike stages II and III which had short root and shoot lengths during storage. Thus, the maintenance of seed moisture content during storage was variable and dependent on the period of collection. Furthermore, the physiological quality differed among earlier and later stages. Early collection favored seed physiological quality, and may be a strategy for better conservation of *A. angustifolia* seeds. Rev. Biol. Trop. 64 (2): 885-896. Epub 2016 June 01.

Key words: recalcitrant seed, storage, seed development, gymnosperms, endangered plants.

Seed development is a process that comprises a set of morphological, cellular, and biochemical/synthetic changes which commences with ovule fertilization to the moment in which the seed is mature (Bewley, Bradford, Hilhorst, & Nonogaki, 2013).

Most seeds undergo a process of “maturation drying” with late stages of development, and these seeds are called orthodox (Roberts,

1973). Before or concomitant with maturation drying, seeds acquire desiccation tolerance, and can be dried to low water content (generally less than 5 %) (Pammenter & Berjak, 2000). On the other hand, recalcitrant seeds, which belong to a group of generally unrelated species, do not undergo maturation drying, nor acquire desiccation tolerance. Instead, they are hydrated and desiccation sensitive throughout

the development and post-shedding (Pamenter & Berjak, 2014).

Araucaria angustifolia (Bert.) O. Kuntze is a conifer species from the Atlantic Forest that presents recalcitrant seeds with significant ecological, economic and social importance in Brazil (Wendling & Brondani, 2015). Due to the intense exploitation of wood resources and expansion of agriculture, its distribution has been reduced to 2-4 % of the original area (Guerra, Silveira, Reis, & Schneider, 2002), and according to the IUCN Red List of Threatened Species, it is a “critically endangered species” (IUCN, 2013). Even though today *A. angustifolia* is legally protected, and its collection for timber is prohibited by law in Brazil, the remaining areas of the species in its natural distribution areas are fragmented and scattered (Wrege et al., 2009). Urgent conservation actions should be implemented, and studies based on genetic and physiological advance should be carried out in order to save this species from possible extinction.

During the development of *A. angustifolia* seeds, changes in the physiological quality were observed, with an increase of viability and vigor at late collection (Shibata, Coelho, & Steiner, 2013). However, there are no reports on the effect of development stages linked to the physiological quality after storage.

The period when the seeds remain viable in storage is determined by genetic and physiological factors, by the seed development stage, and by any deteriorating events or damage prior to or during storage (Barbedo, Centeno, & Ribeiro, 2013; Schmidt, 2007). In general, seed maturity and moisture content are crucial in determining longevity in storage (Barbedo et al., 2013; Lan, Jiang, Song, Lei, & Yin, 2007).

Seeds collected in the early stages of maturity deteriorate quickly during storage (Parisi, Biagi, Barbedo, & Medina, 2013). Furthermore, immature seeds are not able to complete the normal food reserves storage, nor to develop all the needed enzymes and/or growth regulators, nor to complete their full morphological development and cell organization (Bonner, 2008). In recalcitrant seeds, such

as *Inga uruguensis* Willd. subsp. *affinis* (DC.) T. D. Penn., the dry weight of seedlings per mature embryos (44.7 mg) was higher than that of immature ones (29.1 mg) during the whole storage period (65 days at 7°C), demonstrating the higher physiological potential of mature embryos (Parisi et al., 2013). Similarly, *Hopea hainanensis* Merr. et Chun seeds, collected at 173 DAA and after 183 DAA mass maturity, maintained germination higher than those collected at pre-mass maturity (159 DAA) when stored at 4 and 10°C (Lan et al., 2012).

However, early-collected seeds of some species may be able to reach full maturity, including normal storability, if are allowed to follow a post collection maturation (Schmidt, 2000). In *Dalbergia cochichinensis* P. seeds, the viability after one week of storage increased from 92.3 % to 97.5 % for all maturity stages (Hung, 2003). Likewise, *Eugenia pyriformis* C. immature seeds showed an increase of 30 % in germination after 30 days of storage (Santana, 2007). A probable explanation is that immature seeds mature or continue maturing during the beginning of the storage period, while mature seeds start the aging stage faster (Schmidt, 2007), or also even such mature seeds might begin germination despite the seed is still connected to the mother plant (Barbedo et al., 2013), as already showed in *Inga vera* seeds (Caccere, Teixeira, Centeno, Figueiredo-Ribeiro, & Braga, 2013; Parisi et al., 2013).

A. angustifolia seeds should be stored soon after collection, at the maximum moisture content, in order to prevent water loss during this period (Eira, Salomão, Cunha, Carrara, & Mello, 1994). This high moisture content can be obtained with early collections, and before the maximum dry matter accumulation. Storage with high water content is better for the conservation of recalcitrant seeds (Nascimento & Moraes, 2011), this method was successful for *Euterpe edulis* M. (De Andrade, 2001) and *Inga uruguensis* seeds (Bilia, Marcos-Filho, & Novembre, 1998). Despite successful storage of seeds of other recalcitrant species, as reported above, there is currently no reported method for assessing whether the stage of development

of *A. angustifolia* seeds at the time of collection influences the effectiveness of storage and retain high germination after storage. In this study, the physiological quality during storage was evaluated, according to the developmental stage of *Araucaria angustifolia* seeds, with the aim of maintaining viability during storage.

MATERIALS AND METHODS

Seed sampling: Cones were collected from a natural population of approximately 50 trees, located in the town of Curitibanos, Santa Catarina, Brazil (27°18' S - 50°38'12" W, 960 masl). Collections started in March 2012, when megastrobili were immature and embryos were at the cotyledonary stage-control treatment (29th March) (Guerra et al., 2008), and continued on a monthly basis until July, for the I (19th April), II (21th May) and III stages (25th June) (Shibata et al., 2013). A total of 10 cones were collected at each stage.

Measurement of seed size and moisture content: For each development stage, seed length, width, and thickness were measured with calipers, using eight replications of 25 seeds. Three replications of three seeds for each development stage were cut transversally. After that, they were weighed (wet weight), dried at 105 °C ± 3 °C for 24 hours, and reweighed to determine the moisture content (BRASIL, 2009).

Germination test: Seeds were surface-decontaminated with sodium hypochlorite solution (2 %, v/v) for three minutes, and subsequently the tip of each seed was cut off at approximately 3 mm (Moreira-Souza & Cardoso, 2003). Four replications of 25 seeds each were sown in trays with vermiculite, and placed in a germination chamber at 25 °C, with photoperiod of 12 hours. Seeds were monitored for 70 days.

Germination counts were carried out every three days from the beginning of germination, and the germination speed index (GSI) was calculated using the formula: $GSI = G1/$

$N1 + G2/N2 + \dots G_n/N_n$, where G1, G2, G_n were the number of germinated seeds, and N1, N2, N_n were the number of days in the test (Maguire, 1962). At the end of the germination test, the shoot and root length of each seedling were measured.

Tetrazolium test: Four replications of 25 seeds were soaked in water for 18 hours; then, the embryo was separated from seed coat and nutritive tissue for later immersion in 0.1 % tetrazolium solution, at 25 °C, for one hour (Oliveira et al., 2014). Embryos were classified as viable or non-viable, according to the color and appearance of the tissues, to the damage extent, and to the location of the color patches.

Electrical conductivity test: Four replications of 10 embryos were soaked in 75 mL distilled water at 25 °C (Medeiros & Abreu, 2007). Seeds were left to soak for 12 hours (Garcia, Coelho, Maraschin, & Oliveira, 2011). After each period, the electrical conductivity of the solution was measured by a conductivity meter (Quimis-Q795), expressed in µS/cm.g per seed.

Effect of the development stage on post-collect physiological quality: Seeds of each development stage were stored in a refrigerator (8±2 °C) for 60 and 120 days, in plastic bags. The tests carried out were: moisture content, germination, tetrazolium, electrical conductivity, GSI, shoot and root length, as described above, i.e., with the same number of replication/seeds and at the same conditions of tests.

The experimental design for seed development was completely randomized with four treatments (development stages) and four replications for each treatment. The results obtained in percentage terms, such as germination and tetrazolium, were arcsine transformed, and the means were compared by the Tukey test at 5 % significance. The experiment on storage was carried out in a subplot with four stages (cotyledonary, I, II, III) and three periods of storage (0, 60, and 120 days). The results were submitted

to analysis of variance at 5 % probability level, and means were analyzed by regression.

RESULTS

Seed development: Seeds length was shorter at the stages cotyledonary, I and II and it increased to 55.66 mm at stage III. However, seeds width and thick were similar in all stages (Table 1). Seeds moisture content showed a decrease from the cotyledonary stage (66.54 %) to stage I (53.36 %), and remained around 49-47 % at other stages (Fig. 1).

Physiological quality during the development of *A. angustifolia* seeds increased from the cotyledonary stage to the last stage (III). Germination was 74 % at the cotyledonary stage and ± 86% at stages I, II, and III (Fig. 1).

Viability based on the tetrazolium test was similar to the behavior of the germination test, except for the cotyledonary stage, with values were close to 86 % at stages I, II, and III stages.

Differences in viability, according to the test carried out, were observed at the cotyledonary stage (germination or tetrazolium test), presenting 74 % viable seeds by the germination test, and 92 % by the tetrazolium test.

Germination speed index and shoot length were similar to those of the germination test, being the highest values for seeds at stage II

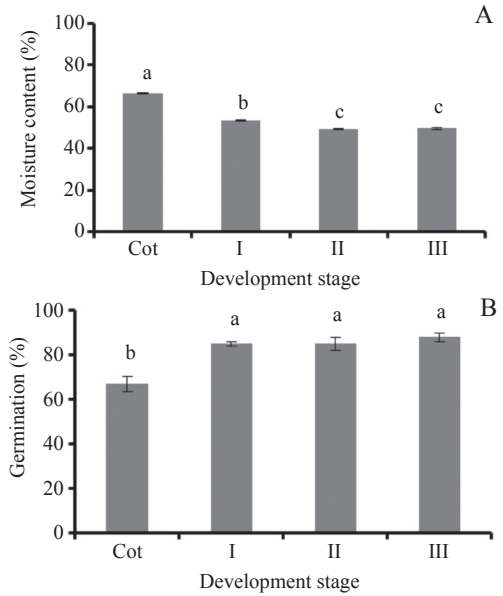


Fig. 1. (A) Moisture content and (B) germination of *Araucaria angustifolia* fresh seeds at different development stages. Means followed by the same letter do not significantly differ (Tukey's test, $P < 0.05$).

and III. However, root length was longer only at stage II (28.32 cm) (Table 1).

The vigor by electrical conductivity, at cotyledonary stage and stage III, showed higher leaching of exudates than at others stages. At cotyledonary stage, seeds were 61.98 $\mu\text{S}/$

TABLE 1
Length, width, thickness, germination speed index (GSI), root and shoot length, electrical conductivity and viability by tetrazolium test obtained by *Araucaria angustifolia* seeds at different development stages

Parameters	Development seed							
	Cotyledonary		I		II		III	
Seed length (mm)	54.35	b	54.22	b	54.33	b	55.66	a
Seed width (mm)	20.03	a	19.43	a	19.60	a	19.06	a
Seed thickness (mm)	16.47	a	16.14	a	16.88	a	16.01	a
GSI	0.22	c	0.36	b	0.4	ab	0.44	a
Shoot length (cm)	7.37	c	9.44	b	13.62	ab	15.47	a
Root length (cm)	14.66	c	10.83	d	28.32	ab	22.82	b
Electric conductivity ($\mu\text{S}/\text{cm.g}$)	61.98	c	41.61	a	46.34	ab	60.58	bc
Viability (%)	92	a	89	ab	83	b	88	ab

Means followed by the same letter do not significantly differ (Tukey's test, $P < 0.05$).
Number of cases, $N = 200$ (seed length, width and thickness) and $N = 100$ (GSI, shoot and root length, electric conductivity and viability).

cm.g, followed by a decrease at stages I (41.61 $\mu\text{S/cm.g}$) and II (46.34 $\mu\text{S/cm.g}$), and by an increase at stages III (60.58 $\mu\text{S/cm.g}$).

Post-collection physiological quality:

Moisture content at the cotyledonary stage was approximately 66 % for fresh seeds, and it was stored by 120 days. At stage I, fresh seeds stored for 60 and 120 days retained 53 % moisture content. On the other hand, at stages II and III, stored seeds showed an average decrease of ± 4 % after 120 days (Fig. 2).

At all development stages in fresh seeds, germination was not observed after 30 days of the beginning of the test. However, when seeds were stored for 120 days, they showed an increase in germination of 79 % and 60 % at cotyledonary stage and stage I, respectively. On the other hand, it was observed 16 % (stage II) and 39 % (stage III) of germination after 60 days of storage, and 10 % (stage II) and 24 % (stage III) of germination after 120 days (Table 2).

At the final evaluation of the germination test (70 days), at the cotyledonary stage, it was observed that the seeds stored for 60 and 120 days had higher viability than fresh seeds.

However, some fresh seeds and seeds stored for 60 days did not germinate, 11 % and 9 % respectively. The cotyledons grew inside the seed, but the embryonic axis elongation did not occur (Fig. 3). At stage I, the seeds had stability in germination (84-88 %), even when stored for up to 120 days. In addition, at stages II and III, seeds showed a decrease in viability after 60 or 120 days of storage (Table 2).

Fresh seeds and seeds stored for 60 and 120 days presented approximately 89 % of viability after the tetrazolium test. At stage II, seeds viability decreased from 83 % (fresh seeds) to 75 % (120 days of storage). At stage III, viability loss was greater than at the other stages, *i.e.*, fresh seeds had 88 % of viability, and after storage, viability decreased to 70 % (60 days) and 56 % (120 days) (Fig. 4).

Electrical conductivity was higher for fresh seeds at the cotyledonary stage (61.98 $\mu\text{S/cm g}$) than for those stored for 60 days (42.57 $\mu\text{S/cm g}$) and 120 days (55.7 $\mu\text{S/cm g}$). However, at other stages, leachates content released after 120 days of storage was higher with the advance of collection period, being 90.57 $\mu\text{S/cm.g}$ (stage I); 141.2 $\mu\text{S/cm.g}$ (stage II), and 160.2 $\mu\text{S/cm.g}$ (stage III).

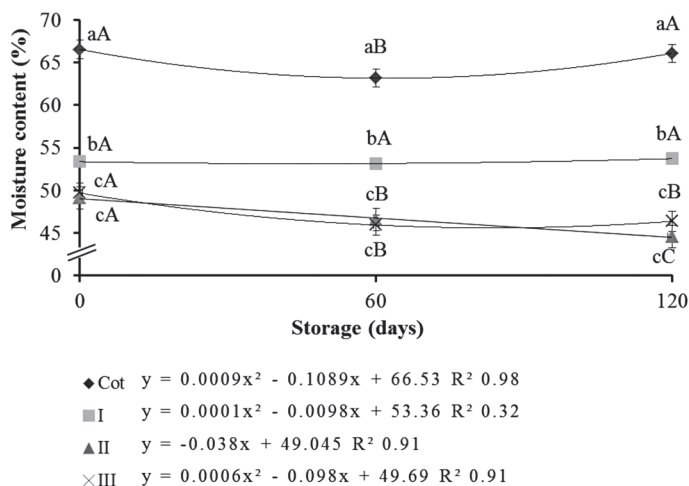


Fig. 2. Moisture content, at the cotyledonary stage, and stages I, II and III, of *Araucaria angustifolia* fresh seeds, and stored in refrigerator condition (8 ± 2 °C) for zero (control), 60 and 120 days. The letters refer to the Tukey test ($P < 0.05$): lowercase letter - differences between the stages of development; uppercase letter - difference between days of storage within each stage. N = 9 seeds/treatment, with three replications in each treatment.

TABLE 2
Germination (%) *Araucaria angustifolia* seeds at cotyledonary stage, and stages I, II and III
after storage for 0, 60 and 120 days (N = 100)

Stage of development	Time of storage (days)	Germination (%)					
		Days after initiation of test					
		30		50		70	
Cotyledonary	fresh seed	0	aCb	12	cBc	74	bAa
	60	8	cBb	79	aAb	86	aAa
	120	79	aAa	90	aAa	91	aAa
I	fresh seed	0	aCc	28	bBb	84	aAa
	60	26	bBb	77	aAa	88	aAa
	120	60	bBa	80	aAa	87	aAa
II	fresh seed	0	aBb	30	aAa	87	aAa
	60	16	bcBa	56	bAb	66	bAb
	120	10	dBab	50	cAb	58	cAb
III	fresh seed	0	aBc	72	aAab	88	aAab
	60	39	aBa	82	aAa	84	aAa
	120	24	cBb	65	bAb	70	bAb

The letters refer to the Tukey's test ($P < 0.05$): lowercase letter - differences in the columns between the stages of development, upper letter - differences in the lines, between days after initiation of germination test and italic letter - differences in the columns between storage times within each stage.

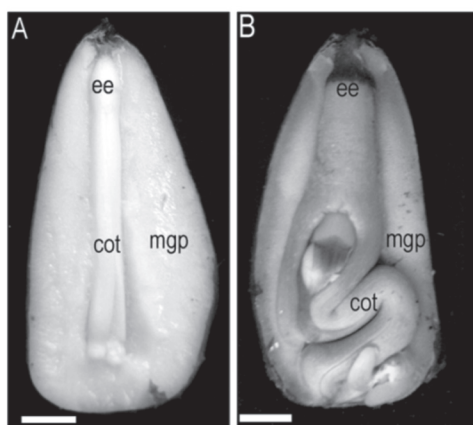


Fig. 3. Longitudinal section of *Araucaria angustifolia* seed at cotyledonary stage after 60 days of storage, showing the difference of seeds in the beginning (A) and in the end (B) of the germination test. The cotyledons grew inside the seed, but the embryonic axis elongation did not occur (B). Integument has been removed. ee: embryonic axis; cot: cotyledons; mgp: megagametophyte. Scale bars correspond to 5 mm.

The values of the germination speed index (GSI) indicated that seeds at cotyledonary stage stored for 120 days (GSI: 0.98) were more vigorous than fresh seeds (GSI: 0.22) and than

those stored for 60 days (GSI: 0.54). At stages I and III, 60 days of storage also favored GSI with an increase of approximately 0.25.

Both shoot and root length showed behavior similar to GSI at the cotyledonary stage: seeds had the highest values with an increase of storage period, reaching 23.39 cm root and 13.16 cm shoot after 120 days of storage. The same occurred at stage I with an increase of the root length from 10.83 cm (fresh seeds) to 28.26 cm (seeds with 120 days of storage). The same did not happen at stages II and III, where short root and shoot lengths were observed during storage (Fig. 4).

DISCUSSION

Morphological and physiological behavior observed during the seed development was different to that found in the previous year by Shibata et al. (2013). An increase of the seed size was observed, instead of a decrease, as shown by the study. It is not surprising that seed characteristics change according to the conditions in which the seeds are formed (Barbedo et al.,

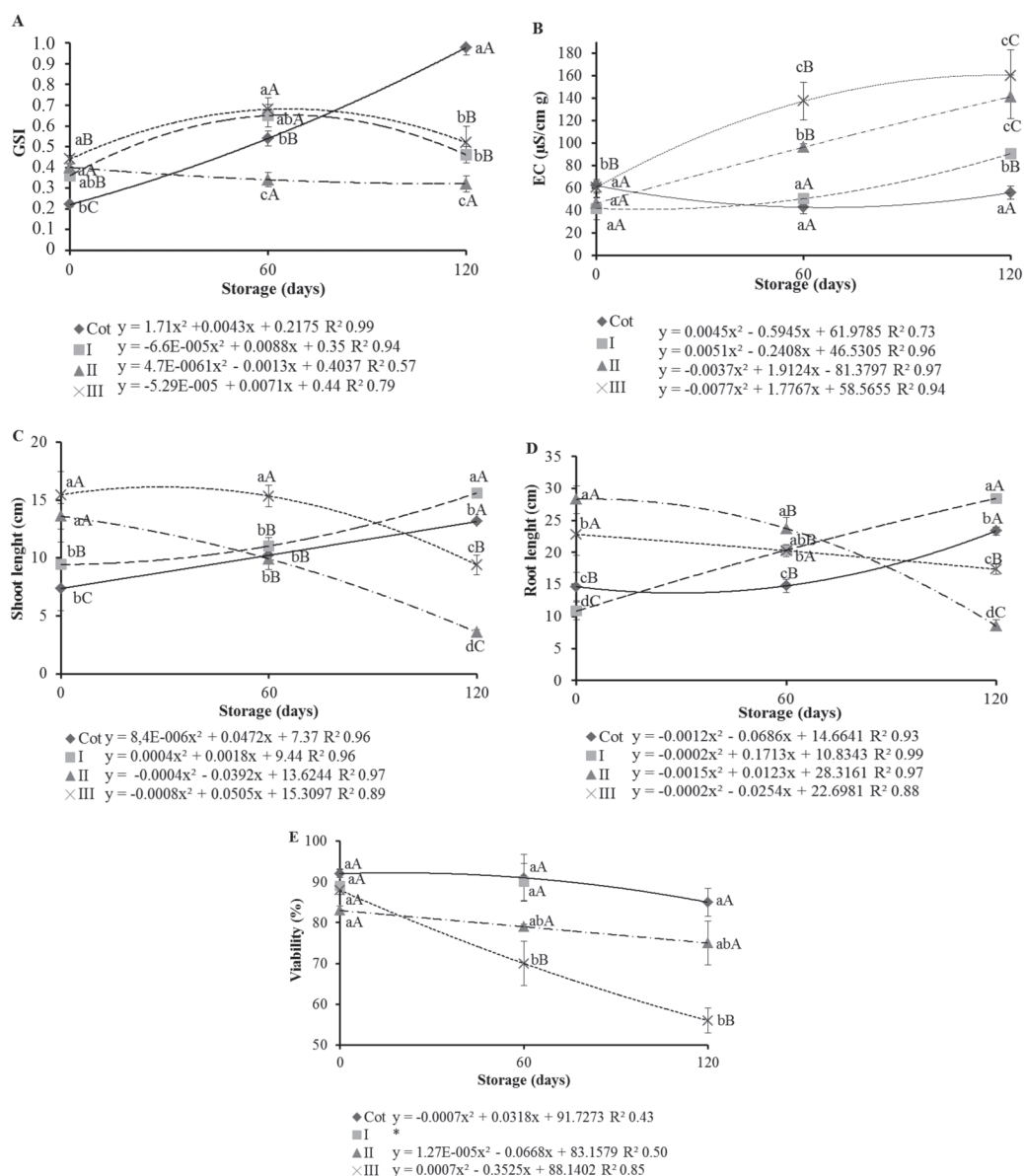


Fig. 4. (A) Germination speed index (GSI), (B) electrical conductivity, (C) shoot and (D) root length and (E) viability by tetrazolium test of *A. angustifolia* seeds at the cotyledonary (Cot) stage, and stages I, II and III stored for 0, 60 and 120 days. The letters refer to the Tukey test ($P < 0.05$). Lowercase letter-differences between the stages of development; uppercase letter- difference between days of storage within each stage. * There were not enough seeds for testing.

2013) and these could be associated with differences in environmental conditions (Lamarca et al., 2013; Mata et al., 2013). Nevertheless, the morphological characteristics were within the values for *A. angustifolia* seeds, varying

from 1.7 to 8.0 cm (length), from 1.0 to 2.5 cm (width), and from 0.9 to 2.0 cm (thickness) (Carvalho, 2002; Krupek & Ribeiro, 2010; Mattos, 2011). This variance is expected because the seeds originated from a different

female cone have large variance in size (Mantovani, Morellato, & Reis, 2004) and seed characteristics can be changed according to the conditions in which they are formed (Barbedo et al., 2013).

The high moisture content observed in the last stage (49.69 %) is common in recalcitrant seeds. Desiccation-sensitive seeds (recalcitrant) are *shed* when they present high moisture contents, and are relatively metabolically active (Farrant, Pammenter, & Berjak, 1993).

The increase in viability and vigor at the last stages were also observed in seeds of *Eugenia pyriformis* and *E. involucrata* DC. (Santana 2007), *Hopea hainanensis* (Lan et al., 2007) and *Inga striata* (Mata et al., 2013).

The vigor by electrical conductivity at the cotyledonary stage showed higher leaching of exudates. This may be associated with incompletely organized membranes at the cotyledonary stage, since the membrane system organizes itself gradually during seed development (Marcos-Filho, 2005). However, at stage III, the apparent loss of membrane integrity may be an indication of structural and biochemical changes or possible phospholipid breakdown (lipid peroxidation).

Seeds physiological activity during storage is influenced by the development stage in *A. angustifolia* seeds. The maintenance of seed moisture content during storage was variable and dependent on the period of collection. Furthermore, the behavior of the physiological quality was different at the early and late stages, *i.e.*, seeds viability and vigor at the cotyledonary stage and stage I increased or remained stable after storage. On the other hand, seeds showed decrease of physiological quality at stages II and III.

Other studies also showed similar behavior for *A. angustifolia* mature seeds: either they presented germination rate 2.5 times above the initial value after 4 and 6 months of storage (Piriz-Carrillo, Chaves, Fassola, & Mugridge, 2003), or they increased of germination from 21 % (fresh seed) to 66 % at 60 days storage (Martins, Tonetti, & Faria, 2011). Farrant, Pammenter and Berjak (1989) observed a slight

increase in germination rate for *A. angustifolia* seeds stored during seven days, and reserve mobilization by ultra-structure analysis. The decrease in physiological quality seems to be more common in recalcitrant seeds (Garcia, Coelho, & Oliveira, 2014; Parisi et al., 2013); however, *A. angustifolia* seeds showed different behavior according to the development stage.

These differences may indicate a desiccation sensibility during storage, according to the stage in which the seeds are collected. Therefore, the level of recalcitrance is related to how far the maturation was achieved, as well as how far the germination advanced before seed shedding (Barbedo et al., 2013). According to Pammenter & Berjak (1999) and Farrant, Pammenter, & Berjak (1986), the development stages in which seeds are collected influence the desiccation response of recalcitrant seeds, and desiccation sensibility may be increased with storage, as *Aesculus hippocastanum* L. (Tompsett & Pritchard, 1998) and *Quercus robur* L. (Finch-Savage, Clay, Blake, & Browning, 1992). Besides the germination increase, it was observed a delay in germination at all stages for fresh seeds, which may be caused by dormancy (Aquila & Ferreira, 1984; Doni Filho, Amaral, & Cervi, 1985; Piriz-Carrillo et al., 2003). Some studies showed that seed germination of *A. angustifolia* was favored by storage at low temperatures (Aquila & Ferreira, 1984; Caçola, Amarante, Fleig, & Mota, 2006).

At cotyledonary stage, some fresh seeds and some seeds stored for 60 days did not germinate. At this stage, seeds have not yet completed their development. A metabolic impediment may have occurred at this stage, especially in the root meristem region, caused by a hormonal imbalance between germination inhibitors (abscisic acid - ABA) and growth promoters (gibberellins - GA). In the hormone-balance model, ABA and GA simultaneously and antagonistically regulate the onset, maintenance and termination of dormancy. Thus, ABA induces dormancy during seed development, and GA promotes the germination of non-dormant seeds (Baskin & Baskin, 2004).

Furthermore, ABA promotes embryo maturation, and mid and late developmental stages reach peak concentrations (Borisjuk et al., 2004; Taiz & Zeiger, 2006). In *A. angustifolia* seeds, the maximum ABA contents were reached at the pre-cotyledonary stage (Silveira et al., 2008). In this study, this hormone was not quantified, but the lowest values of fresh seeds viability at the cotyledonary stage were close to the stage with the highest ABA level found by Silveira et al. (2008). Therefore, seeds probably did not germinate due to peak concentrations of ABA close to the cotyledonary stage, which prevents early germination.

This delay of germination and improvement in physiological quality after storage may support the development of innovative approaches to address the methodological difficulties inherent to *ex situ* conservation of *A. angustifolia*. It is possible that just the early stages of development of *A. angustifolia* seeds, they could really be just seeds (stage cotyledonary and I) and the other ones being germinating seeds (II and III). For this, the seed conservation at stage cotyledonary and I showed better results of conservation of the former ones. For instance, *Inga vera* seeds can disperse the fruits with germinating seeds inside them, sometimes in an advanced process of germination (Caccere et al., 2013; Parisi et al., 2013).

An option for long-term storage of recalcitrant seed is through cryopreservation (Engelmann, 2011) that is a method of high cost that requires a high level of technology (Bonjovani & Barbedo, 2014).

Hence, understanding the phenomenon of recalcitrant seed, and consequently developing conservation practices for species that produce such seeds is of major scientific and practical importance (Berjak & Pammenter, 2008). Future experiments will be necessary to evaluate the anatomical and biochemical changes at pre and post-collection, for a better understanding of the seeds behavior, particularly those collected at the cotyledonary stage.

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RESUMEN

Cosecha temprana aumenta calidad fisiológica de post-cosecha de semillas de *Araucaria angustifolia* (Araucariaceae). *Araucaria angustifolia* es una conífera nativa de Brasil y una especie en peligro de extinción. Sus semillas tienen un corto período de viabilidad, factor que contribuye a su vulnerabilidad. Este estudio tuvo como objetivo evaluar la calidad fisiológica durante el período de desarrollo y post-almacenamiento de semillas de *A. angustifolia*. Conos de *A. angustifolia* fueron recolectados en poblaciones naturales en Curitiba, Santa Catarina, Brasil, en marzo, abril, mayo y junio y clasificados en los estadios de desarrollo cotiledonar I, II y III de acuerdo con el mes de recolecta. Un total de 10 conos fueron recolectados para cada estadio. Las semillas fueron almacenadas en refrigerador durante 60 y 120 días y posteriormente sometidas a pruebas de germinación (25 °C - fotoperíodo de 12 h) siendo evaluados el contenido de humedad, tetraxolol y el vigor (conductividad eléctrica [75 mL de agua destilada a 25 °C], índice de velocidad de germinación, y la longitud de la parte aérea y de la raíz). Durante el desarrollo de las semillas, el contenido de humedad se redujo desde el estadio cotiledonar (66.54 %) al estadio III (47.44%), y el vigor aumentaron en el último estadio. Durante el almacenamiento, el contenido de humedad en el estadio cotiledonar y estadio I fue estable. Entretanto, las semillas almacenadas mostraron una reducción en el contenido de humedad después de 120 días en los estadios II y III. La calidad fisiológica en el estadio cotiledonar mostró un aumento de 86 % y 93 % de germinación después de 60 y 120 días de almacenamiento, respectivamente, a diferencia de los estadios II y III, los cuales mostraron una disminución en la viabilidad de las semillas y en el vigor después del almacenamiento. La conductividad eléctrica fue mayor para las semillas en estadio cotiledonar recién recolectadas que para aquellas almacenadas durante 60 y 120 días. Sin embargo, en otros estadios, el contenido de lixiviados después de 120 días de almacenamiento aumentó con el avance del período de recolecta. El índice de velocidad de germinación y la longitud de la parte aérea y raíz después del almacenamiento eran más altos para las semillas en el estadio cotiledonar y el estadio I, a diferencia del estadio II y III, los cuales tenían raíz y parte aérea de menor longitud.

durante el almacenamiento. Por lo tanto, el mantenimiento del contenido de humedad de la semilla durante el almacenamiento fue variable y depende del período de recolecta. Además, la calidad fisiológica de las semillas difiere entre los estadios más tempranos o tardíos. Una recolección precoz favoreció la calidad fisiológica de las semillas, y puede ser una estrategia para aumentar la conservación de semillas de *A. angustifolia*.

Palabras clave: semillas recalcitrantes, almacenamiento, desarrollo de semilla, gimnospermas, plantas amenazadas.

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