GERMINATION AND OVERCOMING DORMANCY SEED M*imosa bimucronata* (DC.) KUNTZE – LEGUMINOSAE

Thaís Regina Marcon[1]*; Andréa Maria Teixeira Fortes[2]; Lívia Godinho Temponi[2]; Flávia Danieli Rech Cassol[1]; Lorena Camargo de Mendonça[1] e Camila Vanessa Buturi[3]

[1] Universidade Estadual do Oeste do Paraná - UNIOESTE. Pós-Graduação em Engenharia Agrícola - PGEAGRI, Rua Universitária n. 2069, Bairro Universitário, Cascavel, PR. e-mail: thaisregina.marcon@gmail.com; fdrcassol@hotmail.com; l.mendonca@hotmail.com.
[2] Universidade Estadual do Oeste do Paraná – UNIOESTE. Pós-Graduação em Conservação e Manejo de Recursos Naturais, Cascavel, PR, Brasil. e- mail: amtfortes@hotmail.com; liviatemponi@yahoo.com.br.
[3] Universidade Universidade Estadual do Oeste do Paraná, CCBS, Av. Universitária, 2069, Cascavel, PR. e-mail: camila.buturi@gmail.com.

ABSTRACT: The objective of this study was to test treatments to break the Mimosa bimucronata *(DC.) Kuntze seed dormancy. The following were tested for germination: control; immersion in: sulfuric acid for 5 and 10 min; acetone for 15 and 30 min; ethyl ether for 15 and 30 min; water at 80 ºC and 100 ºC, both with cooling in water for 24 hours; permanence in stagnant water (24 hours);dry cold at 5º C (24 hours), dry heat at 65 ºC (24 hours) and cutting the tegument. From the obtained data, we calculated the germination percentage and average germination time and speed. We also carried out the curve of water imbibition, with 50 seeds in three replications per treatment. Treatments with higher germination percentage and germination rate were better in immersion in sulfuric acid (10 min); soaking in acetone for 15 min; cutting the integument and immersion in water at 80 ºC (cooling for 24 hours), and the last treatment had the best result. However, all the treatments can be considered effective for breaking seed dormancy of Mimosa bimucronata by increasing the percentage of germinated seeds and germination rate.*
KEYWORDS: imbibition curve; maricá, seeds physiology.

GERMINAÇÃO E SUPERAÇÃO DA SEMENTE DORMANCY Mimosa bimucronata (DC.) KUNTZE - LEGUMINOSAE

RESUMO: O objetivo deste estudo foi testar tratamentos para superar a dormência das sementes de Mimosa bimucronata *(DC.) Kuntze. Os seguintes tratamentos foram testados: controle; imersão em: ácido sulfúrico por 5 e 10 min; acetona durante 15 e 30 min; éter etílico durante 15 e 30 min; água à 80 ºC e 100 ºC, ambas com resfriamento em água por 24 horas; permanência em água (24 horas), frio seco a 5 ºC (24 horas), calor seco a 65 ºC (24 horas) e corte do tegumento. A partir dos dados obtidos, foram calculados a porcentagem de germinação e o tempo e velocidade média de germinação. Também foi realizada a curva de embebição em água, com 50 sementes em três repetições por tratamento. Os tratamentos com maior porcentagem de germinação e melhores taxa de germinação foram: imersão em ácido sulfúrico (10 min); imersão em acetona por 15 min; corte do tegumento e imersão em água à 80 ºC (resfriamento por 24 horas), sendo o último tratamento o melhor resultado. Entretanto, todos os tratamentos podem ser considerados efetivos para a superação da dormência de sementes de* Mimosa bimucronata*, aumentando a porcentagem de sementes germinadas e a taxa de germinação.*

PALAVRAS-CHAVES: curva de embebição; fisiologia de sementes; maricá.

INTRODUCTION

Mimosa bimucronata, known popularly as maricá, is a tree species native to Brazil that belongs to the Leguminosae family, Mimosoideae subfamily. It is present in vegetation formations of the Cerrado (Savannah) and Atlantic Rainforest and in almost regions of Brazil, the North East, Central West, South East and South (Dutra 2012), especially in semi-deciduous woodlands in Paraná and other countries such as Uruguay (Lorenzi 2002).

It is characteristic of wet and swampy soils, it is a very thorny, branched plant and is cultivated in South Brazil for use as hedging in farm divisions. Its wood has commercial value for use in carpentry and especially in energy production as high quality charcoal that releases a great quantity of heat (Lorenzi 2002).

It is a cespitose plant that is characterized by the capacity to produce several trunks, cork cambium with deep grooves, without evident detachment and with thorns, internal whitish and fibrous bark, branch with persistent stipule, bipinnate leaves and raceme inflorescences with white flowers (Lorenzi 2002, Marcon et al. 2013).

The maricá is ecologically important due to the association with nitrogen-fixing mycorrhiza a characteristic present in the species of the Leguminosae family, where these bacteria are responsible for transforming atmospheric nitrogen (N_2) into ammonia (NH_4), a soluble form that can be used by other species in protein synthesis (Lewis 1987).

In this way the species of the Leguminosae family function as conditioners of the substrate and facilitators of natural succession (Chada et al. 2004) and are recommended for control of erosive processes, plantings in land subject to periodic flooding and in recovery areas in the Atlantic Rainforest (Carvalho 2003, Carvalho 2004, Gris et al. 2012).

Given its great use and importance, the production of *Mimosa bimucronata* in nurseries should be encouraged with the intention of creating a stock of saplings of this species for use both in recovery of areas and in plantations for sustainable exploitation.

However, maricá seeds have dormancy, that is, they may not germinate even with all the conditions necessary for this process to occur; several factors may lead to seed dormancy, including inhibitory substances, mechanical resistance of the embryo external tissues, embryo immaturity or dormancy (Kramer and Krozlowski 1972, Fowler and Bianchetti 2000).

In a natural environment, dormancy may be overcome by altering temperatures, wet or dry heating of the soil or by seed ingestion by dispersal animals, where they suffer the action of acids present in the digestive tract, that enables the entry of water to the inside the seed (Vázquez-Yanes and Orozco-Segovia 1993).

Several methods are tested in the laboratory to overcome dormancy based on natural processes and the most commonly tested treatments are: imbibition in water, subjection to low or high temperatures, mechanical and chemical scarification, usually with acid (Fowler and Bianchetti 2000, Brasil 2009), and each species requires a particular type of treatment.

In Leguminosae seeds, the most common dormancy is tegument impermeability, due to the resistance of the sclereid tissue that covers the seeds and prevents water from entering. Scarification with wearing away of the tegument with sandpaper or other material is the most efficient method to overcome dormancy in the species of this family, allowing water entry and consequently germination (Fowler and Bianchetti 2000).

Often lack of knowledge of seed germination morphology and physiology causes limitations in the multiplication of native forest species, either in the field or in nurseries (Pereira et al. 2011).

Experiments such as the imbibition curve endeavor to complement the information about the germination period and the dormancy type presented by the seed, according to the tegument impermeability and hardness (Lula et al. 2000).

Studies on the seed water imbibition rate can indicate levels of physiological quality, aiming to detail the tegument state and seed hydration level (Nimer et al. 1983).

Through these studies, more efficient techniques can be developed to overcome dormancy in the different species, methods that are easy and financially feasible, to be applied before sowing in the field or nurseries.

Thus, the objective of the present study was to deepen knowledge on the physiology of the germination of *Mimosa bimucronata* seed to improve the methods for overcoming dormancy, ensuring the growth success of this species.

MATERIALS AND METHODS

Obtaining seed

The experiments with *Mimosa bimucronata* seeds were carried out from June to October 2012, in the Plant Physiology Laboratory of the Biological Science and Health Center (Laboratório de Fisiologia Vegetal do Centro de Ciências Biológicas e da Saúde (CCBS) at the State University of theWest of Paraná (Unioeste), campus Cascavel, Paraná.

The seed were obtained from the Arbocenter Company, collected on June 20 2011, in the town of Penápolis-SP, Brazil, batch 00261, latitude 21º 26’ 45.08” South and longitude 50º 8’ 32.36” West. They were beneficiated and selected by hand to choose visually homogeneous and healthy seed.

Maricá seeds are approximately 4.5 mm long, with hard consistency and a flattened, oval shape (Carvalho 2003, Carvalho 2004).

Weight of 1000 seed

The weight of 1000 seeds (g) was obtained by weighing eight 100-seed sub-samples on analytical scales, following the determinations of the Regras para Análises de Sementes (Brasil 2009). The means were calculated based on the weights of the sub-samples multiplied by 10.

Imbibition curve

The imbibition curve was obtained with three replications of 50 seeds immersed in 50 ml distilled water for each treatment. The treatments used for the curve were:

T1- Control

T2- Immersion in sulfuric acid for 5 minutes;

T3- Immersion in sulfuric acid for 10 minutes;

T4- Immersion in acetone for 15 minutes;

T5- Immersion in acetone for 30 minutes;

T6- Immersion in ethylic ether for 15 minutes;

T7- Immersion in ethylic ether for 30 minutes;

T8- Immersion in water at 100 ºC;

T9- Immersion in water at 80 ºC;

T10- Permanence in stagnant water at room temperature for 24 horas;

T11- Exposure to dry cold: seed kept in a refrigerator at 5 ºC, dor 24 horas;

T12- Exposure to dry heat: seeds kept in a chamber at 65 ºC, for 24 horas;

T13- Cutting the tegument: cut with pliers in the region opposite the hilum.

After immersion in warm water, treatments T8 and T9 were kept in water for 24 hours to cool

The seeds in treatments T2, T3, T4, T5, T6 and T7, after being submitted to scarification, were washed in running water for 3 min and then distilled water and dried on paper towel.

The replications were represented by plastic cups (200 ml), duly identified and placed in a germination chamber at 25 °C, with constant aeration on each replication.

The weight was assessed every hour in the first 24 hours, then every two hours until the weight stabilized and the seed germinated. In this process the seed were passed from the cups to sieves, dried on paper towel and weighed.

The treatments that presented germinated seed were removed from the experiment. Imbibition was considered as the increase in final weight compared to the initial weight of the seed.

Test to overcome dormancy

The 13 treatments used to overcome dormancy in *Mimosa bimucronata* seeds were the same as described in the previous topic, imbibition curve, where each treatment was represented by four replications equivalent to the Petri dishes, that contained 25 seeds per experimental unit, totaling 100 seeds per treatment. The Petri dishes, containing three sheets of Germitest paper, were previously autoclaved at 121 °C for 20 minutes and dried in a chamber at 50 °C (Araújo-Neto et al. 2002). The seeds were treated with Captam 1% fungicide for each replication. The petri dishes with the respective treatments were taken to a germination chamber at 25 ºC and 12 hour light photoperiod.

The germination chamber was disinfected previously to prevent contamination of the experiment, using anti-bactericide solution (Lysoform 10%) and antifungus solution (Nistatine 10%) (Bortolini and Fortes 2005).

The same procedure was used before each assessment, to clean the benches and hands. The assessments were carried out daily, and seeds were considered germinated that presented the primary root equal or greater to 2 mm (Hadas 1976), that were removed from the replications.

A completely randomized design was used and the following ere estimated from the data collected: germination percentage (PG) transformed by arc sen $\sqrt{x}/100$ (Banzatto and Kronka 1995), mean germination time (TMG) and mean germination speed (VMG) (Labouriau 1983).

After carrying out the experiments, the data collected were submitted to analysis of variance (Anova) and the means compared by the Tukey test at 5% probability using the Sisvar program version 5.3 (Ferreira 2008).

RESULTS

The weight of 1000 seeds was 8.971 g; the lowest weight obtained was 8.531 g and the highest 9.177 g. Figure 1 shows the data for the water imbibition curve of water of the 13 treatments. The scarification methods via wet heat, with later cooling in water for 24 hours, were the treatments that imbibed most water, that is, immersion water at 100 ºC and immersion water at 80 ºC, but immersion water at 100 ºC was not considered a good treatment because of the low germination percentage (Table 1).

Figure 1 shows the results of the seed imbibition curve for the 13 treatments. Immersions in water at 80 oC, cutting the tegument and immersion in sulfuric acid for 10 minutes were the treatments that presented the greatest water imbibition capacity increasing their mass to approximately 65% (Figure 1), and these treatments presented the best germination results (Table 1).

The treatments to overcome dormancy with chemical scarification, immersion in sulfuric acid for 5 and 10 min, immersion in acetone for 15 min and 30 min, were also important, and presented an addition of approximately twice the initial weight, between 1.0 and 1.1 g (Figure 1).

The control treatment did not present significant mass gain, nor did the other treatments, which absorbed little water and did not reach 1 g after 28 hours imbibition at the end of the experiment.

Table 1 shows the data obtained in the germination tests of the *Mimosa bimucronata* seeds and the different treatments: germination percentage (PG %), mean germination time (TMG), mean germination speed (VMG).

Mimosa bimucronata seed germination started on the second day after setting up the experiments and the assessments were made until the 33rd day, when the germination stabilized for three consecutive days.

The control reached 72% germinated seeds. The treatment that presented the highest germination percentage value was immersion in sulfuric acid for 10 min, with 95% germination, that differed statistically from the control, but did not differ from most of the treatments. The following treatments were also outstanding: immersion in sulfuric acid 5 and 10 min.; immersion in acetone for 15 and 30 min; immersion in a ethylic ether for 15 min; immersion in water at 80 ^{o}C; exposure to dry heat and cutting the tegument.

The treatment immersion in water at 100^{o} C presented only 18% germination, a low value, and differed significantly from the control (72%). The other treatments did not present statistical difference from the control germination percentage.

Regarding the mean germination time, the highest value obtained was for the treatment that remained in stagnant water for 24 hours, with 8.5 days, that did not differ statistically from the control, 8.50 days. Immersion in water at 100^{o} C obtained the shortest mean time (0.52), followed by immersion in water at 80 ^{o}C (2.01). However, the treatment immersion in water at 100 ^{o}C, even though it presented the best time, was not the best treatment, because of the low germination percentage.

Immersion in sulfuric acid for 5 and 10 min.; immersion acetone for 15 minutes; exposure to dry heat and cutting the tegument were important because they presented a short mean germination time, and did not differ from immersion in water at 80 ºC.

For the variable mean germination speed, the control had the lowest speed with 0.12 seed per day, and did not differ statistically from most of the other treatments. The greatest germination speed was immersion water at 100 ºC (1.92), followed by immersion in water at 80 ºC (0.51) and mechanical scarification by cutting the tegument (0.43); immersion in sulfuric acid (0.35) and immersion acetone (0.34) (Table 1).

DISCUTIONS

The weight of 1000 seeds, 8.971 g and 10.08g, obtained in the present study was slightly below the estimated by Ribas et al. (1996), Fowler and Carpanezzi, respectively.

Experiments such as the water imbibition curve are important to help identify the germination periods of the seed and confirm the results of the type of dormancy they present (Lula et al. 2000).

Generally, the treatments that most absorbed water corresponded to the triphasic pattern, as can be observed at the start of phase I (Figure 1), where imbibition appeared quickly in live or dead seeds, driven by the dry seed water potential (Castro and Hilhorst 2004).

Water is absorbed to the plateau level, phase II, a stationary period where the metabolic processes are activated for embryo growth and after this period phase III occurs, where again there is water imbibition giving start to germination (Labouriau 1983, Castro and Hilhorst 2004).

Phase III started between 22 and 28 hours, when the experiment was finished due to protrusion of 2 mm roots in most of the treatments.

The treatments immersion in water at 80 ºC (Mori et al. 2012), cutting the tegument and immersion in sulfuric acid for 10 min presented the best results for germination percentage mean time and speed, as already reported, and these treatments also had the greatest water imbibition capacity increasing their mass by 60-65%, approximately.

These results can be compared with Mantoan et al. (2012) those of a study where seed of the *Adenanthera panoviava* L. species, belonging to the Leguminosae family, presented the triphasic model of the imbibition curve, reaching FIII after 12 hours imbibition, half the time that the seed took in the present experiment of around 24 hours.

Lopes et al. (1998) studying seeds of the leguminosas species *Caesalpinia ferrea* Mart. (Benth.), *Cassia grandis* L. and *Samanea saman* Merrill showed that scarifying the seed promoted a sharp increase in fresh matter.

This fact has been confirmed by several studies of imbibition curves that compared treated and intact Leguminosae seeds and found difference in imbibition between them (Lopes and Matheus 2008, Basqueira et al. 2011, Bortolini et al. 2011, Delgado and Paulilo 2011, Pereira et al. 2011)..

The best methods for overcoming dormancy obtained the greatest germination percentage, speed and mean time (Table 1), did not differ and also presented the greatest water imbibition (Figure 1), compared to the control and the treatments that were not efficacious, showing a correlation between the two tests imbibition and overcoming dormancy.

The control obtained 72% germination, a higher value for dormant seeds compared to other studies on *Mimosa bimucronata*, that obtained 27% and 1% germination for the control, respectively, but the seeds took a long time to germinate Ribas et al. (1996) e Fowler et al. (1998).

The high percentage of germinated seeds in the control can be explained because the seeds were stored a year; they were collected on June 20, 2011 and the experiments took place only one year later.

Similar results have also been reported in other studies with *Mimosa bimucronata* seeds that suggest storage may lead to an increase in tegument permeability, which facilitates seed imbibition and decreases the number of dormant seeds (Ferreira et al. 1992).

This result is in line with Labouriau (1983) who stated that seed genetic factors, maturation conditions and storage can lead to permeability of the seeds in a natural way. But in the imbibition curve, the control seeds absorbed a small quantity of water in a relatively long imbibition period, and did not present significant weight increase until the end of the assessment. This proved that storage for one year caused an increase in the percentage germination of the control seed, but did not increase the mean germination speed or water capacity

The low water imbibition by the control seeds demonstrated that there is resistance imposed by the tegument and suggests that this structure is responsible for the dormancy (Lula et al. 2000).

Similar results were found in other studies on the Mimosoideae (Leguminosae) subfamily where it was observed that seeds without treatment obtained little or no increase in

fresh matter weight on the imbibition curve (Mantoan et al. 2012) that also corroborates experiments with other Fabaceae species (Lopes et al. 1998, Lopes and Matheus 2008, Bortolini et al. 2011).

Immersion in water at 100 ºC with later cooling for 24 hours resulted in the lowest germination percentage, the seeds may have been damaged by the boiling water, a fact already observed in other experiments where immersion in water at initial temperatures higher than 80 °C caused damage to the embryos of a small part of the seeds, reducing germination. Similar results were observed by Lopes et al. (1998) where heat scarification had a lethal effect on the seeds (Fowler et al. 1998).

The treatment immersion in water at 100 ºC also resulted in the highest germination speed (Table 1), in the shortest mean germination time. These would be important data, if the germination had not been so low, thus this treatment is not indicated to overcome dormancy in maricá seeds.

It is also pointed out that this treatment presented significant weight gain at the start of the imbibition curve experiment (Figure 1). This may have been due to the fact that the seeds remained in water for 24 hours, according to the treatment to overcome dormancy. Castro and Hilhorst (2004) stated that when seeds of a determined treatment absorb water very quickly, there is not enough time for the membranes to return to the liquid crystalline state and in this situation there is cell damage and lixiviation, a factor that may also have occurred in the treatment immersion in water at 100 ºC.

However, this damage did not occur in seeds immersed in water at 80 ºC, which also remained in water for 24 hours, after being submitted to high temperature and also absorbing a large quantity of water in a short period. Their membranes may have been able to reorganize, as this was the second treatment that most absorbed water and also presented the best germination percentage, at the highest germination speed in the shortest mean germination time, after that of immersion at 100 ºC. This corroborates the results by Ribas et al. (1996) and Fowler et al. (1998) where immersion in hot water presented the best results, highlighting the initial temperature of 80 °C.

Regarding the chemical scarification treatment using sulfuric acid, it can be stated that it is efficacious in overcoming dormancy in *Mimosa bimucronata* seeds. Similar results were reported by Fowler et al. (1998), who stated that this is the best treatment to overcome dormancy in this species.

In other studies with the *Mimosos*, immersion in sulfuric acid for 4 min was enough to break dormancy in *Mimosa scabrella* Bentham seeds making 60% of the seed germinate

(Barazetti and Sccoti, 2010) while 15 minutes of immersion for *Mimosa caesalpiniaefolia* L. seeds resulted in a germination percentage of almost 100% (Garcia et al. 2002).

However, sulfuric acid can be expensive and difficult to handle, because it requires gloves and care when applying.

Mechanical scarification by cutting the tegument with pliers on the opposite side to the root emission was also outstanding, because it resulted in 90% germination in the present study, accelerating the germination speed and reducing the mean germination time compared to the control, similar to that observed by Ferreira et al. (1992).

In seeds of *Ormosia arborea* (Vell.) Harms (Leguminosae: Papilionoideae) mechanical scarification, with scissors, in the region opposite the hilum increased germination from 15% to 55% (Basqueira et al. 2011).

However, the problem concerning manual mechanical scarification is that it is only viable when small quantities seeds are used, about 1 to 10 kg (Bianchetti et al. 1998) and preferentially on large seeds otherwise its use is hindered by the great demand for time and labor

The present study proved that treatments with capacity to overcome dormancy in *Mimosa bimucronata* seeds are immersion in sulfuric acid 10 min because it resulted in the greatest percentage of germinated seeds, immersion in acetone for 15 min, cutting the tegument and immersion in water at 80°C with cooling for 24 hours, to accelerate the germination speed, the last treatment is the easiest and cheapest to apply.

CONCLUSION

The treatment that is indicated to overcome dormancy in *Mimosa bimucronata* seeds is immersion in water at 80°C, because it increases the germination percentage and accelerates the time that seeds take to germinate, in addition to being a more practical and cheaper method compared to the other treatments analyzed.

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