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Original Paper

Tolerance of *Ormosia arborea* (Fabaceae) seed to water submersion

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Abstract

Soil flooding restricts the availability of the oxygen necessary to activate the embryonic physiological processes that characterize seed germination. However, some species have adaptations that allow them to grow naturally in areas prone to flooding, such as *Ormosia arborea*, a native species of the Atlantic forest and the Cerrado. The objective of this work was to evaluate how seed germination and emergence in this species were affected by submersion in water and scarification. In experiment 1, the seeds were scarified by 15 minutes in sulfuric acid and was subsequently sown in tubes submerged in water for 0, 15, 30 and 45 days. In experiments 2 and 3, seeds were submerged in running water for the same period and, after each time of submersion, were scarified by 0, 5, 10 and 15 minutes in sulfuric acid and sown either in tubes and in Germitest® paper. It was found that, after breaking the tegumental dormancy, 15 days of flooding is enough to derail the germination of *O. arborea* seeds that tolerate submersion in water when they are intact. This response indicates that the hard, impermeable seed coat is an important seed characteristic for this response.

Key words: coat, dormancy, germination, scarification.

Resumo

O alagamento do solo restringe a disponibilidade do oxigênio necessário para ativação dos processos fisiológicos do embrião e que caracterizam a germinação de sementes. No entanto, algumas espécies apresentam adaptações que permitem seu crescimento natural em áreas sujeitas a inundações, como *Ormosia arborea*, espécie nativa da Mata Atlântica e do Cerrado. O objetivo deste trabalho foi avaliar como a germinação e emergência das sementes desta espécie foram afetadas pela submersão em água e escarificação. No experimento 1, as sementes foram escarificadas por 15 minutos em ácido sulfúrico e posteriormente semeadas em tubetes submersos em água por 0, 15, 30 e 45 dias. Nos experimentos 2 e 3, as sementes foram submersas em água corrente pelo mesmo período e, após cada tempo de submersão, foram escarificadas por 0, 5, 10 e 15 minutos em ácido sulfúrico e semeadas em tubetes e em papel Germitest®. Verificou-se que, após a quebra da dormência tegumentar, 15 dias de alagamento são suficientes para inviabilizar a germinação das sementes de *O. arborea* que toleram a submersão em água quando estão intactas. Este resultado indica que o tegumento rígido e impermeável da semente é uma importante característica da semente para essa resposta.

Palavras-chave: tegumento, dormência, germinação, escarificação.

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Introduction

Many areas disturbed by human activity have been able to regenerate naturally after being abandoned, however, under certain conditions, the rate of this process slows significantly and can even halt entirely (Corbin & Holl 2012), which leads to long lasting environmental degradation. In these cases, satisfactory biological methods to speed up the restoration process include the planting of seedlings, direct sowing, the transposition of litter and topsoil, containing the seed bank, or a combination of these and other methods (Martins 2012).

In the case of areas subject to flooding (seasonal or permanent), the key to the success of restoration works may be the selection of species adapted to these waterlogged soil conditions, since, among other effects, the saturation of the soil restricts the availability of oxygen required for the activation of physiological processes that are crucial to germination. This oxygen limitation may provoke intra-specific variation such as increased, reduced, or completely inhibited germination rate, as well as loss of seed viability (Lucas *et al.* 2012).

Among the adaptive mechanisms that seeds uses to tolerate submersion in water, seed dormancy may be advantageous because the onset of germination can be synchronized with that of the dry season, furthermore, the hypoxic period produced by flooding could provide environmental signals involved in breaking this dormancy (Baskin *et al.* 2000a; Lucas *et al.* 2012), in some cases the seasonal regime (wet-dry water regime), may be required (Durant *et al.* 2016).

Ormosia arborea (Vell.) Harms (Fabaceae: Papilionoideae) is a species native to the Atlantic forest and the Cerrado, it can grow in riparian environments or in well-drained soil, and its introduction into plantations for recovery of degraded areas is recommended to stimulate recovery (Junglos *et al.* 2018; Bastos *et al.* 2022). The physical dormancy of its seeds, which is caused by seed coat impermeability, can be overcome by chemical scarification with sulfuric acid for 15 minutes (Silva *et al.* 2014). In addition, the striking red seeds of this species have a small black spot on one side, giving them artisanal value. It is reported that the seeds of this species contain quinolizidine alkaloids, which provide protection against predation without reducing dispersion rates (Guimarães Jr *et al.* 2003).

Although the literature has already established periods of scarification to *O. arborea*, lower periods of *arborea* seed scarification than those reported in the literature were tested to verify that the submersion before scarification influence the start of imbibition, once the 15 minutes could be sufficient to damage to the embryo in seeds. Whereas *O. arborea* is widely found in riparian forest, our hypothesis is that scarified seed should have a lower tolerance independent of time submersion and that only the flooding is not enough to overcome seed dormancy. Consequently, we investigated the effect of different submersion times and scarification on germination and emergence of seeds of *O. arborea.*

Material and Methods

Seeds of *Ormosia arborea* (Vell.) Harms were collected from nine arrays distributed on the left bank of the Ivinhema River (22°03'04.5"S; 53°41'28.2"W), an area prone to flooding, in the municipality of Nova Andradina (state of Mato Grosso do Sul) in June 2014 and a voucher specimen was deposited in the DDMS/UFGD herbarium (registration number 5206). Open fruits with exposed seeds were collected, as recommended by Silva *et al.* (2014). Then the seeds were selected according to their integrity, consistency, and color.

Dissolved oxygen content, temperature (Hanna HI9146 Portable Dissolved Oxygen Meter), and water pH (Hanna HI98128 pH/Temperature Tester with 0.01 pH resolution) were measured at the beginning and end of each seed submersion period.

Experiment 1

The seed was scarified for 15 minutes with concentrated sulfuric acid (98% p.a.), then rinsed in running water for 5 minutes (15). Subsequently, the seeds were sown in 50 × 190 mm tubes (16) at a depth of 1 cm, using a substratum of subsoil and sand at a ratio of 1:1, whereas seedlings of *O. arborea* develop well on substrates with low fertility (Silva & Morais 2013).

The trays with the tubes were deposited in plastic pools that allowed the surface of the substrate to be covered by 5 cm of water for 0, 15, 30, or 45 days. The tubes withdrawn from the flooded conditions and the control tubes (zero days of flooding) were maintained at 70% of the irrigation water retention capacity.

This experiment was conducted in a greenhouse with 30% shade. The climate of the region, which is the Humid Cwa type according to Köppen's classification, had average annual temperatures and precipitation ranging from 20–24°C and 1,250–1,500 mm, respectively.

Ninety days after sowing, the emergence percentage was determined using as a criterion the emission of shoot.

Experiment 2

The seeds were distributed in packaging made from voile fabric and deposited in a box made from Sombrite® fabric, which was submerged in water for 0, 15, 30 and 45 days. The site was the Zezão stream (22°15'56.81"S; 53°52'55.38"W) in the municipality of Ivinhema/MS, which had an average pH of 6.3, an oxygen content of 4.6 oxygen mg/L, and a temperature of 26 °C.

At the end of each submersion time (dive), the seed was scarified by treatment for 0, 5, 10, and 15 minutes with concentrated sulfuric acid (98% p.a.). As was done in previous experiment (1) the seeds were sown in tubes of 50 × 190 mm to 1-cm depth. A substratum containing a 1:1 ratio of subsoil and sand was used and maintained with 70% of the irrigation water retention capacity.

This experiment was conducted in a greenhouse under the same conditions as in experiment 1; the following variables were determined after 90 days of seeding:

- Percentage of emergence (E): a criterion the emission of shoot.

- Emergence speed index (ESI): calculated as the sum of the number of emerged seeds each day, divided by the number of days elapsed between sowing and emergence, according to the formula used by Maguire (1962).

- Mean time of emergence (MTE): calculated as described by Silva & Nakagawa (1995).

- Length of the shoot and root: measured using a ruler with centimeter graduations.

- Total leaf number: measured by counting the total number of leaves per plant.

- Collar diameter: measured with the aid of a digital caliper (0.001 mm), results expressed in millimeters.

- Total fresh mass: obtained by weighing fresh seedlings using a precision analytical balance (0.0001g), results expressed in grams.

- Total dry mass: obtained by weighing seedlings dried in an oven at 60 °C for 48 hours or until fully dry, using a precision analytical balance (0.0001 g), results expressed in grams.

Experiment 3

The seeds were given the same treatment of submersion and scarification as experiment 2. The water used from the brook had an average pH of 6.3, 4.7 mg/L oxygen content and temperature of 29 °C.

However, after each submersion period followed by scarification, seeds were sown in Germitest® paper previously moistened according to the Rules for Seed Analysis (Brasil 2009) and deposited in a B.O.D (Biochemical Oxygen Demand) type germination chamber, with a photoperiod of 12 hours and at a constant temperature of 25 °C (Brancalion *et al.* 2010).

After 40 days of seeding, the following variables were determined:

- Percentage of germination (G): considering the primary root protrusion (Ferreira & Borguetti 2004).

- Percentage of normal seedlings: using as a criterion the emissiom the shoot and root system development.

- Germination speed index (GSI): calculated as the sum of the number of germinated seeds every day, divided by the number of days elapsed between sowing and germination, in accordance with the formula used by Maguire (1962).

- Mean time of germination (MTG): calculated as described by Silva & Nakagawa (1995).

Measurements of the length of the shoot and primary root, collar diameter and total dry and fresh mass were taken from the seedlings used for the germination test, as described in experiment 2.

Statistical design and analysis of data

In experiment 1, a completely randomized design (DIC) was used with 4 times of submersion (0, 15, 30, and 45 days) and 4 repetitions of 25 seeds, totaling 800 seeds.

In experiments 2 and 3, DIC was used in a factorial scheme of 4 times of submersion (day 0, 15, 30, and 45) × 4 times of scarification (0, 5, 10, and 15 minutes) with 4 replicates of 25 seeds, totaling 1,600 seeds by experiment.

The data were subjected to analysis of variance using a two-factor (ANOVA), with the significance level set at $P < 0.05$. So as to percentage of germination and normal seedlings using a regression mong treatments and their interactions. For other parameters the averages were subjected to Tukey's test for scarification and adjusted by regression equations for the submersion time and its interaction with scarification time.

Results

Experiment 1

The seeds of the *O. arborea* which were scarified but not flooded (control treatment) showed 73% seedling emergence; however, seeds that were scarified and subsequently flooded rotted (100%) within the shortest time interval tested (15 days), making the process impossible of germination and seedling formation in these conditions.

Experiment 2

For percentage of emergence and ESI, there was an interaction between the submersion time and scarification time, and unscarified seeds presented low emergence (3%) during the submersion times (Fig. 1a). It should be noted that submersion time appeared to have a positive influence on percentage emergence and ESI when the seed was scarified (5, 10, and 15 minutes) (Fig. 1a-b). However, an increase in room temperature was observed when the seeds were sown at the end of each submersion time (Fig. 2). There was no significant interaction between treatments for MTE; a general average of 50 days was observed.

For growth and biomass accumulation, no interaction was observed between submersion times and scarification times. Only the scarification time influenced these variables, and unscarified seeds showed the lowest results. Seeds subjected to 10 and 15 minutes of scarification produced seedlings with greater root length and number of leaves (Fig. 3).

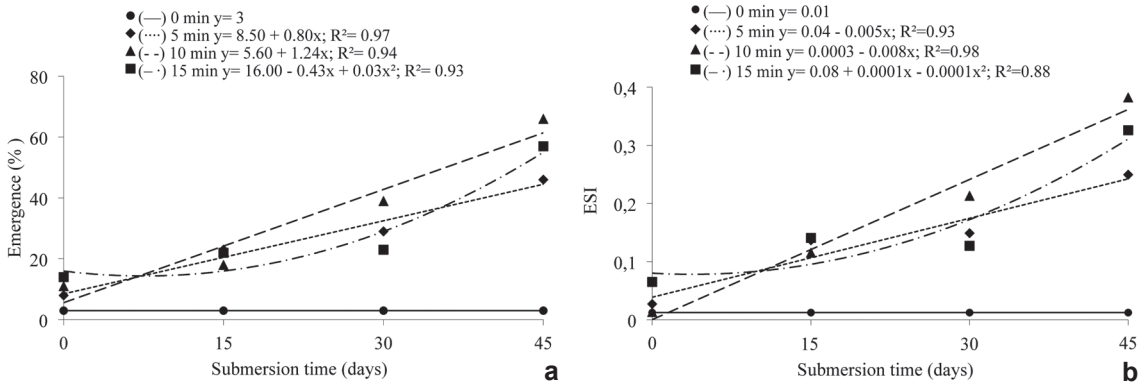

Figure 1 – a-b. Emergence of *Ormosia arborea* seeds subjected to different submersion times followed by scarification and held in greenhouse – a. emergence; b. emergence speed index (ESI).

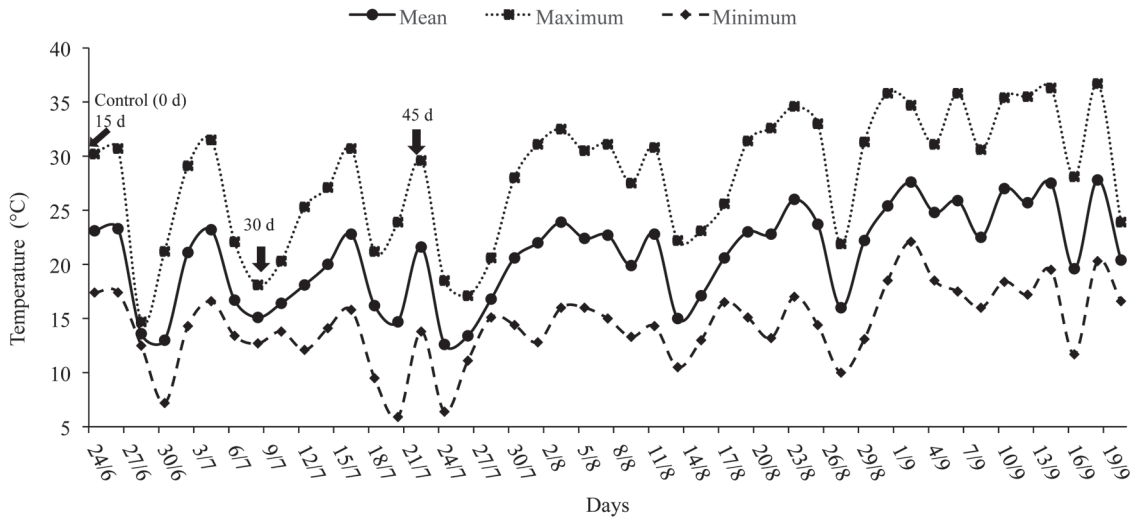

Figure 2 – Room temperature during the period of experiment 2. The arrows indicate when the seeds were sown. Source: Embrapa (2015).

Experiment 3

The interaction between submersion and scarification time was not significant for most of the variables analyzed, except MTG and GSI (Fig. 4). Thus, the submersion time did not affect the percentage of germination, normal seedling formation, initial growth, or biomass gain of *O. arborea*; only isolated scarification times were significant in these cases (Figs. 4a-b; 5).

Unscarified seeds not germinated while evaluated; however, when scarified (5, 10 and 15 minutes), the percentage of germination and normal seedling formation were greater, 93% and 82% respectively (Fig. 4a-b). The largest germination percentages and percentage of normal seedlings occurred in treatments with 10 minutes of seed scarification (Fig. 4a-b). For the length of the shoot and root, collar diameter and total fresh and dry mass, there were only differences between seedlings from scarified (5, 10, and 15 minutes) and unscarified (Fig. 5).

The submersion times positively influenced GSI and MTG; an increase in the GSI was observed and consequently, a reduction in the MTG was found (Fig. 4c-d).

Discussion

Most Cerrado tree species produce dormant seeds, the most common type being physically dormant seeds, especially Fabaceae (Zaidan & Carreira 2008), as in the case of *O. arborea*. This type of dormancy can be broken by chemical scarification resulting in rupture of the coat, allowing the entry of water and oxygen necessary to trigger the process of germination (Zaidan & Carreira 2008; Silva *et al.* 2014, 2021).

However, seeds of *O. arborea* subjected to scarification before flooding lost viability. This may be because of hypoxic conditions, which induce changes in aerobic airways and cause a buildup of toxic lactic acid and ethanol (produced by anaerobic respiration), which can lead to cell death and the loss of seed viability (Lobo & Joly 1998).

Lopes *et al.* (2004) also verified a reduction in germination (4%) with a consequent increase in the percentage of damaged seeds (48%) when the *O. arborea* seeds were mechanically scarified and pre-soaked for 24 hours. The occurrence of sporadic submersion can influence germination performance of a seed lot of *O. arborea* 95% germination was observed in the laboratory and was unsuccessful

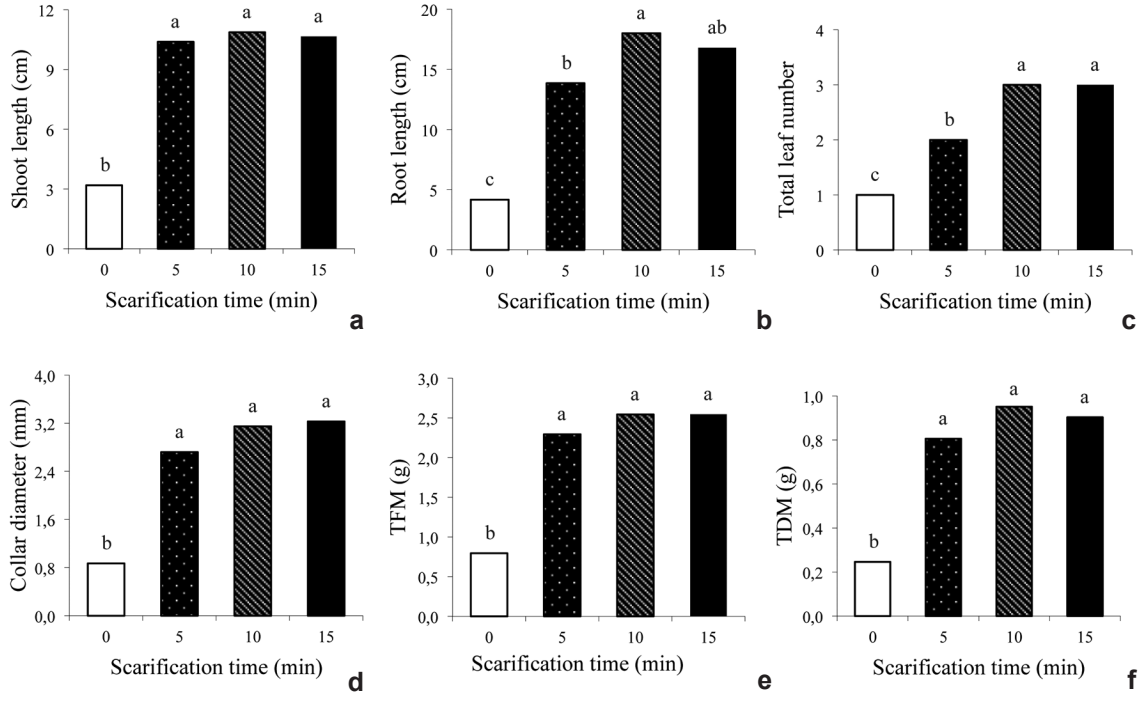

Figure 3 – a-f. *Ormosia arborea* plants from seeds subjected to submersion followed by scarification and grown in and held in greenhouse – a. shoot length; b. root length; c. number of leaves; d. collar diameter; e. total fresh mass; f. total dry mass. Columns with the same letters do not differ based on Tukey's test, 5% probability.

in the field (just 3% emergence) after overcoming the tegumentary dormancy in the work of direct sowing (Isernhagen 2010).

Seeds of *Parkia discolor* Spruce *ex* Benth. (Melo *et al.* 2015) lose viability when scarified and subsequently subjected to flooding. These result indicate that the hard seed coat of these species is important for seed tolerance to flooding (Melo *et al.* 2015), and prevents the onset of the germination process. He relatively thick seed coats but permeable observed did not offer a barrier for seed water uptake (imbibition), but thick seed coats seem to protect seeds from predators and, consequently, enhance seed longevity in the soil, especially in soil from humid areas (Kissmann *et al.* 2012).

Thus, unscarified *O. arborea* seeds have potential to be sown after flooding, since they are not submerged (control - experiment 1) present high emergence rates (73%), and in which case the establishment of the species in temporarily flooded areas will be given to the tolerance of seedlings (Junglos *et al.* 2018).

Unscarified *O. arborea* seeds presented not germination (experiment 3) and low emergence rates (3%) (experiment 2), regardless of whether or not they stayed submerged. Further research with this species also revealed low emergence (1%) (Silva & Morais 2012) and absence of germination in seeds that did not receive pre-germinative treatment (Teixeira *et al.* 2011; Silva *et al.* 2014). Intact seeds that were submerged in water for 72 hours (Teixeira *et al.* 2011) also did not germinate.

However, seeds that were submerged but not scarified remained viable, after all after overcoming dormancy with sulfuric acid (5, 10, and 15 minutes), the seeds showed a high percentage of germination and emergence. This ability of the seeds to withstand extended submersion can be attributed to morphological characteristics, such as the impermeability of the coat, furthermore, some species can not only tolerate prolonged submersion, but also require a period of hypoxia to break seed dormancy (Lucas *et al.* 2012).

In Fabaceae impermeability of the coat is normally associated with the presence of hydrophobic substances in the outer ends of the layers arranged palisade cells with thick lignified secondary walls (Baskin *et al.* 2000b) in *Ormosia paraensis* Ducke the sclerenchyma cells, called

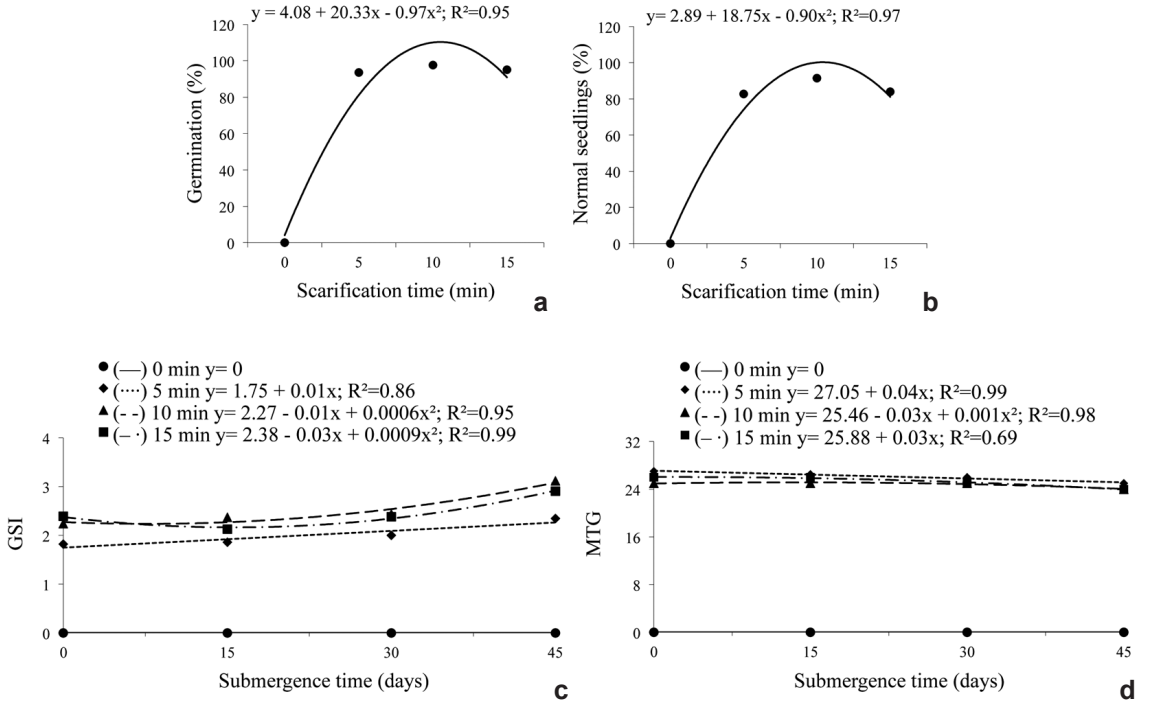

Figure 4 – a-d. Germination of the *Ormosia arborea* seed subject to submersion followed by scarification and sown in B.O.D. (Biochemical Oxygen Demand) – a. germination; b. normal seedlings; c. germination speed index (GSI); d. mean time of germination (MTG).

macrosclereids (Malpighian cells), this form palisade layer, which continuously appears throughout the outer layer, except for the hilum (Silva *et al.* 2015). In this cuticle layer has its integument (coating with hydrophobic substances), hypodermis (hourglass cells, or pillar cells or osteoesclereids) and parenchyma cells (Silva *et al.* 2015).

For the seeds that were kept submerged and subsequently scarified, staying submerged favored some germ responses [E (%), ESI, MTE, and GSI], suggesting that the friction of the water (stream) against the seed surface could carry out slow mechanical scarification of the hard and impermeable seed coat with increasing the time of submersion or the pH slightly acid of stream may be in operation in this process. Baskin *et al.* (2000a) observed that *Schoenoplectus purshianus* (Fernald) M.T. Strong seeds subjected to flooding left the dormancy period faster than non-flooded seeds did. However, Lucas *et al.* (2012) verified that 10 species from the Amazonian floodplains which exhibit dormancy germinated more rapidly when removed from anaerobic conditions (< 3 months).

In the BOD, the submersion appears to have exercised less influence on the GSI and MTG in greenhouse conditions; as submersion time increased the percentage of emergence and the ESI were more expressive. However, in nurseries, external factors are not controlled, and the temperature of the environment may have influenced this response, because in low temperature conditions, the reorganization of cell membranes during imbibition is made difficult, making the process slower (Ferreira & Borghetti 2004), justifying increased germination response with increasing ambient temperature. It is suggested that lower temperatures are not suitable for sowing; because at a temperature of 10 °C for 120 days it did not favor the formation of *O. arborea* plants (Silva *et al.* 2021), being the temperatures of 25 °C are better suited in the Cerrado and Atlantic Forest biomes species (Brancalion *et al.* 2010).

Oliveira *et al.* (2016) verified that for *O. arborea* the temperature of 30 °C is better suited for germination, while 20 °C reduced the germination speed exposing the seeds for a longer period of time to adverse factors, which can lead to a reduction in total germination, vigor and/or MTE, through, for example, slower respiratory rates.

However, it was found that submersion for up to 45 days was insufficient to overcome seed

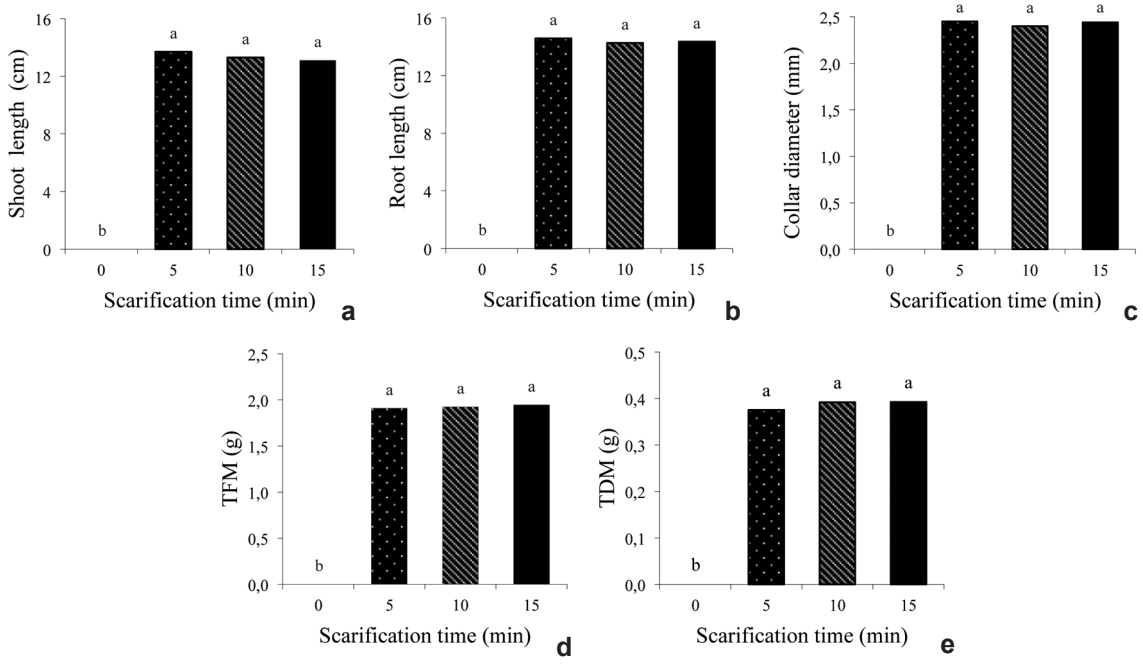

Figure 5 – a-e. *Ormosia arborea* plants from seeds subjected to submersion followed by scarification and grown in and held in B.O.D. (Biochemical Oxygen Demand) – a. shoot length; b. root length; c. collar diameter; d. total fresh mass; e. total dry mass. Columns with the same letters do not differ based on Tukey's test, 5% probability.

dormancy of species and tolerate the hypoxic conditions. The dormancy of the seed before exposure to hypoxia may be advantageous for successful seed germination after the period of flooding (Lucas *et al.* 2012).

Delayed germination and slow emergence can also be beneficial, because in unpredictable habitats, such as savannas, rapid germination may not be advantageous. Therefore, species in this biome commonly exhibit seed dormancy, thus preventing germination under unfavorable conditions (Zaidan & Carreira 2008).

The phenomenon of dormancy in seeds is originated from an adaptation of species to the environmental conditions in which they reproduce. Synchronizing germination with the onset of the most favorable conditions for further development facilitates perpetuation of the species (what guarantees that certain individuals become established) or colonization of new areas (Finch-Savage & Leubner-Metzger 2006).

Thus, whereas in the ecological restoration actions, the germination standards must comply with the natural variability of the species, these findings diverges from the silviculture vision that value standard germination more homogeneous (Martins 2012). Intact *O. arborea* seeds can be used in direct seeding techniques in temporarily flooded areas because their state of physical dormancy allows them to tolerate submersion. This species also exhibit slow germination and can therefore serve to enrich the seed bank.

Especially because depending on the history of specific degraded areas and the distance from the surviving natural vegetation, the seed bank can become highly compromised or even non-existent. These cases in particular require human intervention for regeneration of the ecosystem (Corbin & Holl 2012).

The seeds of *O. arborea* can tolerate submersion in water when intact, but become quickly unviable (15 days) after dormancy has been overcome, indicating that the hard, impermeable seed coat is an important feature that allows the integrity of the embryo to be maintained.

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