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Overcoming seed dormancy and evaluation of viability in *Leucaena leucocephala*

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Abstract

Leucaena leucocephala is an arboreal legume with many applications in agriculture (forage, wood production, charcoal, soil improvement, shading, windbreak, and hedge). Its seeds show dormancy, which is caused by physical blockade through resistant and impermeable integument. The aims of this study were to evaluate and determine efficient methods for overcoming dormancy in *Leucaena leucocephala* seeds. The experiment was carried out in Tangará da Serra city, Brazil, adopting a completely randomized design, with four replications. The treatments consisted of no scarification (T_0), scarification with sandpaper (T_1), immersion in concentrated H_2SO_4 for 5 min (T_2), 10' (T_3), 15' (T_4), and 20' (T_5), and immersion in water at 80°C for 5' (T_6), 10' (T_7), 15' (T_8) and 20' (T_9). The seeds were placed in germination chamber at a temperature of 25ºC and photoperiod of 12 hours day/night over a period of 10 days, for all treatments, using 50 seeds per replicate. The experimental data were submitted to analysis of variance (type I error) by F test ($p<0.05$) with comparison of averages performed by Tukey test ($p<0.05$). The treatment that showed best efficiency in overcoming seed dormancy was immersion in concentrated H_2SO_4 (density of 1.84 and purity of 95%) for 20 min, with 93.0% of viable seed germinated. Immersion of seeds in water at 80ºC for 20 min (0.0% seed germination) showed lowest efficiency in overcoming dormancy of *Leucaena leucocephala* seeds. It was concluded that treatment with immersion in concentrated H_2SO_4 for 20 min is the most efficient in overcoming dormancy of *Leucaena leucocephala* seeds, showing higher values of first germination count, germination percentage, germination speed index and average time of germination.

Keywords: Scarification with sandpaper, immersion in concentrated H_2SO_4, immersion in water at 80°C, *Leucaena leucocephala* seed, dormancy overcoming.
Abbreviations: H_2SO_4_sulfuric acid, DAS_ day after sowing, GSI_germination speed index, ATG_average time of germination, FGC_first germination count, GP_germination percentage.

Introduction

Leucaena leucocephala is an arboreal legume which has a very diversified use, highlighting in reforestation of degraded areas for erosion control. Currently, it is also used for animal feed, green manuring, fence posts, poles, cellulose, and plywood (Osechas et al., 2008). The presence of legumes in tropical grass pastures improves ruminant nutrition due to higher protein contents for development of microorganisms that digest forage (Valente et al., 2016). The tropical legume grasses have shown good results on production of goats (Rubanza et al., 2007), sheep (Santana et al., 2014), buffaloes (Kang et al., 2012) and cattle (Díaz et al., 2009).

In Brazil, the species is popularly known as leucaena (Fonseca and Jacobi, 2011), and is indicated as a good candidate for inclusion in agroforestry systems (Pereyra et al., 2015) due to its rapid growth and high nutritional value of minerals and proteins (Crawford et al., 2015), mainly in semi-arid regions (Walker, 2012). This legume has shown good general characteristics such as high seed production, which facilitates its propagation, if the dormancy of seeds be overcome (Drumond and Ribaski, 2010). The physical impediment such as dormancy increases survival of species, which allows seeds to maintain viability for a longer period of time (Nesi et al., 2016), as well as production of seedlings and obtaining plants with standard development (Drumond and Ribaski, 2010). In legume seeds, the dormancy is promoted by resistant tegument and impermeability to water, preventing water and gas exchanges. It suppresses seed imbibition and absorption of oxygen by embryo (Drumond and Ribaski, 2010). The sowing performance without physical dormancy breaking processes results in slow and irregular emergence, with direct effect on seedling development (Martins and Lago, 1996). According to Teles et

al. (2000) and Paulino et al. (2004) sowing the *Leucaena leucocephala* seeds without overcoming dormancy resulted in a germination index less than 50%, causing slow and irregular emergence with negative consequence on plant density, as well as to promote weed infestation. Therefore, adoption of pre-germination treatments to overcome dormancy is important to accelerate and standardize seed germination (Dapont et al., 2014; Pereira et al., 2013).

The most adopted methods to overcome impermeability to water of legume seeds are through: mechanical scarification, using abrasive surfaces (Mantoan et al., 2012); chemical treatment with use of sulfuric or hydrochloric acids, immersion in acidic substances (Rebouças et al., 2012); and immersion in hot water (Araújo et al., 2012). Thus, research on seed dormancy break provides a fundamental background for scientific communities. Furthermore, the knowledge of seed germination is vital to use in reforestation programs. In addition, genetic diversity between species has promoted greater concern of seed researchers and analysts, in conducting studies that provide information on seed quality, mainly for standardization, improvement and establishment of efficient methods to overcome the seeds.

There is scarcity of information about the characteristics of *Leucaena leucocephala* seeds such as thickness and hardness of tegument. Therefore, this study intends to fill some doubts in knowledge for seedlings production. The present study has chosen practical and cheap methods for overcoming dormancy that can be used in practice by farmers. Thus, the aims were to evaluate and identify methodologies for overcoming dormancy and to promote the germination in *Leucaena leucocephala* seeds for seedlings production.

Results and discussion

Germination of Leucaena leucocephala

The results of first germination count (FGC), germination percentage (GP), germination speed index (GSI) and average time of germination (ATG) are shown in Table 1. In first germination count (FGC), seeds were chemically scarified by immersion in H_2SO_4 for 20 min (T_5). The (T_5) obtained best results with 88.0% seed germination rate possibly due to breaking of seed integument. The treatments with water immersion at 80ºC for 5' (T_6), 10' (T_7), 15' (T_8), and 20' (T_9) caused less beneficial results, lower than scarification (T_1) and chemical treatment (T_2, T_3, T_4, and T_5), being statistically equal to control treatment (T_0).

Oliveira, (2009) studied first count of germination in *Leucaena leucocephala* and observed better results with immersion of seeds in H_2SO_4, compared to treatments with immersion in hot water. The sanding scarification resulted in lower value (62.5%) than described by Cardoso et al. (2012), which recorded value of 97.0% for first count of germination.

For variable germination percentage (GP), most promising treatments were: T_1 (scarification with sandpaper), T_4 (immersion in H_2SO_4 for 15'), and T_5 (immersion in H_2SO_4 for 20') with 82.5%, 75.0%, and 93.0% of germinated normal seedlings, respectively (Table 1). The seed immersion in water at 80ºC for 10' (T_7), and 15' (T_8) did not produce satisfactory results, having a low number of germinated seeds, statistically equal to control (T_0).

The results (Table 1) disagrees with those obtained by Passos et al. (1988), whose reported that immersion in hot water was efficient to overcome dormancy. In our study, it was evident that treatment with immersion at 80ºC water for period of 20' (T_9), produced no seed germination. Perhaps, period and time of treatment with hot water was long enough to cause death of the seed embryo (Xavier et al., 2012).

Alves et al. (2004) and Paulino et al. (2004) observed values of 30% and 70% seed emergence in *Bauhinia divaricata* and *Leucaena leucocephala*, respectively, subjected to treatment with immersion in water at 80ºC.

Souza et al. (2007) observed higher efficiency associated with acid scarification compared to warm water in *Leucaena leucocephala* seeds, especially in seeds exposed to hot water for a longer period. In this case, exposure period has possibly impaired viability of embryo and reduced germination around 30%, compared to the best treatment (H_2SO_4). In a study developed by Teles et al. (2000), they reported seed germination of 97.3% treated with H_2SO_4 for 10', 15', and 20', confirming efficiency of method in overcoming dormancy of *Leucaena leucocephala* seeds.

The application of treatments to overcome seed dormancy does not usually damage embryos if be performed efficiently. Because this procedure is normally promoted in nature by open cracks in bark by action of microorganisms, fungi or acids of soil (Freire et al., 2016). According to Santos et al. (2011) and Costa et al. (2010) as a result of variation in composition and thickness of integuments of each species, it is necessary to carry out studies on type of treatment and time of seed immersion in acid or water to overcome seed dormancy.

From information shown in Table 1, we observe that germination speed index (GSI) was higher than other treatments using immersion in H_2SO_4 for 20', with a value of 89.3%. In contrast, the germination speed index subjected to treatments with hot water for 5' (18.7%), 10' (16.9%), and 15' (12.6%) were lower, only a bit higher than control (3.7%). Similar results were obtained by Oliveira (2009) and Passos et al. (1988), in which overcoming dormancy with H_2SO_4 provided better germination speed index for *Leucaena leucocephala* seeds.

For variable average time of germination (ATG), sandpaper scarification (T_1), and immersion in H_2SO_4 treatments for 10' (T_3), 15' (T_4), and 20' (T_5) obtained values of 6.1, 6.3, 6.2, and 6.0 days, respectively, showing no statistical difference (Table 1). Treatment with T_8 caused a time of 8.4 days for germination of seeds (Table 1).

According to Oliveira (2009), *Leucaena leucocephala* seeds treated with H_2SO_4 revealed best results for average time of germination compared to hot water treatment, due to high temperature used to negatively influence physiological mechanisms of seeds and viability of embryo, delaying germination and inducing seed inactivity.

Material and methods

Plant materials and experimental site

Leucaena (*Leucaena leucocephala*) seeds were collected in April 2013 from trees showing fully mature pods, located at Universidade do Estado de Mato Grosso (UNEMAT), Tangará

Table 1. Influence of methods to overcome dormancy in germination process of *Leucaena leucocephala* seeds.

Treatment	FCG	GP	GSI	ATG
	----------%----------			days
T_0 +	4.0e *	4.5fg	3.7f	7.0c
T_1	62.5b	82.5ab	76.3b	6.1d
T_2	24.5d	34.0d	21.2d	7.0c
T_3	24.0d	62.5c	63.5c	6.3d
T_4	48.5c	75.0b	70.1bc	6.2d
T_5	88.0a	93.0a	89.3a	6.0d
T_6	10.5e	24.0de	18.7de	7.6b
T_7	8.5e	15.0ef	16.9de	7.8b
T_8	1.0e	12.0efg	12.6e	8.4a
T_9	0.0e	0.0g	0.0f	0.0e
CV(%)	17.78	16.97	9.42	3.26

* Means followed by the same letter in the column do not differ by the Tukey test at 5% probability. First count of germination (FCG), germination percentage (GP), germination speed index (GSI), and average time of germination (ATG). + T_0 – no scarification (control); T_1 – scarification with sandpaper; T_2 – immersion in H_2SO_4 for 5'; T_3 – immersion in H_2SO_4 for 10'; T_4 – immersion in H_2SO_4 for 15'; T_5 – immersion in H_2SO_4 for 20'; T_6 – immersion in water at 80°C for 5'; T_7 – immersion in water at 80°C for 10'; T_8 – immersion in water at 80°C for 15'; T_9 – immersion in water at 80ºC for 20'.

da Serra city, State of Pará, Brazil (14°37'10'' S e 57°2 9'09'' W). After collection, seeds were dried in shade for a period of one week for later layout of experiment, being conducted in Phytopathology Laboratory of UNEMAT.

Dormancy breaking treatments

The experimental design was a completely randomized design with four replications, consisting of the following treatments:
T_0: without scarification;
T_1: scarification with sandpaper;
T_2: immersion in concentrated H_2SO_4 (density of 1.84 and purity of 95%) for 5';
T_3: immersion in concentrated H_2SO_4 for 10';
T_4: immersion in concentrated H_2SO_4 for 15';
T_5: immersion in concentrated H_2SO_4 for 20';
T_6: immersion in H_2O at 80°C for 5';
T_7: immersion in H_2O at 80°C for 10';
T_8: immersion in H_2O at 80°C for 15';
T_9: immersion in H_2O at 80°C for 20'.

Experiment information

A common sandpaper type was used for scarification treatment with sandpaper. Seeds were sanded manually on both sides, without damaging the embryo (Cardoso et al., 2012), while, immersion treatment in H_2SO_4 was used as proposed by Xavier et al. (2012), in which seeds were treated with H_2SO_4 (density of 1.84 and purity of 95%) for 5', 10', 15' and 20' (min), respectively, then, washed in running water for 5' for removal of acid residues and after drying on paper towels (Nautiyal et al., 2014).

In hot water treatment at 80ºC, seeds were immersed in beakers with water, and remained in water bath equipment until their treatment periods were reached, which were 5', 10', 15', and 20'. Afterwards, seeds were pulled out from heat source, remaining in water until reaching ambient temperature (Pacheco et al., 2014).

To evaluate influence of treatments on seed germination, four replicates of 50 seeds of each were disinfested by immersion in sodium hypochlorite (2%) for 5', washed in running water and placed to dry on germitest paper in the shade. The seeds were distributed on a paper substrate in the form of rolls, previously moistened with distilled water, in an amount equivalent to 2.5 times the weight of dry paper (Paiva et al., 2016; Zimmer et al., 2016). Then, paper was organized in roll form and incubated in germination chamber (Biochemical Oxygen Demand – B.O.D.) at a constant temperature of 30°C, with photoperiod of 12 hours for 10 day (Brasil, 2009).

Evaluated characteristics

The germination was evaluated daily, starting on the 4th day after sowing (DAS) and finished in 10th DAS, through percentage of normal seedlings, being considered germinated seeds that showed process of emission of essential structures of embryo.

The germination speed index (GSI) was expressed as a percentage, were calculated as outlined below (Vange et al., 2016):

$$GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \cdots + \frac{G_n}{N_n}$$

Being:
G_1, G_2, G_n = number of seedlings germinated in first, second, until last count;
N_1, N_2, N_n = number of days after sowing from first, second, to last count.

The average time of germination (ATG) was obtained using the formula, in which results expressed in days (Yu et al., 2008):

$$ATG = \frac{G_1 \times T_1 + G_2 \times T_2 + \cdots + G_n \times T_n}{G_1 + G_2 + \cdots + G_n}$$

Being:
G_1, G_2, G_n = number of seedlings germinated in first, second, until last count;
T_1, T_2, T_n = number of days after sowing from first, second, to last count.

Statistical analysis

Initially, the experimental data were submitted to the analysis to test the normality and homogeneity of variance. Then treatment means were submitted to analysis of variance (type I error) by F-test ($p<0.05$) with comparison of

averages performed by Tukey test at 5% of probability, using SISVAR version 5.3 software (Ferreira, 2011).

Conclusion

The treatment of immersion in H_2SO_4 for 20 min is the most efficient in overcoming dormancy of *Leucaena leucocephala* seeds, showing higher values of first germination count, germination percentage, germination speed index and average time of germination. The immersion of *Leucaena leucocephala* seeds in water at a temperature of 80ºC does not promote overcoming of dormancy efficiently because causes inactivity in embryo of *Leucaena leucocephala*.

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