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Germination and Viability of Seeds of *Caesalpinia pulcherrima* Newly Harvested and Stored

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Abstract

Caesalpinia pulcherrima is an exotic shrub species, belonging to the Fabaceae family, that has medicinal properties, and is widely used for urban afforestation. The objective of this research was to evaluate the overcoming of the *C. pulcherrima* seed dormancy, the influence of temperature, storage, and water quantity on the substrate in the germination of the species, as well as the use of the tetrazolium test for viability analysis. The analyzes were carried out at the Plant Propagation Laboratory at the Agricultural Sciences Center of the Federal University of Alagoas, in the municipality of Rio Largo, Brazil, and at the Laboratory of Plant Physiology, Arapiraca Campus, at the same University. The experiments were performed under a completely randomized design, with four replicates of 25 seeds. The results demonstrated that during storage the seeds developed a possible secondary dormancy, which was overcome with a time of twelve months of storage. The *C. pulcherrima* seeds subjected to the test of light qualities presented a significant difference in their germination percentage when verified with the time of storage. In the far-red quality, the newly harvested seeds had a germination percentage of (98%), higher than seeds with 12 months of storage (80.5%). The water volume 3.5-fold the weight of the dry paper provides (90%) germination when compared to other volumes. The tetrazolium salt concentrations of 0.075% and 0.1%, under the temperature of 30°C within 2 hours, are indicated for the viability analysis of *Caesalpinia pulcherrima* seeds.

Keywords: *Caesalpinia pulcherrima*, flamboyant mirim, forest seed, longevity, numbness

1. Introduction

The lack of information about seeds germination and physiological quality has been one of the main factors responsible for the inadequate formation of forest species seedlings, resulting in negative impacts in the establishment and uniformization of populations (Gasparin et al., 2013). This is due to the use of seeds of unknown quality, without any information about their germinative capacity and establishment potential.

Some species have survival mechanisms, such as seed dormancy, which results in a long time for germination. Moreover, the lack of certain information has resulted both in high costs in reforestation projects in degraded areas and in the substitution by species with lower potential (Araújo Neto et al., 2002). Seed storage is also an important factor in germination, helping to understand the propagation and consequent conservation of species (Marchiori, 2015).

The *Caesalpinia pulcherrima* (L.) (SW) forest species, known as marvel, is a woody shrub, originated in Central America, commonly found in all northeastern states of Brazil. The species has tolerance to heat and drought (Gilman & Watson, 2003). It belongs to the Fabaceae family, and it is commonly used in afforestation and living fence because of its small size and inflorescence diversity. In addition to having medicinal properties, its parts are used for febrile, infections, mouth ulcers, wounds and eye irritation treatments (Gilman & Watson, 2003).

Species from the Fabaceae family are known to present integumentary dormancy. However, studies conducted

by Gilman and Watson (2003), Oliveira et al. (2010), Araújo Neto (2014), Naverrete-Tindall (2002), and Belinni et al. (2011) have shown variation in the *C. pulcherrima* species dormancy intensity. In the face of the need of information in order to optimize this species germination process, it is essential to know its behavior against environmental conditions, which can be determinant for its success in degraded areas environments.

Among the determinant environmental conditions, temperature, light, and substrate humidity are listed, which are the determinants in the germination process. In this sense, to explore the germinative capacity, before these factors, provides knowledge of information, which allows the use and maintenance of forest species, as temperature affects the germination capacity of seeds and the rate at which it occurs. Thus, it is extremely important to know the temperature limits in which a species can germinate, these limits being known by the cardinal temperatures (maximum, minimum and optimal) (Marcos Filho, 2015).

Light is one of the external factors that affect the growth and development of plants in different ways, from germination to dormancy overcoming. The analysis of the influence of light intensity contributes to the knowledge and classification of the species as for its photoblastism, so it is possible to analyze if the species has properties to germinate from the forest edge at full sun to its inside (Rossatto & Kolb, 2010).

Besides the knowledge of germination conditioning factors, the evaluation of the seeds physiological quality is of fundamental importance for the knowledge of their physical and physiological attributes. In this sense, one of the tests that can be used to provide faster results is the tetrazolium test, which allows the observation of the staining of living tissues, indicating the seed viability (Oliveira, 2016).

Thus, the objective of this study was to evaluate the germination capacity of *Caesalpinia pulcherrima* species seeds under temperatures, pre-germination treatments, storage, water quantity in the substrate and verification of seed viability.

2. Material and Methods

2.1 Place of Conducting Work

The tests were carried out at the Plant Propagation Laboratory, at the Agricultural Sciences Center (CECA), Federal University of Alagoas, in the municipality of Rio Largo-AL (09°28′S, 35°49′W and 127 m), and at the Plant Physiology Laboratory, Arapiraca Campus, at the same University, in Arapiraca-AL (09°42′S, 36°41′W and 264 m altitude).

The seeds were harvested from *Caesalpinia pulcherrima* plants located in the Arapiraca region (09°74′S, 36°65′W, 260 m altitude). At the laboratory, the newly harvested seeds were taken to a forced circulation oven to determine the initial water content using the methodology described by the Rules for Seed Analysis (Brasil, 2009). After this stage, the seeds were stored in a dry chamber at 22 °C, with 60% relative humidity until the beginning of the tests.

2.2 Pre-germinative Treatments

For the beginning of the tests, all the seeds were subjected to asepsis, with the immersion of the seeds in alcohol (70%) (Rocha et al., 2008) for one minute, then washed with distilled water.

In the first test, two seed batches were used: one batch composed of newly harvested seeds and the other composed of seeds stored for one year in a regulated dry chamber (22 °C and 60% UR). These seeds were subjected to pre-germination treatments: intact seeds (control) and mechanical scarification with the use of number 80 sandpaper in the region opposite the hilum (Oliveira et al., 2010). The seeds were placed in plastic boxes (1 × 11 × 3.5 cm) followed by soaking, with twice the weight of the dry substrate, according to the Seed Analysis Rules (Brasil, 2009), and placed in germination chambers under temperatures of 30 and 20-30 °C.

The germination evaluation was performed daily, being considered as germinated the seeds that presented primary root with 2 mm in length (Carreira & Zaidan, 2007). At the end of the test, which lasted 15 days, the percentage of germination and germination velocity index (IVG) was determined by the sum of the number of seeds with primary root protrusion (G1, G2, G3, ... Gn) each day, divided by the number of days elapsed (N1, N2, N3 ... Nn) between sowing and germination, according to the formula described by Maguire (1962):

$$IVG = G1/N1 + G2/N2 + G3/N3 + ... + Gn/Nn \quad (1)$$

The Germina Quant 1.0 software (Marques et al., 2015) was used in this test to obtain the synchrony and uncertainty variables (Laboriau & Valadares, 1976).

The results for the relative frequency (Fr) were determined according to Labouriau and Valadares (1976).

For the statistical analysis, a completely randomized design was used in a factorial scheme (2 × 2 × 3) (two

temperatures, two pre-germination treatments, three storage periods: freshly harvested seeds with six and 12 months of storage). The Sisvar software was used to obtain the variables.

2.3 Light Quality

In the second experiment, the physiological behavior of the seeds was verified according to temperature, light quality, and storage periods. For this, the seeds were placed in plastic boxes, then soaked with 2.5-fold the weight of the dry substrate (Brasil, 2009) and incubated at temperatures of 30 °C and alternated at 20-30 °C under white LED light (5,000/10000K) red (630nm), far-red (670/730 nm) and dark. The dark condition was obtained by the use of gerbox painted with black paint. For treatments in wavelengths at red, far-red bands and absence of light, the asepsis, sowing and daily germination evaluation procedures were performed in a dark camera under a safety green light.

The design was completely randomized in a factorial scheme (4 × 2 × 2) (white, red, extreme red light, the absence of light, two temperatures, and two storages) with four replicates of 25 seeds. The results were subjected to analysis of variance, and the averages were compared by the Tukey test at 5% probability.

2.4 Substrate Water Volume

In the third experiment, seed germination and the germination speed index were analyzed according to the water content to be made available on the germination substrate, that is, newly harvested seeds were placed on the substrate germitest paper moistened with amounts of water equivalent to 1.5, 2.0, 2.5, 3.0 and 3.5-fold the dry paper weight and kept in germination chambers at temperatures of 30 and 20-30 °C (Amaro, 2014).

In this experiment, the trial design was a completely randomized design, in a 5 × 2 factorial scheme (five water volumes and two temperatures), using four replicates of 25 seeds per treatment. The effects of the water volumes were studied by regression analysis, choosing the appropriate models to represent them according to their biological behavior, the coefficients significance, the model and the value of the determination coefficient (R^2) and the effects of temperature were studied by the Tukey test at 5% significance.

2.5 Tetrazolium Test

For the tetrazolium test, the *Caesalpinia pulcherrima* seeds were placed in plastic boxes, followed by soaking and placed in germination chambers under the temperature of 30 °C for 48 hours. Then, with the aid of a scalpel, longitudinal sections were cut in the seeds, obtaining the endosperm portion containing the embryo which was immersed in plastic cups containing 50 ml of a triphenyl tetrazolium chloride solution of 2, 3, 5 at four concentrations of 0.075, 0.1, 0.5 and 1.0%, for three staining periods (2, 4 and 6 hours) in a chamber set at 30 °C in the dark. Four replicates of 25 seeds were used for each concentration, according to Oliveira (2016), with modifications.

3. Results

3.1 Pre-germinative Treatments

The interaction between the pre-germination treatments, storage and temperatures were statistically significant for the germination percentage (Table 1).

Table 1. Percentage of seed germination of *Caesalpinia pulcherrima*, submitted to two temperature regimes, storage and pre-germination treatments.

Pre-germinative Treatments	Storage (months)					
	0		6		12	
	Temperature °C					
	30	20-30	30	20-30	30	20-30
Scarified	91aAa	89aAa	42aBa	31aBa	85aAa	88aAa
Intact	89aAa	80aAa	1.0bBb	20aBb	76aAb	90aAa
Value of "F" for interaction (A × G × T)	3.883*					
CV (%)	15.00					

Note. Surgery the same lowercase letter (for pre-germinative treatments and temperature) in the line and uppercase in the column (to storage) is not stand by the boat of Tukey, 5% probability.

* Significant by Tukey test at 5% probability.

3.2 Pre-germinative Treatments IVG

There was a significant interaction between factors, temperature, pre-germination treatments, and storage for the seed germination rate index (Table 2).

Table 2. Rate of germination rate of *Caesalpinia pulcherrima* submitted to two temperatures, storage and pre-germination treatments

Pre-germination Treatments	Storage (months)					
	0		6		12	
	Temperature °C					
	30	20-30	30	20-30	30	20-30
Scarified	1.21bBb	1.51bBb	1.49bBb	1.31bBb	1.50bBb	1.42bBb
Intact	1.39bBb	1.57bBb	0.79aAa	1.30bBb	1.19aBb	1.43bBb
Value of "F" for interaction (A × Q × T)	6.549*					
CV (%)	13.08					

Note. *Significant by the Tukey test at 5% probability.

Means followed by the same lower-case letter in the row (for pre-germination treatments and temperature) and upper case (for storage) in the column do not differ by Tukey test at 5% probability.

3.3 Pre-germinative Treatments Uncertainty of Germination

The interaction between storage temperature and pre-germination treatments had an influence on the *C. pulcherrima* germination synchronicity and uncertainty, as a consequence of the conditions imposed on the

seeds germination. Asynchronicity was recorded when the germination of the intact seeds and the seeds with scarification were analyzed (Table 3).

Table 3. Uncertainty of germination *of Caesalpinia pulcherrima* seeds submitted to two temperatures, storage and pre-germination treatments

Pre-germinative Treatments	Storage (months)					
	0		6		12	
	Temperature °C					
	30	20-30	30	20-30	30	20-30
Scarified	2.58aAa	2.11aAb	1.56aBa	1.31aBa	2.22aAa	2.05aAa
Intact	2.68aAa	2.17aAb	0.70bCb	1.22aBa	2.16aAa	2.28aAa
Value of "F" for interaction (A × G × T)	3.923*					
CV (%)	10.66					

Note. * Significant by Tukey test at 5% probability.

Means followed by the same lowercase letter in the row (for pre-germinative treatments and temperature) and upper case in the column (for storage) do not differ from each other by the Tukey test at 5% probability.

3.4 Pre-germinative Treatments Length of Caesalpinia Pulcherrima Seedlings

Based on the seedlings total length values (Table 4), it was possible to identify that seeds with six months of storage presented smaller lengths when compared to six-month storage newly harvested seedlings. This decrease in the six-month storage intact seedling growth shows the induced dormancy that this storage time provided. In the scarified seeds, these lower averages can be explained by a small decrease in the germination percentage that occurred in this treatment, when compared to the others (Table 4).

Table 4. Length of *Caesalpinia pulcherrima* seedlings, submitted to pre-germinating temperatures and storage

Storage (months)		
0	6	12
1.309a	0.836 b	1.159 a
Value of "F" for storage (A)	12.85*	
CV (%)	24.46	

Note. * Significant by Tukey test at 5% probability .Means followed by the same lowercase letter in the row (for pre-germinative treatments and temperatures) and upper case in the column (for storage) do not differ from each other by the Tukey test at 5% probability.

3.5 Pre-germinative Treatments Dry Mass of Caesalpinia Pulcherrima Seedlings

The interaction between the factors was not significant in the total accumulation of the dry matter mass. However, storage influenced the values, showing higher averages in the newly harvested seeds and with twelve months of storage (Table 5), probably due to induced dormancy, which may have caused a delay in the seeds imbibition process and, consequently, the germination decreased.

Table 5. Dry mass of *Caesalpinia pulcherrima* seedlings, submitted to different storage conditions

Light Qualities	Storage (months)	
	0	12
White	90.0aA	86.5aA
Red	98.0aA	92.5aA
Extreme red	98.0aA	80.5bA
No light	80.aB	82.0aA
Value of "F" (A × luz)	3.410*	
CV (%)	10.26	

Note. * Significant by Tukey test at 5% probability.

Averages followed by the same lowercase letter in the row and upper case in the column do not differ from each other by the Tukey Test at 5% probability.

3.6 Light Quality

The *C. pulcherrima* seeds showed a light-insensitive behavior, considering that the germinability occurred in all the photoblastic treatments evaluated, being considered neutral photoblastic (Table 6).

Table 6. Percentage of germination of *Caesalpinia pulcherrima* seeds, submitted to different light qualities, and storage

Pre-germinative Treatments	Storage (months)					
	0		6		12	
	Temperature °C					
	30	20-30	30	20-30	30	20-30
Scarified	9.71aAa	9.69aAa	8.90aBa	7.33aBb	10.57aAa	10.43aAa
Intac	9.42aAa	9.85aAa	0.00bBb	6.64aBa	9.93aAa	9.88aAa
Value of "F" for interaction (A × Q × T)	6.549*					
CV (%)	13.08					

Note. * Significant by Tukey test at 5% probability.

Averages followed by the same lowercase letter in the row and upper case in the column do not differ from each other by the Tukey Test at 5% probability.

3.7 IVG Quality of Light

As for the germination speed index, the interaction between storage, temperature and light factors was not significant, only storage and light qualities had an influence on the *Caesalpinia pulcherrima* seeds IVG (Table 7).

Table 7. Rate of seed germination rate of *Caesalpinia pulcherrima*, submitted to different light qualities, storage types and temperature

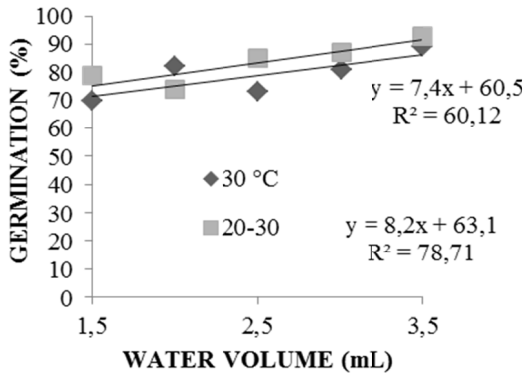
Light Qualities	Storage (months)	
	0	12
White	2.35aA	2.14bA
Red	1.22bB	1.53aB
Extreme red	1.22aB	1.24aC
No light	2.30aA	2.28aA
Value of "F" (Storage × temperature)	0.158	
Value of "F" (Storage × light)	5.763*	
CV (%)	10.08	

Note. * Significant by Tukey test at 5% probability.

Averages followed by the same lowercase letter in the row and upper case in the column do not differ from each other by the Tukey Test at 5% probability.

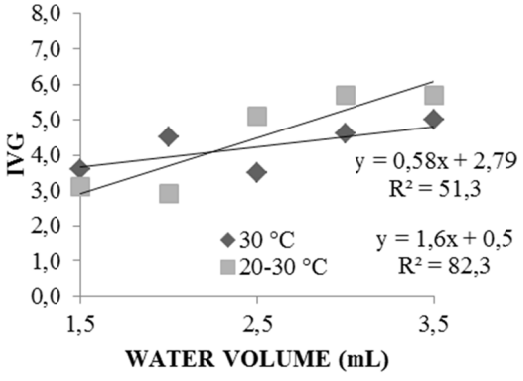
3.8 Substrate Water Volume

As for substrate moisture, it was verified that the interaction between water volume in the substrate and temperature influences the *C. pulcherrima* seeds germination (Figure 1A).



1(A)

Figure 1(A). Percentage of germination of *Caesalpiniapulcherrima* submitted to five volumes of distilled water in the germination substrate and at two temperatures



1(B)

Figure 1(B). Rate of seed germination rate of *Caesalpinia pulcherrima* submitted to water volumes and at two temperatures

3.9 Tetrazolium Test

The results for the percentage of viable seeds by the tetrazolium test at different concentrations, time of exposure and germination test, conducted at 30 °C in the *C. pulcherrima* seeds are shown in Table 8. It was observed that the interaction between the staining period and the concentrations of the tetrazolium salt solution were statistically significant. The concentrations of 0.075% (92% of viable seeds) and 0.1% (91% of viable seeds) allowed to verify greater viability in the period of two and four hours of staining (Table 8), providing estimates similar to germination tests (91%).

Table 8. Viability of *Caesalpinia pulcherrima* seeds obtained by the germination test

Coloring periods (hours)	Concentrations of tetrazolium solution (%)			
	0.075	0.1	0.5	1.0
2	92 aAz	91 aAz	11 bBy	10 bBy
4	92 aAz	92 aAz	13 bAy	11 cAy
6	23 bBy	54 aBy	0cCy	0cCy
	Germination = 91 z			
Value of "F" for periods (P)	418.00**			
Value of "F" for concentrations (C)	1347.22**			
Value of "F" for interaction (P × C)	75.73**			
Value of "F" for additional vs factorial	702.76**			
Value of "F" for treatments	502.84**			
CV (%)	8.17			

Note. Means followed by the same uppercase letters (A, B, C) in the row and Lower case letters (a, b, c) in the column do not differ significantly at 5% probability.

Means followed by the same letter (z, y) between germination (control-germination test) and viability obtained in the tetrazolium test did not differ significantly from the 5% probability by the Dunnett test.

3.9.1 Tetrazolium Test

The staining patterns were observed through the tetrazolium test in *Caesalpinia p.* seeds (Figure 2). The seeds that presented pink color throughout the length indicate viable seeds (Figures 2A, 2B and 2C), demonstrating the tissues vigor. However, seeds exposed to the tetrazolium solution showed intense red staining (Figure 2D), indicating tissue deterioration, while milk-white color (Figure 2E) showed non-viability (dead tissue).

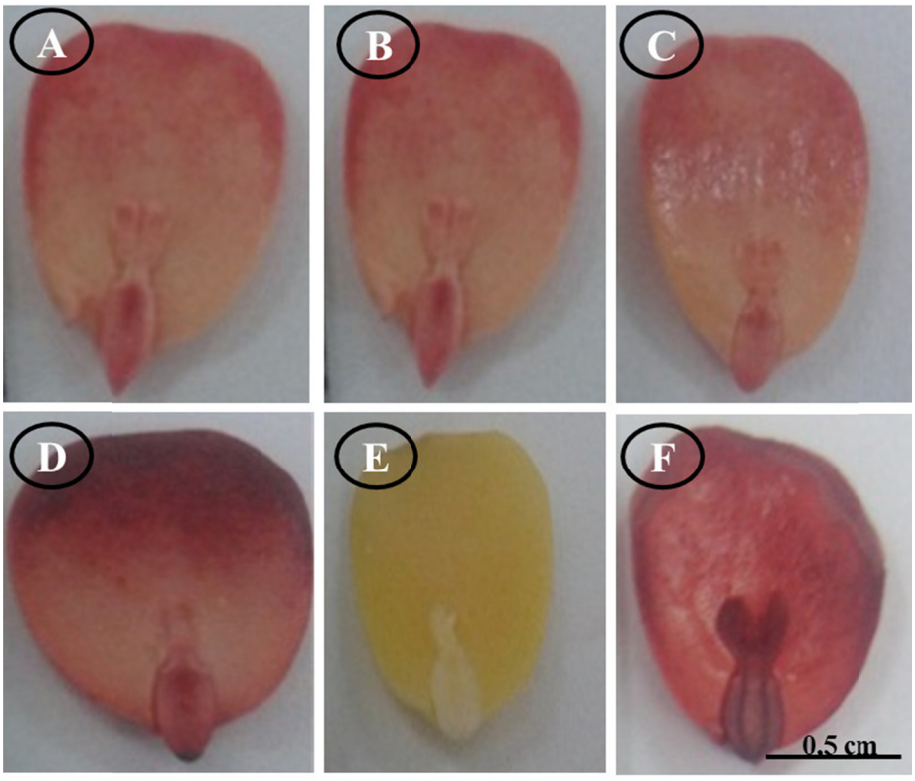


Figure 2. Viable seeds of *Caesalpinia pulcherrima* embryo with light pink color (A, B, C); hypocotyl-radicle seeds with intense red coloration in the cortex but not reaching the central cylinder (D). Inviable seeds: embryo with discolored/milky white areas (E); embryo with intense red color throughout its extension or hypocotyl-radicle axis with intense red color reaching the central cylinder (F)

4. Discussion

The *Caesalpinia pulcherrima* seeds presented a peculiar behavior, where the dormancy-breaking method did not influence the germination of the newly harvested seeds, that is, they did not present dormancy, having a germination percentage above 80% in the intact seeds (Table 1). However, seeds with six-month storage demonstrated an inhibition of the germinability potential with a percentage of 1% of germination at 30 °C. This loss is surpassed by seeds with twelve-month storage, where the percentage increases again, reaching 90% of germination. This behavior can be related to a possible dormancy induced in the seeds with six months of storage, which is surpassed by the twelve-month storage.

This behavior can also be justified by the intensity dormancy variation among seeds of the same species.

It is believed that this difference in the *Caesalpinia pulcherrima* seed behavior may be related to the climatic conditions during the seed maturation stage, seed genetics, drying conditions, or environmental variations under which the seeds were collected (Guedes et al., 2015). Probably there are individual variations within the same species due to environmental influences during seed development and genetic variability (Turnbull, 1975; Santos et al., 2009).

The influence of the pre-germination treatments between intact seeds and those subjected to mechanical scarification at the alternating temperature of 20-30 ºC (Table 1) in the three storages was not verified, which shows that the alternating temperatures resemble temperatures from the natural environment, and in this way, it can act as a dormancy-breaking method. According to Copeland and McDonald (1995), some species show better behavior in the germination process when subjected to temperature alternation.

Naverrete-Tindall (2002), and Belinni et al. (2011) mention that this species does not require pre-germination treatments and that intact, freshly harvested seeds reach 90 to 100% germination. However, studies by Gilman and Watson (2003), Oliveira et al. (2010) Araújo Neto (2014), Jozef et al. (2010), and Jayasuriya et al. (2013). found that the species presented dormancy and that the mechanical scarification method was efficient to overcome it, since the seeds subjected to this method had a higher germination percentage than those from the control treatment. This suggests that environmental conditions, in addition to storage conditions after the harvest period, may cause changes in their metabolism, since the seeds were collected in different regions as well as different climate, this being a sign for a possible response to a certain species behavior.

In the six-month storage period, the scarified seeds presented germination speed index higher than the intact seeds at 30 °C. At the alternating temperature 20-30 °C, there was no statistical difference between scarified and intact seeds regarding the germination speed (Table 2).

Data from this research show that the 30 °C temperature, along with storage time, decreases the *Caesalpinia pulcherrima* seed germination rate, probably due to dormancy induction. However, the newly harvested seeds had higher IVG than seeds with six and twelve months of storage at 30 °C (Table 2).

Results from Araújo Neto (2014) verified that the 30 °C temperature provides higher IVG in the *C. pulcherrima* seeds when compared to the temperature of 25 °C. Oliveira et al. (2010) studying dormancy breaking methods in the same species, observed that the favorable condition for germination and IVG is under the temperature of 30 °C. Novembre et al. (1999) reported that *Mimosa caesalpinia efolia* seeds presented higher germination speed at 30 °C, and Fernández and Jimeénez (2014).

It is noted that intact seeds with six months of storage, under the temperature of 30 °C, indicate lower U values (Table 3). These values, however, did not induce greater synchrony of the germination process, under these conditions, but they can be related to the germination percentage, lower than other treatments.

In contrast, the alternating temperature 20-30 and the temperature of 30 °C in freshly harvested seeds and seeds with 12 months of storage did not differ statistically, resulting in a unimodal behavior, which may suggest that these conditions may develop a physiological similarity in the seeds, causing uniformity in the germination. Dorneles, Ranal, and Santana (2013) declare that the lower asynchronicity in *Anadenanthera colubrina* germination and emergence values derive from the greater stability of thermal and humidity conditions of the germination environment.

Data from Rossatto and Kolb (2010) verified that lower temperatures caused loss of synchrony in the *Pyrostegia venustae* germination; greater synchrony in the optimal temperature between 30 and 35 °C. *Melanoxylon braunna* seeds showed higher synchronization in germination at temperatures of 25, 30 and 35 °C (Flores et al., 2014). Therefore, the temperature is a determining factor in the germination process, which may affect germination uniformity (Carvalho & Nakagawa, 2012).

This decrease in the six-month storage intact seedling growth shows the induced dormancy that this storage time provided. In the scarified seeds, these lower averages can be explained by a small decrease in the germination percentage that occurred in this treatment, when compared to the others.

Guedes et al. (2015) verified significant differences in the *Amburana cearenses* seeds germination and seedling growth, collected during the same period and kept under the same storage conditions. J. R. O. Silva, Albuquerque, and I. C. O. Silva (2014) observed a decrease in *Parkia pendula* root growth with four months of storage.

These data corroborate with those of J. R. O. Silva, Albuquerque, and I. C. O. Silva (2014), where they verified that the *Parkia pendula* seeds showed an increase of the dry matter mass according to the storage time.

The inclusion of a seed in the classification of light sensitive/insensitive depends on the conditions of maturation, storage, imbibition and incubation temperature and osmotic treatment Ladeira et al. (1987), El-Keblawy et al. (2018). The occurrence of the germination process in all light qualities may be related to the capacity in which the *C. pulcherrima* seeds have to germinate from the forest edges at full sun to its inside, under the canopy (Rossatto & Kolb, 2010). These data corroborate with data observed by Holanda et al. (*Mimosa caesalpiniifolia* Benth.), which germinated in any light environment, considered to be neutral photoblastic.

The influence of light on plant germination is mediated by phytochrome which corresponds to a class of photoreceptor pigments. These pigments' way of action depends on the means of incident radiation. The photosensitive seeds' germination derives from the active means of phytochrome that is caused by light with a high red/far-red ratio Takaki (2001), Benech-Arnold et al. (2000). Thus, seeds that present light insensitivity, such as the *Caesalpinia pulcherrima* species, must possess enough active means to induce germination in the absence of light, in the fiA way, which controls through a very low creep response (Menezes, 2004).

The temperature had no influence on germination in this experiment. The interaction between storage time and light quality was significant for germination, showing that the newly harvested seeds had a germination percentage (98%) higher than seeds with twelve months of storage (80.5%) under far-red light (Table 6). With these results, it can be considered that this light quality, along with storage time, influenced this species' the germination.

Although there was germination in all light qualities, it was observed that white, red and far-red light statistically provided higher results than the germination percentage found in the absence of light in the newly harvested seeds. However, this difference is non-existent in the seeds with 12 months of storage (Table 6). This may induce that the newly harvested seeds present a photodormency caused by the absence of light, which is overcome after storage.

In this sense, it can be considered that for the *C. pulcherrima* seeds the pre-existing phytochrome B no longer acts in the germination process during storage. There is, from this moment on, the action of type A phytochrome, which is, according to Takaki (2001), the type of phytochrome that acts on seeds of the neutral photoblastic type.

According to Marcos Filho (2015), the influence of light diminishes as the seeds age, revealing a natural alternative for survival, given that over time the deterioration phenomenon is irreversible and unavoidable.

Different from other studies on forest species, such as the *Mimosa scabrella*, *Chorisias peciosa*, *Tabebuia avellanedae* and *Esenbeckia leiocarpa*, where seed germination is higher in the dark, decreasing in the following order: red, blue, white and far-red light (Dias et al., 1992).

Data from Ferraz-Grande Takaki (2006) pointed out that *Caesalpinia* peltofhoroides seeds did not present photosensitivity, with no variation in the germinability percentage in the presence or absence of light, as well as in other photoblastic treatments.

In white light, the freshly harvested seeds had IVG superior to the seeds with 12 months of storage, demonstrating that the presence of white light and storage may reduce seed vigor.

Nogueira et al. (2014) observed that the *Dalbergia cearensis* seeds IVG showed no significant statistical difference between light and dark, stating that the *Dalbergia cearensis* seeds germination speed seems to be more influenced by temperature than by light quality.

The results were linearly presented, with the percentage above 70% in the volume of 1.5-fold the dry substrate weight, showing linear growth with volume increase 2.0, 2.5, 3.0, reaching more than 90% in the volume of 3.5-fold the weight of the dry substrate. Vicente Noronha and Silberschmidt (1969) observed that the most favorable germination might not be induced only by one volume, but by several volumes, demonstrating the amplitude in the moistening range of the species. These results are in agreement with data from Ramos; Varela;

and Melo (2006), who verified that the *Ochroma pyramidale* species had high germination index in all water volumes. In addition, it was necessary to add 3.0-fold the weight of dry substrate in order to obtain maximum germination (Amaro et al., 2014). Varela et al. (2005) studying the forest species from the Amazon states that the Angelim-stone (*Dinizia excelsa*) germination is not influenced by the amount of water in the substrate neither by the temperature.

The forest species *Schizolobium amazonicum* (paricá) presented better results in the volumes of 2.5 and 3.0-fold the dry substrate weight, with a percentage of 85% germination at 30 °C (Ramos, Varela, & Melo, 2006).

In the results of the germination speed index (IVG) the interaction between temperature and water volumes was significant, presenting linear growth as the water volume increased (Figure 1B).

The temperature of 30 °C provided higher seed germination speed in the volume of 1.5-fold the substrate weight (Figure 1B). The volume of water was 3.5-fold the weight of the dried substrate, which allowed a better germination speed, both at 30 °C and 20-30 °C. However, the volume of 1.5-fold the dry paper weight at the alternating temperature shows lower IVG when compared to other volumes. Thus, volumes larger than 2.0-fold the substrate weight are indicated for germination tests of this species at alternate temperature.

The water volume did not influence the *Ochroma pyramidale* seed germination speed index, although, higher temperatures favored the process (Ramos, Varela, & Melo, 2006).

Paricá seeds presented similar behavior with germination speed indexes of 5.3 and 5.8 in the volumes of 2.5 and 3.0, respectively, at 30 °C (Ramos, Varela, & Melo, 2006). Varela et al. (2004) state that seed germination rate of *D. excelsa* was neither influenced by the water volumes in the substrate nor the temperatures.

Analyzing the relative frequency in different volumes of water in the substrate, it was observed that at 20-30 °C the germination has a unimodal character with germination peaks in the volumes of 1.5 and 2.0-fold the substrate weight. Nevertheless, in the volumes of 1.5, 2.0, 2.5, 3.0 and 3.5 at 30 °C the seeds germinated in a shorter time, as well as in the volumes of 2.5, 3.0 and 3.5 at alternating temperature, resulting in a polymodal distribution aspect (data not shown).

This way, the use of these concentrations in the two-hour period, at 30 °C is suggested for this species, for laboratory viability analyzes since it allows one to obtain results faster than germination tests. Studies with Fabaceae found that lower concentrations allow more appropriate viability results to be verified with germination tests than with higher tetrazolium salt concentrations, as it is with *Pterodon pubescens* (Ferreira et al., 2001), *Senna multijuga* and *Senna macranthera* seeds (Ferreira et al., 2004).

These results are consistent with Oliveira's (2016) claims that *Simira gardneriana* seeds present higher viability averages as concentrations increase, at temperatures of 30 °C, but not in the two nor in the four-hour periods. The concentration of 0.075% in the period of two hours also allowed better results in brazilwood (*Caesalpinia echinata* Lam.) Seeds (Lamarca, Leduc, &, Barbedo, 2009).

The main characteristic after seed exposure to tetrazolium solution is the difference in tissue staining, which allows interpreting test results (Oliveira et al., 2016). The color intensity of the seeds after the tetrazolium test varies among species. As well as in the exposure period, in forest seeds, there are variations to perform the tests and staining evaluations. For *Erythrina velutina Willd* seeds, 6 hours of imbibition with 3 hours of immersion at a concentration of 0.075% tetrazolium salt is the ideal time for conducting the tetrazolium test (Cunha & Gomes, 2015).

Thus, the concentrations of 0.075% and 0.1% tetrazolium salt solution for 2 hours, at 30 °C, are indicated to analyze the viability of *Caesalpinia pulcherrima* seeds in the tetrazolium test.

5. Conclusions

Mechanical scarification with sandpaper (No. 80) did not influence the germination of *Caesalpinia pulcherrima*.

C. pulcherra seeds are neutral photoblastic.

Moistening the substrate in water volumes of 3.5-fold the dry substrate weight gives better results for the species.

Tetrazolium salt concentrations of 0.075% and 0.1% at 30 °C for 2 hours are efficient for seed viability analysis.

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