

DORMANCY OVERCOMING AND PRECONDITIONING IN *Mimosa caesalpiniiifolia* Benth. SEEDS¹

HOHANA LISSA DE SOUSA MEDEIROS², CLARISSE PEREIRA BENEDITO^{2*}, NADJAMARA BANDEIRA DE LIMA DANTAS², JORGE RICARDO SILVA DO COUTO JÚNIOR², LUIRLA BENTO RAMALHO²

ABSTRACT - Seeds of 'sabiá' (*Mimosa caesalpiniiifolia* Benth.) have coat dormancy, which hampers the absorption of water and oxygen, thereby delaying germination. Thus, this study aimed to evaluate methods for overcoming dormancy associated with priming on germination and vigor of seeds of 'sabiá', considering the efficiency, practicality and cost of treatment. The experimental design was completely randomized, in a 4 x 5 factorial scheme (four methods for overcoming dormancy x five types of priming), constituting 20 treatments with four replicates of 25 seeds. The methods for overcoming dormancy used were tip removal, seed immersion in hot water at 100 °C for three minutes, immersion in sulfuric acid for 10 minutes and intact seeds. For the determination of physiological conditioning of seeds, the imbibition curve with the different priming agents was constructed. Priming was done on filter paper moistened with solutions of mannitol at the potentials of -0.2 MPa (16 hours), -0.4 MPa (24 hours) and -0.6 MPa (36 hours) and only with distilled water for the hydropriming (12 hours) and seeds without priming. The variables analyzed were first count of germination, germination, root length, shoot length and dry weight of seedlings. The data were subjected to analysis of variance by F test and Tukey test at 5% probability. The dormancy of *M. caesalpiniiifolia* seeds should be overcome with the use of hot water (100 °C) for three minutes, without the need for priming.

Keywords: Fabaceae. Caatinga. Forest seeds. Dormancy. Priming.

SUPERAÇÃO DE DORMÊNCIA E PRÉ-CONDICIONAMENTO EM SEMENTES DE *Mimosa caesalpiniiifolia* Benth.

RESUMO - Sementes de sabiá (*Mimosa caesalpiniiifolia* Benth.) apresentam dormência tegumentar, dificultando a absorção de água e oxigênio, consequentemente retardando a germinação. Dessa forma, objetivou-se avaliar métodos para superação de dormência associados ao condicionamento fisiológico sobre germinação e vigor de sementes de sabiá, considerando a eficiência, praticidade e custo do tratamento. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 4 x 5 (quatro métodos de superação de dormência x cinco tipos de condicionamento fisiológico), constituindo vinte tratamentos, com quatro repetições de vinte e cinco sementes. Os métodos de superação de dormência utilizados foram desponte, imersão das sementes em água quente a 100 °C por três minutos; imersão em ácido sulfúrico por 10 minutos e sementes intactas. Para determinação do tempo de condicionamento fisiológico das sementes, realizou-se a curva de embebição com os diferentes agentes condicionantes. O condicionamento fisiológico foi feito em papel filtro umedecido com soluções de manitol nos potenciais de -0,2 MPa (16 horas), -0,4 MPa (24 horas) e -0,6 MPa (36 horas) e apenas com água destilada para o hidrocondicionamento (12 horas) e sementes sem condicionamento. As variáveis analisadas foram primeira contagem de germinação, germinação, comprimento radicular, comprimento da parte aérea e massa seca de plântulas. Os dados foram submetidos à análise de variância pelo teste F e teste de Tukey a 5% de probabilidade. A superação de dormência de sementes de *M. caesalpiniiifolia* deve ser realizada com o uso de água quente (100 °C) durante três minutos, sem necessidade do condicionamento fisiológico.

Palavras-chave: Fabaceae. Caatinga. Sementes florestais. Dormência. Priming.

*Corresponding author

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²Department of Agricultural Sciences and Forestry, Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil; hohanalissa@hotmail.com - ORCID: 0000-0001-6616-8058, clarisse@ufersa.edu.br - ORCID: 0000-0002-2846-1162, nadybandeira@hotmail.com - ORCID: 0000-0002-1261-2181, jorgericardosej@gmail.com - ORCID: 0000-0002-8076-5788, luiirla_ramalho@hotmail.com - ORCID: 0000-0002-4231-0029.

INTRODUCTION

The multiplication of native species has been more valued in recent times, aiming mainly at the production of seedlings for the restoration of degraded areas and restoration of landscapes, due to a greater concern with the preservation of the environment and biodiversity.

Among the potential native species for these activities, there is *Mimosa caesalpinifolia* Benth., popularly known in Portuguese as 'sabiá', 'unha-de-gato' or 'sansão-do-campo', belonging to the Fabaceae family and of natural occurrence in the Northeast region (MAIA, 2012). This species has ornamental characteristics, and its wood is used in external environments in the form of stakes, hedges and also in the production of firewood and charcoal. The leaves are used as fodder for livestock, with high protein content (CALDAS et al., 2010). In addition, it can be used in reforestation of degraded areas (MAIA, 2012).

The multiplication of *M. caesalpinifolia* is undermined by the fact that the seeds have coat dormancy (COSTA et al., 2018), which hampers the absorption of water and consequently delays germination (SILVA et al., 2012). In general, when it comes to break coat dormancy, the most commonly used methods are: mechanical scarification (tip removal or abrasion with sandpaper), chemical scarification (sulfuric acid and hydrochloric acid) or physical scarification (immersion in hot water) (MONTANHA et al., 2018). Factors such as time of exposure, temperature, concentration of the product and species influence the success of treatment. In addition, at the time of the choice of the best method, one should consider the financial cost, risk to the operator, convenience and efficiency.

The Instruction Manual for Forest Species (BRASIL, 2013) recommends for *M. caesalpinifolia* the tip removal in the region opposite to the hilum, performed manually with the use of pliers. Despite being recommended for the species and having low cost, this method is not practical when it comes to large quantities of seed, because it requires more time and labor for its implementation.

In addition to methods for overcoming dormancy, it has also been suggested the use of other techniques that aim to favor the germination and emergence of seedlings, especially the physiological conditioning, which consists in the controlled absorption of water by the seed, encouraging the metabolism of seeds during the phases I and II of imbibition, without the occurrence of primary root protrusion (MARCOS-FILHO, 2015). This

technique has been used mainly with the aim of increasing the speed of germination and uniformity of seedling grown from small seeds of vegetables or flowers, being still little used on forest seeds.

Priming can be done through the hydropriming (using water) and the osmotic conditioning (using solutions of polyethylene glycol, mannitol and salts) (KUBALA et al., 2015). Factors such as osmotic agent, concentration, temperature and time can directly influence the efficiency of the treatment. In a study carried out by Pellizzaro et al. (2011) with seeds of 'amendoim-bravo' (*Pterogyne nitens* Tul.), it was found that the seed scarification with sandpaper or sulfuric acid followed by the osmotic conditioning in mannitol at -0.41 MPa promoted higher germination percentages compared with the non-primed seeds. Studies that involve methods for overcoming dormancy and priming are still scarce, mainly for forest species.

In this context, this study aimed to evaluate methods to overcome dormancy and the effects of priming on the germination and vigor of *M. caesalpinifolia* seeds, considering the efficiency, practicality and cost of treatment.

MATERIAL AND METHODS

The seeds were obtained from mature fruits collected in approximately 20 parent trees, located in the city of Lagoa Nova, Rio Grande do Norte, Brazil (06 05'45.6"S; 36 28'08.4"W and 650 m altitude), in July 2017. After collection, the seeds were manually processed, placed in plastic bottle and stored in a controlled environment for eight months (17 °C; 55% RH).

After processing, the moisture content of seeds was determined by the method of the oven at 105 °C ± 3 for 24 hours (BRASIL, 2009), with two replicates of four grams. The results were expressed in percentage (wet basis).

To set the time duration of priming in each priming agent, the curve of seed imbibition was constructed at potentials of 0.0; -0.2; -0.4 and -0.6 MPa at 25 °C, with four replicates of 25 seeds, after overcoming dormancy with tip removal in the region opposite to the hilum (BRASIL, 2013). For the potential 0.0 MPa, distilled water was used, and the amount of mannitol in grams was calculated using the van't Hoff equation: $\Psi_{\text{Osm}} = -RTC$, where Ψ_{Osm} = osmotic potential (atmosphere), R = universal gas constant = 0.082 mol L⁻¹ atm K⁻¹, T = temperature (K), C = molar concentration (mols of solute/ 1000 g of water) (Table 1).

Table 1. Amount of mannitol in g L⁻¹ of distilled water used to prepare the solutions with different levels of osmotic potential.

Level (MPa*)	Mannitol (g L ⁻¹)
0.00	0.00
-0.2 MPa	14.7
-0.4 MPa	29.4
-0.6 MPa	44.1

*1 MPa= 10 bar; 1 bar= 0.987 atm.

The seeds were placed between two sheets of filter paper previously moistened with the respective priming agents using a volume equivalent to 2.5 times the dry weight of the papers placed in Petri dishes, maintained in a germination chamber at 25 °C with a photoperiod of 12 hours. The hydration of seeds was evaluated by weighing the seeds every hour in the first 12 hours, every three hours in the following intervals: between 22 and 34 hours; between 46 and 58 hours, between 60 and 73 hours after the start of imbibition. At each weighing, the seeds were removed from the moist substrate, removing the excess water through fast drying in filter paper, and weighed on precision scale. After weighing, the seeds were put back in the moist substrate.

Before the germination test, dormancy was overcome using the following methods: tip removal in the region opposite to the hilum, hot water at 100 °C for 3 minutes; sulfuric acid for 10 minutes and without overcoming dormancy (intact). Tip removal was done with the use of pliers, performing a cut in the region opposite to the hilum. For immersion in hot water, 300 mL of water were poured into an aluminum pan heated until reaching 100 °C; then, the water was transferred to a beaker containing seeds, which remained under this condition for 3 minutes. For the treatment with sulfuric acid, the seeds were placed in a beaker, fully immersed in sulfuric acid for 10 minutes and were subsequently rinsed in tap water with the use of a sieve with aluminum mesh until removing all the product and then rinsed with distilled water.

After that, the seeds were placed on paper moistened with distilled water (0.0 MPa) for 12 hours, with mannitol at -0.2 MPa for 16 hours, -0.4 MPa for 24 hours and -0.6 MPa for 36 hours, in addition to the seeds without priming. The experimental design was completely randomized (CRD), in a 4 x 5 factorial scheme (four methods for overcoming dormancy x five types of priming), totaling 20 treatments with four replicates of 25 seeds.

After the treatments of seeds, the germination test was conducted. For this, Gemitest® paper sheets were previously sterilized in an oven at 105 °C for one hour and then moistened with distilled

water using a volume equivalent to 2.5 times the weight of the dry paper. Soon after, the seeds were distributed on two sheets of paper, covered with a third sheet, arranged in the form of a roll, and then placed in a germinator at 25 °C. The following variables were evaluated: a) first count: by counting the number of normal seedlings on the fifth day after the installation of the test, with results expressed in percentage with one decimal place (BRASIL, 2013); b) normal seedlings: counted at 10 days, and the results were expressed in percentage with one decimal place (BRASIL, 2013); c) shoot length and root length: measured with a ruler graduated in millimeters, considering shoot length as the distance from the collar to the apex of the seedling and root length as the distance from collar to the tip of the main root, both with results expressed in centimeters; d) dry mass of seedlings: normal seedlings were dried in an oven with forced air circulation at 65 °C until reaching constant mass. Subsequently, the seedlings were weighed on an analytical balance (0.000 g) and the results were expressed in grams.

The analysis of variance was performed by means of F test and, when significant, comparisons between the means of the treatments were performed by Tukey test at 5% probability level (qualitative variables), using the statistical software Sisvar (FERREIRA, 2011).

RESULTS AND DISCUSSION

The imbibition curve in different types of priming is represented in (figure 1), in which the average initial moisture content was 9.2%. In the initial twelve hours, there was rapid absorption of water in all treatments, whose average increase was 3.0% per hour of soaking. Between 12 and 22 hours of imbibition, a reduction was observed in the absorption of water in all treatments, with average increases of moisture per hour of 1.0%, 1.2%, 0.75% and 1.3% for the hydropriming, mannitol -0.2, -0.4 and -0.6 MPa, respectively. The germination, of at least one seed, occurred after 25, 31, 34 and 49 hours of imbibition for hydropriming, mannitol -0.2, 0.4 and 0.6 MPa, respectively.

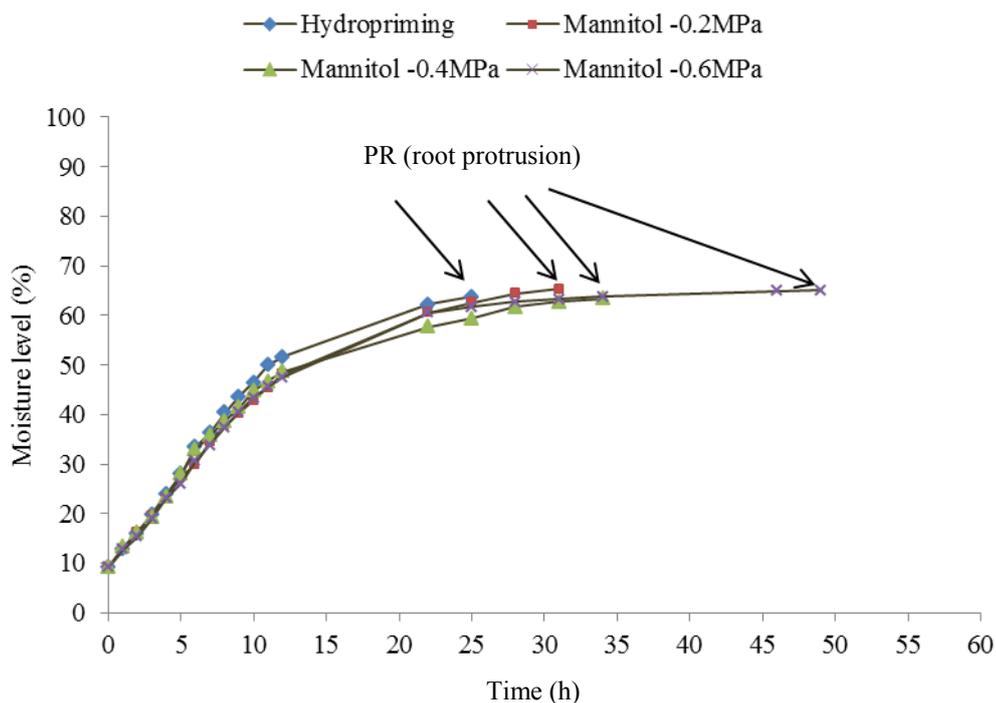


Figure 1. Imbibition curve for 'sabiá' (*Mimosa caesalpinifolia* Benth.) seeds submitted to different levels of osmotic solutions with mannitol.

Thus, the imbibition curve followed the triphasic pattern proposed by Bewley and Black (2013), whose phase I is characterized by the rapid absorption of water and increase of seed metabolism; in the second phase, the imbibition remains constant, during the occurrence of metabolic events required for embryo development. Finally, in phase III the seeds absorb water quickly again, and then there is

the production of the primary root in viable and non-dormant seeds.

According to the analysis of variance, there was a significant interaction between the different types of conditioning and methods for overcoming dormancy for the majority of the analyzed variables, except for root length, which had only single effects for both factors (Table 2).

Table 2. Summary of the analysis of variance for first count (FC), germination (G), root length (RL), shoot length (SL) and dry weight of seedlings (DWS) of 'sabiá' (*Mimosa caesalpinifolia* Benth.) grown from seeds submitted to priming (P) and methods for overcoming dormancy (OD).

Sources of variation	DF	FC	G	RL	SL	DWS
Priming (P)	4	6.79*	8.12*	27.14*	9.31*	6.61*
Overcoming dormancy (OD)	3	507.02*	435.64*	9.20*	6.25*	19.24*
P x OD	12	5.096*	4.51*	0,249n.s	2.22*	2.49*
CV		9.91	9.78	17.14	9.43	14.68
Mean		69	71.0	3.47	4.67	0.2378

*significant at 5% probability by the F test. n.s not significant at 5% probability by F.
DF: Degrees of freedom; CV: Coefficient of variation.

Based on the results of the first count, there was efficiency in all methods of overcoming dormancy used compared to the intact seeds, since they showed statistically higher means. It was also verified for the intact seeds that the different types of

priming were not able to increase the germination at first count, with values below 30% in all types of conditioning, confirming the presence of coat dormancy (Table 3).

Table 3. Means of the first count and germination of ‘sabiá’ (*Mimosa caesalpinifolia* Benth.) seeds submitted to different methods of conditioning and dormancy overcoming.

Priming	First count (%)			
	Intact	Hot water	Sulfuric acid	Tip removal
Without priming	13 AC	87 aA	74 bB	86 abAB
Hydropriming	19 AC	83 aB	97 aA	98 aA
Mannitol -0.2 MPa	21 aB	88 aA	91 aA	87 AbA
Mannitol -0.4 MPa	13 AC	69 bB	91 aA	83 bA
Mannitol -0.6 MPa	24 AC	67 bB	94 aA	99 aA
	Germination (%)			
Without priming	18 abB	87 aA	75 bA	86 aA
Hydropriming	23 abB	87 aA	97 aA	99 aA
Mannitol -0.2 MPa	30 aB	92 aA	92 aA	89 aA
Mannitol -0.4 MPa	16 abC	71 bB	91 aA	86 aA
Mannitol -0.6 MPa	28 abC	69 bB	94 aA	99 aA

*Means followed by the same letters, lowercase in columns and uppercase in rows, did not differ statistically by Tukey test at 5% probability level.

With the use of hot water and tip removal, there was also no statistical difference between the seeds without priming, subjected to hydropriming and to mannitol -0.2 MPa; however, the values of the first count were above 80%, clearly showing the efficiency of the methods for overcoming dormancy. However, for the seeds with pre-treatment in hot water and subjected to conditioning at potentials of -0.4 and -0.6 MPa, there was a reduction in the values of the first count (Table 3). Probably the seeds were more sensitive after the breaking of dormancy with hot water, which could be noted when they were primed at more negative potentials, because there was a reduction in the values of the first count. This occurred due to the increase in the period of phase II of the germination process (BEWLEY; BLACK, 2013), in which the increase in the osmotic tension of the solution inhibited the synthesis of alpha amylase, thereby reducing the metabolism in the cells of the aleurone layer, consequently decreasing the values of germination. In a study carried out by Pellizzaro et al. (2011) with seeds of *P. nitens* Tul., the authors found that mannitol concentrations above -0.4 MPa for seed priming resulted in a reduction in seed germination percentage.

For the treatment with sulfuric acid and without priming, germination was lower than 80%; however, when seeds were subjected to the methods of conditioning, the values of the first count remained above 90% (Table 3). In this case, the physiological conditioning favored seeds whose dormancy was broken with sulfuric acid, because the means were statistically higher compared to those of seeds without priming. Possibly, the time of immersion in acid (10 minutes) may have caused damage to the seeds, and the treatment with hydropriming and osmotic conditioning in mannitol were beneficial, promoting significant increases in germination.

In general, it has been observed that the

benefits of priming are attributed mainly to the action of repair mechanisms, which leads to reorganization of the system of membranes, in addition to favoring the mobilization of reserves used in germination. The mobilization of carbohydrates, lipids and proteins and the synthesis of enzymes from the beginning of imbibition prepares the seeds for the rapid germination shortly after the post-conditioning imbibition is resumed. The concentration of mobilized components enables the reduction of the water potential of seeds, facilitating the absorption of water as soon as they are exposed to hydration and promoting rapid germination (MARCOS-FILHO, 2015).

Opposite results were obtained by Silva et al. (2012), who found that the treatment with sulfuric acid for 10 minutes in seeds of *M. caesalpinifolia* promoted more than 90% of germination, suggesting that the intensity of dormancy can vary between seeds of the same species. In accordance with Santana et al. (2015), part of the seeds of some species are dispersed and germinate readily, while another part is dispersed with impermeable seed coat, revealing distinct intensities of dormancy, and this mechanism interferes with the efficiency of pre-germinative treatments, especially in the methods for overcoming dormancy.

For seeds of *Parkia platycephala* Benth., Nascimento et al. (2009) used various treatments for overcoming dormancy and found that the most efficient treatments to break the seed coat were mechanical scarification with sandpaper and immersion in sulfuric acid (15 to 45 min), leading to higher averages in the first count. Nogueira et al. (2013) evaluated methods for overcoming dormancy in seeds of *M. caesalpinifolia* and verified that tip removal enabled the opening of cracks on the seed coat, allowing for a higher water absorption by seeds, consequently promoting higher germination percentage in the first count.

In studies performed by Masetto et al. (2013) and Bittencourt et al. (2004) with *Sesbania virgate* (Cav.) Pers and *Asparagus officinalis* L. using solutions of PEG, it was also possible to obtain positive effects of priming on germination of seeds of these species.

For germination percentage, the treatments showed similar results to those of the first count, in which the comparison of the methods for overcoming dormancy to intact seeds showed once more that the germination of intact seeds was low, regardless of the type of conditioning. In other words, the methods used for the conditioning showed no positive effects on the intact seeds (Table 3).

The method of hot water promoted high germination for seeds without priming, hydroprimed and osmoprimed at -0.2 MPa, but seeds were more sensitive when subjected to osmopriming in mannitol at -0.4 MPa and -0.6 MPa, showing a reduction in the germination values (Table 3). Masetto et al. (2014) also observed, in seeds of *Dimorphandra mollis* Benth., that as the concentration of osmotic solutions increased, there was a reduction of water absorption by seeds and a lower percentage of germination.

Likewise, the method of sulfuric acid without priming led to lower germination when compared to

hot water and tip removal. However, when the seeds were primed (hydropriming or osmopriming in mannitol -0.2, -0.4 and -0.6 MPa), there was an increase in the values of germination, showing once again that, with sulfuric acid, all types of priming were favorable to increase the averages of this variable (Table 3).

On the other hand, for seeds subjected to tip removal, there were no statistical differences between the treatments of priming. In other words, after tip removal there is no need to perform the conditioning in seeds of *M. caesalpinifolia*. Mendonça et al. (2005) and Pereira and Martins Filho (2012) found similar results in seeds of *Triplaris americana* L. and *Solanum sessiliflorum* Dunal., respectively, in which the osmotic conditioning did not influence the percentage of germination.

By analyzing the single effect of methods of conditioning on the root length, it was observed that the hydropriming had a positive effect on seedling root growth, being superior to the other treatments (Table 4). Seedling root growth is a characteristic related to the initial vigor of seeds, so seeds that were initially hydroprimed showed greater vigor than seeds from the other treatments.

Table 4. Single effect of priming methods on the root length of seedlings of 'sabiá' (*Mimosa caesalpinifolia* Benth.).

Priming	Root length (cm)
Without priming	2.67 c
Hydropriming	4.67 a
Mannitol -0.2 MPa	3.11 c
Mannitol -0.4 MPa	3.00 c
Mannitol -0.6 MPa	3.89 b

Means followed by the same letter did not differ statistically by Tukey test at 5% probability level.

The results of the single effect of the methods for overcoming dormancy on root length show that the seedlings grown from seeds treated with tip removal, sulfuric acid or hot water obtained greater root growth in comparison to the intact seeds, i.e., it confirms once more the need for overcoming

dormancy in seeds of this species, also contributing to root growth (Table 5). A similar result was found by Pacheco and Matos (2009), who concluded that all treatments to overcome dormancy of *Apeiba tibourbou* Aubl. promoted good root development of its seedlings when compared to the control.

Table 5. Single effect of methods for overcoming dormancy on the root length of seedlings of 'sabiá' (*Mimosa caesalpinifolia* Benth.).

Overcoming dormancy	Root length (cm)
Intact	2.85 b
Tip removal	3.57 a
Sulfuric acid	3.71 a
Hot water	3.73 a

Means followed by the same lowercase letters in the column did not differ statistically by Tukey test at 5% probability level.

In regard to shoot length, for seeds pre-treated with hot water and tip removal, there were no differences between the methods of conditioning. On the other hand, when seeds were pre-treated with sulfuric acid, the osmopriming in mannitol at -0.2 MPa promoted greater shoot growth, compared to the seeds without priming (Table 6). Therefore, once more there was a positive effect of priming on seeds treated with sulfuric acid. These results differ from those found by Silva et al. (2012), also for *M.*

caesalpinifolia, as these authors noted the lack of influence of methods for overcoming dormancy on the length of seedlings. Generally, during the osmotic conditioning there are specific metabolic processes for the beginning of cell division and expansion, accelerating the germination, seedling growth and consequently leading to higher biomass accumulation (DELL AQUILA; TARANTO, 1986; SMITH; COBB, 1992).

Table 6. Shoot length and dry weight of seedlings of 'sabiá' (*Mimosa caesalpinifolia* Benth.) grown from seeds submitted to different methods of conditioning and dormancy overcoming.

Priming	Shoot length (cm)			
	Intact	Hot water	Sulfuric acid	Tip removal
Without priming	3.73 bB	4.15 aAB	4.16 bAB	4.83 aA
Hydropriming	4.25 bB	4.86 aAB	4.85 abAB	5.09 aA
Mannitol -0.2 MPa	4.14 bB	4.82 aAB	5.12 aA	4.65 aAB
Mannitol -0.4 MPa	5.42 aA	5.02 aA	4.94 abA	5.18 aA
Mannitol -0.6 MPa	4.39 b AB	4.21 aB	4.38 abAB	5.18 aA
	Dry weight of seedlings (g)			
Without priming	0.064 aB	0.284 abA	0.245 cA	0.301 aA
Hydropriming	0.0817 aB	0.293 abA	0.332 aA	0.335 aA
Mannitol -0.2 MPa	0.0980 aB	0.309 aA	0.318 abA	0.299 aA
Mannitol -0.4 MPa	0.055 aB	0.227 bcA	0.262 bcA	0.282 aA
Mannitol -0.6 MPa	0.090 aC	0.213 cB	0.321 abA	0.341 aA

*Means followed by the same letters, lowercase in columns and uppercase in rows, did not differ statistically by Tukey test at 5% probability level.

With regard to the dry weight of seedlings, the seeds responded positively to the treatments with hot water, sulfuric acid and tip removal in comparison to intact seeds. By analyzing the effects of priming in each method of overcoming dormancy, it was found that in intact seeds and in those subjected to tip removal, there was no effect of the different methods of conditioning, since the averages of seeds without priming were statistically equal to those of the other treatments. For seeds pre-treated with hot water, there was a reduction in dry weight of seedlings when seeds were primed at -0.6 MPa mannitol. In contrast, when seeds were pre-treated with sulfuric acid and osmoprimed in mannitol at -0.2 and -0.6 MPa, there were increases in the values of the dry weight of seedlings (Table 6).

In general, there were positive effects of both hot water and tip removal on the germination and vigor of seeds of *M. caesalpinifolia*, without the need for priming. On the other hand, the sulfuric acid led to satisfactory results when associated with the physiological conditioning. However, some points such as practicality, cost, risk to the operator and quantity of seeds to be treated must be considered when choosing the best method. Although tip removal has low cost and risk in handling, this method requires more labor when it comes to large quantities of seeds, because it must be performed on

each seed individually when no devices are available, compromising the uniformity in the application of this treatment. Sulfuric acid, in contrast, has a higher cost and greater risk to the health of the operator, compared to the other treatments, and should be recommended only when other treatments are not successful. Thus, the method of hot water becomes more feasible as it has a low risk, low cost and can also be used for large quantities of seeds in a uniform manner.

CONCLUSION

Dormancy of *M. caesalpinifolia* seeds should be overcome with hot water (100 °C) for three minutes, without the need for priming.

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