

Biometry and germination of *Balfourodendron riedelianum* Eng. Eng.¹

Daniele Rodrigues Gomes^{2*}, Maristela Machado Araujo²,
Ubirajara Rossi Nunes², Suelen Carpenedo Aimi²

ABSTRACT - This study investigated the biometry, pre-germination treatments and substrates for the germination of diaspores of *Balfourodendron riedelianum*. The diaspores were characterized during germination according to their length, width, thickness, thousand kernel weight (TKW) and water content. Dormancy overcoming was tested by diaspore scarifying with sandpaper, sulfuric acid immersion, water and water at 100 °C; together with the control (untreated), they were associated to different substrates (including paper (EP), sand (EA) and vermiculite (EV)). Germination tests were conducted in a germination chamber at 25 °C. The physiological quality of seeds was performed through the first count, germination and germination speed index (GSI). Biometric data were analyzed in frequency classes and the pre-germination treatment by analysis of variance. Diaspores are on average 18.59 mm long; 9.03 mm wide and 9.38 mm thick. The treatment of immersion in cold water for 48 hours and the substrate vermiculite (EV) were effective to overcome dormancy and recommended for germination tests.

Index terms: seed analysis, morphology, dormancy, forest species.

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RESUMO - O presente trabalho objetivou estudar a biometria, tratamentos pré-germinativos e substratos para germinação de diásporos de *Balfourodendron riedelianum*. Os diásporos foram caracterizados na germinação quanto ao comprimento, largura, espessura, peso de mil sementes (PMS) e teor de água. Foram empregados os métodos de escarificação com lixa e ácido sulfúrico, imersão em água fria e em água a 100 °C, e o controle (sem tratamento), associados a diferentes substratos (entre papel (EP), entre areia (EA) e, entre vermiculita (EV), sendo conduzidos em câmara de germinação a 25 °C. A caracterização fisiológica foi realizada por meio da primeira contagem, germinação e índice de velocidade de germinação (IVG). Os dados biométricos foram analisados em classes de frequências e os tratamentos pré-germinativos por meio da análise de variância. Os diásporos apresentam em média 18,59 mm de comprimento; 9,03 mm de largura e 9,38 mm de espessura. O tratamento de imersão em água fria por 48 horas e o substrato entre vermiculita (EV) foi efetivo para a superação da dormência e o mais adequado para o teste de germinação da espécie.

Termos para indexação: análise de sementes, morfometria, dormência, espécie florestal.

Introduction

Balfourodendron riedelianum Eng. Eng. (Rutaceae family), known as Brazilian maple and ivory maple, is a forest species native from Brazil. Since it is not endemic, its propagation occurs through seeds; however, due to the difficulty in removing the seeds from inside the fruits, they are used as propagules in seedling nurseries. Brazilian maple is a species that can be used as wood resource, for landscaping purposes and for the recovery of riparian degraded areas; during its adult stage, it reaches from 6 to 30 m of height and 30 to 90 cm of diameter, and in some cases, it may reach 35 m

and 100 cm, respectively (Backes and Irgang, 2002; Carvalho, 2003; Lorenzi, 2008). Despite the appealing characteristics of the species, the lack of information on its propagation makes it harder for it to be used, and it is fundamental to conduct studies through germination analysis, production of seedlings and in-field growth, allowing its use on forestry programs.

Studies related to the biometry of the diaspores, methods to overcome dormancy, need for light, water, temperature and substrate are fundamental to know the germination process of vegetable species. The inherent knowledge to the germination process regarding the size of diaspores, water content, dormancy overcoming, substrate, among others, are

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²Departamento de Ciências Florestais, Universidade Federal de Santa Maria, 97105-900 – Santa Maria, RS, Brasil.

*Corresponding author <eng.danielegomes@gmail.com>

important information for the propagation of forest species; however, they may vary from species to species.

The methods to conduct the germination tests are shown on the Rules for Seed Testing (RAS) (Brasil, 2009), and through the Guidelines for the Analysis of Seeds of Forest Species (Brasil, 2013). The latter contemplates 319 forest species; however, among them, only 50 have validated tests (Brasil, 2010; Brasil, 2011; Brasil, 2012). However, *Balfourodendron riedelianum* is not among the group of validated species, showing the lack of studies on this species.

The study on whether the dormancy of the seeds occurs or not is vital, since it is common on several forest species. There are several manners for the manifestation of dormancy and to determine the methods to overcome it, depending on the endogenous and exogenous dormancy methods. Biruel et al. (2007) used a mechanical scarification to overcome the dormancy of seeds of *Caesalpinia leiostachya*, Oliveira et al. (2012) used chemical and mechanical scarification to overcome the physical dormancy on seeds of *Parkia gigantocarpa*, Seneme et al. (2012) and Alexandre et al. (2015) used physical and chemical treatments on seeds of *Peltophorum dubium* and *Enterolobium contortisiliquum*, respectively. In addition, methods such as immersion on hot water (Azeredo et al., 2010) and the use of hormones, such as gibberellic acid, may also be used (Campos et al., 2015).

For the germination of the seeds, each species requires specific conditions regarding its hydration, aeration, temperature and luminosity. Nogueira et al. (2014) and Godoi and Takaki (2004) evaluated the light and temperature effects on seeds of *Piptadenia stipulacea* and *Cecropia hololeuca*, respectively, and determined that the germination is indifferent to luminosity, and that the best temperatures are between 20 and 30 °C on a constant or alternating basis, while seeds of *C. hololeuca* germinated in the dark when the temperature was alternated (20-25; 20-30 and 20-35 °C). Dresch et al. (2012) evaluated the influence of the temperature and humidity of the substrate on the germination of seeds of *Campomanesia adamantium* and reached a substrate humidity of 2.5 times the mass of the dry paper, at a temperature of 25 °C.

In that sense, the substrate acts directly, due to its physical functions related to the structure, aeration, capacity to retain water, propensity to infestation by pathogens, among others (Martins et al., 2012). Santos and Aguiar (2000), when evaluating the germination of seeds of *Sebastiania commersoniana* due to the substrate and the temperature regimen, observed that the most adequate substrate was on sand, which offered maximal germination, at an alternating temperature of 20-30 °C. Guedes et al. (2010), when evaluating substrates and temperature to test the germination and vigor of seeds of *Amburana cearensis*, concluded that the temperature of 35 °C was the most adequate

to conduct the tests, regardless of the substrate used.

On the Rules for Seed Testing, some substrates are recommended, such as paper (buffer, towel and filter), sand and soil (Brasil, 2009). However, for most forest species, there are no standardized procedures, and other substrates have been tested, as it is the case of vermiculite (Brasil, 2013).

Considering the above, the objective of this paper was to study aspects of the biometry, establishing pre-germination treatments and determining the substrates that favor the germinability of diaspores of *Balfourodendron riedelianum*, a potential species for forestry activities.

Material and Methods

The diaspores (fruits) of *Balfourodendron riedelianum* were collected in July, 2013, from four matrix trees in forest fragments located in the district of Caemborá (29°28'18.9"S and 53°18'03.4"W), in the municipality of Nova Palma, RS, Brazil. Due to the lack of uniformity on the fructification, the standard used were matrixes that showed similar characteristics as to the fruit maturation, when they changed from the green color (2.5GY 5/4) to beige-yellow (2.5Y 7/6). The coloration of the diaspores was determined based on the chart of colors of vegetable tissue (Munsell, 1976).

After the collection, the material was stored on individualized plastic bags identified by matrix, and transported to the forest seedling nursery. There, the diaspores went through natural pre-drying in a covered location with air circulation (19 °C and 75.8% RH), and they were arranged on trays placed on tables, for three days, with daily turning over. This material was processed in order to remove branches and leaves. Then, the winged part of the diaspore was removed, with the help of pruning shears. The use of diaspores for the laboratory tests is due to the difficulty in removing the seeds from them without compromising and/or damaging the structure of the embryo.

After processing, the seed lot with the diaspores was constituted, and they were allocated into two paper packages, inside kraft paper cylinders, stored in a cold and moist chamber (temperature \pm 8 °C and relative humidity (RH) around 80%).

Lot characterization

A sample was collected before storage in order to characterize the diaspores according to Rules for Seed Testing (Brasil, 2009) and the Guidelines for the Analysis of Seeds of Forest Species (Brasil, 2013). In order to determine one thousand diaspores weight (TDW), eight replications of 100 diaspores were used, obtaining results expressed in grams. The evaluation of the water content was conducted using the oven method, at 105 °C (\pm 3)

for 24 hours, with two replications of approximately five grams of intact diaspores, and the results were expressed based on the humid weight of the diaspores (Brasil, 2009).

The biometric description of the diaspores was conducted using 100 units, randomly removed from the lot (five replications with 20 diaspores), measuring the length (mm), width (mm) and thickness (mm), obtained with a digital caliper rule (precision of 0.01 mm).

Pre-germination treatments

The germination test was conducted in the laboratory. Initially, the superficial asepsis was done, which consisted in the immersion of the diaspores in alcohol 70% and in a commercial sodium hypochlorite solution (2.5% Cl) at 1% (p/p), consecutively, both for two minutes; then, they were washed in distilled water for two minutes.

The pre-germination treatments were: Control – consisting of intact diaspores; Immersion in water – immersion in cold water at 10 °C for 24, 36 and 48 horas; Immersion in boiling water – immersion in water at a temperature of 100 °C for five minutes; Chemical scarification in sulphuric acid (H₂SO₄) for five minutes, followed by rinsing in running water to remove the residues, for two minutes; Mechanical scarification with sandpaper n. 60 on the opposed region to the peduncle; Mechanical scarification with sandpaper, followed by immersion in water at room temperature (19 °C) for 24 hours; Mechanical Scarification with sandpaper, followed by immersion in gibberellic acid GA₃ at a concentration of 500 mg.L⁻¹. The GA₃ solution was prepared according to the methodology described by Brasil (2009).

The feasibility of the diaspores was determined through the germination test. After the pre-germination treatments, four replications with 20 diaspores were planted on three different substrates: sand, vermiculite and paper, arranged on transparent plastic boxes with the following dimensions: 11 cm x 11 cm x 3.5 cm.

Previously to the germination test, the substrates were sterilized on an autoclave (120 °C for 60 minutes) and, afterwards, they were taken to the forced air circulation oven at 70 °C for two hours and 24 hours, for the drying of the paper, vermiculite and sand, respectively. The sand and vermiculite substrates were moistened with distilled water at 60% of their retention capacity, which corresponded to 43 and 47 mL, respectively, while the paper substrate was moistened with distilled water at a quantity equivalent to 2.5 times the dry mass of the paper, which is equivalent to 14 mL (Brasil, 2013).

The plastic boxes were maintained in Mangelsdorf-type germination chambers, at a temperature of 25 (± 1 °C) and photoperiod of 8-16 hours (light/dark). The evaluations

were conducted at every three days, between the 18th and the 45th day, a period that corresponds to the beginning and the stabilization of the germination. The seedlings that were considered as normal were the ones that showed all the essential structures (primary root, hypocotyl, cotyledons and epicotyl), and the seedlings considered as abnormal were the ones that did not show all of these structures, according to the photographic record (Figure 2) (Brasil, 2009).

The diaspore was considered a multiple seed unit (MSU), considering that it may produce more than one normal seedling. Therefore, when the diaspore (seed unit) produced more than one seedling (normal or abnormal), only the first one to germinate was counted in order to determine the germination percentage. The diaspores considered as hard diaspores were the ones that did not show at least one seedling. The results were expressed in germination percentage (Brasil, 2009) and the germination speed index (GSI), determined according to the formula suggested by Maguire (1962).

Experimental design and data analysis

The biometry data of the diaspores were analyzed by the adjustments of statistical distributions and descriptive statistics, which comprehended position measurements (mean) and dispersion measurements (variation coefficients) (Silva et al., 2013).

The pre-germination treatment data were conducted on a completely randomized design (CRC) with four replications, on a 9 x 3 factorial (overcoming of dormancy x substrate). They were evaluated as to the normality assumptions of the residues, according to the Shapiro-Wilk test, and variance homogeneity, according to the Bartlett test. When not met, they were subjected to data transformation, considering the water content, germination percentage, hard diaspores on $\arcsin \sqrt{x/100}$; GSI and abnormal seedlings $\sqrt{x+0.5}$, considering x as the value obtained for the observed variable. The transformed data was used only for the statistical analysis, and the original data was maintained for the presentation of the results.

The statistical analysis was conducted with the help of the SISVAR software (Ferreira, 2011), subjecting the data to analysis of variance (ANOVA) and, when a difference was observed among the treatments, the means were compared by the t and Scott-Knott tests at 5% of error probability.

Results and Discussion

The lot constituted by *B. riedelianum* diaspores showed one thousand diaspores weight (TDW) value of 361.43 g with a variation coefficient (VC) of 2.61%, which is equivalent to 2,766 fruits per kilogram, and moisture content of 17.13%.

When studying the species, Donazzolo et al. (2013) found a humidity equivalent to 14.7%, and the thousand kernel weight showed similar results than the ones observed by Lorenzi (2008) and Grings and Brack (2011), with values that varied between 2,200 and 2,900 fruit.Kg⁻¹ and 2.460 fruit.Kg⁻¹, respectively.

This variation on the water content of the diaspores, as well as on the thousand kernel weight, may be associated to different factors, such as, for example, the genotype and the origin region. The quality and vigor obtained from the lot sample are directly related to the dimensions of the diaspores, and they are associated not only to environmental factors, but also to the reactions of the population to the establishment on a new environment, mainly when the species has a broad

distribution (Rodrigues et al., 2006).

The frequency distribution of the biometric data of the diaspores of *B. riedelianum*, in relation to the length and width, showed means of 18.59 ± 1.71 mm for length; 9.03 ± 1.12 mm for width, and 9.38 ± 1.40 mm for thickness (Figure 1). The most representative frequency class was 19.87-21.57 mm (41%) for length; 9.51-10.38 mm (36%) for width, and 10.87-11.96 mm (28%). These results are similar to the interval determined by Carvalho (2003), when characterizing the diaspore of *B. riedelianum*, during its mature phase, with dimensions that vary from 5 to 25 mm wide by 20 to 25 mm long. However, they were superior than the observed by Grings and Brack (2011) for the species, with biometric values that vary from 3 to 4 cm long and 2.5 to 3.0 cm wide.

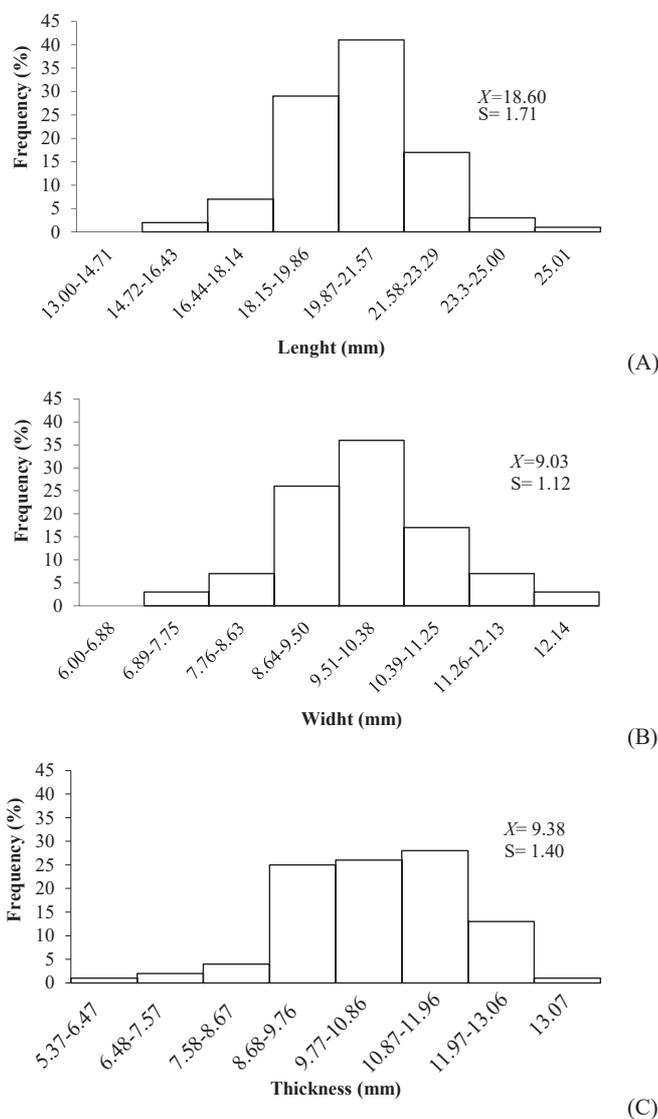


Figure 1. Frequency, mean (\bar{X}) and standard deviation (S) of the length (A), width (B), and thickness (C) of the diaspores of *Balfourodendron riedelianum*.

The position of the seed on the mother plant may affect the size, morphology and germination, which is mediated by environmental factors that act during the development and maturation of the fruit (Gutterman, 1992). The biometric characteristics of the diaspores show little variation, as it may be observed by the standard deviation, varying from 13.24 to 24.1 mm (1.82 times) in length and 6.05 to 12.18 mm (2.01 times) wide, respectively, which may be a reference that indicates diaspores collected on the region of Nova Palma, RS.

Physiological characteristics

On the analysis of variance of the data, a significant interaction was observed among pre-germination treatments and substrates for the germination and germination speed index variables; however, for the abnormal seedling and hard diaspores, there was a significant effect only of the dormancy overcoming treatments. For the first counting variable, the descriptive analysis of the data was conducted.

The germination is epigeal phanerocotylar, starting on the

18th day after the installation of the test, a period in which it was possible to conduct the first counting. The first seedlings were observed on the treatments with immersion in cold water for 24 and 48 hours, on the vermiculite and sand substrates, and the last substrate was only for immersion for 48 hours. The germination stabilization occurred on the 45th day. Brasil (2013) and Figliolia and Piña-Rodrigues (1995) recommend that the first counting for *B. riedelianum* is conducted on the 20th to the 25th day after installing the test; on this study, the sample used allowed this evaluation to be conducted within a shorter period of time, after overcoming the dormancy imposed by the membrane.

Based on the data related to the germination percentage means of the diaspores of *B. riedelianum*, higher values were observed on the treatment with immersion in water for 48 h, regardless of the substrate used (Table 1). The pre-germination treatments, immersion in boiling water and sulphuric acid for five minutes, regardless of the substrate, did not offer the germination process of the seeds, which occurred only when the diaspores were treated with sulphuric acid and cultivated on vermiculite (9%).

Table 1. Pre-germination treatments on diaspores of *Balfourodendron riedelianum* associated to different substrates as the index of physiological quality through the following variables: germination and germination speed index (GSI).

| Treatments | Germination (%) | | |
|--|-----------------|---------|-------------|
| | Substrate | | |
| | Paper | Sand | Vermiculite |
| Control | 51 Bb | 51 Bc | 73 Aa |
| Immersion on cold water 10 °C for 24 h | 56 Bb | 62 Bb | 79 Aa |
| Immersion on cold water 10 °C for 48 h | 80 Aa | 84 Aa | 83 Aa |
| Immersion on cold water 10 °C for 72 h | 61 Ab | 49 Ac | 60 Ab |
| Immersion on boiling water at 100 °C 5 min | 0 Ae | 0 Ad | 0 Ae |
| Chemical scarification with sulphuric acid 5 min | 0 Be | 0 Bd | 9 Ad |
| Mechanical scarification with sandpaper | 31 Bd | 34 Bc | 53 Ab |
| Mechanical scarification with sandpaper and immersion on water for 24 h | 46 Ac | 46 Ac | 55 Ab |
| Mechanical scarification with sandpaper and immersion on GA ₃ | 43 Ac | 44 Ac | 33 Ac |
| Treatments | GSI | | |
| | Substrate | | |
| | Paper | Sand | Vermiculite |
| Control | 0.47 Ac | 0.49 Ac | 0.60 Aa |
| Immersion on cold water 10 °C for 24 h | 0.82 Aa | 0.89 Aa | 0.66 Ba |
| Immersion on cold water 10 °C for 48 h | 0.62 Ab | 0.69 Ab | 0.80 Aa |
| Immersion on cold water 10 °C for 72 h | 0.54 Ac | 0.60 Ac | 0.65 Aa |
| Immersion on boiling water at 100 °C 5 min | 0.00 Af | 0.00 Ae | 0.00 Ac |
| Chemical scarification with sulphuric acid 5 min | 0.00 Af | 0.00 Ae | 0.05 Ac |
| Mechanical scarification with sandpaper | 0.24 Ae | 0.36 Ad | 0.38 Ab |
| Mechanical scarification with sandpaper and immersion on water for 24 h | 0.65 Bb | 0.87 Aa | 0.63 Ba |
| Mechanical scarification with sandpaper and immersion on GA ₃ | 0.34 Bd | 0.60 Ac | 0.43 Bb |

*Means followed by the same letter on the row (uppercase) and on the column (lowercase), not different from each other according to the Scott-Knott Test at 5% of error probability.

The treatment with immersion in sulphuric acid for five minutes had most of the diaspores rigid (hard) at the end of the evaluation period of the germination test, indicating that the

scarification time was insufficient to increase the permeability of the membrane, allowing the water absorption and even causing damages to the embryo of the seed. A similar effect

was observed by using the pre-germination treatment with immersion in boiling water for five minutes, whose water temperature possibly caused damages to the embryonic tissues, resulting on the lack of germination.

The mechanical (physical) dormancy is frequent on forest species, in which the growth of the embryo is limited due to an impermeable structure, which prevents the water absorption even under favorable environmental conditions (Souza et al., 2012; Venier et al., 2012; Nasr et al., 2013).

Previous studies recommended, for the *B. riedelianum* species, the use of immersion in water for 24 hours and mechanical scarification (Carvalho, 2003; Mori et al., 2012; Donazzolo et al., 2013). However, they found from 12 to 37% of germination, values that are expressively lower than the ones obtained on this research.

The use of mechanical scarification, as a pre-germination treatment, on this research, even when conducted on the region to the side of the peduncle, offered a reduction of the germination percentage, when compared to the methods of immersion in cold water, indicating that this procedure caused damages to the internal structure of the seeds, or it was not sufficient to overcome the barrier for water absorption.

Regardless of the treatments to overcome dormancy, on the mechanical scarification treatments with moistening of the substrate on a GA_3 solution, there was no significant difference among the other substrates. The efficiency and facility to use vermiculite as the substrate on the germination test corroborates with the recommendation described for the *B. riedelianum* species on the Guidelines for the Analysis of Seeds of Forest Species (Brasil, 2013).

The GSI results (Table 1) showed the same trend observed for the germination with the treatments to overcome the dormancy, observing that the treatments of immersion in water at 100 °C and sulphuric acid for five minutes were responsible for the lower indexes for all substrates.

It is observed that the treatments to overcome the dormancy promoted different germination speed indexes for each substrate used. For the paper substrate, the highest GSI is attributed to the treatment of immersion in water for 24 hours. With sand, the immersion in water for 24 hours and scarification and immersion for 24 h stand out; for vermiculite, most of the treatments showed no differences in relation to each other.

In that sense, with the purpose of reducing the time to conduct the germination test, the treatment with immersion in cold water for 48 hours associated to the use of the vermiculite substrate is suggested for the germination test, since it resulted on a GSI of 0.80 and mean germination of 83%.

The normal seedlings of *B. riedelianum* on the 24th day after the installation of the experiment are represented on

Figure 2 A. According to the illustration of the morphology of these seedlings, all of them show an adequately developed primary root, hypocotyl, open cotyledons and leaf primordia. Some diaspores that emitted a radicle, but which did not emit the other essential structures, were considered as abnormal at the end of the experiment (Figure 2C). The main types of abnormalities found on the different treatments were: atrophied primary root (Figure 2C - I); lack of primary root (Figure 2C - II); disproportional primary root in relation to the other structures of the seedling (Figure 2C - III); short and thick primary root with negative geotropism (Figure 2C - IV); lack of defined primary root, and disproportional to the above-ground part (Figure 2C - V) (Brasil, 2009). In addition to the abnormalities mentioned, the necrosis and malformation of the radicle could be observed; this may have occurred due to the loss of humidity by the substrate, mainly on the paper substrate, harming the development of the seedlings.

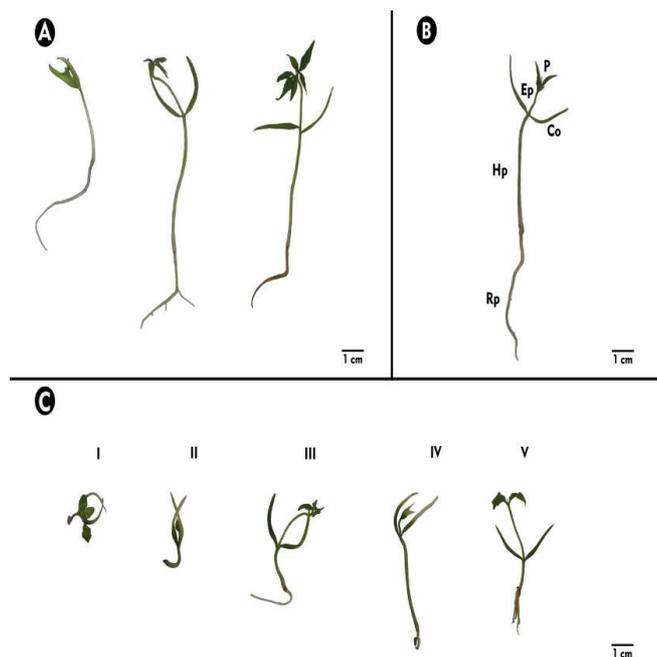


Figure 2. Seedlings of *Balfourodendron riedelianum* obtained on the germination test: A – Normal seedlings; B – Normal seedling with primary root (Rp), hypocotyl (Hp), open cotyledons (Co), Epicotyl (Ep), protophil and first pair of leafs (P); C – Abnormal seedlings with atrophied primary root (I), lack of primary root (II), primary root not proportional in relation to the other structured of the seedling (III), short and thick primary root with negative geotropism (IV), lack of defined primary root, not proportional to the above-ground part (V). (Bars: 2 cm).

On seedlings considered as abnormal (Table 2), it was not possible to determine the direct relationship with the pre-germination treatment. However, the treatments with immersion

in cold water (24 and 48 hours) and mechanical scarification with posterior immersion in cold water for 24 hours showed the highest GSI values (Table 1) and abnormal diaspores.

Table 2. Pre-germination treatments on diaspores of *Balfourodendron riedelianum* associated to different substrates as an index of the physiological quality through the following variables: abnormal seedlings and hard diaspores.

| Treatments | Abnormal seedling (%) | Hard diaspores (%) |
|---|-----------------------|--------------------|
| Control | 2.61 a | 31.56 b |
| Immersion on cold water at 10 °C for 24 h | 3.95 a | 37.72 c |
| Immersion on cold water at 10 °C for 48 h | 3.52 a | 14.40 a |
| Immersion on cold water at 10 °C for 72 h | 2.97 a | 40.37 c |
| Immersion on boiling water at 100 °C 5 min | 0.00 b | 100.00 e |
| Chemical scarification with sulphuric acid 5 min | 0.08 b | 97.00 e |
| Mechanical scarification with sandpaper | 1.64 b | 59.19 d |
| Mechanical scarification with sand paper and water immersion | 3.59 a | 47.25 c |
| Mechanical scarification with sandpaper and GA ₃ immersion | 2.29 a | 58.13 d |

*Means followed by the same (lowercase) letter on the column, not different from each other according to the Scott-Knott Test at 5% of error probability.

The percentage of hard diaspores is directly related to the efficiency of the methods used to overcome the dormancy; therefore, treatments with low germination indexes resulted on high percentages of hard diaspores.

Conclusions

The dimensions of the diaspores of *Balfourodendron riedelianum* are, on average, 18.59 mm long, 9.03 mm wide and 9.38 mm thick.

The dispersing unit of *Balfourodendron riedelianum* shows, as its dormancy mechanism, the inability of the wrap to soaking of water, which is overcome with immersion in cold water (8 ± 2 °C), for 48 hours, and the use of vermiculite as substrate for the germination test.

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