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Can Seeds Storage Conditions Influence the Quality of *Cedrela fissilis* Seedlings?

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ABSTRACT

Seeds vigor defined in the laboratory does not always reflect the final quality of seedlings produced under nursery conditions or even their survival in plantations. So, we studied the influence of *Cedrela fissilis* seed storage conditions on its emergence and the morphological quality of produced seedlings. Seeds were collected in October/2011, benefited, conditioned in closed glasses, and stored in three environments for a period of 515 days. Treatments consisted of: I - seedlings produced from seeds without storage (control); II - seedlings produced from seeds stored in a dry chamber; III - seedlings produced from seeds stored in a humid chamber; IV - Seedlings produced from seeds stored in an uncontrolled environment (laboratory). Sowing was performed in 50 cm³ plastic tubes filled with decomposed pinus bark and coconut fiber (50/50 v/v) and packed in a glasshouse. The study analyzed the percentage of seedlings emergence, mean seedlings emergence time, stem diameter, total height, the ratio between total height and stem diameter, shoot length, root length, total, root and shoot dry biomass, and Dickson quality index. Seed storage proved to be a negative factor for the emergence and vigor of *C. fissilis* seedlings, regardless of the storage environment. In addition, seeds vigor is a preponderant factor to increase seedlings' morphological quality, such as stem diameter and height.

1. Introduction

The demand for native forest seedlings is growing in Brazil due to legal issues, mainly for restoring legal reserve and recovery of degraded ecosystems [1 and 2]. Due to this situation, the need for large-scale seedlings production with quality and reduced costs is increasing [3].

Storage may be an excellent alternative to avoid the shortage of seeds, aiming at maintaining or minimizing the loss of physiological quality until sowing [4]. However, seeds tend to undergo physiological and biochemical changes, as a consequence of storage conditions, with negative consequences in their quality conservation [5].

The practice of seed storage may generate new perspectives for species such as *Cedrela fissilis* Vell. (Meliaceae), popularly known as “pink cedar” or “white cedar”. It is a secondary species with natural occurrence in South and part of Central America, with characteristics attractive to the timber industry, among which stands out a height of up to 40 meters and DBH (diameter at breast height) of 300 cm when adult [6], as well as being present in the list of vulnerable species [7].

Seedling quality can be evaluated by measuring morphological characters such as shoot height, stem diameter, root system development, and stem lignification [8 and 9]. The use of these morphological characters is of extreme importance in determining the quality of seedlings produced under similar environmental conditions [10]. Together, they generate indices with a high capacity to represent the morphological quality of seedlings, of which two are frequently applied for this purpose; the first is represented by the ratio between total height and stem diameter H/DC) [11 and 12]. The second, named Dickson quality index (IQD), calculated from morphological variables height, stem diameter, and dry biomass, indicating that the higher the value of the index, the better is the quality of evaluated seedling [13].

Few studies have been developed to correlate the quality of seedlings with the effects generated by forest species seeds storage [5 and 20]. Thus, based on the importance of seed storage in the viability of forest seedlings production, we evaluate the influence of *C. fissilis* seeds storage conditions on its emergence and seedlings' morphological quality.

2. Materials and Methods

Seeds were collected in a non-certified seed production area in October 2011 to conduct the experiments. They contained 42 mother trees, located at the experimental field of EMPAER, in São José dos Quatro Marcos, Mato Grosso, Brazil (15º39' S and 58° 19' W, 223 m). From this area, 12 productive mother trees located in the center of the plot were selected to avoid interference from the border. The region's climate is Aw type, according to Köppen classification, characterized as tropical, with an average temperature of the coldest month above 18 ºC. The rainy season is present in summer, from November to April, with a clear dry season in winter.

After collection, the seeds were sent to Seed Laboratory from the Forest Engineering Department of the Federal University of Paraná for the initial characterization of the lot. Therefore, the number of seeds per kilogram (seeds Kg^{-1}), dimensions of the major and minor axes (mm), and purity (%) were evaluated, following methodologies established by the Brazilian Rules of Seeds Analysis [14].

Prior to the storage of the seeds, they were disinfected by immersing them for 3 minutes in a 1% sodium hypochlorite solution (NaClO). Afterwards, the seeds were placed in hermetic containers and stored in three different environments: humid chamber at 5±2 ºC and 80% relative humidity; dry chamber at 20±2 ºC and 60% relative humidity, and dry chamber at 20±2 ºC and 60% relative humidity, and laboratory environment under uncontrolled conditions.

After 515 days of storage, the moisture content of the seeds (wet basis) was determined in each environment, using five replicates of 10 seeds, which were placed in a forced air circulation oven at 105±3 ºC, for a period of 24 hours [14].

Afterwards, the seeds were put to germinate in plastic tubes of 50 cm^3, according to the following treatments: I - Seedlings produced from seeds without storage (control); II - Seedlings produced from seeds stored in a humid chamber for 515 days; III - Seedlings produced from seeds stored in a dry chamber for 515 days; and IV - Seedlings produced from seeds stored in an uncontrolled environment (laboratory) for 515 days. The tubes were filled with substrate containing decomposed pine bark and coconut fiber (50/50 v/v), with the addition of 1.3 kg m^{-3} of Osmocote® 18-5-9 Mini Prill (5M). Sowing was done manually, distributing one seed per tube, covered with a substrate layer with approximately twice the seed thickness. After sowing, the containers were kept in a glass-house (three daily irrigations of 10 minutes, with a flow rate of 144 L $hour^{-1}$), where they remained for 92 ± 6 days.

During this period, the percentage of seedling emergence (PSE) and mean seedlings emergence time (MSET) were evaluated every two days until the seedlings presented the first differentiated leaves (leaf primordia) (Figure **1**).

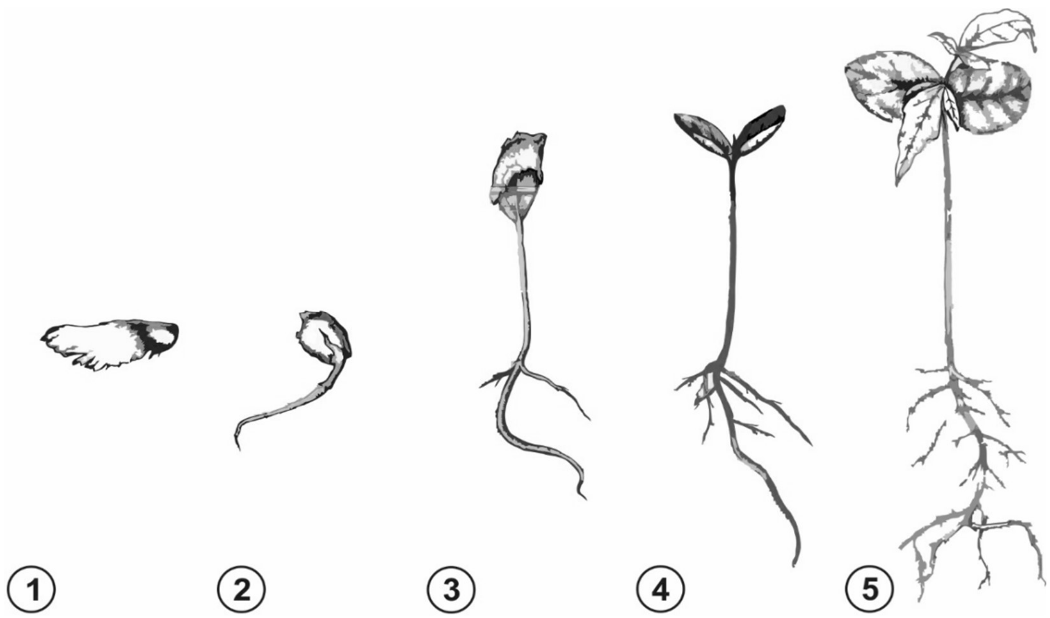

Figure 1: Morphological aspects of germination and emergence of *Cedrela fissilis* seedlings: 1 - Whole seed; 2 - Seed showing the emission of primary root; 3 - Seed with approximately 2.0 cm radicle; 4 - Seed in the final stage of germination with emission of the main root and apparent cotyledons; 5 - Seedling in the emergency final stage with emission of the main root and secondary roots and eophiles. Author: Laila Carvalho.

At 92±6 days after the installation of the experiment, the morphological quality of the seedlings and vigor of the seeds was verified by evaluating the stem diameter (SD), total height (H), shoot length (SL), root length (RL), total dry biomass (TDB), root dry biomass (RDB) and shoot dry biomass (SDB). For the evaluation of dry biomass, ten plants were selected per experimental unit and submitted to drying in an oven for 24 hours at 105±2 °C until constant weight, with subsequent weighing on an analytical balance of 0.001 g precision. From the collected data, the sturdiness quotient (H/SD) was calculated, and the Dickson quality index (IQD) [13] was determined by the following formula:

$$DQI = TDB/(H/SD + SDB/RDB)$$

Where:

TDB - Total dry biomass (g);

RDB - Root dry biomass (g);

SDB – Shoot dry biomass (g);

H/SD – Sturdiness quotient.

To evaluate the morphological quality of the seedlings, a completely randomized design with four storage environments and five replications of ten plants per experimental unit was adopted. Treatment variances were evaluated for homogeneity by the Bartlett test, and the variables that showed significant differences by the F test had their means compared by Tukey's test at the 5% probability level. To verify the influence of MSET on morphological variables, Pearson's correlation analysis was applied ($p<0.01$ and $p<0.05$).

3. Results

In the initial characterization of the seed lot, carried out at the time of storage, the average weight of 11,088.6 seeds Kg^{-1} was obtained, presenting dimensions of 11.5 mm for the major axis and 7.0 mm for the minor axis. Regarding the analysis of purity, a percentage of 93.5% was obtained.

After the storage period, the highest moisture content of seeds was obtained in the control treatment, with 11.5%, followed by the treatment of humid chamber, with 8.8%, dry chamber with 8.6%, and the uncontrolled environment with 8.1%. For the percentage of seedling emergence, the highest value was obtained in the control treatment, with 71.2%, differing statistically from the other treatments, which presented values below 30% (Table **1**). Likewise, a better mean germination time (TMEP) was obtained for seeds not stored (control), with 27.51 days, with a significant increase in germination time in the other treatments, regardless of the storage condition (Table **1**). It is noteworthy that in the control treatment, the emergence of seedlings started at 20 days, while in the other treatments, the process began only at 25 days. The end of the seedling emergence process occurred at 28 days and 33 days, respectively.

Table 1: Percentage of seedlings emergence (PSE) and mean seedlings emergence time (MSET) in *Cedrela fissilis* seeds as a function of storage conditions.

Treatments	PSE (%)	MSET (days)
T-I	71.20 a	27.51 b
T-II	25.60 b	32.92 a
T-III	28.80 b	32.65 a
T-IV	16.80 c	31.44 a
Means	35.60	31.13
C.V. (%)	7.22	2.85

Means followed by the same letter in column do not differ between them by Tukey's test ($p<0.05$). T-I: Seeds without storage (control); T-II: Seeds stored in a humid chamber for 515 days; T-III: Seeds stored in a dry chamber for 515 days; T-IV: Seeds stored in an uncontrolled environment (laboratory) for 515 days. C.V. (%): Coefficient of variation.

Regarding the morphological quality of *C. fissilis* seedlings, again, the seedlings from the control treatment were the ones that showed better development, statistically superior to the others in all variables, except for SD (Table **2**). In general, progressively lower values were obtained for seeds stored in a humid chamber, dry chamber, and uncontrolled environment.

These results are reinforced by correlation analyzes between the mean seedlings emergence time and morphological variables, which showed negative values above 0.80 for all variables, except for SD, although this also showed a negative correlation (Table **3**).

4. Discussion

The average number of C. fissilis seeds per kilo obtained was lower than the values found in other studies [15 and 16], although a considerable variation regarding the dimensions of the seeds is recognized due to the different edaphoclimatic conditions in which the mother plants are found. Purity, on the other hand, was higher

[15 and 16]. Other studies reinforce this variation present in seeds from different origins, which is also repeated for the degree of moisture, which can vary from 6.7% to 13.6%, with the values found in this study within the range of values described [17].

Table 2: Stem diameter (SD), total height (H), Sturdiness quotient (H/SD), Dickson quality index (DQI), shoot length (SL), total dry biomass (TDB), root dry biomass (RDB), shoot dry biomass (SDB) for *C. fissilis* seedlings at 92±6 days as a function of seeds storage conditions.

Storage Conditions	SD	H	H/SD	DQI	SL	TDB	RDB	SDB
	mm	cm			cm	--- (g) ---		
T-I	3.80 a	27.17 a	7.14 a	1.25 a	14.69 a	10.37 a	5.11 a	5.25 a
T-II	3.68 a	20.94 b	5.68 c	0.94 b	8.06 b	6.35 b	3.14 b	3.21 b
T-III	3.43 a	20.50 bc	6.01 bc	0.89 bc	7.69 b	6.27 b	3.11 b	3.16 b
T-IV	2.92 b	18.86 c	6.44 b	0.81 c	6.60 c	6.09 b	3.04 b	3.04 b
Means	3.46	21.87	6.32	0.97	9.26	7.27	3.60	3.67
C.V. (%)	6.32	4.45	5.10	6.34	5.38	5.55	4.54	4.72

Means followed by the same letter in column do not differ between them by Tukey's test ($p<0.05$). T-I: Seeds without storage (control); T-II: Seeds stored in a humid chamber for 515 days; T-III: Seeds stored in a dry chamber for 515 days; T-IV: Seeds stored in an uncontrolled environment (laboratory) for 515 days. C.V. (%): Coefficient of variation.

Table 3: Correlation between mean seedlings emergence time (MSET) and morphological variables evaluated in *Cedrela fissilis* seedlings.

Variables	Coefficient of Correlation
MSET X SD	- 0.33 ^{ns}
MSET X H	- 0.83 **
MSET X H/SD	- 0.80 **
MSET X DQI	- 0.80 **
MSET X SL	- 0.85 **
MSET X TDB	- 0.90 **
MSET X RDB	- 0.91 **
MSET X SDB	- 0.90 **

** and * Significant at 5% and 1% probability, respectively; ^{ns} Not significant. SD: stem diameter; H: total height; H/SD: Sturdiness quotient; DQI: Dickson quality index; SL: shoot length; TDB: total dry biomass; RDB: root dry biomass; SDB: shoot dry biomass.

The higher moisture content obtained for *C. fissilis* seeds without storage, in turn, is possibly related to the higher seedling emergence and lower mean seedlings emergence time, also verified in this treatment. These results indicate high sensitivity to long-term storage, regardless of the environment, although, under uncontrolled conditions, the seeds had lower germination. It is noteworthy that the high percentages of seedling emergence in non-stored seeds (71.2%) (Table **1**) reflect the collection carried out at the appropriate physiological maturation point. After physiological maturation, the process of seeds' progressive deterioration begins, resulting in a consequent reduction in germination potential [18 and 19]. This deterioration is favored by environment humidity, which depends on air relative humidity, i.e., the higher air humidity increases the moisture content of seeds, leading to a more significant deterioration of seeds [20]. The effect of this deterioration is evident in the low PEP obtained for seeds stored in an uncontrolled environment (Table **1**).

Likewise, the higher values for PEP in seeds stored in a dry chamber (28.8%), compared to those stored in a humid chamber (25.6%), reinforce the need for environments with low relative humidity and temperature for orthodox seeds, such as *C. fissilis*, conditions in which the embryo can maintain a lower metabolic activity [21 and 22]. Furthermore, the loss of physiological quality of seeds as a consequence of storage directly reflects on their viability [23].

In addition to PEP, the increase in MSET has direct implications on seedlings' quality since the initial vigor of seedlings is lost, which is desirable for forest species seedlings production. This reduction in vigor is a consequence of storage, where seeds quality cannot be improved, only preserved, when placed in favorable conditions, such as humidity and temperature, reducing its metabolism and consequently the attack by pathogenic microorganisms [21].

The SD value is essential for evaluating forest species seedlings’ quality. There is evidence that, within a single species, plants with higher SD tend to have a higher capacity for root formation and growth in field conditions [24], becoming a key variable for evaluating the potential for survival and post-plant growth [25], besides being characterized as an easy variable to evaluate in the nursery [26].

Seed vigor is a complex physiological characteristic, essential to ensure rapid and uniform emergence of seedlings under adverse environmental conditions and depends basically on resistance to degenerative effects caused by storage [27]. The effect of *C. fissilis* seed vigor on seedlings' quality is evident when, except for SD, all morphological variables evaluated negatively influenced seeds storage (Table **2**).

The H/SD and DQI indexes indicate the quality of seedlings at the time of nurseries shipment, both of which have been influenced by the storage of *C. fissilis* seeds. The H/SD relation has relevance in determining the proportional growth of seedlings [11 and 12]. In addition to being a good reference index for reserves accumulation, it may represent a higher resistance and fixation of seedlings to the substrate [28].

Differing from H/SD, considering root system, DQI may reflect the structural quality of produced seedlings since plants with less root development or with excessive stem growth may be less resistant to stresses generated after planting, resulting in fewer seedlings survival in the field [3]. Based on this assumption, seedlings with the highest quality and potential for field survival are those produced from non-stored seeds (Table **2**), which presented greater vigor in relation to those stored (Table **1**). This hypothesis is reinforced by a negative correlation between variables MSET and H/SD and MSET and DQI (Table **3**).

Variables TDB, RDB, and SDB are also influenced by seeds storage, being higher in non-stored seeds (Table **2**). Higher values for TDB may indicate appropriate conversion of solar energy in assimilated compounds, especially carbohydrates [29], with influence not only on the stem, but in different vegetative organs, making it extremely important in efficiency and growth potential evaluation of seedlings produced in the field. The highest values for SDB are a direct relation with photosynthetically active leaf surface, constituting a significant variable to determine the survival capacity of seedlings after planting [30].

In general, results presented in this study demonstrate the importance of seeds vigor for *C. fissilis* seedlings' quality. This hypothesis is even more evident when, except for SD, all variables present a strong negative correlation with MSET, indicating a strong influence of this variable on final seedlings' quality (Table **3**).

5. Conclusion

Seeds storage for a period of 515 days proved to be a negative factor for the emergence and vigor of *C. fissilis* seedlings, regardless of the storage condition evaluated. The vigor of seeds is a preponderant factor to increase the morphological quality of produced seedlings.

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