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Different Treatments for Breaking Dormancy of Leucaena Seeds (*Leucaena leucocephala*)

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Abstract

The objective of the study was to evaluate the influence of methods for breaking dormancy the seeds of tropical forage legume *Leucaena leucocephala* (Lam.). The seed treatments were: (T1) water at 100 ºC/10 min; (T2) water at 100 ºC/1 min; (T3) Acetone (10 min); (T4) Ethyl alcohol (10 minutes); and (T5) intact seeds (control). Data were analyzed using a completely randomized design with eight replications, and it was adopted the Tukey test at 5% significance level. The treatment T1, had the highest speed of germination of seeds GSI, differed ($P < 0.05$). The T5 treatment (control) was the slowest response, that treatments that passed through some break dormancy until the seventh day after the beginning of the test (Germ7), with an amount of 20% accumulation of germinated seeds. The worst result of germination it was T5, with 30% of non-germinated seeds after 15 days of sowing.

Keywords: acetone, boiling water, germination, seed physiology

1. Introduction

The *Leucaena leucocephala* known as leucaena is a perennial legume shrub of subtropical regions in which the forage shows favourable characteristics for livestock production, whose body weight gain in ruminants are superior than the most other forage systems (Garcia et al., 1996). The presence of legumes in tropical grass pastures improves ruminant nutrition, due to the higher protein necessary for the development of microorganisms that digest forage (Gomes et al., 2011; Valente et al., 2015, 2016a). With good results of tropical grass pastures production with goats (Kanani et al., 2006; Rubanza et al., 2007), sheep (Karda, 2007; Santana et al., 2014), buffaloes (Kang et al., 2012; Hung et al., 2013) and cattle (Lourenço et al., 2001; Díaz et al., 2009).

The consortium with tropical grasses increases the nitrogen in pasture. Consequently, this measurement increases forage supplies at certain times of the year. It improves the nutritional quality of pasture, it reduces the annual variation of forage supply, it increases diversity of grassland beyond reduce fertilizers use (Valente et al., 2016b). The leucaena can be used in animal nutrition, because it is a legume with good range of crude protein (CP) between 20.9-29.5% (Aganga & Tshwenyane, 2003). Thus, further recent work suggests that leucaena mitigates enteric methane emissions in ruminant, implying that the shrub may also reduce greenhouse gas emissions at the whole farm level (Harrison et al., 2015) due to the effects of condensed tannins from Leucaena on rumen fermentation and decrease populations of methanogens and protozoa (Tan et al., 2011, Soltan et al., 2013). However, these legumes are more difficult to spread seeds in comparison to forage grasses. A limiting factor for spreading the legume forages is the deep seed dormancy, which results in slow and desuniform germination. This fact happens in reason to the impermeability of the tegument with water. This phenomenon is one of the most common causes of dormancy in legumes. It can be demonstrated by the low percentage of germinated seeds observed in intact

seeds (control) (Bruno et al., 2011). Seeds of legumes are generally considered to have physical dormancy, various kinds and combinations of dormancy due storage behaviour in seeds family, second Jayasuriya et al. (2013). Tropical legumes have a high percentage of seeds that do not germinate soon after sowing. The percentage of seeds can reach up to 90%. This dormancy is due to the presence of a water-impermeable cover that prevents germination (Valente et al., 2016c). Recent studies show that manipulations can improve dormancy breaking by increasing water permeability, and consequently increases seed sensitivity to light and temperature. Factors such as gas permeability, and removal of inhibitors influence seed metabolism, improving dormancy (Mayer & Poljakoff-Mayber, 1989).

To breaking dormancy, various methods have been reported in the literature and the most commons are immersion in water, removal of the seed coat, cut tegument, pierce the tegument, mechanical scarification, soaking in hot or cold water, hydrogen peroxide, chemical scarification sulfuric acid, hydrochloric acid, soda, acetone and alcohol (Deminicis, 2005). For this study were chosen practical and cheap methods for breaking dormancy that can be used in practice by farmers. Knowledge of the influence of temperature through boiling water may be an alternative, as it is a quick technique to be applied. Solvents such as acetone and alcohol can be used in the farm routine, so increased interest in your response. Due to the size of the seed the scarification is not a viable option (Paulino et al., 2004), and the use of sulfuric acid to break dormancy, which is not common practice, to have high corrosive power are required special care during handling, due to the danger, it is only realized in laboratory setting, beyond the high cost and the need for a special license to use sulfuric acid in the farm.

The objective of the study was to evaluate the influence of methods for breaking dormancy the seeds of tropical forage legume *Leucaena leucocephala*.

2. Materials and Methods

2.1 Study Location

The experiment was conducted in the laboratory of IFGoiano Campus Posse GO, Brazil. The leucaena seeds (*Leucaena leucocephala*) were tested for different dormancy breaking methods.

Seeds with or without the pre-germination treatments were germinated at constant temperatures of 25 ºC. The germination test was installed on two sheets of paper moistened with distilled water (an amount equivalent to 2.5 times its dry weight) in transparent plastic box 11 × 11 × 3 cm, with cover, 8 repetitions of 30 seeds and eight hours photoperiod, it was used an equipment germinating seed of solab® brand installed in the dependence of IFGoiano Campus Posse-GO, Brazil.

2.2 Germination Seeds

The number of germinated seeds was evaluated daily at the germination criterion radicle protrusion (growth, with about 2 cm long, the emerged seedlings of all). After the counting of the number of germinated seeds daily, the following characteristics were evaluated:

Step 1: Germination count which represents the cumulative percentage of germinated seeds on the third day after the start of the test (Germ3);

Step 2: Percentage of germinated seeds that correspond to the total percentage of seeds that germinate until the a seventh day after start of the test (Germ7);

Step 3: Percentage of germinated seeds that correspond to the total percentage of seeds that germinate until the fifteenth day after start of the test (Germ15);

Step 4: Germination speed index (GSI), which was calculated with the formula proposed Maguire (1962). Formula below,

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \frac{G3}{N3} \quad (1)$$

Where,

G1, G2, G3 … Gn = number of germinated seeds to the nth observation; N1, N2, N3 … Nn = number of days after sowing.

Step 5: Total count of seeds do not germinate after 15 days (NGerm).

2.3 Treatments

The seed treatments were: (T1) water at 100 ºC/10 min; (T2) water at 100 ºC/1 min; (T3) Acetone ReagentPlus® ≥ 99.0% purity (10 min); (T4) Ethyl alcohol 90% (10 minutes); and (T5) intact seeds (control).

The immersion in solvents such as acetone and alcohol, corresponds to at least 2.5 times the size of the seed.

2.4 Statistical Procedures and Model Evaluation

Data were analyzed using a completely randomized design with two replications. It was used, according to the Yij = μ + Ti + eij model, where: Yij is the value observed in the jth experimental unit that received the ith treatment; μ is the overall mean; Ti is the fixed effect of the ith treatment; eij is the experimental error related to the experimental unit. The data were subjected to statistical analysis through Analysis System variance – ASSISTAT version 7.7 (Silva & Azevedo, 2009). And was adopted the Tukey test at 5% significance level. The GSI data were transformed into log (X + 0.5) and to check the normal distribution, the Shapiro-Wilk test was applied for alpha = 0.5 % normality.

3. Results and Discussions

Germination counting represents the cumulative percentage of germinated seeds on the third day after the start of the test (Germ3). Within 3 days 75% of the seeds germinated in T1, differing ($P < 0.05$), while T2 = 24%, T3 = 21%, T4 = 13% and T5 = 5% germination (Table 1). The faster the seed germinates the greater the likely seed prosper (Deminicis, 2009). The speed and uniformity of seedling emergence depended on the seed vigor and the ambient conditions. It is of practical interest to know the intrinsic physiological quality of each seed (Paiva et al., 2008). As noted in this experiment, intact seeds spent more time to germinate to three days (Germ3).

Table 1. Average values of germination of seeds of leucena (*Leucaena leucocephala*), Germ3, Germ7, Germ15, GSI and NGerm after different treatments of dormancy breaking

Treatment	Germ3	Germ7	Germ15	GSI	NGerm
T1 = water at 100 ºC/10 min	75[a]	90[a]	97[a]	0.78[a]	3[a]
T2 = water at 100 ºC/1 min	24[b]	35[b]	80[b]	0.51[b]	20[b]
T3= Acetone (10 min)	21[b]	40[b]	80[b]	0.37[c]	20[b]
T4 = Ethyl alcohol (10 min)	13[b]	30[b]	75[b]	0.33[c]	25[b]
T5 = intact seeds (control)	5[b]	20[b]	70[b]	0.31[c]	30[b]
F test	79.250*	16.555*	26.000*	66.584*	26.000*
P-value	< .0001	0.0043	0.0013	< .0001	0.0013
Coefficient of Variation %	17.20	22.06	3.90	7.52	16.6

Note. * Significant to 1%. Different letters in columns indicate significant difference. According to Tukey test ($P < 0.05$).

The accumulated % germination until the fifteenth day after sowing (Germ15) can be seen in Figures 1, respectively representing the treatments T1 to T5. The T1 treatment was the only one had difference ($P < 0.05$) in germination after 15 days of sowing.

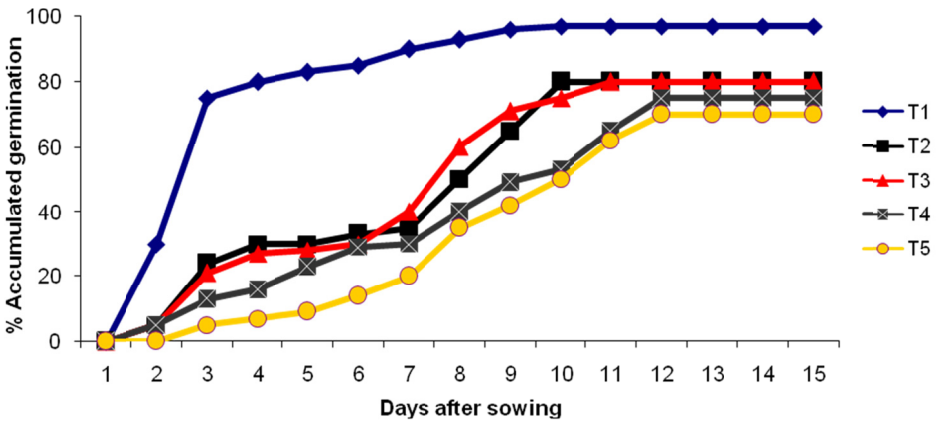

Figure 1. Treatment T1 to T5 % accumulated germination until Germ15

The T5 treatment (control) illustrated the slowest response, whose treatments passed through some break dormancy until the Germ7, with solely 20% accumulation of germinated seeds.

The T1 treatment was the only one which had significant difference ($P < 0.05$) in germination after 15 days of sowing (Germ15). For intact seeds (T5) the dormancy was 30% according Bewley and Black (1994), this value is within the normal range of legume dormancy. Nevertheless, Teles et al. (2000) found the critical values of 32.7% for intact germinating seeds (untreated). What highlights the need for treatments for breaking dormancy.

The treatment T1 had the highest speed of germination of seeds GSI, differed ($P < 0.05$). Treatment T2 also differed ($P < 0.05$), the worst GSI was the treatments T3, T4 and T5, which were not statistically different from each other.

In the treatment T1 it was calculated only 3% of non-germinated seeds (NGerm), the treatments T2, T3, T4 and T5 did not differ ($P > 0.05$) between them. The worst result of germination was for the treatment intact seeds (control), with 30% of non-germinated seeds after 15 days of sowing.

The germination process is conceptualized after the end of the physiological seed repose period. After the termination of morphogenetic events that result in the transformation of embryo seedling; a number of processes that transforms the seed, from a relatively inert structure to another active growth. An ordered sequence of metabolic events whose results reflects in the restart of embryo development, yielding a seedling; or simply the return of mature seed embryo growth (Santos et al., 2011). Since the germination depends on the same environmental conditions, which depends on vegetative growth, water availability and oxygen, the temperature must be appropriate and should not be inhibitory substances in the soil. Despite, in many seeds germination it is impeded due to the presence of a hard out tegument or because of the presence of inhibitory substances, and often also by external factors, all of which require the dormancy state. Thus, even a viable seed can not germinate, even with all the favorable environmental conditions. Seed dormancy leads to a time delay in the germination process (Deminicis, 2009).

The most typical tropical legumes have a high percentage of hard seeds, or seeds that do not germinate after sowing. As evidenced percentage of hard seeds between 69-90%. This dormancy is in reason to the presence of a waterproof cover for water penetration, preventing the germination to a certain extent, so that some seeds to germinate in each period and contribute to ensure the survival of the species (Bewley & Black, 1994). Recent studies show that manipulations can improve the breaking of dormancy as the case of seed coat rupture to increase the water permeability, which can induce an increased sensitivity to light and temperature, permeability to gases, removal inhibitors, and influences the metabolism of the seeds and thus the dormancy (Kumar et al., 2015).

The treatments for dormancy breaking tegumentary seed were efficient because they promoted the rupture of the impermeable layer in the tegument for T1, thus, enhancing the water absorption by the seeds and speeding up the germination process. The treatment of immersion in water for 24 hours is one of the cheapest techniques, with the main objective anticipate germination breaking dormancy, but is only effective when water enters quickly in the tegument. The technique of hot water to break dormancy is very simple to do, but the results are mixed for most legumes (Nascimento & Oliveira, 1999). In this study treatment T1 was the most efficient. Despite Teles et al. (2000) water at 80 °C for 5 min was efficient to break seed dormancy and did not influence the germination and vigor of leucaena seeds.

The use of hot water at 60 °C or 80 °C associated with mechanical scarification did not affect the germination of the leucaena seeds (Paulino et al., 2004). Nevertheless, Gonçalves et al. (2011) found different results for breaking dormancy of a leguminous tree, treatment with water at 100 °C had the worst result to compare with this experiment.

Second Amritphale et al. (1993), acetone can be effective to break the dormancy the legumes. However, it was not sufficiently accurate data to improve the germination of seeds leucaena. This response may vary depending on the hardness of the cover legume seed coat and the time of contact with the reagent.

4. Conclusion

Leucaena seeds treated with water at 100 °C/10 min before cultivation is enough for ensure germination.

Leucaena seeds without any treatment has germination hampered by to the delay in germination and natural seed dormancy.

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