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Physiological and biochemical changes in *Cedrela fissilis* seeds during storage

Abstract – The objective of this work was to evaluate the effect of storage on the physiological quality of cedar (*Cedrela fissilis*) seeds, as well as to correlate the germination and vigor of the seeds with their main biochemical changes. The experiments were carried out in a completely randomized design, in a 3×5 factorial arrangement (three environments × five storage periods). Seeds were stored for 0, 135, 280, 381, and 515 days in: a humidity chamber at 5±2ºC and 80% relative humidity, a drying chamber at 20±2ºC and 60% relative humidity, and an uncontrolled environment (laboratory) at 16±10ºC and 60±25% relative humidity. In all storage periods, the content of moisture on a wet basis and the percentages of proteins, lipids, total carbohydrates, and ash were evaluated. For the viability and vigor tests, the percentage of germination and mean germination time were calculated. At sampling time, seeds showed 11.5% water content, 85.5% germination, and mean germination time of 13.5 days, and all were negatively influenced by storage period. Protein percentage showed a downward trend, while that of carbohydrates increased as the storage period was extended. Seed germination and vigor reduce drastically with storage.

Index terms: *Cedrela fissilis*, biochemical components, forest seeds, pink cedar, seed storage conditions.

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Alterações fisiológicas e bioquímicas de sementes de *Cedrela fissilis* durante o armazenamento

Resumo – O objetivo deste trabalho foi avaliar o efeito do armazenamento na qualidade fisiológica de sementes de cedro (*Cedrela fissilis*), bem como correlacionar a germinação e o vigor das sementes com suas principais alterações bioquímicas. Os experimentos foram conduzidos em delineamento inteiramente casualizado, em arranjo fatorial 3×5 (três ambientes × cinco períodos de armazenamento). As sementes foram armazenadas por 0, 135, 280, 381 e 515 dias, em: câmara úmida a 5±2ºC e 80% de umidade relativa, câmara seca a 20±2ºC e 60% de umidade relativa e ambiente não controlado (laboratório) a 16±10ºC e 60±25% de umidade relativa. Em todos os períodos de armazenamento, foram avaliados o teor de umidade em base úmida e as percentagens de proteínas, lipídios, carboidratos totais e cinzas. Para os testes de viabilidade e vigor, foram calculados percentagem de germinação e tempo médio de germinação. No momento da coleta, as sementes apresentaram 11,5% de água, 85,5% de germinação e tempo médio de germinação de 13,5 dias, e todas foram negativamente influenciadas pelo período de armazenamento. A percentagem de proteína mostrou tendência de queda, enquanto a de carboidratos aumentou com o aumento do período de armazenamento. A germinação e o vigor das sementes reduzem drasticamente com o armazenamento.

Termos para indexação: *Cedrela fissilis*, componentes bioquímicos, sementes florestais, cedro-rosa, condições de armazenamento de sementes.

Introduction

The demand for native forest seedlings in Brazil has increased considerably in the last decade (Ribeiro-Oliveira & Ranal, 2014), especially for the restoration of degraded ecosystems, timber production, forest by-products, and the integrated crop-livestock-forest system. To avoid seed shortage, storage may be an excellent alternative, aiming to maintain or reduce the loss of seed physiological quality until sowing time (Vijay et al., 2015).

For short-term storage, dry and cold environments can be used, often without extensively damaging seed germination and vigor, whereas, for long-term storage, facilities with specific climate control should be adopted. Variations in temperature, relative air humidity, and oxygen concentration are the main environmental factors responsible for causing seed damage during storage (Selvi & Saraswathy, 2018). Therefore, controlling these factors allows generating conditions to store seeds for prolonged periods (Motlagh & Shaban, 2014).

When stored in improper conditions, seeds tend to suffer physiological and biochemical changes, which negatively impact the preservation of their quality (Silva et al., 2011). Deterioration, for example, has a negative effect on seed germination and vigor, often making germination impossible (Rajjou & Debeaujon, 2008), being associated with seed resistance to degradation, an intrinsic characteristic of each species (Mohammadi et al., 2012).

Another type of seed damage is aging, which is influenced by different factors related to: environmental conditions, such as temperature and humidity; genetics; and the used material, including seed moisture content and quality at storage time (Rajjou & Debeaujon, 2008; Zhou et al., 2020). Aging is common during storage because reserve substances, such as proteins, lipids, and carbohydrates, are significantly affected (Ghasemnezhad & Honermeier, 2009), hindering the formation of the embryo, which uses them as a source of energy and substrate for its cellular structures (Pontes et al., 2002). Among the main physiological consequences observed, stand out those linked to losses of seed viability and vigor of germination (Toledo et al., 2009; Donadon et al., 2015).

Despite these challenges, seed storage is still an important strategy for ex situ genetic conservation, aiming at germplasm conservation (Flores et al., 2018), breeding, and mass propagation (Rajjou & Debeaujon, 2008; Borges et al., 2009). This generates new perspectives for species such as *Cedrela fissilis* Vell. (Meliaceae), popularly known as "pink cedar" or "white cedar", which is listed as vulnerable to extinction (Barstow, 2018) and has great potential as a phytoremediator for ecological restoration (Covre et al., 2020). This secondary species occurs naturally in South America and in part of Central America, and, specifically in the Atlantic Rainforest in Brazil, its populations are found from 20 m to more than 1,600 m above sea level (Mangaravite et al., 2016). The species presents attractive characteristics to the timber industry, including a height of up to 40 m and a diameter at breast height of 300 cm in adulthood (Carvalho, 2003).

Knowing how storage affects the biochemical and physiological quality of seeds is, therefore, relevant when working with neotropical species, whose seasonal fruit production and genetic variability are compromised by the constant fragmentation of their natural habitat (Rajjou & Debeaujon, 2008; Mangaravite et al., 2019).

The objective of this work was to evaluate the effect of storage on the physiological quality of cedar seeds, as well as to correlate the germination and vigor of the seeds with their main biochemical changes.

Materials and Methods

The seeds were collected from a noncertified production area, located in the experimental field of Empresa Mato-grossense de Pesquisa, Assistência e Extensão Rural, in the municipality of São José dos Quatro Marcos, in the state of Mato Grosso, Brazil (15º39'S, 58º19'W, at 223 m altitude). The area of seed production was considered a set of 42 plants, from which 12 productive matrices were selected from the center of the plot.

The experiments were carried out in a completely randomized design in a 3×5 factorial arrangement (three environments × five storage periods). Seeds were stored for 0 (after collection), 135, 280, 381, and 515 days, in the following three environments: a humidity chamber at 5±2ºC and 80% relative humidity; a drying chamber at 20±2ºC and 60% relative humidity; and a laboratory (uncontrolled environment) at 16±10ºC and 60±25% relative humidity (Table 1).

Because *C. fissilis* has dehiscent fruits with predominant dispersion by anemochory, the point of maximum physiological maturity of the seeds was evaluated at intervals of 30 days, and fruit were collected when their color was greenish brown to clear. After collected, seeds were packed in plastic containers, placed in cardboard boxes, and sent to the Seed Laboratory of the Forest Engineering Department of Universidade Federal do Paraná, located in the municipality of Curitiba, in the state of Paraná, Brazil. For the initial characterization of the seed lot, the number of seeds per kilogram, the longest and shortest lengths (mm), and purity (%) were evaluated following the method established by the rules for seed analysis in the country (Brasil, 2009). Afterwards, seeds were placed in closed glass containers and stored in the three environments. The viability and vigor tests were performed after each storage time in each environment.

Moisture content on a wet basis and as a function of the treatments was determined in five replicates of ten seeds, which were placed in a drying oven at 105±3ºC for 24 hours under forced-air circulation (Brasil, 2009). Before the viability and vigor experiments were carried out, seeds were immersed for 3 min in a 1% sodium hypochlorite (NaClO) solution.

Germination and vigor were evaluated by ten replicates of 20 seeds, which were sown in acrylic boxes (gerbox type) containing vermiculite and placed in a germinator at 25±2ºC. Seeds with 1.0±0.5-cm root length – observed daily – were considered germinated. Vigor was assessed together with the germination test through mean germination time (MGT) (Labouriau, 1983) and calculated according to the formula:

$$MGT = \sum n_i \times t_i / \sum n_i$$

where n_i is the number of seeds germinated in a day and t_i is the number of days after sowing.

Biochemical analyses were carried out at the Laboratory of Technology for Non-Timber Forest Products of Embrapa Florestas, located in the municipality of Colombo, in the state of Paraná, Brazil. The seeds were initially peeled using locking pliers and cut into small segments, then milled in a cyclone mill with a 0.5-mm mesh. Each sample, corresponding to a storage period, was composed of approximately 200 g ground seeds, which were packed in sealed vials and stored in a freezer at -18°C.

To obtain moisture content, samples were oven dried at 105ºC for 24 hours, with three replicates of 1.0 g, and later weighed on a precision analytical scale (Brasil, 2009). After the percentage of water content was determined, samples were placed in a muffle furnace at 550ºC for 5 hours and weighed again to quantify ash content. To calculate the percentage of total proteins, the total nitrogen content in the seeds was determined by the micro-Kjeldahl method (Latimer Jr., 2016) and transformed into protein using a correction factor of 6.25. Lipids were extracted by a Soxhlet extractor, using diethyl ether as a solvent, at 40ºC, in two replicates. The fiber volume fraction was determined enzymatically using the Total Dietary Fiber kit (Megazyme International Ireland Ltd., Wicklow, Ireland) (AOAC, 1995). All methods applied followed the official methodologies of Instituto Adolfo Lutz (Zenebon & Pascuet, 2005), with results expressed on a wet and dry basis (g 100 g^{-1}). Total carbohydrates (TC) were determined according to the formula:

TC = 100% - (water content + ash + proteins + lipids + fiber)

The variances of the treatments were evaluated for homogeneity by Bartlett's test, and the means of the variables that differed significantly by the F-test were compared by Tukey's test, at 5% probability. Pearson's correlation analysis at 1% and 5% probability was applied to verify the influence of the main biochemical components – analyzed in triplicate – on the percentage of germination.

Table 1. Storage environments and storage period for *Cedrela fissillis* seeds, as well as experimental period.

Storage environment[(1)]	Storage period (days)	Experimental period[(2)]
Drying chamber	0	Oct. 24 to Oct. 27, 2011
Humidity chamber	135	Jan. 17 to Jan. 23, 2012
Laboratory	280	May 25 to May 29, 2012
(uncontrolled	381	Oct. 06 to Oct. 11, 2012
conditions)	515	Feb. 20 to Feb. 25, 2013

[(1)]Drying chamber at 20±2ºC and 60% relative humidity; humidity chamber at 5±2ºC and 80% relative humidity; and laboratory, considered as uncontrolled conditions, at 16±10ºC and 60±25% relative humidity. [(2)]The experiments were setup on October 23, 2011.

Results and Discussion

On average, 11,089 seeds per kilogram showed a length of 11.5 mm in the major axis and of 7.0 mm in the minor axis, as well as 93.5% purity. The mean seed size was smaller than those found by Pereira et al. (2017), which were of 24.7 to 27.2 mm in the major axis and of 9.1 to 12.1 mm in the minor axis. The difference in these values may be attributed to the high genetic variability of the morphological characteristics of the reproductive organs of the mother tree (Araújo et al., 2015), influenced by biotic and abiotic factors during seed development (Santos et al., 2009; Pereira et al., 2017). The size of the seeds is an important indicator of their physical quality and has direct effects on vegetative plant performance (Kumar et al., 2016).

When the experiments were installed, seed moisture content was 11.5% for the control; however, 515 days later, it decreased to 8.8% in the humidity chamber, 8.6% in the drying chamber, and 8.1% in the laboratory (Figure 1). It should be noted that, at a constant temperature, the storage of seeds with a high moisture content may promote deterioration (McDonald, 1999). For *C. fissilis*, this value is of 12.4% moisture, which, at 10 and 20ºC, does not influence seed viability up to 360 days (Martins & Lago, 2008). However, the moisture content of *C. fissilis* seeds collected at the beginning of natural dehiscence varies significantly, with values of: 22.4% (Corvello et al., 1999), 12.4% (Martins & Lago, 2008), 13.3 to 20.8% (Lazarotto et al., 2013), and 23.9% (Pereira et al., 2017).

Up to 135 days of storage, there was a rapid reduction in seed moisture content and a subsequent stabilization in all environments (Figure 1). During this period, seeds stored in the drying and humidity chambers maintained almost constant percentages of water content, while those stored in the laboratory showed a downward trend up to 515 days, confirming the influence of the storage environment on the hygroscopic balance of seeds. Therefore, the temperature and moisture content of the storage environment are preponderant variables to guarantee the prolonged physiological quality of forest seeds (Motlagh & Shaban, 2014).

The viability of *C. fissilis* seeds before storage was 85.5%, considered high, slightly decreasing up to 381 days and then reducing drastically, compromising the use of the seeds during their collection (Figure 1). Regarding germination percentage, there was no significant interaction between environment and storage period. However, higher means were found for shorter storage periods: 85.5% for 0 day and 82.1% for 135 days, followed by 74.5% for 280 days and 68.8% for 381 days, from when all treatments presented lower values for this variable, culminating at 515 days with a mean germination of 30.5%. Viability was of 73.7 and 69.7% in the humidity and drying chambers, respectively, significantly higher than that of the laboratory environment, which was of 61.5%.

Soon after physiological maturation, the process of seed progressive deterioration begins, consequently reducing germination percentages (Girardi et al., 2013). This deterioration is increased by the humidity and temperature of the storage environment, i.e., a higher humidity and temperature favor the acceleration of physiological processes, leading to a greater seed deterioration (Selvi & Saraswathy, 2018).

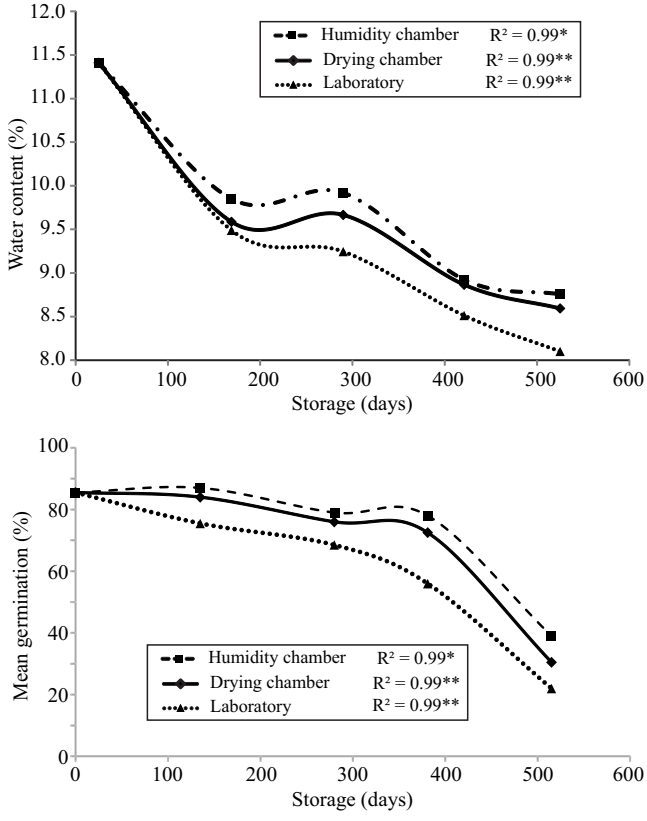

Figure 1. Moisture content (%) of *Cedrela fissillis* seeds as a function of storage (A) and mean germination of *C. fissillis* seeds as a function of storage period (B). ** and *Significant by the F-test, at 5 and 1% probability, respectively.

None of the environments was able to maintain seed viability after 381 days. This reduced viability can be attributed to the effects of the environmental conditions on the physiological quality of *C. fissilis* seeds. In addition, germination percentages did not differ significantly between the different environments, probably due to a better condition (lower temperatures) for the maintenance of *C. fissilis* seed viability (Figure 1), as already observed for this species when stored at 10 and 20°C (Martins & Lago, 2008).

Seed mean germination time (MGT) showed a significant interaction between environments and storage period, with a significant influence of storage environments from 350 days onwards and a lower MGT for seeds stored in the laboratory (Table 2). As the storage period increases, there is a decrease in the germination percentages (Figure 1) and an increase in MGT (Table 2), with a significant negative correlation of -0.82 between both variables. This increase in MGT is a result of seed deterioration during the storage period, since the rate of deterioration depends directly on environmental conditions and on the length of the storage period (Salinas et al., 2001). The predisposition to deterioration is influenced by different factors, among which stand out the genetic and morphological ones. Certain characteristics of some species result in a greater or lower viability during storage, and small morphological differences, such as seed size, may lead to a greater deterioration due to the higher ratio between surface area and volume, making seeds more susceptible to water absorption (Motlagh & Shaban, 2014).

MGT reached a stability point between 90 and 350 days for seeds stored in the laboratory, slightly decreasing to 16.66 days at 350 days, when compared with 16.92 days at 210 days (Table 2). Since the experiments were installed in October, the influence of the lower temperatures of mild seasons is evident, affecting the maintenance of seed vigor, even under uncontrolled conditions, such as those of the laboratory.

The percentage of proteins in *C. fissilis* seeds showed a decreasing trend as the storage period was extended, regardless of the storage environment (Table 3). This reduction may be a consequence of deterioration events, including loss of membrane integrity, reduced energy metabolism, impaired RNA and protein synthesis, and DNA degradation (Kibinza et al., 2006). Moreover, seed qualitative parameters, which include biochemical components, such as oils, fatty acids, and proteins, are strongly influenced by storage conditions (Ghasemnezhad & Honermeier, 2009). Therefore, after storage, among the most common physiological symptoms caused by seed deterioration stand out the reduction of germination percentage and vigor (Toledo et al., 2009; Donadon et al., 2015). This result was confirmed by the positive correlation of 0.64 for mean germination x proteins and the negative correlation of -0.71 for MGT x proteins (Table 4).

Between 0 and 515 days of storage, there was a reduction of 28.5% in protein contents, with a reduced variation among environments. The stability of protein contents between 0 and 169 days was emphasized, from which there was a drastic reduction in mean values, followed by stability between 290 and 525 days (Table 3). This reduction in seed protein content may be related to deterioration, directly affecting the oxidation of the proteins present in the seeds and, consequently, leading to the loss of the functional properties of proteins and enzymes (Rajjou et al., 2008).

Lipid and ash contents remained close to those found at the beginning of the experiments. Lipid contents increased by 4.7%, on average, in the drying and humidity chamber environments. Both the stability and slight increase observed in lipid contents can be explained by the adequate temperature and humidity

Table 2. Mean germination time of *Cedrela fissilis* seeds as a function of storage period[(1)].

Storage environment	Storage period (days)				
	0	90	210	350	470
Drying chamber	13.59aC	15.26aC	16.05aC	19.80aB	28.19aA
Humidity chamber	13.59aC	15.25aC	16.41aBC	19.56aB	25.34bA
Laboratory	13.59aC	15.41aBC	16.92aB	16.66bBC	20.77cA
CV (%)			17.76		

[(1)]Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ by Tukey's test, at 5% probability.

conditions, respectively, in the humidity and drying chambers. However, ash content showed a decrease of 8.6% in the drying chamber, followed by one of 15.7% in the laboratory environment (Table 3). Ash content represents the organic residue remaining after organic matter is burned, but does not necessarily have the same mineral composition due to losses caused by volatilization or by interactions between constituents (Ocloo et al., 2010). Therefore, ash content depends directly on the soil-climatic conditions to which the mother trees were exposed (Vale et al., 2011). It should also be pointed out that the chemical composition of seeds influences their mechanical properties, varying according to moisture content and increasing seed resistance as the percentages of protein and ash increase (Kuźniar et al., 2016).

The percentages of total carbohydrates showed a gradual increase as the storage period was extended, regardless of the storage environment (Table 3). The increase in total carbohydrate content in *C. fissilis* seeds may be associated with the increased solubilization of reserves, a result already reported for other orthodox seeds under storage conditions (Garcia et al., 2006). Therefore, the increase in the percentages of total carbohydrates during the storage period proved to be unfavorable for the germination and vigor of *C. fissilis* seeds, with a significant correlation of -0.66 for mean germination and of 0.71 for MGT (Table 4).

The obtained results are indicative that the storage of *C. fissilis* seeds for periods of up to 381 days maintains their viability, regardless of the storage environment, with a germination greater than 50%. These findings will expand the perspectives and use of the species in silvicultural systems, both from an ecological and from an economic point of view.

Table 4. Correlation between germination percentage (GP) and biochemical components of *Cedrela fissilis*.

Variable[(1)]	Correlation
GP x Proteins	0.64*
MGT x Proteins	(-)0.71**
MGT x Total carbohydrates	0.71**
MG x Total carbohydrates	(-)0.66**
MGT x Lipids	0.44^{ns}
MG x Lipids	(-)0.45^{ns}
MGT x Ash	(-)0.37^{ns}
GP x Ash	0.64**

[(1)]MGT, mean germination time. * and **Significant at 5 and 1% error probability, respectively. ^{ns}Nonsignificant by the F-test, at 5% error probability.

Table 3. Percentages of proteins, lipids, total carbohydrates, and ash in *Cedrela fissilis* seeds, on a dry basis and as a function of storage environments and period.

Storage environment[(1)]	Storage period (days)	Proteins	Lipids	Total carbohydrates	Ash
		(g 100 g^{-1})			
Drying chamber	0	25.16±0.18	19.21±0.51	49.95±2.62	5.68±0.24
	135	25.25±0.32	18.21±0.89	50.69±1.28	5.85±0.12
	280	19.92±0.03	19.92±0.98	54.40±3.12	5.59±0.04
	381	18.29±0.43	20.51±0.51	54.93±1.90	6.27±0.98
	515	18.39±0.27	20.28±0.90	55.85±1.76	5.48±0.46
Humidity chamber	0	25.16±0.18	19.21±0.51	49.95±2.62	5.68±0.24
	135	25.06±0.04	19.15±0.20	49.65±2.92	6.15±0.09
	280	18.53±0.88	18.53±0.22	54.88±2.84	6.11±0.19
	381	17.45±1.14	19.11±0.39	58.16±2.30	5.28±0.26
	515	17.52±1.02	20.63±1.74	56.54±2.17	5.31±0.68
Laboratory	0	25.16±0.18	19.21±0.51	49.95±2.62	5.68±0.24
	135	25.59±0.34	17.20±1.46	51.09±1.76	6.12±0.20
	280	18.51±0.11	18.51±0.99	56.59±3.29	5.15±0.25
	381	19.34±0.34	21.82±1.67	53.46±1.55	5.38±0.37
	515	18.08±0.08	19.43±0.46	57.70±3.05	4.79±0.99

[(1)]Drying chamber at 20±2ºC and 60% humidity; humidity chamber at 5±2ºC and 80% humidity; and laboratory, considered as uncontrolled conditions, at 16ºC±10 and 60%±25 relative humidity.

Conclusions

1. *Cedrela fissilis* seed germination and vigor reduce drastically with storage.

2. The viability of *C. fissilis* seeds is affected by storage periods greater than 381 days.

3. The vigor of *C. fissilis* seeds is impaired by storage period and conditions, with a greater damage in the uncontrolled environment (laboratory) at 515 days.

4. The decrease in total protein content and the increase in carbohydrate content are unfavorable for *C. fissilis* seed germination and vigor.

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