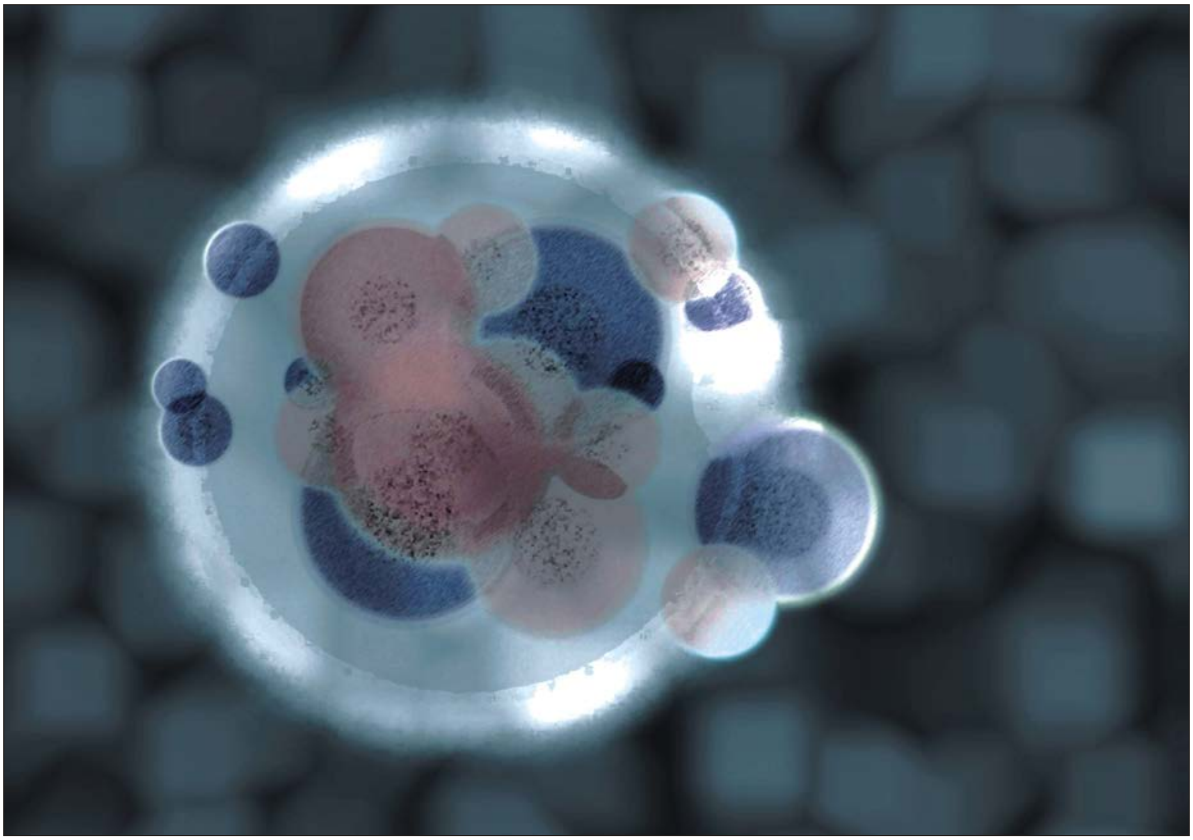

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 10 (6): 541-544, 2010
ISSN 1727-3048

Effects of Scarification Chemical Treatments on the Germination of *Crotalaria retusa* L. Seeds

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Abstract: This study was conducted to determine the effectiveness of scarification treatments in the germination of *Crotalaria retusa* to propose an effective and practical method for breaking seed dormancy. Seeds were collected on December 2009 from disturbed forests in coastal dunes and tested with different times of exposure (0, 5, 10, 20 and 30 min) to sulfuric acid immersion on February 2010, assays were done in the Seed laboratory of Natural Sciences Faculty a day/night regime of 32/18°C was taken into consideration along with a 12 h photoperiod. The germination was observed daily early in the morning for a period 30 days. All the treatments initiated their germination three days after the start of the experiment and reached their maximum value between the days twenty-six and twenty-nine. Seeds treated with sulphuric acid, responded positively in all cases but, the immersion in acid for thirty or twenty minutes were the treatment that gave the best results, for purposes of re-colonization in forest areas is recommended the use of these treatment.

Key words: Sprout, scratches, legumes, acid, pretreatments

INTRODUCTION

Crotalaria L. is a large genus comprising of more than 600 species scattered all over the tropics and sub-tropics (Bhatt *et al*., 2009). This genus shows largest species diversity in tropical Africa followed by Southeast Asia and Central America (Pandey *et al*., 2010). It is not known the number of species for Mexico. For the Campeche state there are two species, *Crotalaria mucronata* and *Crotalaria retusa*, both growing in areas degraded by the erosion in infertile grounds and of bad quality in coastal dunes vegetation characterized by a mosaic of habitats in which environmental factors changes (Auda, 2010). Dune habitats are formed by the sandy beach, embryo dunes and foredunes in which sand movement is the dominating environmental factor (Labuz and Grunewald, 2007).

The function of the seed coat is to protect the embryo and endosperm from desiccation, mechanical injury, unfavorable temperatures and attacks by bacteria, fungi and insects (Ko *et al*., 2004). Similarly to many other taxa of legumes, the seeds of *Crotalaria* plants have a low and irregular germination which is attributed principally to the impermeability of the seeds to water (Jurado and Flores, 2005; Tauro *et al*., 2009). These seeds remain in a state of physical dormancy until the seed coat is made permeable by some environmental factors in natural conditions (Wen *et al*., 2009). Many research have been done by testing methods to overcome the physical dormancy of *Crotalaria* seeds (Scott *et al*., 2010).

For most species with physical dormancy, previous research has largely been limited to describing the initial level of physical dormancy, subsequent release of seed dormancy and effect of artificial procedures such as acid scarification and boiling water, rather than examining mechanisms that are likely to occur naturally (Baskin *et al*., 2006). Generally, the mechanism and environmental factors contributed to physical dormancy release under natural conditions are poorly understood (Baskin *et al*., 2000).

Seed dormancy can be caused by embryo immaturity or poor of water and gas exchange through the seed coat. In the latter case, such as with *C. retusa*, dormancy can be broken by treating those hard coverings.

In the wild, physical dormancy release could be not only the effects of the gastric juice of birds and rodents that eat the seeds, but it could also be influenced by the partial chewing and high temperatures and humidity to which the seeds are exposed when are in contact to feces (Baskin and Baskin, 2004; Ness and Bressmer, 2005).

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Other studies related with germination deal with large numbers of species with dormant seeds and classification of dormancy with respect to phylogeny, physiology, anatomy and biogeography (Baskin and Baskin, 2004).

Physical dormancy exists in the seeds of *C. retusa* as in many legumes, however its germination can be improved after the application of pregerminative treatments by softening the seeds coat. The potential use of these plants like enhancers of the fertility of grounds (Alderete-Chavez *et al*., 2009; Oenema *et al*., 2004) and the need to know the environment conditions of growth, justify the search of methods to improve the germination of its seeds and to achieve the efficient propagation of the species in the field.

Taking in consideration the preceding, the objective of this research was to investigate the effect of scarification with sulphuric acid on the germination potential of *Crotalaria retusa*, to propose an effective and practical method for breaking seed dormancy.

MATERIALS AND METHODS

The seeds of *Crotalaria retusa* were obtained from disturbed forests in coastal dunes, Municipality of Carmen, Campeche State, Mexico (18° 38' 36" N and 91° 49' 51" W), at 2 m above sea level, on slopes of 2-4%. The mature seeds were collected from 400 plants on December 2009.

The laboratory assays were done in the Seed laboratory of Natural Sciences Faculty, Universidad Autónoma del Carmen, Campeche State, Mexico, between January and February 2010. A day/night regime of 32/18°C was taken into consideration along with a 12 h photoperiod. Pregerminative treatment considered the immersion of seeds in 98% Sulphuric acid (H_2SO_4), for 0 (control), 5, 10, 20 and 30 min, after which seeds were washed with distilled water.

A total of 300 seeds (ten seeds with 6 replications for treatments) were sown in each experimental unit (petri dishes with Agrolite as substrate). The germination was observed daily early in the morning for a period 30 days. The seeds were considered germinated when the radicle reached the length of the seed. Seeds with roots or cotyledons malformed were not considered for the test. The seeds were irrigated with distilled water and a 3% Captan solution.

The experimental design used was completely randomized blocks with six repetitions. Germination was registered by replica per treatment during he duration of experiment. Germination frequencies for the same treatment were grouped and expressed as percentage. Results per treatments were analyzed through Kruskal-Wallis ANOVA by ranks and Wilcoxon Matched pairs test with significance level $p<0.05$.

Final results are shown as germination percentages per treatment considering cumulative frequency for each treatment. Excel was used for the data primary treatments and Statistica (v 8.0, Statsoft) for the rest of analysis.

RESULTS

All treatment applied were statistically significant ($p = 0.05$). The *C. retusa* radicles started to emerge two days after the establishment of the experiment. The seeds with immersion in 98% H_2SO_4 treatment responded positively in all cases compared with the control when the temperature was under 32/18°C day/night Fig. 1. When the H_2SO4 is used, germination percentage is over 64% at day 29 in the all seed treated in contrast with 35% obtained at the control Fig. 2.

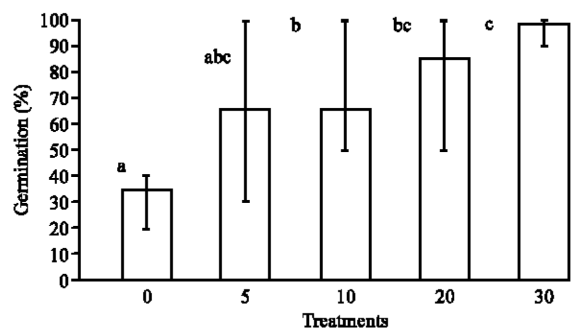

Fig. 1: Frequency of germination by chemical scarification (H_2SO_4) treatment expressed as mean percentage. Error bar denote range of germination percentage including 6 replicates. Different letters means significant difference

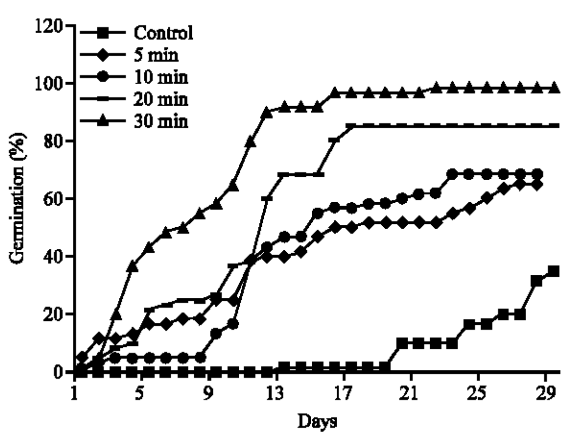

Fig. 2: Accumulated germination frequency (%) of *C. retusa* seeds with chemical scarification during 30 days of experiment

In concordance with statistical analysis, seeds exposed to immersion for 30, 20 or 5 min show similar behavior at the end of the experiment, however when the seed are treated for 30 min, the range of germination percentages by replica are very short in contrast with the other treatments and germination rate is increased, reaching more than 20% at day 3 while the same result were obtained after 9 days for the rest of the assays. The other treatments get no more than 68% of germination at the end of the assay. All treated seeds had significantly higher germination percentages than the control which only obtained 35% of germination at the end of the assays (Fig. 2).

The trend observed at different treatments shown that conditions proposed for the experiments are adequate for germination and the application of 98% H_2SO_4 for 30 and 20 min, significantly increased the germination of *C. retusa*.

DISCUSSION

The seeds of *Crotalaria* obtained from a natural environment and the H_2SO_4 scarification treatments simulated pass of the seeds through the digestive tract of animals (birds and rodents) (Chavez *et al*., 2010; Righini *et al*., 2004), which under natural conditions execute chemical scarification (Carpinellia *et al*., 2005).

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate (Finch-Savage and Leubner-Metzger, 2006). Otherwise, characteristics of seeds as coat thickness and coat hardness, time and conditions where they are stored and treatment previously offered are involved at sprouting.

Effect of dormancy breaking method on germination rate depends on the plant species, thus it can be suggested that the most effective method in breaking the dormancy of forage legumes should be determined (Balouchi and Sanavy, 2006) by the application or each method with different period, concentration or degree combinations to the seeds in order to maximum germination rate. Our results demonstrate that H_2SO_4 treating were able to enhance germination because seed dormancy in *C. retusa* is reduced when the acid act over the seeds coat improving germination percentage.

Similar trends were found by Acosta-Percastegui and Rodriguez-Trejo (2005) in *L. montanus,* who reported higher percentage of germination with chemical scarification (Sulphuric acid) for 15 min at temperatures of 20/15°C with light obtaining 100% germination. Chavez *et al.* (2010), reported higher percentage of germination with chemical scarification (Sulphuric acid) for 15 min at temperatures of 25/15°C with light (82.3% of germination) in *L. leptophyllus*. Chemical germination was found in seeds of *Calystegia soldanella* pretreated with H_2SO_4 for 3 h (Ko *et al*., 2004). Chemical applications also improve germination in such species as S. obtusifolia. A positive effect of H_2SO_4 are seen when the seeds were pretreatments with reaching about 90% germination. (Baskin *et al*., 1998). Applications also improve germination in such species as *Capparis spinosa,* where positive effects are seen with pretreatments of either H_2SO_4 for 20 (Sozzi and Chiesa, 1995).

From the results of this research that simulates the effects of the pass throughout animal digestive tract in germination and from the literature reviewed it is inferred that *C. retusa* have about 35% germinative efficiency in natural conditions (without treatment) and when the seeds are consumed by rodents and other animals, the digestion process helps scarification and defecation the dispersion of the seeds throughout the ecosystem.

The positive response to sulfuric acid treatments indicates that the impermeable coat is responsible for a low germination rates from intact seeds as seen at experimental untreated controls. By overcoming the physical dormancy with chemical pretreatment, seed coats are softened and water uptake is enabled. Both actions are crucial to sustaining the life cycle of hard-seeded species.

CONCLUSIONS

Germination was affected by all H_2SO_4 scarification treatments. The treatment of immersion in sulphuric acid for 30 min gave 98.3% of germination after 30 days seeds were sown but germination rate was the highest during the two first weeks of experiment. For purposes of re-colonization in forest areas is recommended the use of these treatments.

ACKNOWLEDGMENT

Special thanks to PROMEP granting the internal project number PROMEP/103.5/09/4162, without who's financing the development of this research would not be possible.

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