https://doi.org/ 10.32854/agrop.v14i2.1966

Scarification treatments in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.) used to improve their germination

Rojas-García Adelaido R.[1], Maldonado-Peralta María de los Á.[1*], Sánchez-Santillán Paulino[1], Ayala-Monter Marco A.[1], Álvarez-Vázquez Perpetuo[2], Ramírez-Reynoso Omar[3]

[1]Universidad Autónoma de Guerrero, Facultad de Medicina Veterinaria y Zootecnia N. 2, Cuajinicuilapa, Guerrero, México. [2]Universidad Autónoma Agraria Antonio Narro, Departamento de Recursos Naturales, Saltillo, Coahuila, México. [3]Universidad Autónoma de Guerrero, Centro Regional de Educación Superior de la Costa Chica. Cruz Grande, Guerrero.
***Corresponding Author**: mmaldonado@uagro.mx

ABSTRACT

Objective: The objective was to evaluate different scarification treatments to improve germination in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.).

Design/Methodology/Approach: The study was established in the School of Veterinary Medicine and Zootechnics N. 2 of the Universidad Autónoma de Guerrero. The chepil seeds were weighed and counted; 2 experiments were established through a CRD with 4 treatments of 4 repetitions each. Imbibition and germination were evaluated. The data were analyzed with the statistical software package SAS® 9.0.

Results: The use of water at different temperatures and times presented positive results in imbibition and increased the germination percentages. The treatment with water at 100 °C until cooling reached a germination of 80%, and the control of 12.3%.

Study Limitations/Implications: Chepil is a wild species that has seeds with physical dormancy, which is something that requires more research in order to accelerate and increase the germination percentages.

Findings/Conclusions: The imbibition and germination was affected by the treatments applied. Chepil seeds presented physical or superficial dormancy that may be eliminated with the use of heat treatments; however, evaluations still need to be performed to accelerate and find a higher percentage of germination.

Keywords: legume, emergence, nutrition, quality.

INTRODUCTION

Endemic or vulnerable species must be conserved, and for this purpose, it is necessary to deepen knowledge and seek adequate techniques for their multiplication (Camarillo-Castillo and Mangan, 2020; Zapata *et al*., 2017). The germplasm of small or shrub species that develop in the tropics is strongly affected by environmental conditions and factors such as felling, overgrazing, burning, etc., which increases the possibility of extinction (Javed *et al*., 2013; Solh and van Ginkel, 2014); therefore, their conservation is necessary.

Agroproductividad: *Vol. 14, Núm. 2, febrero. 2021. pp: 67-72.*
Recibido: *agosto, 2020.* **Aceptado:** *febrero, 2021.*

Chepil, chipil or chipilín (*Crotalaria longirostrata* Hook. & Arn.) is a legume of the Fabaceae family, originally from southern Mexico and Central America; it is used for both human and animal diets (Arias *et al*., 2003). Species of the *Crotalaria* genus develop throughout the year, in tropical and subtropical zones (Casimiro *et al*., 2013; Gómez Sosa, 2000). Chepil is a wild plant that contains a large amount of protein (Laguna, 2016), with functional properties for animal nutrition; however, there is scarce bibliographical information recorded about its agronomic behavior, other than its leaves substituting the scarcity of grasses during the drought season (Sanabria *et al*., 2003).

On the other hand, huaje (river tamarind, *Leucaena leucocephala*), parota (guacanaste, *Enterolobium cyclocarpum*), carob (*Ceratonia siliqua*), chepil (*C. longirostrata*), crotalaria (*C. Juncea* L.), etc., are very important alternatives for shepherding communities, because they improve the quality of the diet and contribute nitrogen to the soil (Boschi *et al*., 2016; Sevillal and Fernández, 1991), whether cultivated as sources of protein or in associations (Benítez-Bahena *et al*., 2010; Castillo *et al*., 1993). To achieve the implantation of a fodder legume species, it does not only depend on management practices, but rather on the form of propagation and the availability of genetic material (botanical or vegetative seed); in addition, the hardness of the seed depends on the species (Zimmermann *et al*., 2003), since the seeds do not always generate a plant quickly and in some cases they take up to four months or more to germinate (Sevillal and Fernández, 1991).

Legumes are propagated primarily by seed, which in some cases presents viability; however, the so-called dormancy makes the germination percentages small, because even when there are favorable conditions, they are incapable of absorbing and emerging (Doria, 2010; Sanabria *et al*., 2003). Latency is a mechanism for defense and survival; it can be reduced by using mechanical, physical or chemical scarification, which allows accelerating the process of germination (Kimura and Islam, 2012). Studies carried out about germination of legume seeds show that physical scarification is positive when germination is minimal (Juárez and Lagunes, 2014; Sánchez-Paz and Ramírez-Villalobos, 2006; Villagra *et al*., 2004); in addition, the use of treatments with hot water is effective for some species (Navarro *et al*., 2010; Zapata *et al*., 2017). The hypothesis of this study is that chepil seeds present dormancy, which must be overcome with some pre-germination treatment. Therefore, the objective of this study was to evaluate different scarification treatments to improve germination in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.), in the dry tropics.

MATERIALS AND METHODS

The study was carried out in the fodder area of the School of Veterinary Medicine and Zootechnics N. 2, located in Cuajinicuilapa, Guerrero, Mexico, at 16° 28' 28'' LN and 98° 25' 11.27'' LW, at an altitude of 46 m; this is a zone with dry tropical climate and summer rains, average precipitation of 1,129 mm, which is distributed in the months of June to October and mean annual temperature of 28.4 °C (García, 2004).

The experiment was established in January, 2020. Prior to this, dry chepil fruits (*Crotalaria longirostrata* Hook. & Arn.) were harvested, the pericarps opened, and seeds were obtained; they were left under the shade at room temperature for 3 days and conserved in a closed glass container at 4 °C, for 15 days.

The seeds were counted and weighed in an electric-analytical scale (Scientech® ZSA 120), and 4 treatments were carried out with 4 repetitions of 250 each, using a Completely Random Design (CRD). Each repetition was placed in a cloth (tulle) bag, for treatments: 1) the bags were submerged in water at 100 °C until cooling; 2) they were submerged in boiling water (100 °C) for one minute, and after that time they were dried and placed in water at room temperature (cold) for 3h (during which time all the treatments were at room temperature); 3) consisted in placing the seeds in water at 70 °C until reaching room temperature; and 4) control treatment that consisted in intact seeds. All the treatments were carried out by duplicate, using one for imbibition and the other for germination.

For imbibition, each repetition was placed on cotton inside Petri dishes, they were moistened with 25 ml distilled water, without flooding, covered and placed on a table at room temperature. The seeds were weighed at 5, 12, 24, 36 and 48 h, moment when the emergence of the radicle began, the number of emerged seeds was counted. The imbibition rate was calculated by subtracting the initial weight (g) from the final weight (ISTA, 2003).

For the germination, 4 l plastic trays were used, they were labeled and 3 l of a mixture of sand, peat and soil (1:1:1 v/v/v) were added, they were watered to field capacity. The seeds from each repetition were dispersed homogeneously according to the treatment established, and they were covered with 0.5 cm of the mixture used. Irrigation was applied every 2 days, to field capacity, during the whole cycle of the experiment. The variables evaluated were the weight of 1, 100 and 1,000 seeds, percentage of germination $\left(PG = \frac{NTSG \times 100}{NTSS}\right)$ which consisted in the rate of the total number of germinated seeds (NTSG) divided by those sown (NTSS), and the speed of germination $\left(VG = \sum(ni/t)\right)$ with *ni* being the number of germinated seeds on day *i* in relation to the time (*t*) of germination, performed from the moment when the first plant emerged and, later, counted every 3 and 5 days.

For the analysis, the percentages of the data were transformed with the function ARCSIN $\sqrt{X/100}$, then they were subjected to analysis of variance (ANOVA) and means comparison (Tukey α=0.05), using the statistical package SAS® 9.0 (SAS, 2009). The graphs were made with the Microsoft Excel 2010® software.

RESULTS AND DISCUSSION

The different treatments applied to the chepil seeds showed significant differences (α=0.05), in the imbibition rate and the number of emerged seeds (Figure 1). It was found that the control treatment presented lower rates of absorption, remaining minimal; this is considering that it was constant during the 48 h of evaluation, but without exceeding or equaling the other treatments.The emergence of the radicle began in some seeds during this time; however, the control presented 98.7% of intact seeds and the use of water at 100 °C until 81.4% cooling, finding at plain sight that the seeds presented physical or superficial latency. This agrees with studies performed by Ramorinoa giraloe, where they mention that the physical dormancy present in the seeds restricts the process of imbibition (Zapata *et al*., 2017).

Lastiri *et al*. (2007) evaluated the emergence and germination of chickpea (*Cicer arietinum*) and alfalfa (*Medicago sativa* L.) in saline conditions, and they found that each species absorbs differently, the result was reflected in the germinating capacity; in addition, when seeds present dormancy, they do not absorb even when the conditions are adequate (Sanabria *et al*., 2003) which agreed with this study, since the control (without heat treatment) showed low absorption rates.

On the other hand, the treatment where the seeds were submerged in water at 70 °C until cooling presented better absorption; however, it remained constant during the whole process, which shows that these treatments accelerate germination and decrease dormancy; in addition, the physical treatments in seeds increase the number of plants. Studies in different species show that the volume of the seeds increases discretely and the imbibition increases considerably (Méndez *et al*., 2008; Monroy-Vázquez *et al*., 2017; Souza-Pavia *et al*., 2006); this coincides with the present study.

The water absorbed increased according to the time of imbibition, situation that can be observed in this study; in this sense, in the treatment where water was used at 100 °C until cooling there was higher absorption. Each seed seemingly absorbed 0.01 g; however, some did not show an effect from water absorption while others drastically increased their size (they swelled) (Bonner, 1974), and therefore they presented timely emergence. The use of hot water decreased the physical latency, which depends on time because when placing the seeds for 1 min in water at 100 °C, 95.3% of them did not absorb.

This study culminated at 48 h, due to the emergence of the radicle of the seeds, in all the imbibition treatments; when using water at 100 °C until cooling, 186 (18.6%) emerged seeds were found, treatment that had the greatest effect.The others were lower, according to the treatment applied, where seeds were placed during 1 min in the water at 100 °C; the emergence was 4.7% and the control barely reached 1.3% (Bar in Figure 1).

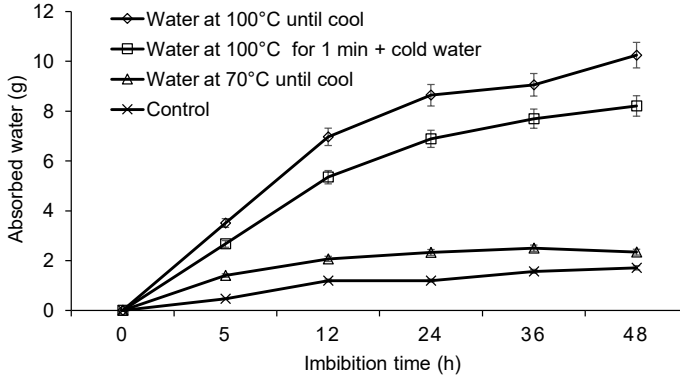

Figure 1. Water absorbed over time and onset of emergence of chepil seeds (*Crotalaria longirostrata* Hook. & Arn.), with heat treatment.

Chepil seeds are orthodox, since they are conserved and dispersed when they have low percentage of humidity (Camacho, 1994; Doria, 2010); in addition, they present physical dormancy, which prevents germination from taking place under low success conditions for development, and at the same time, this causes for seeds not to germinate in some cases, even when the conditions are adequate and favorable, because there are times when they lose their ability to emerge (Chong *et al*., 2002), situation caused by the latency they present.

The water applied in the different treatments increased the absorption and germination percentages (Table 1). At 30 days after the start of germination, the treatment with higher amount of germinated seeds was 1, with 80.0%, followed by treatment 2 with 69.2%, treatment 3 with 29.1%, and lastly, the control with 12.3%, corroborating that the species evaluated presents waterproof testa (Juárez and Lagunes, 2014); in addition, Zuloaga *et al*. (2011) mention that the heat shock at 100 °C breaks the dormancy and increases the germination percentages of the species.

The germinated plants in treatments 1, 2 and 3 showed the first true leaves, rolled, small and deformed; meanwhile, those from the control were normal, probably because subjecting them to heat treatments accelerated and improved the germination, but showed a negative effect in the initial development of the seedling.

The average weight of a chepil seed is 0.0096 g, 100 seeds weigh 0.9 to 1 g and 1,000 9.6 g, respectively (α=0.05). Dormancy was observed clearly in the control treatment (Figure 2), which presented lower germination percentages. This shows that the wild seeds of this species require scarification treatments to accelerate and increase the emergence. The control sown in trays began germination at 9 days, with 1.2%, while treatments 1, 2 and 3 began at 3 days after the experiment was established, with 12, 9.3 and 0.3 %, respectively; at 6 days, treatment 2 presented an accumulated value of 23% and 1 of 28%; at the same time, treatment 3 reached 1.9%. Studies performed by Zapata *et al*. (2017) in tree-like legumes mentioned that the use of hot water significantly improves the germination; however, when it is compared with mechanical scarification, more time is required in order to reach 70% of germination.

Table 1. Imbibition and germination of chepil (*Crotalaria longirostata* Hook. & Arn.) seeds with heat treatment.

Treatments	Imbibition (g)	Germination (%)
1 Water at 100 °C until cool	10.25 a	80.0 a
2 Water at 100 °C for 1 min + cold water	8.21 b	69.2 ab
3 water at 70 °C until cool	2.34 b	29.1 b
4 Control	1.71 b	12.3 b

Equal letters do not differ significantly from each other, according to Tukey's test (α=0.05).

It is important to mention that there were differences in the time of emergence of the radicle at the moment of absorbing water in the Petri dishes, compared to germination in the trays with a mixture of soil, sand and peat. In the imbibition of seeds from all the treatments, the emergence began at 2 days, while in the trays the onset of the germination happened at different times, at 3 days in treated seeds and in the control it extended until 9 days. This could be due to the availability of water.

CONCLUSIONS

Chepil seeds presented physical or superficial dormancy, the testa is waterproof; however, the physical treatments accelerated and increased the percentages of water absorbed and of germination.

The use of water at 100 °C until cooling showed better imbibition rates; this treatment presented

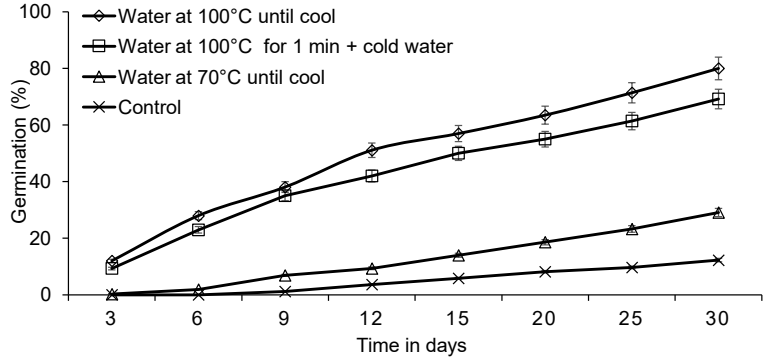

Figure 2. Germination percentages, accumulated, in chepil seeds (*Crotalaria longirostrata* Hook. & Arn.).

80.0% of germination. In addition, it began at three days in treatments 1, 2 and 3, but the first true leaf was deformed and rolled. On the other hand, in the control the emergence began at 9 days, but the plants were normal.

REFERENCES

Arias, L., Losada, H., Rendon, A., Grande, D., Vieyra, J., Soriano, R., Rivera, J., y Cortés J. (2003). Evaluation of Chipilín (*Crotalaria longirostrata*) as a forage resource for ruminant feeding in the tropical areas of Mexico. Livestock Research for Rural Development, 15 (30): 1–9.

Benítez-Bahena, Y., Bernal-Hernández, A., Cortés-Díaz, E., Vera, C. G., y Carrillo, A. F. (2010). Producción de forraje de guaje (*Leucaena* spp.) asociado con zacate (*Brachiaria brizantha*) para ovejas en pastoreo. Revista Mexicana de Ciencias Agrícolas, 1(3): 397–411.

Bonner, F. T. (1974). Seed Testing. In Seeds of woody plants in the United States, Agriculture Handbook No. 450. For. Service, USDA, Wáshington D.C.

Boschi, F., Latorre, P., Saldanha, S., Machado, J., Betancur, O., y Moure, S. (2016). Importancia de las semillas duras en leguminosas forrajeras producidas en Uruguay. Nota técnica. Agrociencia Uruguay, 20 (2): 43–50.

Camacho, F. (1994). Dormición de semillas: causas y tratamientos. México, DF: Editorial Trillas, 128 p.

Camarillo-Castillo, F., y Mangan F. X. (2020). Fijación biológica de nitrógeno en chipilín (*Crotalaria longirostrata* Hook. & Arn.), una fuente sostenible de nitrógeno para la producción comercial. Revista Chapingo, Serie Horticultura, 26 (2): 125-141.

Casimiro, D., Fechine, J. T., Ferreira dos Santos, P., Vieira-Sobral, C. M., Agra, M., Lima-Subrinho, F., Braz-Filhob, R., y Sobral da Silva, M. (2013). Structural elucidation and NMR assignments of a new pyrrolizidine alkaloid from Crotalaria vitellina Ker Gawl. Magn. Reson. Chem., 51 (1): 497– 499.

Castillo, E., Ruiz, T. E., Febles, G., Barrientos, a., Puente, R., Díaz, E., y Bernal, E. (1993). Utilización de huaje (*Leucaena leucocephala*) para la producción de carne bovina en sistemas de bancos de proteína con libre acceso. Revista Cubana de Ciencia Agrícola, 27: 39–44

Chong, C., Bible, B. B., y Hak-Yoon Ju. (2002). Germination and emergence. [online] En: M. Pessarakli (Ed.). Handbook of plant and crop physiology. 2a. ed. [online] New York: Marcel Dekker Inc. 1–11 pp.

Doria, J. (2010). Generalidades sobre las semillas: su producción, conservación y almacenamiento. Revisión bibliográfica. Cultivos Tropicales, 31 (1): 74–85.

García, E. (2004). Modificaciones al sistema de clasificación climática de Koppen. 4ed. Universidad Nacional Autónoma de México. México, D. F. 217 p.

ISTA, (International Seed Testing Association, CH). (2003). International Rules for Seed Testing. Edición 2003. Basserdorf, Switzerland. ISTA.

Gómez-Sosa, E. (2000). Las especies argentinas de Crotalaria (Leguminosae-Crotalarieae): novedades, descripciones y clave. Gayana Bot., 57 (1): 67-87

Javed, S. B., Anis, M., Khan, P. R., Aref, I. M. (2013). *In vitro* regeneration and multiplication for mass propagation of *Acacia ehrenbergiana* Hayne: a potential reclaiment of denude arid lands. Agroforestry Systems, 87(3): 621–629.

Juárez, F. B., Lagunes, E. L. E. (2014). Germinación como un proceso que mejora la calidad nutritiva de leguminosas tropicales. II. Congreso Internacional y XVI Congreso Nacional de Ciencias Agronómicas, 23-25 abril, Chapingo, México, 217–218 pp.

Kimura, E., y Islam, M. A. (2012). Seed scarification methods and their use in forage legumes. Research Journal of Seed Science, 5(2): 38–50.

Laguna, G. J. P. (2016). Determinación de la actividad biológica y caracterización de extractos de chipilín (*Crotalaria longirostrata*) con potencial aplicación en alimentos. Tesis para obtener el título de Ingeniero en Ciencia y Tecnología en alimentos. Buenavista, Saltillo. Coahuila, México. 1–70 pp.

Lastiri, H. M. A., Álvarez, D. B., Soria, M. L. H., Ochoa, E. S., y Cruz-Cárdenas, G. (2017). Efecto de la salinidad en la germinación y emergencia de siete especies forrajeras. Revista Mexicana de Ciencias Agrícolas, 8 (6): 1245–1257.

Mendez, N. J. R., Merazo, P. J. F., y Montaño, M. N. J. (2008). Relación entre la tasa de imbibición y el porcentaje de germinación en semillas de maíz (*Zea mays* L.), caraota (*Phaseoulus vulgaris* L.) y quinchoncho (*Cajanum cajan* (L.) Mill.). Revista UDO Agrícola, 8 (1): 61–66.

Monroy-Vázquez, M. E., Peña-Valdivia, C. B., García-Nava, J. R., Solano-Camacho, E., Campos, H., y García-Villanueva E. (2017). Imbibición, viabilidad y vigor de semillas de cuatro especies de *Opuntia* con grado distinto de domesticación. Agrociencia, 51 (1): 27-42.

Navarro, M., Febres, G., Torres, V., y Noda, A. (2010). Efecto de la escarificación húmeda y seca en la emergencia de plántulas de *Albizia lebbeck* (L.) Benth. Pastos y Forrajes, 33 (3): 1–14.

Sanabria, D., Silva, R., Oliveros, M., y Manrique, U. (2003). Germinación de semillas de las leguminosas arbustivas forrajeras *Cratylia Argentea* y *Cassia Moschata* sometidas a inmersión en ácido sulfúrico. Bioagro, 16 (3): 225–230.

Sánchez-Paz, Y., y Ramírez-Villalobos, M. (2006). Tratamientos pregerminativos en semillas de *Leucaena leucocephala* (Lam.) de Wit. y Prosopis juliflora (Sw.) DC. Revista de la Facultad de Agronomía de la Universidad de Zulia, 23(3): 257–272.

SAS, Institute. (2009). SAS/STAT® 9.2. User's Guide. SAS Institute. Cary, NC, USA. 1848 pp.

Sevillal, G. H., y Fernández, O. N. (1991). Leguminosas forrajeras herbáceas: emergencia y establecimiento de plántulas [En línea]. 8p. Consultado 18 octubre 2016. Disponible en: http://www.produccionanimal.com.ar/produccion_y_manejo_pasturas/pasturas%20artificiales/69-emergencia_y_establecimiento_ plantulas.pdf.

Solh, M., y van Ginkel, M. (2014). Drought preparedness and drought mitigation in the developing world's drylands. Weather and Climate Extremes 3 (): 62–66.

Sousa-Paiva, E. A., Lemos-Filho, J. P., and Trombert-Oliveira, D. M. (2006). Imbibition of *Swietenia macrophylla* (Meliaceae) seeds: The role of stomata. Annals of Botany, 98 (1): 213–217.

Villagra, P. E., Cony, M. A., Mantován, N. G., Rossi, B. E., González-Loyarte, M. M., Villalba, R., Marone, L. (2004). Ecología y manejo de los algarrobales de la Provincia Fitogeográfica del Monte. In Arturi MF, JL Frangi, JF Goya eds. Ecología y manejo

de bosques nativos de Argentina. La Plata, Argentina. Editorial Universidad Nacional de La Plata. Presentación multimedia en CD.

Zapata, R. M., Azagra M. C., y Karlin M. S. (2017). Tratamientos pregerminativos para la ruptura de la dormición en semillas de tres poblaciones de *Ramorinoa girolae*, leñosa endémica de zonas áridas en Argentina. Bosque, 38 (2) 237–245.

Zimmermann, L. R., Galussi, A. A., Martinelli, A. H. M., Férnandez, A. P. (2003). Viablilidad y longevidad de las semillas duras de leguminosas forrajeras. Ciencia, Docencia y Tecnología, (26): 231–257.

Zuloaga, A. S., Briones, O., and Orozco, S. A. (2011). Seed Germination of Montane Forest Species in Response to Ash, Smoke and Heat Shock in Mexico. Acta Oecologica, 37 (1): 256–262.