Germination of *Miconia ligustroides* (Melastomataceae) diaspores submitted to different treatments for dormancy overcoming

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ABSTRACT – (Germination of *Miconia ligustroides* (Melastomataceae) diaspores submitted to different treatments for dormancy overcoming). To assess the germination behavior of *Miconia ligustroides* (DC.) Naudin, diaspores were submitted to different regimes of temperature, light and substrate. Due to intrinsic low germination of the diaspores, they were submitted to treatments that aimed to overcome endogenous and exogenous dormancy. *Miconia ligustroides* presents complex dormancy and to overcome it is recommended to soak the diaspores in H_2SO_4 diluted at 75% for 5 minutes, wash it and then soak it in a solution of 400 mg L^{-1} of GA_3 for 12 hours. Seeding should be done on Germtest® paper at 25 °C.

Key words - complex dormancy, germinability, *jacatirão-do-brejo*

RESUMO – (Germinação de diásporos de *Miconia ligustroides* (Melastomataceae) submetidos a diferentes tratamentos de superação de dormência). Para avaliar o comportamento germinativo de *Miconia ligustroides* (DC.) Naudin, diásporos foram submetidos a diferentes regimes de temperatura, luz e substrato. Devido à baixa germinabilidade dos diásporos, foram realizados tratamentos de superação de dormência endógena e exógena. *Miconia ligustroides* apresenta dormência complexa e para superá-la recomenda-se a imersão dos diásporos em H_2SO_4 diluído a 75% por 5 minutos, seguida de lavagem e embebição em solução de 400 mg L^{-1} de GA_3 por 12 horas. A semeadura deve ser realizada em papel Germtest® à 25 °C.

Palavras-chave - dormência complexa, germinabilidade, jacatirão-do-brejo

Introduction

Ensuring the survival of plant species is directly linked to the existence of seeds, which symbolize its continuity and diversity. However, besides containing the genetics traits of the parents, the seeds are capable of receiving stimuli from the environment during or after their formation, allowing them to change their behavior after their release from the mother plant. So, after adapting to the environment, many plants species started to develop, in evolutionary terms, mechanisms that allow their survival. Among these, seed dormancy represents one of the key skills of plant species to ensure their survival and perpetuation, being related to the life cycle and hardiness of the species (McIvor & Howden 2000).

Dormancy is a phenomenon in which the seeds of some species, even though viable and possessing all the environmental conditions to start the germination process, do not germinate (Bewley & Black 1994). Based on the putative mechanisms involved, dormancy can be classified into two major groups: endogenous and exogenous (Cardoso 2004). Endogenous dormancy is caused by some germination blockage related to the embryo itself, but eventually may involve extra-embryonic tissues, and can be divided into: physiological, morphological, and morphophysiological. Morphological dormancy occurs in seeds that are shed from the parent plant when their embryos are not fully developed, since they are rudimentary or torpedo-shaped (Arteca 1996).

Exogenous or extra-embryonic dormancy is caused primarily by the integument, the endocarp, the pericarp and/or by extrafloral organs, and usually by little or no direct participation of the embryo in its overcoming. In general, the mechanisms responsible for this type of dormancy are related to impermeability, mechanical effect, and/or to presence of inhibitory substances in the tissues. They can be divided into physical, chemical and mechanical (Cardoso 2004). Importantly, the same species may show different degrees and types of dormancy. For species that present more than one type of dormancy, the dormancy is often said to be complex or combined.

According to Zaidan & Barbedo (2004), the importance of different degrees of dormancy is to prevent a rapid and uniform germination of all seeds produced in a given time, which may result in competition among

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seedlings or in the death of all of them immediately after its emergence, in case of occurrence of a drastic change in the environmental conditions. Still, dormancy is often a detrimental issue to the activities of a nursery, where large quantities of seeds are expected to germinate in a short time, allowing the production of uniform seedlings. In addition, the seed lots that have some type of dormancy may have its viability underestimated, when low values of germination percentage are obtained. In this case, knowledge of the dormancy causes is of significant practical importance, allowing the application of an appropriate treatment to ensure a better germination (Melo *et al.* 1998).

Miconia ligustroides (DC.) Naudin (Melastomataceae), popularly known as *jacatirão-do-brejo*, is widely distributed in Brazil, occurring from Ceará to Santa Catarina (Martins *et al.* 1996). Generally it occupies savannahs, fields, swamps and wet locations (Goldenberg 2004). It can be characterized as a bush or a 0.5 to 4.5 m tree. Its fruits are dispersed by animals, being a major attraction for wildlife. Often, it also has a large number of bromeliads attached to its stems (Bonnet & De Queiroz 2006). *Miconia ligustroides* has analgesic (Cunha *et al.* 2003) and trypanocidal (Cunha *et al.* 2006) qualities. Although the species present medical and ecological importance, there are only a few studies concerning its sexual propagation, which justify the development of an efficient technology that facilitate its management in large scale, especially in regard to dormancy overcoming.

Material and methods

To assess the influence of light and temperature on germination, the diaspores were sown on two sheets of Germtest® paper moistened with distilled water (2.5 fold the weight of the paper) and placed in Petri dishes (Brasil 2009). Later they were placed in BOD incubator at constant temperatures of 20 °C, 25 °C, 30 °C and 35 °C and 20-30 °C, with relative humidity around 60% ± 3%, at photoperiod of 12 hours. To simulate the dark conditions, the Petri dishes were wrapped with two sheets of aluminum foil and then covered with polystyrene bags, in order to guarantee dark condition. Germination behavior was evaluated under green safe light (Santos *et al.* 2005).

The influence of substrate on germination was tested by sowing on two sheets of Germtest® paper, on sand, in a vermiculite or on Plantmax®, kept in a BOD incubator regulated to maintain the temperature at 25 °C, 12 hours photoperiod and 60% ± 3% relative humidity. The experiments were conducted in a complete randomized design (CRD) with four replicates of 50 diaspores each. Germination evaluations

were performed daily, and using as a parameter the protrusion of ≥ 1 mm of radicle for the diaspores sown on paper and the emergence of the hipocotyledonar hook for the diaspores sown on other substrates. We calculated the total percentage of germination and germination speed index (GSI) according to Maguire (1962). Statistical analysis was performed by means of an analysis of variance. Means were further compared by the Tukey test ($P \leq 0.05$). For these analyses we used the statistical program Sisvar (Ferreira 1999).

To evaluate the presence of endogenous dormancy in the diaspores they were submitted to the following treatments that aimed to overcome dormancy: immersion and agitation for 12 hours in gibberellic acid (GA_3) solution at concentrations of 200, 400, 600 and 800 mg L^{-1}, in potassium nitrate (KNO_3) solution at concentrations of 1 m and 0.2 M, and the combination of these two substances at concentration of 800 mg L^{-1} of GA_3 and 0.1 m KNO_3. As a control, diaspores were immersed and agitated in water, for 12 hours. Later, the diaspores were placed to germinate on Petri dishes covered with Germtest® paper in BOD incubator at 25 °C, with 12 hours photoperiod and 60% ± 3% RH. Diaspore germination was evaluated daily with the aid of a magnifying glass. The experiment was conducted in a complete randomized design (CRD) with four replicates of 50 diaspores each. Statistical analysis was performed by means of an analysis of variance. Means were further compared by the Scott-Knott test ($P \leq 0.05$) using the software Sisvar.

The seeds were analyzed by scanning electron microscopy (SEM) to evaluate the presence of morphological dormancy, according to the protocol established by Alves (2004). This protocol suggests that once fixated, the diaspores should be frozen in liquid nitrogen and sectioned longitudinally to observe the internal structures. The samples were analyzed by scanning electron microscope (LEO EVO 40 XVP Cambridge, United Kingdom).

The presence of physical or mechanical dormancy was analyzed by subjecting the diaspores to treatments with sulfuric acid (H_2SO_4) and thermal shock. Diaspores were treated with H_2SO_4 75% for 5, 10 and 15 minutes and concentrated H_2SO_4 for 30 and 60 seconds, then thoroughly washed in running water. For the heat shock test, diaspores were quickly dipped in hot water and then in cold water. The diaspores were placed to germinate under the same conditions of treatments for dormancy overcoming previously described, and assessed in the same way.

The assessment of combined dormancy was performed by combining the best results of the previous treatments (400 mg L^{-1} of GA_3 plus H_2SO_4 75% for 5 minutes). The germination test was performed in the same conditions as previously described, and assessed in the same way.

Results and discussion

Regardless of the temperature tested, there was no germination in darkness, therefore *M. ligustroides*

can be considered essentially as photoblastic positive. According to Carreira & Zaidan (2007), other species of *Miconia* (*M. albicans* (Sw.) Triana, *M. langsdorffii* Cong., *M. rubiginosa* (Bonpl.) DC. and *M. stenostachya* Schr. & Mart. ex DC.) present the same characteristic. Godoi & Takaki (2007), studying *M. theaezans* found that its seeds have strong dependence on the presence of white light to induce germination with daily need of 4-6 hours of light for maximum induction of the process.

The germination of *M. ligustroides* was higher at 25 °C and lower at 20-30 °C, and no germination at all occurred at 35 °C. However, there was no significant difference in germination when the diaspores were subjected to constant temperatures of 20 °C and 30 °C (figure 1). The GSI followed the same pattern found in the germination. All protruded seeds gave rise to normal seedlings.

According to Bewley & Black (1994), temperature influences the speed and the final percentage of germination, affecting both water absorption by the seeds and the biochemical reactions that regulate the metabolism involved in this process. Seeds are able to germinate under a wide range of temperatures, being set for each species a minimum and a maximum temperature, above and below which germination does not occur (Bewley & Black 1994). Such behavior indicates the adaptive potential of *M. ligustroides*, since it demonstrates an ability to establish greater than that found when there is thermal restriction (Thompson, 1970, Townsend & McGinnies 1972). In this context, the diaspores of *M. ligustroide* subjected to thermal regimes of 20 °C and 30 °C had lower %G and GSI when compared to 25 °C, probably because the variation in temperature slowed the process,

due to the reduction on the activity of enzymes involved in the metabolism of the seed (Simon *et al*. 1976, Bewley & Black 1994). The increase in temperature to 35 °C, on the other hand is probably beyond the maximum temperature for germination. Alternating temperatures (20-30 °C) decreased the %G and GSI when compared to 25 °C. Mayer & Poljakoff-Mayber (1989) reported that alternating temperatures may be more effective in promoting germination than each temperature applied alone, a fact that has not been demonstrated in seeds of *M. ligustroides*, neither in seeds of species studied by Carreira & Zaidan (2007) nor in seeds of *M. chamissois* Naudin (Válio & Scarpa 2001).

Temperature alters the embryo growth potential and influences hormonal levels. At higher temperatures termoinhibition may occur due to induction of abscisic acid (ABA) synthesis (Leymarie *et al*. 2008; Toh *et al*. 2008) and giberellin (GA) repression (Toh *et al*. 2008).

Diaspores germinated on paper showed higher %G and GSI (figure 2). The results in other substrates did not differ. The substrate used in the germination tests has a great influence on the germination, since factors such as structure, aeration, water retention capacity, pathogen infestation may vary depending on the type of material used (Popinigis 1977). The choice must be made according to the requirements of the seed, regarding its size and shape (Brasil 2009). Vermiculite and Plantmax® have a good capacity to absorb and hold water, while sand is difficult for maintaining moisture, because it is uneven in terms of water retention and distribution. Thus, seeds of *M. ligustroides* prefer substrates with lower water retention, such as paper (preferably) and sand.
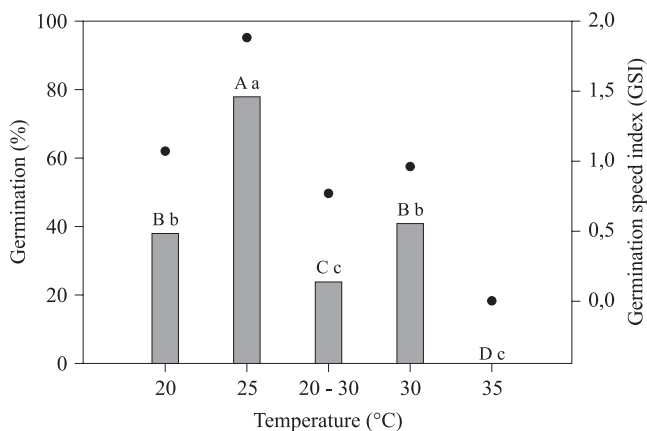

Figure 1. Germination behaviour of *Miconia ligustroides* diaspores subjected to different temperatures. Upper case letters compare % germination (bars) and lower case compare GSI (dot).
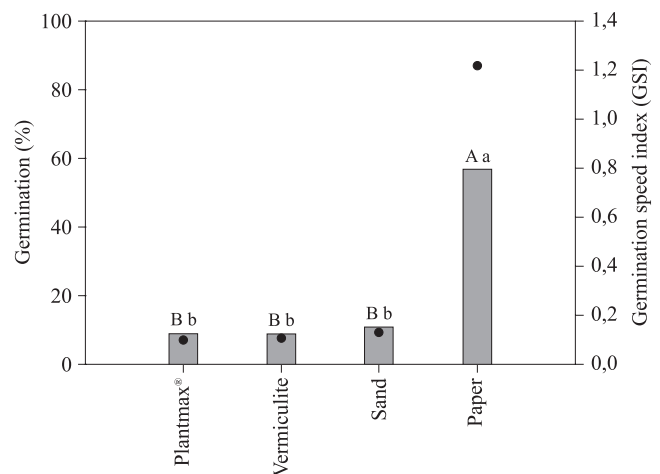

Figure 2. Germination behaviour of *Miconia ligustroides* diaspores subjected to different substrates. Upper case letters compare % germination (bars) and lower case compare GSI (dot).

According to the literature, Melastomataceae species occurring in the Atlantic Forest germinate around 30% (Amaral & Paulilo 1992, Zaia & Takaki 1998, Carreira & Zaidan 2007). In *M. ligustroides* the germination percentage was 55%, a relatively low value.

Regarding the treatments used to overcome physiological dormancy, higher results of %G were obtained with solutions of 400 mg L^{-1} GA$_3$ (96%), followed by immersion in 0.1 and 0.2 M KNO$_3$ solutions (figure 3). These were followed by the treatments with GA$_3$ at 200 mg L^{-1}, and 800 mg L^{-1} plus 0.2 M KNO$_3$ and immersion and agitation in water for 12 hours, which did not differ among them. Soaking in GA$_3$ at 800 mg L^{-1} and the control treatment provided the lowest values. The same pattern was observed in the GSI, which is an indicative of physiological quality and germination uniformity of the seeds. GSI increased with treatments for dormancy overcoming in relation to the control, but the values remained very low. This justifies the development of studies that aim to increase GSI, as will be described later in the works about acid scarification.

The morphophysiological dormancy was characterized by the presence of immature embryos, all in the globular stage of development, observed by SEM (figure 4-7). The embryo of *M. ligustroides* is con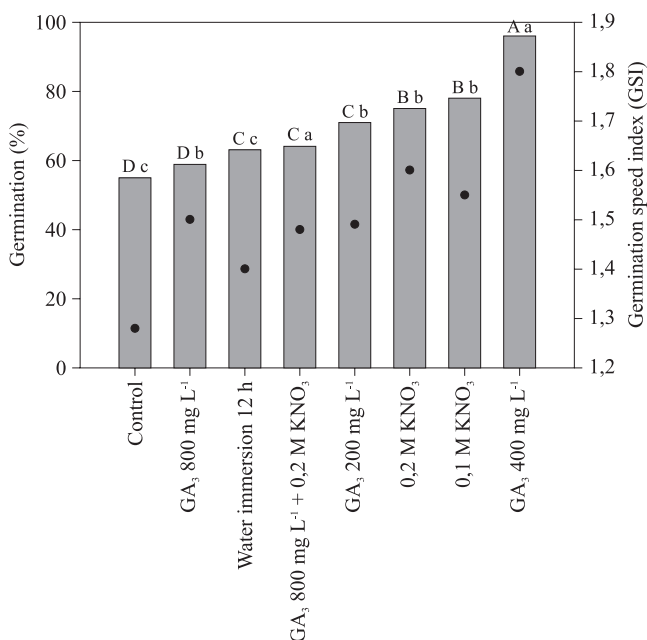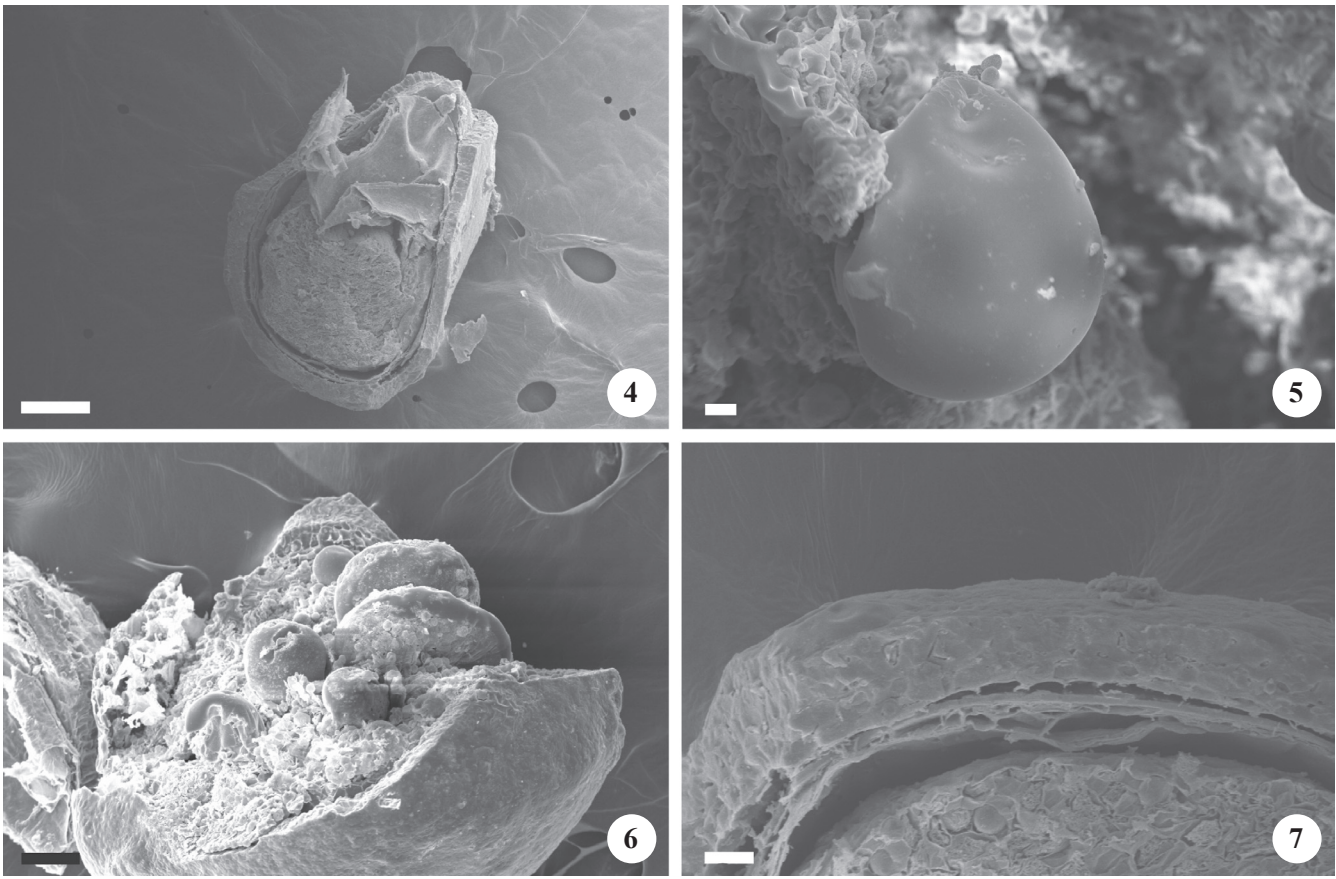stituted, at the moment of dispersal, of a mass of hyaline cells, in which it is not possible to identify the cotyledons or the axis, even under high resolution (figure 5). The immaturity of embryo delays germination until the complete development of the embryo.

Regarding the GSI, the best treatment to overcome dormancy was immersion in H$_2$SO$_4$ 75% for 5 minutes (figure 8). However, the results of immersion in H$_2$SO$_4$ 75% for 10 minutes and immersion in H$_2$SO$_4$ 75% for 15 minutes did not differ between them. The control and heat shock treatment did not differ between them also, they being, however, lower than the treatments mentioned above. The immersion in H$_2$SO$_4$ for 30 and 60 seconds had the worst results. Acid scarification promoted an increase of GSI, resulting in the synchronization of germination. This is an especially important factor for the production of seedlings in nurseries, which demands a rapid germination.

Importantly, the germinability of the diaspores submitted to these treatments was lower than that found in the treatment of endogenous dormancy (lower than 60%). However, the GSI of the diaspores soaked in H$_2$SO$_4$ diluted to 75% for 5 minutes was higher than all other treatments, demonstrating that, besides the presence of morphophysiological dormancy, seeds of *M. ligustroides* also possess a type of physical dormancy, therefore characterizing a complex dormancy. For treatments tested to overcome endogenous dormancy it was also observed a higher timing of germination compared to control.

The presence of physical dormancy is probably due to the presence of the thick integument of *M. ligustroides* diaspores, as observed by SEM (figures 4, 7). The thick outer mantle hinders absorption of water and oxygen by the seeds, retarding germination. The acid treatment, probably promoted the partial degradation of the outer envelope allowing a faster imbibition and thus an early root protrusion. On the other hand, the lower %G may has been due to the fact that only embryos at later stages of development have sprouted. Although we couldn´t find any study that aimed the dormancy overcoming in species of *Miconia*, several studies reporting success in overcoming this kind of dormancy can be found, especially using immersion in H$_2$SO$_4$, (Alves 2000, Albuquerque *et al.* 2007, Azania *et al.* 2009, Benedito *et al*. 2009).

The combination of the treatments for endogenous and exogenous dormancy overcoming promoted high values of %G (98%) and GSI (3.00). The partial degradation of the outer mantle, probably allowed a faster imbibition of GA$_3$.

Figure 3. Germination behaviour of *Miconia ligustroides* diaspores subjected to different treatements aiming endogenous dormancy overcoming. Upper case letters compare % germination (bars) and lower case compare GSI (dot).

Figures 4-7. Electron-micrographs of *Miconia ligustroides* seeds. 4. Aspect of the seed, with thick integuments and embryo cell mass. 5. Embryo at the globular stage of development. 6. Polyembryony evidenced by the presence of six embryos in the globular stage of development. 7. Thick integument of the seeds, which are characteristic of species that possess exogenous dormancy. Bar = 200 µm (4,5); 100 µm (6); 300 µm (7).
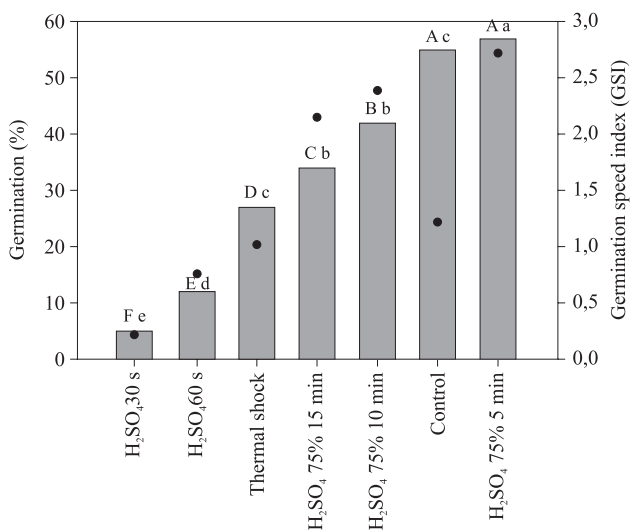

Figure 8. Germination behaviour of *Miconia ligustroides* diaspores subjected to different treatements aiming exogenous dormancy overcoming. Upper case letters compare % germination (bars) and lower case compare GSI (dot).

To overcome the complex dormancy presented in *M. ligustroides* diaspores, they must be treated with H_2SO_4 75% for 5 minutes and 400 mg L^{-1} of GA_3 for 12 hours. The germination test must be done on Germtest® paper at 25 °C.

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