Revista Mexicana de Ciencias Forestales Vol. 13 (69)
Enero – Febrero (2022)

DOI: https://doi.org/10.29298/rmcf.v13i69.844

Article

Efectos del pretratamiento con *Trichoderma* y *Bacillus* en la germinación de semillas de *Agave victoriae-reginae* T. Moore
Effects of *Trichoderma* and *Bacillus* pre-treatments on the germination of *Agave victoriae-reginae* T. Moore seeds

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Resumen
La aplicación de tratamientos pregerminativos es fundamental para mejorar las tasas de germinación de las semillas de especies forestales, entre los cuales el uso de microorganismos es uno de ellos. En este estudio se evaluó el porcentaje de germinación de semillas de *Agave victoriae-reginae* tratadas con *Trichoderma* spp. y *Bacillus* spp. Se probaron tres tratamientos: T_1 (*Trichoderma*), T_2 (*Bacillus*) y T_3 (Testigo), con tres repeticiones de 100 semillas cada una. Las semillas se sumergieron en una solución de 1×10^6 UFC (tratamientos T_1 y T_2), y en agua corriente (T_3), luego se sembraron sobre *Sphagnum* peat moss y se registró el porcentaje de germinación diariamente. La germinación comenzó a los 5 días después de la siembra, lo que significa que las semillas no presentaron latencia. Entre el 8° y 12° día se registró un aumento acelerado de la germinación en los tres casos, hasta que la el proceso finalizó a los 26 días, con 85 % de germinación total para semillas tratadas con *Trichoderma*, 86.7 % con *Bacillus* y 74 % con el testigo. Se observó un efecto significativo del tratamiento sobre el porcentaje de germinación; incluso el uso de ambos microorganismos aceleró el proceso de germinación con respecto al testigo. Estos resultados sugieren que el uso de *Trichoderma* spp y *Bacillus* spp. como tratamientos pregerminativos puede mejorar la germinación de *A. victoriae-reginae* y su conservación a largo plazo, lo que contribuye a la permanencia de esta especie en peligro de extinción.

Palabras clave: Agave, especie en riesgo, latencia, noa, tratamiento pregerminativo, viabilidad de semilla.

Abstract
Application of germinative treatments is key to improve germination rates of forest species seeds, among which is found the use of microorganisms. In this study, the percentage of germination of *Agave victoriae-reginae* seeds treated with *Trichoderma* spp, and *Bacillus* spp was evaluated. Three treatments were tested: T_1 (*Trichoderma*), T_2 (*Bacillus*), and T_3 (control), with three replications of 100 seeds each one. The seeds were immersed in a solution 1×10^6 CFU (treatments T_1 and T_2), and water in T_3, then they were sowed, and the germination percentage was recorded daily. The germination began 5 days before the sown, which indicated that seeds did not present dormancy. Between 8° and 12° day an accelerated increase of germination was recorded in the three cases, until it ended at day 26, with 85 % as total germination for seeds treated with *Trichoderma* spp., 86.7 % with *Bacillus* spp., and 74 % with control. A significant effect of treatment on the germination percentage was found; even the use of both microorganisms accelerated the germination process compared to control. These results suggest that the use of *Trichoderma* spp, and *Bacillus* spp. as pregerminative treatments can improve the germination of *A. victoriae-reginae* and its long-term conservation, which contributes to the preservation of this endangered species.
Key words: Agave, endangered species, dormancy, noa, germinative treatment, seed viability.

Fecha de recepción/Reception date: 24 de agosto de 2021
Fecha de aceptación/Acceptance date: 26 de noviembre de 2021

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Introduction

The collection and commercialization of non-timber forest resources is one of the main economic activities in the arid and semi-arid zones of northeastern Mexico (Castillo *et al*., 2015). However, the inadequate management of the exploited species, as well as clandestine collection, have had effects on the diversity, the area of distribution and the abundance of these species, to the extent of leading them to different levels of risk of extinction (Durán and Núñez, 2015). Among the threatened species in Mexico is *Agave victoriae-reginae* T. Moore (Asparagaceae) (Tropicos, 2019), known as "noa" or "queen's agave", a perennial species, endemic to the *Chihuahua*n Desert, distributed in northern Mexico, in the states of *Coahuila, Durango* and *Nuevo León* (Durán and Núñez, 2015).

From its peculiar beauty, this species has been the focus of attention by collectors for ornamental use (González *et al.,* 2011). This situation has led this taxon to be included in the Official Mexican Standard NOM-059-SEMARNAT-2010 under the category of "endangered species" (Semarnat, 2010), as well as in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (CITES, 2017). Therefore, the assisted reproduction of *A. victoriae-reginae* seedlings and the subsequent reforestation in their habitat constitutes an alternative to mitigate the loss of natural populations.

Germination is the most crucial stage of the plant cycle, as it depends on a set of environmental conditions and resources such as soil and nutrients, which limit or promote the establishment of the seedling on a substrate, and on a broader scale, the regeneration of a plant population (Donohue *et al.,* 2005). Therefore, understanding the germination process is key to improving nursery production (Barnett and Varela, 2004) and thus promoting the conservation of forest populations.

Maturity, viability (period during which seeds retain their germination capacity) and dormancy (inability to germinate under optimal environmental conditions and resources) are intrinsic factors on which seed germination depends, as well as extrinsic factors such as temperature, substrate, light intensity and humidity (Khurana and Singh, 2001; Doria, 2010). The use of pregerminative treatments allows to accelerate and homogenize germination, among which are mechanical, thermal and chemical scarification, dehydration, imbibition, and the use of growth regulators (Cubillos *et al.,* 2011; Hernández *et al.*, 2017).

There is evidence that certain microorganisms have the ability to stimulate seedling germination and growth (Cubillos *et al.,* 2011). On the one hand, the use of fungi such as *Trichoderma* spp. reduces the mechanical resistance of the testa in the seeds and facilitates the breaking of dormancy (Delgado-Sánchez *et al.,* 2013). On the other hand, the use of bacteria such as *Bacillus* spp. allows the solubilization of nutrients such as phosphates, which improves the nutrition of the embryos (Cabra *et al*., 2017).

In general, the use of pregerminative treatments in seeds of *Agave* species has been limited and has focused on evaluating the effect of environmental conditions such as temperature in eight species of this genus, including *Agave lechuguilla* Torr. and *Agave cupreata* Trel. *et* Berger (Ramírez *et al*., 2012), the origin of the seed in *Agave potatorum* Zucc. (Rangel *et al.,* 2015), or the substrate in *A. victoriae-reginae* (Sánchez *et al*., 2017). Furthermore, in this genus, the use of microorganisms as germination facilitators has not been evaluated. Therefore, the objective of this study was to evaluate the effect of pregerminative treatments with *Trichoderma* spp. and *Bacillus* spp. on the percentage of germination of seeds of *A. victoriae-reginae*. The hypothesis of this research is that the use of *Trichoderma* spp. and *Bacillus* spp. as pregerminative treatments, the GP can be increased compared to sowing without these microorganisms.

Materials and Methods

Studied species

Agave victoriae-reginae T. Moore. Small, compact plant, simple or furrowed or cespitose, acaulescent with short stem (highly variable under cultivation). Short, green leaves with striking white markings, generally very intertwined, 15-20 (-25) × 4-6 cm. linear ovate, rounded at the apex, stiff, thick, flat to concave at the top, rounded to sharp at the bottom; corneal margin white, generally toothless, 2-5 cm wide, continuous to base; terminal spines 1-3, 1.5-3 cm long, trine-conical, subulate, very broad at the base, with broad base, widely concave on the upper face, with rounded keels below, black: inflorescence spiky, erect 3-5 m high, densely flowered in the upper half of the axis, the peduncle with long attenuated deltoid chartaceous bracts; flowers in pairs or short triads, forked, sturdy pedicels, 40-46 mm long; thickly fusiform, with a short neck; shallow tube, extended, 3 × 8-10 mm; tepals almost equal, 18-20 × 5-6 mm, linear, rounded apiculate, spreading, then enveloping the filaments in the post-anthesis and erect, the interior strongly keeled, filaments 45-50 mm long, inserted at the edge of the tube; anthers 18-21 mm long, yellow or tan, centric or eccentric; capsules ovoid to oblong, 17-20 × 10-13 mm, rounded at the base, apiculate: seed 3-5 × 2.5-3.5 mm, hemispherical to lacriform, veined on both sides, lower marginal wing (Gentry, 1982).

Germplasm collection

A mass harvest of *A. victoriae-reginae* seeds was carried out directly from the capsules that indicated the dehiscence of specimens (plants) from the botanical garden of the *La Sauceda* Experimental Site, which belongs to the *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)* (National

Institute of Forest, Agricultural and Livestock Research (INIFAP), located in the *Ramos Arizpe* municipality, state of *Coahuila*, between 101°18'58.45'' W and 25°50'48.45'' N, at 1 024 masl. The environmental conditions for this place according to the Köppen climatic classification, modified by García (1973), the climate is predominantly dry, semi-warm and temperate (BWK) with an average annual temperature of 18.5 °C and average annual rainfall of 198.7 mm; annual average evaporation of 1 349 mm (Díaz *et al*., 2007). The germplasm was collected in 2018 and stored in a transparent plastic container (10x10 cm) and hermetic at room temperature (25 °C average) for a year, within the *Saltillo* Experimental Field of INIFAP, in the *Saltillo* municipality, *Coahuila*.

Pregerminative treatments

Three pregerminative treatments were applied to the seeds: T_1 (Consortium of *Trichoderma* species), T_2 (Consortium of *Bacillus* species) and T_3 (control based on only direct running tap water). The microorganisms were purchased in the form of commercial biostimulant complexes; for *Trichoderma*, it was Prevence™ (*Trichoderma harzianum* Rifal (1969) *T. asperellum* Samuels, Lieckf. & Nirenberg and *T. yunnanense* Z. F.Yu & K. Q. Zhang, equivalent to 1×10^{12} spores ml^{-1}) and for *Bacillus* it was Bioshield-R™ (*Bacillus subtilis* (Ehrenberg 1835) Cohn 1872*, B. amyloliquefaciens* Priest *et al.,* 1987*, B. licheniformis* (Weigmann 1898) Chester 1901, *B. megaterium* de Bary 1884 and *B. mycoides* Flügge 1886, equivalent to 1×10^9 CFU ml^{-1}). *Agave* seeds were soaked in a final solution composed of 1×10^6 CFU for one minute, and then allowed to dry at room temperature.

Experimental design

A completely random experimental design was used with three replications of 100 seeds each. The seeds treated with the microorganisms were sown in 25 × 10 cm transparent plastic containers with a cover, and *Sphagnum* peat moss (Premier™) was used as substrate in a 5 cm thick layer moistened at field capacity. After sowing, each of the containers was kept at a temperature of 26 ± 2 °C and conditions of 12 h light and 12 h of darkness, under laboratory conditions.

The number of germinated seeds was recorded every 24 hours to determine the germination percentage (PG), which was calculated using the equation:

$$GP = \frac{SG}{ST} x100$$

Where:

GP = Germination percentage (%)

SG = Number of germinated seeds

ST = Total number of seeds

Statistical analysis

An analysis of covariance was performed to evaluate the effect of pregerminative treatments and the time after sowing on the GP of the treated seeds (α = 0.05), using the statistical package R (3.4.3) (R Core Team, 2017).

Results

The analysis of covariance showed that the treatment and the time had a significant influence on the germination of the seeds (Table 1). the *Trichoderma* and *Bacillus* consortium (*P* <0.001) treatment determined a final PG statistically higher than control, with an increase in germination of 14.8 % and 17.1 %, respectively. The germination time after sowing also influenced GP (*P* <0.001), as during the first days a notable increase in germination was observed, to follow afterwards a stabilization trend after the 12th day of sowing.

Table 1. Effect of the treatment and time in the germination percentage of *Agave victoriae-reginae* T. Moore.

Variation factor	GL	SC	PSC	F value	P value
Treatment	2	2 989	1 495	13.221	<0.001
Time	1	34 515	34 515	305.297	<0.001
Treatment - time	2	244	122	1.081	0.345
Residuals	75	8 479	113		

GL=degrees of freedom; SC= sum of squares; PSC= SC average.

Germination of *A. victoriae* seeds began on the 5th day after sowing, with 29.6 %, 19.6 % and 12.6 % for the seeds treated with *Trichoderma* spp., *Bacillus* spp. and control, respectively. Between the 8th and 12th day, an exponential increase in germination was observed, and later, the increase in germination began a relative stabilization period until the end of 26 days, when the GP reached 85 % for seeds treated with *Trichoderma* spp., 86.7 % with *Bacillus* spp. and 74 % with the control treatment (tap water) (Figure 1).

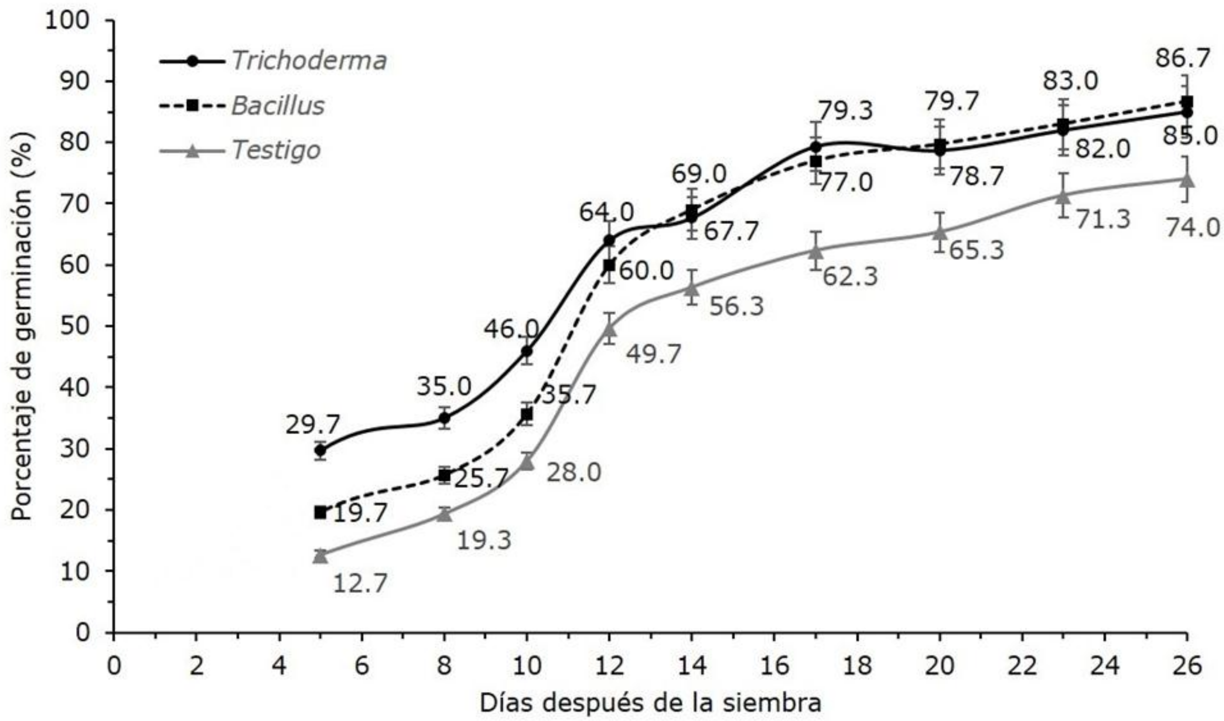

Figure 1. Percentage of germination of seeds of *Agave victoriae-reginae* T. Moore according to the treatment with respect to time.

The type of treatment did not influence the germination time (*P*> 0.345), since the three treatments followed similar temporal trajectories, but there was significant evidence that the use of microorganisms accelerated the GP in the first days, for example, on the 5th day, the GP with *Trichoderma* spp. was 29.7 %, a figure higher than that registered with the control on the 10th day (28 %). Also in the last days of germination, the control reached a GP of 74 % at 26 days, while with microorganisms this value was exceeded at 17 days (79.3 % with *Trichoderma* spp. and 77 % with *Bacillus* spp.). During the first 12 days, there were significant differences between the three treatments. After this period, the GPs with the microorganisms were similar, but they did differ in contrast to the control.

Discussion

The total GP of the three treatments were higher than those calculated for *Agave mapisaga* Trel. (70 %) and *A. angustifolia* Haw. subsp. *tequilana* (F.A.C. Weber), with 28 % germination (Ramírez *et al.*, 2016), in which no pre-germination treatments with microorganisms were used. Also, the seeds of *A. victoriae-reginae* were viable after one year of being collected. Since metabolic processes such as perspiration and metabolisms that cause natural aging occur in stored seeds, and, consequently, viability tends to decrease over time (Moncaleano *et al.,* 2013), it is possible that if *A. victoriae-reginae* seeds are sown during the year of collection, they might achieve higher GPs. However, the results of this study suggest that it is possible to store the seeds for at least one year, and have acceptable GPs.

In addition, under the environmental conditions during the experiment, it can be stated that seeds did not show dormancy, characteristic of other A*gave* species from arid areas, such as *Agave lechuguilla* Torr. (Freeman *et al*., 1977) and *Agave parryi* Engelm. var. *parryi* (Freeman, 1975). The absence of dormancy in the evaluated seeds in this study is similar to that known for seeds stored for two years of *Agave salmiana* Otto ex Salm-Dyck, which had lower dormancy than those sown in the same year of collection; such differences are attributable to the effect of the storage temperature (Peña *et al.,* 2006).

The germination start time was similar to that reported in another study with *A. victoriae-reginae* (Sánchez *et al.,* 2017), which varied between 3 and 4 days. The time after sowing significantly influenced the GP in which significant differences were identified between the three treatments before 12 days, expressed in a period of acceleration of germination; meanwhile, in the second half of the evaluated time there were no differences between *Bacillus* spp. and *Trichoderm*a spp., but they were found with the control. This behavior is similar to that of other *Agave* species, with a fast-growth period followed by a saturation of the germination curve (Ramírez *et al.,* 2012; Ramírez *et al.,* 2016).

The increase in GP during the first days of the experiment could be attributed to the fact that the largest seeds could have been the first to germinate, as they were genotypically the most vigorous (Sánchez *et al.,* 2011). Another factor could be the high moisture due to the covered container used, which favored the ideal humidity conditions to promote germination (Castillo *et al.,* 2014).

The response of A*. victoriae-reginae* to the microorganisms added as pregerminative treatments was satisfactory, indicating that the use of *Trichoderma* spp. and *Bacillus* spp. can improve the nursery production of this species. These results coincide with those recorded in *Opuntia streptacantha* Lem, another plant from semi-arid areas, in which fungi have the ability to dissolve the testa, without affecting the endocarp, which facilitates the emergence of the embryo (Delgado-Sánchez *et al.,* 2010). In addition, fungi and bacteria release radical exudates, among which are sugars, mucilage, organic acids and amino acids, which can provide nutrients for the embryos, and even promote the subsequent growth of seedlings (Ahmad *et al.,* 2008; Gómez *et al.,* 2013).

In addition, some of the biocomposites produced by these inoculants, such as antimicrobial substances or that stimulate the seedling's immune system, inhibit the development of phytopathogens, which represents an advantage for new seedlings (Guillén *et al.,* 2006). Therefore, the management of *Trichoderma* spp. and *Bacillus* spp., widely used in agriculture (Moreno *et al.,* 2018), is highly recommended to promote the germination of *A. victoriae-reginae* seeds, since they increase the GP.

In forest plantations with coniferous species, the incorporation of microorganisms such as mycorrhizae has also shown increases in seedling survival (Gómez *et al*., 2013). This confirms the great role that microorganisms play during the first stages of the life cycle of plant species such as pine trees and also agaves, through biotic interactions such as mutualism (Sieber, 2007). For this reason, it is of vital importance to introduce this type of microorganisms in the production of forest species in nurseries (Ortega *et al.,* 2004).

Because the seeds of *A. victoriae-reginae* respond favorably to the use of *Trichoderma* spp. and *Bacillus* spp., the nursery production of this species is feasible, which means a great opportunity for the recovery of the populations of this endangered species. In subsequent studies, it will be important to evaluate the effect of other environmental conditions, such as humidity, temperature and light, on the germination of the seeds of this species and others at risk.

Conclusions

The germination of seeds of *A. victoriae-reginae* responded favorably to treatment with *Trichoderma* spp. and *Bacillus* spp., increasing the PG. These characteristics represent a great opportunity for the assisted reproduction of this species at risk and the recovery of natural populations in the long term.

Conflict of interests

The authors declare no conflict of interest.

Contribution by author

Francisco Castillo Reyes: experimental design, data analysis, writing of the manuscript, David Castillo Quiroz: germplasm collection, direction and establishment of the experiment, taking parameters, writing and review of the manuscript. Jesús Eduardo Sáenz Ceja, Agustín Rueda Sánchez and J. Trinidad Sáenz Reyes: data analysis, writing and review of the manuscript.

References

Ahmad, F., I. Ahmad and M. S. Khan. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research 163(2):173-181. Doi:10.1016/j.micres.2006.04.001.

Barnett, J. P. and S. Varela. 2004. A review of chemical treatments to improve germination of longleaf pine seeds. Native Plants Journal 5(1):18-24. Doi:10.2979/NPJ.2004.5.1.18.

Cabra C., T., C. A. Rodríguez G., C. P. Villota C., O. A. Tapasco A. y A. Hernández R. 2017. Efecto de *Bacillus* sobre la germinación y crecimiento de plántulas de tomate (*Solanum lycopersicum* L). Acta Biológica Colombiana 22(1):37-44. Doi:10.15446/abc.v22n1.57375.

Castillo R., F., J. D. Sánchez C., S. E. Rangel E. y J. Canul K. 2014. Efecto de microorganismos en la promoción de la germinación de semillas de la cactácea *Echinocactus platyacanthus* Link & Otto. Interciencia 39(12):863-867. www.redalyc.org/articulo.oa?id=33932786006 (10 de abril de 2020).

Castillo Q., D., D. Y. Avila F., F. Castillo R., A. Antonio B. y O. U. Martínez B. 2015. *Nolina cespitifera* Trel. recurso forestal no maderable de importancia económica y social del noreste de México. Interciencia 40(9):611-617. https://www.redalyc.org/articulo.oa?id=33940998005 (3 de mayo de 2020).

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). 2017. Lista de especies CITES. http://checklist.cites.org/#/es/search/output_layout=alphabetical&level_of_listing=0&show_synonyms=1&show_author=1&show_english=1&show_spanish=1&show_french=1&scientific_name=Agave+victoriaereginae&page=1&per_page=20 (10 de agosto de 2020).

Cubillos H., J. G., A. Páez R. y L. Mejía D. 2011. Evaluación de la capacidad biocontroladora de *Trichoderma harzianum* Rifai contra *Fusarium solani* (Mart.) Sacc. asociado al complejo "Secadera" en Maracuyá, bajo condiciones de invernadero. Revista Facultad Nacional Agronomía Medellín 64(1): 5821-5830. http://www.scielo.org.co/pdf/rfnam/v64n1/a08v64n01.pdf (6 de mayo de 2020).

Delgado-Sánchez, P., M. A. Ortega-Amaro, J. F. Jiménez-Bremont and J. Flores. 2010. Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (Cactaceae). Plant Biology 13(1):154-159. Doi:10.1111/j.1438-8677.2010.00333.x.

Delgado-Sánchez, P., J. F. Jiménez-Bremont, M. L. Guerrero-González and J. Flores. 2013. Effect of fungi and light on seed germination of three *Opuntia* species from semiarid lands of central Mexico. Journal of Plant Research 126: 643–649. Doi:10.1007/s10265-013-0558-2.

Díaz P., G., J. A. Ruíz C., G. Medina G., M. A. Cano G., V. Serrano A. e I. Sánchez C. 2007. Estadísticas climatológicas básicas del estado de Coahuila. (Periodo 1961 – 2003). Libro Técnico N° 16. Campo Experimental Cotaxtla. CIRGOC-INIFAP Medellín de Bravo, Ver., México. 159 p.

Donohue, K., L. Dorn, C. Griffith, E. Kim, A. Aguilera, C. R. Polisetty and J. Schmitt. 2005. The evolutionary ecology of seed germination of *Arabidopsis thaliana*: Variable natural selection on germination timing. Evolution 59(4): 758-770. Doi: 10.1111/j.0014-3820.2005.tb01751.x.

Doria, J. 2010. Generalidades sobre las semillas: Su producción, conservación y almacenamiento. Cultivos Tropicales 31(1):74-85. http://scielo.sld.cu/pdf/ctr/v31n1/ctr11110.pdf (5 de junio de 2020).

Durán, M. C. y H. G. Núñez P. 2015. Utilización de un sistema de inmersión temporal (SIT) para multiplicar plantas ornamentales de *Agave victoriae-reginae*. Jóvenes en la Ciencia 1(2): 66-71. http://www.jovenesenlaciencia.ugto.mx/index.php/jovenesenlaciencia/article/view/381 (13 de junio de 2020).

Freeman, C. E. 1975. Germination responses of a New Mexico population of Parry agave (*Agave parryi* Engelm. var. *parryi*) to constant temperature, water stress and pH. The Southwestem Naturalist 20(1):69-74. Doi: 10.2307/3670012.

Freeman, C. E., R. S. Tiffany and W. H. Reid. 1977. Germination responses of *Agave lechuguilla, A. parryi,* and *Fouquieria splendens.* The Southwestern Naturalist *22(2):*195-204. Doi: 10.2307/3669810.

García, E. 1973. Modificaciones al sistema de clasificación climática de Köppen, Segunda Edición. Instituto de Geografía, UNAM. México, D.F., México. 146 p.

Gentry, H. S. 1982. Agaves of Continental Norht American. The University of Arizona Press. Tucson, AZ, USA. 670 p.

Gómez R., M., J. Villegas, J., C. Sáenz R, C. y R. Lindig C. 2013. Efecto de la micorrización en el establecimiento de *Pinus pseudostrobus* en cárcavas. Madera y Bosques 19(3):51-63. Doi: 10.21829/myb.2013.193327.

González E., M. S., M. González E., I. L. López E., L. Reséndiz R., J. A. Tena F. y F. I. Retana R. 2011. El complejo *Agave victoriae-reginae* (Agavaceae). Acta Botánica Mexicana 95:65-94. Doi: 10.21829/abm95.2011.268.

Guillen, C. R., F. D. Hernández C., G. Gallegos M., R: Rodríguez H., C. N. Aguilar G., E. Padrón C. y M. H. Reyes V. 2006. *Bacillus* spp. as biocontrol in infested soils with

Fusarium spp., *Rhizoctonia solani* Kühn and *Phytophthora capsici* Leonina and its effect on development and yield of pepper (*Capsicum annuum* L.). Revista Mexicana de Fitopatología 23:105-113. https://www.redalyc.org/articulo.oa?id=61224204 (9 de mayo de 2020).

Hernández M., S., R. Novo S., M. A. Mesa P., A. Ibarra M. y D. Hernández R. 2017. Capacidad de *Trichoderma* spp. como estimulante de la germinación en maíz (*Zea mays* L.) y frijol (*Phaseolus vulgaris* L.). Revista de Gestión del Conocimiento y el Desarrollo Local 4(1):19-23. https://revistas.unah.edu.cu/index.php/RGCDL/article/view/898 (10 de agosto de 2020).

Khurana, E. and J. S. Singh. 2001. Ecology of seed and seedling growth for conservation and restoration of tropical dry forest: A review. Environmental Conservation 28(1):39-52. Doi:10.1017/S0376892901000042.

Moncaleano E., J., B. C. F. Silva, S. R. D. Silva, J. A. A. Granja, M. C. J. L. Alves and M. F. Pompelli. 2013. Germination responses of *Jatropha curcas* L. seeds to storage and aging. Industrial Crops and Products 44: 684-690. Doi:10.1016/j.indcrop.2012.08.035.

Moreno R., A., V. García M., J. L. Reyes C., J. Vásquez A. y P. Cano R. 2018. Rizobacterias promotoras del crecimiento vegetal: una alternativa de biofertilización para la agricultura sustentable. Revista Colombiana de Biotecnología 20(1):68-83. Doi: 10.15446/rev.colomb.biote.v20n1.73707.

Ortega, U., M. Duñabeitia, S. Menendez, C. González M. and J. Makada. 2004. Effectiveness of mycorrhizal inoculation in the nursery on growth and water relations of *Pinus radiata* in different water regimes. Tree Physiology 24: 65-73. Doi:10.1093/treephys/24.1.65.

Peña V., C. B., A. B. Sánchez U., J. R. Aguirre R., C. Trejo, E. Cárdenas and A. Villegas M. 2006. Temperature and mechanical scarification on seed germination of ´maguey´(*Agave salmiana* Otto ex Salm-Dyck). Seed Science and Technology 34: 47-56. Doi: 10.15258/sst.2006.34.1.06.

R Core Team. 2017. R project 4.3.4. https://www.r-project.org/ (16 de febrero de 2017).

Ramírez T., H. M., C. B. Peña V., J. R. Aguirre R., J. A. Reyes A., A. B. Sánchez U. and S. Valle G. 2012. Seed germination temperatures of eight Mexican *Agave* species with economic importance. Plant Species Biology 27:124-137. Doi: 10.1111/j.1442-1984.2011.00341.x.

Ramírez T., H. M., R. Niño V., J. R. Aguirre R., J. Flores, J. A. De-Nova V. and R. Jarquin G. 2016. Seed viability and effect of temperature on germination of *Agave angustifolia* subsp. *tequilana* and *A. mapisaga*; two useful *Agave* species. Genetic Resources and Crop Evolution 63: 881-888. Doi: 10.1007/s10722-015-0291-x.

Rangel L., S., A. Casas and P. Dávila. 2015. Facilitation of Agave potatorum: An ecological approach for assisted population recovery. Forest Ecology and Management 347:57-74. Doi: 10.1016/j.foreco.2015.03.003.

Sánchez U., A. B., I. Ortega, I. Cano, A. González, C. B. Peña V., G. Rivero, G. Sthormes y D. Pacheco. 2011. Efecto de la escarificación de la semilla y del sustrato sobre el crecimiento de plántulas de *Agave salmiana*. Revista de la Facultad de Agronomía 28: 40-50. https://produccioncientificaluz.org/index.php/agronomia/article/view/26979/27604 (20 de agosto de 2020).

Sánchez S., J., J. Flores, E. Jurado, J. Sáenz M., P. Orozco F. y G. Muro P. 2017. Hidrocoria en semillas de *Agave victoriae-reginae* T. Moore, especie en peligro de extinción: Morfología y anatomía como facilitadores de la hidro-dispersión y germinación. Gayana Botánica 74(2):251-261. Doi:10.4067/S0717-66432017000200251.

Secretaria de Medio Ambiente y Recursos Naturales (Semarnat). 2010. Norma Oficial Mexicana NOM-059-ECOL-2010. Protección ambiental Especies nativas de México de flora y fauna silvestres. Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio. Lista de especies en riesgo. Diario Oficial de la Federación. 30 de diciembre de 2010. México, D.F., México. 40 p.

http://dof.gob.mx/nota_detalle.php?codigo=5173091&fecha=30/12/2010 (5 de agosto de 2020).

Sieber, T. N. 2007. Endophytic fungi in forest trees: Are they mutualist? Fungal Biology Review 21: 75-89. Doi: 10.1016/j.fbr.2007.05.004.

Tropicos. 2019. Tropicos.org. Missouri Botanical Garden. http://www.tropicos.org. (6 de agosto de 2020).