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Influence of pre-germination treatments on germination seed in *Melanoxylon brauna* Schott

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This study evaluated the presence of integument dormancy in *Melanoxylon brauna* seeds by testing the influence of nine pre-germination treatments on seed germination compared to a control (no pre-germination) treatment. Pre-germination treatments include; immersion in sulfuric acid for 5 min, 10 min, 15 min and 30 min, hot water at 80°C without soaking, hot water at 80°C with soaking for 30 min, 90 min, 24 h, and mechanical scarification. The experiment consisted of a completely randomized design, with four replicates and 25 seeds per experimental unit. Percentage germination, germination speed, number of normal and abnormal seedlings, and mortality rate were evaluated. Seeds began to germinate at four days, and germination varied from 33 to 89%, with the highest percentage found in hot water with or without soaking for 30 min, which not differed statistically from control. Immersion in sulfuric acid for longer than 10 min reduced seed germination and increased mortality rate. We conclude that *M. brauna* seeds do not undergo seed dormancy, and there is no need to apply pre-germinating treatments to its seeds.

Key words: Dormancy, seed technology, Leguminosae.

INTRODUCTION

Melanoxylon brauna, popularly known as brauna, brauna-preta or garauna, is an arboreal species of the Leguminosae Family - Caesalpinoideae, endemic to Brazil and native to the Caatinga, Cerrado, and Atlantic Forest biomes. It occurs naturally in the states of Bahia, Goiás, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, and Paraná (Lorenzi, 2009; Carvalho, 2010). The species has very heavy and compact wood suitable for external and hydraulic works, fence posts and civil construction (Carvalho, 2010). Due to intense extractive exploitation of its wood for use in construction, the species is currently categorized as vulnerable on the endangered species list (Martinelli and Moraes, 2013). It has ornamental potential due to its intense yellow

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flowering and is thus used in landscaping projects and afforestation of squares/parks. It has symbiotic association with nitrogen-fixing bacteria (De Faria et al., 1984), however it is little used in environmental restoration projects due to the difficulty of obtaining its seeds and their slow growth during the seedling phase and in the field.

Understanding the main processes involved in germination is of vital importance for multiplying native species, considering that the seminal route is often the only form of propagating these species, as in the case of Brauna. Germination consists of a complex and orderly set of biochemical and physiological events which begin with water absorption by the seeds that activates metabolism culminating in the emergence of the embryonic axis (Labouriau, 1983). This process is influenced by several intrinsic and extrinsic factors which can act in isolation or in interaction with others.

Several species of the Leguminosae Family have waterproof seed coatings/integument, which causes physical or exogenous dormancy. This characteristic is determined by the presence of substances such as suberin, lignin, cutin and mucilage that can concentrate on different parts of the seeds such as its coating/integument, pericarp and nucellar membrane, which differ from one species to another (Mayer and Poljakoff-Mayber, 1982). The waterproof coating/integument can act in two ways on seed dormancy; inhibiting water absorption and preventing seed soaking, or by reducing oxygen availability to the embryo.

Dormancy is ecologically important for preventing germination when the environmental conditions for the species' growth are not adequate by increasing the prospects of success for its establishment and survival. Under natural conditions, physical dormancy can be broken by scarification processes, animal ingestion, microorganism activity, natural soil acidity and by burning (Mayer and Poljakoff-Mayber, 1982).

Seeking methodologies for analyzing forest seeds has a fundamental role in scientific research and is of diverse interest. For Brauna, the effects of temperature, substrate and seed size on germination and vigor have already been investigated (Flores et al., 2014a, b). Large dark seeds put to germinate on a paper roll substrate in temperatures ranging between 30 to 35.8°C showed better performance. However, no studies have addressed the influence of pre-germination treatments on the germination of this species. The objective of this study was to verify the influence of different pre-germination methods on seed germination and vigor in *Melanoxylon brauna*.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Leguminous Forests, Embrapa Agrobiology, Seropédica, Rio de Janeiro.

Melanoxylon brauna seeds used in the experiment were harvested in July, 2015 in Leopoldina, Minas Gerais and remained stored in a cold room at 5ºC for one year. Then the moisture content of the seeds was determined by the drying oven method at 105 ± 3°C for 24 h, adopting two replicates of 5 g of each seed, and then 1000-seed weight was also determined, consisting of weighing eight samples of 100 seeds (Brasil, 2009).

For the germination test, seed asepsis was first performed by immersion in 5% (v/v) sodium hypochlorite for 10 min, followed by rinsing under running water. The treatments used to break dormancy were:

Chemical scarification

The seeds were immersed in sulfuric acid with 98% concentration for 5 min (T1), 10 min (T2), 15 min (T3) and 30 min (T4), and then rinsed in running water.

Thermal shock

The seeds were immersed in water at 80°C without soaking (T5), and with soaking for 30 min (T6), 90 min (T7) and 24 h (T8).

Mechanical scarification (T9)

The region opposite to the axis of the embryo was manually rubbed using sandpaper P150, until a slight change of color was obtained in that area of the coating/integument.

Control (T10)

Intact seeds without any treatments

A germitest paper roll was used as substrate, with three papers per repetition, moistened with distilled water 2.5 times its weight (47.5 ml per roll). The rolls were placed in transparent polyethylene plastic bags with dimensions of 40 cm x 60 cm with 0.033 mm thickness, which were then well tied with crochet thread to avoid drying out. The rolls of paper with plastic were kept upright in a BOD germinating chamber at a temperature of 25ºC, and a photoperiod of 12 h. The experimental design was completely randomized with four replicates and 25 seeds per experimental unit.

Counts were carried out daily in the first week until the first germinated seeds were observed. The evaluations were weekly from the second week on with the following characteristics evaluated: percentage of normal and abnormal seedlings, percentage of hard or non-germinated seeds and dead seeds. Germinated seeds were considered those whose radicles were equal to or greater than 2 mm. Also, the Speed Germination Rate (SGR) was calculated according to the formula proposed by Maguire (1962).

The experiment lasted 25 days, when all treatments presented a stabilized germination percentage. The data of the evaluated variables were subjected to analysis of variance (ANOVA) and the means that originated from the treatments were compared by the Scott-knott test, at 5% probability. There was a need for transformation in arc sin sen$(\sqrt{x/100})$ of the normal seedlings variable values because they did not present a normal distribution or homogeneity of residue variance, which are necessary assumptions to perform ANOVA. Non-parametric Kruskal Wallis test was used for the variable percentage of abnormal seedlings, as the residues did not fit such assumptions even after transformation. Analyzes were performed with the help of SAEG 9.1 (SAEG, 2007),

Table 1. Summary of the analysis of variance with the sources of variation and probabilities of significance.

Source of variation	GL	G (%)			SGR			PLAN (%)			PLAA (%)			M (%)		
		QM	F	P	QM	F	P	QM	F	P	SQ	F	P	QM	F	P
Treatment	9	9384.4	12.43	0.0000	16.45	6.22	0.0001	1007.11	5.083	0.0003	18.71	1.823	0.1052	1042.7	12.43	0.0000
Error	30	2516.0			2.64			198.13			10.26			83.86		
CV (%)		13.33			14.23			50.27			188.48			20.26		

(P) for the analyzed variables corresponding to germination percentage (G), normal seedlings (PLAN), abnormal seedlings (PLAA), mortality (M) and speed germination rate (SGR).

Table 2. Germination percentage values (G%), speed germination rate (SGR), percentage of normal seedlings (PLAN%), percentage of abnormal seedlings (PLAA%) and percentage of mortality (M%) of *Melanoxylon brauna* seeds subjected to different pre-germination treatments after 25 days of testing under controlled conditions.

Treatment		G %	SGR	PLAN %	PLAA %	M %
T1	Immersion in sulfuric acid 5 min	73^{b}	13.43^{a}	33^{a}	0^{a}	27^{c}
T2	Immersion in sulfuric acid 10 min	70^{b}	12.70^{a}	27^{a}	3^{a}	30^{c}
T3	Immersion in sulfuric acid 15 min	51^{c}	10.09^{b}	4^{c}	1^{a}	49^{b}
T4	Immersion in sulfuric acid 30 min	33^{d}	6.84^{c}	1^{c}	0^{a}	67^{a}
T5	Hot water 80°C without soaking	80^{a}	12.16^{a}	33^{a}	4^{a}	20^{d}
T6	Hot water 80°C with 30 min soaking	89^{a}	13.81^{a}	42^{a}	0^{a}	11^{d}
T7	Hot water 80°C with 90 min soaking	73^{b}	11.58^{a}	42^{a}	6^{a}	27^{c}
T8	Hot water with 24 hours soaking	67^{b}	10.67^{b}	17^{b}	0^{a}	33^{c}
T9	Mechanical Scarification	53^{b}	10.55^{b}	34^{a}	0^{a}	31^{c}
T10	Control	82^{a}	12.37^{a}	47^{a}	3^{a}	18^{d}

Mean followed by equal letters in the column did not differ among each another by Kruskal-Wallis test (PLAA%) at 5% significance.

SISVAR (Ferreira, 2011) and R (The R Project for Statistical Computing, version 3.3.2.) software programs.

RESULTS AND DISCUSSION

The 1000 seed weight for *Melanoxylon brauna* was 136.70 g, meaning 1 kg corresponds to 7,135 seeds. The moisture content of the seeds was 7.42%. The 1000 seed weight reported by Flores et al. (2014) for the species was very close to what was found: 133.43 g (7,494 seeds per kilogram), with 13% humidity. Carvalho (2010) reported values of 7,800 to 30,000 seeds per kilo for the species, and Lorenzi (2009) found 30,000 seeds per kilo, values well above what we found.

The analysis of variance showed a highly significant difference between treatments for germination ($F = 12.433$; $p < 0.0000$), speed germination rate ($F = 11.42$; $p < 0.0001$), normal seedlings ($F = 5.083$, $p < 0.0003$), and mortality ($F = 12.433$, $p < 0.0000$). The only exception occurred for the abonormal seedlings ($F=1.823$, $p<0.1052$), which was not significant. Thus, it was possible to statistically distinguish the treatments for breaking dormancy (Table 1).

The highest percentages of germination were found in hot water treatment with 30 min soaking (T6), control (T10) and hot water without soaking (T5) (Table 2). Germination was 89%, 82% and 80%, respectively for these treatments, not differing among them. The second best results were obtained by treatments T1, T2, T7, T8, T9,

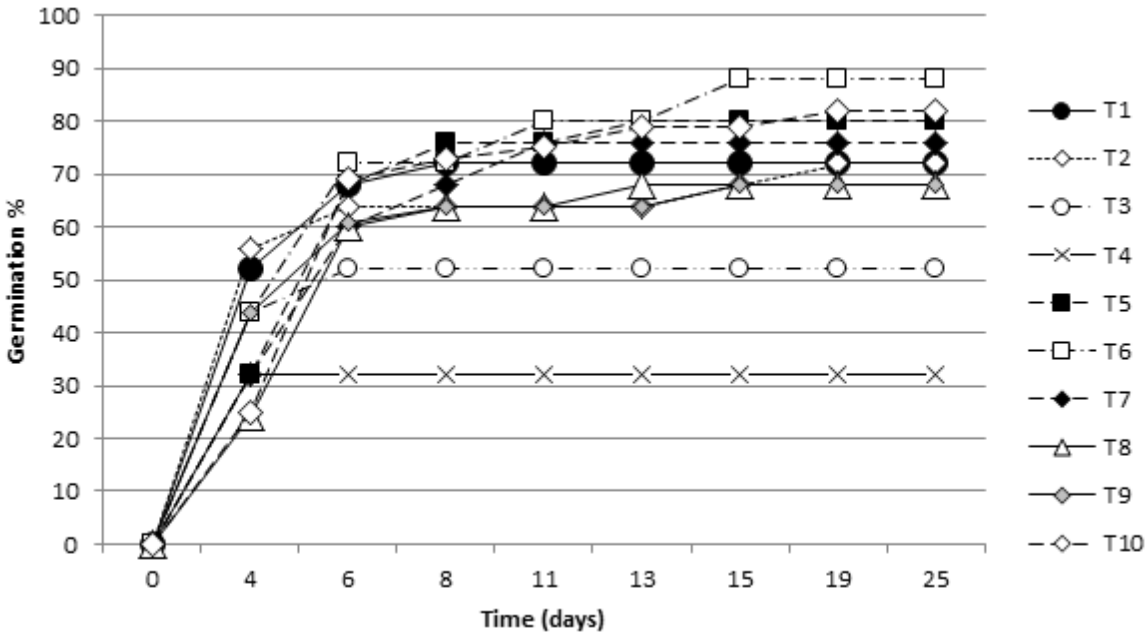

Figure 1. Germination time of *Melanoxylon brauna* seeds subjected to different pre-germination treatments under controlled conditions.

which presented germination varying from germination of 67 to 73%, respectively, with no statistical difference. The lowest percentage of germination was found for T4 - Immersion in sulfuric acid for 30 min (33%). The treatments of immersion in sulfuric acid for 15 min (T3) showed germination values of 51%. Speed germination rate formed a first group with treatments T1, T2, T5, T6, T7, T10, a second group with T3, T8, T9, and lastly the T4 treatment (Table 2).

The seeds began to germinate on the fourth day of the experiment for all treatments. All treatments reached a germination percentage greater than 50% at 6 days, except for immersion in sulfuric acid for 30 min (T6), which reached 32% persisting throughout the experiment period (Figure 1).

In general, the mortality of Brauna seeds was quite high, varying from 11% to 67%. The lowest value was found for T6 (seeds soaked in hot water 80°C by 30 min), not differing significantly from control (T10) and hot water without soaking (T5).

A high incidence of fungi in the seeds was observed and may have contributed to its high mortality, especially when combined with treatments that caused seed damage. Although fungal attack causes an increase in mortality, its presence is a factor that can positively influence the germination process, since it leads to deterioration of the coating/integument. It was not measured in this experiment, but have already been observed between *Alternaria*, *Aspergillus* fungi and *Astragalus utahensis* seed (Long et al., 2012; Eldredge et al., 2016) and between corn and *Fusarium subglutinans* (Rheeder et al., 1990).

Immersion in sulfuric acid for longer than 10 min impaired germination and increased seed mortality. Although, chemical scarification leads to integument/coating degradation allowing water to enter. Excessive immersion/soaking can cause the breakdown of essential cells, thereby inducing mechanical damage and fungi invasion, which in turn hinder the emergence of seedlings (Rolston, 1978).

Immersion in hot water for 24 h also impaired Brauna seed germination, reducing speed germination rate and increasing mortality. Excess water can impair the germination process by promoting pathogenic microorganism proliferation, restricting oxygen entry and absorption. When set to soak for an extended period, the seeds may suffer irreversible damage to the membrane system, and consequent leaching of cellular contents that negatively affects germination. Similar to Brauna, *Parkia pendula* seeds do not seem to tolerate long periods of soaking, and a reduction in germinability and percentages of normal seedlings can be observed when soaked for 24 h after the heat treatment (Pinedo and Ferraz, 2008). These authors found that a soaking greater than 20% of their mass after about 4h significantly reduced the emergence of seedlings. This reduction was observed in *Parkia multijuga* when soaking seeds exceeded 45.4% in water content (Calvi et al., 2008).

Variable results in relation to the same chemical treatments in different plant species can be observed due to the germination peculiarities of each species and the

characteristics of each integument/coating. *Colubrina glandulosa* Perk germinated well with sulfuric acid for 30 to 90 min, however germination losses were observed for 120 and 150 min (Brancalion et al., 2010). *Libidibia ferrea* (Mart. ex Tul.) LP Queiroz) had good results with sulfuric acid for 20 and 40 min (Matos et al., 2015). *Samanea tubulosa* (Benth.) Barneby & JW Grimes germinated with sulfuric acid for 5 and 10 min (Muniz Giachini et al., 2010). *Parkia panurensis* e *P. velutina* had a good performance with immersion in sulfuric acid for 30 min (Melo et al., 2011). *Enterolobium contortisiliquum* (Vell.) Morong germinated with immersion in sulfuric acid for 30 to 50 min without significant difference between immersion/soaking periods (Lozano et al., 2016). *Gledistchia amorphoides* Taub seeds subjected to chemical scarification for 1 or 2 h had germination increased by 70% and anticipated in 15 days (Bortolini et al., 2011).

Although mechanical scarification has been indicated as a good method to overcome seed dormancy in many Leguminosae species (Guedes et al., 2011; Dayrell et al., 2015), in this study it caused a decrease in brauna speed germination rate and percentage. Based on the results, it can be assumed that the seeds of Brauna do not have seed dormancy.

Conclusion

Treatments with seed immersion in 80°C hot water with or without soaking for 30 minutes show the highest germination, speed germination rate and lowest mortality values. These treatments are not statistically different from each other or the control group, concluding that brauna seeds do not undergo physical dormancy and do not require pre-germination treatments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Bortolini MF, Koehler HS, Zuffellato-Ribas KC, Malavasi MDM, Fortes AMT (2011). Overcoming of seed dormancy in *Gleditschia amorphoides* Taub. Cienc. Rural 41(5):823-827.

Brancalion PHS, Mondo VHV, Novembre ADDLC (2011). Escarificação química para a superação da dormência de sementes de saguaraji-vermelho (*Colubrina glandulosa* Perk.-Rhamnaceae). Rev. Árvore 35(1):119-124.

Brasil (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasília: Mapa/ACS P 399.

Calvi GP, Audd FF, Vieira G, Ferraz IDK (2008). Tratamentos de pré-embebição para aumento do desempenho da germinanção de sementes de *Parkia multijuga* Benth. Rev. For. Latinoam. 23(2):53-65.

Carvalho PER (2010). Espécies florestais brasileiras. Colombo: Embrapa-CNPF; Brasília: DF: Embrapa Informação Tecnológica; Colombo, PR: Embrapa Florestas 644p.

De Faria SM, Franco AA, Menandro MS, de Jesus RM, Baitello JB, de Aguiar OT, Dobereiner J (1984). Levantamento da nodulação de leguminosas florestais nativas na região sudeste do Brasil. Pesqui. Agropecu. Bras. 19(13):143-153.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciência e agrotecnologia 35(6):1039-1042.

Dayrell RLC, de Jesus Gonçalves-Alvim S, Negreiros D, Fernandes GW, Silveira FAO (2015). Environmental control of seed dormancy and germination of Mimosa calodendron (Fabaceae): implications for ecological restoration of a highly threatened environment. Braz. J. Bot. 38(2):395-399.

Eldredge SD, Geary B, Jensen SL (2016). Seed isolates of *Alternaria* and *Aspergillus* fungi increase germination of *Astragalus utahensis*. Nativeplants 17(2):89-94.

Flores AV, Borges EEL, Gonçalves JFC, Guimarães VM, Ataíde GdaM, Barros DP, Pereira MD (2014). Efeito do substrato, cor e tamanho de sementes na germinação e vigor de *Melanoxylon brauna.* Pesqui. Flor. Bras. 34(78):141-147.

Flores AV, Borges EEDL, Guimarães VM, Ataíde GDM, Castro RVO (2014). Germinação de sementes de *Melanoxylon brauna* schott em diferentes temperaturas. Rev. Árvore 38(6):1147-1154.

Labouriau LG (1983). A germinação das sementes. Washington: Secretaria Geral da Organização dos Estados Americanos 174p.

Long Y, Tan DY, Baskin CC, Baskin JM (2012). Seed dormancy and germination characteristics of *Astragalus arpilobus* (Fabaceae, subfamily Papilionoideae), a central Asian desert annual ephemeral. S. Afr. J. Bot. 83:68-77.

Lorenzi H (2009). Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil. Nova Odessa: Instituto Plantarum 1:368.

Lozano EC, Zapater MA, Mamani C, Flores CB, Gil MN, Sühring SS (2016). Efecto de pretratamientos en semillas de *Enterolobium contortisiliquum* (Fabaceae) de la selva pedemontana argentina. Bol. Soc. Argent. Bot. 51(1):79-87.

Maguire JD (1962). Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2(2):176-177.

Martinelli G, Moraes MA (2013). Livro vermelho da flora do Brasil. Rio de Janeiro: Andrea Jakobsson: Instituto de Pesquisa Jardim Botânico do Rio de Janeiro 1100p.

Matos ACB, Ataíde GDM, Borges EEL (2015). Physiological, physical, and morpho-anatomical changes in *Libidibia ferrea* ((Mart. ex Tul.) LP Queiroz) seeds after overcoming dormancy. J. Seed Sci. 37(1):26-32.

Mayer AM, Poljakoff-Mayber A (1982). The germination of seeds. 3. ed. Oxford: Pergamon Press.

Melo MDGG, Mendonça MS, Nazário P, Mendes MAS (2011). Superação de dormência em sementes de três espécies de *Parkia* spp. Rev. Bras. Sement. 33(3):533-542.

Muniz Giachini R, Lobo FA, Albuquerque MCF, Ortiz CER (2010). Influência da escarificação e da temperatura sobre a germinação de sementes de *Samanea tubulosa* (Benth.) Barneby & JW Grimes (sete cascas). Acta Amaz. 40(1):75-80.

Pinedo GJV, Ferraz IDK (2008). Hidrocondicionamento de *Parkia pendul*a (Benth. ex Walp.): sementes com dormência física de árvore da Amazônia. Rev. Árvore 32:39-49.

Rheeder JP, Marasas WFO, Vanwyk PS (1990). Fungal associations in corn kernels and effect on germination. Phytopathology 80:131-134.

Rolston MP (1978). Water impermeable seed dormancy. Bot. Rev. 44(33):365-396.

SAEG (2007). SAEG: sistema para análises estatísticas, versão 9.1. Viçosa: UFV, http://www.scielo.br/scielo.php?script=sci_nlinks&ref=000207&pid=S1516-35982010000900024000049&lng=pt