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# Imbibition of *Swietenia macrophylla* (Meliaceae) Seeds: The Role of Stomata

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• *Background and Aims* The occurrence of stomata in seed coats is uncommon and there is limited information about their function(s). The aim of this study was to verify the distribution of stomata in seed coats of *Swietenia macrophylla* and to relate it to the imbibition process and aspects of the structure of the outer integument layers.
• *Methods* For the structural and ultrastructural studies, the seeds were processed using the usual techniques and studied under light and scanning electron microscopes. Histochemical tests were employed to identify the cell wall composition in the different seed coat portions. To assess the role of the stomata in the imbibition, non-impervious seeds were compared with partially impervious ones, in which only the embryo, median or hilar regions were left free. Further, the apoplastic pathway marker was employed to confirm the role of the stomata as sites of water passage during imbibition.
• *Key Results* A positive relationship was observed between seed coat thickness and stomata density. The stomata were devoid of movement, with a large pore. They occurred in large numbers in the embryo region and extended with lower frequency towards the wing. Imbibition rates were related to stomata density, suggesting that the stomata act as preferential sites for water entry in the *S. macrophylla* seeds.
• *Conclusions* At maturity, the stomata in the seed coat play a significant role in seed imbibition. The data may also infer that these permanently opened stomata have an important role in gas exchange during seed development, aiding embryo respiration.

**Key words:** Embryo, germination, integument, mahogany, Meliaceae, seed, seed anatomy, seed coat, seed imbibition, stomata, *Swietenia macrophylla*.

## INTRODUCTION

The Meliaceae is a family in which the diversity in fruits and seeds outweighs in complexity the floral details, which are the basis of angiosperm classification (Corner, 1976). Nevertheless, according to Corner (1976), it is necessary to examine fruit and seed structure in order to understand specific alliances within and between genera.

The species of *Swietenia* are known for their wood quality (Pennington *et al*., 1981). Mahogany (*Swietenia macrophylla*) is the only species of the genus found in Brazil, and is one of the most valuable wood species in South America. According to Lemes *et al*. (2003), it is one of the most exploited and valued neotropical trees and, because of intense exploitation, is threatened with extinction (Alvarenga and Flores, 1988; Barbosa, 1992).

*Swietenia macrophylla* seeds are practically exalbuminous, with a prominent wing. The outer integument is variable in thickness, being thick in the region that contains the embryo and thin on the wing (Corner, 1976). According to Alvarenga and Flores (1988), mahogany seeds are anemochoric with low density because of the spongy trait of the seed coat.

The occurrence of stomata in seeds is uncommon (Jernstedt and Clark, 1979), and studies regarding their distribution and functions are scarce. If present, the stomata are usually scattered over the whole surface of the seed or are more restricted to certain zones, such as the dorsal and dorsal–lateral parts, antiraphe and raphe sides (Werker, 1997). The occurrence of stomata in *S. macrophylla* seeds was reported by Corner (1976), but data on their distribution and functional aspects were not reported. According to Corner (1976), stomata are not always present in seeds of Meliaceae and there are reports only on the *Melia* and *Swietenia* genera (Werker, 1997).

Considerations of the function of stomata in the seed coat have appeared in the literature over the years. However, their exact function has not been determined with any degree of certainty. It is also unclear whether the stomata are functional in young, immature seeds with living seed coat cells or in the dry mature seeds with mostly dead cells, or both, having a different function at each stage (Werker, 1997). One of the principal functions of these stomata appears to be to facilitate gas exchange, especially in photosynthesizing seeds (Flint and Moreland, 1943; Jernstedt and Clark, 1979). Werker (1997) suggests that the stomata also facilitate water uptake during imbibition.

Seed coat permeability is a major factor controlling the rate of water uptake (Woodstock, 1988). During imbibition, the movement of water into the seed is due to diffusion and capillary action, with water moving from a region of higher to lower water potential. The morphological and chemical properties of the seed coat interact with seed size in determining the ability of the seed to take up water (Harper and Benton, 1966). An impervious seed coat must possess one

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or more layers of very tightly packed cells, with no pores, intercellular spaces or stomata between them. Repellent materials, such as cutin, phenolic compounds or lignin, must constitute their cell walls or impregnate them and/or be deposited from within or from outside (Werker, 1997).

Considering the small number of studies about the functionality of stomata in the seed coat, the aim of this study was to verify the distribution of stomata in mahogany seeds and to relate this to the imbibition process.

## MATERIALS AND METHODS

Seeds of *S. macrophylla* were collected from plants growing at the Federal University of Minas Gerais State (UFMG) campus, Minas Gerais, Brazil. For the anatomical studies, seeds at physiological maturity, obtained from pre-dehiscent fruits, were collected and fixed in FAA50 (Johansen, 1940), dehydrated in an ethyl series and embedded in hydroxy-ethyl-metacrylate (Leica). Transverse (5 μm) sections were cut using a microtome and stained with toluidine blue (O'Brien *et al*., 1964). To observe the stomata and determine their density, seeds were cleared according to Fuchs (1963) and analysed under a light microscope. The following histochemical tests were performed: phloroglucinol + HCl to detect the lignified walls (Sass, 1951); Sudan IV to detect lipids; and 10 % ferrous chloride aqueous solution with the addition of a small amount of calcium carbonate to detect phenolic compounds (Johansen, 1940). On paradermal sections of immature seeds, glycerin was employed to observe stomata movements (Jernstedt and Clark, 1979).

For scanning electron microscopy (SEM) studies, unimbibed mature seeds were collected. Portions of the seed coat, in the median region of the seeds, were removed with a razor blade, dehydrated in a chamber at 60 °C, placed on stubs and gold sputtered (Robards, 1978). The seed coat was fractured after dehydration to study the seed coat. The samples were examined using a Zeiss DSM950 scanning electron microscope at 20 kV.

To determine the role of the stomata in water uptake, in the different regions of the seed, *S. macrophylla* seeds were collected from five plants and held at 30 °C for 48 h to standardize the moisture contents. Selected intact seeds of uniform size were separated into the following groups, with 10 replicates of each: seeds with completely exposed seed coat; seeds with the distal region exposed, where the embryo is located; seeds with the median region exposed; and seeds with the hilar region exposed. The unexposed areas in each treatment were made impervious by covering these areas with liquid Paraplast (60 °C). In a preliminary trial, seeds completely covered with Paraplast were immersed in water and the absence of imbibition was observed, demonstrating the efficacy of the method to render seeds impermeable. After the initial preparation, the seeds were completely immersed in distilled water in a germination chamber at 25 °C. Imbibition curves of the seeds in the different treatments were obtained by weighing at the following times 0, 0·5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 60 h. The impervious areas and the total area of each seed were determined using a digital screen pad.

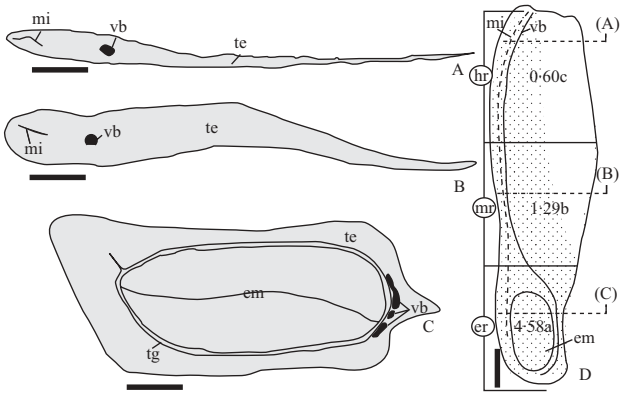


FIG. 1. Diagram showing *S. macrophylla* mature seed. (A–C) Transverse section of a seed, highlighting the seed coat thickness at the hilar, median and embryo regions, respectively. (D) Distribution of stomata (dots show the stomata density); the dashed lines indicate the position of the sections of the other diagrams. In each region, stomata density (stoma $mm^{-2}$) is represented, being different by Tukey test at $P \leq 0{\cdot}05$. em, embryo; er, embryo region; hr, hilar region; mi, micropyle; mr, median region; te, testa; tg, tegmen; vb, vascular bundle. Scale bars = 3 mm in (A–C), 1 cm in (D).

To study the effect of the embryo on water uptake, the seed coats of 10 seeds were cut to remove the embryo. After removing the embryo, the cut surface was sealed with epoxy glue and the seeds placed for imbibition, together with the same number of intact seeds. The imbibition curves of the seeds with and without an embryo were compared.

To confirm the role of the stomata as preferential sites for water entry during imbibition, a 1 % aqueous safranin solution was employed (Seago *et al*., 2000). Intact seeds were placed to imbibe in this solution for 2 h, then washed in distilled water and samples of the seed coat were removed. Freehand cross-sections were obtained from the samples, which were analysed under a light microscope.

## RESULTS

### *Seed structure*

The seeds of *S. macrophylla* are bitegmic. The seed coat thickness varied in different parts of the seed (Fig. 1A–C). In the region where the embryo is located (distal region), the seed coat was thick, reaching 2 mm thickness, and the total thickness, including the embryo, ranged from 6 to 8 mm (Fig. 1C). On the other hand, on the wing, the tegmen was absent and the testa thickness is smaller, decreasing towards the hilar region (proximal) and the raphe face towards the opposite face (Fig. 1A and B). The mesotesta thickness decreased towards the face opposite the raphe, reaching a single cell layer and even disappearing.

Stomata were observed in the exotesta with voluminous guard cells, and a wide pore with an elliptic to circular contour (Fig. 2A, B). The stomata were more concentrated in the embryo region, and extended with lower frequency towards the wing (Fig. 1D). On the wing, the stomata were

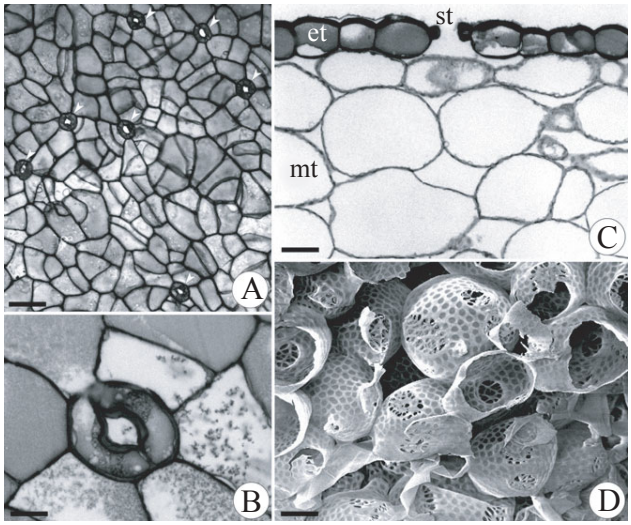


FIG. 2. *Swietenia macrophylla* seed coat. (A) Stomata (arrowhead) distribution in the embryo region. (B) Detail of one stoma; note the large pore. (C) Transverse section of a seed coat, showing exotesta and mesotesta. (D) SEM photograph showing cells of a fractured mesotesta; note the reticulated wall. et, exotesta; mt, mesotesta; st, stoma. Scale bars = 100 μm in (A), 20 μm in (B) and 45 μm in (C and D).

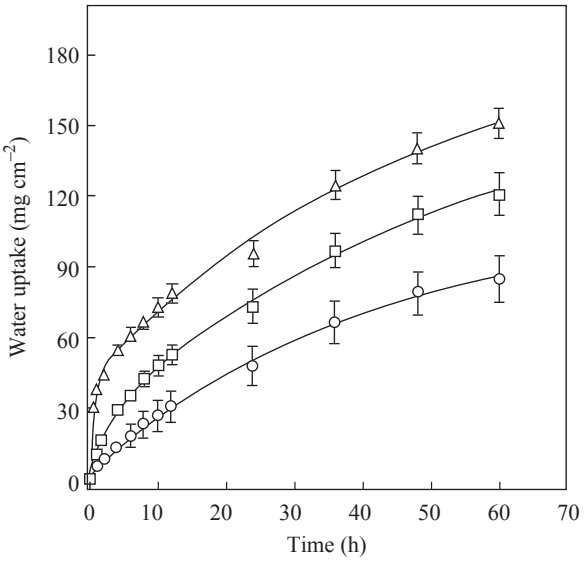


FIG. 3. Imbibition curves of seeds with embryos. The treatments consisted of partial seed coating with Paraplast, to render it impermeable, in order to expose only the embryo (triangles), median (squares) or hilar (circles) region to imbibition. Bars = s.e., $n = 10$.

fewer with an irregular distribution, being more numerous close to the vascularized region and rare on the opposite face (Fig. 1D). Tests with glycerin on immature seeds showed that the stomata of the seed coat were always open even under plasmolysing conditions. The common cells of the exotesta had a thin cuticle and walls impregnated by phenolic substances, while the guard cells present pecto-cellulosic walls.

The mesotestal cells were globe shaped and dead at maturity. The cell wall was lignified and showed reticulated reinforcements; the intercellular spaces were wide, conferring low density and spongy appearance to the tissue (Fig. 2C and D).

The tegmen presented few cell layers of parenchymatic nature and loose appearance, and were restricted to the region that encloses the embryo.

*Seed imbibition*

The start of imbibition was fast, and water entry in the seeds was detected 30 min after their immersion in water (Fig. 3). The volume of water absorbed by the seed coat was greater for the seeds with the embryo region exposed, at all the time points studied, followed by those with the median and hilar region exposed, respectively (Fig. 3). In the seeds with the embryo region exposed, the water uptake reached 30 mg cm$^{-2}$, remaining fastest at 60 h (Fig. 3). In the other seed regions, the imbibition was slower.

During the first 2 h in water, the imbibition rate (mg $H_2O$ cm$^{-2}$ h$^{-1}$) was markedly greater in the region that contained the embryo compared with the other regions studied (Table 1). In addition, in this period, the rate of imbibition was greater in the seeds with the median region exposed, compared with those seeds with the hilar region exposed. At 2 h after the start of imbibition, the water uptake rate was similar for all the studied seed regions (Table 1).

TABLE 1. *Water uptake rate in seeds of* S. macrophylla *(mg $H_2O$ cm$^{-2}$ h$^{-1}$)*

| | Interval | | |
|---|---|---|---|
| Seed portion | 0–2 h | 2–10 h | 10–60 h |
| Embryo region | 44·21$^{a}$ | 3·55$^{cd}$ | 1·57$^{d}$ |
| Middle region | 16·35$^{b}$ | 3·97$^{cd}$ | 1·45$^{d}$ |
| Hilar region | 7·57$^{c}$ | 2·40$^{d}$ | 1·16$^{d}$ |

Different letters within each column indicate significantly different means ($P \leq 0{\cdot}05$, Tukey test).

When seeds with and without an embryo were compared (Fig. 4), similar imbibition rates were observed until 24 h. After this time, the imbibition rates of the seeds with an embryo were greater compared with those without an embryo, which demonstrates that the seed coat stored a considerable amount of water before the embryo started to grow (Fig. 4). During imbibition, the increase in seed weight was considerable, although the alteration in volume was discrete. A great accumulation of air in the seed coat was observed in all treatments, especially in the testa, even 60 h after the start of imbibition.

Analysis of the transverse sections of the seed coat from seeds imbibed in aqueous safranin solution showed that the stain penetrated at different points, which corresponded to the stomata. Stain diffusion through the common epidermis cells was not observed.

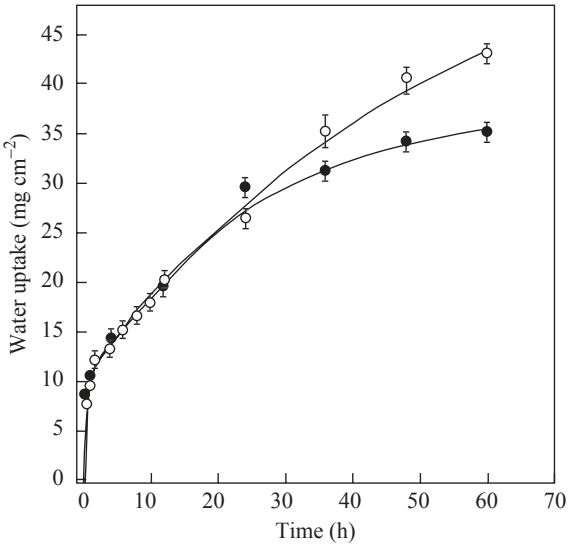


FIG. 4. Imbibition curve of seeds with (open symbols) and without an embryo (filled symbols). Bars = s.e., $n = 10$.

## DISCUSSION

The absence of protoplast, and the thinness of the cell walls and intercellular spaces of the *S. macrophylla* mesotesta were all characteristics that confer a higher permeability to water and gases to this layer. However, the presence of cuticle and phenolic compounds in the exotesta seemed to restrict this permeability. Furthermore, the large volume and structural organization of the mesotesta conferred a very low density to the seed coat, which permits anemochoric, and possibly hydrochoric, dispersion. According to Alvarenga and Flores (1988), mahogany seeds are anemochoric, but the low density and high capacity to reserve air suggest that hydrochory could be important to *S. macrophylla*, an observation reinforced by the fact that the species can be found in a variety of soils that are well drained or at times subject to flooding (Pennington *et al*., 1981).

The thicker testa at the embryo region and differences in testa thickness around the seed, as we observed, were first described by Corner (1976) in *Swietenia* seeds, but the differences in testa thickness were not correlated to stomata distribution.

In *S. macrophylla*, the distribution of the stomata in the seeds was related to the seed coat thickness, and there was greater stomata frequency in the thicker seed coat region, which is occupied by the embryo and by the vascular bundle of the wing. The occurrence of stomata in seeds is uncommon, but they have been reported occasionally in seeds from species scattered among dicot and monocot families (Rugenstein and Lersten, 1981; Werker, 1997). Although the presence of stomata in the *S. macrophylla* seed coat had already been reported by Corner (1976), this is the first time that the variation in their distribution in the seed has been confirmed, relating this to seed coat thickness.

Stomata occur in the epidermis of the endocarp of *S. macrophylla* fruits (E. A. S. Paiva, unpubl. res.), which make gas exchange through the pericarp possible and suggest that the stomata in seeds may be important in gas exchange during embryo development. If locule air can be replaced, the gas exchange by the embryo may become more efficient, but this hypothesis remains to be tested. According to Werker (1997), when chlorophyll is lacking in the seed, as we observed in *S. macrophylla* seeds, the stomata apparently provide a passage for respiratory gas exchange for the growing embryo and endosperm. Intercellular spaces within the seed coat also take part in aeration of the developing seed (Corner, 1976).

The fact that, even after imbibition, the presence of a large volume of air was detected in the seed coat is relevant because, in addition to conferring lightness and permitting the seed to float or to be wind dispersed, it forms an air reservoir that permits embryo respiration, before the seed coat ruptures. Among the possible roles of the stomata in seeds, facilitating gaseous exchange (Flint and Moreland, 1943; Jernstedt and Clark, 1979; Werker, 1997) and acting as preferential sites for water entery during imbibition (Werker, 1997) stand out.

The data obtained during seed imbibition showed a relationship between imbibition rates and stomata density, which suggests that the stomata may act as preferential sites for water entry in the *S. macrophylla* seeds. The greater quantity of water stored in the seed coat of the seeds with an exposed embryo region, at all the time points studied, was a function of the greater imbibition rates in the first 2 h for this region of the seed, where the stomata density is significantly greater. One of the advantages conferred by the presence of a greater number of stomata in the embryo region is the higher rate of water uptake that these structures seem to provide in the first hour of imbibition. Rapid water uptake in the first 2 h and water availability to the embryo may make the germination process independent from the continuity of water supply, which could signify an adaptive advantage.

The initial imbibition rates, until 24 h after the start of the process, seemed to be little influenced by the presence of the embryo, because seeds with and without an embryo presented similar rates. After this time, however, the increase in the mass of the intact seeds could be interpreted as the start of water uptake by the embryo. Our data showed that in the *S. macrophylla* seeds, the weight gain of the embryo occurred only after a considerable water accumulation in the seed coat. This suggests that the growth of the embryo only started with a guaranteed water supply. Thus the seed coat of the imbibed seeds would serve as a water reservoir for germination, acting to protect the embryo against desiccation. According to Harper and Benton (1966), the biggest seeds would be more susceptible to desiccation because they have less contact surface with the substrate; in the present case, the seed coat of the mahogany seeds seems to minimize this effect.

The use of aqueous safranin as an apoplastic pathway marker, as suggested by Seago *et al*. (2000), showed that in the *S. macrophylla* seed coat the stomata are preferential sites for water entry during imbibition. Although the

cuticle is thin, the presence of phenolic compounds in the exotesta may explain the impermeability of the cells to water (Suarez and Engleman, 1980; Werker, 1997) as we observe in the first 2 h of imbibition.

The data obtained in this study permitted the conclusion that, at maturity, the stomata in the *S. macrophylla* seed coat play a significant role in seed imbibition. The restriction of stain penetration to stomata and the direct relationship between imbibition rates and stomata density clearly support this position. Our analyses indicated further that the permanently open stomata during seed development might develop an important role in gas exchange, favouring embryo respiration.

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