International Journal of Botany

ISSN: 1811-9700

International Journal of Botany 6 (1): 64-68, 2010
ISSN 1811-9700

Effects of Different Scarification Treatments on the Germination of *Lupinus leptophyllus* Seeds

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Abstract: This study was conducted to determine the effectiveness of scarification treatments in the germination of *Lupinus leptophyllus* Schlecht and Cham; seeds were tested with different times of exposure to sulfuric acid immersion and heat. All the treatments induced germination two days after the start of the experiment and reached their maximum value between the days twenty-seven and twenty-nine, almost at the end of the experiment. Seeds treated with sulphuric acid, responded positively in all cases, the immersion in acid for fifteen minutes was the treatment that gave the best results. The treatment with heat at 80°C had lower germination than the control. However, in all the other cases, the seeds responded positively and at temperatures of 140 and 110°C for 2 min the best results were obtained.

Key words: *Lupinus leptophyllus*, seeds, chemical scarification, heat

INTRODUCTION

Lupinus leptophyllus Schlecht and Cham, is a short lived annual plant with erect or ramified stems and a solid pith. It is more than 1 m in height, has 5 to 12 mm long purple stipules and 2 to 8 cm long petioles and 9-15 mm wide with an acute apex. The flowers are purple with whitish wings and scattered with a feathery apex that disappears with age. The fruits are hairy pods 4-5 cm long and have 8-9 mm mature seeds per pod. Blooming starts in the last days of January and the first mature seeds (dark to dark brown) appear in March. It can be found in agricultural lands from 2981 to 3179 meters above sea level, slopes of 2-30%, in disturbed *Abies religiosa* H.B.K. forests and cultivated plots in the Northeast of the state of Mexico (Alderete *et al*., 2008; Calderón and Rzedowski, 2005).

Similarly to many other taxa of legumes, the seeds of *Lupinus leptophyllus* have a low and irregular germination which is attributed principally to the impermeability of the seeds to water (Jurado and Flores, 2005). Diverse research has been done by testing methods to overcome the physical dormancy of *Lupinus* seeds.

The general process of germination has been more documented for legumes, the seeds of these plants present physical dormancy and after being consumed and released in the feces of animals they may germinate (Olesen and Valido, 2003; Schauer *et al*., 2004). However, these results could be due not only to the effects of the gastric juices, but also to the partial chewing, high temperatures and humidity to which the seeds are exposed when they make contact with feces (Baskin and Baskin, 2004). Furthermore, rodents can collaborate in seed dispersion (Jones and Longland, 1999), by depositing them in micro sites subjected to disturbance and high temperatures like those used in this experiment (25/15°C day/night).

The seeds of *L. leptophyllus* were obtained from a natural environment and the H_2SO_4 scarification treatments simulated pass of the seeds through the digestive tract of animals (Righini *et al*., 2004; Godínez-Alvarez and Valiente-Banuet, 2000), which under natural conditions execute chemical scarification (Carpinellia *et al*., 2005).

In natural forest conditions seeding species tend to produce a greater proportion of viable seeds (Ooi *et al*., 2007), than species which are capable of reproducing following fire. However, fire could act as a scarification agent for several species of *Lupinus* and temperature is an important factor for germination in these conditions.

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The heat treatments are associated with forest fires at different levels of intensity, indicating that forest fires are one of the most important ecosystem disturbances. After fire, the open environments are covered with ash, which could affect both germination and seedling development (Reyes and Casal, 2004; Rodriguez and Fulé, 2003) and during fire seeds are subjected to heat that weakens the seed coat, this is, causes a thermo scarification.

The exposure of seeds to dark conditions, light, red light and temperature for germination was analyzed by Hoffmann (1999). Seed size has been studied in connection with many other aspects of plant biology (Guo *et al*., 2000). It has been predicted that species with lighter seeds will be more likely to have some form of dormancy and empirical data support this evidence in most cases (Carter and Ungar, 2003), but not in all environments.

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 *L. leptophyllus* 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.

Therefore, the objective of this research was to investigate the effect of temperature, scarification with sulphuric acid on the germination potential of the specie and its improvement in germination.

MATERIALS AND METHODS

The seeds were obtained from the Tláloc Mountain in the Nevada mountain range, Municipality of Texcoco, Mexico State, at 2891 meters above sea level, on slopes of 2-30%, 19° 26' 43.8" N and 98° 46' 16.8" W, in disturbed *Abies religiosa* forests. The mature seeds were collected from 100 plants in a colony which occupied and area of approximately 1000 m^2.

The germination was observed daily for 30 days. The seeds were considered germinated when the radicle reached the length of the seed. Seeds with malformed roots or cotyledons were not considered for the test. The seeds were irrigated with distilled water and a 3% captan solution.

The laboratory work was done in the Forest Seeds laboratory of the Forest Sciences Division, Chapingo Autonomous University, Mexico, between September and October of 2007. A day/night regime of 25/15°C was taken into consideration along with a 12 h photoperiod. Also two pregerminative treatments were considered: 1) the immersion of seeds in 98% sulphuric acid (H_2SO_4), for: 0 (control), 7, 15 and 30 min, then later washing them with distilled water; 2) Heat treatment at; room temperature 18°C (control), 80, 110 and 140°C at different exposure times of 0 (control), 1, 2 and 5 min.

Ten seeds were sown in each experimental unit (Petri dishes with agrolite as substrate), with 6 repetitions for each combination of treatments. The experiment was conducted in a controlled environment chamber equipped with fluorescent light bulbs.

The experimental design used was completely randomized blocks with six repetitions. The SAS (2003) program (v. 2003) for microcomputers, was employed to conduct the ANOVA tests (Proc Mixed) and mean comparisons (significant minimum difference), also with the SAS program. The treatments were considered as fixed effects, the blocks as random effects.

RESULTS

All treatments applied were statistically significant ($p = 0.05$). The *L. leptophyllus* 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 when the temperature was under 25/15°C day/night (Fig. 1).

The treatment using a 15 min immersion had the best results with a germination of 82.3%, followed by the treatments 30 and 7 min, with 55.7 and 54% of germination,

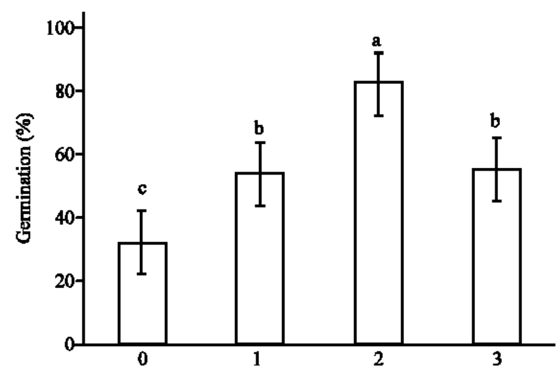

Fig. 1: Benchmark tests of averages and significant minimum differences in seeds of *Lupinus leptophyllus* put under treatments of chemical scarification (H_2SO_4) to different exposure times 0: Control, 1: immersion by 7′, 2: Immersion by 15′, 3: Inmersion by 30′. Different letters shows significant difference

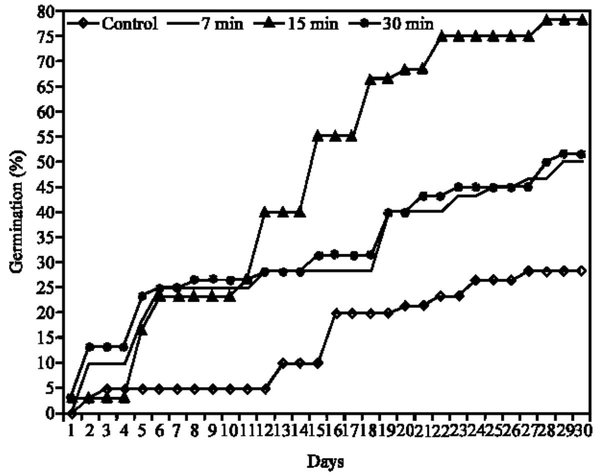

Fig. 2: Accumulated germination of *L. leptophyllus* seeds with chemical scarification under laboratory conditions

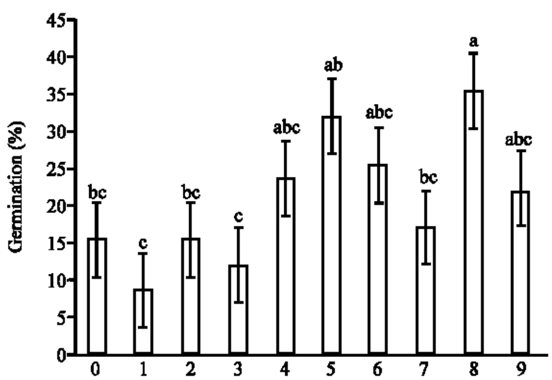

Fig. 3: Benchmark tests of averages and significant minimum differences in seeds of *Lupinus leptophyllus* put under treatments of scarification by application of heat, different exposure times 0: Control (room temperature 18 °C), 1: 80°C-1′, 2: 80°C-2′, 3: 80°C-5′, 4: 110 °C-1′, 5: 110°C-2′, 6: 110°C-5′, 7: 140°C-1′, 8: 140°C-2′, 9: 140°C-5′. Different letters shows significant difference

respectively. Those percentages of germination where higher than the control, that had 32.3% of germination (Fig. 2).

The seeds subjected to temperature treatments at 80, 110 and 140°C during one, two and 5 min, presented the following results. At 80°C all the treatments did not responded positively with results poorer than the control treatment (room temperature 18°C), so this treatment seems not to be sufficient to stimulate the germination (Fig. 3).

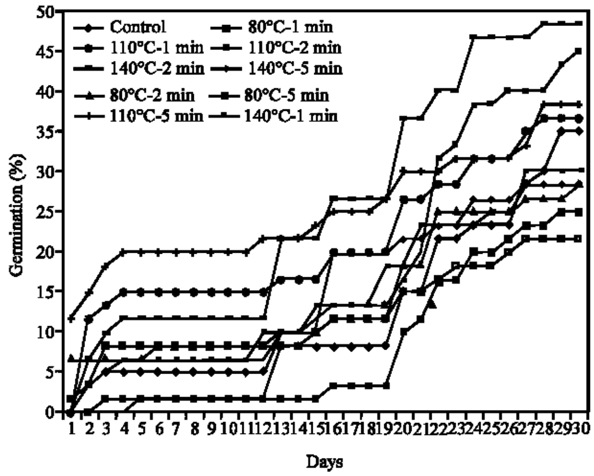

Fig. 4: Accumulated germination of *L. leptophyllus* seeds with different heat treatments and exposure times

All the other treatments were efficient, but the best ones were treatments at 140 and 110°C with two minutes of exposure, that had the highest percentage of germination with 35.4 and 32%, respectively. Those percentages of germination where higher than the control, that had 15.4% of germination (Fig. 4).

The trends observed show that the day/night temperature regime of 25/15°C is adequate for germination and the application of 98% H_2SO_4 for 15 min, or the exposition to heat (110 or 140°C) for two minutes, increased the germination of *L. leptophyllus*. For the manager of a forest nursery we recommend the use of the chemical scarification.

DISCUSSION

Seed physical dormancy is associated with a hard seed coat. Present results indicated that the treatment of physical scarification with heat at different degrees and expositions and chemical scarification with sulfuric acid at different immersion timings generally increases the percentage of germination in relation to the seeds without it, therefore the evaluated treatments increase the germination of the seeds of *Lupinus leptophyllus*; similar trends were found by Acosta-Percástegui and Rodríguez-Trejo (2005) in *L. montanus,* who reported higher percentage of germination at temperatures of 20/15°C with chemical scarification (Sulphuric acid) for 15 min, with light (100% germination). But the treatments with heat are only efficient and increase the germination of *Lupinus* to temperatures higher than 110°C. From the ecological point of view it is confirmed that the heat due to the fire, only aids the germination of the seeds if it is this intense, these results exhibit similar trends to previous reports by

Briggs and Morris (2008) in seeds of *Grevillea linearifolia* and Travlos and Karamanos (2007) in seeds of *Vitex agnus castus* L.

In other cases is not the fire the scarification factor, but the nitrogen oxide contained in the smoke of the fire, like in the annual plant *Emmenanthe penduliflora*, that grows in the Californian chaparral (Keeley and Fotheringham, 1997, 1998).

CONCLUSIONS

Germination was affected by temperature and other scarification treatments, in a day/night temperature regime of 25/15°C and a 12 h photoperiod. The treatment of immersion in sulphuric acid for 15 min gave the best results with 82.3% of germination. The heat treatments with 140°C and 110°C for two minutes gave the best results with 35.4 and 32% germination. For purposes of re-colonization in forest areas after fire is recommended the use of these treatments for the scarification of seeds.

From the results of this research that simulates the conditions of forest fire and animal digestive tract in germination and from the literature review it is inferred that *Lupinus leptophyllus* have a higher germinative efficiency in natural conditions, were the seeds are consumed by rodents and other agents and the digestion process helps scarification and the animals the dispersion of the seeds throughout all the ecosystem.

On the other hand, a favorable response to temperature observed in the treatments with heat suggests that germination could be favored by the occurrence of forest fires, that promotes more efficient germination allowing this lupines species to be the firth species to emerge as secondary vegetation with a reduced competency of other species, this produce a more rapid colonization reducing the risk of erosion.

ACKNOWLEDGMENTS

Special thanks to CONACYT for granting the scholarship number 177441, to the improvement program and to the faculty for granting the scholarship for high quality studies number PROMEP/103.5/06/1973, without who's financing the development of this research would not be possible.

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