

# Seed structures in water uptake, dormancy release, and germination of two tropical forest Fabaceae species with physically dormant seeds

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**Abstract** The morphoanatomy and germination of dormant seeds of *Peltophorum dubium* (Spreng.) Taub. and *Mimosa bimucronata* (DC) O. Kuntze, Atlantic forest Fabaceae species, were examined. Temperature treatments for breaking physical dormancy were applied, and the effects of three forest environment on seed germination were examined. The location of water inlet was carried out soaking in water nondormant seeds with parts of the integument waterproofed with glue. Scanning electron microscopy and optical microscopy were employed to observe the morphoanatomy of seed coat's surface. Wet shocks of 40 and 50 °C were effective in breaking the physical dormancy of seeds. Higher temperatures in forest clearing had greater effect on germination and emergence of seedlings. Both species presented external and internal seed morphology similar to that of most Fabaceae species. In *P. dubium* seeds, the water inlet appears to occur not only by the lens, but also by the micropyle and hilum. In *M. bimucronata*, the sites of water intake were the micropyle and lens.

**Keywords** Atlantic forest · Temperature treatment · Water gaps

## Introduction

In most Fabaceae species, water impermeability of the coat is caused by the presence of one or more layers of elongated, lignified Malpighian cells that are tightly packed

together and impregnated with water-repellant chemicals. This results in physical dormancy of the seeds (Morrison et al. 1998; Smith et al. 2002; Baskin 2003). The hydrophobic substances vary based on the species and can be suberin, lignin, or callose, as well as other substances (Kelly et al. 1992; Serrato-Valenti et al. 1993; Jayasuriya et al. 2007). One characteristic of physically dormant seeds is the presence of a specialized anatomic region that develops an opening, into which water can enter (Baskin and Baskin 2001). In Fabaceae with physically dormant seeds, different structures, depending on the species, act as the water inlet, including, for example, the lens (Serrato-Valenti et al. 1995; Baskin 2003; Hu et al. 2008; Paula et al. 2012; Delgado et al. 2015). In addition, under natural conditions, temperature is an important environmental factor that influences the breaking of physical dormancy (Vázquez-Yannes and Orozco-Segovia 1982; Moreno-Casasola et al. 1994; Kondo and Takahashi 2004; Jayasuriya et al. 2008).

This work aimed to examine the morphoanatomy and germination of two Atlantic Forest Fabaceae species with physically dormant seeds, focusing on (1) the structure and the chemical composition of the seed coat, (2) the structures of water inlet, and (3) the effect of temperature on breaking physical dormancy.

The study used seeds of *Peltophorum dubium* (Spreng.) Taub. (Caesalpinioideae) and *Mimosa bimucronata* (DC) O. Kuntze (Mimosoideae). Both exhibit characteristics of pioneer species (Durigan and Nogueira 1990; Carvalho 2003). *Peltophorum dubium* occurs in the semideciduous Atlantic Forest, in deep, not too wet, clayey soil (Carvalho 2003; Saueressig 2014). *Mimosa bimucronata* is found in the Atlantic Rainforest and semideciduous Atlantic Forest, in moist, sandy, poorly drained soil (Saueressig, 2014). The semideciduous Atlantic Forest has a seasonal climate with

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relatively a severe dry season, and the Atlantic Rainforest has a warm and wet climate without a dry season (Morellato and Haddad 2000).

## Materials and methods

### Seed collection

*Peltophorum dubium* seeds were donated by the Cantareira Forest Nursery, located in Pedreira, SP (22°44'31"S, 46°54'05"W). *Mimosa bimucronata* seeds were obtained from fruits collected from five trees growing in the Atlantic Forest, in Florianópolis, Santa Catarina, Brazil (27°35'36"S, 48°35'60"W). The seeds were newly collected.

### Imbibition and germination of the seeds

To evaluate the germination of intact and treated seeds, four replicates of 25 intact and thermally scarified seeds were added to Petri dishes with sheets of filter paper moistened with distilled water. The thermal treatment was done with the seeds being kept in a beaker containing water at a temperature of 95 °C for *P. dubium* (Oliveira et al. 2008) and at 80 °C for *M. bimucronata* (Fowler and Carpanezzi 1998) until the entire water reached the cooling condition. The seeds were then stored in Petri dishes for 30 days at 25 °C under the 12-dark and 12-h light (40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) condition, kept in a germination chamber. Dark condition was created by putting the seeds inside a black gerbox container. After this period, nongerminating seeds were subjected to the tetrazolium testing.

### Germination of seeds in the field

To evaluate the influences of the forest gap, edge, and understory on germination and seedling emergence, four replications of 20 seeds for each environment were tested. The seeds were planted in ceramic pots with 0.5 kg of substrate (forest soil) and placed in each environment in an Atlantic Forest fragment in the municipality of Florianópolis. The pots remained in their respective environments for 34 days during the summer. They were watered daily, and the intensity of photon flux was measured at noon on a clear cloudless day using a quantameter (250 Li-Cor). The measurements were taken in nine positions for each replicate. The photon flux intensities measured were for forest interior 21.30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; forest edge, 510.29  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; and forest gap, 1380.40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The temperatures at the sites were measured using a maximum and minimum thermometer (Incoterm), and the data are illustrated in Table 1. The recorded minimum temperatures

**Table 1** Maximum and minimum temperatures in different environments of a segment of Atlantic forest

Environment	T <sub>maximum</sub> (°C)	T <sub>minimum</sub> (°C)
Forest gap	47 (38–42)	14 (20–24)
Forest edge	34 (25–30)	13 (19–24)
Forest interior	31 (22–27)	18 (20–25)

Inside the parentheses: the temperature interval with frequency of 70% or more during the experiment

occurred in the last three days of the experiment. The maximum temperatures for the three environments were recorded in a single time, on the fourth day of the experiment for the forest gap and in the middle of the experimentation period for the other two environments. The number of temperature classes and their frequency were calculated using BioEstat 5.0 (Ayres et al. 2007).

### Influence of temperature on breaking physical dormancy

To evaluate the effect of temperature on breaking the physical dormancy under laboratory conditions, the most frequent temperatures recorded in the field served as the basis for experiments, except the temperature shock of 50 °C, which has been tested to be possible to occur in field. The germination conditions for the seeds were created by a germination chamber (Dist 300-220 EF) with a cycle of 12 h of dark and 12 h of light (40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at a constant temperature of 25 °C for seeds before treatment with temperature shock, or at alternating temperature for seeds treated with alternating temperature (see treatments below). Each treatment had four replicates of 20 seeds placed in Petri dishes on two sheets of filter paper moistened with 5 mL of distilled water. The temperature shock treatments were (a) 4 h at 50 °C for one day and (b) 4 h at 40 °C for one day. The shock treatments of 50 and 40 °C were applied by putting the seeds in a Samrello seed germination chamber without light. Every two days, the seeds were analyzed for germination. After shock temperature treatments, the seeds were placed for 40 days in a germination chamber. The treatments with alternating temperatures were (a) 40 °C/20 °C, (b) 35 °C/20 °C, and (c) 30 °C/20 °C with the seeds, during all time of the experiment, 40 days, being kept in a germination chamber under environmental conditions described above.

### Location of the water inlet

Based on results obtained for germination with thermal shocks (see “Results”), to break physical dormancy of *P. dubium* and *M. bimucronata*, the seeds were soaked in water in a germination chamber at 40 °C for 4 h. Next,

seed coats were impermeabilized with Super Bonder glue (Henkel, Jundiai, Brazil). For *P. dubium*, the following areas were impermeabilized: (1) the hilar region, including the hilum, lens, and micropyle; (2) just the hilum; (3) just the lens; and (4) just the micropyle. For *M. bimucronata*, the following areas were impermeabilized: (1) the hilar region, including the hilum, lens, and micropyle; and (2) the pleurogram region. The control group consisted of nondormant, nonblocked seeds. Three replicates of five seeds were made for each treatment. Seeds were placed in Petri dishes on two layers of filter paper with 10 mL of distilled water. The seeds were stored at 25 °C with a photoperiod of 12 h/12 h dark/light. Incubated seeds were counted daily to evaluate germination.

### Structural analysis of seeds

The morphology of the seed coat's surface of intact seeds was observed using an EZ4D Leica stereoscope microscope (Wetzlar, Germany). For analysis of tissue and cell constituents of the tegument, ten fresh seeds were embedded in Leica Historesin<sup>®</sup> (Wetzlar, Germany) and sectioned (30–35 µm thick) using a Leica RM 2125RT rotary microtome (Wetzlar, Germany). The sections were observed using a Leica MPS 30 DMLS optical microscope (Wetzlar, Germany). The cell dissociation method (Franklin 1945) was used to observe individual cells. Dissociated material was stained with toluidine blue at 6.8 pH (O'Brien et al. 1964) and observed under light microscopy. For histochemical study of tissues, ten fresh seeds of each species were split manually with a razor blade, and the sections were exposed to the following reagents: sudan IV for evidence of suberin, oils, waxes, and cutin (Gerlach 1984); acidified phloroglucinol (Costa 1982) and toluidine blue for evidence of lignin (O'Brien et al. 1964); and toluidine blue for evidence of tannins and cellulose (O'Brien et al. 1964). The sections obtained were mounted between slides and cover slips and studied. Images were taken using a digital camera coupled to a Leica MPS 30 DMLS optical microscope (Wetzlar, Germany). Aniline blue was used to look for evidence of callose (Ruzin 1999). The sections were observed under blue/ultraviolet epifluorescence using an Olympus BX41 microscope (Tokyo, Japan) with excitation at 330/385 nm and emission at 420 nm. Images were taken using a QImaging digital camera (3.3 megapixel QColor 3C) and the QCapture Pro 5.1 software (QImaging, Surrey, British Columbia, Canada).

### Effect of temperature on seed integument and structures

To compare seed integuments and structures before and after treatment by thermal shock (4 h or 1 h at 40 °C,

according the methodology described above), a scanning electron microscope (SEM) was used. Five scarified and five nonscarified seeds of each species were fixed in 2.5% glutaraldehyde with a 0.1 M sodium phosphate buffer at 7.2 pH for 2 h. Following this, the samples were washed three times in the same buffer and dehydrated in a graded ethanol series. The samples were then dried using a Leica EM CDP 300 CO<sub>2</sub> critical point dryer (Horridge and Tamm 1969). The dried samples were mounted on aluminum stubs, coated with gold (20 nm thick), and viewed using a Jeol JSM 6390 LV scanning electron microscope at LCME (Laboratório Central de Microscopia Eletrônica, UFSC).

### Data analysis

Analyses of the germination data were made by means of BioEstat 5.0, using the Student's *t* test for comparison of two treatments and ANOVA for three treatments. The Kruskal–Wallis test was used to verify the normality of the distribution of data and heteroscedasticity (Ayres et al. 2007).

## Results

### Germination of the seeds

Intact seeds of *P. dubium* and *M. bimucronata* showed 8.0 and 18.0% germination in light and 0 and 7% germination in dark, respectively, after 30 days of incubation. However, the hot scarified seeds of *P. dubium* showed 73% germination after 10 days of incubation in light, while those of *M. bimucronata* showed 80% germination after three days of incubation in light.

In the outdoor experiment, the average percentages of seedling emergence for *P. dubium* were 40% in clearing, 11% at forest edge, and 14% in forest understory. For *M. bimucronata*, the percentages of seedling emergence were 53% in clearing, 13% at forest edge, and 8% in forest understory. The results obtained for both species from the clearing and the other two environments were different ( $P \leq 0.05$ ). Nongerminated seeds were pinkish in color after the tetrazolium test, indicating that they were alive.

The wet and dry shock treatments of 40 or 50 °C for 4 h provoked some germinations of seeds of both species compared with untreated seeds (Table 2). For seeds of *P. dubium*, shock with both temperatures had a similar effect, while for *M. bimucronata*, germination was higher for seeds treated at 40 °C (Table 2). For *M. bimucronata*, wet shock improved germination compared with dry shock, but not for *P. dubium* (Table 2). The effect of alternating temperatures provoked higher germination in seeds of both species compared with control seeds. Alternating

**Table 2** Percentages of germination of *Peltophorum dubium* and *Mimosa bimucronata* seeds treated with wet and dry heat shock and alternating temperatures

Treatments	<i>Peltophorum dubium</i> (%)	<i>Mimosa bimucronata</i> (%)
Wet shock		
40 °C	60.0 <sup>b</sup>	84.4 <sup>a</sup>
50 °C	55.0 <sup>b</sup>	57.0 <sup>b</sup>
Dry shock		
40 °C	38.3 <sup>c</sup>	49.0 <sup>b</sup>
50 °C	28.3 <sup>c</sup>	26.7 <sup>c</sup>
Alternating temperature		
30 °C/20 °C	38.3 <sup>c</sup>	78.5 <sup>b</sup>
35 °C/20 °C	38.5 <sup>c</sup>	85.0 <sup>a</sup>
40 °C/20 °C	81.9 <sup>a</sup>	90.0 <sup>a</sup>
25 °C (control)	8.0 <sup>d</sup>	18.4 <sup>c</sup>

Letters compare values for the same species (ANOVA-Tukey)  $P \leq 0.05$ . Different letters show statistically significant differences. Shocks were applied for 4 h

temperature of 40 °C/20 °C had the best effect for *P. dubium*, while for *M. bimucronata*, 35 °C/20 °C and 40 °C/20 °C had the same effect (Table 2).

### Structural analysis of the seeds

The seeds of *P. dubium* and *M. bimucronata* have a sub-apical hilum. The micropyle, hilum, and lens are aligned, with the hilum between the two other structures (Figs. 1–2). The seed testa of both species consists of one palisade layer, composed of compacted macrosclereid cells, one layer of osteosclereids, several layers of sclerified parenchyma and a few layers of white cells (Figs. 3–4). A light line can be seen in the middle of the palisade layer in the extrahilar region, and a cuticle is above the palisade layer. The light line is impregnated with callose, and the cuticle exhibits waxy substances, as observed during the histochemical tests.

The macrosclereid walls are cellulosic in *P. dubium* and lignified in the basal portion in *M. bimucronata*, which was also observed during histochemical tests. The osteosclereids of both species showed a positive reaction for lignin.

Two layers of macrosclereids can be seen in the hilar regions of *P. dubium*, and in *Mimosa bimucronata*, the hilar region is a single layer of macrosclereids (Figs. 5–6). *Mimosa bimucronata* possesses a structure called a pleurogram in the testa, which is formed by a crack in the palisade layer that reaches the light line (Figs. 7–8).

### Effects of thermal shock on seed integument and structures

Seeds of *P. dubium* that underwent thermal shock of 40 °C had a ruptured lens, but the hilum and micropyle were unaffected (Fig. 9–10). However, seeds of *M. bimucronata* after thermal shock of 40 °C had a wider micropyle,

cracked lens (Figs. 11–12) and wider fissure of the pleurogram (Figs. 13–14) measuring 7.5 µm in untreated seeds and 164.0 µm in thermally shocked seeds.

### Location of water inlet

Seeds of *P. dubium* treated with thermal shock to block hilum, micropyle, and lens alone, or together, showed very low germination, while seeds treated with thermal shock, but not blocked, had germination rates that reached 95% after eight days of imbibition (Fig. 15). In contrast, seeds of *M. bimucronata* treated with thermal shock to block hilum, micropyle, and lens did not germinate at all. Seeds with blocked pleurogram germinated, although germination was lower compared to not blocked seeds (Fig. 16). After six days of imbibition, germination was 95% for not blocked seeds, 70% for seeds with blocked pleurogram, and 5% for seeds with blocked hilum, micropyle and lens.

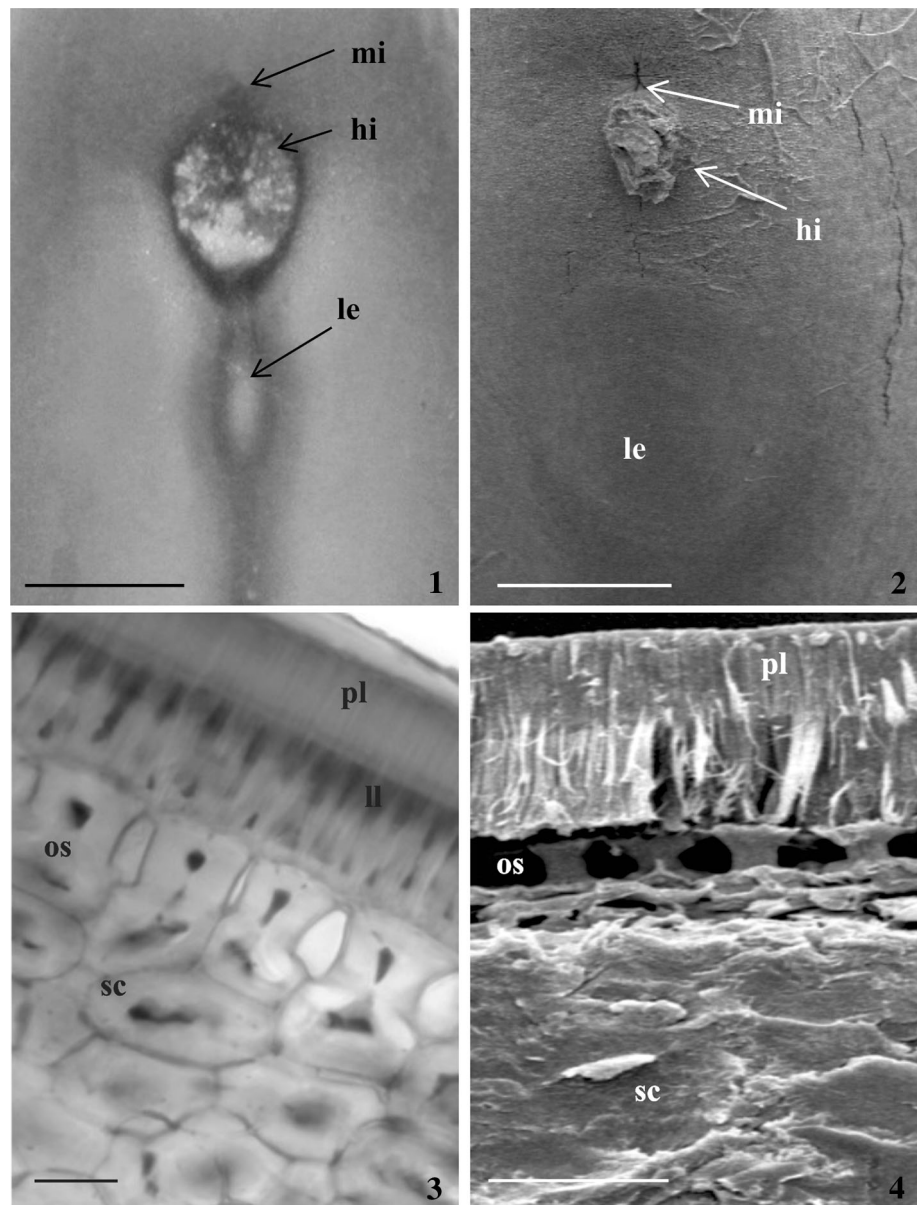
## Discussion

### Seed germination

Species with wide distributions, such as *P. dubium* and *M. bimucronata*, have seeds that germinate under a wide range of conditions, resulting in plasticity that contributes to plant survival (Pereira et al. 2013). Thus, seeds collected in different regions could present different germination requirements relative to environmental conditions, such as temperature. Therefore, it is important to evaluate different seed lots when determining germination characteristics of a species. The presence of physical dormancy of seeds found in this study has been confirmed by Piroli et al. (2005) and Oliveira et al. (2008) and for *P. dubium* and by Fowler and Carpanezzi (1998) and Ribas et al. (1996) for *M.*



**Figs. 1–4** Optical micrographs of seed coat of *Peltophorum dubium* (1–3) and electron micrographs of *Mimosa bimucronata* (2–4). Front view (1–2), longitudinal sections (3–4). Legend: *le* lens, *hi* hilum, *mi* micropyle, *mc* macrosclereids, *ll* light line, *os* osteosclereids, *sc* sclerenchymatous tissue, *pl* palisade layer. Bars: 2 mm (1); 200  $\mu$ m (2); 10  $\mu$ m (3); 25  $\mu$ m (4)



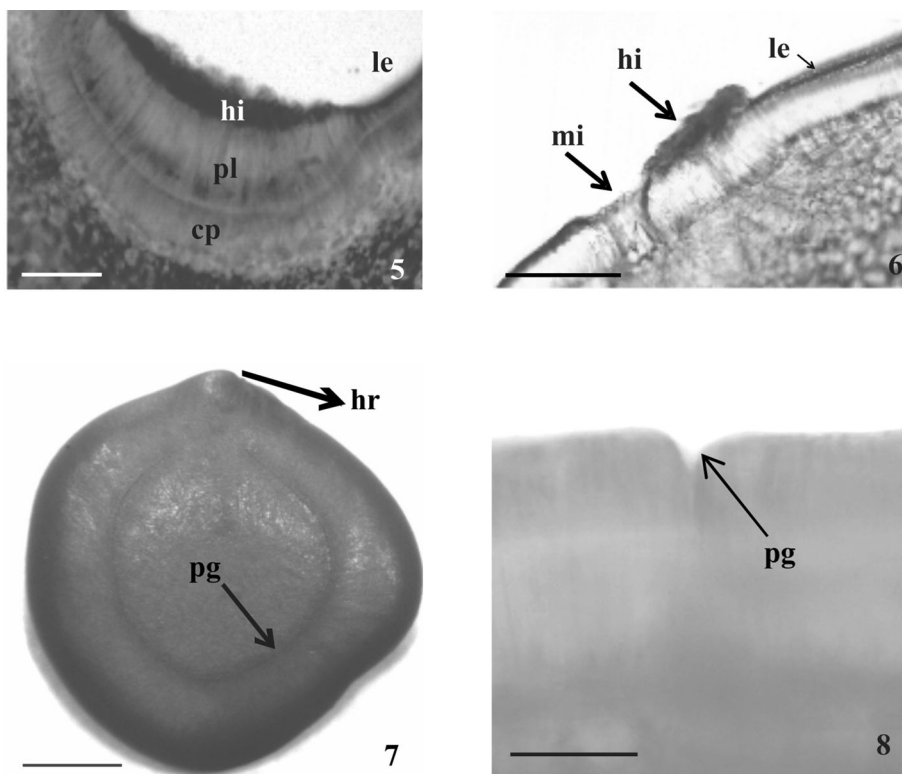
*bimucronata*. In the present work, the seeds of *P. dubium* and *M. bimucronata* reached 60 and 84% of germination, respectively, at the temperature of 25 °C. The optimal temperature for seed germination of these species is around 35 °C; however, they germinated well at 25 °C (Carvalho 2003; Pereira et al. 2013).

The literature describes more on the action of thermal shock to break the physical dormancy in seeds of plants environments subject to the passage of fire or prolonged droughts (Baskin and Baskin 2001; Tieu et al. 2001; Ribeiro and Borghett 2013; Silveira and Overbeck 2013); however, there are some papers reporting the influence of thermal shock in tropical plants moist environments (van Klinken and Flack 2005; van Kinken et al. 2006, 2008). For both species, wet shocks were better than dry shocks.

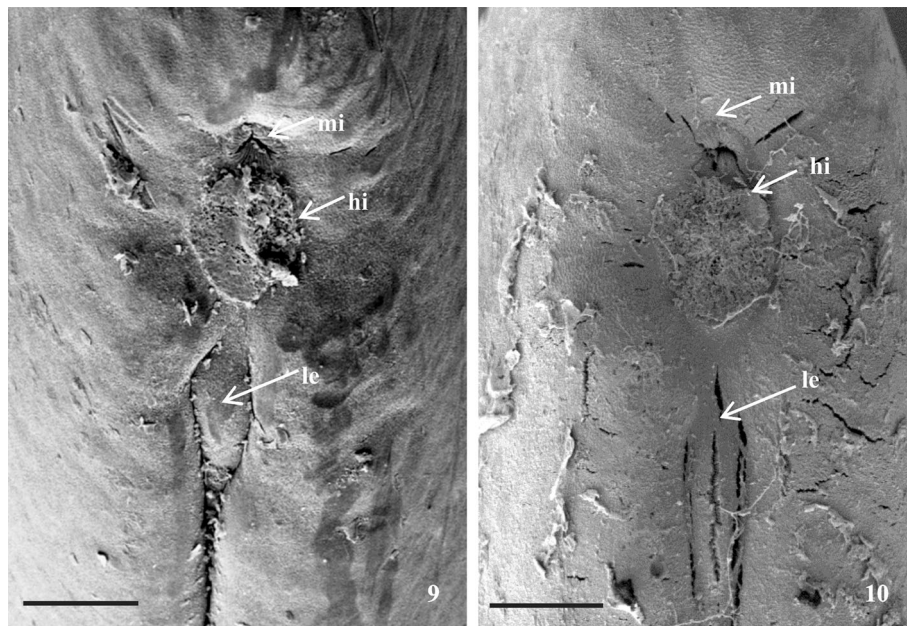
Atlantic forest are typically warm and wet for significant periods of the year (Morellato and Haddad 2000) and warm wet conditions might therefore be an important mechanism for dormancy release under tropical conditions (van Klinken and Flack 2005; van Klinken et al. 2008). The wet heat shock for a short time tested here is likely to occur in the field as seen here in the experiment in clearing and in summer in Atlantic Forest gaps (Ewers and Banks-Leite 2013).

*Mimosa bimucronata* showed under the treatments of alternating temperatures (40 °C/20 °C, 35 °C/20 °C, 30 °C/20 °C) similar germination of the seeds treated with wet shock of 40 °C. However, 4 h under 40 °C was enough to break seed physical dormancy as seen in this study for this species, then the breaking of seed dormancy under

**Figs. 5–8** Optical micrographs of *Peltophorum dubium* (5–7) and *Mimosa bimucronata* (6–8) seed coat. Longitudinal sections (5–6). Frontal view (7) and longitudinal section of the pleurogram (8). *Legend:* *le* lens, *hi* hilum, *mi* micropyle, *hr* hilar region, *pl* palisade layer, *cp* counterpalisade, *pg* pleurogram. *Bars:* 10  $\mu\text{m}$  (5); 100  $\mu\text{m}$  (6); 1 mm (7); 25  $\mu\text{m}$  (8)



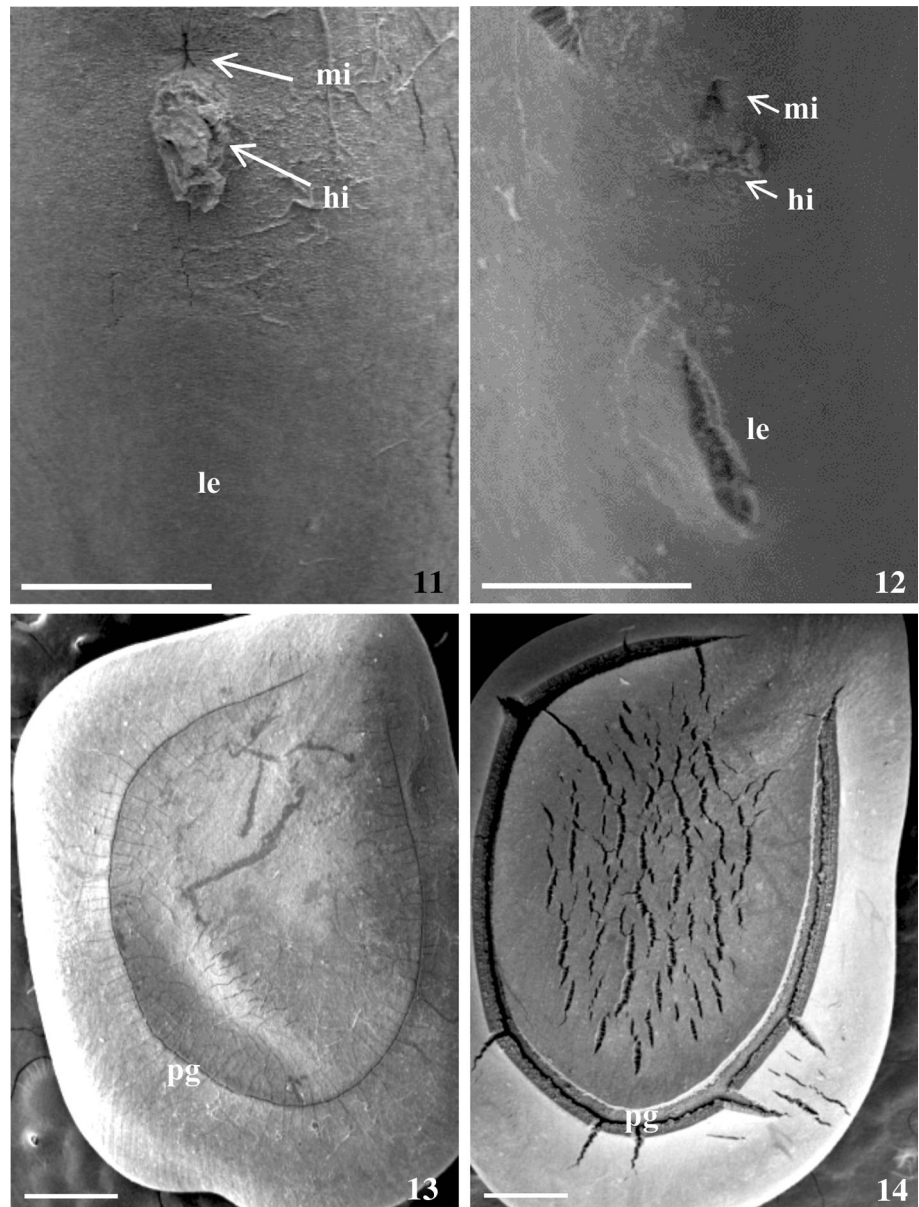
**Figs. 9–10** Electron micrographs of *Peltophorum dubium* seeds before (9) and after (10) treatment to break dormancy (40 °C for four hours). *Legend:* *le* lens, *hi* hilum, *mi* micropyle. *Bars:* 200  $\mu\text{m}$



alternating temperature of 40 °C/20 °C was in fact the effect of 40 °C. The results of other alternating temperatures on the break of seed physical dormancy do not allow to draw any conclusions, since it has not been tested the isolated temperature of 35 and 30 °C on physical dormancy breaking of the seeds. Seeds of *P. dubium* under alternating temperatures of 40 °C/20 °C markedly improved

germination over that of seeds of wet shocked at 40 °C. However, this result cannot be due to a larger number of seeds with broken dormancy, but due to heat accumulation during the time of germination of the seeds. Heat accumulation on seed germination was observed for other species by several authors as Moreno-Casasola et al. (1994), del Monte and Tarvis (1997), Cave et al. (2011)

**Figs. 11–14** Electron micrographs of *Mimosa bimucronata* seeds before (11–13) and after (12–14) treatment to break dormancy (40 °C for four hours). Legend: *le* lens, *hi* hilum, *mi* micropyle. Bars: 200  $\mu\text{m}$  (11–12) and 500  $\mu\text{m}$  (13–14)



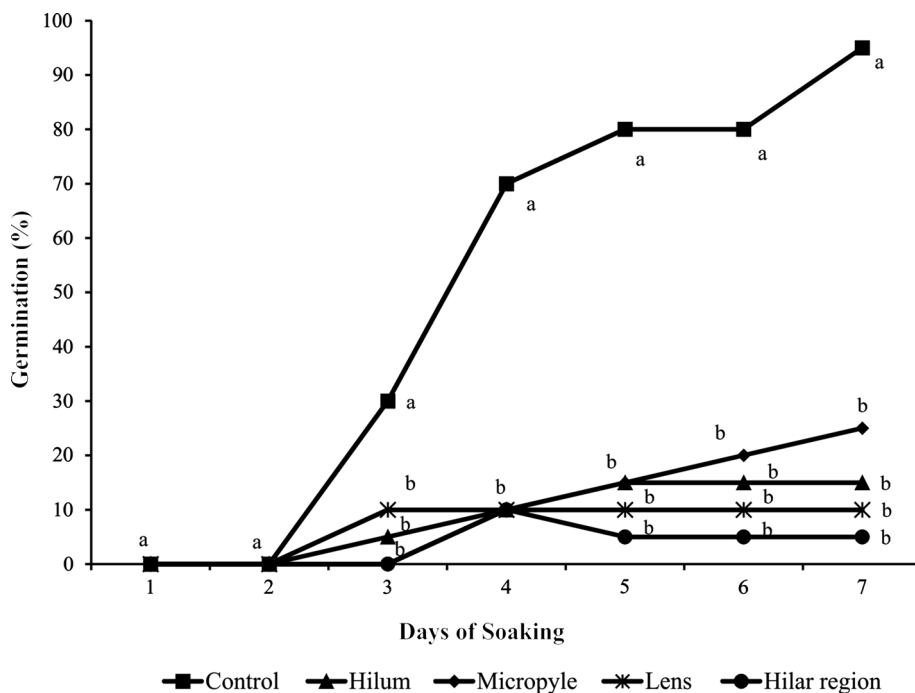
and Cristaudo et al. (2014). The result observed here for *P. dubium* and *M. bimucronata* is consistent with the observation of van Klinken and Goulier (2013) that species with physical seed dormancy will have habitat-specific dormancy-release mechanisms. The different conditions of release dormancy and germination of species belong to different niches of the Atlantic Forest should allow these species occur at the same time (Hudson et al. 2015).

In outdoor experiments, the temperature was higher in clearings, and both breaking of physical dormancy and seedling emergence were higher in this area compared with the forest edge and understory, in complete agreement with the pioneer status of these species. There is a greater amount of light in clearings than in the other forest

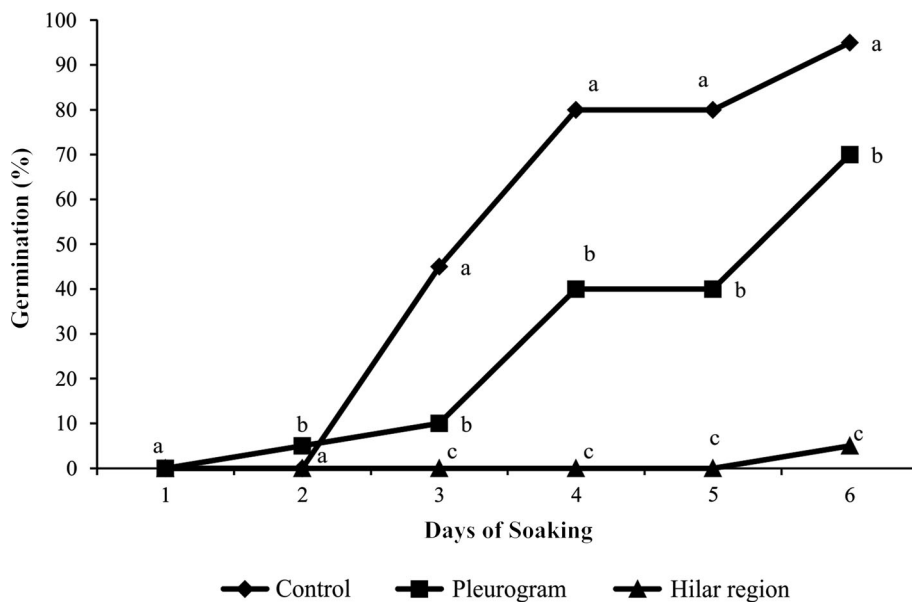
environments, and thus, it might be supposed that the light could be involved in breaking physical dormancy in seeds of these species. Based on the results of laboratory experiments in this work, we find that light proved to have a negligible effect on breaking dormancy. On the other hand, van Klinken et al. (2006) found that in the wet-dry tropics the physical dormancy-release mechanism was demonstrated to result in low rates of dormancy release in situations where the environment was buffered from temperature extremes, such as under dense herbaceous or foliar cover, or deep burial. In previous work, it was found that after breaking dormancy of seeds of *P. dubium* (Perez et al. 2001) and *M. bimucronata* (Carvalho 2003), the germination occurs in light and dark.



**Fig. 15** Germination curves of *Peltophorum dubium* seeds with waterproofing hilum, micropyle, and lens, all the three structures together (hilar region) and seeds not waterproofed. Letters compare different curves. Different letters indicate statistical difference (ANOVA-Tukey,  $P \leq 0.05$ )



**Fig. 16** Germination curves of *Mimosa bimucronata* seeds with waterproofing together lens, hilum, and micropyle (hilar region), pleurogram, and seeds not waterproofed (control). Letters compare different curves. Different letters indicate statistical difference (ANOVA-Tukey,  $P \leq 0.05$ )



### Structural analysis of seeds

*Peltophorum dubium* and *M. bimucronata* seeds were found to be morphologically similar, both externally and internally, to most Fabaceae species. For example, the micropyle, hilum and lens are in a line with the hilum between the two other structures (Corner 1951), and a palisade layer of macrosclereids is followed by a layer of osteosclereids and sclerified parenchyma (Corner 1951; Esau 1997). *P. dubium* has smaller cells in the palisade layer in the lens region as

found in some other Caesalpinioideae (Serrato-Valenti et al. 1995; Souza et al. 2012). *Mimosa bimucronata* has a pleurogram, which is a fine groove that is U-shaped on both sides of the seed coat. This structure is only found in the Cucurbitaceae and Fabaceae, and it has taxonomic value (Barroso et al. 1999). In Fabaceae, a pleurogram occurs in the subfamilies Mimosoideae and Caesalpinioideae. It is an inverted U-shape in Mimosoideae and closed and circular in Caesalpinioideae (Corner 1951, 1976; Gunn 1981; Barroso et al. 1999). In Mimosoideae, the pleurogram consists of a



disruption in the palisade cells, which forms a slot (Corner 1951, 1976), as found in *M. bimucronata*.

Lignin and callose are reported to be impermeable to water (Serrato-Valenti et al. 1993; Baskin 2003; Jayasuriya et al. 2007). Fabaceae seeds do not necessarily possess both substances, as found in *P. dubium*, which only has callose in the palisade layer located in the light line. *Mimosa bimucronata*, on the other hand, has lignin in the basal portion of macrosclereids and callose in the light line region. The absence or presence of lignin in the palisade layer has been found in seeds of other Fabaceae species; for example, it is absent in *Cassia cathartica* Benth. (Souza 1982), *Sesbania punicea* (Cav.) Benth. (Bevilacqua et al. 1987), *Stylosanthes scabra* Vog. (Serrato-Valenti et al. 1993), *Leucaena leucocephala* (Lam.) De Wit (Serrato-Valenti et al. 1995) and *Schizolobium parahyba* (Vell.) Blake (Souza et al. 2012), but present in *Glycine max* (L.) Merrill (Alvarez 1997), *Cassia grandis* L., *Anadenanthera macrocarpa* (Benth.) Brenan, *Enterolobium contortisiliquum* (Vell.) Morong (Costa et al. 2011), *Cassia leptophylla* Vog., *Senna macranthera* (DC. ex Collad.) H. S. Irwin & Barnaby (Paula et al. 2012), *Sophora tomentosa* L., and *Erythrina speciosa* Andr., (Delgado et al. 2015). The presence of callose in the light line, as found in *P. dubium* and *M. bimucronata*, also occurs in other Fabaceae, such as *Trifolium subterraneum* L. (Bhalla and Slaterry 1984), *Sesbania punicea* (Bevilacqua et al. 1987), *Stylosanthes scabra* (Serrato-Valenti et al. 1993), *Leucaena leucocephala* (Serrato-Valenti et al. 1995), *Cassia leptophylla*, *Senna macranthera* (Paula et al. 2012), *Schizolobium parahyba* (Souza et al. 2012), *Sophora tomentosa* and *Erythrina speciosa* (Delgado et al. 2015). Aside from their impermeability to water, callose and lignin are substances that contribute to long-term seed persistence and provide an effective barrier against predation and microbes, particularly in warm and moist environments (Souza and Marcos Filho 2001).

### Location of water inlet

In physically dormant seeds of Fabaceae species, the lens has been reported as the site of initial water intake (Manning and Van Staden 1987; Serrato-Valenti et al. 1995; Baskin et al. 2000; Hu et al. 2009), although other seed structures may play this role (Gama-Arachchige et al. 2013; Delgado et al. 2015).

Based on structure-blocking experiments, the water inlet in *P. dubium* seeds appeared to be the lens, micropyle, and hilum. Cracks on the sides and center of the lens, as seen in SEM micrographs of seeds with broken physical dormancy, support the finding that the lens is involved in water intake. However, the micrographs showed no

modifications of the hilum and micropyle after physical dormancy had been broken. The cracks in the micrographs seen in the extrahilar region were not involved in letting water into the seed, as shown by the experiment that blocked these structures.

Scanning electron microscope analysis of *M. bimucronata* showed that the sites of water intake could be the micropyle and the lens, which exhibited structural changes after seed dormancy was broken. The micropyle has also been shown to be involved in water absorption in Fabaceae species (Rangaswamy and Nandakumar 1985; Bhattacharya and Saha 1990; Paula et al. 2012).

Mello-Pinna et al. (1999) suggested that the pleurogram was involved in water absorption in Fabaceae seeds with physical dormancy, which was based on monitoring changes in this structure during times of drought and high temperatures. In *M. bimucronata*, as seen from the blocking experiment, when pleurogram is blocked, nearly 70% germination can be observed. This is an indication of the permeability of seeds even after pleurogram is blocked. However, when hilum is blocked, seeds have remained impermeable. If water enters through the pleurogram, even after blocking the hilum, seeds should imbibe water. Thus, there is no evidence to support the involvement of pleurogram in water intake. The SEM images revealed a widening of pleurogram after heat shock. However, heat shock can damage a part of palisade cells or the cuticle layer, but the seed must have remained impermeable, when taking into account the data from blocking experiment.

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