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Treatments for breaking dormancy of the crotalaria seeds (*Crotalaria ochroleuca*)

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The objective of the study was to evaluate the influence of methods in breaking dormancy in the seeds of tropical forage legume *Crotalaria ochroleuca*. The seed treatments were: (T1) water at 100ºC/10 min; (T2) immersed in water for 24 h at ambient temperature, after immersion in water at 100°C/1 min; (T3) acetone (10 min); (T4) ethyl alcohol (10 min); and (T5) intact seeds = control treatment. Data were analyzed using a completely randomized design with two replications, and was adopted for the Tukey test at 5% significance level. Seeds immersed in water for 24 h at ambient temperature before cultivation is enough to ensure germination. The treatments T1, T2 and T5 had the highest speed germination of seeds GSI, which differed (P<0.05) from T3 and T4. The T3 treatment was the only one with difference (P<0.05) in germination which was 15 days after sowing (Germ15). The T1 and T2 treatment had the highest amount of seed germination (P<0.05). The acetone affected the embryo in the tegument negatively.

Key words: Acetone, germination, seed physiology.

INTRODUCION

The presence of legumes in tropical grass pastures increases livestock production, due to the higher protein necessary for the development of microorganisms that digest forage (Gomes et al., 2011; Valente et al., 2015, 2016a). The consortium with tropical grasses can be an alternative for the recovery of degraded grasslands and increase in the pasture's nitrogen which consequently increases forage supplies at certain times of the year. This improves the nutritional quality of pasture, reduces the annual variation of forage supply, increases diversity of grassland beyond reduction in fertilizer usage (Carvalho and Pires, 2008; Valente et al., 2016b). The crotalaria can be used in animal nutrition, because it is a legume with good amount of crude protein (CP) in the range of 13.8 to 19.3%. It is an alternative legume that is widely used in semi-arid regions (Kallah et al., 2000). A study compared used only *Chloris gayana* hay or hay supplemented with *Crotalaria ochroleuca* on the feed intake, growth rate and feed utilization of growing sheep, and the concluded supplementation had a significant ($P < 0.01$) effect on the total and daily gain with the increasing level of crotalaria in the diet. The supplementation

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increases dry matter digestibility and the organic matter of hay with the CP digestibility about three times that of the sheep unsupplemented (Sarwatt, 1990). However, these legumes are more difficult to seed propagation in relation to forage grasses.

A limiting factor to the spread of forage legumes is the deep seed dormancy, which results in slow and uneven germination. This fact is due to the impermeability of the tegument with water, this phenomenon is considered by Popinigis (1985) as one of the most common causes of dormancy in legumes. This can be demonstrated by the low percentage of germinated seeds observed in undamaged seeds (control) (Kramer and Kozlowski, 1972; Bruno et al., 2011).

Secondly, Jayasuriya et al. (2013) seeds of legumes are generally considered to have physical dormancy, but most seed biologists are unaware of the various kinds and combinations of dormancy and storage behaviour in seeds of this family.

To breaking dormancy, various methods have been reported and the most common 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. The treatment with sulfuric acid was done with a highly corrosive product and the tests show that it is capable of scarifying seeds of most legumes. However, the use of sulfuric acid to break dormancy is not common practice, since its high corrosive power require special care during handling, due to the danger, and can only be realized in a laboratory setting (Deminicis, 2005).

The objective of the study is to evaluate the influence of methods for breaking dormancy in the seeds of tropical forage legume *C. ochroleuca*.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of IFGoiano Campus Posse GO - Brazil. The crotalaria seeds (*C. ochroleuca*) were tested for different dormancy breaking methods.

The seeds were collected from the Experimental Station of the Campus Posse, and the seeds after the harvest were selected for the germination tests. The pods with legume seeds were harvested manually during the first hours of the day. The pods were dried in the plant and no chemical product was used to make the drying. The seeds were not stored for any period of time. Seeds with or without the pre-germination treatments were germinated at a constant temperature of 25ºC. The germination test was installed on two sheets of paper moistened with distilled water, the amount equivalent to 2.5 times its dry weight in transparent plastic box 11×11×3 cm, with cover, 2 repetitions of 20 seeds and eight hours photoperiod. It was used as germination chamber for germinating the seed of solab® brand installed in the dependence of IFGoiano Campus Posse-GO, Brazil.

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

Step 1: Germination count 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 fifteenth day after the test (Germ15).
Step 3: Germination speed index (GSI), which was calculated with the formula proposed Maguire (1962):

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \frac{Gn}{Nn}$$

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

Step 4: Total count of seeds do not germinate after 15 days (NGerm).

The seed treatments were: (T1) Water at 100°C/10 min; (T2) immersed in water for 24 h at ambient temperature, after immersion in water at 100°C/1 min; (T3) acetone 40% (10 min); (T4) ethyl alcohol 90% (10 min); and (T5) intact seeds = control treatment.

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

Statistical procedures and model evaluation

Data were analyzed using a completely randomized design with two replications 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 and Azevedo, 2009). Means were compared by 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.

RESULTS AND DISCUSSION

Germination count represents the cumulative percentage of germinated seeds on the third day after the start of the test (Germ3). Within 3 days, 100% of the seeds germinated in T1 and T2, while T5 had 90% germination differs ($P < 0.05$) and T3 to T4 treatment were respectively 65 and 40% germination (Table 1). The faster the seed germinates the better the chance of the seed to prosper (Deminicis, 2009). The speed and uniformity of seedling emergence depend 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, seeds treated with acetone or alcohol had the germination rate affected for three days (Germ3).

The T3 treatment was the only one with difference ($P < 0.05$) in germination after 15 days of sowing (Germ15). Nasreen et al. (2015) opine that breaking of dormancy is effective when using 25% acetone in sunflower seeds. It differs from this experiment which had the acetone concentration at 40% higher. The T3 treatment had

Table 1. Average values for seeds germination of crotalaria (*Crotalaria ochroleuca*), Germ3, Germ15, GSI and NGerm after different treatments for breaking dormancy.

Treatment	Germ3	Germ15	GSI	NGerm
T1 = Water at 100ºC/10 min	100[a]	100[a]	0.99[a]	-
T2 = Immersed in water for 24 h at ambient temperature, after immersion in water at 100°C/1 min	100[a]	100[a]	1.02[a]	-
T3= Acetone (10 min)	40[b]	50[b]	0.63[b]	50[b]
T4 = Ethyl alcohol (10 minutes)	65[b]	85[a]	0.73[b]	15[a]
T5 = Intact seeds	90[a]	90[a]	0.96[a]	10[a]
F test	10.461*	17.000*	22.983*	17.000*
P-value	0.012	0.004	0.002	0.004
Coefficient of Variation %	14.43	8.32	6.23	47.14

*Significant to 1%. Different letters in line indicate significant difference according to Tukey test (P<0.05).

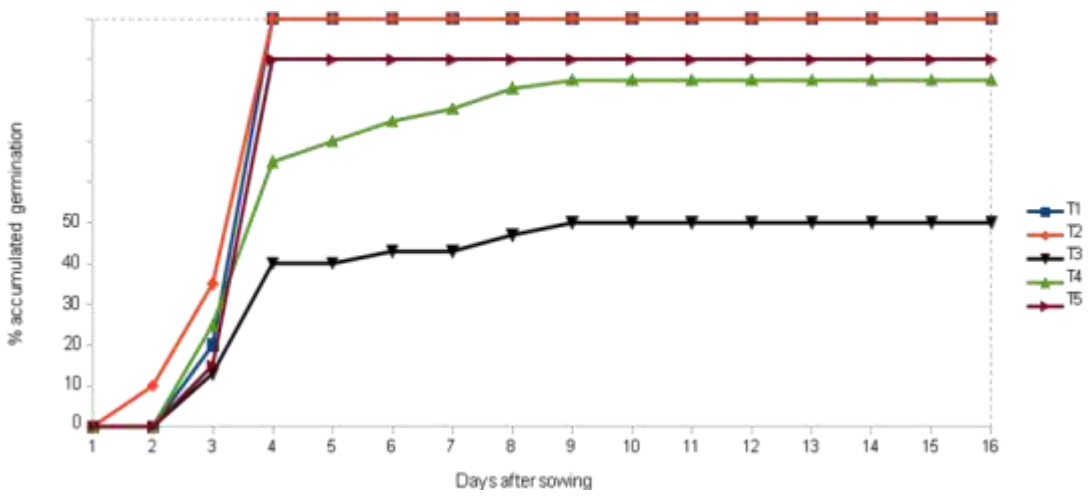

Figure 1. % accumulated germination in all treatments.

decreased germination (P <0.05) and the acetone negatively affected the embryo in the tegument.

The treatment T1, T2 and T5 had the highest speed germination for seeds GSI which differed (P <0.05) between T3 and T4. Treatment T2 had the best result, 100%, of the seeds germinated with a better GSI. The treatments for breaking dormancy in tegumentary seed were efficient because they promoted the rupture of the impermeable layer in the tegument for T1 and T2, thus, enhancing the water absorption by seed and the germination process. The T2 for having been immersed in water for 24 h at ambient temperature had advantage at GSI. Gonçalves et al. (2011) found different results for breaking the dormancy of a leguminous tree, treatment with water at 100°C had the worst result to compare with this experiment. In Figure 1, the representation of % germination during the trial period.

The treatment of immersion in water for 24 h 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 (Seiffert, 1982). 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 transform the seed from a relatively inert structure other active growth; an ordered sequence of metabolic events that results in the restart of embryo development, yielding a seedling; or simply the return of mature seed embryo growth (Santos et al., 2011; Nascimento and Oliveira, 1999). Since the germination dependent on the same environmental conditions depends on vegetative growth, water availability and oxygen, the temperature must be appropriate and should not be inhibitory substances in the soil (Smith, 2013). However, many seed germination is impeded due to the presence of a hard out tegument or due to the presence of inhibitory substances, and often by external factors, all of which require the dormancy state. Thus, even a viable seed cannot germinate, even

with all the favorable environmental conditions. Seed dormancy leads to a time delay in the germination process (Deminicis, 2009).

The T3 treatment had 50% of non-germinated seeds (NGerm). There was no difference between the T1, T2, T4 and T5 treatments (P>0.05). Acetone can be effective to break the dormancy (Amritphale et al., 1993). However, the level of 40% was detrimental to germination *C. ochroleuca*, this response is variable depending on the hardness of the cover legume seed coat.

The most tropical legume has a high percentage of hard seeds, or seeds which do not germinate after sowing. The evidenced percentage of hard seeds is between 69 and 90%. The response to the germination of 90% of the control treatment (T5) is due to the fact that the seeds are used soon after the harvest. In this way, they were easier to germinate due to the lower maturity of the seed, consequently lower hardness of the tegument protection cover. This dormancy is due to the presence of a waterproof cover for water penetration, preventing the germination to a certain extent, so that some seeds germinate in each period and contribute to ensure the survival of the species (Bewley and Black, 1994). Recent studies show that manipulations can improve the breaking of dormancy as the case of rupture of the tegument to increase the water permeability, 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 (Mayer and Poljakoff-Mayber, 1989, Kumar et al., 2015).

Conclusion

Seeds immersed in water for 24 h at ambient temperature, after immersion in water at 100°C/1 min before cultivation is enough to ensure germination. Acetone decreases germination and the seeds negatively affect the embryo in the tegument.

Conflict of Interests

The authors have not declared any conflict of interests.

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