PRE-TREATMENTS, STRATIFICATION, AND EMERGENCE OF SEEDS OF *ILEX PARAGUARIENSIS* A.ST.-HIL.

Mara Cíntia Winhelmann[1*], Leo Jaime de Vargas[2], Julia Gastmann[3], Lucas Massena de Oliveira[4], Elisete Maria de Freitas[5], Claudimar Sidnei Fior[6]

[1]*Universidade Federal do Rio Grande do Sul, Programa de Programa de Pós-graduação em Fitotecnia, Porto Alegre, RS, Brazil - marawinhelmann@gmail.com
[2]Universidade do Vale do Taquari, Laboratório de Botânica, Lajeado, RS, Brazil - leo.vargas@universo.univates.br
[3]Universidade do Vale do Taquari, Laboratório de Botânica, Lajeado, RS, Brazil - julia.gastmann@universo.univates.br
[4]Universidade do Vale do Taquari, Laboratório de Botânica, Lajeado, RS, Brazil - lucas.oliveira@universo.univates.br
[5]Universidade do Vale do Taquari, Laboratório de Botânica, Lajeado, RS, Brazil - elicauf@univates.br
[6]Universidade Federal do Rio Grande do Sul, Programa de Programa de Pós-graduação em Fitotecnia, Porto Alegre, RS, Brazil - csfior@ufrgs.br

Received for publication: 06/04/2021 – Accepted for publication: 02/02/2022

Resumo

Pré-tratamentos, estratificação e emergência de sementes de Ilex paraguariensis A.St.-Hil. A propagação da erva-mate é realizada por sementes. Na natureza as aves são as principais dispersoras e a passagem das sementes pelo sistema digestivo auxilia na germinação. A simulação em laboratório das condições proporcionadas pela passagem das sementes no trato digestivo pode ser uma alternativa de pré-tratamento. Assim, o objetivo do trabalho foi avaliar o efeito de pré-tratamentos, associando ambiente ácido na estratificação, sobre a emergência de sementes de *I. paraguariensis*. Foram testados dois pré-tratamentos (PT): solução aquosa acidificada (PT1) e uma sequência de soluções aquosas (neutra + ácidas) (PT2). As sementes pré-tratadas foram submetidas a estratificação em areia (T1 e T2), além de sementes sem pré-tratamento que foram estratificadas em areia (T3) e sementes estratificadas em areia e enterradas (T4). Aos 90, 135 e 180 dias foram retiradas sementes da estratificação e levadas ao laboratório. Aliado a estes tratamentos, incluiu-se um tratamento controle (sementes sem pré-tratamento e sem estratificação) e sementes pré-tratadas (PT1 e PT2) sem estratificação (T5 e T6) que foram submetidas à emergência e acompanhadas semanalmente durante 180 dias. Após este período foi realizado o teste de tetrazólio nas sementes não emergidas. Sementes estratificadas por 90 dias não emergiram, com 135 dias obteve-se 0,9% de emergência e o tratamento com estratificação em areia e enterrado (T4) apresentou a maior emergência (4,25%). Com 180 dias de estratificação obteve-se emergência média de 9,6% e a maior porcentagem foi verificada no T2 (pré-tratamento seguido de estratificação), com 33% de emergência.
Palavras-chave: dormência, erva-mate, teste de tetrazólio, tratamento ácido, viabilidade.

Abstract

The propagation of yerba mate is carried out by seeds. In nature, birds are the main dispersers and the passage of seeds through the digestive system helps in germination. The laboratory simulation of the conditions provided by the passage of seeds in the digestive tract can be a pre-treatment alternative. Thus, the objective of the work was to evaluate the effect of pre-treatments, associating acid environment in the stratification, on the emergence of *I. paraguariensis* seeds. Two pre-treatments (PT) were tested: acidified aqueous solution (PT1) and a sequence of aqueous solutions (neutral + acid) (PT2). The pretreated seeds were subjected to stratification in sand (T1 and T2), in addition to seeds without pretreatment that were stratified in sand (T3) and stratified in sand and buried (T4). At 90, 135, and 180 days, seeds were removed from the stratification and taken to the laboratory. In addition to these treatments, a control treatment (seeds without pre-treatment and stratification) and pretreated (PT1 and PT2) seeds without stratification (T5 and T6), which were submitted to emergency and followed up weekly for 180 days, were included. After this period, the tetrazolium test was performed on non-emerged seeds. Seeds stratified for 90 days did not emerge, with 135 days there was 0.9% emergence, and the treatment with stratification in sand and buried (T4) had the greatest emergence (4.25%). With 180 days of stratification, an average emergency of 9.6% was obtained and the highest percentage was seen in T2 (pre-treatment followed by stratification), with 33% of emergence.
Keywords: dormancy, yerba mate, tetrazolium test, acid treatment, viability.

INTRODUCTION

Ilex paraguariensis A.St.-Hil. (Aquifoliaceae), known as yerba mate, is a dioecious arboreal species with one of the abortive sporangia (CARVALHO, 2003; PIRES *et al*., 2014). Flowering occurs from September to December and fruiting is from December to mid-May (PIRES *et al*., 2014). The unit of dispersion is the pyrenes, usually in the number of four in each fruit, consisting of a woody endocarp and a seed. The seed, in turn, is composed of the tegument, endosperm and embryo (CARVALHO, 2003; WENDLING; SANTIN, 2015; GALÍNDEZ *et al*., 2018).

In Brazil, yerba mate is grown mainly by family farmers, so it has a great economic, social, cultural, and environmental importance, besides being part of one of the most balanced forest systems in Brazil, with increasing findings of new uses of its raw material (WENDLING; SANTIN, 2015). Propagation is carried out mainly through by seeds (CARVALHO, 2003; WENDLING; SANTIN, 2015); however, the seeds have low genetic and physiological quality, low germination power, uneven germination, and morphophysiological dormancy (WENDLING; SANTIN, 2015; GALÍNDEZ *et al*., 2018). Morphophysiological dormancy is identified as one of the main causes of low seed germination rates (WENDLING; SANTIN, 2015), which are characterized by having an embryo with incomplete development (globular stage to late cordiform heart-shaped stage) and the presence of inhibitory compounds (BEWLEY *et al*., 2013; BASKIN; BASKIN, 2014).

The most widespread method to overcome seed dormancy in yerba mate is stratification. In short, it is the maintenance of seeds mixed or interspersed in layers of moist sand for 180 days (WENDLING; SANTIN, 2015). This process favors embryonic development by promoting the overcoming of morphological dormancy and allows the leaching of inhibitory compounds, favoring the overcoming of physiological dormancy (BEWLEY *et al*., 2013; BASKIN; BASKIN, 2014). Even so, germination is slow and uneven (from 100 to 360 days) with low rates, usually less than 20% (WENDLING; SANTIN, 2015).

A period of approximately 18 months is necessary to obtain the yerba mate seedlings. This period is comprised of the collection of seeds, stratification, sowing, germination, and seedling development. What contributes to this prolonged period is the non-evolution of dormancy overcoming techniques. In this sense, the use of pre-treatments in seeds associated with stratification may be a viable alternative, to standardize and increase germination rates, reduce the stratification period, and, consequently, the time for seedling formation.

In nature, numerous plant species, especially those with fleshy fruits, are adapted for dispersal by animals, especially birds. The consumption of fresh fruits, with subsequent seed dispersal, is considered a key process in plant ecology (SILVEIRA *et al*., 2012). In the specific case of yerba mate, which has a fleshy drupe-like fruit, its dispersion is of the zoochoric type, by ornithochory syndrome, especially for birds of the *Turdus* genus, such as the thrush (CARVALHO, 2003). These birds eat the whole fruit (COLUSSI; PRESTES, 2011), and the seeds can be dispersed up to about 2.0 km away (DIAZ *et al*., 2013), evidencing the importance of avifauna in this process. The passage of seeds through the digestive system has a mechanical and/or chemical action, which may affect the structure and/or permeability of the tegument (TRAVESET, 1998; LEHOUCK *et al*., 2011). Birds can also aid to overcome dormancy, mainly by removing substances such as suberins present in the internal and external cuticle of the tegument by the passage through the digestive system (BEWLEY *et al*., 2013), in addition to affecting other seed structures and contributing for the removal of inhibitors (TRAVESET, 1998).

Seeds of *Xymalos monospora* (Harv.) Baill. ex Warb. (Monimiaceae), which had passed through the digestive tract of three bird species, showed a higher percentage and shorter mean germination time when compared to seeds without this treatment, showing the efficiency of birds in the germination induction process (LEHOUCK *et al*., 2011). However, most studies focus on identifying the species of birds that disperse the seeds of a given plant and some evaluate *in situ* the seeds that have passed through the digestive system. Few studies evaluated, in the laboratory, forms similar to the conditions provided by the passage of seeds in the digestive tract of animals, which would be a pre-treatment alternative, being an accessible methodology to overcome the dormancy of yerba mate seeds.

In this sense, this work was based on the hypothesis that the simulation of the digestive tract of birds can help to overcome the dormancy of yerba mate seeds and reduce the time required for the production of seedlings. Therefore, the objective of this work was to evaluate the effect of pre-treatments, associating an acid environment in the stratification, on the emergence of *I. paraguariensis* seeds, in a commercial nursery.

MATERIAL AND METHODS

Although the species' dispersion unit is a pyrene (Figure 1A), the term seed will be used over this work to designate a diaspore, constituted by the endocarp and the seed (Figure 1B).

Seed collection

Seeds from two mother plants located in the municipality of Arvorezinha, Rio Grande do Sul, Brazil (28º 50' 56'' S 52º 14' 14'' W) were used. In this region, according to the Köppen and Geiger classification, the climate is Cfb (humid temperate climate with temperate summer) with an average temperature of 16.9 °C and an average annual rainfall of 1704 mm (CLIMATE, 2021).

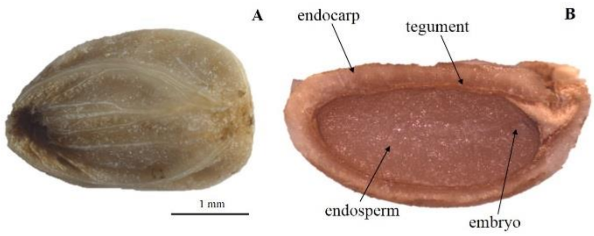

Figure 1: Yerba mate pyrene (A) consisting of the endocarp and the seed (composed of the tegument, endosperm and embryo) (B).
Figura 1: Pirênio de erva-mate (A) constituído pelo endocarpo e a semente (composta pelo tegumento, endosperma e embrião) (B).

In January 2018, naturally dispersed fruits (abscised) were collected on plastic canvases arranged in the projection of the canopy of the mother plants before fruit maturation began. The collection of these fruits was carried out in a dispersion interval of three days. Subsequently, they were taken to the laboratory and macerated in running water. The resulting mass, consisting of seeds and pulp, was submerged in a container with water, and then the supernatant seeds were discarded (WENDLING; SANTIN, 2015), which corresponded to approximately 10%. Then, the seeds remained to dry on paper towels on a laboratory bench for five days, after which they were homogenized, composing a single batch. Afterward, the water content was determined through the percentage difference in mass after drying in an oven at 105 ± 2 °C for 24 hours, using three repetitions of 0.5 g (BRASIL, 2009). The initial viability of the batch was also evaluated by the tetrazolium test. Subsequently, the seeds were stored in a glass *snap cap* and kept at a controlled temperature of 5 ± 2 °C for four months.

Pre-treatments of seeds in the laboratory

Part of the seeds was exposed to two pre-treatments:

- Pre-treatment 1 (PT1): seeds soaked in acidified aqueous solution (pH 2.78) for 48 hours at 40 °C.

- Pre-treatment 2 (PT2): seeds soaked in neutral aqueous solution (pH 6.95) for two hours at 40 °C. Subsequently, the pH of the water was adjusted to 3.5 with hydrochloric acid (1 N), remaining in this acidified aqueous solution for eight hours at a temperature of 40 °C. During this period, the pH was monitored every 30 minutes and, when necessary, hydrochloric acid or potassium hydroxide (1 N) was added to keep it constant. After, the seeds were immersed in acidified aqueous solution with pH 5.9, also at 40 °C, for 30 minutes. Then the seeds were transferred and kept in sieved and autoclaved sand with 60% of the water retention capacity (WRC), at a temperature of 25 °C for approximately 40 hours.

Stratification of seeds established in commercial nursery

The pre-treated seeds were immediately transferred to the nursery and, together with seeds without pre-treatment, were submitted to the stratification treatments:

- Treatment 1 (T1): the seeds went through pre-treatment 1 (PT1) (as described in the previous item) and were sand-stratified.

- Treatment 2 (T2): the seeds went through pre-treatment 2 (PT2) (as described in the previous item) and were sand-stratified.

- Treatment 3 (T3): seeds without pre-treatment and sand-stratified.

- Treatment 4 (T4): seeds without pre-treatment, stratified in the sand and buried at a depth of 20 cm in a well-drained site in the commercial nursery. The soil at this location has 21% clay, pH 4.8; 2.2% organic matter; 10.7 mg dm^{-3} of phosphorus, and 151 mg dm^{-3} of potassium.

For stratification, white polyethylene trays (192 x 116 x 64 mm) were used, where the seeds were placed on a black polyethylene screen with a 50-mesh sieve, and placed between two layers of sand, approximately 3 cm high each (1.0 L each). The sand was moistened with water at 60% of the WRC. The bottom of each tray was previously perforated (each tray with 12 holes of 3 mm in diameter) to enable drainage and, to avoid the loss of sand, a polyethylene screen was placed on the bottom of the tray and another on the sand top layer. The stratification treatments (T1, T2, T3, and T4) were maintained under the conditions of manual irrigation and management that the nursery worker adopts on farms, with daily monitoring, so that the sand remained with humidity close to 60% of the WRC during the entire experimental period. Each treatment consisted of four replications of approximately 1,000 seeds. The experimental design was completely randomized and the stratification period was 180 days.

Seed emergency

At 90, 135, and 180 days of stratification, a seed sample was taken from each stratification treatment (T1, T2, T3, and T4). These seeds were transferred to the laboratory where they were washed in autoclaved ultra-purified water. A control treatment (CT) - seeds without pre-treatment and without stratification, stored in a glass *snap cap* at a controlled temperature of 5 ± 2 °C. Seeds submitted to the two pre-treatments 1 and 2 (PT1 and PT2) were also used, but without stratification, which was denominated treatments 5 and 6 (T5 and T6) respectively, totaling seven treatments (Table 1) (Figure 3).

For all treatments, four replications of 100 seeds were used, which were submitted to the emergence in gerbox-type plastic boxes properly disinfected with 70% alcohol and 1.5% sodium hypochlorite (a. i.). Each gerbox-type received 250 g of sieved and autoclaved medium sand, moistened with autoclaved ultra-purified water to 60% of the WRC.

The gerbox-type were kept in Mangelsdorf-type seed germinator chambers at a temperature of 25 ± 1 °C and relative humidity > 90% without photoperiod control, in a completely randomized design. Emergence was monitored weekly for 180 days, and the seed that emitted any visible structure on the sand surface was considered to have emerged (BRASIL, 2009). The percentage of emergence was calculated based on the initial viability of the seed lot (64%).

Table 1. Description of the seed emergence treatments of *Ilex paraguariensis* A.St.-Hil.
Tabela 1. Descrição dos tratamentos de emergência de sementes de *Ilex paraguariensis* A.St.-Hil.

Treatment	Code	Pre-treatment	Stratification
Control treatment	CT	not applied	not applied
Treatment 1	T1	acidified aqueous solution - pH 2,78/48h	in sand
Treatment 2	T2	neutral aqueous solution - pH 6,95/2h, acidified aqueous solution - pH 3,5/8h and pH 5,9/30 min.	in sand
Treatment 3	T3	not applied	in sand
Treatment 4	T4	not applied	in sand and buried
Treatment 5	T5	acidified aqueous solution - pH 2,78/48h	not applied
Treatment 6	T6	neutral aqueous solution - pH 6,95/2h, acidified aqueous solution - pH 3,5/8h and pH 5,9/30 min.	not applied

Seed viability analysis

For each treatment, after completing 180 days in emergence, the tetrazolium test of the remaining seeds was performed. For that, the non-emerged seeds that remained in the gerbox-type were sieved and washed in autoclaved ultra-purified water to remove the excess sand. Then, a longitudinal section was performed with the aid of tweezers and a scalpel and subjected to a 0.1% tetrazolium solution for 24 hours at 35 °C (Souza *et al.*, 2020). Afterward, the seeds were analyzed with the aid of a Leica EZ4 HD stereoscopic microscope, with a magnification of 20 to 30 times, and evaluated for the reaction to the solution. Those that presented firm and colored endosperm, with visible embryo, without damage, and with a firm consistency were considered viable (Figure 2). All seeds that did not show these characteristics were classified as non-viable (BRASIL, 2009).

Data on the percentage of emergence, percentage of viable and non-viable seeds for 90, 135, and 180 days of stratification were submitted to analysis of variance (ANOVA) in a two-factor scheme (treatment x stratification time) and the means were compared by the test of LSD-Fisher at a 5% error probability level using the statistical programs Costat 6.4 and Sigmaplot 11.0. The data on the percentage of viable seeds at 180 days of stratification did not meet the ANOVA assumptions, so they were transformed to $\sqrt{x}$, and the results were shown in their original values.

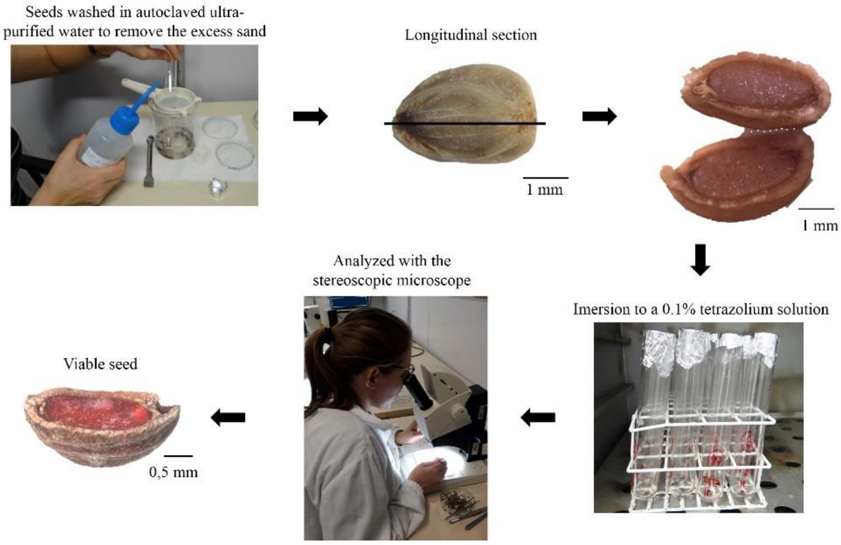

Figure 2: Analysis of the viability of *Ilex paraguariensis* A.St.-Hil. seeds.
Figura 2: Análise da viabilidade das sementes de *Ilex paraguariensis* A.St.-Hil.

RESULTS

The batch had 5.7% water content and the analysis of variance showed interaction ($p < 0.001\%$) between treatments and stratification time for the percentage of emergence, considering the initial viability of the batch of 64%. Table 2 shows the results of the percentage of emergence after 180 days, where it can be seen that the seeds stratified for 90 days did not emerge. Seeds stratified for 135 days showed an average emergence percentage of 0.9%, and the treatment with stratification in the sand and buried (T4) differed statistically from the others and showed the highest value (4.25%). At 180 days of stratification, an average emergence of 9.6% was obtained, with emphasis on the seeds exposed to a pre-treatment followed by stratification (T2), which presented 33% of emergence, followed by stratification in the sand and buried (T4) (19.28%) and sand stratification (T3) (10.46%).

Table 2. Mean seed emergence percentage of *Ilex paraguariensis* A.St.-Hil. at 180 days for the different treatments and periods of stratification.
Tabela 2. Porcentagem média de emergência de sementes de *Ilex paraguariensis* A.St.-Hil. aos 180 dias, para os diferentes tratamentos e tempos de estratificação.

Periods of stratification	90 days	135 days	180 days
Treatment	---------------% emergency ---------------		
CT - Seeds stored at 5 °C	0 a A	0 b A	0 e A
T1 - Seeds soaked in acidified aqueous solution + sand-stratified	0 a B	0 b B	4.25 d A
T2 - Seeds soaked in neutral aqueous solution + soaked in acidified aqueous solution + sand-stratified	0 a B	0.98 b B	33 a A
T3 - Seeds sand-stratified	0 a B	0.98 b B	10.46 c A
T4 - Seeds stratified in the sand and buried	0 a C	4.25 a B	19.28 b A
T5 - Seeds soaked in acidified aqueous solution	0 a A	0 b A	0 e A
T6 - Seeds soaked in neutral aqueous solution + soaked in acidified aqueous solution	0 a A	0 b A	0 e A
Mean	0	0.9	9.6

CT=Control treatment; T=Treatment; means followed by distinct lowercases in the same column and distinct uppercases in the same line are different between themselves by the LSD-Fisher test with a 5% error probability.
CT=Tratamento controle; T=Tratamento; médias seguidas por letras minúsculas distintas na coluna e letras maiúsculas distintas na linha diferem entre si pelo teste de LSD-Fisher a 5% de probabilidade de erro.

There was no interaction between treatments and stratification time for the percentage of viable and non-viable seeds. The emergence test with 90 days of stratification showed mean values of 11.75% of viable seeds and 88.25% of non-viable seeds (Table 3). For this stratification time, the treatment in which the seeds were stratified in the sand (T3) had the highest percentage of viable seeds (33.25%), followed by the control treatments (CT), stratified and buried seeds (T4), and pre-treatment in acidified aqueous solution with stratification (T1), which did not differ from each other.

For the emergence test with 135 days of stratification, a higher percentage of viable seeds (19.75%) was found in the control treatment (CT), but there was no statistical difference between the treatments where the seeds were buried and stratified (T4) and the pre-treatments followed by stratification (T1 and T2). Also, these three treatments did not differ from the seeds that were stratified in the sand (T3).

In the emergence test at 180 days of stratification, the control treatment (CT) showed the highest percentage of viable seeds (19.75%), followed by stratification in the sand (T3) and with pre-treatment in acidified aqueous solution followed by stratification (T1) (12.5% and 9.5%, respectively), followed by buried stratification (T4) and seeds pre-treated in a sequence of aqueous solution and stratified (T2), both with 5.5%.

Table 3. Viable and non-viable seed percentage of *Ilex paraguariensis* A.St.Hil. after 180 days of emergence, considering 90, 135 and 180 days of stratification.

Tabela 3. Porcentagem de sementes viáveis e não viáveis de *Ilex paraguariensis* A.St.Hil. após 180 dias de emergência, considerando os tempos 90, 135 e 180 dias de estratificação.

Periods of stratification	90 days		135 days		180 days	
Treatment	Viable	Non-viable	Viable	Non-viable	Viable	Non-viable
CT - Seeds stored at 5 °C	17.25 b	82.75 b	19.75 a	80.25 c	19.75 a	80.25 d
T1 - Seeds soaked in acidified aqueous solution + sand-stratified	14.75 b	85.25 b	14 ab	86 bc	9.5 b	90.5 bc
T2 - Seeds soaked in neutral aqueous solution + soaked in acidified aqueous solution + sand-stratified	0 c	100 a	13.75 ab	86.25 bc	5.5 c	94.5 b
T3 - Seeds sand-stratified	33.25 a	66.75 c	13.5 b	86.5 b	12.5 b	87.5 c
T4 - Seeds stratified in the sand and buried	17 b	83 b	14.5 ab	85.5 bc	5.5 c	94.5 b
T5 - Seeds soaked in acidified aqueous solution	0 c	100 a	0 c	100 a	0 d	100 a
T6 - Seeds soaked in neutral aqueous solution + soaked in acidified aqueous solution	0 c	100 a	0 c	100 a	0 c	100 a
Mean	11.75	88.25	10.79	89.21	7.54	92.46
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

CT=Control treatment; T=treatment; means followed by distinct lowercases in the same column are different between themselves by the LSD-Fisher test with a 5% error probability.

CT=Tratamento controle; T=Tratamento; médias seguidas por letras minúsculas distintas na coluna diferem entre si pelo teste de LSD-Fisher a 5% de probabilidade de erro.

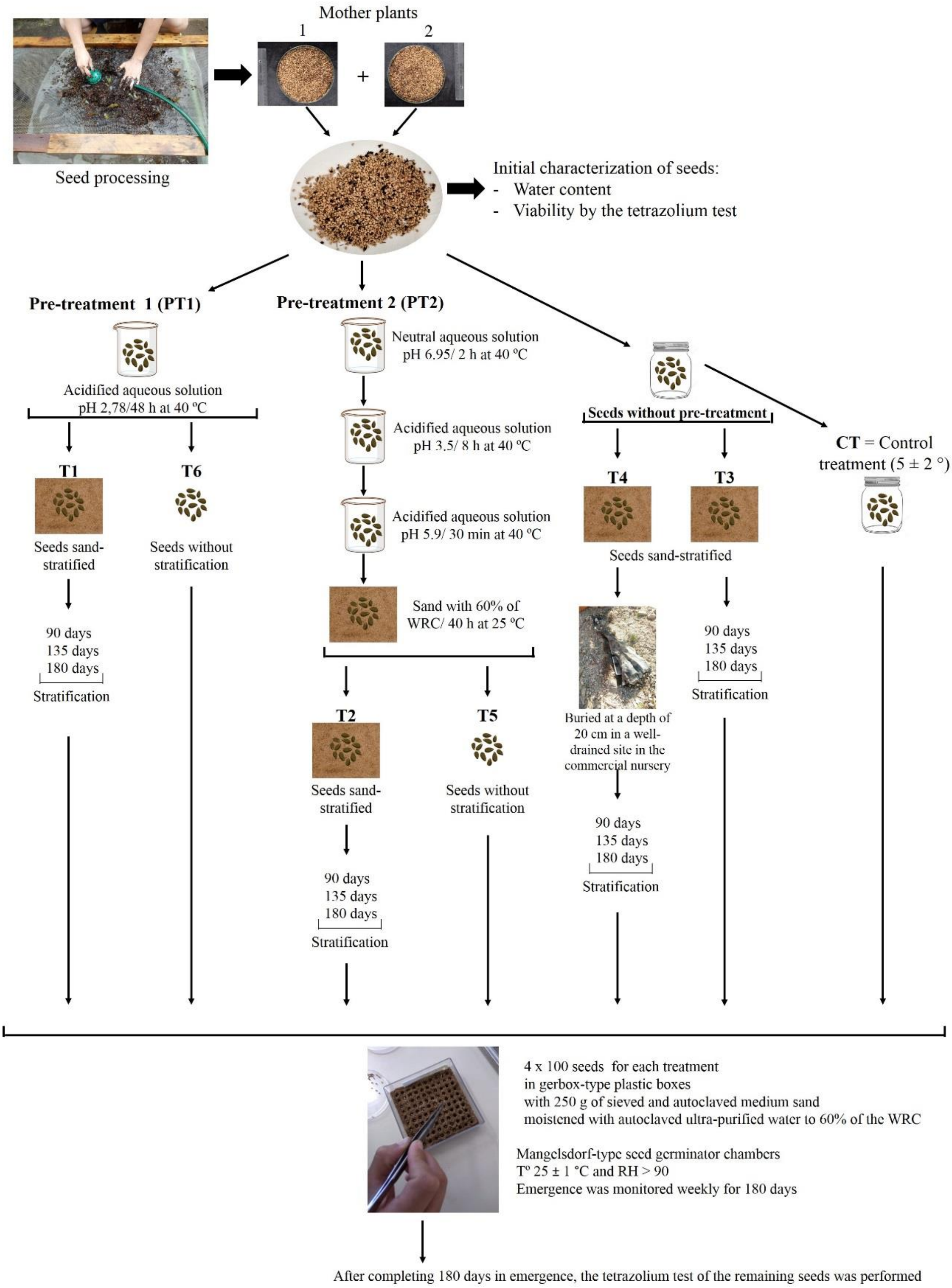

Figure 3: General scheme of material and methods.
Figura 3: Esquema geral do material e métodos.

DISCUSSION

Literature has no records of the use of pre-treatments with acidified aqueous solutions to overcome the dormancy of yerba mate seeds. Some studies tested pre-treatments, such as immersion in hot water (50 and 100 °C), immersion in distilled water, and coconut water for 24 hours, but the germination percentage was ≤ 1% (SCHAPARINI; VIECELLI, 2011).

Pre-treatments with seed imbibition in a sequence of aqueous solutions (T2 and T6) tried to simulate the digestive system of a bird, as in nature, birds are the main dispersers of yerba mate seeds (CARVALHO, 2003; COLUSSI; PRESTES, 2011). On the germination of *Copaifera langsdorffii* Desf. there was an increase of 50% in the acid treatment that simulated the effects of a disperser, presenting a satisfactory result when compared to seeds that did not undergo any treatment (SOUZA *et al*., 2015). In this work, the increase in emergence was 33% when there was a pre-treatment with neutral aqueous solution, followed by acid combined with stratification (T2).

The action of acid treatments is proven in overcoming physical dormancy, as it helps in softening the tegument. However, there is not much information on the effect on other types of dormancy. For most species that have drupe-type diaspores, such as yerba mate, the seeds do not have physical dormancy (BEWLEY *et al*., 2013; BASKIN; BASKIN, 2014). Thus, exposing the diaspore to acid helps soften the endocarp, which is extremely hard (BASKIN; BASKIN, 2014). The endocarp has several layers of lignified cells, which is the characteristic responsible for impairing water to enter the seeds (GRIGOLETTI JÚNIOR *et al*., 1999) and, consequently, for tissue expansion and embryo growth (DOLCE *et al*. 2010).

The effect of acid treatments may go beyond the physical effects on the seed tegument with physiological dormancy, as seed scarification is not necessary for germination (BASKIN; BASKIN, 2014). The action of intestinal acids can trigger several physiological processes that act directly on seed embryos and contribute to increasing germination (BRAVO *et al*., 2020). In seeds with morphological/morphophysiological dormancy, a significantly higher percentage of germination was observed after passage through the intestine of birds when compared to seeds without this passage (SOLTANI *et al*., 2018). In general, how the passage through the intestine affect's dormancy remains unclear (BRAVO *et al*., 2020), as well as the action of treatments with aqueous solutions that try to simulate these effects.

In seeds with morphophysiological dormancy, the tissues that surround the embryo (endosperm, tegument, pericarp) must undergo loosening and thus they become more fragile, constituting a key process for the removal of dormancy, as it allows the extension and protrusion of the radicle through these layers (BEWLEY *et al*., 2013). The endosperm of the mature seed may also be involved in the control of germination, serving mainly as a barrier to root development. When germination is stimulated, several enzymes are activated, the micropylar endosperm is weakened, causing the radicle to expand into the adjacent tissues (LINKIES *et al*., 2010). The pre-treatment, with a sequence of aqueous solutions, may have contributed to the weakening of the endosperm and mobilization of reserves for embryo nutrition, with consequent development and growth. Stratification aided in the removal of inhibitory compounds such as polyphenols (MIRESKI *et al*., 2018) which culminated in higher emergence rates observed in the T2 treatment.

The darkening of the endocarp was also observed in many seeds, which may be the result of the presence of fungi that act in its degradation (SOUZA *et al*., 2019) and contributed to significantly increasing the percentage of non-viable seeds in all treatments. During stratification and emergence, the development of fungi is promoted by the high humidity that occurs during the process (GRIGOLETTI JÚNIOR *et al*., 1999).

In seeds submitted to pre-treatments without stratification (T5 and T6), a high percentage of non-viable seeds (100%) was observed, in addition to the no emergence, confirming that yerba mate seeds need a period of stratification to overcome morphophysiological dormancy. In the seeds of these treatments (T5 and T6), it was verified that the endocarp was softened, with gelatinous or liquid endosperm and in many cases with a dark color. This may be caused by the pre-treatment associated with constant temperature (25 °C) without stratification, since the greater deterioration of yerba mate seeds was also observed in the stratification in the sand at 25 °C compared to those that were stratified at -2 °C (MENEGUETI *et al*., 2004). In the control treatment, a higher percentage of viable seeds was observed, that is, a greater presence of dormant seeds after 180 days of emergence, again showing the importance of stratification in overcoming dormancy.

It should be pointed out that the outcome achieved in this work was due to the association of the effects of the pre-treatment in a sequence of neutral and acidic aqueous solutions with the stratification (T2) as the action of only one of the factors was not effective, proving the initial hypothesis of this study. In addition, these results are linked to the specific environmental conditions existing during the stratification period, for this reason, further studies involving pre-treatments are needed to obtain higher germination rates. However, this work is pioneering and presented a high rate of emergence (33%) when compared to the works available in the literature that present values lower than 20%.

In general, the pre-treatment of the neutral aqueous solution, followed by acidified aqueous solutions associated with stratification (T2) contributed to the softening of the endocarp and tegument, promoting the input of oxygen and water, helping to mobilize reserves for the development, in addition to facilitating the removal of inhibitory compounds, thus overcoming morphophysiological dormancy and culminating in higher emergence rates.

Further studies are needed to increase the percentage of initial viability of the seed batch, as recent studies indicate that the low rates of the twinning of the species cannot be completely attributed to morphophysiological dormancy, but to the initial quality of the batch, which may have a high percentage of empty and deteriorated seeds (Souza *et al*., 2020). Thus, it is important to carry out seed processing studies, in addition to associating pre-treatments and chemical elements, particularly sources of nitrates in the stratification, because, in nature, the effect of fertilization through the deposition of fecal material around the seed can help germination and seedling establishment (LEHOUCK *et al*., 2011; SOLTANI *et al*., 2018).

CONCLUSIONS

It can be inferred through the analyses carried out in this experiment that:

- Pre-treatment with a neutral aqueous solution, followed by acidified aqueous solutions combined with stratification promotes the emergence of yerba mate seeds.

ACKNOWLEDGMENTS

We are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), to the nursery João Luís Citron which allowed seed collection and the installation of the stratification experiment, and to the Secretaria de Desenvolvimento Econômico, Ciência e Tecnologia (SDECT) of the State of Rio Grande do Sul government for financing the project.

REFERENCES

BASKIN, C. C.; BASKIN, J. M. **Seeds: ecology, biogeography, and evolution of dormancy and germination**. 2nd. ed. San Diego: Elsevier/Academic Press, 2014. 1586 p.

BEWLEY, J. D., BRADFORD, K. J.; HILHORST, H. W. M.; NONOGAKI, H. **Seeds: physiology of development, germination and dormancy.** 3. ed. New York: Springer, 2013. 392 p.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes.** Secretaria de Defesa Agropecuária. Brasília, DF: MAPA/ACS, 2009. 399 p.

BRAVO, C.; CHAMORRO, D.; HIRALDO, F.; SPEZIALE, K.; LAMBERTUCCI, S. A.; TELLA, J. L.; BLANCO, G. Physiological dormancy broken by endozoochory: Austral parakeets (*Enicognathus ferrugineus*) as legitimate dispersers of calafate (*Berberis microphylla*) in the Patagonian Andes. **Journal of Plant Ecology**, Oxford, v. 13, n. 5, p. 538-544, 2020.

CARVALHO, P. E. R. **Espécies arbóreas brasileiras.** v. 1. Brasília, DF: Embrapa Informação Tecnológica. 2003. 1039 p.

CLIMATE. **Dados climáticos para cidades mundiais.** Oedheim, 2021, Disponível em: http://pt.climate-data.org/. Accessed: 06 de apr. 2021.

COLUSSI, J.; PRESTES, N. P. Frugivoria realizada por aves em *Myrciaria trunciflora* (Mart) O. Berg. (Myrtaceae), *Eugenia uniflora* L. (Myrtaceae) e *Ilex paraguariensis* St. Hil. no norte do estado do Rio Grande do Sul. **Revista Brasileira de Ornitologia**, Basingstoke, v. 19, n. 1, p. 48-55, mar. 2011.

DIAZ, A. V. S.; SEOANE, C. E. S.; KAGEYAME, P. Y.; SEBBENN, A. **Diversidade genética, estrutura genética espacial e fluxo gênico em populações de erva-mate (*Ilex paraguariensis* A. St. Hil.) na área de entorno do Parque Nacional do Iguaçu.** Documento, 247. Colombo, PR: Embrapa Florestas, out. 2013. 48 p.

DOLCE, N. R.; MROGINSKI, L. A.; REY, H. Y. Endosperm and endocarp effects on the *Ilex paraguariensis* A. St. -HiL. (Aquifoliaceae) seed germination. **Seed Science and Technology**, Bassersdorf, v. 38, n. 2, p. 441-448, jul. 2010.

GALÍNDEZ, G. C. D.; BUBILLO, R.; LINDOW-LÓPEZ, L.; MALAGRINA, G.; ORTEGA-BAES, P.; BASKIN, C. C. Three levels of simple morphophysiological dormancy in seeds of *Ilex* (Aquifoliaceae) species from Argentina. **Seed Science Research**, Cambridge, v. 28, n. 2, p. 131-139, may 2018.

GRIGOLETTI JÚNIOR, A.; ZANON, A.; AUER, C. G.; FOWER, J. A. P. Efeito de fungicidas aplicados nas sementes, na emergência de plântulas de erva-mate (*Ilex paraguariensis* St. Hil.). **Boletim de Pesquisa Florestal**, Colombo, v. 9, p. 31-39, jul./dec. 1999.

LEHOUCK, V.; SPANHOVE, T.; LENS, L. Avian fruit ingestion differentially facilitates seed germination of four fleshy-fruited plant species of an Afrotropical forest. **Plant Ecology and Evolution**, Meise, v. 144, n. 1, p. 96-100, mar. 2011.

LINKIES, A.; GRAEBER, K.; KNIGHT, C.; LEUBNER-METZGER, G. The evolution of seeds. **New Phytologist**, Hoboken, v. 186, n. 4, p. 817-831, apr. 2010.

MENEGUETI, J. C. B.; NÓBREGA, L. H. P.; SMANHOTTO, A.; SILVEIRA, J. C. M. da. Superação da dormência de sementes de erva-mate (*Ilex paraguariensis* St. Hill) por estratificação. **Revista Varia Scientia**, Cascavel, v. 4, n. 8, p. 157-182, dec. 2004.

MIRESKI, M. C.; BÜHRER, C. de B.; HELM, C. V.; WENDLING, I.; VIEIRA, E. S. N.; SANTOS, A. F. dos; NOGUEIRA, A. C. Polyphenols in yerba mate seeds: potential inhibitors of germination. **Floresta**, Curitiba, v. 48, n. 4, p. 593-600, oct./dec. 2018.

PIRES, E. Z.; STEDILLE, L. I. B.; MACHADO, S.; MANTOVANI, A.; BORTOLUZZI, R. L. da C. Biologia reprodutiva de erva-mate (*Ilex paraguariensis* A. St. Hill.) em remanescente de Floresta Ombrófila Mista Altomontana. **Revista de Ciências Agroveterinárias**, Lages, v. 13, n. 2, p. 171-180, 2014.

SCHAPARINI, P. S.; VIECELLI, C. A. Superação de dormência de sementes de erva mate. **Cultivando o Saber**, Cascavel, v. 4, n. 4, p. 163-170, 2011.

SILVEIRA, F. A. O.; MAFIA, P. O.; LEMOS-FILHO, J. P.; FERNANDES, G. W. Species-specific outcomes of avian gut passage on germination of Melastomataceae seeds. **Plant Ecology and Evolution**, Meise, v. 145, n. 3, p. 350-355, nov. 2012.

SOLTANI, E.; BASKIN, C. C.; BASKIN, J. M.; HESHMATI, S.; MIRFAZELI, M. S. A meta-analysis of the effects of frugivory (endozoochory) on seed germination: role of seed size and kind of dormancy. **Plant Ecology**, Bassersdorf, v. 219, n. 11, p. 1283-1294, sep. 2018.

SOUZA, A. C.; OLIVEIRA, L. M.; SOUZA, G. F.; SCHMIDT, S. S.; LIESCH, P. P. Causes of low seed quality in *Ilex paraguariensis* A. St. Hil. samples (Aquifoliaceae). **Floresta e Ambiente**, Seropédica, v. 27, n. 4, e20170960, jul. 2020.

SOUZA, G. F.; OLIVEIRA, L. M. de; AGOSTINETTO, L.; PUCHALE, L. Z.; SÁ, A. C. S. Efeito da estratificação em substrato esterilizado na qualidade sanitária de sementes de *Ilex paraguariensis*, **Ciência Florestal**, Santa Maria, v. 29, n. 2, p. 854-862, abr./jun. 2019.

SOUZA, M. L.; SILVA, D. R. P.; FANTECELLE, L. B.; LEMOS FILHO, J. P. de. Key factors affecting seed germination of *Copaifera langsdorffii*, a Neotropical tree. **Acta Botanica Brasilica**, Brasília, DF, v. 29, n. 4, p. 473-477, oct./dec. 2015.

TRAVESET, A. Effect of seed passage through vertebrate frugivores' guts on germination: a review. **Perspectives in Plant Ecology, Evolution and Systematics**, Amsterdam, v. 1, n. 2, p. 151-190, 1998.

WENDLING, I.; SANTIN, D. **Propagação e nutrição de erva-mate.** Brasília, DF: Embrapa, 2015. 195 p.