

Priming of *Mimosa bimucronata* Seeds – A Tropical Tree Species from Brazil

P.H.S. Brancalion¹, A.D.L.C. Novembre¹, R.R. Rodrigues¹ and D. Tay²

¹ Escola Superior de Agricultura “Luiz de Queiroz”, University of São Paulo, Piracicaba, Brazil

² Ornamental Plant Germplasm Center, The Ohio State University, Columbus, OH 43210, USA

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Abstract

Priming is a technique used to improve seed performance. *Mimosa bimucronata* is one of the most important tree species used for tropical green area restoration in Brazil, as it is fast growing. This species is also planted for firewood, living fence, landscaping and honey production. The aim of this research was to evaluate the priming efficiency in *M. bimucronata* seeds. Before priming treatments, dormancy was overcome by cutting the seed coat extremity with a sharp blade. Seeds were primed in water for 11h and in aqueous PEG 8000 solution with osmotic potential of -0.8 MPa for 24, 48, 72 and 96h. After priming, the seed moisture content (MC) of all the treatments was approximately 50%. Half of the seed of each treatment was dried to the initial MC (6.6%) and both fresh primed and dried primed seeds were tested. Eight replicates of 25 seeds were used for each treatment. Germination percentage and speed of germination were evaluated, and seedling growth and uniformity were evaluated by using The Ohio State University's Seed Vigor Imaging System (SVIS-OSU). Electrical conductivity of seed leakage was also analyzed. The results showed that the PEG primed seeds, mainly fresh seeds primed for 48h, presented higher germination percentage and speed of germination, higher seedling growth and uniformity, and lower electrical conductivity. The benefits obtained with priming were partially lost with seed drying, since dried primed seeds had lower performance and higher electrical conductivity than fresh primed seeds. In conclusion, priming of *M. bimucronata* seeds is efficient to improve seed performance and it can be used as a methodology to improve seedling production and stand establishment.

INTRODUCTION

Priming is the controlled hydration of seed, in which there is the metabolic preparation for the germination process, but without primary root protrusion (Heydecker et al., 1975; Bradford, 1986). In priming, seeds can be put in contact with water or aqueous solutions with low osmotic potentials, which can be prepared using polyethylene glycol (PEG). The osmotic potential of the solution regulates the amount of water that can be absorbed by seeds in their initial germination phases (Phases I and II), but without primary root protrusion (Phase III), according to the three-phased pattern proposed by Bewley and Black (1978). As the preparatory metabolism was initiated before sowing, seed germination and seedling establishment are faster and thus improve seedling emergence in seedbed.

Mimosa bimucronata is one of the most important tree species used for tropical green area restoration in Brazil, as it is fast growing. In a green area restoration project evaluated in Brazil by Nave (2005), this species showed the largest canopy diameter and height. This species is also planted for firewood, living fence, landscaping and honey production (Lorenzi, 2002).

M. bimucronata has small flat seed (5 mm length × 3 mm width), with an average of 90,000 seeds per kg. The seed coat is brownish, smooth and shining, and as for most *Mimosa* species, it is impermeable to water.

The aim of this research was to evaluate the priming efficiency of *M. bimucronata* seeds.

MATERIAL AND METHODS

The research was done with *Mimosa bimucronata* (Fabaceae-Mimosoideae) seeds, which were collected from 14 wild mother trees in Piracicaba, São Paulo State, Brazil.

M. bimucronata has seed with impermeable seed coat which prevents water uptake and consequently seed germination. Therefore, before the experiments, seed dormancy was overcome by cutting the seed coat extremity (opposite to the micropyle end) with a sharp blade (Ferreira et al., 1992), including seeds of the unprimed control.

Seeds were primed at the osmotic potentials, 0.0 and -0.8 MPa. Deionized water was used for 0.0 MPa and aqueous solution of PEG 8000, which was prepared in accordance with Michel and Kaufmann (1973), was used for -0.8 MPa. The seeds were set on top of three round blotter papers, which were soaked with 15ml of water or PEG solution in covered petri dishes. The petri dishes were incubated in a germinator at 20°C in constant light, until the seeds reach the desired seed moisture content (MC).

The amount of water absorbed was monitored periodically, by weighing the seeds on an analytical balance. MC was determined according to International Seed Testing Association (ISTA) methods (1999). Before weighing, the seeds were dried on a paper towel to remove any free liquid on seed coat surface.

Based on previous imbibition curves (data not showed), the stabilized MC in -0.8 MPa PEG solution at 20°C during imbibition was 50% and the time required to reach this MC was 24h. The MC of 50% was thus selected for the priming experiment.

Seeds were primed in water for 11h (time required for the seeds to reach the 50% MC in water) and in -0.8 MPa PEG solution for 24, 48, 72 and 96h. Fresh primed seeds had approximately 50% MC immediately following all the priming treatments. In order to study the effects of drying back the seeds after priming, half of the primed seeds of all priming treatments was submitted to fast drying at 30°C with constant air flow until the seeds reached the initial 6.6% MC. It took five hours to dry seeds down to this MC. As the result, eleven treatments were tested: non-primed seeds (control); seeds primed in water for 11h, submitted or not to drying; seeds primed in PEG solution for 24, 48, 72 and 96h, submitted or not to drying. Eight replicates of 25 seeds each were used for each treatment.

Germination tests were done according to the Ohio State University's Seed Vigor Imaging System (SVIS) (Hoffmaster et al., 2003), where seeds were sowed between two rectangular blotter papers (24cm × 13cm) intercalated with one wax paper, saturated with deionized water in a rectangular plastic box in a plastic bag to prevent water loss. The boxes were placed in a germinator at 25°C in constant light. This is the optimal temperature for *M. bimucronata* seed germination (Fowler and Carpanezzi, 1998).

After four days, the boxes were opened for seedling growth and uniformity evaluation by SVIS. Images were captured with inverted scanner at a resolution of 150 dpi. From this day forward, germination was evaluated daily, and the germination speed index, according to the formula described by Maguire (1962), and the germination percentage were calculated. The germination was evaluated by counting the normal and abnormal seedlings, according to ISTA rules (1999).

Both seedling growth and uniformity were obtained with SVIS and the SVIS growth and uniformity values were used to compare the treatments. These values are indices (have no measurement unit) and, therefore, the higher is the value, the better is the seedling growth or uniformity.

For the electrical conductivity of seed leakage analysis, eight replicates of 25 seeds from all of the treatments were immersed in clean beakers with 20 ml of deionized water, set in a germinator at 20°C for 24h. The measurement was carried out with a conductivity meter, YSI 3100 (YSI Incorporated).

For variance analysis, the percentage data was transformed with arcsin of square root of x/100 and means were compared with Tukey test, at 5% confident level, by using

SAS (SAS Institute, 1990).

RESULTS AND DISCUSSION

The results showed that priming improved seed performance, mainly when it was done in an aqueous PEG solution at -0.8 MPa for 48h and without drying (Table 1). In this treatment, seedling growth and uniformity, as well as germination percentage and germination speed index, were higher, indicating the suitability of this methodology for *M. bimucronata* seed priming.

The vigor tests (SVIS and germination speed index) gave similar results in identifying the treatment, PEG solution at -0.8 MPa for 48h and without drying, as the best priming method. Based on this, both germination speed index and SVIS (seedling growth and uniformity) could be used to determine the best methodology for seed priming. However, electrical conductivity of seed leakage seemed to be more related to the washing off of seed solutes during priming than to seed vigor, as further described below.

Several works have shown the improvement in seed performance after priming. This includes increasing seed germination speed (Ruan et al., 2002), uniformity (Bodsworth and Bewley, 1981) and seedling growth (Khan et al., 1978). This improvement is a consequence of physiological changes during the priming process which affects protein (Smith and Cobb, 1991; Fujikura and Karssen, 1992), enzyme (Dell'Aquila and Spada, 1992; Sung and Chang, 1993), DNA (Ashraf and Bray, 1993) and RNA (Coolbear et al., 1990; Davison et al., 1991) synthesis and activity. However, these benefits are commonly lost with seed drying following priming (Carpenter, 1989; Parera and Cantilife, 1992; Heydecker and Coolbear, 1977), as also shown in our results.

Seed leakage electrical conductivity was reduced following priming, i.e., as the period of priming (hours) increased, the electrical conductivity was lower. The reduction of seed leakage electrical conductivity has been frequently reported in the literature as one of the main results of priming. One of the explanations for this reduction is attributed to the cellular membrane reorganization during priming, which reduces solutes leakage (Pandey, 1989; Choudhuri and Basu, 1988). Besides that, it is possible that priming reduces the level of imbibition injuries caused by rapid water absorption by the seeds, since the process is slower (Tilden and West, 1985).

Copeland and McDonald (2001) suggested that part of seed solutes is washed during priming, which thus reduces the amount of solutes being washed off into the solution that is used for seed leakage electrical conductivity analysis. The reduction of seed leakage diminishes the incidence of pathogens on primed seeds, which can result in higher rates of seedling survival (Osburn and Schroth, 1988).

Seed leakage electrical conductivity of primed seeds after drying was higher in all of the treatments when compared with fresh primed seeds. This could be due to the occurrence of mechanical damages during seed drying, as previously reported by Huang and Zou (1989) for drying primed seeds of *Pinus sylvestris* and *Larix gmelinii*. In fact, it was possible to see cracks on the seed coat and cotyledons of dried *M. bimucronata* primed seeds, which demonstrate the presence of mechanical damages. This problem was also described for soybean when drying primed seeds (Armstrong and McDonald, 1992).

CONCLUSION

Priming was effective in improving *M. bimucronata* seed performance. The best result was achieved in PEG aqueous solution at -0.8 MPa for 48h and without drying where increasing seedlings growth and uniformity, germination percentage and speed, as well as reducing electrical conductivity values of seeds leakage, were observed.

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Tables

Table 1. Effects of different seed priming treatments on *Mimosa bimucronata* seed germination percentage (G%), germination speed index (GSI), electrical conductivity of seed leakage (E.C.), seedling growth and seedling uniformity. (seed dormancy of all the treatments, including the control, was overcome by cutting the seed coat extremity (opposite to the micropyle end) with a sharp blade).

Treatments	G%	GSI	E.C. ($\mu\text{S}/\text{cm}/\text{g}$)	Growth	Uniformity
control	68e	12f	555a	339ab	196bc
0.0MPa fresh - 11 hours	86abc	16bcd	365b	349a	181bc
0.0MPa dried - 11 hours	78cde	14cde	354b	341ab	191bc
-0.8MPa fresh - 24 hours	85bc	18ab	242cd	335abc	246ab
-0.8MPa dried - 24 hours	78cde	17bc	403b	264de	238ab
-0.8MPa fresh - 48hours	96a	21a	207d	333abc	281a
-0.8MPa dried - 48 hours	89ab	17bc	394b	305bcd	244ab
-0.8MPa fresh - 72 hours	86abc	17bc	177d	332abc	202abc
-0.8MPa dried - 72 hours	77cde	14de	332b	291cde	176cd
-0.8MPa fresh - 96 hours	80bcd	13ef	206d	334abc	211abc
-0.8MPa dried - 96 hours	72de	11f	319b	244e	139d

