

Kinetics of Imbibition and Light Quality in *Echinocactus platyacanthus* LK & O Germination

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Abstract: Given that germination is a crucial stage in the development of plants, it is essential to carry out studies on the factors that affect this phase. The optimal conditions for each species should also be defined, especially those that, by their nature, present such problems in their propagation and conservation that render them vulnerable. Many species of the cacti family belong to this group, so this research aims to contribute to the knowledge of some factors that impact the germination of *Echinocactus platyacanthus*. The first purpose of this work was to describe the kinetics of scarified seeds imbibition at 25, 30, and 35 °C and their impact on the germination response. The evaluation of the effects of red light on the germinative response was established as a second purpose. The results showed that the imbibition process in scarified seeds fits a curve of the Weibull model, with 15.03, 15.62, and 18.84 hours α coefficients, and 0.08, 0.09, and 0.45 β coefficients for temperatures of 25, 30, and 35°C, respectively. In non-scarified seeds, the coefficients were 10.85, 13.11, and 13.06 hours α , and 0.12, 0.10, and 0.08 β for temperatures of 25, 30, and 35°C, respectively. Scarification and temperature favor rapid water uptake. The imbibition behavior is similar under both conditions, unlike the final germination percentage. Temperature influences the imbibition percentage and favors germination since the temperature of 35 °C registered over 90%. The seeds irradiated with red light recorded 96% germination at 144 and 168 hours, confirming that light quality influences this response. A possible physical latency and mechanical dormancy are ruled out, demonstrating positive photodormancy.

Key words: photodormancy, cactus, red light, scarification, germination

1. Introduction

Cactaceae are a source of potentially usable resources. Still, this family is threatened by losing or modifying their natural habitats, over-harvesting, illegal trading, and possibly climate change. Actually, the 48 genera and 563 species native to Mexico have decreased in recent decades. *Echinocactus platyacanthus* is a species that has been used to produce *acitrón* as feed for cattle in times of drought and, currently, as an ornamental plant [1, 2]. The irrational exploitation and its slow growth — estimated at 100 years — have placed it on the list of species “subject to protection”, according to the Official Mexican Standard NOM-059-SEMARNAT-2010. Accordingly, it is necessary to carry out studies about the factors that

affect the germination of its seeds as a conservation measure. Currently, pregermination treatments are being used to interrupt the different types of dormancy; for example, irradiation interrupts photodormancy; soaking and scarification dissolve the seed coat, interrupting physical and mechanical latency, and leaching inhibitors in the testa. In addition, it is possible to conduct a bioassay for abscisic acid that indicates if the seed shows chemical latency [3]. This study used soaking treatments at different times, chemical scarification, and irradiation with red light. This research aimed to describe the imbibition kinetics of scarified seeds at 25, 30, and 35 °C, its influence on the germination response, and to assess the effect of red light on the germination response. These factors will

determine the possible dormancy of the *Echinocactus platyacanthus* LK & O seeds.

2. Material and Methods

2.1 Biological Material

The seeds were obtained from mature fruits of *Echinocactus platyacanthus* plants from the cacti collection of the Botanical Garden of the Faculty of Higher Studies, Cuautitlán, UNAM (FESC). Forty-five fruits were collected and left to dry for two months in paper bags in a dry and cool place, at an average temperature of $26 \pm 1^\circ\text{C}$ during the drying period, in the FESC Plant Physiology Laboratory. The seeds were extracted and left in 9 cm diameter Petri dishes for two months to degrade the testa inhibitors (if any). The seeds were selected, discarding those that did not have an embryo (flat appearance), and were distributed in batches of 50 seeds.

2.2 Statistical Analysis

The Weibull model was applied to adjust the relationship between imbibition time and seed hydration with and without scarification to obtain imbibition kinetics. On the other hand, to perform the mean comparison test for the germination percentage in Experiment 1, the t-student statistical test was used with 95% confidence. In Experiment 2, red light irradiation of *E. platyacanthus* seeds, a completely randomized experimental design with five replications, was used. Tukey's test ($\alpha = .05$) was run to find means comparisons.

2.2.1 Experiment 1

Before applying any treatment on scarified or non-scarified seeds, 20 samples -containing 50 seeds each- were weighed individually to obtain the average initial weight. The *E. platyacanthus* seeds were immersed in a 25% sulfuric acid solution for 15 minutes for scarification before starting the imbibition process. Fifteen imbibition times (0.25, 0.5, 1, 1.5, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours) at three imbibition temperatures (25, 30, and 35°C) were

applied to both batches of seeds (with and without scarification). Every treatment was repeated five times, and each repetition consisted of a small cloth sack with 50 seeds each, placed in one of three beakers with water covered with aluminum foil. Each beaker contained 75 small sacks (5 repetitions per imbibition time) for non-scarified and scarified seeds. Treatment 1 was set at room temperature (25°C); Treatment 2 in an oven at 30°C ; Treatment 3 in an oven at 35°C , respectively. At the end of each treatment, the final weight was recorded. Subsequently, the seeds of each repetition were distributed evenly in an adequately labeled Petri dish with a double layer of absorbent paper, previously sterilized and moistened with distilled water. Constant humidity was maintained throughout germination, and a daily record was kept.

The dishes with the seeds were incubated under laboratory conditions at room temperature, an average of $26 \pm 1^\circ\text{C}$. Seeds were considered germinated when the radicle length reached 2 to 4 mm. The maximum germination percentage was considered reached when the number of germinated seeds remained constant for five consecutive days.

2.2.2 Experiment 2

Seven treatments were applied for the red light irradiation experiment (0, 24, 72, 96, 120, 144, and 168 hours of irradiation), with five repetitions each. The Led lamps, the light source for these red light treatments, were covered with a double layer of red cellophane paper and placed at a distance of 35 cm from the material to be irradiated. Before the irradiation treatments, the seeds were imbibed in the dark for 15 minutes at 35°C ; optimal conditions were determined in Experiment 1. The seeds were exposed to a light intensity of 0.09 klx (90 Lux), constant humidity was maintained throughout germination, and a daily record was kept. Seeds were considered germinated when the radicle length reached 2 to 4 mm. The response variable was the seed germination percentage.

3. Results and Discussion

Seed imbibition is a water uptake process affected by the difference between the seed inside and outside water potentials. It can be explained on the basis of low seed metabolism, high degree of dehydration, and low water potential. When the seed comes into contact with water, it tends to absorb a significant amount to restore its balance since its internal water potential is lower than the external one, as observed in the seed under study (Fig. 1 and Fig. 2).

The Weibull distribution is a good indicator of the seed water uptake during imbibition in a given time. The increased fresh weight recorded is evidence of water absorption during seed hydration, implying that

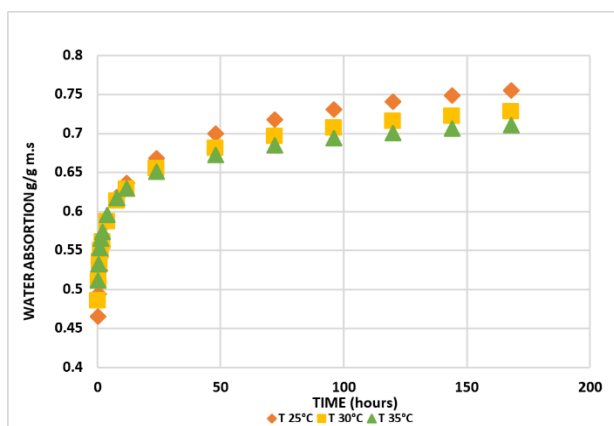


Fig. 1 Imbibition kinetics of *E. platyacanthus* seeds without scarification at 25, 30, and 35 °C, modeled using the Weibull distribution.

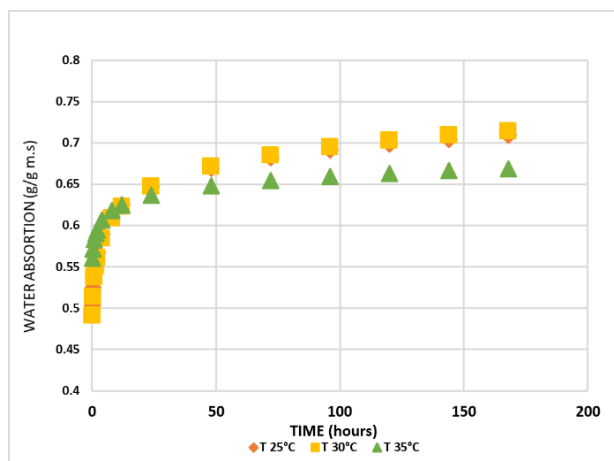


Fig. 2 Imbibition curves of *E. platyacanthus* seeds with scarification at 25, 30, and 35 °C, modeled using the Weibull distribution.

the imbibition percentage of *E. platyacanthus* gradual gain depends on the imbibition time and temperature (Experiment 1). Thus, it is reasonable to expect since the imbibition speed depends on the water potential gradient, which is impacted by time and temperature [3, 4].

In Table 1 shows constants α and β of the Weibull model and the imbibition coefficients of determination (R^2) of the *E. platyacanthus* seeds at the three different temperatures, with and without scarification. The lowest values of constant β were observed in the scarified seeds (0.0455, 0.0874, and 0.095), in contrast to those obtained without scarification, which were higher. These values suggest that the imbibition process was affected by scarification, registering faster incorporation of water in scarified seeds. It is possible because chemical scarification promotes destabilization of the seed coats components, for instance, the epicuticular waxes and wax granules [5], favoring the entry of water and, consequently, accelerating the imbibition process.

Godínez-Álvarez and Valiente-Banuet (1998) [6] mention that imbibing some cacti seeds does not increase the germination percentage and, in some species, it is significantly reduced, particularly within 24 and 48 hours. In contrast, the germinative response of the *E. platyacanthus* seed was not affected up to 168 hours of imbibition. On the other hand, temperature exerts a favorable effect on imbibition. As observed at

Table 1 Coefficients α and β of the Weibull distribution corresponding to the imbibition of *E. platyacanthus* seeds at 25, 30, and 35 °C with and without scarification and coefficients of determination.

Scarified			
Temperature	α (h)	β	R^2
25 °C	15.034	0.0874	0.7235
30 °C	15.624	0.0950	0.7246
35 °C	18.848	0.0455	0.7240
Non-Scarified			
25 °C	10.851	0.1245	0.7248
30 °C	13.110	0.1032	0.7254
35 °C	13.061	0.0844	0.7236

35 °C in both conditions (Table 1), the values of constant β are lower than those recorded at 30 and 25°C, which suggests that hydration results in a synergistic effect of temperature and scarification, translating into increased germination percentages. A similar response was observed by Reino et al. (2011) [7] in legume seeds combining chemical scarification and temperature. It indicates that the response depends on the species, even belonging to the same family [8, 9].

Another factor influencing the imbibition process is the seed's anatomical and morphological characteristics and natural habitat conditions. It can be explained because *E. platyacanthus* is a cactus that grows in arid and semi-arid climates [10]; under limited access to water, cacti maximize water management. They have developed a finely reticulated texture with relief on their outer layer [5], which increases the contact surface and therefore enhances water absorption. Their ovoid shape also contributes to expanding the absorption surface.

The proteinaceous material deposited in the testa epidermis of some seeds from arid and semi-arid regions improves water absorption and increases germination with a minimum amount of available water, as described by Bregman and Graven (1997). Grajales (2004) [3] indicates that the protein material acts as a matrix that reduces the hydric potential of the seed, favoring the water uptake and, consequently, the imbibition percentage. The high values of β observed in seeds without scarification at 30 and 25 °C (Table 1) imply that hydration was slower than in scarified seeds.

The highest values of the α factor correspond to the seeds under scarification conditions: 15.03 (at 25 °C), 15.62 (at 30 °C), and 18.85 (at 35 °C). These values represent the time required to achieve 63% progress in the imbibition process [11].

The imbibition process in *E. platyacanthus* seeds displays a behavior similar to that observed by Monroy-Vázquez et al. (2017) [12] in 9 species of opuntias that reported accelerated imbibition in the first hours and up to 68 hours, reaching maximum

imbibition of 60%. Likewise, Domínguez-Domínguez et al. (2007) [13] also found this behavior in Jamaican seed varieties.

Because scarification is a pregermination treatment used to soften or dissolve the testa, releasing the possible mechanical or physical dormancy determined by the cutin or suberin in the cell walls of the seed coat [3], it follows that *E. platyacanthus* seeds do not show these types of dormancy. Now, in the hydric behavior found in *E. platyacanthus* (Figs. 1-2), the seed can carry out the second water uptake, manifested by the increased fresh weight over time; therefore, it does not have a waterproof testa [3].

The final germination percentage recorded is not statistically significant between the treatments at 25 and 30 °C ($P < 0.05$), with and without scarification (Fig. 3). However, the germination percentage observed at 35 °C shows a highly significant statistical difference ($P < 0.05$) concerning the data recorded at 25 and 30 °C, with and without scarification (data not shown). As shown, the germination percentage in all imbibition times registers percentages greater than 70%, which are not unfavorable for seeds without scarification and scarified (Fig. 2). The germination percentage obtained at 35 °C shows values greater than 90% and 95%, reached after 15 minutes of imbibition. In this regard, Mazzola et al. (2013) conclude that water availability and temperature are critical to obtaining high germination percentages.

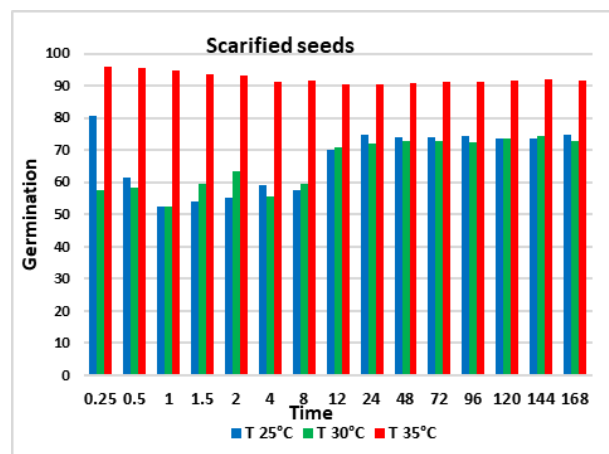


Fig. 3 Germination percentage of *E. platyacanthus* seeds subjected to three temperatures with 15 imbibition times.

The final germination percentage is not affected by scarification but by imbibition temperatures higher than 35 °C. These results coincide with the observations found in two species of *Mammillaria*, where scarification does not affect the germination percentage but the speed [14].

Irradiated *E. platyacanthus* seeds responded well to red light stimulation; as the exposure time increased, the germination percentage grew until it reached 96% during the 144 and 168-hour treatments (Table 3). The variance analysis showed statistically significant differences among all the treatments, except those with irradiation times of 144 and 168 hr (Tables 2 and 3). The treatment with 0 hours of irradiation was eliminated from the variance analysis because it presented 0% germination.

The germination response to seed irradiation is frequent in species with positively photoblastic seeds [15, 16], a protection mechanism when light conditions are not favorable for plant establishment. The answer coincides with other studies carried out on cacti species, such as *Stenocereus queretaroensis* [1, 16].

The germination response to the stimulus of red light is possibly due to the participation of phytochrome, which presents an absorption spectrum similar to the

wavelength of red. This chromoprotein has two conformations: Pr (red phytochrome) and Pfr (far red phytochrome), the latter being the physiologically active form that in turn regulates the function of phytohormones, including gibberellins, which are responsible for initiating the germination metabolic processes [3]. Positively photoblastic seeds contain high levels of Pr, so when irradiated, the photointerconversion of the phytochrome is activated, and it becomes Pfr until reaching the photoequilibrium of its two conformations, triggering the molecular events behind germination.

4. Conclusion

The imbibition kinetics of the *E. platyacanthus* seeds were obtained at three temperatures in seeds without scarification and with scarification. The results revealed that the imbibition process describes a curve that fits the Weibull model with a determination coefficient of $R^2 = 72\%$.

The analysis concluded that both time and temperature determine the water imbibition percentage reached by the *E. platyacanthus* seeds. The imbibition temperature influences the final germination percentage, greater than 90% at 35 °C.

The *E. platyacanthus* seeds do not show physical or mechanical dormancy; a positive photodormancy, though, is confirmed. The germination response is influenced by the quality of light and the irradiation time.

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Table 2 Variance analysis for *E. platyacanthus* seeds subjected to seven irradiation times.

Tratamientos (horas de irradiación)	Porcentaje de germinación promedio
144	96.77 A
168	96.42 A
120	83.56 B
96	59.58 C
72	49.23 D
24	34.37 E
0	0.00 F

Table 3 Comparing averages of *E. platyacanthus* seeds subjected to seven irradiation times (Tukey = 0.05).

Fuentes de Variación	G. L.	S.C.	C.M.	F
Entre tratamientos	6	38031.31	6338.55	10063.62
Dentro de los tratamientos	28	17.63	0.62	

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