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Seed dormancy and germination in Uvaia (*Eugenia pyriformis* Cambess)

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Abstract: South Brazil has a great diversity of alimentary fruit species. The uvaia (*Eugenia pyriformis* Cambess), along with other native species, has great economic potential due to its fruits and as a feedstock for the food industry. However, there is little information on seed dormancy for this species and neither a defined official protocol regarding dormancy overcoming, a necessary requisite for large-scale production. This work aimed to verify the presence of dormancy in *E. pyriformis* seeds, also proposing a possible method to overcome it. The applied seed breaking treatments were: mechanical scarification with sandpaper, chemical scarification using concentrated sulfuric acid, thermal shock using hot water, chemically induced germination using gibberellic acid, a combination of sulfuric acid and gibberellic acid treatments, a combination of hot water and gibberellic acid treatments, and a control (seed without treatment). The seeds were kept incubated in a BOD incubator for 60 days at 25±5 °C and a photoperiod of 8:16 h (light:dark). The percentage of germination and germination speed index (GSI) were evaluated. The results indicated that mechanical scarification induced faster germination (GSI values of 0.637 for mechanical scarification and 0.187 for the control) and germination percentages of 77%, while the control treatment presented 36%. The fresh collected *E. pyriformis* seeds may present dormancy, which was overcome by mechanical scarification.

Keywords: Dormancy overcoming methods, Gibberellic acid, Scarification, Thermal shock.

Resumo: *O Sul do Brasil apresenta grande diversidade de espécies nativas alimentícias. A uvaia (Eugenia pyriformis Cambess.), junto com outras espécies nativas, tem grande potencial econômico devido a seus frutos e como matéria-prima para a indústria alimentícia. No entanto, existe pouca informação sobre a existência de dormência de sementes para esta espécie, nem um protocolo oficial estabelecido em relação à superação de dormência, um requisito necessário para a produção desta espécie em larga escala. Este trabalho teve como objetivo verificar a presença de dormência em sementes de E. pyriformis, propondo também um possível método para superá-la. Os tratamentos de superação de dormência empregados foram: escarificação mecânica utilizando uma lixa, escarificação química com ácido sulfúrico concentrado, choque térmico utilizando água quente, indução química da germinação utilizando ácido giberélico, uma combinação dos tratamentos com ácido sulfúrico e giberélico, uma combinação dos tratamentos com água quente e ácido giberélico, e o controle (semente não tratada). As sementes foram incubadas por 60 dias em estufa BOD a 25±5 °C e fotoperíodo de 8:16 h (claro:escuro). A porcentagem de germinação e o Índice de Velocidade de Germinação (IVG) foram avaliados. Os resultados indicaram que a escarificação mecânica induziu uma germinação mais rápida (IVG de 0,637 para a escarificação mecânica e 0,187 para o controle) e porcentagem de germinação de 77%, enquanto que o controle apresentou 36%. As sementes de E. pyriformis recém-coletadas podem apesentar dormência, que foi superada com a escarificação mecânica.*

Palavras-chave: *Métodos de superação de dormência, Ácido giberélico, Escarificação, Choque térmico.*

1. INTRODUCTION

Uvaia (*Eugenia pyriformis* Cambess.), also commonly called ubaia and uvalha, is a Myrtaceae fruit plant from the Atlantic Forest; it can be found in Brazil, Argentina, and Paraguay. In Brazilian territory, it is distributed from the state of São Paulo to the state of Rio Grande do Sul [1-2].

The tree has a medium to big size, it can present height from 6 to 13 m, rounded treetop, trunk generally erect, with a diameter ranging between 30 to 50 cm. It presents disease resistance and rapid growth. In its native occurrence range, *E. pyriformis* blooms in August and September; fruiting occurs from September to November [1,3].

E. pyriformis propagation occurs by seed dispersion. The fruit is eaten by the wild birdlife and the seeds are dispersed. Its fruits are big fleshy drupes, with yellow or orange color. Each fruit has, generally, from one to three seeds. The seeds have nut-brown color and a relatively slender tegument, with about 1.0 to 1.5 cm [2-3].

E. pyriformis presents ornamental and economic potential due to its foliage characteristics, with silvery color, white flowers, and yellow/golden fruits. The *E. pyriformis* wood has characteristics of being hard, resistant, and heavy, being used by local populations to produce poles, stakes, firewood, and coal. Its fruits have a velvety taste, presenting a great economic potential to be consumed *in natura* or as juice, wine and vinegar, candies, jams, and others [3-4].

Andrade and Ferreira [4], besides citing the presence of some dormancy mechanism in *E. pyriformis* seeds, also cited this seed as being of a recalcitrant kind, i.e., the seed loses viability if it is dried beyond a critical humidity threshold. In this case, not necessarily a dormancy mechanism may be associated with the slow and non-uniform germination of *E. pyriformis*, but the death of the embryo occurs because of seed humidity loss during its storage [2].

The classical definition of seed dormancy corresponds to the blocking of the germination of a seed that is intact and is viable, in conditions that are favorable for the seed to germinate. Thus, the seed needs a post-maturation treatment or a dormancy

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overcome for its development to start and allow germination. However, dormant seeds present metabolic activity, although it is reduced [5-7].

The source of dormancy mechanism generally varies among species and habitat and the dormancy phenomenon may be a set of two or more dormancy mechanisms. It is necessary to know and understand the several dormancy mechanisms to determine the more adequate method to overcome it [5,8].

Seed dormancy can be divided into two big groups: dormancy from endogenous origin, and dormancy from exogenous origin. The dormancy from endogenous origin is associated with events bound to the embryo, while in exogenous dormancy the phenomenon is associated with the external structures, such as the tegument, the endosperm, or with the barriers imposed by the fruit [6,9].

Seed dormancy can be also classified as primary (or natural) and secondary (or induced). Primary dormancy presents itself as a seed's characteristic, developed when still in the mother plant, and remaining after the dispersion. On the other hand, secondary dormancy corresponds to the induction of dormancy due to unfavorable conditions to germination in non-dormant seeds or in the seeds whose primary dormancy has already been overcome [6-8].

Several dormancy overcoming mechanisms may be employed; however, the most suitable ones are linked to the dormancy kind present in the seed [10]. When there is tegumentary dormancy, caused by an impermeable seed tegument, methods aiming to soften and render the tegument more water-permeable are needed, such as water imbibition or mechanical/chemical scarification. In cases where the embryo has to be exposed to environmental conditions to complete its development, cold/hot stratification or light exposure may need to be used. For embryos that have germination inhibitors present in the cotyledons or that need to be exposed to heat to release germination factors, thermal shock using hot water may be employed. The induction of germination by imbibition in gibberellin solutions may also be carried out to enhance seed germination and vigor [6,10-11].

Several native species from South Brazil of the Myrtaceae botanical family and the *Eugenia* genus are regarded as having seed dormancy, but there are divergences in the literature. As examples can be cited the araçá (*Psidium cattleyanum* Sabine), guamirim (*Eugenia hiemalis* Cambess), pitangueira (*Eugenia uniflora* L.), guabiyu (*Myrcianthes pugens* Berg.), guabiroba (*Campomanesia xanthocarpa* O.Berg.), and the cherry of the Rio Grande (*Eugenia involucrata* DC.) among others [12-14].

Currently, there is no specific official protocol to test *E. pyriformis* seeds relative to dormancy overcoming. However, the use of an improper dormancy breaking method may harm the seed, reducing its vigor and fitness, or even rendering it non-viable. In this sense, it is important to determine/verify the mechanism or the main mechanism of dormancy, to use a suitable method to overcome it with and keeping the seed vigor as much as possible [6-7,12-13].

Due to the scarce literature on *E. pyriformis* seed dormancy and the methods to overcome it, this work aimed to verify the existence of seed dormancy in freshly collected *E. pyriformis* seeds and, if dormancy exists, the most efficient dormancy overcoming procedure for this species.

2. MATERIAL AND METHODS

Mature *E. pyriformis* fruits were collected in the 'Serra do Nordeste do Rio Grande do Sul' (Northeast Mountain Range of Rio Grande do Sul) region, in the municipality of Nova Roma do Sul, at the geographical coordinates 28°59'45" S, 51°26'57" W, and an altitude of 584 m. This region is classified as Cfa (subtropical humid climate), according to the Köppen classification [15].

The fruits were collected from a local plant population composed of ten *E. pyriformis* plants in an approximate area of 2,500 m^2 (50x50 m) of red latosol. The fruits were collected from the plants only (not from the ground). Approximately 200 fruits were collected from each plant, totaling 2,000 fruits. The fruits were considered to be mature when presenting a uniform yellow color. Immediately after collection, the fruits were pulped, and the seeds were washed with tap water. Posteriorly, the seeds were disinfected by bathing in a sodium hypochlorite solution at 1% v/v for 5 min. The seeds were washed with distilled water and dried away from sunlight at room temperature for 24 h. The germination tests were carried out immediately after seed drying.

The following dormancy overcoming methods were used: T1 - chemical scarification by immersion in concentrated (98%) sulfuric acid (Labsynth, Brazil) for 15 s; T2 - thermal shock by immersion in hot water at 80 °C for 1 min; T3 - chemical scarification with sulfuric acid for 15 s + immersion in a 500 $mg \cdot L^{-1}$ gibberellic acid solution for 24 h (ProGibb®,10 wt.% active ingredient, Sumitomo, Brazil) for 24 h; T4 - a combination of the sulfuric acid and gibberellic acid treatments; T5 - immersion in a 500 $mg \cdot L^{-1}$ gibberellic acid solution for 24 h; T6 - mechanical scarification using 150-grit sandpaper; and T7 - a control (seed without treatment). These treatments were based on the work of Barbiero et al. [16].

The germination tests were based on the procedures described in the Instructions for the Analysis of Forest Species and the Rules for Seed Analysis, with modifications [12-13]. Each treatment was divided into four replicates of 50 seeds each replicate, totaling 200 seeds for each treatment. The seeds were selected randomly from all plants. Each replicate was put in a transparent plastic gerbox with three sheets of blotting paper moistened with distilled water. The seeds were incubated in a CT-705.330 BOD incubator (Cientec, Brazil) for 60 days at 25±5 °C and a photoperiod of 8 h of light and 16 h of dark. The evaluations were carried out every 15 days up to 60 days (four evaluations). The germination percentage was calculated using Equation 1. The

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seeds were considered germinated when the radicle presented a length greater than 2 mm.

$$Germination\ (\%) = 100x\ \frac{G_n}{T} \qquad (1)$$

Being 'G_n' the number of seeds that germinated in the 'n'-th evaluation, and 'T' the total number of seeds in the replicate (50 seeds). The germination speed index (GSI) was calculated according to the formula proposed by Maguire [17], presented in Equation 2.

$$IVG = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \frac{G_3}{N_3} + \ldots + \frac{G_n}{N_n} \qquad (2)$$

Being G_x the number of normal seedlings counted in the 'x'-th evaluation and N_x the number of days since the seeds were sowed relative to the 'x'-th evaluation.

The data underwent Levene's normality test, followed by analysis of variance (ANOVA) and the means were compared by Fisher's Least Significant Difference (LSD) test at 5% probability. The statistical analysis was carried out using the Agrostat® software. The seed samples were registered in SISGEN (*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado*) system under the protocol AD8650D.

3. RESULTS AND DISCUSSION

After 60 days, the chemical scarification (T1), chemical scarification plus chemical germination induction (T3), thermal shock (T2), and thermal shock plus chemical germination induction (T4) did not present any seed that germinated. Table 1 presents the germination percentages for the treatments in each evaluation.

Table 1: Germination percentages of treated *E. pyriformis* seeds on each evaluation.

Treatment	Days after incubation			
	15	30	45	60
T1	0.00 b	0.00 b	0.00 c	0.00 c
T2	0.00 b	0.00 b	0.00 c	0.00 c
T3	0.00 b	0.00 b	0.00 c	0.00 c
T4	0.00 b	0.00 b	0.00 c	0.00 c
T5	0.00 b	10.67 b	24.00 b	33.33 b
T6	6.67 a	40.00 a	74.67 a	77.33 a
T7	0.00 b	4.00 b	16.00 b	36.00 b
CV (%)	91.65	55.88	32.41	25.88

Means in the same column followed by the same letter do not present statistical difference by Fisher's LSD test at 5% probability (α = 0.05). T1 – chemical scarification with sulfuric acid for 15 s; T2 – thermal shock with hot water (80 °C) for 1 min; T3 - chemical scarification with sulfuric acid for 15 s + immersion in a 500 mg·L^{-1} gibberellic acid solution for 24 h; T4 - thermal shock with hot water (80 °C) for 1 min + immersion in a 500 mg·L^{-1} gibberellic acid solution for 24 h; T5 - immersion in a 500 mg·L^{-1} gibberellic acid solution for 24 h; T6 – mechanical scarification with 150-grit sandpaper; T7 – control (seed without treatment). CV - Coefficient of variation.

According to Table 1, the mechanical scarification (T6) presented a statistical difference at all evaluations when compared to the control. The gibberellic acid treatment (T5) did not differ from the control, indicating the treatment does not overcome seed dormancy. It is also noteworthy the zero germination of the treatments T1-T4, even after 60 days of incubation. The high coefficient of variation observed at the start of the experiment (15 and 30 days) was due to most of the treatments having zero or low germination percentages at these evaluation times.

According to Barbiero et al. [16], who worked with Brazilian guava or araçá (*Psidium cattleyanum* Sabine - Myrtaceae) seed germination, the treatment of thermal shock using hot water also presented zero germination. The excessive heat may have led to the death of the embryo by protein denaturation. Since sulfuric acid dissolution in water is very exothermic, probably the seed was burned during the washing with distilled water. Since both GSI and germination percentages were zero for these treatments, they were not presented in the figures.

Regarding *E. pyriformis* seed germination, Silva et al. [18] (2003) reported germination percentages ranging from 98 to 100%, for untreated fresh collected seeds. On the other hand, Silva et al. [19], also working with the germination of fresh collected seeds over several substrates, reported germination percentages ranging from 30 to 80%. Andrade and Ferreira [4] reported a germination percentage of 67% for fresh seeds. Prataviera et al. [20] reported germination percentages of *E. pyriformis* seed samples, collected from several places of São Paulo and Paraná states, ranging between 57 and 100%. Costa et al. [21], working with 13 different *E. pyriformis* seed matrices, reported germination percentages ranging from 83 to 100%.

Considering the very different germination percentages reported by the literature, it is difficult to definitively establish if this species has seed dormancy or if dormancy is restricted to some plant populations, however, Andrade and Ferreira [4] stated that *E. pyriformis* presents seed dormancy. Nevertheless, several factors may influence seed germination, which may explain the great variation between results. The germination percentage obtained by mechanical scarification (77%) lies within the range commonly reported by other works (50-100% germination).

However, the germination percentage of the studied seeds, taking into account the control treatment (36%), was relatively low, and this may indicate a plant population that may have seed dormancy or low vigor seeds due to genetic or edaphoclimatic conditions. Andrade and Ferreira [4] also cited the *E. pyriformis* seed as recalcitrant, where the moisture content of the seed is critical for embryo survival and further development. In this sense, excessive drying of *E. pyriformis* seeds (seed moisture smaller than 15 wt.%) may render them non-viable.

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Figure 1 presents the evolution of the germination for the treatments T5, T6, and T7 in the four evaluations.

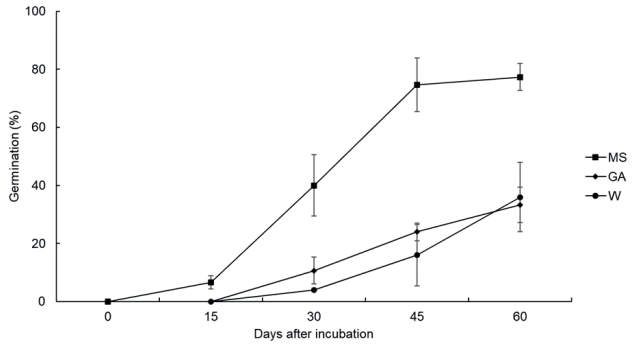

Figure 1 : Germination percentage of *E. pyriformis* seeds for the three dormancy breaking tests on each measurement. MS – mechanical scarification using sandpaper (T6); GA – immersion in gibberellic acid 500 mg·L^{-1} for 24 h (T5); W – control (T6). Source: Authors (2020).

By analyzing Figure 1, it is possible to see the germination curve of mechanical scarification (MS; T6) presented a greater slope, which indicates faster germination than the other treatments. About 50% of the mechanically scarified seeds germinated between 30-45 days, whereas both control (W; T7) and gibberellic acid (GA; T5) treatments presented approximately 30-36% after 60 days. Andrade and Ferreira [4] reported 60-70 days as the germination time for most fresh *E. pyriformis* seeds; the storage in cold chamber increases the germination time. On the other hand, Costa et al. [21] reported that the germination time for most fresh *E. pyriformis* seeds ranged between 7 and 30 days after incubation. Therefore, a 'population' effect may be present and affect seed vigor and fitness for germination.

Table 2 presents the GSI values for each treatment in each evaluation.

Table 2: GSI values of treated *E. pyriformis* seeds on each evaluation.

Treatment	Days after incubation			
	15	30	45	60
T1	0.000 b	0.000 b	0.000 c	0.000 c
T2	0.000 b	0.000 b	0.000 c	0.000 c
T3	0.000 b	0.000 b	0.000 c	0.000 c
T4	0.000 b	0.000 b	0.000 c	0.000 c
T5	0.000 b	0.089 b	0.163 b	0.201 b
T6	0.111 a	0.378 a	0.626 a	0.637 a
T7	0.000 b	0.033 b	0.104 b	0.187 b
CV (%)	90.82	69.92	27.56	24.13

Means in the same column followed by the same letter do not present statistical difference by Fisher's LSD test at 5% probability ($\alpha = 0.05$). T1 – chemical scarification with sulfuric acid for 15 s; T2 – thermal shock with hot water (80 °C) for 1 min; T3 - chemical scarification with sulfuric acid for 15 s + immersion in a 500 mg·L^{-1} gibberellic acid solution for 24 h; T4 - thermal shock with hot water (80 °C) for 1 min + immersion in a 500 mg·L^{-1} gibberellic acid solution for 24 h; T5 - immersion in a 500 mg·L^{-1} gibberellic acid solution for 24 h; T6 – mechanical scarification with 150-grit sandpaper; T7 – control (seed without treatment). CV - Coefficient of variation.

By analyzing Table 2, it can be seen that mechanical scarification (T6) induced higher GSI values (0.637), which means a faster germination rate relative to the gibberellic acid (0.202 - T5) and control (0.187 - T7) treatments. The gibberellic acid treatment (T5) has not differed from the control (T7), indicating that this dormancy breaking method is ineffective in accelerating seed germination. The high coefficient of variation, also observed in germination percentages (Table 1) at the start of the experiment (15 and 30 days) was due to most of the treatments having zero GSI values at these evaluation times.

Regarding GSI values, Silva et al. [18] reported GSI ranging from 0.539 to 0.699 for untreated, fresh-collected seeds. On the other hand, Silva et al. [19] reported GSI ranging between 0.202 and 0.579, also for fresh collected seeds. Costa et al. [21] reported a GSI of 1.13 for an incubation period of 35 days. The observed GSI value after 60 days for mechanical scarification (0.637) lies within most of the literature reports, but for untreated seeds. Taking into account the control treatment (0.187), it was quite low relative to other literature reports. In this sense, a possible 'population' effect may be present, as cited previously, which may render less vigorous and less fit seeds. Other factors may have also played a role during seed development that may render it less fit for germination [7,16].

Figure 2 presents the GSI values for each dormancy breaking treatment at each measurement.

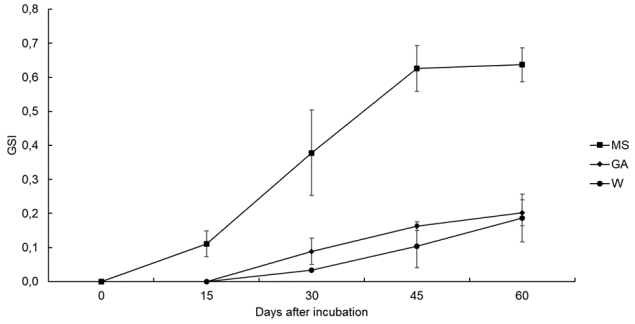

Figure 2 : Germination speed index (GSI) for the three dormancy breaking treatments on each measurement. MS – mechanical scarification using sandpaper (T6); GA – immersion in gibberellic acid 500 mg·L^{-1} for 24 h (T5); W – control (T7). Source: Authors (2020).

By analyzing Figure 2, it is possible to observe that the GSI value for mechanical scarification is more than three times the GSI of gibberellic acid treatment and the control, indicating that this treatment induced earlier germination. Despite the low germination percentage at 15 days after incubation (about 6%),

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Costa et al. [21], who evaluated 15 *E. pyriformis* matrices from São Paulo state, Southeast Brazil, cited that *E. pyriformis* seeds can germinate as earlier as one week, in contrast to Andrade and Ferreira [4], who cited that *E. pyriformis* seed does not germinate before 30 to 40 days after incubation; these authors evaluated *E. pyriformis* seeds collected in the municipality of Santa Maria, Rio Grande do Sul state (South Brazil). It is also remarkable the relatively low GSI of gibberellic acid and control treatments, indicating that the seeds may present a poor vigor when compared to seeds from other places.

Considering that scarification induces a decrease in tegument impermeability to water, allowing the water to enter in the seed and initiate germination; and gibberellic acid is a chemical germination inductor, which needs to surpass the tegument barrier to enter the seed and act in the embryo, very probably the *E. pyriformis* seed presents an exogenous dormancy, result of tegument impermeability [2,4].

This may indicate that treating the *E. pyriformis* seed with gibberellic acid to break seed dormancy or even accelerate germination without removing the impermeable tegument barrier does not have an impact on the seed, rendering the treatment ineffective.

A phenomenon noted in this work was the infection of the non-germinated seeds with fungi, probably Aspergillus sp., mainly between 30 to 45 days after incubation (mainly the seeds treated with the procedures that presented zero germination), even with the disinfection treatment. Andrade and Ferreira [4] cited the same phenomenon occurring in their work and stated that the fungi infection may be indicative of the non-viability of the seed.

The mechanical scarification provided a high germination percentage and a high GSI value, indicating that *E. pyriformis* seed dormancy most probably is due to tegument impermeability. Since the tegument is impermeable, an impaired water absorption very probably delayed the germination or even hindered it. The chemical scarification with sulfuric acid and thermal shock with hot water probably killed the embryo, since there was no growth in any of these treatments. The embryo death may be attributed to the excessive heat liberated over it during the dormancy overcome treatment. By analyzing the obtained results with literature data, it not possible to establish if *E. pyriformis* seeds have primary dormancy, however, the presence of dormancy cited in the literature may be also a secondary dormancy induced by storage or even the death of the seed due to excessive drying.

4. CONCLUSIONS

Mechanical scarification induced higher germination and GSI values, whereas all other treatments have not differed from the control. The treatments T1-T4 presented zero germination, very probably due to harm to the embryo, and, therefore, are not recommended to be used to overcome seed dormancy in *E. pyriformis*. The treatment using only gibberellic acid (T5) was ineffective in increasing both germination percentage and GSI. Thus, mechanical scarification of seed tegument using sandpaper may be employed as a treatment to overcome seed dormancy in fresh collected *E. pyriformis* seeds.

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