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Methods for overcoming dormancy in *Stryphnodendron pulcherrimum* seeds

Adriano Gonçalves Pereira[1], Eniel David Cruz[2]*, Hellen Síglia Demétrio Barros[3]

[1]Museu Paraense Emilio Goeldi, Av. Magalhães Barata, 328, CEP 66060-281, Belém, PA, Brasil

[2]Embrapa Amazônia Oriental, Travessa Dr. Enéas Pinheiro S/N, C P 48, CEP 66017-970, Belém, PA, Brasil

[3]Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Agronômicas, Rua José Barbosa de Barros, 1780, C P 237, CEP 18610-307, Botucatu, SP, Brasil

*Autor correspondente:
eniel.cruz@embrapa.br

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Abstract - Seed dormancy is a phenomenon observed in several tropical species. This condition causes low and non-uniform germination. The present study was designed to identify an efficient method of breaking seed dormancy in *Stryphnodendron pulcherrimum*. Seeds of four mother plants were subjected to the following treatments: immersion in sulfuric acid for 2, 4, 6, 8, 10 and 12 min and scarification on 150-grit sandpaper. Seeds were sown on substrate containing sand and sawdust (1:1). It was evaluate the days to onset seedlings emergence, seedlings emergence (SE), emergence speed index (ESI), germination (G), hard seeds (HS), dead seeds (DS), dormant seeds (DMS), abnormal seedlings (AS) and dry mass of aerial part (DMAP) and roots (DMR). The experimental design was completely randomized with four replications of 25 seeds for each treatment. Data were subjected to analysis of variance and means compared by Tukey's test ($p < 0.05$). Significant differences among treatments were observed for ESI, SE, G, HS, DMAP and DMR. Highest HS was observed in control treatment (85%). Highest G was observed in seeds scarified with sulfuric acid for 10 min (82%) and 12 min (74%). These treatments also showed highest ESI, DMAP and DMR, indicating that these scarification treatments were the most efficient in overcoming dormancy.

Métodos para superação da dormência em sementes de *Stryphnodendron pulcherrimum*

Resumo - A impermeabilidade do tegumento das sementes é um fenômeno observado em várias espécies tropicais. Essa condição causa germinação baixa e desuniforme. O presente estudo objetivou identificar métodos para superar a dormência em sementes de *Stryphnodendron pulcherrimum*. Sementes de quatro matrizes foram submetidas aos seguintes tratamentos: imersão em ácido sulfúrico por 2, 4, 6, 8, 10 e 12 min e escarificação com lixa. As sementes foram semeadas em substrato de areia e serragem (1:1). Avaliou-se os dias para iniciar a emergência de plântulas (DIE), emergência de plântulas (EP), índice de velocidade de emergência (IVE), germinação (G), sementes duras (SD), sementes mortas (SM), sementes dormentes (SDM) plântulas anormais (PA), massa seca da parte aérea (MSPA) e massa seca das raízes (MSR). O delineamento utilizado foi inteiramente casualisado com quatro repetições de 25 sementes por tratamento. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey ($p < 0,05$). Diferenças significativas entre tratamentos foram observadas para IVE, EP, G, SD, MSPA e MSR. Maior porcentagem de SD foi observada nas sementes não escarificadas (85%). Maior porcentagem de germinação foi observada nas sementes escarificadas em ácido sulfúrico por 10 min (82%) e 12 min (74%). Esses tratamentos também mostraram maiores IVE, MSPA e MSR, indicando serem os mais eficientes para superar a dormência.

Introduction

Seeds are considered the main or even the only propagation mean for many native tropical species. Research aiming to generate information about seed germination of these species is essential, since many of them are used for restoration of degraded areas, forest plantations or ecosystems conservation.

Seeds of many species, although viable and under favorable conditions, have non-uniform and slow germination and sometimes they remain as dormant and do not germinate. Immature embryos, presence of substances that inhibit germination and impermeability of seed coat to water and gases, which restricts embryo growth, are among the most frequent causes of seed dormancy (Metivier, 1979; Fowler & Bianchetti, 2000; Rodrigues et al, 2009).

Seed coat impermeability to water and gas exchange is a mechanism of dormancy in Fabaceae (Schmidt, 2007), but it is also founded in others families (Ballard, 1973; Atwater, 1980; Schmidt, 2007). In a study of 260 legume species Rolston (1978) and Maranho & Paiva (2012) noted that about 85% had seed coat totally or partially impermeable to water.

In order to overcome this kind of dormancy several treatments are recommended as scarification with sulfuric acid, scarification on abrasive surface and soaking in hot water (Schmidt, 2007). However each treatment has advantages and disadvantages and the method to be used should consider feasibility and cost of pre-treatment (Eira et al., 1993).

Stryphnodendron pulcherrimum (Willd.) Hochr. (Fabaceae) known in Brazil as "paricazinho", "faveira-camuzé" or "paricarana", has a limited natural occurrence area in South America, being the Amazon region probably the area with the highest concentration of the species (Occhioni, 1990).

According to Lorenzi (2002), *S. pulcherrimum* is a pioneer species recommended for reforestation, urban forestry and beekeeping. Its wood is moderately heavy and it is used for plywood, cable tools, firewood and charcoal. This species is also important for restoration of riparian vegetation (Oliveira et al., 2012).

Despite its importance *S. pulcherrimum* has limitations for seeds germination and consequently for seedlings production, because of hard-seediness, causing a slow and non-uniform germination.

The objective of this study was to identify an effective method to overcome dormancy of *S. pulcherrimum* seeds. The working hypothesis was that germination increases when seeds are scarified.

Materials and methods

Ripe fruits of four mother plants of *Stryphnodendron pulcherrymum* were collected on the ground, in a native forest area in municipality of Moju, Pará State (Table 1), during dry season. The fruits were transported in polypropylene bags to Belém, PA (01°26'14"S; 48°26'29"W) where experiment was carried out. The fruits were fumigated with Gastoxin to prevent insect damage and left at room environment for two months, until processing. After this period the fruits were manually opened and the seeds removed with tweezers. Seeds were stored in plastic bags with no control of air temperature and relative humidity during four months, until the beginning of the study.

Seeds were mixed proportionally. Seed moisture content was quantified in four replications of 10 seeds, in an oven with temperature of 105 ± 3 °C during 24 h (Brasil, 2009). Results were expressed as percentage of water content (fresh weight basis). Seeds were treated with sodium hypochlorite 2% for 3 min, washed in running tap water for 1 min and dried over paper towels for 3 h.

Table 1. Location, height, and diameter at 1.30 m above ground level (DBH) of *Stryphnodendron pulcherrimum* trees.

Mother plant	Latitude	Longitude	Height (m)	DBH (cm)
EDC 387	02°10'30.8"S	048°48'08.5"W	8.0	34.0
EDC 393	02°09'14.2"S	048°47'47.1"W	15.0	45.0
EDC 412	02°10'53.8"S	048°47'42.2"W	12.0	39.0
EDC 432	02°10'44.5"S	048°48'07.1"W	12.0	52.8

The following treatments were evaluated: control treatment (untreated seeds) immersion in sulfuric acid (H_2SO_4) for 2, 4, 6, 8, 10 and 12 min and scarification on 150-grit sandpaper (metal sandpaper). Scarification was on both cotyledons. In sulfuric acid treatments it was used 15 mL of acid for 110 seeds. After scarification, seeds were washed in running tap water during 10 min and dried over paper towels for 3 h.

Seeds were sown at depth of 1.0 cm in plastic pots (18 x 13 x 11 cm), using substrate containing sand and sawdust (1:1), previously heated at 100 °C for 2 h to reduce microbial contaminants (Cruz & Carvalho, 2003). The pots were irrigated every day.

It was evaluate days to onset seedlings emergence, seedlings emergence, emergence speed index, germination, hard seeds, dormant seeds, dead seeds, abnormal seedlings, dry mass of aerial part (stem + leaf) and dry mass of roots. Seedlings emergence was counted daily for 15 days. A seedling was considered emerged when the cotyledons stood at 0.5 cm above of the substrate surface.

At the end of seedlings emergence test the substrate was washed to quantify germination (normal seedlings) and dead, dormant and hard seeds. Normal seedlings were divided in aerial part and roots, placed in paper bags and weighted after dried at 65 °C for 48 h.

Experimental design was completely randomized with four replications of 25 seeds for each treatment. Data were subjected to Levene test for homogeneity of variance. The variables germination and hard and dead seeds were transformed using *Arcosen* $\sqrt{Vx/100}$ and roots dry mass was transformed using Log (x+1). Data were subjected to analysis of variance and means compared by Tukey's test ($p \leq 0.05$) using *Statistica* software. Data were back transformed for presentation.

Results and discussion

Seed moisture content of *Stryphnodendron pulcherrimum* was 11.6%. Significant differences ($p \leq 0.05$) between treatments were observed for number of days to the onset seedling emergence (DOE), emergence speed index (ESI), seedlings emergence (SE), germination (G) and hard seeds (HS) (Tables 2 and 3). Seedlings required 6 to 9 days to start emergence. The rate of seedling emergence was lower in seeds from control (4%) and scarification with sulfuric acid for 2 min (20%) treatments. The highest seedling emergence rates were observed in seeds scarified with sulfuric acid for 10 (83%) and 12 min (74%). These treatments also showed the highest emergence speed index.

Scarification with sulfuric acid for 10 min lowered in 25% the time required to start seedling emergence. According to Lorenzi (2002), seeds of this species when subjected to chemical scarification show improved seedling emergence.

In addition to improving seedlings emergence, scarification also contributed to increase germination, especially in treatments with sulfuric acid for 10 and 12 min (Table 3), resulting in better uniformity of seedlings. Cruz et al. (2007) reported a similar result in *Schizolobium amazonicum* Huber ex Ducke seeds scarified with sulfuric acid for 60 min.

Table 2. Number of days to the onset of seedlings emergence (DOE), seedlings emergence speed index (ESI), and seedlings emergence (SE) in *Stryphnodendron pulcherrimum* in response to different scarification methods.

Treatments	DOE	SE (%)	ESI
Control	9.0 b	4 d	0.115 c
Scarification in H_2SO_4 for 2 min	8.5 ab	20 d	0.528 c
Scarification in H_2SO_4 for 4 min	7.0 ab	58 bc	1.720 b
Scarification in H_2SO_4 for 6 min	8.0 ab	58 bc	1.532 b
Scarification in H_2SO_4 for 8 min	7.0 ab	52 c	1.518 b
Scarification in H_2SO_4 for 10 min	6.8 a	83 a	2.396 a
Scarification in H_2SO_4 for 12 min	7.0 ab	74 ab	2.290 a
Scarification on sandpaper	7.0 ab	65 bc	1.962 ab

Means presenting the same letter within columns are not significantly different by Tukey's test ($p > 0.05$).

Table 3. Germination (G), abnormal seedlings (AS), hard seeds (HS), dormant seeds (DMS) and dead seeds (DS), in *Stryphnodendron pulcherrimum* in response to the scarification method.

Treatments	G	AS	HS	DMS	DS
			(%)		
Control	4 d	*	85 f	7	4
Scarification in H_2SO_4 for 2 min	19 d	1	65 d	1	11
Scarification in H_2SO_4 for 4 min	58 bc	*	28 bc	*	14
Scarification in H_2SO_4 for 6 min	58 bc	5	28 bc	*	9
Scarification in H_2SO_4 for 8 min	52 c	1	34 c	2	11
Scarification in H_2SO_4 for 10 min	82 a	4	7 a	2	5
Scarification in H_2SO_4 for 12 min	74 ab	5	2 a	1	18
Scarification on sandpaper	65 bc	3	20 b	2	10

Means presenting the same letter within columns are not significantly different by Tukey`s test ($p > 0.05$). *Zero values excluded from analysis of variance.

Seeds treated with sulfuric acid for 10 and 12 min showed lower percentage of hard seeds, while no difference among treatments could be detected for abnormal seedlings and dormant and dead seeds (Table 3). In general, the increase in seeds exposition to acid reduced the hardseedness. Although the scarification with sandpaper increased germination rate, this treatment was inefficient in 20% of seeds. Varela et al. (1991) studying this species obtained 75% of germination when seeds

were scarified with sandpaper and remained in water for 6 h. According to Dias (2005) the intensity of dormancy for the same species may vary according to genotype and environment in which seeds are produced, besides others factors.

The efficiency of scarification with sulfuric acid to overcome seed coat impermeability and increase seed germination has been reported for different species as *Bowdichia virgilioides* Kunth. (Albuquerque et al., 2007), *Ceiba glaziovi* (Kuntze) K. Schum (Nascimento, 2012), *Senna silvestres* (Vell.) H.S. (Maranho & Paiva, 2012), *Centrosema plumieri* Benth. (Gama et al., 2011), *Sesbania virgata* (Cav.) Pers. (Silva et al., 2011), *Colubrina glandulosa* Perk. (Brancalion et al., 2011), *Stryphnodendron adstringens* (Mart.) Coville (Martins & Nakagawa, 2008; Martins et al., 2008) and *S. polyphyllum* Mart. (Martins et al., 2008). However, the efficiency of this treatment varies with the acid concentration, plant species and treatment duration (Baskin & Baskin, 1998).

Although scarification with sulfuric acid for 10 and 12 min has provided the highest germination rates and the lowest hard seeds rates, it was observed on seeds scarified for 12 min an increase of dead seeds rate, probably due to damage on embryos caused by sulfuric acid. According to Rolston (1978), during chemical scarification seed coat is degraded and the increase of seeds exposure may cause cell rupture, favoring mechanical injuries and fungi penetration that affects seed germination.

For most scarification treatments the maximum seedlings daily emergence was recorded on the 8th day after sowing (sulfuric acid for 4 min, 8 min, 10 min and sandpaper). Maximum emergence was observed on the 7th and 9th day, when seeds were scarified with sulfuric acid for 12 min and 6 min, respectively. Seeds scarified with sulfuric acid for 2 min showed maximum emergence from 8th to 10th day after sowing (Figure 1).

Aerial part and roots dry mass were higher for plants originated from seeds treated with sulfuric acid for 10 and 12 min (Table 4), indicating that these scarification treatments were the most efficient to overcome dormancy. The efficiency of these treatments is related to greater accumulation of aerial part and roots dry mass of seedlings due to higher seed germination.

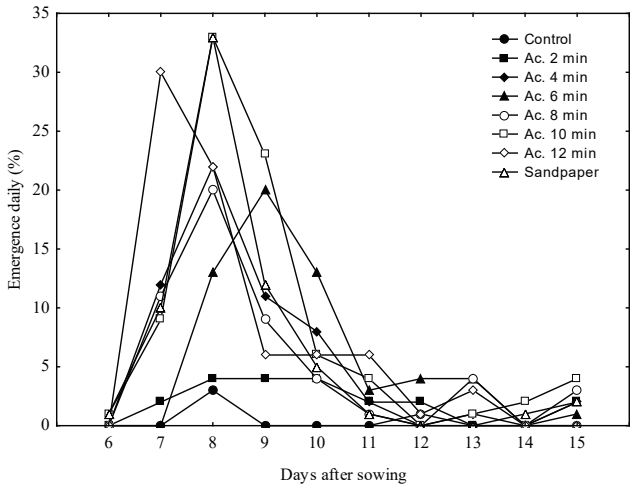

Figure 1. Emergence daily in *Stryphnodendron pulcherrimum* seeds in response to the scarification method.

Tabela 4. Dry mass of aerial part (DMAP) and of roots (DMR) in seedlings of *Stryphnodendron pulcherrimum*.

Treatments	DMAP (g.seedling^{-1})	DMR (g.seedling^{-1})
Control	0.0109 c	0.0024 c
Scarification in H_2SO_4 for 2 min.	0.0321 c	0.0060 c
Scarification in H_2SO_4 for 4 min.	0.1648 ab	0.0348 ab
Scarification in H_2SO_4 for 6 min.	0.1449 b	0.0276 ab
Scarification in H_2SO_4 for 8 min.	0.1327 b	0.0208 b
Scarification in H_2SO_4 for 10 min.	0.2090 a	0.0399 a
Scarification in H_2SO_4 for 12 min.	0.2163 a	0.0301 a
Scarification on sandpaper	0.1897 ab	0.0323 ab

Means presenting the same letter within columns are not significantly different by Tukey`s test ($p > 0.05$).

Scarification with sandpaper is feasible for small number of seeds to be practical, inexpensive and safe (Hermansen et al., 2000; Santos et al., 2004). However, scarification of large amount of seeds using this treatment may be unfeasible due to its difficulty on executing (Oliveira et al., 2003).

On the other hand, sulfuric acid scarification is useful for seeds of small size (Cortines et al., 2010), such as *S. pulcherrimum*. Despite its efficiency, sulfuric acid is difficult to acquire (Martins et al., 2008) and it must be used with special care, as it is highly corrosive hazardous substance, which can cause severe burns on contact with skin or eyes (Cruz & Pereira, 2014).

Conclusion

Dormancy of *Stryphnodendron pulcherrimum* can be effectively overcome by scarification with sulfuric acid for 10 and 12 min.

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