

Seed anatomy and water uptake and their relation to seed dormancy of *Ormosia paraensis* Ducke¹

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ABSTRACT - *Ormosia paraensis* Ducke has ornamental seeds widely used in the manufacture of bio-jewels and wood used in the furniture industry. For seedling production, the information on its seed physiology is scarce. Thus, the aim of this study was to assess methods for breaking dormancy and relate them to integument structure and water uptake by *O. paraensis* seeds. Seed dormancy-breaking was performed by mechanical scarification and soaking in sulfuric acid for 0, 15, 30, 60, 120, and 240 minutes. Dormancy-broken seeds were compared with intact seeds. Seed integument is formed by a cuticle (hydrophobic substances), epidermis (macroscleroids of the palisade layer.), hypodermis (osteosclereids), and parenchyma cells. Intact seeds did not absorb water after 72 hours of soaking. The highest percentages and rates of seed germination were observed in treatments with mechanical scarification and soaking in sulfuric acid for 60 or 120 minutes. Seed soaking in sulfuric acid (H₂SO₄ p.a. 98.08%) for 60 or 120 minutes or mechanical scarification are adequate to overcome physical dormancy associated with *O. paraensis* seed integument impermeability to water or gases.

Index terms: breaking dormancy, germination, scarification, Fabaceae (Leguminosae).

Anatomia de sementes e absorção de água e sua relação com a dormência de sementes de tento (*Ormosia paraensis* Ducke)

RESUMO - *Ormosia paraensis* Ducke, conhecida popularmente por tento, possui sementes ornamentais amplamente usadas na confecção de bijóias, assim como, sua madeira é usada na indústria moveleira. Para produção de mudas, as informações sobre a fisiologia das sementes de tento são escassas. Desta forma, o objetivo da presente pesquisa foi avaliar métodos para a quebra de dormência, assim como, relacioná-los com a estrutura do tegumento e com a absorção de água pelas sementes. Para a quebra de dormência das sementes, foi utilizada a abrasão com lixa e a imersão em ácido sulfúrico por 0, 15, 30, 60, 120 e 240 minutos em comparação com sementes intactas. A semente de tegumento é formada pela cutícula (substâncias hidrofóbicas), epiderme (camada paliçada - macrosclerides), hipoderme (osteoesclerides) e células parênquimáticas. As sementes intactas não absorveram água após 72 horas de imersão. As maiores porcentagens e taxas de germinação de sementes foram observadas nos tratamentos de escarificação mecânica e imersão em ácido sulfúrico por 60 ou 120 minutos. A imersão em ácido sulfúrico (H₂SO₄ - p.a. - 98,08%) por 60 ou 120 minutos ou a abrasão mecânica com lixa são adequadas para a superação de dormência física associada à impermeabilidade do tegumento das sementes de tento a água ou gases.

Termos para indexação: quebra de dormência, germinação, escarificação, Fabaceae (Leguminosae).

Introduction

Timber use is a very small fraction of the multiple non-timber forest uses, such as handicrafts, production of oils,

resins, and dyes, folk medicine and holotherapy, food, and afforestation and landscaping, among others, of Fabaceae species (Ferreira et al., 2005; Chevreuil et al., 2011). However, there is a shortage of information on the Amazonian forest

¹Submitted on 03/26/2017. Accepted for publication on 04/11/2018.

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species aiming at the domestication and potentialization of their use for the Amazon and Brazilian economy.

Among the Fabaceae forest species, *Ormosia paraensis* Ducke is a characteristic tree species of the dense ombrophilous and secondary forests of the Amazon used for timber purposes in the civil construction and furniture production, recovery of degraded areas, and production of handicrafts with seeds (Barros et al., 2009; Castro et al., 2012; Ribeiro et al., 2013).

Seeds of *O. paraensis* are stenospermic, bitegumented, exalbuminous, rounded, bicolored (red and black), have average dimensions of $12.36 \times 9.68 \times 8.03$ mm (length \times width \times thickness) and dry matter content of 0.45 g. seed⁻¹. Seed integument is formed by four distinct layers: cuticle (coating with hydrophobic substances), epidermis (with a layer of compact palisade consisting of radially elongated macrosclereids and densely thickened cell walls), hypodermis (hourglass cells or osteosclerotids), and parenchyma cells (Silva et al., 2015).

Most of the *Ormosia* Jacks seeds are ornamental, hard, and red-colored, commonly with a black spot and rarely yellow. Because of their hardness and resistance, seeds of several *Ormosia* species are used for making handicrafts. However, this hardness hinders seed analysis and seedling production (Lopes et al., 2006; Gonçalves et al., 2011; Baigts, 2009; Teixeira et al., 2009; Basqueira et al., 2011; Curiel and Morais, 2011; Gurski et al., 2012; Silva and Moraes, 2012; Silva et al., 2014; Silva et al., 2015; Vargas-Simón et al., 2017).

According to Carvalho and Nakagawa (2012) and Marcos-Filho (2015), seeds require a series of endogenous (maturity and hormonal balance) and exogenous factors (water, temperature, and substrate) to germinate. If these factors are favorable for germination and viable seeds do not germinate, they are in a state of dormancy. In seeds, dormancy is caused by integument impermeability to water or gases, presence of germination inhibiting or promoting substances, occurrence of immature embryos, and special requirements of light and temperature, among others (Carvalho and Nakagawa, 2012; Baskin and Baskin, 2014).

Seed dormancy is a natural strategy that maximizes the probability of seedling establishment, limits short-term germination opportunities, and avoids germination when conditions are not favorable (Baskin et al., 2006; Van-Klinken and Goulier, 2013; Baskin and Baskin, 2014).

According to Deminicis et al. (2006), Sautu et al. (2006; 2007), Hu et al. (2009), Van-Klinken and Goulier (2013), and Andreani-Junior et al. (2014), several Fabaceae species are known to have, in most cases, seed integument resistant and impermeable to water or gases. Seed integument impermeability in Fabaceae species is an important feature for species permanence in the field under adverse climatic

and pathogen conditions, thus being able to remain viable in the soil for a long period composing a seed bank (Souza and Marcos-Filho, 2001; Deminicis et al., 2006).

Under natural conditions, seeds with impermeability are subject to the action of abiotic (thermal amplitude, precipitation, soil pH, light exposure, and abrasion with soil particles) and biotic (fungi, bacteria, and animals) factors on seed integument, promoting the necessary opening for water uptake and hence germination (Carvalho and Nakagawa, 2012; Baskin and Baskin, 2014). However, seed dormancy in the nursery is one of the main problems for seedling production of forest species (Carvalho and Nakagawa, 2012).

For the seedling production of *O. arborea* (Vell.) Harms, Silva et al. (2014) indicated seed soaking in sulfuric acid (100%) for 15 minutes to obtain 91% germination, while Lopes et al. (2006) recommended seed soaking in sulfuric acid for 10 minutes or mechanical abrasion to achieve 96% germination in *O. nitida* Vogel seeds. Moreover, for *O. macrocalyx* Ducke, the indication is the isolated use of mechanical scarification or mechanical scarification followed by gibberellic acid (GA₃) application to overcome seed dormancy (Vargas-Simón et al., 2017).

Considering the diversity of indications of *Ormosia* Jacks seeds aiming at seed analysis and seedling production, the aim of this study was to assess methods for breaking seed dormancy and relate them to integument structure and water uptake by *O. paraensis* seeds.

Material and Methods

Fruits were harvested from ten individuals of *O. paraensis* from the Arivaldo Gomes Barreto Natural Park of Macapá, AP, Brazil. Subsequently, ripe fruits were collected directly from the trees before dispersion, as in Silva et al. (2015).

These fruits were dried in the shade for 24 hours in the Department of Landscaping and Urban Afforestation (SEMAM-PMM, Macapá, AP) and then seeds were manually removed. Subsequently, seeds were sent to the Laboratory of Seeds located at UNESP, Campus of Jaboticabal, SP, Brazil.

Seed water content was determined by drying in an oven at $105 \text{ }^{\circ}\text{C} \pm 3$ for 24 hours, as recommended by the Rules for Seed Testing (RAS) (Brasil, 2009), but with three replications of 10 seeds broken with a plier.

Water uptake curve was determined by soaking in water at $30 \text{ }^{\circ}\text{C}$ for 0, 1, 3, 6, 12, 24, 48, and 72 hours (Lima et al., 2006) for three replications of 10 seeds without scarification and with mechanical (abrasion with sandpaper) and chemical scarification (soaking in H₂SO₄ p.a. 98.08% for 0, 15, 30, 60, 120, and 240 minutes). Subsequently, the percentage of water uptake by seeds was calculated according to the equation UP =

$[(Mf - Pi)/Pi] \times 100$, where UP is the percentage of water uptake by seeds, Mf is the fresh seed mass after soaking, and Pi is the fresh seed mass before soaking (Baskin et al., 2006).

For seed germination, the following treatments were performed: intact seed (control), mechanical scarification in the hilum with sandpaper, and chemical scarification by soaking in sulfuric acid (H_2SO_4 p.a. 98.08%) for 0, 15, 30, 60, 120, and 240 minutes and subsequent washing for 10 minutes under running water.

In order to assess seed integument after physical and chemical scarification, seeds were dried at ambient temperature for 24 hours and then mounted on aluminum structures and covered with an 18-nm palladium/gold layer using a Hitachi E102 ion sputter and analyzed in a JEOL JSM 500 scanning electron microscope operated at 2,500 V (Santos, 1996).

Germination tests were set up with five replications of 20 seeds placed between paper sheets in plastic boxes, moistened with 2.5 times their dry weight (Brasil, 2009) with 0.1% Maxin XL[®] aqueous solution, and maintained in germination chambers at 30 °C and with a photoperiod of eight hours (Silva et al., 2015). At the end of germination test, the percentage of germinated, hard, and dead seeds were assessed and calculated according to Brasil (2009) and Silva et al. (2015).

For germination test, the percentage and average germination time were calculated according to $PG = (\sum n_i / N) \times 100$, where PG is the percentage of germination (%), n_i is the number of seeds germinated on the day, and N is the total number of germinated seeds (Labouriau, 1983), and $AT = \sum n_i \times t_i / \sum n_i$, where AT is the average germination time (days), n_i is the number of seeds between $t_i - 1$, and t_i is the total number of germinated seeds (Labouriau and Agudo, 1987). Seed germination rate was estimated by $GR = \sum n_i / \sum n_i \times t_i$, where GR is the seed germination rate ($days^{-1}$), n_i is the number of seeds between $t_i - 1$, and t_i is the number of days between experiment set up and the observation of the i -th day (Hong et al., 2005). Subsequently, the relative frequency of germination was calculated according to Labouriau and Valadares (1976).

The experimental design was a completely randomized design with 5 replications of 20 seeds for germination test and 3 replications of 10 seeds for the soaking curve. The analysis of variance was performed by means of the F test; when F was significant, mean comparisons were carried out by applying the Tukey's test at 5% probability level. The respective regression equations were determined for the periods of seed soaking in H_2SO_4 . Statistical analyses were performed by using the R program (R Core Team, 2014) through the vegan library (Oksanen et al., 2013).

Results and Discussion

Seeds of *O. paraensis* showed a water content of $10 \pm 1.5\%$. Similarly, Silva et al. (2014) and Lopes et al. (2006) reported about 18 and 9% water content for seeds of *O. arborea* (Vell.) Harms. and *O. nitida* Vog., respectively.

Intact seeds of *O. paraensis* practically did not absorb water after 72 hours of soaking. However, water uptake in seeds mechanically scarified with sandpaper and soaking in H_2SO_4 for 60, 120, and 240 minutes stabilized gradually after 24 hours (Figure 1). For *O. nitida*, seed water uptake stabilization was observed in 24 hours (Lopes et al., 2006).

Soaking of scarified *O. paraensis* seeds presented a three-phase water uptake pattern, as the concept of Bewley and Black (1994), since a rapid water uptake (phase 1 or log phase) was observed in the first 24 hours, a little water uptake (phase 2 or stationary phase) was observed between 24 and 48 hours, and in the last hours, seeds restarted to absorb water (phase 3) (Figure 1). Similar results were observed by Curiel and Moraes (2011), Basqueira et al. (2011), and Silva et al. (2014) in scarified seeds of *O. arborea*, but with smaller or larger soaking periods.

According to Silva et al. (2015), seed integument of *O. paraensis* has four distinct layers: cuticle (coating with hydrophobic substances), epidermis (with a layer of compact palisade consisting of radially elongated macrosclereids and densely thickened cell walls), hypodermis (hourglass cells or osteosclerotids), and parenchyma cells (Figures 2A–F). According to Choudhury et al. (2009), Hu et al. (2009), Carvalho and Nakagawa (2012), Baskin and Baskin (2014),

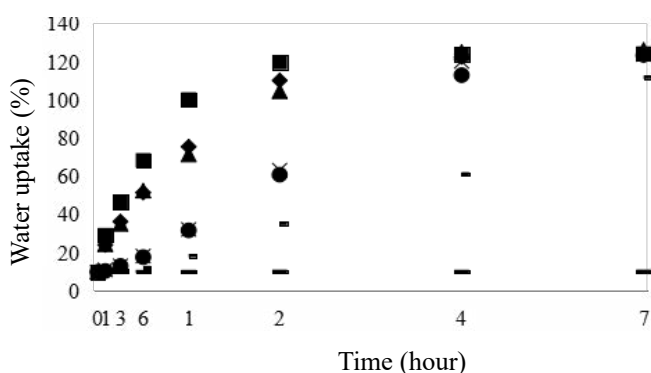


Figure 1. Water uptake curve for intact (control) and chemical (H_2SO_4 p.a. 98.08%) and mechanically (sandpaper 8) scarified seeds of *Ormosia paraensis* Ducke maintained immersed in water at 30 °C. Legend: (—) intact, (●) sandpaper, (-) H_2SO_4 15', (×) H_2SO_4 30', (▲) H_2SO_4 60', (◆) H_2SO_4 120', and (■) H_2SO_4 240'.

Hudson et al. (2015), and Silva et al. (2015), chemical composition, arrangement, and intercellular substances of the palisade layer influence seed water uptake. Lewis and Yamamoto (1990) reported that lignin is a natural polymer present only in seed coat. However, waxes, suberin, tannin, or even lignin can be found in the cell wall of plants (McDougall et al., 1996).

In non-scarified seeds, the integument was intact (Figures 2A–B). However, water uptake by *O. paraensis* seeds was observed after mechanical scarification due to integument

rupture and a consequent exposure of parenchyma cells of the embryo cotyledon (Figures 1 and 2C–D). Vargas-Simón et al. (2017) observed the highest percentages of germination in seeds of *O. macrocalyx* Ducke by means of mechanical scarification or its combination with gibberellic acid, which presented values of 68.0 and 61.3%, respectively.

In seeds chemically scarified with H_2SO_4 for 15 minutes, we observed cuticle ruptured and corrosion of macroscleireids of the palisade layer of the integument epidermis (Figures 2E–F). From 30 minutes, we observed cuticle and epidermis

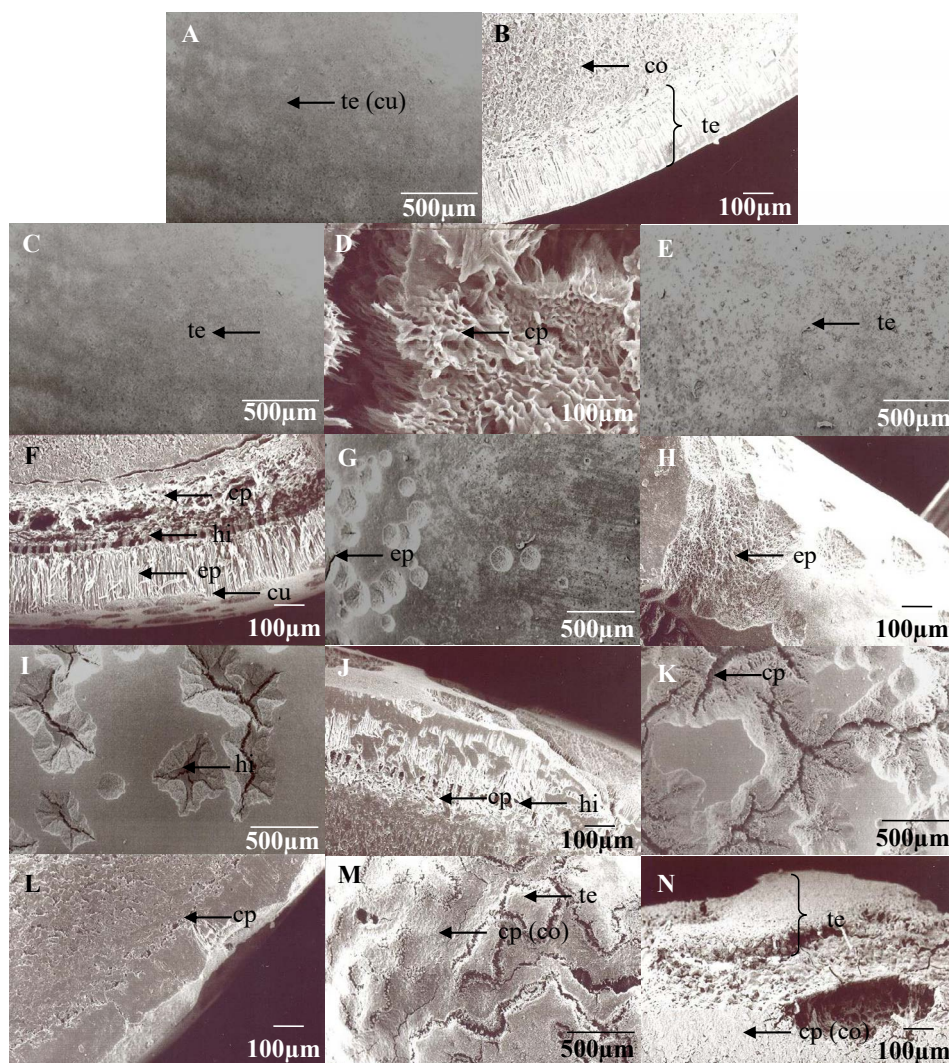


Figure 2. Scanning electron micrographs of seeds of *Ormosia paraensis* Ducke: intact seed (A and B), mechanically scarified seed with sandpaper (C and D), and chemically scarified seed with sulfuric acid (H_2SO_4 p.a. 98.08%) for 15 (E and F), 30 (G and H), 60 (I and J), 120 (K and L), and 240 (M and N) minutes. Legend: A, C, E, G, I, K, and M – external view; B, D, F, H, J, L, and N – cross-section; E and F – cuticle rupture and corrosion of the epidermis; G and H – cuticle and epidermis rupture; I and J – cuticle, epidermis, and hypodermis rupture and corrosion of the integument parenchyma cells; and K and N – integument rupture and corrosion of the cotyledon parenchyma cells. te – integument; co – cotyledon; cu – cuticle; ep – epidermis; hi – hypodermis; and cp – parenchyma cells.

rupture and corrosion of the hypodermis (Figures 2G–H). From soaking in sulfuric acid for 60 minutes, we observed cuticle, epidermis, and hypodermis rupture and corrosion of parenchyma cells (Figures 2I–J). However, from 120 minutes, we observed integument rupture and corrosion of parenchyma cells of the embryo cotyledon in *O. paraensis* seeds (Figures 2K–M) (Silva et al., 2015).

Percentage of germination of *O. paraensis* seeds was low (Table 1) due to the cuticle and palisade layers (Figures 2A–B) and hence low water uptake (Figure 1) of seeds not scarified. According to Lopes et al. (2006), Gonçalves et al. (2008), Silva and Morais (2012), and Vargas-Simón et al. (2017), seeds of *O. nitida*, *O. arborea*, and *O. macrocalyx* presented low percentages of germination due to the tegument impermeable to water or gases.

The highest percentages of germination (Table 1) were observed in *O. paraensis* seeds scarified with sandpaper and soaked in H_2SO_4 for 15, 30, 60, and 120 minutes. For seeds of *O. nitida*, *O. arborea*, and *O. macrocalyx*, Lopes et al. (2006), Gonçalves et al. (2008), and Vargas-Simón et al. (2017) recommended mechanical scarification with sandpaper. However, the recommended soaking period in H_2SO_4 is variable according to the species, i.e. a period of 15 to 30 minutes for *O. arborea* (Teixeira et al., 2009; Silva e Moraes, 2012; Silva et al., 2014) and 30 minutes for *O. nitida* (Lopes et al., 2006). Probably, the observed behavior regarding soaking in H_2SO_4 among species is associated with the chemical and morpho-anatomical composition of the seed integument (Carvalho and Nakagawa, 2012; Baskin and Baskin, 2014).

Seeds soaked in H_2SO_4 for 240 minutes had their germination abruptly reduced since this prolonged period damaged the embryo (Figures 2M–N and Table 1). Similarly,

Silva et al. (2014) reported a reduction in seed germination of *O. arborea* from 60 minutes of soaking in H_2SO_4 .

For percentage of germination, the maximum point for soaking in H_2SO_4 was 113.02 minutes (Figure 3A). Thus, there was a tendency of reducing the percentages of germination in shorter or longer periods of soaking in H_2SO_4 due to a lower water uptake and, consequently, a lower seed germination related to integument impermeability (Figures 1 and 2A–B) or damages to seed embryo (Figures 2M–N).

Scarification promoted by H_2SO_4 reached seed surface indistinctly (Figures 2E–N) and several areas for water uptake arose. However, in mechanically scarified seeds, with abrasion carried out in seed hilum (Figures 2C–D), we observed a slower initial water uptake, reaching values similar to those observed in seeds soaked in H_2SO_4 for 60 and 120 minutes after 72 hours (Figure 1).

Due to differences in water uptake (Figure 1), the highest germination rates were observed in seeds scarified with sandpaper or soaked in H_2SO_4 for 60 and 120 minutes when compared to intact seeds and those soaked in H_2SO_4 for 15 and 30 minutes (Table 1 and Figure 3B). Seed germination rates were extremely low for seeds soaked in H_2SO_4 for 240 minutes when compared to the other soaking periods (Table 1 and Figure 3B).

Seed germination rate presented a maximum point for soaking period in H_2SO_4 of 122.15 minutes (Figure 3B). Thus, a tendency of reduction of germination rates was observed in lower or higher soaking periods in H_2SO_4 due to a slow water uptake (Figures 1 and 2A–B) or damages to seed embryo (Figures 2M–N).

Due to the lower water uptake (Figure 1), non-scarified seeds had low germination or its delay, occurring on average at 17.8 days (Figure 4A). Similarly, seeds treated by soaking in H_2SO_4 for 15, 30, and 60 minutes (Figures 4C–E) showed higher average germination times when compared to

Table 1. Percentage of germination (PG), hard seeds (HS), dead seeds (DS), and germination rate (GR) of intact (control), chemically (H_2SO_4 p.a. 98.08%), and mechanically (sandpaper) scarified seeds of *Ormosia paraensis* Ducke maintained between paper sheets in plastic boxes at 30 °C and photoperiod of 8 hours.

Treatment	PG (%)	GR (day ⁻¹)	HS (%)	DS (%)
Control	6 c	0.070 d	87 a	7 c
Mechanical scarification (sandpaper)	80 a	1.998 a	0 b	20 bc
H_2SO_4 15'	75 a	1.362 b	0 b	25 b
H_2SO_4 30'	81 a	1.372 b	0 b	19 bc
H_2SO_4 60'	77 a	1.720 ab	0 b	23 bc
H_2SO_4 120'	79 a	2.076 a	0 b	21 bc
H_2SO_4 240'	27 b	0.662 c	0 b	73 a
LSD	16.7*	0.548*	5.7*	17.6*
CV (%)	13.8	20.6	23.0	32.6
F test	68.8**	35.4**	658.1**	27.4**

**Significant at 1% probability by the F test. *Means followed by the same letter do not differ from each other by the Tukey's test at 5% probability.

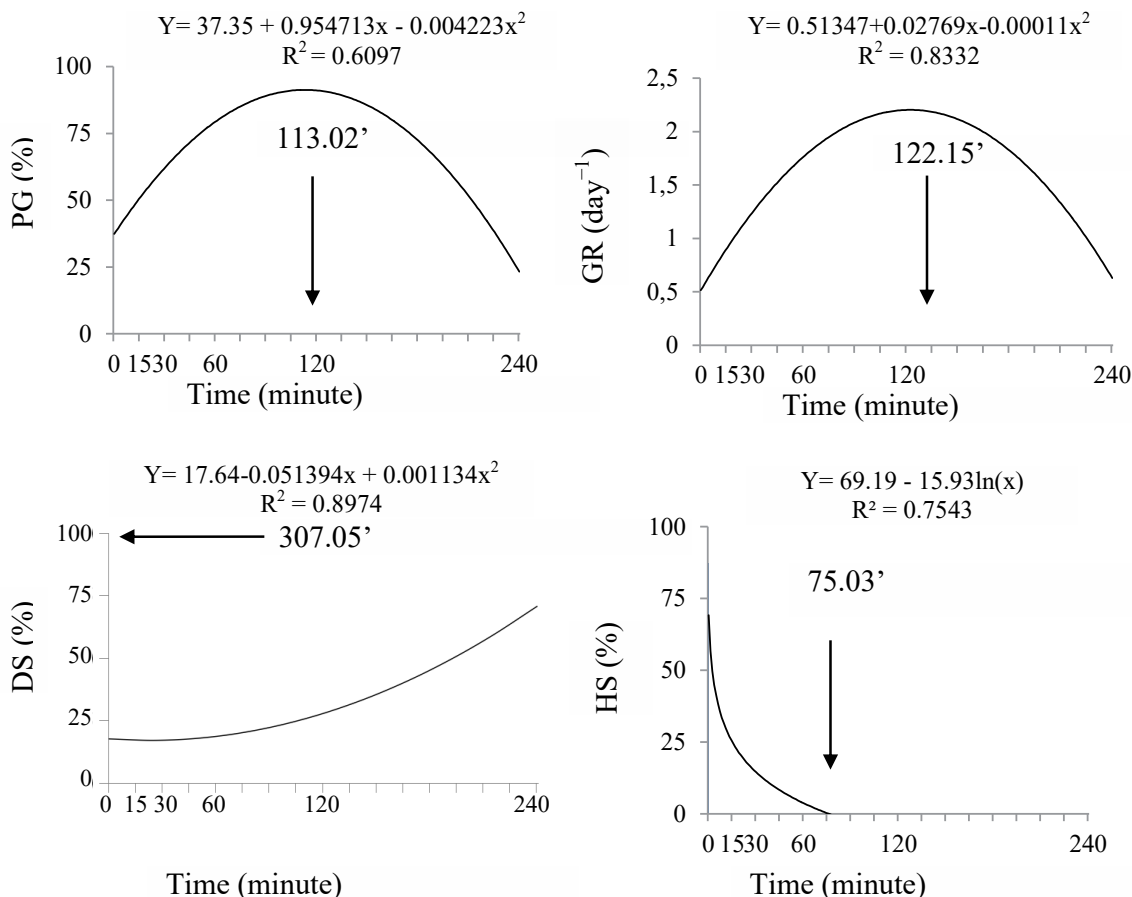


Figure 3. Percentage of germination (PG), germination rate (GR), percentage of hard (HS) and dead (DS) seeds of *Ormosia paraensis* Ducke. Intact and chemically (H_2SO_4 p.a. 98.08%) scarified seeds for 15, 30, 60, 120 or 240 minutes, maintained between paper sheets in plastic boxes at 30 °C and photoperiod of 8 hours. Test F at 5%.

sandpaper scarification and soaking in H_2SO_4 for 120 and 240 minutes (Figures 4B, F–G). Similarly, Lopes et al. (2006) observed that *O. nitida* seeds treated by soaking in H_2SO_4 for 1, 5, 10, 15, 20, 25, and 30 minutes presented average germination times similar to and lower than those observed in intact seeds.

The highest synchronies for germination were observed in seeds treated with sandpaper scarification and soaking in H_2SO_4 for 120 and 240 minutes (Figures 4B, F–G). Thus, the frequencies of germination of *O. paraensis* seeds in these treatments were more unimodal, while polymodal in other treatments (Figure 4). For *O. arborea* seeds, the average germination time ranged from 8.2 to 14.8 days for sandpaper scarification plus soaking in water for 5 days and soaking in H_2SO_4 for 15 minutes. These treatments exhibited the highest and lowest synchronization, respectively (Silva et al., 2014).

The percentage of hard seeds in both mechanical and chemical scarifications was zero (Figure 3C) whereas non-

scarified seeds presented about 87% hard seeds since they did not absorb water (Figure 1 and Table 1). A similar result was observed by Lopes et al. (2006) in non-scarified seeds of *O. nitida*, as their integument remained intact and hence did not allow water uptake.

The percentage of dead seeds increased quadratically as the soaking period in H_2SO_4 increased (Figure 3D) since H_2SO_4 passed through the integument and corroded seed embryo (Figures 2M–N). In addition, after about 307.05 minutes of soaking, seeds were totally killed by the corrosive action of H_2SO_4 (Figure 3D). According to Lopes et al. (2006), the H_2SO_4 action also increased the number of dead seeds in *O. nitida*.

Seeds chemically or mechanically scarified absorbed water and most of them germinated. In contrast, intact seeds had a low percentage of uptake and germination (Figure 1 and Table 1). Thus, an exponential tendency was observed for dormancy release, whose point of intersection of the equation

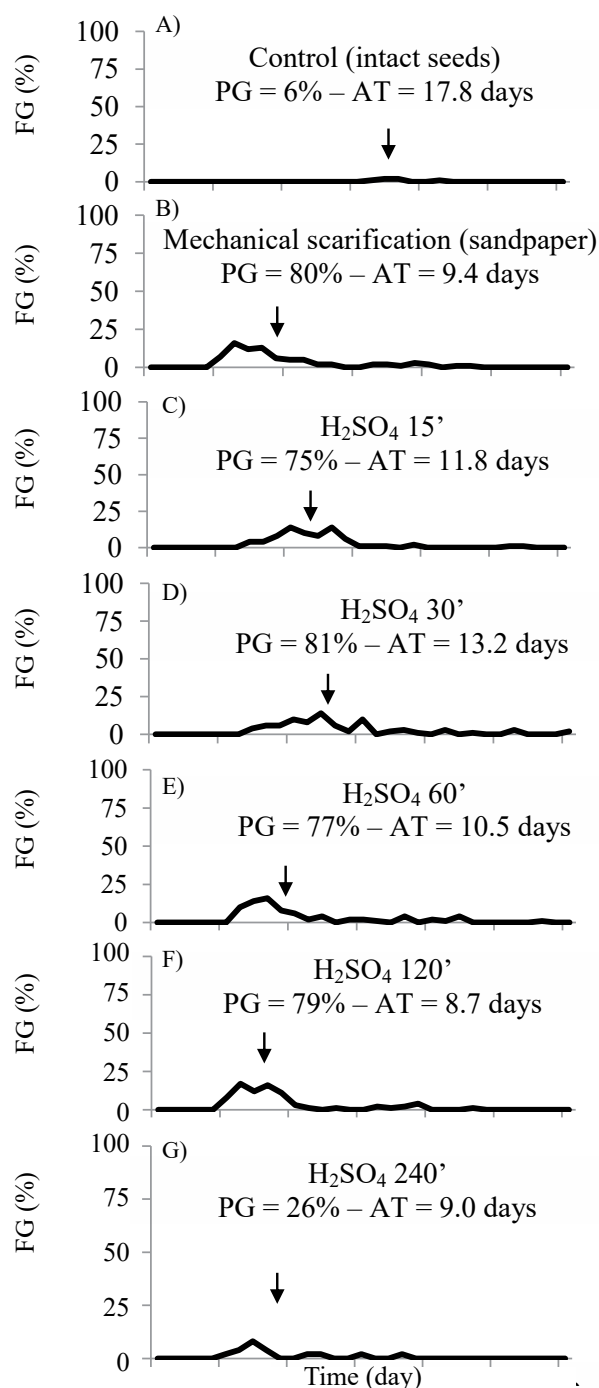


Figure 4. Frequency of germination (FG) of intact (A), mechanically (sandpaper) (B), and chemically scarified (H_2SO_4 p.a. 98.08%) seeds of *Ormosia paraensis* Ducke for 15 (C), 30 (D), 60 (E), 120 (F), or 240 (G) minutes maintained between paper sheets in plastic boxes at 30 °C and photoperiod of 8 hours.

was about 75.03 minutes of soaking in H_2SO_4 (Figure 4C).

Conclusions

Seed soaking in sulfuric acid (H_2SO_4 p.a. 98.08%) for 60 or 120 minutes or mechanical abrasion with sandpaper are adequate to overcome physical dormancy associated with *O. paraensis* seed integument impermeability to water or gases.

Acknowledgments

To UNESP for the facilities, equipment, and reagents. To CAPES and CNPq for granting the doctoral scholarship to the first author and the research productivity scholarships to the last two authors. To UEAP for granting of financial assistance for publication.

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