Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Evaluation of scarification in seed germination and characterization of the early stages of seedling development of *Rubus erythroclados*

Avaliação da escarificação na germinação de sementes e caracterização dos estágios iniciais de desenvolvimento das plântulas de *Rubus erythroclados*

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Abstract

The shrub *Rubus erythroclados* Mart. ex Hook.f. is endemic to Brazil and has high nutritional and medicinal potential. The objective of this study was to test scarification methods in the seed germination of *R. erythroclados* and to characterize the early stages of seedling development. The seeds were submitted to four treatments: 1) distilled water; 2) sodium hypochlorite (5% NaOCl); 3) sulfuric acid (80% H_2SO_4) and 4) sulfuric acid (98% H_2SO_4). The experimental design used was completely randomized. Each treatment (150 seeds) contained three replications with 50 seeds each, totaling 600 seeds. The seedling phase was also monitored weekly, from emergence to the loss of cotyledons. Furthermore, young plants were monitored until they acquired 5-leaflets. Seed germination had a general average of 46.3%. The treatment 5% NaOCl showed the highest mean germination (88.7%), followed by 80% H_2SO_4 (66.7%), both higher than the distilled water (30.0%). No seeds germinated in the 98% H_2SO_4 treatment. The seedling emergence time in the substrate was, on average, 54.4 days, ranging from 22.3 to 107.3 days, after sowing. The 5% NaOCl and 80% H_2SO_4 treatments increased the percentage of seed germination by scarification and reduced the seedling emergence time, being indicated for the cultivation of *R. erythroclados*. On the other hand, the 98% H_2SO_4 treatment is not indicated, as it possibly damaged the seeds. In the seedling phase, the growth in height was slow and the average number of leaves increased over the weeks. This phase lasted on average 107.9 ± 14.3 days. Pentafoliate leaves were observed on average 398.5 ± 54.7 days and lateral sprouting on average 435.7 ± 54.7 days, both after seedling emergence. These results indicate that plants produced by seeds may take some years to become sexually reproductive.

Keywords: medicinal plant, sexual reproduction, unconventional food plant.

Resumo

O arbusto *Rubus erythroclados* Mart. ex Hook.f. é endêmico do Brasil e apresenta alto potencial nutricional e medicinal. O objetivo deste estudo foi testar métodos de escarificação na germinação de sementes de *R. erythroclados* e caracterizar os estágios iniciais de desenvolvimento das plântulas. As sementes foram submetidas a quatro tratamentos: 1) água destilada; 2) hipoclorito de sódio (NaClO 5%); 3) ácido sulfúrico (H_2SO_4 80%); e 4) ácido sulfúrico (H_2SO_4 98%). O delineamento experimental utilizado foi o inteiramente casualizado. Cada tratamento (150 sementes) continha três repetições com 50 sementes cada, totalizando 600 sementes. A fase de plântulas também foi monitorada semanalmente, desde a emergência até a perda dos cotilédones. Além disso, plantas jovens foram monitoradas até adquirirem cinco folíolos. A germinação das sementes apresentou média geral de 46,3%. O tratamento NaClO 5% apresentou a maior média de germinação (88,7%), seguido por H_2SO_4 80% (66,7%), ambos superiores a água destilada (30,0%). Nenhuma semente germinou no tratamento H_2SO_4 98%. O tempo de emergência das plântulas no substrato foi, em média, de 54,4 dias, variando de 22,3 a 107,3 dias, após a semeadura. Os tratamentos NaClO 5% e H_2SO_4 80% aumentaram a porcentagem de germinação das sementes por escarificação e reduziram o tempo de emergência das plântulas, sendo indicados para o cultivo de *R. erythroclados*. Por outro lado, o tratamento com H_2SO_4 a 98% não é indicado, pois possivelmente danifica as sementes. Na fase de plântulas, o crescimento em altura foi lento e o número médio de folhas aumentou ao longo das semanas. Essa fase durou, em média, 107,9 ± 14,3 dias. Folhas pentafoliadas foram observadas, em média, 398,5 ± 54,7 dias e brotações laterais, em média, 435,7 ± 54,7 dias, ambas após a emergência das plântulas. Esses resultados indicam que plantas produzidas por sementes podem levar alguns anos para se tornarem sexualmente reprodutivas.

Palavras-chave: planta medicinal, reprodução sexuada, planta alimentícia não convencional.

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Received: April 13, 2024 – Accepted: September 30, 2024

1. Introduction

The genus *Rubus* (Rosaceae) is a highly diverse, including more than 750 species (Thompson, 1995) and has a global distribution (Hummer et al., 2019). Mostly the cultivation of plants of the genus *Rubus* is carried out in a vegetative way, however the use of seeds is of great importance for the genetic improvement and conservation programs (Clark et al., 2007). Due to the great diversity of *Rubus* species, a single standard protocol is unlikely to be useful for germinating seed of wild germplasm (Daubeny, 1996).

Seeds of this genus generally show dormancy of two natures: embryonic dormancy induced by specific germination inhibitors within the embryo or the presence of a dormant embryo, causing delay or even absence of germination; and/or dormancy imposed by the structure surrounding the embryo (rigid or impermeable integument), isolating the seed from the external environment, preventing its imbibition and gas exchange (Taylor, 2005; Zasada and Tappeiner III, 2003; Wada and Reed, 2011a).

In Brazil there are seven native species of *Rubus* (Carpanezzi et al., 2019). The native species of *Rubus* may have characteristics of interest for cultivation due to their hardiness and adaptation to climatic conditions, but none of the Brazilian species was domesticated. The domestication of native species, including those already known and used on a small scale by local and regional populations, still has little national or international market, and represents a great opportunity to be explored (Coradin et al., 2011). Furthermore, *Rubus* species are typical of early successional stages, indicating a potential for environmental recovery (Carpanezzi et al., 2019).

Among the native species, *Rubus erythroclados* Mart. ex Hook. f. is cited since the European arrival in Brazil, being its fruits known as apé at the time, already aroused the interest of colonizers (Hue, 2008). At the moment is popularly known as amora-verde, amora-branca or capinuriba-verde.

The species *R. erythroclados* is endemic to Brazil, occurring in the Atlantic Forest (Ombrophylous Mixed Forest and Campos) and in Cerrado Biomes, occurring in the South (Rio Grande do Sul, Santa Catarina and Paraná) and Southeast (São Paulo, Rio de Janeiro and Minas Gerais) regions of the Brazil (Simão-Bianchini, 2015). It is considered one of the three most important species of the genus *Rubus* in southern Brazil (Reitz and Klein, 1996). *R. erythroclados* small shrub, reaching up to two meters in height, with loose, red branches armed with numerous aculeus, its lower leaves 5-foliolate and the upper 3-foliolate, ovate-lanceolate leaflets, narrow, acuminate, flowers with white color and small, arranged in terminal panicles, fruit with green color, subglobose (Pio Corrêa, 1984; Baroni et al., 2023). It is described as an unconventional food plant, due to its green fruits with high sugar content and pleasant taste (Kinupp and Lorenzi, 2014; Bueno et al., 2018; Baroni et al., 2023). This species has medicinal properties, being used in popular phytotherapy (Cechinel Filho, 2000; Waltrich, 2014). Recent studies have demonstrated that the propagation of *R. erythroclados* by stem cuttings is viable (Balestrin et al., 2020, 2021). There are no data in the literature on its germination and seedling development. Thus, the objective of this study was to evaluate scarification in seed germination and to characterize the early stages of seedling development of *R. erythroclados*.

2. Material and Methods

2.1. Experimental location and conditions

The experiment was carried out from January 2021 to March 2023 in the Federal Institute of Rio Grande do Sul (IFRS) – *Campus* Sertão. The first part of the experiment (seed germination) was carried out at the Natural Sciences Laboratory, and the second part (seedling evaluation) was carried out in a greenhouse.

The seeds were collected from a home orchard located in the municipality of Getúlio Vargas, South Brazil (27° 52' 37" S, 52° 13' 35" W and 665 m.a.s.l.). The climate of the region, based on the Köppen classification, is *Cfa* (humid subtropical) with an average temperature of 17.7 °C and annual rainfall of 1,803.1 mm (Ramos et al., 2009). The local soil is classified as dystrophic Red Latosol (Oxisol) (Santos et al., 2018).

2.2. Treatments and experimental design

The seeds were submitted to four treatments: 1) distilled water – immersion in distilled water for 48 hours, adapted from Rawat et al. (2011); 2) sodium hypochlorite (5% NaOCl) – immersion in sodium hypochlorite for 16 hours and 32 hours in distilled water, according to Vásquez et al. (2019); 3) sulfuric acid (80% H_2SO_4); and 4) sulfuric acid (98% H_2SO_4) – both with immersion for one hour and 47 hours in distilled water, adapted from Bonilla Ponluisa (2013).

The experimental design used was completely randomized. Each treatment (150 seeds) contained 3 repetitions with 50 seeds each, totaling 600 seeds. The seeds were sown in gerbox containing sand as substrate and were kept in BOD germination chambers, with a temperature of 20 °C and a photoperiod of 12 hours.

After emergence, the seedlings were transplanted to plastic pots containing an organic substrate. We consider as seedling all phase with cotyledons. In this phase the following stages of seedling development were identified, namely: I – seedling with two cotyledons; II – seedling with two cotyledons and one leaf; III – seedling with two cotyledons and two leaves; IV – seedling with two cotyledons and three leaves; V – seedling with two cotyledons and four leaves; VI – seedling with two cotyledons and five leaves.

After loss of cotyledons, 10 young plants were monitored to evaluate the presence of compound leaves and the number of leaflets was counted. The emission of lateral sprouting was also evaluated.

2.3. Variables analyzed

The following variables were analyzed: seed germination (%), seedling emergency (days), stage duration (days),

seedling height (mm), hypocotyl diameter (mm), cotyledon length and width (mm); and leaf length and width (mm).

2.4. Statistical analysis

The data were submitted to the Shapiro-Wilk and Bartlett tests to verify the normality of the residues and the homogeneity of the variances, respectively. Subsequently, the data were submitted to ANOVA and Tukey's test (α = 0.05). Analyzes were performed using the Sisvar 5.6 software. In addition, young plant data were analyzed by descriptive statistics (mean and confidence interval, with α = 0.05).

3. Results

Seed germination showed an overall average of 46.3%. The 5% NaOCl treatment showed highest germination (88.7%), followed by 80% H_2SO_4 (66.7%), both being higher than the distilled water (30.0%). None seeds germinated in the 98% H_2SO_4 treatment.

The emergence time, from sowing to emergence of seedlings in the substrate, was on average 54.4 days, ranging from 23.3 to 107.3 days. Regarding the emergence time of the seedlings in the substrate, the treatments 5% NaOCl (23.3 ± 0.7 days) and 80% H_2SO_4 (32.7 ± 0.8 days) showed a shorter seedling emergency time in relation to distilled water (107.3 ± 24.2 days) (Table 1).

At all stages of seedlings there were no significant differences for the evaluated morphologic variables between treatments (Table 1). The different stages of seedling development (Figure 1) were described, namely:

Stage I – The mean duration of phase I was 9.6 days. At this stage, the mean was 6.0 mm for seedling height and 0.6 mm for hypocotyl diameter. The mean cotyledon length was 3.8 mm and the mean cotyledon width was 2.4 mm;

Stage II – The mean duration of phase II was 10.0 days. At this stage, the mean was 8.0 mm for seedling height and 0.6 mm for hypocotyl diameter. The mean cotyledon length was 4.5 mm and the mean cotyledon width was 3.0 mm. At this stage, the seedlings showed mean leaf length of 3.5 mm and mean leaf width of 2.5 mm;

Stage III – The mean duration of phase III was 14.8 days. At this stage, the mean was 9.7 mm for seedling height and 0.7 mm for hypocotyl diameter. The mean cotyledon length was 4.8 mm and the mean cotyledon width was 3.3 mm. At this stage, the seedlings showed mean leaf width of 3.5 mm and mean leaf length of 4.4 mm;

Stage IV – The mean duration of phase IV was 19.4 days. At this stage, the mean was 9.7 mm for seedling height and 0.6 mm for hypocotyl diameter. The mean cotyledon length was 4.9 mm and the mean cotyledon width was 3.5 mm. At this stage, the seedlings showed mean leaf length of 4.0 mm and mean leaf width was of 2.9 mm;

Stage V – The mean duration of phase V was 21.1 days. At this stage, the mean was 11.5 mm for seedling height and 0.6 mm for hypocotyl diameter. The mean cotyledon length was of 5.2 mm and the mean cotyledon width was of 3.5 mm. At this stage, the seedlings showed mean leaf length of 4.7 mm and mean leaf width of 3.5 mm;

Stage VI – The mean duration of phase VI was 31.5 days. At this stage, the mean was 12.0 mm for seedling height and 0.6 mm for hypocotyl diameter. The mean cotyledon length was of 4.5 mm and the mean cotyledon width was of 3.4 mm. At this stage, the seedlings showed mean leaf length of 3.3 mm and mean leaf width of 2.7 mm.

In *R. erythroclados*, in the seedling phase, the growth in height was slow (Figure 2a) and the average number of leaves increased over the weeks (Figure 2b). The duration of the all seedling phase, from seedling emergence in the substrate to loss of cotyledons by the seedling, lasted on average 107.9 ± 14.3 days, ranging from 55 to 203 days.

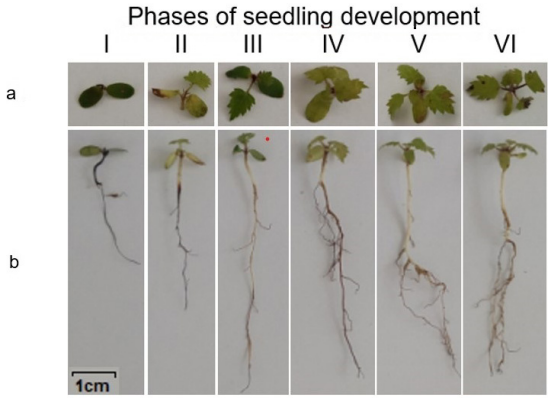

Figure 1. Phases of seedling development (I, II, III, IV, V, VI) of *Rubus erythroclados*. Illustrates a top view of the seedlings (a); and illustrates a side view of the seedlings (b).

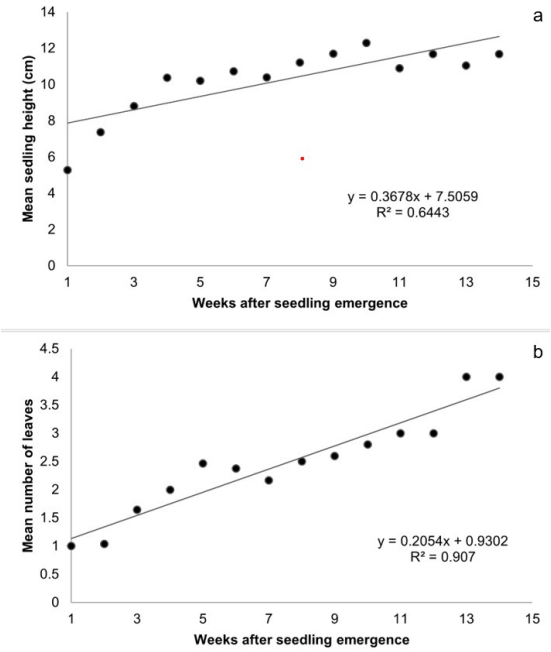

Figure 2. Mean height (a) and mean number of leaves (b) during the seedling development of *Rubus erythroclados* after their emergence on the substrate in all scarification treatments.

Table 1. Average germination and morphological attributes on stages of seedling development in different germination scarification treatments of *Rubus erythroclados*.

Stages	Morphologic attributes	Treatments				Overall mean	Coefficient of variation (%)
		Distilled water	5% NaOCl	80% H_2SO_4	98% H_2SO_4		
I	Seeds	150	150	150	150	150	-
	Germination	*n* = 45	*n* = 133	*n* = 100	*n* = 0	*n* = 92.7	-
	%	30.0b	88.7a	66.7a	0c	46.3	18.4
	Seedling emergency (days)	107.3b ± 24.2	23.3a ± 0.7	32.7a ± 0.8	-	54.4	88.1
	Stage duration (days)	8.6a ± 1.2	9.4a ± 0.7	10.9a ± 1.1	-	9.6	11.8
	Seedling height (mm)	5.2a ± 0.5	6.1a ± 0.4	6.7a ± 0.6	-	6.0	12.5
	Hypocotyl diameter (mm)	0.5a ± 0.03	0.5a ± 0.02	0.8a ± 0.3	-	0.6	31.5
	Cotyledon length (mm)	3.8a ± 0.3	4.1a ± 0.1	3.7a ± 0.3	-	3.8	6.1
	Cotyledon width (mm)	2.4a ± 0.2	2.6a ± 0.1	2.3a ± 0.2	-	2.4	5.9
II	Stage duration (days)	10.6a ± 1.2	8.7a ± 0.6	10.7a ± 0.8	-	10.0	10.5
	Seedling height (mm)	5.9a ± 0.5	8.5a ± 0.4	9.8a ± 0.6	-	8.0	12.7
	Hypocotyl diameter (mm)	0.6a ± 0.03	0.6a ± 0.03	0.6a ± 0.03	-	0.6	15.5
	Cotyledon length (mm)	4.6a ± 0.2	4.3a ± 0.1	4.6a ± 0.2	-	4.5	6.0
	Cotyledon width (mm)	3.1a ± 0.2	2.8a ± 0.1	3.2a ± 0.2	-	3.0	9.4
	Leaf length (mm)	3.9a ± 0.4	3.0a ± 0.2	3.8a ± 0.2	-	3.5	13.0
	Leaf width (mm)	2.8a ± 0.2	2.1a ± 0.1	2.8a ± 0.2	-	2.5	11.9
III	Stage duration (days)	19.4a ± 3.7	12.2a ± 0.9	12.9a ± 1.4	-	14.8	34.0
	Seedling height (mm)	8.1a ± 0.6	10.1a ± 0.5	10.9 ± 0.8	-	9.7	16.2
	Hypocotyl diameter (mm)	0.7a ± 0.03	0.7a ± 0.03	0.7a ± 0.04	-	0.7	11.3
	Cotyledon length (mm)	5.1a ± 0.2	4.5a ± 0.1	4.7a ± 0.2	-	4.8	5.2
	Cotyledon width (mm)	3.3a ± 0.2	3.0a ± 0.1	3.4a ± 0.1	-	3.3	5.5
	Leaf length (mm)	4.8a ± 0.3	4.1a ± 0.2	4.2a ± 0.2	-	4.4	6.9
	Leaf width (mm)	3.9a0.3	3.2a ± 0.2	3.4a ± 0.2	-	3.5	12.3
IV	Stage duration (days)	21.0a ± 5.8	18.7a ± 1.6	18.4a ± 2.4	-	19.4	7.4
	Seedling height (mm)	8.9a ± 1.2	9.5a ± 0.4	10.7a ± 0.7	-	9.7	21.1
	Hypocotyl diameter (mm)	0.6a ± 0.02	0.6a ± 0.02	0.6a ± 0.02	-	0.6	1.7
	Cotyledon length (mm)	5.2a ± 0.01	4.8a ± 0.1	4.7a ± 0.3	-	4.9	5.6
	Cotyledon width (mm)	3.3a ± 0.02	3.8a ± 0.1	3.5a ± 0.3	-	3.5	7.9
	Leaf length (mm)	4.2a ± 0.4	4.0a ± 0.1	3.9a ± 0.3	-	4.0	3.9
	Leaf width (mm)	2.5a ± 0.2	3.1a ± 0.1	3.1a ± 0.2	-	2.9	10.8
V	Stage duration (days)	28.0a ± 6.1	16.0a ± 0.9	19.3a ± 2.1	-	21.1	29.4
	Seedling height (mm)	10.9a ± 1.2	12.4a ± 0.3	11.1a ± 1.1	-	11.5	9.8
	Hypocotyl diameter (mm)	0.7a ± 0.02	0.5a ± 0.01	0.7a ± 0.03	-	0.6	14.5
	Cotyledon length (mm)	5.3a ± 0.02	5.0a ± 0.1	5.5a ± 0.2	-	5.2	4.8
	Cotyledon width (mm)	3.1a ± 0.02	4.0a ± 0.1	3.4a ± 0.04	-	3.5	11.9
	Leaf length (mm)	5.2a ± 0.4	4.5a ± 0.1	4.5a ± 0.2	-	4.7	8.9
	Leaf width (mm)	3.8a ± 0.3	3.3a ± 0.1	3.5a ± 0.3	-	3.5	7.1
VI	Stage duration (days)	31.5a ± 6.2	28.0a ± 1.2	35.0a ± 4.9	-	31.5	11.1
	Seedling height (mm)	11.3a ± 1.2	12.8a ± 0.6	11.9a ± 0.7	-	12.0	11.0
	Hypocotyl diameter (mm)	0.6a ± 0.03	0.7a ± 0.01	0.5 ± 0.01	-	0.6	15.7
	Cotyledon length (mm)	4.5a ± 0.2	6.0a ± 0.1	3.1a ± 0.1	-	4.5	32.6
	Cotyledon width (mm)	3.4a ± 0.1	4.2a ± 0.1	2.6a ± 0.1	-	3.4	23.1
	Leaf length (mm)	3.9a ± 0.4	4.7a ± 0.2	4.5a ± 0.2	-	3.3	39.3
	Leaf width (mm)	2.9a ± 0.2	3.6a ± 0.1	3.1a ± 0.1	-	2.7	34.3

The young plants showed, on average, bifoliate leaves in 283.5 ± 21.8 days, trifoliate leaves in 260.6 ± 21.4 days, tetrafoliate leaves in 381.8 ± 114.8 days, and pentafoliate leaves in 398.5 ± 54.7 days, after seedling emergence in the substrate. The young plants showed lateral sprouting on average 435.7 ± 54.7 days after seedling emergence in the substrate.

4. Discussion

The 5% NaOCl and 80% H_2SO_4 treatments increased seed germination of *R. erythroclados*, possibly due to the scarification of the integument. Most blackberry and raspberry (*Rubus*) seed exhibit delayed or poor germination because of a deep double dormancy (Wada and Reed, 2011b). The effectiveness of sulfuric acid for *Rubus* seed scarification is likely due to degradation of proanthocyanidins (PAs) in the testa (Wada et al., 2011). In *R. glaucus* Benth, the cultivars 'Castilla' and 'Andimora' treated with 5.25% sodium hypochlorite had high germination percentages (~80%) (Vásquez et al., 2019). Scarification with sodium hypochlorite (15% NaOCl) is recommended for sand blackberry seeds (*R. cuneifolius* Pursh) (Campbell et al., 1988). On the other hand, in dry seeds of six *Rubus* species, seeds scarified with NaOCl germinated more slowly and in significantly lower percentages for most species than seeds scarified with H_2SO_4 (Wada and Reed, 2011b).

Wada and Reed (2011b) when evaluating the germination of 17 species of *Rubus*, concluded that most of the freshly collected moist seeds from the species did not germinate, confirming that primary dormancy occurs in this genus. The dormancy of 'Castilla' blackberry seeds can be released by reducing coat impermeability through scarification with 5.25% sodium hypochlorite applied for 16 to 21 h (Díaz Diez et al., 2013).

In *R. coreanus* seeds the best germination was achieved by stratifying seeds for 30-45 days prior scarifying with H_2SO_4 for 15 minutes. The germination was completed in two stages, (a) cracking of coat and (b) rupturing of endospermic layer. Scarification with 95% sulfuric acid generates a higher number of seeds germinated in *R. idaeus*, in a shorter period time (first weeks), in comparison to others concentrations of sulfuric acid (0, 5, and 50%) (Contreras et al., 2016).

None seed of *R. erythroclados* germinated in 98% H_2SO_4 treatment possibly due to seed damage. The scarification time in H_2SO_4 can cause damage to the seeds (Hummer, 1996; Wada and Reed, 2011b; Díaz Diez et al., 2013). In *R. glaucus* sulfuric acid led to a germination percentage similar to the control as it could cause direct damage to the seed, affecting its emergence (Vásquez et al., 2019). On the other hand, the best treatment to scarify seeds of our *R. idaeus* crosses was with 95% sulfuric acid (Contreras et al., 2016). The genus *Rubus* is especially difficult due to the disparity in seed-coat thickness and structure (Daubeny, 1996).

Rubus species exhibited testa structure generally typical for the genus; however anatomical differences and unique composition of the cells, cell shapes, numbers and structure of layers, were noted among the species. These differences relate to the protective functions and germination inhibiting qualities of the seed coat (Wada et al., 2011). The observed differences in requirements for germination, were the thickness and hardness of the tegument key factor in studies carried out with eight wild species of *Rubus* (*R. arcticus*, *R. chamaemorus*, *R. leucodermis*, R. *niveus*, *R. odoratus*, *R. parviflorus*, *R. sanctus, R. urticifolius*) (Wada and Reed, 2011a).

There was no difference between the treatments for the variables (duration, height, diameter of the epicotyl, seedling height and width of the cotyledons and leaves), since the treatments used act only on germination.

The seedlings of *R. erythroclados* had simple leaves and, later on, the young plants added compound leaves with two, three, four or five leaflets. Breeding plants of *R. erythroclados* have digitate composite leaves with 3 (cauline apex) and 5-leaflets (Carpanezzi et al., 2019; Baroni et al., 2023).

In conclusion, in addition to slow germination, seedlings (107.9 ± 14.3 days, after emergence in the substrate) and young plants (435.7 ± 54.7 days for compound leaves to acquire 5 leaflets, after seedling emergence in the substrate) of *R. erythroclados* also showed slow development. These results indicate that plants produced by seeds may take some years to become sexually reproductive. In distilled water, *R. erythroclados* seeds had dormancy with low and slow germination. Scarification with 5% sodium hypochlorite and 80% sulfuric acid increased germination, possibly due to the thickness and hardness of the seed coat found in the genus *Rubus*.

Acknowledgements

Juliana Marcia Rogalski, Talissa Baroni, Luis Henrique Rigo, and Eduardo Bedin Pasquetti received a scholarship from PET Conexões – Licenciatura em Ciências Agrícolas (MEC/PET); Angela Julia Dorn received scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (PIBIC/CNPq); and Júlio Tagliari Balestrin received scholarship from Fundação de Amparo à pesquisa do Estado do Rio Grande do Sul (PROBIC/FAPERGS).

References

BALESTRIN, J.T., LAMB, R.O., FERRARI, V. and ROGALSKI, J.M., 2020. Estaquia caulinar de *Rubus erythroclados* Mart. ex Hook. f em diferentes concentrações de ácido indolbutírico. *RevUergs*, vol. 6, pp. 193-197.

BALESTRIN, J.T., BARONI, T. and ROGALSKI, J.M., 2021. Effect of cutting size on the performance of exogenous auxin IBA in vegetative propagation of *Rubus erythroclados* Mart. ex Hook. f. *Journal of Experimental Agriculture International*, vol. 43, pp. 56-66. http://doi.org/10.9734/jeai/2021/v43i830726.

BARONI, T., BALESTRIN, J.T., RIGO, L.H. and ROGALSKI, J.M., 2023. Biologia reprodutiva de *Rubus erythroclados* Mart. ex Hook. f.: uma espécie com potencial de uso. In: G. SEABRA, ed. *Terra: paisagens e sociobiodiversidade*. Ituiutaba: Barlavento, pp. 712-721.

BONILLA PONLUISA, W.E., 2013. *Escarificación química de la semilla de mora de castilla (Rubus glaucus Benth) variedad colombiana*

sin espinas. Cevallos: Facultad de Ingeniería Agronómica, Universidad Técnica, 124 p. Tesis en Ingeniería Agronómica.

BUENO, P.M.C., BIASI, L.A. and TOFANELLI, M.B.D., 2018. Micropropagation protocol for the wild Brazilian greenberry (*Rubus erythroclados*). *Revista Colombiana de Ciencias Horticolas*, vol. 12, no. 2, pp. 405-415. http://doi.org/10.17584/rcch.2018v12i2.7226.

CAMPBELL, P.L., ERASMUS, D.J. and VAN STADEN, J., 1988. Enhancing seed germination of sand blackberry. *HortScience*, vol. 23, no. 3, pp. 560-561. http://doi.org/10.21273/HORTSCI.23.3.560.

CARPANEZZI, A.A., ZANONA, K. and VOLTZ, R.R., 2019. *Separação botânica de espécies de Rubus da Região Metropolitana de Curitiba.* Curitiba: Embrapa Florestas. Documentos, no. 326.

CECHINEL FILHO, V., 2000. Principais avanços e perspectivas na área de produtos naturais ativos: estudos desenvolvidos no NIQFAR/Univali. *Química Nova*, vol. 23, no. 5, pp. 680-685. http://doi.org/10.1590/S0100-40422000000500017.

CLARK, J.R., STAFNE, E.T., HALL, H. and FINN, C.E., 2007. Blackberry breeding and genetics. In: J. JANICK ed. Plant Breeding Reviews. Portland: Timber Press, vol. 29, pp. 19-144. http://doi.org/10.1002/9780470168035.ch2.

CONTRERAS, E., GREZ, J. and GAMBARDELLA, M., 2016. Scarification and stratification protocols for raspberry (*Rubus idaeus* L.) seed germination. *Acta Horticulturae*, no. 1133, pp. 153-158. http://doi.org/10.17660/ActaHortic.2016.1133.23.

CORADIN, L., SIMINSKI, A. and REIS, A., 2011. *Espécies nativas da flora brasileira de valor econômico atual ou potencial*. Brasília: MMA.

DAUBENY, H.A., 1996. Brambles. In: J. JANICK and J.N. MOORE, eds. *Fruit breeding: vine and small fruits.* New York: John Wiley & Sons, vol. II, 477 p.

DÍAZ DIEZ, C.A., LOBO ARIAS, M., CARTAGENA VALENZUELA, J.R. and MEDINA CANO, C.I., 2013. Dormancy and germination of Castilla blackberry seeds (*Rubus glaucus* Benth). *Revista Facultad Nacional de Agronomía*, vol. 66, pp. 6855-6864.

HUE, S.M., 2008. *Delícias do descobrimento: a gastronomia brasileira no século XVI*. Rio de Janeiro: Zahar.

HUMMER, K., 1996. *Rubus* diversity. *HortScience*, vol. 31, no. 2, pp. 182-183. http://doi.org/10.21273/HORTSCI.31.2.182.

HUMMER, K.E., WILLIAMS, K.A. and BUSHAKRA, J.M., 2019. North American crop wild relatives of temperate berries (Fragaria L., Ribes L., Rubus L., and Vaccinium L.). In: S. GREENE, K. WILLIAMS, C. KHOURY, M. KANTAR and L. MAREK, eds. North American Crop Wild Relatives. London: Springer, vol. 2, pp. 283-327. http://doi.org/10.1007/978-3-319-97121-6_9.

KINUPP, V.F. and LORENZI, H., 2014. *Plantas Alimentícias Não Convencionais (PANC) no Brasil: guia de identificação, aspectos nutricionais e receitas ilustradas.* 1ª ed. São Paulo: Instituto Plantarum de Estudos da Flora.

PIO CORRÊA, M., 1984. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas.* Rio de Janeiro: Imprensa Nacional.

RAMOS, A.M., SANTOS, L.A.R. and FORTES, L.T.G., 2009. *Brazilian climate normals*. Brasilia: Inmet, pp. 1961-1990.

RAWAT, J.M.S., RAWAT, V. and TOMAR, Y.K., 2011. Seed germination and seedling performance of *Rubus ellipticus* (Smith) as influenced by various pre-treatments. *Journal of Hill Agriculture*, vol. 2, pp. 119-121.

REITZ, R. and KLEIN, R.M., 1996. Rosáceas. In: R. REITZ, ed. *Flora Ilustrada Catarinense*. Itajaí: Herbário Barbosa Rodrigues, pp. 1-135

SANTOS, H.G., JACOMINE, P.K.T., ANJOS, L.H.C., OLIVEIRA, V.A., LUMBRERAS, J.F., COELHO, M.R., ALMEIDA, J.A., ARAUJO FILHO, J.C., OLIVEIRA, J.B. and CUNHA, T.J.F., 2018. *Brazilian system of soil classification.* 5th ed. Brasília: Embrapa.

SIMÃO-BIANCHINI, R., 2015 [viewed 6 July 2023]. Rosaceae. In: JARDIM BOTÂNICO DO RIO DE JANEIRO, ed. *Lista de espécies da flora do Brasil* [online]. Rio de Janeiro: JBRJ. Available from: http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB32508

TAYLOR, K., 2005. Biological flora of the British Isles: *Rubus* vestitus Weihe. *Journal of Ecology*, vol. 93, no. 6, pp. 1249-1262. http://doi.org/10.1111/j.1365-2745.2005.01076.x.

THOMPSON, M.M., 1995. Chromosome numbers of *Rubus* species at the National Clonal Germplasm Repository. *HortScience*, vol. 30, no. 7, pp. 1447-1452. http://doi.org/10.21273/HORTSCI.30.7.1447.

VÁSQUEZ, W., PUPIALES, P., VITERI, P., SOTOMAYOR, A., FEICAN, C., CAMPAÑA, D. and VIERA, W., 2019. Chemical scarification and use of gibberellic acid for seed germination of blackberry cultivars (*Rubus glaucus* Benth). *Interciencia*, vol. 44, pp. 159-164.

WADA, S. and REED, B.M., 2011a. Optimized scarification protocols improve germination of diverse *Rubus* germplasm. *Scientia Horticulturae*, vol. 130, no. 3, pp. 660-664. http://doi.org/10.1016/j.scienta.2011.08.023.

WADA, S. and REED, B.M., 2011b. Standardizing germination protocols for diverse raspberry and blackberry species. *Scientia Horticulturae*, vol. 132, pp. 42-49. http://doi.org/10.1016/j.scienta.2011.10.002.

WADA, S., KENNEDY, J.A. and REED, B.M., 2011. Seed-coat anatomy and proanthocyanidins contribute to the dormancy of *Rubus* seed. *Scientia Horticulturae*, vol. 130, no. 4, pp. 762-768. http://doi.org/10.1016/j.scienta.2011.08.034.

WALTRICH, C.C., 2014. *Etnoconhecimento e hábitat de amoreiras-brancas (Rubus spp.).* Lajes: Universidade do Estado de Santa Catarina, 56 p. Dissertação de Mestrado em Produção Vegetal.

ZASADA, J.C. and TAPPEINER III, J.C., 2003. *Rubus* L. blackberry, raspberry. In: UNITED STATES DEPARTMENT OF AGRICULTURE, ed. *The woody plant seed manual.* Washington: USDA, pp. 984.