Acta Botanica Mexicana

Research article

Effect of preconditioning treatments and seed storage time on *Homalocephala parryi* (Cactaceae) germination in greenhouse conditions

Efecto de tratamientos de preacondicionamiento y tiempo de almacenamiento de semillas sobre la germinación de *Homalocephala parryi* (Cactaceae) en condiciones de invernadero

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Abstract:

Background and Aims: *Homalocephala parryi* is an endemic and threatened Mexican cactus whose survival is at risk due to the adverse effects of anthropogenic activities in its habitat. Implementing successful germination procedures is fundamental for propagating threatened plants, establishing living collections, and reintroducing individuals. This study aimed to evaluate the effects of preconditioning treatments and seed storage time on the germination of *H. parryi*.

Methods: The effects of three seed preconditioning treatments (24 hours water imbibition, 48 hours water imbibition, and 20 days of 5 °C stratification) were evaluated on germination percentage and germination rate in seeds stored one month and in seeds stored one year. In addition, fruit and seed morphology, as well as the germination process and seedling development, are described.

Key results: Significant differences were found between experiments conducted with seeds stored for one month and those with seeds stored one year (P<0.05). The highest germination percentages (44 and 32%) and germination rates (8.04 and 7.09) were obtained from stratified seeds stored with one year and one month, respectively.

Conclusions: Experiments showed better effects on germination using stratified seeds with one year of storage. This may be due to seed dormancy during the cold, dry seasons, which is probably broken almost a year later during the hot, rainy season. Seed stratification may support the *ex situ* conservation of *Homalocephala parryi*.

Key words: biological conservation, seed imbibition, seed stratification.

Resumen:

Antecedentes y Objetivos: *Homalocephala parryi* es un cactus mexicano, endémico y amenazado, cuya supervivencia está en riesgo debido a los efectos adversos de actividades antropogénicas en su hábitat. Implementar procedimientos de germinación exitosos es fundamental para propagar plantas amenazadas, establecer colecciones vivas y reintroducir individuos. Los objetivos de este estudio fueron evaluar los efectos de tratamientos de preacondicionamiento y del tiempo de almacenamiento de las semillas sobre la germinación de *H. parryi*.

Métodos: Se evaluaron los efectos de tres tratamientos de preacondicionamiento de semillas (imbibición en agua por 24 horas, imbibición en agua por 48 horas y estratificación a 5 °C por 20 días) sobre el porcentaje y la tasa de germinación en semillas con un mes y un año de almacenamiento. Además, se describe la morfología del fruto y la semilla, así como el proceso de germinación y el desarrollo de plántulas.

Resultados clave: Se obtuvieron diferencias significativas entre los experimentos realizados con semillas almacenadas por un periodo de un mes y semillas almacenadas durante un año (P<0.05). Los porcentajes de germinación (44 y 32%) y las tasas de germinación (8.04 y 7.09) más altos se obtuvieron con semillas estratificadas con un año y un mes de almacenamiento, respectivamente.

Conclusiones: Las semillas estratificadas y almacenadas durante un año presentaron mejores efectos sobre la germinación. Esto podría deberse a la latencia de las semillas durante la temporada fría y seca, la cual, probablemente, se rompe casi un año después, durante la temporada cálida y lluviosa. La estratificación de semillas puede apoyar la conservación *ex situ* de *Homalocephala parryi*.

Palabras clave: conservación biológica, estratificación de semillas, imbibición de semillas.

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Received: October 31, 2025.
Reviewed: December 12, 2025.
Accepted by Marie-Stéphanie Samain: February 12, 2026.
Published Online first: March 17, 2026.
Published: Acta Botanica Mexicana 133 (2026).

To cite as: Vargas-Luna, M. D., G. Lazo-Anchondo, S. De La Torre, J. F. Aristizábal and C. Figueroa. 2026. Effect of preconditioning treatments and seed storage time on *Homalocephala parryi* (Cactaceae) germination in greenhouse conditions. Acta Botanica Mexicana 133: e2529. DOI: https://doi.org/10.21829/abm133.2026.2529

e-ISSN: 2448-7589

Introduction

The barrel cactus of Guzmán Lake, *Homalocephala parryi* (Engelm.) Vargas-Luna & Bárcenas is a cactus species of the tribe Cacteae (Vázquez-Sánchez et al., 2013). The species was historically classified within the genus *Echinocactus* Link & Otto. However, it is now considered in *Homalocephala* Britton & Rose, together with *H. polycephala* (J.M. Bigelow & Engelm.) Vargas-Luna & Bárcenas and *H. texensis* Britton & Rose (Vargas-Luna et al., 2018; Korotkova et al., 2021). *Homalocephala parryi* is endemic to the Chihuahuan Desert, specifically to Chihuahua, Mexico, with a restricted geographic distribution of approximately 100 km^2, in the municipalities of Ahumada, Ascensión, and Juárez. The species generally inhabits sedimentary and metamorphic hills, between 1200 and 1300 m a.s.l., with grasslands and xerophytic scrub. Nevertheless, a few individuals have been observed growing at 1800 m a.s.l. in the higher parts of the Sierra de Samalayuca in xerophytic vegetation mixed with some individuals of *Juniperus arizonica* (R.P. Adams) R.P. Adams (Anderson, 2001; Hernández and Gómez-Hinostrosa, 2011).

Homalocephala parryi adults are solitary (Fig. 1A), sometimes branched with a globose to slightly cylindrical grayish-green stem, usually with 13 acute ribs, 15-45 × 15-30 cm. Areoles have four robust central spines covered with grayish trichomes, and a reddish epidermis visible in new spines and in wet mature spines. Spines are reflexed, the largest up to 4-6 cm long. Flowers are yellow with a reddish base, 5-7 cm in diameter (Fig. 1B). Fruits are fleshy and green in the initial stage of their development, becoming dry and brownish with age, with basal dehiscence, 2-4 cm in length (Fig. 1C). Seeds are black, lenticular to rounded, hilum circular, deeply excavated, 2.5-3.1 mm in length (Bravo-Hollis and Sánchez-Mejorada, 1991; Chamberland, 1997; Anderson, 2001; Vázquez-Sánchez et al., 2012).

In Mexico, *Homalocephala parryi* is considered a threatened species, while the IUCN Red List classifies it as Near Threatened (IUCN, 2025). However, it lacks the risk analysis that verifies the threatened categorization (SEMARNAT, 2010). To conserve endemic and endangered plants, the ideal is to develop *in situ* conservation actions. Nevertheless, this is complicated due to the rapid fragmentation and destruction of habitats (Anderson, 2001). Therefore, the foremost conservation actions for these plants are *ex situ* through seed banks, propagation of plants in greenhouses, and subsequent reintroduction to their habitats (Birnbaum et al., 2011). It is imperative to know the treatments that promote the germination of cacti and other plants of interest for their potential conservation (Rojas-Aréchiga and Vázquez-Yanes, 2000).

The germination of certain cacti species is enhanced by seed preconditioning treatments, such as imbibition in water and temperature stratification (Masini et al., 2014; Calderón-García et al., 2021; Ritthidechrat and Anuwong, 2024). In this context, 24-hour imbibition of *Echinocactus platyacanthus* Link & Otto seeds resulted in a germination percentage of up to 70% (Gómez-Serrano et al., 2021). Also, in this species, chemically scarified seeds exhibited a 60% increase in germination compared to the control (Rosas-López, 2002). In *E. horizonthalonius* Lem., 5 °C stratification for 15 days produced 25% additional germination compared to the control, which generated only 5% (Arredondo-Gómez et al., 2007). *In vitro* culture of mechanically scarified seeds in *H. parryi* had up to 96% germination (García-González et al., 2022).

Temperature stratification seems to have better effects on breaking dormancy, specifically on species adapted to seasonal variations in temperature (summer *vs.* winter), and in humidity (dry *vs.* wet season) (Baskin and Baskin, 2014). It is known that *Echinocactus platyacanthus*, which is closely related to *Homalocephala parryi*, has patterns of dormancy cycles, with high germination percentages in spring and winter (62.36 and 62.61%), contrary to low percentages in summer and autumn (37.5 and 19.59%), respectively (Aragón-Gastélum et al., 2018). Dormancy could occur in *Homalocephala parryi*, which liberates its seeds during the cold, dry season, and these probably germinate until the next hot and wet season. This topic could be clarified with germination experiments and the factors that affect it.

Seed storage time may affect germination capacity. However, this condition varies among species and is also affected by the temperature and humidity in which the seeds are stored (Barrios et al., 2020; Briseño-Sánchez et al., 2024). Additionally, in Cactaceae, some seeds require a post-ripening period, which could be shown in stored seeds

Figure 1: Adult plants of *Homalocephala parryi* (Engelm.) Vargas-Luna & Bárcenas with reproductive structures. A. lateral view showing the globose to slightly cylindrical stem; B. flowers with yellow tepals, reddish at the base; C. pubescent fruits retained in the stem apex. Scale bars=5 cm. Photographs by Coyolxauhqui Figueroa.

(Rojas-Aréchiga and Vázquez-Yanes, 2000). This research aimed to 1) evaluate the effects of three preconditioning treatments (24 h water imbibition, 48 h water imbibition, and 20 days of 5 °C stratification) and seed storage time on germination of *H. parryi*, 2) predict which interaction of factors (treatments *vs.* seed storage time) would promote seed germination, and 3) describe seed and fruit morphology, as well as the germination process and seedling development.

Materials and Methods

Field work

In May 2021 and 2022, ten mature fruits (five per year) from different individuals of *Homalocephala parryi* were collected in a wild population in Ahumada, Chihuahua, Mexico. Fruits were collected in paper bags, followed by manual extraction of the seeds in the laboratory, quantifying the number of seeds per fruit. To generate completely random experiments, all seeds were mixed in two paper bags, one with seeds from 2021 and another with seeds from 2022. Seeds from 2021 were stored for one year (1 yr), and seeds from 2022 were stored just one month (1 M) at room conditions (24 °C and less than 10% relative humidity), until sowing in June 2022. To prevent illegal collecting and preserve this small population (<50 individuals), the location of the study site is omitted.

Fruit and seed morphology

Fruits were measured with a caliper, and images of the structures were captured with a camera (Canon EOS 6D Mark II, Canon Inc., Tokyo, Japan). Seeds were observed under a stereoscope (VELAB UV S5, Velab Co., McAllen, USA) and photographed using a Sony Cyber-shot camera (Tokyo, Japan). Seed length and width (n=20) were measured using an electronic digital caliper and weighed in an analytical balance (AND HR-120, Tokyo, Japan). To analyze their internal morphology, seeds were cut longitudinally by hand and dehydrated with ethanol at graded concentrations from 50 to 100% for one hour, then dried to a critical point with an Emitech K850CO2 dryer (Quorum Technologies Ltd, Lewes, East Sussex, UK). Seeds were fixed to an aluminum sample holder and coated with gold in a Quorum Q150R ES ionizer (Quorum Technologies Ltd, Lewes, East Sussex, UK). The coated seeds were observed in a Hitachi S-2460 N scanning electron microscope (Hitachi, Tokyo, Japan) operated at 15 kv at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Seed morphology was designated based on the terminology of Barthlott and Hunt (2000) and the botanical dictionary of Font Quer (1963). The germination process and seedling development were described from the analyses of photographs captured weekly in the stereoscope during the first six months of age.

Preconditioning treatments, germination, and seedling development

To evaluate germination, we measured the effects of two factors, preconditioning treatments and storage time (1 M and 1 yr). Treatments correspond to: 24 h water imbibition, 48 h water imbibition, 20 days of 5 °C stratification, and control (without treatment). These treatments were applied twice, once to seeds from 1 M and once to seeds from 1 yr of storage time. This factorial design yielded 40 replicates, with five repetitions (seed trays) per combination. Each replicate consisted of 10 seeds and represented the experimental unit, with 400 seeds used in total. The germination percentage was obtained from each seed tray. These preconditioning treatments were selected because stratification at low temperatures may be a prerequisite for the germination of some plants (Kathpalia and Bhatla, 2018), and hydration (imbibition) promotes testa softening, washes inhibitory substances, accelerates germination, and facilitates radicle emergence, effectively stimulating germination in some species of cacti (Rojas-Aréchiga and Vázquez-Yanes, 2000; Contreras-Quiroz et al., 2016; Calderón-García et al., 2021; Monteon-Ojeda et al., 2021). Each seed tray was established under the same substrate, temperature, and humidity as described below in the text. Because of the low availability of seeds, we did not perform indirect viability tests, such as the 2,3,5-triphenyl tetrazolium chloride test (TZ).

Seeds were dipped in fungicide (metalaxyl + chlorothalonil) 1 g l^{-1} for one hour to avoid fungal infection. They were then sown in transparent plastic containers perforated on both surfaces (lid and bottom), each with 10 seeds, in a substrate mixture of coconut fiber and tezontle (1:1). The experiment was carried out in the greenhouse of the Plant Production Unit of the Universidad Autónoma de Ciudad Juárez (UACJ). During germination, from 1 June to 20 July 2022 (50 days), the temperature ranged from 20 to 40 °C, and the humidity inside the germinator container was >50%. Irrigation took place daily for 50 days, and germinated seeds were counted. In this study, we followed the criterion of Kathpalia and Bhatla (2018) to determine that a seed had germinated when the radicle emerged. After the first 50 days, irrigation was gradually spaced (twice a week). Seedlings of 1 cm in height were transplanted into independent pots, each containing one to three individuals. Description of the germination process and the development of seedlings was carried out from the analysis of photographs captured in the stereoscope weekly during the first six months of age.

Data analyses

To test the efficiency of each treatment, we generated germination curves and calculated the germination rates according to Maguire (1962). Germination rates (germination speeds) were calculated by summing the divisions of the number of germinated seeds by the time in days. Also, to evaluate the effect of the pre-conditioning treatments and seed storage time on germination, a generalized linear model (GLM) with a binary logistic distribution suitable for proportional data using the function glm() from *stat* package (Cayuela and De La Cruz, 2022). This approach is appropriate since the dependent variable (germination) is measured as a proportion of germinated seeds out of the total number of seeds (germinated *vs.* non-germinated). The model was designed to evaluate the interaction between the two factors and identify the most effective combination of levels to explain germination proportions. For the selected model, the relationships between variables were calculated by the extraction of the *p-value* and the slope of the GLM test. Type III sum of squares (or marginal sum of squares) was used in the function Anova() from the *car* package (Fox and Weisberg, 2019) as it is appropriate for testing the interaction effect in factorial models. In the GLM framework, effects were assessed based on likelihood-based tests (e.g., deviance or Wald tests) (Cayuela and De La Cruz, 2022). To compare the selected model with the null model, we used likelihood ratio tests via Anova (test: “Chisq”) from stats (R Core Team, 2022). Subsequently, we plotted the proportions of germination means with a 95% confidence interval for each combination of levels to predict the best combination (i.e. interaction of variables) that increases the proportion of germination and were estimated using the function emmeans() plotted using emmip() from the *emmeans* package that include tukey test for level significance differences (Lenth, 2023). GLMs do not assume normality in the residuals; instead, they directly model the distribution of the response variable (e.g., binomial for proportions). Given the factorial design with five seed trays per factor combination, the GLM accounts for the experimental design's structure (Cayuela and De La Cruz, 2022). To assess the validity of our analysis, we examined residual plots for patterns, evaluated dispersion, and tested for overdispersion using the function gof() from *aods3* package (Lesnoff et al., 2024), and checked for residual autocorrelation with dwtest() from the *lmtest* package (Zeileis and Hothorn, 2002). GLM analysis was performed using the *stats* and *car* packages in R v. 4.2.2 (Fox and Weisberg, 2019; R Core Team, 2022), and the prediction of germination values was estimated and plotted using the *plotrix* package (Lemon, 2006).

Results

Fruit and seed morphology

Fruits are slightly cylindrical, 2.5 cm (±0.53 SD) length × 2 cm (±0.24 SD) width, covered with abundant trichomes that emerge from acute bracts, dehiscent by a basal operculum (Fig. 2A-C), with 233.3 (±55.0 SD) average seeds per fruit. Seeds are reniform to lenticular, with black testa, medium size, 1.99 mm (±0.07 SD) length × 1.22 mm (±0.14 SD) width, and a weight average of 3.16 mg (±1.75 SD). Testa irregular with a convex dome-shaped relief, lustrous with a dorsal keel (Fig. 2D-F). Concave hilum, pubescent, separated from the micropyle by a ridge on the testa (Fig. 2E), and adhered to it is the brown, papyraceous tegmen. The curved embryo occupies most of the seed, and an air chamber is present (Fig. 2F). Elliptical perisperm, slightly curved, and contiguous with the lower portion of the hilum-micropylar region (Fig. 2F).

Germination and seedling development

Sown seeds absorbed water, then the testa broke, and the radicle emerged (germination). After 10 to 20 days of sowing, abundant hyaline root hairs are observed. Then, the hypocotyl grew acropetally in the distal portion, showing the acute cotyledons, which are first contiguous but later, at about 25 to 30 days after germination, they separate (Fig. 2G, H). The primary root began to form lateral roots. After the separation of the cotyledons, the first areole is formed 30-40 days after sowing. This has trichomes and four to six spines, glabrous, hyaline, whitish, slightly yellowish, and even reddish, and is followed by the formation of other areoles 50-60 days after sowing. At this stage, four stem tubercles are visible (Fig. 2I-L). In addition, the cotyledons were indistinguishable from the stem; these appear to integrate into the stem tissue. Seedlings at six months pass into their juvenile stage, shown by the formation of lignified spines, with more than six acute ribs and the pith (Fig. 2M).

Germination responses to storage time and seed preconditioning treatments

A positive effect of treatment and storage time on germination percentage was found (Fig. 3; Table 1). There were significant differences between control and stratification ($P<0.001$) and 24 h imbibition ($P<0.01$), with stratification showing higher germination (Estimation = 11.3). Also, seeds stored for 1 year had higher germination proportions than seeds stored for 1 month (Estimate = 1.5, $P<0.05$), averaged across treatments. Notably, for the interaction between treatment levels, the stratification method differs from the control at 1 month and imbibition at 48 h.

Predictions made by the model on the interactions between factor levels are similar, e.g., 24 h imbibition in 1 M and 1 yr of storage, with control 1 yr (abc); 48 h imbibition with 1 M and 1 yr of storage (ab) (Fig. 4). The interaction stratification / 1 yr of storage is significantly different ($P< 0.01$) from all (c), as well as control / 1 M of storage with the lowest germination. Also, it is worth noting that the model predicts that if stratification is used on 1 yr storage seeds, the mean germination ratio will be approximately 0.4 (with confidence intervals), as the best combination. The germination curve (Fig. 5) and germination rates (Fig. 6) showed that stratification at 5 °C had the most positive effects in both 1 M (32 and 7.09%) and 1 yr (44 and 8.04%) storage seeds.

Discussion

The highest germination percentages were obtained in 1 yr storage seeds with 5 °C stratification (44%), followed by 24 h imbibition (28%). Similar results were reported in *Astrophytum myriostigma* Lem. (Sánchez-Salas et al., 2006). These authors determined that the best treatments for germination were 24 h imbibition (72.66%) and 5 °C stratification for 12 h (66.66%), while the treatment with the lowest ger-

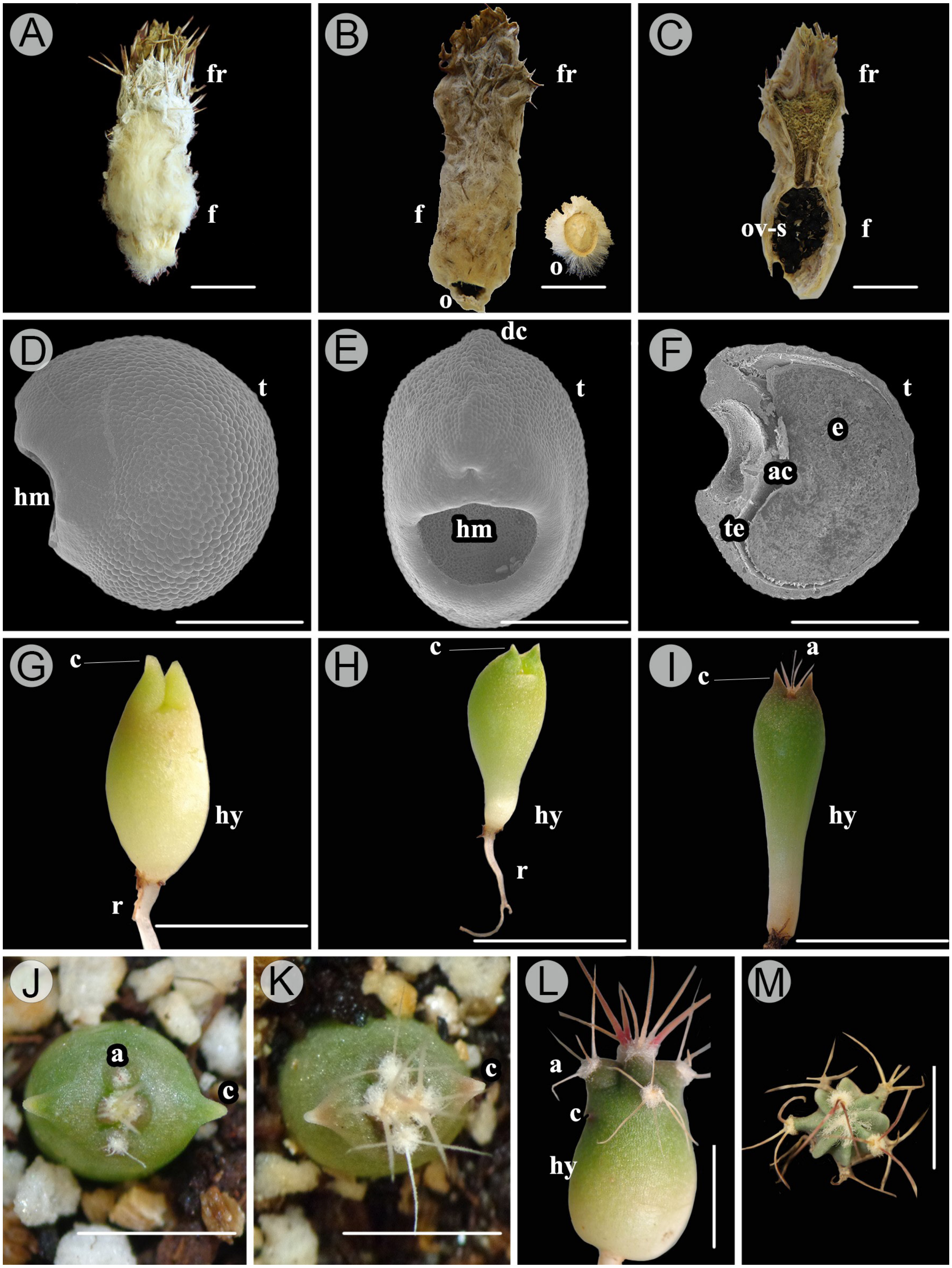

Figure 2: Fruit and seed morphology as well as the seedling development of *Homalocephala parryi* (Engelm.) Vargas-Luna & Bárcenas. A. mature fruit; B. fruit showing operculum; C. fruit longitudinal section with flower remnants; D. seed lateral view, note dome ornamentation, and the hilum-micropylar region; E. seed front view showing the hilum-micropylar region and dorsal crest; F. seed longitudinal section, note curved embryo; G. seedling with contiguous cotyledons and an elongated hypocotyl; H. separated cotyledons; I. first areole emergence; J. seedling with three areoles and still with cotyledons; K. seedling with four areoles; L. two-month-old seedling, note tubercles, and spines; M. two-year-old juvenile plant, note lignified spines. a=areole, c=cotyledons, ac=air chamber, dc=dorsal crest, e=embryo, fr=flower remnants, f=fruit, hy=hypocotyl, hm=hilum-micropylar region, o=operculum, ov-s=ovarium with seeds, r=root, t=testa, te=tegmen. Scale bars: A-C=1 cm; D-F= 1 mm; G-I=1 cm; J-L=0.5 cm; M=2 cm. Stereo microscope photographs by Coyolxauhqui Figueroa; SEM photographs by Berenit Mendoza Garfias.

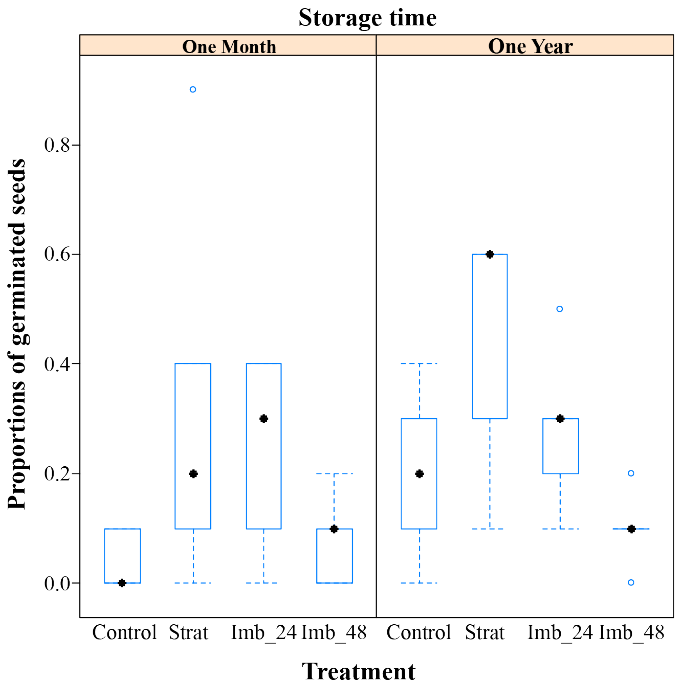

Figure 3: Conditional boxplot showing proportions of seed germination of *Homalocephala parryi* (Engelm.) Vargas-Luna & Bárcenas as a function of treatments used (Strat: 5 °C Stratification; Imb_24=24 h Imbibition; Imb_48=48 h Imbibition) and seed storage time (One Month and One Year). The black circle represents the mean, and the blue circle represents the outliers; the bars show the minimum and maximum values.

Table 1: GLM results analyzing the effect of treatments, storage time, and the interaction factor on the germination ratio of *Homalocephala parryi* (Engelm.) Vargas-Luna & Bárcenas seeds. Ctrl=Control, Strat=Stratification, Imb_24=24 h imbibition, Imb_48=48 h imbibition.

Factor	Level/Contrast	Estimate	z-value	P
Treatment				
	Stratification	11.3	3.1	<0.001
	Imb_24	7.6	2.6	<0.01
Storage time (year)				<0.05
	1	1.5	2.2	<0.05
Interaction Treatment: storage time				<0.05
	Ctrl_1M : Strat_1M	-2.4	-3.1	< 0.05
	Ctrl_1M : Strat_1yr	-2.9	-3.8	<0.01
	Imb48_1M : Strat_1yr	-2.2	-3.7	<0.01
	Strat_1yr : Imb48_1yr	2.0	3.5	<0.01

mination percentage was mechanical scarification (17.33%). However, it is notable that the control showed a higher germination percentage than the treatments (74.66%), so the promoting effect of preconditioning is doubtful. In *Myrtillocactus geometrizans* Console, seeds subjected to stratification treatments at 4 °C for one and two weeks obtained germination percentages of 83.33 and 87.08%, respectively. However, their control obtained a germination percentage of 83.70%, so they did not obtain significant differences (Calzada-Tlapalamatl, 2017). Villanueva et al. (2016) reported that, when applying dormancy-breaking treatments on seeds of *Echinocactus platyacanthus*, the highest germination rates and speed of germination were in imbibition treatments in hot water at 50 °C for 10 minutes (30%).

It would be relevant to evaluate the effect of different light and temperature ranges on the germination percentages of *Homalocephala parryi* seeds, because in other barrel-shaped species phylogenetically close to this species, such as *Echinocactus platyacanthus,* these factors have a considerable impact on germination. The latter species shows higher germination percentages when white light and a temperature of 25 °C are used (Rojas-Aréchiga et al., 1997, 1998). According to Rojas-Aréchiga and Vázquez-Yanes (2000), most cactus seeds germinate best at 20 °C. However, each species has different responses to temperature, so it would be interesting to test the temperature ranges at which *H. parry* seeds germinate best, considering that this species is more adapted to extreme climates concerning *E. platyacanthus*. For some cacti, the best germination percentages can be obtained at constant temperature, and in others with alternating temperatures (Lindow-López et al., 2018). In *E. platyacanthus*, germination percentages increase when the seeds are exposed to temperatures between 25 and 36 °C, and in combination with medium water potential levels, at -0.4 MPa (Flores et al., 2017). Seeds of the latter species can even germinate once exposed for two hours to temperatures

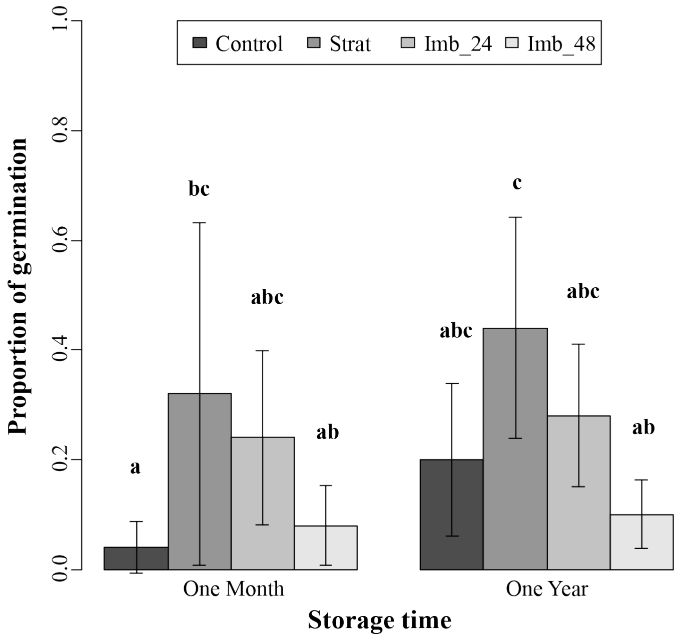

Figure 4: The proportion of germination predicted by the model for combinations of levels of the factors: treatments (Strat=5 °C Stratification; Imb_24=24 h Imbibition; Imb_48=48 h Imbibition and storage time (One Month and One Year). Bars represent the mean gemination proportion of *Homalocephala parryi* (Engelm.) Vargas-Luna & Bárcenas with a 95% confidence interval. The letters above the bar indicate the statistical difference obtained with a Tukey test between combination levels (equal letters=no statistical difference between contrasts).

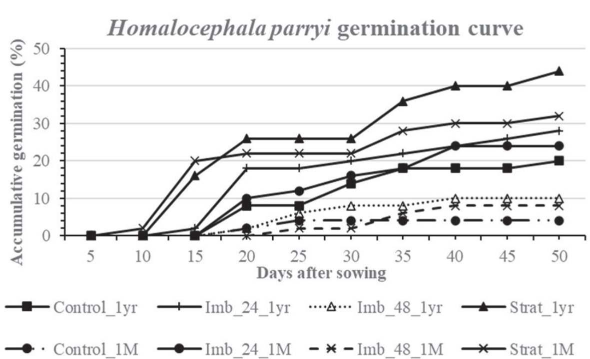

Figure 5: Germination curve of one year (1yr) and one month (1M) storage seeds per treatment (Strat=5 °C Stratification; Imb_24=24 h Imbibition; Imb_48=48 h Imbibition).

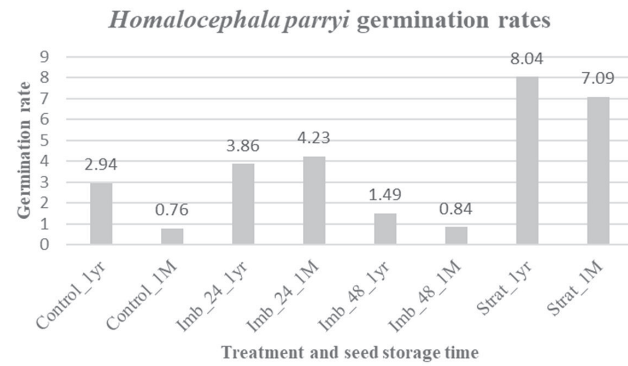

Figure 6: Germination rates in one year (1yr) and one month (1M) storage seeds per treatment (Strat=5 °C Stratification; Imb_24=24 Imbibition; Imb_48=48 h Imbibition).

of 40 °C and 70 °C, which indicates that the embryos are highly tolerant to high temperatures (Pérez-Sánchez et al., 2011).

In this study, we evaluated the germination of *Homalocephala parryi* of one population. It would be interesting to assess seed quality among populations of *H. parryi,* since it is known that quality may vary between populations of the same species (Ruiz-Pérez et al., 2021). Knowing which populations of *H. parryi* produce the best quality seeds would help identify sites with priority for conservation, since these could function as a germplasm reservoir. It would also be pertinent to analyze the viability of seeds found in aerial and soil banks, secondary dormancy, and germination responses to extreme temperatures, to determine the effect of these factors on population dynamics and to generate future projections that consider global warming (Aragón-Gastélum et al., 2018).

According to Baskin and Baskin (2014), for dormant seeds to germinate, they must enter a non-dormant period. Some species have seeds with non-deep physiological dormancy; that is, before germinating, they can repeatedly transition between a dormant and non-dormant state. These transitions occur gradually in response to climatic changes. To date, only seeds with non-deep physiological dormancy are known to be capable of returning to dormancy, establishing annual cycles between these states. In *Homalocephala parryi*, higher germination percentages were observed in seeds after one year of storage, so it seems that seeds likely required a post-maturation time, as occurs in some other cacti species (Rojas-Aréchiga and Vázquez-Yanes, 2000). These results suggest that the species has primary dormancy; nevertheless, it is required to conduct more studies to evaluate possible dormancy cycles in response to climatic fluctuations present in its natural habitat. These authors reported that six-year-old seeds of *Ferocactus latispinus* Britton & Rose and *Echinocactus platyacanthus*, stored in glass containers at room temperature (20-23 °C), maintained more than 50% germination. However, the optimal conditions for the long-term storage of most cactus seeds are unknown (Gurvich et al., 2021). The latter authors analyzed the germinability of seeds with different storage times (0, 3 and 7 years) for 13 species of cacti from Córdoba, Argentina. They found that the majority of seeds lose their germination capacity at 3 and 7 years of storage. Notably, these 13 species inhabit humid subtropical climates, contrary to those of *F. latispinus* and *E. platyacanthus,* which inhabit cold to temperate semiarid climates and apparently have a long-term persistence (Kottek et al., 2006).

In vitro propagation of *Homalocephala parryi* has previously been performed by somatic embryogenesis and seed sowing without testa, which increased germination percentages up to 70% with respect to the control (García-González et al., 2021; 2022). *In vitro* propagation has shown that the germination percentage in *Echinocactus platyacanthus* increased (70%) compared to soil cultivation (60%), and an acceleration in seedling growth was observed with the use of cytokines and auxins (Gómez-Serrano et al., 2021). Recently, Osuna-Avila et al. (2025) reported that mechanical scarification in medium-size seeds of *Kroenleinia grusonii* (Hildm.) Lodé, cultivated *in vitro*, increased germination percentage from 38% in the control to 66%. However, *in vitro* cultivation has the disadvantage that plants are highly susceptible to contamination by fungi or bacteria, as they usually do not have a cuticle. Likewise, the presence of vitrification was frequent in *E. platyacanthus*. In addition, plant mortality during the acclimatization process was high (Villanueva et al., 2016). In this research, we observed that *H. parryi* seeds presented a relatively good germination percentage under greenhouse conditions and that germination increased if the seeds were stored for one year and stratified. With these preconditioning treatments, the established seedling survival was 90% at six months of age, and plants look resistant and apparently morphologically well adapted to climate for possible reintroduction.

The pregerminative treatments applied to seeds and storage time in this study positively affected the germination of *H. parryi.* The low-temperature stratification treatment on one-year-old seeds had the highest germination percentage. This could be related to the large climatic fluctuations in *H. parryi* habitat. The production of fruits is in July-August, and the seeds most likely germinate until the following year during the rainy season, suggesting that the seeds of this species probably require low temperatures after fruiting to break down dormancy.

Homalocephala parryi is an endemic cactus threatened with extinction, mainly due to land use changes, since the region where it lives is being impacted by urban development and illegal extraction. The preconditioning treatments used in this study, in particular stratification at 5 °C and 24 h water imbibition, showed that they are effective in increasing the germination percentage of *H. parryi.* Their application will allow for more efficient propagation of the species under greenhouse conditions and the availability of acclimatized plants for their future reintroduction to their habitat.

Author contributions

Project administration: MDV, CF; Conceptualization: MDV, CF; Data curation: MDV, GLA, JFA, CF; Formal analysis: JFA; Investigation: MDV, GLA, CF; Methodology: MDV, GLA, JFA, SD, CF; Resources: MDV, CF; Writing – original draft: MDV, JFA, CF; Writing – review and editing: MDV, JFA, CF.

Funding

This study was partially supported by the Secretaría de Ciencias, Humanidades, Tecnología e Innovación of Mexico (SECIHTI) postdoctoral scholarship to MDV (BPPA-20240530104315268,8217409) and the master in science scholarship to GLA (4052427).

Acknowledgments

We thank the Departamento de Ciencias Químico-Biológicas of the Universidad Autónoma de Ciudad Juárez for the facilities granted for experiments. The Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) for the collection permit (SBRA/DGVS/00156/25). To B. Mendoza-Garfias of the Institute of Biology of the UNAM for the images from the Scanning Electron Microscope, and to Y. Granados for her support in the field, and O. López for manuscript revision. Finally, we thank reviewers and editors for their comments and editing.

Data Availability Statement

The dataset supporting the results of this study was submitted to SciELO Data and can be accessed at https://doi.org/10.48331/SCIELODATA.XLGXAI.

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