Seed analysis of *Lupinus albescens* Hook. & Arn.[1]

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ABSTRACT - The objective of this study was to describe the dispersion, color, size and morphological characteristics of fruits and seeds of *Lupinus albescens*, as well as pre-germinative treatments, aspects of luminosity and seed conservation. Above twenty populations were collected in São Francisco de Assis/RS and Alegrete/RS, pre-germinative treatments (seven pre-germinative methods, scarification times and temperatures) were analyzed; photoblastic test (seeds scarified between sandpapers, submitted to 25ºC); imbibition curve (control and scarification treatments between sandpapers); seeds conservation (cold room in plastic bag and natural environment in paper bag). Evaluations: description of fruits, seeds and viability analysis, using a completely randomized design. The results showed dispersion by autocoria, brown coloration of fruits and seeds and some striped seeds. Superior pre-germinative treatment was between sandpapers, for 40 seconds and under 25ºC. Seeds are classified as neutral photoblasts and without dormancy. Natural environment and paper bag were suitable for storage.

Keywords: native species, Pampa, seed physiology

RESUMO - **Análise de sementes de *Lupinus albescens* Hook. & Arn.** O objetivo do estudo foi descrever dispersão, coloração, tamanho e características morfológicas dos frutos e sementes de *Lupinus albescens*, além de tratamentos pré-germinativos, aspectos de luminosidade e conservação das sementes. Acima de vinte populações foram coletadas em São Francisco de Assis/RS e Alegrete/RS, analisaram-se tratamentos pré-germinativos (sete métodos pré-germinativos, tempos de escarificação e temperaturas); teste fotoblástico (sementes escarificadas entre lixas, submetidas a 25ºC); curva de embebição (tratamentos controle e escarificação entre lixas); conservação das sementes (câmara fria em saco plástico e ambiente natural em saco de papel). Avaliações: descrição de frutos, sementes e análises de viabilidade, utilizando um delineamento inteiramente casualizado. Os resultados demonstraram dispersão por autocoria, coloração marrom de frutos e sementes e algumas sementes listradas. Tratamento pré-germinativo superior foi entre lixas, durante 40 segundos e sob 25ºC. Sementes classificam-se como fotoblásticas neutras e sem dormência. Ambiente natural e saco de papel foram adequados para o armazenamento.

Palavras-chave: espécie nativa, Pampa, fisiologia de sementes

INTRODUCTION

There are records of 766 genera of the Fabaceae family in the Brazilian flora. Among them, *Lupinus* stands out (Byng *et al.* 2016). It is represented in Rio Grande do Sul state (RS) by thirteen species with natural occurrence widely distributed (Iganci & Miotto 2016). Species of this genus have important environmental characteristics, mainly because they grow under adverse conditions in areas of dry soils and low fertility (Elbandy & Rho 2014, Martínez-Alcalá *et al.* 2010). In general, they are plants living in sunny open places for they have intolerance to shading. In forested areas, they grow in clearings, especially as a pioneer plant in recently disturbed soils (Gladstones 1998). Among such species, we can highlight *Lupinus albescens* Hook. & Arn., which is found only in the Litoral,

Campanha and Missões regions of the RS state in Brazil (Freitas *et al.* 2010). It is an herbaceous species with an erect growth, and it develops in habitats with a high solar incidence. It has adaptability to sandy texture soils and can be found even in coastal dunes (Pinheiro & Miotto 2001). For this reason, this species presents favorable characteristics for the recompositing of degraded areas, becoming an important indicator of soil fertility recovery (Pillar *et al.* 2009).

The beginning of the germination process of *L. albescens* may last between 10 and 20 days in the natural environment, which results in the formation of uneven stands (Cremonez *et al.* 2013). Due such unevenness, there is a need for scientific studies aiming to accelerate its germination process. Such studies should address seed morphological and physiological aspects. Thereby, the

description of seeds morphological and physiological aspects is extremely important for the interpretation and standardization of the analyzes (Abud *et al.* 2010), and also for the integration of the feasibility studies, which make possible the understanding of the germination or deterioration processes of the seeds.

The germination of a viable seed occurs only in suitable environmental conditions. The main ones are humidity, temperature, oxygen and occasionally light (Carvalho & Nakagawa 2012). The germination test is the main parameter for the evaluation of seed physiological quality, where ideal conditions can be simulated, allowing an understanding of germinability of lots and/or accessions (Carvalho & Nakagawa 2012, Marcos Filho 2015). Thereby, studies related to seed analysis have drawn attention of the scientific environment since they allow obtaining information to safely assess seed physiological quality (Felippi *et al.* 2012b). Thus, the objective of this study is to describe the dispersion, color, size and morphological characteristics of fruits and seeds of *L. albescens*, as well as pre-germination treatments, aspects of luminosity and seed conservation.

METHODS

The experiment began in December 2015 with the collection of *L. albescens* fruits *in situ* from mother plants of three different populations. The first population was found on a road slope near a sand focus in the São Francisco de Assis, RS (29°35'02''S, 55°21'49''W) (pop. 1). The second population was found on a road slope in front of the bridge Cerro do Tigre (29°39'56''S, 55°23'31''W) (pop. 2). The third population is located in a sandy area in the Fazenda Cerro do Tigre (29°39'29''S, 55°24'02''W) (pop. 3). The populations 2 and 3 were found in Alegrete, RS, 1.5km apart in a straight line. After the collection, the fruits were taken to the Biotechnology Laboratory of the Department of Horticulture and Forestry of the Federal University of Rio Grande do Sul, Porto Alegre, RS. The fruits were arranged on greenhouse benches for drying and final dehiscence. Then, the beneficiation process was carried out by manually collecting seeds, which the homogenization in lots occurred according to place of origin.

Fruit analysis

The fruit analysis was performed before beneficiation, by means of four replicates of 50 fruits, which were randomly collected from each of the three sampled populations. The characteristics evaluated were total number of *loci* per fruit, fruit size (cm), number of intact seeds, number of seeds damaged by insects, number of malformed seeds (deformities in their shape), number of empty *loci* and number of viviparous seeds. Therefore, after the beneficiation process, the variables below were evaluated.

Seed size

In each population collected, four replicates of 50 seeds were selected for dimension analysis (length, width and thickness) and measuring using a digital caliper (mm).

Seed water content and thousand seeds weight

On the day after the collection, were used samples from all three populations for tests. To evaluation of water content, three replicates of 0.5g of seeds were selected using the percentage difference method after drying in an oven at 105ºC ± 3ºC until constant weight (Brazil 2009). The thousand seeds weight was evaluated using eight replicates of 100 seeds according to Brazil (2009) and the final result was calculated for number of seeds in 1.0kg.

In some of the following works, only pop. 2 seeds were used due to the results found in experiments 1 and 2 of the pre-germination treatments.

Imbibition curve

The seeds of pop. 2 were submitted to the treatments control (without pre-germination treatment) and mechanical scarification between sandpapers nº120 for 40 seconds. After the treated seeds were stacked at a constant temperature environment (25ºC) and a photoperiod of 16 hours inside a Biochemical Oxygen Demand (BOD) incubator.

During imbibition curve monitoring, the seeds were evaluated by successive weighing at every 2 hours in the first 12 hours, and every 12 hours in the remaining time until stabilization (156 hours). In each weighing, the seeds were removed from the gerbox boxes, placed on paper towels to remove excess water, and weighed using a precision scale (0.001g). After weighing, they were placed back in their boxes, and remained inside the controlled environment. After the last weighing, the seed water content was determined by the method mentioned above, according to Brazil (2009). The mass percentage increase, related to the initial mass for each evaluation time, was calculated using the equation below.

$$Mass\ increase\ (\%) = \frac{Current\ weight \times 100}{Dry\ weight}$$

Pre-germination treatments (PGT) – Experiment 1, 2 and 3

The pre-germinative treatments were divided into three experiments, which were conducted in 16-hour photoperiod at 2000 Lux of light intensity (fluorescent lamps) inside BOD incubation chambers.

In experiment 1, pop.2 seeds were subjected to the following treatments: control (without pre-germinative treatment), mechanical scarification between sandpapers nº 120 for 20 seconds, mechanical scarification with sandpapers nº120 on the opposite side of the embryo, immersion in water at 80ºC for 30 seconds, immersion

in water at 80°C to room temperature, immersion in 6% NaOH solution for 10 minutes and immersion in water at 60°C to room temperature.

The sanding process was carried out manually with sandpapers nº120, at defined times, with two friction movements per second, through the total length (or width) of the sandpapers, with a pressure that does not exceed the weight of the loose hand on the sandpaper's material.

After the pre-germination treatment was defined, the experiment 2 was implemented. The seeds were randomly selected from the three populations and submitted to the pre-germinative treatment of mechanical scarification between sandpapers nº120, varying the times of 0, 20 and 40 seconds. In experiments 1 and 2, the seeds were placed to germinate in a BOD incubation chamber with a constant temperature of 25°C.

In experiment 3, pop.2 seeds were submitted to the pre-germinative treatment with mechanical scarification between sandpapers nº120 for 40 seconds, being tested the temperatures of 20, 25 and 30ºC.

Photoblastic test

Pop. 2 seeds were subjected to pre-germination test with mechanical scarification between sandpapers nº120 for 40 seconds. They were sown at a constant temperature of 25ºC and a photoperiod of 16-hour under white light (2000 lux) and green light (1300 lux) inside a BOD incubator chamber. White light was provided by fluorescent lamps available in the BOD and the green light was cast by wrapping plastic gerbox boxes wrapped with green cellophane leaves. The green light was used to inhibit white light in seed germination. As performed in other studies, the green light was used to evaluate the germination process (Garcia *et al.* 2006, Pereira *et al.* 2013). A Panlux (Gossen Eletronic 2) luminometer was used to determine luminous intensity.

Seed conservation test

Seed conservation (mixing of three lots) was tested in two storage environments: 1 - cold room with temperature between 5 and 8ºC, with seeds packed in transparent polyethylene bags (capacity of 2.0 liters) filled to 1/4 of its volume and closed with a zip lock system; 2 - in a laboratory environment, monitoring weekly temperature and relative humidity using a digital thermo-hygrograph. In this environment, seeds remained in a closed Kraft paper package.

At 15, 30, 60 and 90 days from the start of storage, the following were evaluated: seed water content (as described above) and the viability of the seeds was through the tests of germination and electrical conductivity. For germination tests, seeds were subjected to pre-germination treatment with mechanical scarification between sandpapers nº120 for 40 seconds and stacked at a constant temperature of 25°C and a photoperiod of 16-hour inside a BOD incubator. To the electrical conductivity test, four replicates of 15 seeds were used. They were previously weighed and packed

in plastic cups (200 mL) containing 75 mL of deionized water. Later, electrical conductivity was measured using a digital conductivity meter at 0, 24, 48, 72, 96, 120-hour after the beginning of evaluations. Throughout the period, the seeds remained immersed into distilled water in an environment with a constant temperature (25ºC) inside a BOD incubator. After the analysis, the value of each conductivity reading was divided by the respective mass of the sample, expressing the electrical conductivity in $\mu Scm^{-1}g^{-1}$ of seeds.

For all seed viability tests, in addition to imbibition curve, four replicates of 25 seeds were used, totaling 100 seeds per treatment. Before sowing, the seeds were submitted to disinfestation by immersion into 70% (v/v) ethanol for 30 seconds, followed by 1% (v/v) sodium hypochlorite for 10 minutes. To the removal of residues from disinfestation agents, the seeds were rinsed three times in autoclaved deionized water. The sowing was performed on germitest paper, with the exception of photoblastic tests and embedding curve, for which blotting paper was used. In each repetition, two sheets of paper (previously autoclaved) were used moistened with autoclaved distilled water at the proportion of 2.5 times the dry paper mass (Brazil 2009). After sowing, the papers were rolled and packed. Photoblastic tests and the imbibition curves were conducted in transparent gerbox plastic boxes, individually packed in transparent polyethylene bags.

Seed viability evaluations consisted in the daily counting of germinated seeds and normal seedlings formed (NSF) using the protrusion of the radicle with about 2mm in length as a germination parameter. As a parameter for seedling formation were considered seedlings that presented normal shoot and root systems (Brazil 2009). At the end of the germination test (15 days), the following variables were calculated: percentage of germination (G) and seedlings formed (SF), according to the total number of normal seedlings; germination rate index (GR), according to the formula suggested by Maguire (1962); germination mean time (MTG) and seedling formation mean time (MTS), according to the formula proposed by Silva & Nakagawa (1995). In addition, measurements of root and shoot length of normal seedlings were made using a graduated ruler (mm), and the seedling total root volume was calculated by immersing the roots in a beaker containing 20mL of water, measuring the volume of water displaced. Afterwards, seedling shoots and roots were placed in Kraft paper bags and subjected to oven-drying at 65ºC until constant weight, after the dry matter weight of these structures was calculated (SDM).

Statistical analysis

The experimental design was completely randomized. After evaluations, data were submitted to tests of normality and equality of variances, followed by comparison of means with MSD test (Minimum Significant Difference) using the CoStat 6.4 software. Data on root length, seedling fresh and

dry matter referring to the second experiment of the test (pre-germination treatment) were analyzed by Generalized Linear Model using the SPSS 23.0 software. In addition, seed conservation test data were submitted to analysis of variance (ANOVA) and polynomial regression using the SigmaPlot 11.0 software. The variables that did not meet the ANOVA assumptions had their data transformed, as specified in the results tables, but the averages are in real values.

RESULTS AND DISCUSSION

Fruit analysis and seed size

In this study, we observed that *L. albescens* disperses its seeds by autochory through fruits called explosives. This also occurs with other species of the genus *Lupinus,* such as *L. albus* L., *L. angustifolius* L. and *L. arboreus* Sims. (Gustavo *et al.* 2011). In species of the family Fabaceae it is common the dispersion by autochory, because in dry and dehiscent fruits, as is the case of some vegetables, the opening of the fruit can occur by pressures exerted by the pericarp after the water loss in its maturity (Souza *et al.* 2006). In the case of *L. albescens*, it was found that its fruits abruptly open in helical form, spinning on its axis and throwing the seeds out of the mother plant, according to Van der Pijl (1982), this dispersive capacity of propagules is called an autochoric dispersion.

The species hasmature fruits and seeds are dark brown. Some seeds may have clear stripes (bursts). In the three populations of this species, the total number of *loci* showed ranged from six to eight *loci* per fruit, and the fruit size was on average 5.89cm. The variables number of whole seeds and number of seeds per fruit showed averages of 5.7 and 6.69 units, respectively. In the case of the biometric measurements of the seeds, higher values were found for

population 1, such as 5.97, 4.72 and 2.43 mm for length, width and thickness, in that order. The number of decayed, malformed, vacuous and viviparous *locus* were similar for the three populations, with a mean of 0.425, 0.945, 0.085 and 0.017 units per fruit.

Seed water content and thousand seeds weight

At the time of collection, the water content of the fruits of the three populations of *L. albescens* was 8.2, 8.48 and 9.9%, respectively, being verified the highest content for the population 3, however, the weight of one thousand seeds was higher for population 1, with 54.64 g (p<0.01). The population with the highest seed water content was not the same as that with the highest thousand seeds weight. According to Marcos Filho (2015), water content directly interferes with seed weight. However, it may vary according to the conditions of the collection site, seed age and degree of maturation. By analyzing the data concerning seed biometry, it can be observed that population 1 seeds have a greater width and thickness, and consequently a greater accumulation of dry matter, which justifies the greater 1.000 seeds weight even with a lower water content.

Imbibition curve

There was imbibition both in the control treatment and the pre-germination treatment. However, scarified seeds showed a higher and faster mass gain in the three germination phases when compared to the control (Fig. 1). Germination is composed of three phases: the phase I comprises seed water imbibition; phase II comprises metabolic processes activated for the growth of the embryo; and in the phase III the embryo begins to grow (Ferreira & Borghetti 2004). The duration of each phase may vary according to the permeability of the integument, the seed size and the conditions of the soaking process, such as
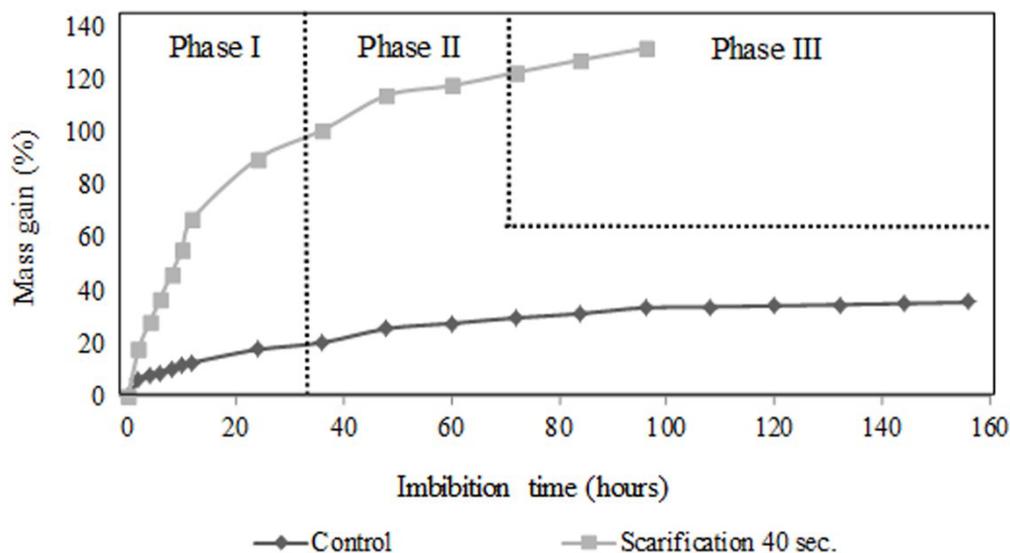

Fig. 1. Imbibition curve of the *Lupinus albescens* seeds submitted to treatments of 0 and 40 seconds of mechanical scarification time between number 120 sandpaper.

temperature and substrate (Carvalho & Nakagawa 2012). Therefore, the imbibition curve allows stating that *L. albescens* seed coats is not impermeable, only requiring a longer period for the imbibition process.

Thus, the pre-germination treatment with scarification between sandpapers contributes to foster conditions of water absorption and, consequently, the germination process. According to Shimizu *et al.* (2011), the scarification method with sandpapers causes small fractures in the seed coat, contributing to a greater permeability during imbibition. As a result, water absorption occurs faster in tissues, and consequently there is an accelerated depletion of endosperm reserves.

Studies on ideal conditions during the germination process are extremely important especially for the different responses of each species, such as viability studies and environmental conditions involving water, light, temperature and oxygen (Carvalho & Nakagawa 2012). Most species belonging to the Fabaceae family have seeds with impermeable and resistant teguments (Piña-Rodrigues *et al.* 2007), in which the presence of such barriers prevents the absorption of water and the subsequent biochemical reactions in its interior. This means an obstacle during the spread of a large number of plant species of this family. However, the results of this study allow inferring that *L. albescens* does not present integument dormancy since its seeds germinate in a period shorter than 28 days after sowing according to specific environmental conditions (Baskin & Baskin 2014).

PGT - Experiment 1

The ANOVA showed significant results among the treatments tested for the variables germination percentage and seedling formation (p>0.05). However, mean, fresh and dry mass of seedlings did not differ significantly between treatments (p<0.05).

The results found for germination percentage, seedling formation, germination série rate index and mean germination time were higher for the T3 treatment (mechanical scarification with sandpapers nº120 on the opposite side of the embryo) (Tab. 1). However, seedlings grew longer in shoot length in the T1 treatment (7.82 cm) (control: no pre-germination treatment), and in primary roots in the T6 treatment (8.25cm) (immersion in 6% NaOH solution for 10 minutes) (Tab. 1). According to the data obtained in the first experiment, we observed for all variables analyzed that the T2 treatment (scarification between sandpapers for 20 seconds), although it did not differ from T3 and T6, was more favorable due to practicality in performing the experiment and less dangerous (Tab. 1).

PGT - Experiment 2

The seeds of the population 2 presented a maximum germination (87%) in the treatment with 40 seconds of scarification between sandpapers (Tab. 2). Mechanical scarification causes cracks in the seed coat, increasing the permeability and facilitating the imbibition process (Guedes *et al.* 2013b), and therefore, the beginning of the germination process. The method of mechanical scarification with sandpapers also shows good results in promoting the germination of different species, such as *Dimorphandra mollis* Benth. (Freitas *et al.* 2009), *Schizolobium parahyba* (Vell.) Blake (Pereira *et al.* 2011), *Plathymenia foliolosa* Benth. (Lopes *et al.* 2010) and *Centrosema plumieri* Benth. (Gama *et al.* 2011).

Seeds with impermeable teguments have a low water content due to the presence of this physical barrier, which reduces the intensity of metabolism, minimizing respiratory activity and, therefore, decreasing the consumption of essential reserves for germination and initial seedling growth (Zaidan & Barbedo 2004). For percentage of seedling formation (shoot and root formed), there were high results

Table 1. Average data from germination (G) and seedling formation percentage (SF), index of germination rate (GR), shoot and root length, and mean time of germination (MTG), under seven pre-germination treatments to accelerate the germination process of *L. albescens* seeds.

Trea.	G (%)	SF (%)	GR	Shoot length (cm)	Root length (cm)	MTG (days)
T1	84bc	84 bc	11.47 b	7.82 a	4.48 d	2.2 ab
T2	90 ab	89 ab	11.22 b	5.24 ab	7.71 ab	2.3 ab
T3	99 a	99 a	15.02 a	3.7 b	6.58 c	2.02 a
T4	77 bc	78 bc	8.32 c	3.9 b	6.84 bc	2.8 c
T5	78 bc	78 bc	9.3bc	4.09 b	7.69 ab	2.5 bc
T6	90 ab	90 ab	10.9 bc	4.77 b	8.25 a	2.5 bc
T7	74 c	74 c	8.36 c	4.02 b	7.37 abc	2.5 bc
CV (%)	14.21	14.7	16.11	36.79	10.73	12.10
p-value	0.018	0.030	0.002	0.017	<0.001	0.025
Transformed variables	*	*	–	Log x/10	–	–

Trea. = treatments; T1 = control; T2 = mechanical scarification between sandpapers nº120 during 20s; T3 = mechanical scarification with sandpaper nº120, on the opposite side to the embryo; T4 = immersion in water at 80 ºC during 30 seconds; T5 = immersion in water at 80ºC up to room temperature; T6 = immersion in a 6% NaOH solution during 10 minutes; T7 = immersion in water at 60ºC up to room temperature; CV = coefficient of variation; *Kruskall-wallis test = test for the non-parametric analysis of variance. In the column, averages followed by the same letters do not differ from each other by the MSD test (5%).

for population 2 under scarification for 40 seconds (87%) (Tab. 2). Rosseto *et al.* (2009) reported, for seeds of *Parkia pendula* (Willd.) Benth. ex Walp., that the mechanical scarification process using sandpapers provided a high percentage of seedling formation, with a mean of 99% of normal seedlings formed. To the germination rate index was higher in the seeds of the population 2 after 40 seconds of scarification, with a mean of 8.02 days (Tab. 2). This was also the case of seeds of *Schizolobium parahyba* (Vell.) Blake (Pereira *et al.* 2011), *Centrosema plumieri* Benth. (Gama *et al.* 2011) and *Poincianella bracteosa* (Benth.) (Ferreira *et al.* 2014), which showed a high germination rate index after mechanical scarification with sandpapers.

Seedling dry matter is an important variable because it analyzes growth and indicates the transfer of seed reserves to the embryonic axis, so that it estimates seed vigor proportionally to the dry matter content of seedlings (Custódio 2005, Gama *et al.* 2010). *L. albescens* seedlings had a high dry matter content after scarification between sandpapers for 40 seconds, especially seeds collected from the population 2 (Tab. 2). However, the values obtained for the variable seedling fresh matter did not show significant differences among the treatments analyzed (Tab. 2) because fresh matter was obtained for each seedling, thus presenting

similar data among scarification times, but with superior results for the 40-second period.

Shoot length, root length and mean time for seedling formation did not differ significantly among scarification times between sandpapers, only among accessions. To the population 2, a high seedling length (5.08cm) and the shortest mean time for seedling formation (8.92 days) were observed. However, the primary root growth was higher in the population 1, with a length of 7.61cm (Tab. 3). According to Fellipi *et al.* (2012a), genotypic characteristics and environmental conditions can affect the seed production phase within the same species, causing variability between lots and matrices from different locations. This may justify the difference found for seeds of different accesses, since the populations were collected in three areas with different environmental characteristics. The first area is characterized by a low slope surface and native country vegetation, being adjacent to a sandstone focus. In the same way, the second area is with small slope and native vegetation formation, however, on the edge of a road. And the third area is located inside a site affected by the sandstone phenomenon, with bare soil and very susceptible to the action of erosive wind and water processes.

The species rate of occupancy in a community may depend on the mean time of germination (Ferreira & Borghetti 2004). Characteristics of accelerated germination

Table 2. Average data from germination (G) and seedling formation percentage (SF), index of germination rate (GR), seedling fresh (SFM) and dry (SDM) mass for the three populations, under different times of mechanical scarification among sandpapers in *L. albescens* seeds.

Population	ST (s)	G (%)	SF (%)	GR	SFM (g)	SDM (g)
	0	19 aA	19 aA	0.80 aA	0.35[ns]	0.037 aA
1	20	16 aB	16 aB	0.63 aB	0.36	0.048 aA
	40	17 aC	17 aC	1.10 aC	0.39	0.040 aB
	0	8 cB	8 cB	0.54 bA	0.34	0.031 bA
2	20	69 bA	69 bA	7.47 aA	0.36	0.038 abAB
	40	87 aA	87 aA	8.02 aA	0.39	0.065 aA
	0	13 bA	13 bA	0.70 bA	0.34	0.041 aA
3	20	57 aA	57 aA	5.88 aA	0.35	0.026 aB
	40	50 aB	50 aB	6.25 aB	0.36	0.018 aB
p-value	–	<0.001	<0.001	<0.001	0.669	0.023
Transformed variables	–	$\sqrt{x}$	$\sqrt{x}$	$\sqrt{x}$	–	–

[ns] not significant at 5% probability of error; ST = scarification time in seconds; In the column, comparison of means of scarification times with lowercase letters and comparison of averages with capital letters to collection points, where the same letters do not differ from one another by the MSD test (5%).

Table 3. Average data from shoot and root length, mean time of seedling formation (MTS) and mean time of germination (MTG), according to the three populations collected and the MTG, according to the times of mechanical scarification among sandpapers in the seeds of *L. albescens*.

Population	Shoot length (cm)	Root length (cm)	MTS (days)	MTG (days)	ST (s)	MTG (days)
1	2.62 b	7.61 a	10.48 b	6.21 b	0	6.24 b
2	5.08 a	6.28 b	8.92 a	4.75 a	20	5.16 ab
3	4.86 a	6.01 b	9.61 ab	4.84 a	40	4.31 a
p-value	<0.001	0.001	0.002	0.039	–	0.017

ST = scarification time in seconds. In the column, averages followed by the same letters do not differ from each other by the MSD test (5%).

and emergence of seedlings are important attributes for the formation of seedlings (Martins *et al.* 2009). Mechanical scarification is a simple, low cost and fast execution method that favors homogeneity in germination (Albuquerque *et al.* 2007, Marcos Filho 2015). The results for germination mean time did not show an interaction between populations and mechanical scarification times between sandpapers. The lowest germination mean time was verified for seeds of the populations 2 and 3, with no statistical differences between them (Tab. 3). Similar results were observed for *Schizolobium parahyba* (Vell.) Blake, in which there was a short germination mean time by using mechanical scarification with sandpapers (Pereira *et al.* 2011).

The results found for the first and second experiments were decisive to define the treatments of the third experiment, where different temperatures were tested using the seeds of the population 2 and the scarification method between sandpapers for 40 seconds.

PGT - Experiment 3

Temperature has an important influence on seed germination, according to Baskin & Baskin (2014), maximum or minimum temperatures, in relation to the optimal temperature, presuppose a reduction in the speed of the germination process, leaving the seedlings exposed to adverse factors for longer periods. This may lead to a decrease in the total number of germinated seeds. In the third experiment, the germination percentage was not significantly different among treatments at 20 and 25°C, with 78-87% of germinated seeds, respectively (Tab. 4). Similar results were found for *Parkia pendula* (Willd.) Benth. Ex Walp (Rosseto *et al.* 2009), *Poecilanthe parviflora* Bentham (Valadares & Paula 2008), *Dimorphandra mollis* Benth. (Freitas *et al.* 2009) and *Caesalpinia ferrea* Mart. ex Tul. (Fonseca & Jacobi 2011), which showed a high percentage at 25°C.

A normal seedling formation is very important for the definition of an optimum temperature, since seedling development may vary according to thermal regime (Carvalho & Nakagawa 2012). As in germination, the percentage of seedling formation was high at 20 and 25°C, with no statistical differences between them (Tab. 4). The results reflect what was reported for the seedling formation of *Parkia pendula* (Willd.) Benth. Ex Walp (Rosseto *et al.*

2009) and *Poecilanthe parviflora* Bentham (Valadares & Paula 2008). To the germination rate index showed high values at 25°C, with an average of 8.02 (Tab. 4). As in seeds of *Poecilanthe parviflora* Bentham (Moraes 2007), *Caesalpinia ferrea* Mart. ex Tul. (Fonseca & Jacobi 2011), *Cedrela fissilis* Vell. (Oliveira & Barbosa 2014) and *Mimosa caesalpiniifolia* Benth. (Holanda *et al.* 2015), we found a high germination rate index at 25°C.

To the determination of seed vigor, simple tests can be used, such as germination mean time, which is based on the assumption that the most vigorous seeds germinate faster (Piña-Rodrigues *et al.* 2004). However, in the case of *L. albescens*, the germination mean time did not show significant differences at the temperatures analyzed (Tab. 4), evidencing an adaptation plasticity of the species under study. Furthermore, the formation of seedlings (normal shoots and roots formed) showed a shorter mean time at 30°C, with a mean of 7.06 days (Tab. 4). However, at this temperature, seedlings were dehydrated and damaged. Thereby, due to the difference found in the germination rate index (GR), it can be inferred that the temperature of 25°C is able to provide better environmental conditions for seeds to express their vigor, since the GR was higher at that temperature.

In the *L. albescens* species, shoot length was significantly higher in the treatment at 25°C (Tab. 4). Similar results were verified for *Parkia pendula* (Willd.) Benth. Ex Walp (Rosseto *et al.* 2009), in which a temperature of 25°C provided a higher shoot growth, as well as for *Clitoria fairchildiana* Howard, which also showed a higher root development in the seedlings (Alves *et al.* 2013). The seedling growth test can assist in the determination of seed vigor, as well as, in the study by Guedes *et al.* (2013a), the seedling length test evidenced a great efficiency to separate lots into vigor levels when it was applied to evaluate seedlings based on field emergence test. Thus, the test to evaluate seedling growth shows a good ability to highlight lots inside vigor categories and may be applied to both controlled and field environments.

The variables seedling fresh and dry matter were higher for seeds germinated at 20°C (Tab. 4). These results are in agreement with Valadares & Paula (2008), who also found a high fresh and dry matter at 20°C for *Poecilanthe parviflora* Bentham.

Table 4. Germination (G) and seedling formation percentage (SF), index of germination rate (GR), mean time of germination (MTG), and mean time of seedling formation (MTS), shoot and root length, seedling fresh (SFM) and dry (SDM) mass, submitted to different temperatures in *L. albescens* seeds.

Temp.	G (%)	SF (%)	GR	MTG (days)	MTS (days)	Shoot length (cm)	Root length (cm)	SFM (g)	SDM (g)
20 ºC	78 a	78 a	7.64 b	3.36^{ns}	10.05 c	3.3 b	7.0 a	0.410 a	0.034 a
25 ºC	87 a	87 a	8.02 a	4.45	8.70 b	5.2 a	6.3 a	0.295 b	0.018 b
30 ºC	63 b	63 b	7.23 c	4.05	7.06 a	4.6 c	4.8 b	0.053 c	0.004 c
p-value	<0.001	<0.001	<0.001	0.063	0.001	<0.001	<0.001	<0.001	<0.001
TV	–	–	x^2	x^2	$1/x^2$	–	–	–	–

^{ns} not significant at 5% probability of error; Temp. = temperatures; TV = transformed variables; In the column, averages followed by the same letters do not differ from each other by the MSD test (5%).

Photoblastic test

Photoblastic test data did not show significant differences among treatments for the variables analyzed (p<0.05), with average values of 79% germination and 70% seedling formation, because this species seeds presented pioneer species characteristics, *i.e.*, it was able to germinate under two conditions of luminosity, conferring a great capacity of adaptation. Different species of lupine (American, European and African) have a universal characteristic of inhabiting forested sites, growing in clearings, especially as pioneering plants, in recently disturbed soils (Gladstones 1998).

The seed response to luminosity allows classifying them into positive photoblastic (germination benefited by the induction of light), negative photoblastic (germination impaired by the induction of light) and non-photoblastic or neutral (indifferent or insensitive to light) (Marcos Filho 2015). Thus, seeds of *L. albescens* may be classified as neutral photoblastic. Since, light is one of the main environmental factors of fundamental importance in the control of seed germination (Mendes & Carvalho 2015). The seed sensitivity to light is quite variable according to the species, with seeds controlled by the presence or the absence of light, besides seeds indifferent to this factor. It is an ecophysiological response of certain species (Gonçalves *et al.* 2006, Manhone 2010).

Seed conservation test

L. albescens seeds stored in a cold chamber had their water content reduced throughout the storage period, presenting a minimum of 7.4% at 90 days. For seeds stored in an environment without temperature and humidity control, there was a small variation of the water content during storage, initially from 9.99% to 9.65% at 90 days. Seed water content allows determining appropriate conditions during the storage period, as well as relative humidity, which are influenced by environment temperature and packaging type (Toledo *et al.* 2009). Similar results were found for *Psidium cattleianum* Sabine (Silva *et al.* 2011) and *Parkia pendula* (Willd.) Benth. ex Walp. (Silva *et al.* 2014a) seeds in laboratory environment conditions. They showed little variation in water content during the storage period.

Due to the high number of *L. albescens* fruits collected in the field, the processing was carried out gradually. Seeds were gradually stored in a refrigerator at 5-8ºC. Thus, the seeds of this study remained for 15 days in refrigeration until the end of processing, and then they were subjected to conservation test at two storage locations. Possibly, this cooling period provided stabilization of the seeds' physiological processes, decreasing their germination rate, since seeds stored in the natural environment resumed their physiological functions, increasing germination over time (Fig. 2A).
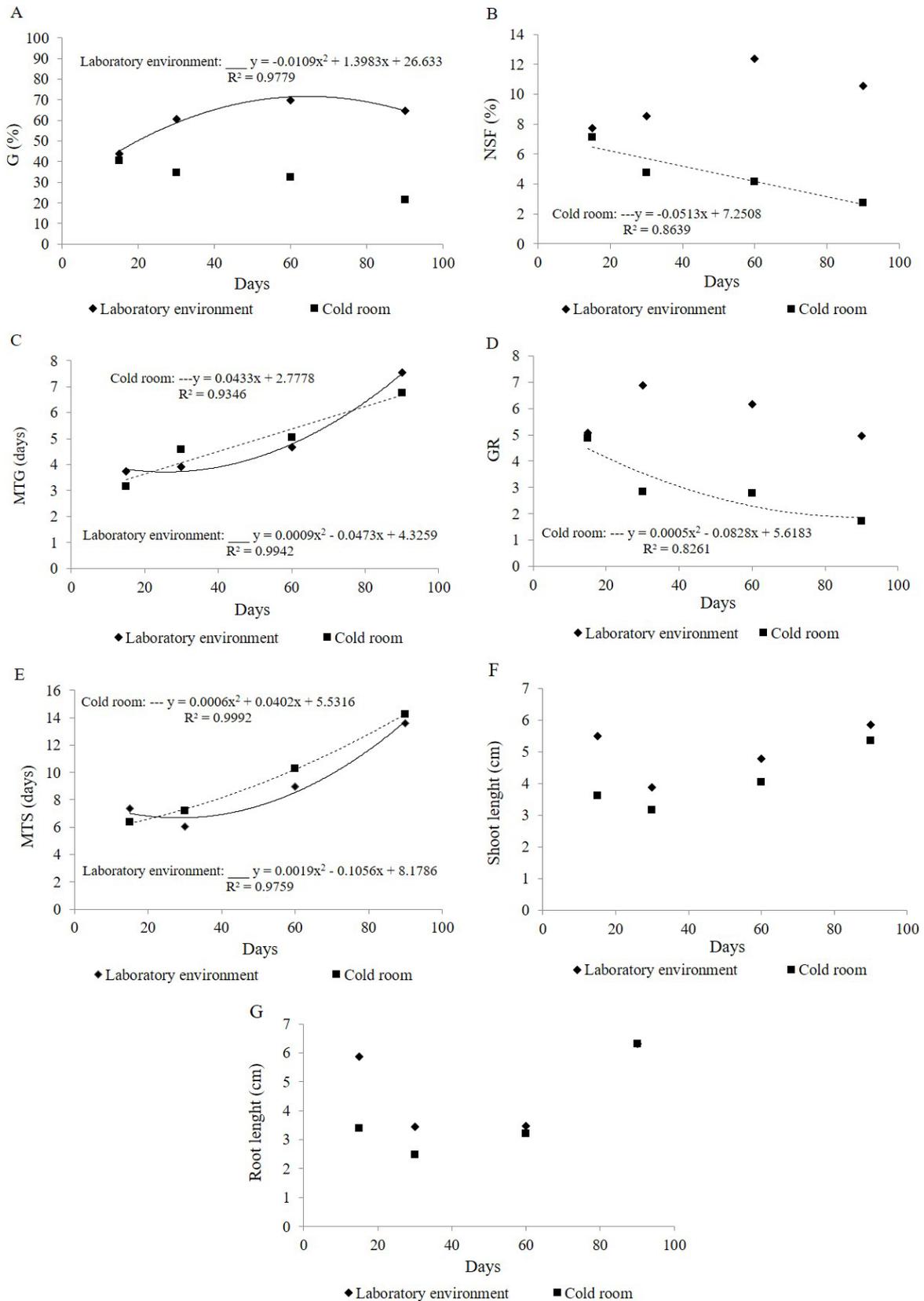
The initial seed germination was approximately 40% under laboratory conditions. However, there was an increase in the percentage of germinated seeds at the end of the experiment (close to 70%) (Fig. 2A). In contrast, there was a marked decrease in the germination of seeds stored in cold room, reaching 20% of germination (Fig. 2A). Thus, *Apeiba tibourbou* AUBL. (Matos *et al.* 2008), *Anadenanthera colubrina* (L.) Speg, *Enterolobium contortisiliquum* (Vell.) Morong. e *Cedrela fissilis* Vell. (Lima *et al.* 2007) showed an increase in the germination percentage after a storage period in an environment without temperature and humidity control and packed in Kraft paper bags. In agreement with the data obtained in the germination test, the seeds initially conditioned in a laboratory environment showed a low percentage of normal seedling formation. However, during the storage period, there was an increase in the number of seedlings formed (Fig. 2B). However, cold storage resulted in a reduced seedling development inversely proportional to storage time (Fig. 2B).

For germination mean time, there was a growing quadratic tendency for seeds stored in a laboratory environment. However, for seeds stored in the refrigerator, we observed a linear tendency increase (Fig. 2C). An increase in the germination mean time of *L. albescens* seeds over the storage period was observed for both storage locations. In the case of the germination rate index, there was a significant decrease for the seeds stored in cold room. However, for seeds stored in the laboratory environment, there was superiority in the results (Fig. 2D). Similar to those results, *Apeiba tibourbou* AUBL. seeds also showed a decrease in the germination time index when stored in cold room for up to 90 days (Matos *et al.* 2008). Moreover, the mean time for seedling formation showed a growing quadratic tendency in both storage locations. However, in the laboratory environment with Kraft paper packaging, there was a lower average time compared to the cold room (Fig. 2E). With similar results, *Parkia pendula* (Willd.) Benth. ex Walp. seeds showed a low average seedling time at room temperature wrapped in paper packaging (Silva *et al.* 2014a).

Shoot length data did not present a significant tendency by regression analysis in relation to the storage time. However, we observed that seeds kept in the laboratory environment resulted in a superior growth in relation to the cold room using plastic packaging, indicating a decrease in length (Fig. 2F). Also, *Parkia pendula* (Willd.) Benth. ex Walp. (Silva *et al.* 2014a) seeds showed a decrease in shoot length when subjected to a refrigeration environment using plastic packaging during the storage period. The mean root length at the beginning of storage was 5.8 and 3.4 cm for laboratory and cold room, respectively, and a decrease in this variable was verified at 15 and 30 days for seeds stored at both locations (Fig. 2G).From 60 days, there was a growth of seedling roots, reaching approximately 6.3cm for both storage locations (Fig. 2G).Similarly, *Parkia pendula* (Willd.) Benth. ex Walp. (Silva *et al.* 2014a) and *Apeiba tibourbou* AUBL. (Matos *et al.* 2008) seeds packed in paper bags showed variations in seedling root length according to type of storage environment during the experiment and until 90 days, respectively.

In sum, the results of the variables analyzed during the storage period showed, for the native species *L.*

A

Laboratory environment: ___ y = -0.0109x² + 1.3983x + 26.633
R² = 0.9779

♦ Laboratory environment   ■ Cold room

B

Cold room: ---y = -0.0513x + 7.2508
R² = 0.8639

♦ Laboratory environment   ■ Cold room

C

Cold room: ---y = 0.0433x + 2.7778
R² = 0.9346

Laboratory environment: ___ y = 0.0009x² - 0.0473x + 4.3259
R² = 0.9942

♦ Laboratory environment   ■ Cold room

D

Cold room: --- y = 0.0005x² - 0.0828x + 5.6183
R² = 0.8261

♦ Laboratory environment   ■ Cold room

E

Cold room: --- y = 0.0006x² + 0.0402x + 5.5316
R² = 0.9992

Laboratory environment: ___ y = 0.0019x² - 0.1056x + 8.1786
R² = 0.9759

♦ Laboratory environment   ■ Cold room

F

♦ Laboratory environment   ■ Cold room

G

♦ Laboratory environment   ■ Cold room

Figs. 2A-G. **A**. Germination of the *Lupinus albescens* seed. **B**. Normal seedling formation percentage; **C**. Mean time of germination; **D**. Index of germination rate; **E**. Mean time of seedling formation; **F**. Shoot length; **G**. Root length of the *L. albescens* seeds during the storage.

albescens, that the conditions of the laboratory natural environment and the use of a dark Kraft paper bag were the most suitable for the conservation of its seeds. In the same way, *Anadenanthera colubrina* (L.) Speg, *Enterolobium contortisiliquum* (Vell.) Morong. (Lima *et al.* 2007), *Apeiba tibourbou* AUBL. (Matos *et al.* 2008), *Psidium cattleianum* Sabine (Silva *et al.* 2011) and *Parkia pendula* (Willd.) Benth. ex Walp. (Silva *et al.* 2014a) showed less loss of viability and vigor with storage in a natural laboratory environment wrapped in paper packaging, being more appropriate for the conservation of seeds.

The beginning of seed deterioration process is characterized by the de-structuring of the cell membrane system. The electrical conductivity test provides information together with other vigor analysis during the storage period (Milani *et al.* 2012, Dalanhol *et al.* 2014). The values for electrical conductivity of *L. albescens* seeds, after 24 and 48 hours, showed no significant differences between storage locations (laboratory and cold room temperature).

The electrical conductivity of seeds imbibed for 72 hours presented only a significant regression to the laboratory environment. EC values were directly proportional to the storage time, showing a quadratic trend (Fig. 3A). During this period, the imbibition promoted a higher solute leaching rate in 45 days. Afterwards, a decrease in the presence of solutes during the conservation test was evidenced. To the 96-hour period, there was no significant regression for seeds stored in the laboratory environment. However, the location of the refrigerator caused a linear decrease throughout the analysis

period (Fig. 3B). Thus, there was an interaction between the factors time and storage local. We observed for both locations that there was a high electrical conductivity at 30 days, after which there was a decrease in leachate contents until the end of evaluations. After 120 and 144 hours, the electrical conductivity tests recorded a superiority in relation to 15-day test. However, from 30 days, there was a decrease in the contents of leached solutes both in laboratory environment and under refrigeration (Fig. 3C and 3D).

According to the results of seed imbibition analysis for 96, 120 and 144 hours, the electrical conductivity test evidenced a higher volume of leached solutes at the beginning of the storage of seeds wrapped in a plastic zip lock bag stored in the refrigerator. According to Silva *et al.* (2014b) and Delazeri *et al.* (2016), the high volume of solute leached in the solution, and consequently the high electrical conductivity value, are directly related to a lower seed vigor. On the other hand, from 30 days, as the electrical conductivity contents decrease, we verified an increase in the vigor of seeds stored in laboratory and in refrigerator, that is, an increase in the percentage of germination. However, by the germination test, *L. albescens* presented a high germination rate for seeds stored in a laboratory environment. Thus, the application of such vigor tests, together with a constant water content for the species under study, showed more vigor for seeds stored under laboratory conditions.

One of the main challenges during the recovery of degraded areas, especially in areas with arenization, such
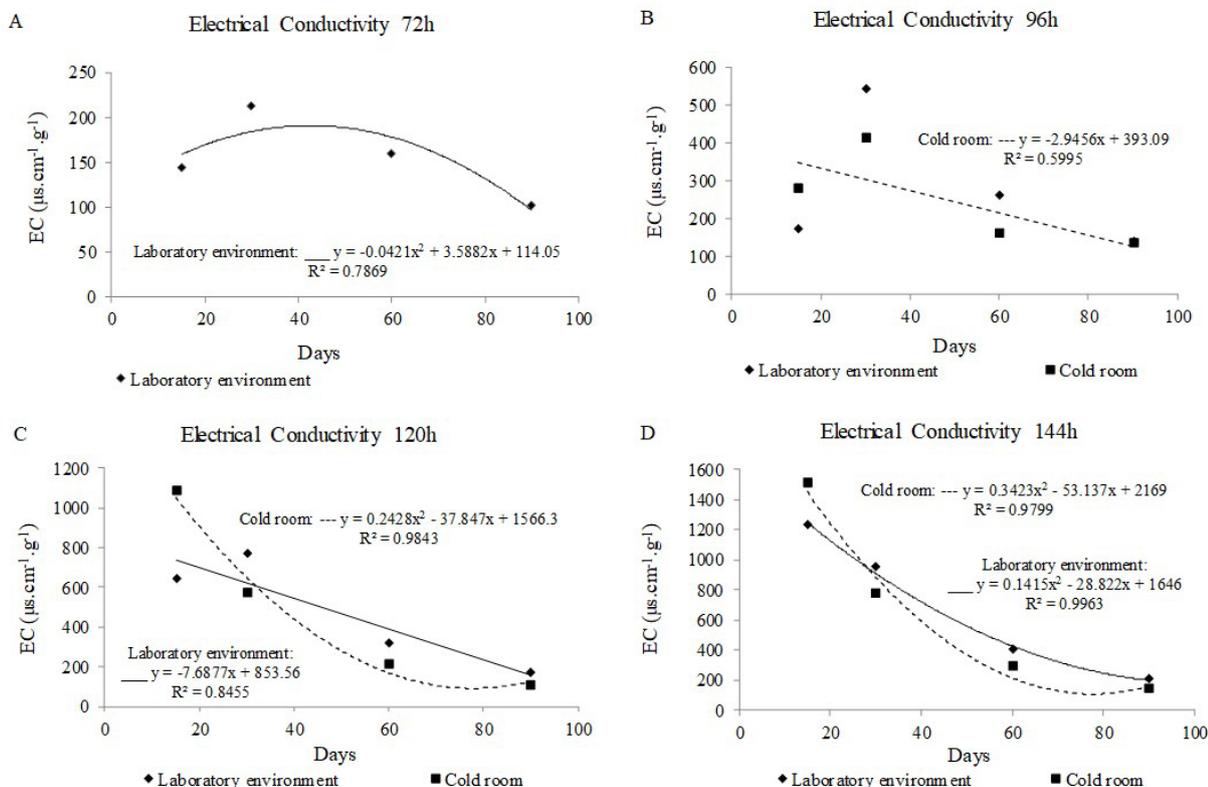
Figs. 3A-D. Determination of the electrical conductivity of *Lupinus albescens* seeds under the imbibition times of **A**. 72h; **B**. 96h; **C**. 120h; **D**.144h, during the storage period of 90 days, at laboratory environment and cold room locations.

as in the southwest of Rio Grande do Sul, is the choice of promising plant species with adaptability to impacted sites seeking satisfactory results. In this context, the use of native species of the place of origin becomes an alternative to accelerate the recovery process of these areas, such as *L. albescens*, which presents itself as a possibility, due to its potential for good soil cover, dry matter production and nitrogen fixation. Pinheiro & Miotto (2001) mentioned its development in places with a high solar radiation, adaptability to soils with a sandy texture and even coastal dunes. It is a Fabaceae species located in the phytogeographical domain of the Pampa Biome (Iganci & Miotto 2016), as well as in the southwest region of RS and in some areas affected by arenization.

However, this native species has not yet undergone genetic improvement techniques, presenting heterogeneity in its developmental stages. One can find it at several phases within a same population. Thus, for the species *L. albescens*, there are few studies on the behavior and aspects of its fruits and seeds. According to Silva *et al.* (2012) and Moura *et al.* (2013), there is great relevance in the knowledge of such characteristics since they contribute in the studies of interpretation and conduction of germination tests in the laboratory, orient with cultivation techniques for the production of seedlings and in the comprehension of the processes of planting in natural conditions. In addition, they provide subsidies for the study of the mechanisms of dispersion, succession and natural regeneration of the species (Almeida Júnior *et al.* 2010).

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