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Conditions for the germination and the early growth of seedlings of *Polaskia chichipe* (Goss.) Backeberg and *Echinocactus platyacanthus* Link and Otto fa. *grandis* (Rose) Bravo-Hollis (Cactaceae)

(with 2 tables & 2 figures)

Rosas-López[1] Ulises, Margarita Collazo-Ortega[1]

Abstract. The effect of different combinations of chemical scarification, soaking and substrates (*ex vitro* and *in vitro*) on seed germination of *Polaskia chichipe* (Goss.) Backeberg and *Echinocactus platyacanthus* Link & Otto fa. *grandis* (Rose) Bravo-Hollis, collected in the Tehuacan Valley, Mexico, were studied. The *P chichipe* seeds did not require either chemical scarification or soaking, whereas *E platyacanthus* required chemical scarification with strong acids. Growth and maintenance of the seedlings was evaluated in two different concentrations of Murashige-Skoog medium (50% and 100% of their components). It was found that MS 50% stimulated germination in both species and promoted seedling growth.

Key words: *Polaskia chichipe, Echinocactus platyacanthus*, cacti, seed germination, establishment, seedling growth

The study of the germination and initial growth of cacti seedlings has become important due to the fact that nowdays propagation is essential to their conservation. These studies have contributed with information to knowledge of their physiology about the factors that influence their germination as well as the dormancy and water uptake by seeds.

Depending on the type of dormancy, the seeds can germinate immediately or delay the process for months to years. The seeds of many species of cacti are ingested and dispersed by birds and mammals. Several

[1] Dpto. Biología Comparada. Facultad de Ciencias, UNAM. México 04510 México, D. F. Telephone number (52-55) 56-22-49-16, Fax number (52-55) 56-22-48-28. E-mail bioyunuen@hotmail.com

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works have demonstrated the effect that the ingestion of fruits by animals has over the coat, allowing to the seeds to germinate if conditions are suitable (2, 8). When fruits are swallowed, seeds pass through the digestive tract, they receive an acid treatment that softens the coat and prepared them for germination, when finally they are deposited in faeces.

To stimulate the natural conditions to which the seeds are exposed, one of the methods usually used in germination experiments to break dormancy is chemical scarification.

A quick immersion of the seeds in acid solutions at low concentrations increases the germination in some species like *Pachycereus pringlei* (7), *Pachycereus hollianus* (3), *Ferocactus histrix* (2) among others. Nevertheless, for *Pilosocereus chrisanthus*, *Cephalocereus column-trajanii*, *Ferocactus latispinus*, *Stenocereus stellatus* and *Wilcoxia viperina* the treatment by chemical scarification did not increase germination (1). Similar results were found for *Neobuxbaumia tetetzo*, *Coryphanta pallida*, *Echinocactus platyacanthus*, *Ferocactus flavovirens*, and *Opuntia puberula* (3).

Given that there is no general methodology for the germination of cacti seeds, we decided to carry out two germination experiments to establish which scarification treatment triggers in each species a fast and synchronous germination, and the culture medium that support the best result of germination and early growth of the seedlings (100 days old) in *Polaskia chichipe* (Goss.) Backeberg and *Echinocactus platyacanthus* Link and Otto fa. *grandis* (Rose) Bravo-Hollis.

MATERIALS & METHODS

Seed collection. *Polaskia chichipe* and *Echinocactus platyacanthus* fruits were collected in localities of the Tehuacan-Cuicatlán Valley, Mexico. Those of *P. chichipe* were collected during May, 2001, in a population near the highway Huajuapan-Tamazulapan-Oaxaca, km 7 (N 17º45.155´, W 97º44.739´; GPS Garmín 45XL, error 15 m), belonging to the Huajuapan Valley. Those of *E. platyacanthus* were collected during March, 2001, in a population proximal to the highway Mexico-Huajuapan, km 13 (N 18º24.483´, W 97º26.207´), belonging to the Zapotitlán Valley. The seeds were stored in waxed paper bags during 4 months at room temperature.

Germination test with scarification treatments. Five treatments of scarification were applied, with strong acids and soaking as follow:

I. Without scarification (*Control*)
II. Scarification with strong acids (concentrate H_2SO_4 or concentrated HCl) pH 1 during 1 hour (H^+)
III. Soaking in distilled water at 50 ºC for 5 min and later in distilled water at 24 ºC for 24 h (*Soaking*)
IV. Treatment II plus III (H^+ + *soaking*)
V. Scarification with concentrated H_2SO_4 to 15 s (H_2SO_4)

After scarification, seeds were cultured on two substrates: soil-tepojal (volcanic rock) (S-T) 1:1 (*ex vitro*) and water-agar 1% (W-A) (10 g L^{-1}) pH 5.7 (*in vitro*). In the latter one the seeds were first washed with Tween 80 (3 drops in 50 mL^{-1}) for 30 min, then disinfected with a solution of colloidal silver (Mycrodin 3 drops in 50 mL^{-1}) for 15 min, ethanol 70% for 2 min, commercial sodium hypochlorite 6% of active chlorine (20%) for 15 min and 3 rinsings with sterilized distilled water (modified from López-Escamilla, 5).

Experiment was a 2 (species) x 5 (scarification treatments) x 2 (substrates) factorial, with 3 replicates of 10 seeds each one.

***In vitro* germination assays.** According to the best treatment of scarification obtained for each species in first experiment, seeds were cultured in mediums as follows (pH 5.7):

I. Water-bacteriological agar 1% (10 g L^{-1}) (W-*A*)
II. Murashige & Skoog medium (6) at 50% of its components, water-bacteriological agar 1% (10 g L^{-1}) (*MS 50%*)
III. Murashige & Skoog medium (6) at 100% of its components, water-bacteriological 1% (10 g L^{-1}) (*MSC*)

Experiment was a 2 (species) x 3 (culture treatments) factorial, with 3 replicates of 25 seeds each one.

In both tests the cultures were maintained in a controlled environment chamber to 25º C ± 1, photoperiod 16 hours (11960-15640 mmol m^{-2} s^{-1}). In the first test daily counts of germination (radicle protrusion) were made until 37 days after the sowing; in the second test daily counts were made until the 17th day and punctuals counts in the 30th day for *P. chichipe*, and to the 35 day for *E. Platyacanthus*. Germination capacity (Gc) and time in which the first seed germinates (G) and the accumulated seed germination were calculated (4). In second experiment the state of vigor of all seedlings (turgency and color) was registered until 35th day after germination.

Data were subjected to Kruskall-Wallis ANOVA by Ranks. Wilcoxon Matched Pairs test was used to compare means for significant differences.

RESULTS

Germination test with scarification treatments. There were no significant differences among species in the time for germination (G) ($H_{1,45}$=1.52, p=0.2167), while there were significant among substrates ($H_{1,45}$=8.31, p=0.0039) and treatments ($H_{1,45}$=16.17, p=0.0028). In both species the shortest time for germination was observed in *S-T* substrate: in *E. platyacanthus at Control* treatment (3 days), in *P. chichipe* at H^++ *soaking* treatment (4 days) (Table 1).

Significant differences in germination capacity (Gc) among species ($H_{1,60}$=5.23, p=0.0222) and treatments ($H_{4,60}$=11.06, p=0.0259) was obtained, but not among substrates ($H_{1,60}$=2.6081, p=0.1063). In *P. chichipe* there was response at *Control*, H^+ and H_2SO_4 treatments in W-A substrate, and in all treatments in S-T substrate, except in H^+; highest germination capacity was obtained at Control in both substrates, 60 and 50% in *W-A* and S-T respectively. In *E. platyacanthus* there was response in all treatments of W-A (between 80 and 100% except H^+ + *soaking*), and in *Control*, H^+ and H^+ + *soaking* treatments in *S-T*, with highest value in H^+ (90%) (Table I).

In *P. chichipe* there was also a rapid increment in the germination rate in *Control* treatment (Figure 1a). In *W-A* substrate 60% of germination was reached at 6 days. In *S-T* substrate a 50% of germination was reached but in 36 days (Figure 1a). In *E. platyacanthus* (Figure 1b), in *W-A*, the treatments *Control* and *Soaking* reached 80% to 20 and 24 days respectively, in H_2SO_4 and H^+ 100% germination was reached to 21 and 23 days respectively. In the *S-T* substrate the H^+ treatment reached 90% of accumulated germination until 24 days.

***In vitro* germination assays.** The best germination results in scarification treatments (Table I) were obtained for both species in the *W-A* substrate, in *Control* treatments for *P. chichipe* and with strong acids (H^+ and H_2SO_4) for *E. platyacanthus*. That is why in the *in vitro* experiments the seeds of *P. chichipe* were not scarified, and those of *E. platyacanthus* were scarified as described to H_2SO_4.

Table I.– Effect of the scarification treatment and substrate in the time to which the first seed germinates (G, days) and germination capacity (Gc, %) of *P. chichipe* and *E. platyacanthus*. *W-A*: Water-Agar; S-T: Soil-Tepojal.

Variable	Species	Treatment	*Control*	H^+	*Soaking*	H^+ + *soaking*	H_2SO_4
	Substrate						
G	*P.chichipe*	*W-A*	6_a*	36_b	nr**	nr	13_c
		S-T	5_a	Nr	19_b	4_c	11_d
	E. platyacanthus	*W-A*	13_a	13_a	16_b	30_c	14_d
		S-T	3_a	7_b	Nr	27_c	
Gc	*P. chichipe*	*W-A*	60_a	20_b	0_c	0_c	20_b
		S-T	50_a	0_b	30_c	30_c	40_d
	E. platyacanthus	*W-A*	80_a	100_b	80_a	25_c	100_b
		S-T	30_a	90_b	0_c	10_d	0_c

*Different subscript letters means significant differences (p<0.05) among treatments in each substrate.
Among substrates there were differences (p<0.05) in all treatments in both species, as between species for treatment in each substrate.
**nr: no response

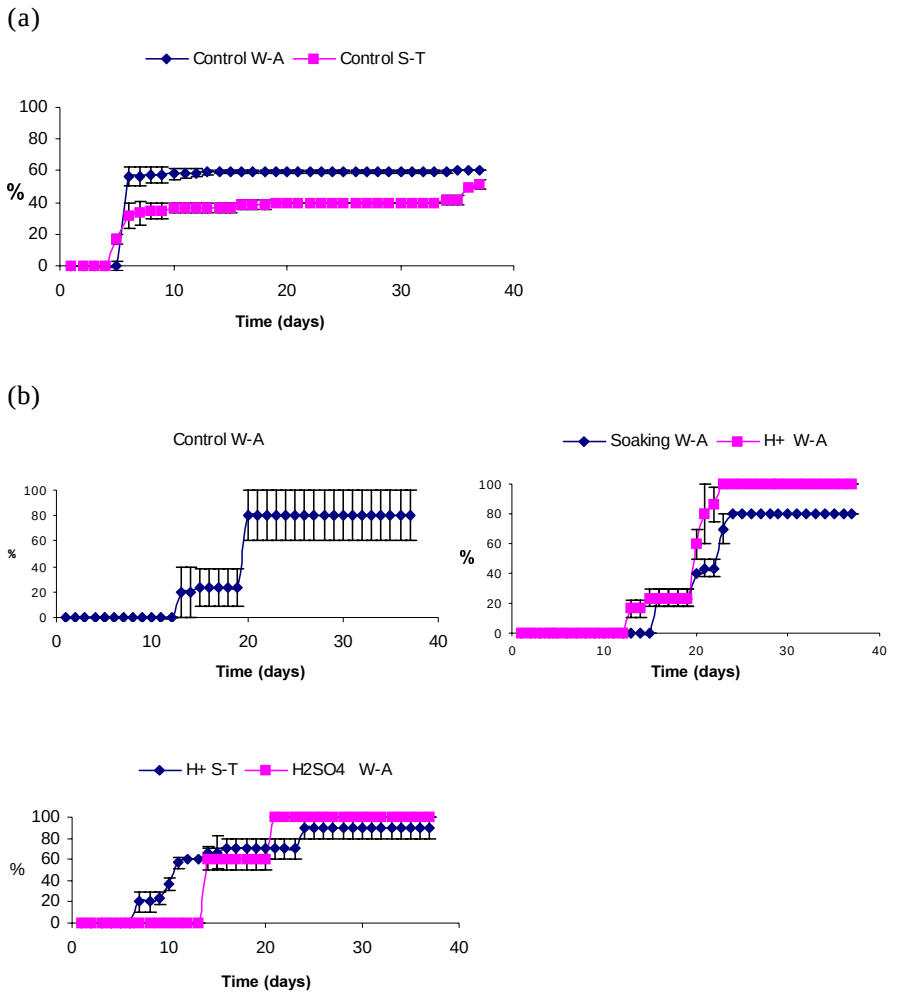

Fig. 1.– Effect of the scarification treatment and substrate in the accumulated germination (%). a) *P. chichipe* b) *E. platyacanthus*. *W-A*: Water-Agar; *S-T*: Soil-Tepojal

There were significant differences among substrates in time for germination (G) ($H_{2,18}$=15.54, p=0.0004) and germination capacity (Gc) ($H_{2,18}$=6.80, p=0.0334), but no between species neither G ($H_{1,18}$=16.19, p=0.6874) nor Gc ($H_{1,18}$=1.19, p=0.6874). In all the treatments, response for both species was obtained (Table II). In *P chichipe* the best response was in *W-A* with a germination time of 5 days, and a maximum germination capacity of 90%; in the other substrates the time for germination was an average of 12 days and the germination capacity did not exceed 50%. In *E. platyacanthus*, in *W-A* as well as in *MS 50%* substrates, ger-

Table II.– Effect of substrate in the time to which the first seed germinates (G, days) and germination capacity (Gc, %) of *P. chichipe* and *E. platyacanthus*. W-A: Water-Agar; *MS50%*: Murashige & Skoog medium to 50% of its components; *MSC*: Murashige & Skoog medium to the 100% of its components.

Variable	Species	W-A	MS 50%	MSC
G	*P. chichipe*	5_a*	11_b	13_c
	E. platyacanthus	7_a	8_b	14_c
Gc	*P. chichipe*	90_a	12_b	24_c
	E. platyacanthus	84_a	88_b	57_c

*Different subscript letters means difference ($p<0.05$) among substrates in each species for *G* and *Gc*.

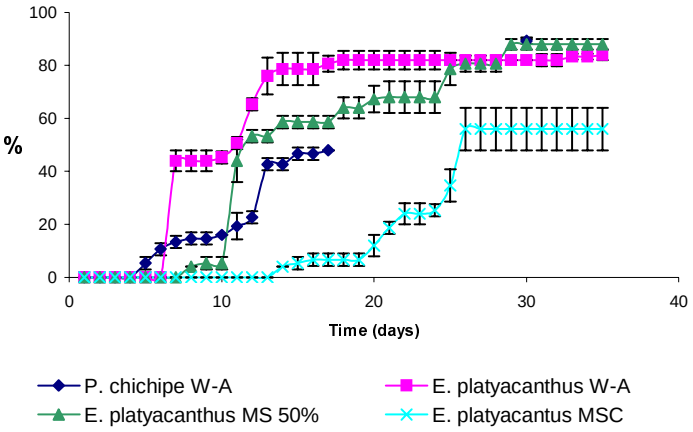

Fig. 2.– Effect of the substrate in the accumulated germination (%) of *P. chichipe* and *E. platyacanthus*. *W-A*: Water-Agar, *MS 50%*: Mu-rashige & Skoog medium to 50% of its components, *MSC*: Murashige & Skoog medium to the 100% of its components.

mination time was similar (7 and 8 days respectively) and also the germination capacity was 84 and 88% for each substrate; in *MSC* the germination time was duplicated, although the germination capacity exceeded 50%.

In *P. chichipe* there was a slow germination rate in *W-A* treatment (Figure 2), reaching a germination capacity of 50% until 18th day, the 90% was reached at the 30th day. In *E. platyacanthus* 50% of germination was

reached between the 11 and 12 days in substrates *W-A* and *MS 50%* respectively, but in the first, maximum germination was reached more quickly. Nevertheless, in this species as well as in *P. chichipe*, the seedlings did not grow and/or had little vigor in *W-A*, whereas in *MS 50%* they grew more and were roughly more vigorous.

DISCUSSION

P. chichipe always presented low values of germination, only in *Control* treatment it was slightly superior to 50%, which could mean that, added to the detrimental effect of the scarification, maybe not all the seeds were viable, or the embryo was not mature enough, or water did not penetrate the coat in 50% of the seeds. In *E. platyacanthus* the chemical scarification intensified the germination; nevertheless its fruits are not fleshy, which reduces the possibility that an animal could ingest the seeds and scarify them inside the digestive tract as it was reported (2) for *Ferocactus latispinus*. It is important to carry out more studies on the dispersion of the seeds of this species.

The fact that the greatest percentage of germination in both species has been obtained in the *W-A* substrate is attributed to the absence of solutes in the medium, which causes the hydric potential to be greater so that the seeds have more water availability. Nevertheless this only accelerates the germination but not the growth of the seedlings due to a deficit of nutriments. In *E. platyacanthus* a similar percentage of germination in *W-A* and *MS 50%* were obtained, but at almost double the time in the latter. In *P. chichipe* a similar situation happened: in *MS 50%* a germination of 80% was registered until the 50 days (data no shown).

This situation can probably be attributed to the solutes of the medium *MS 50%* which diminish the hydric potential and restrict the water availability, nevertheless it favors the growth of seedlings by the availability of nutriments. In *MSC* medium germination was less than the others substrates, the seedlings showed yellowish and low rates of growth due to the greater amount of solutes and less water availability.

CONCLUSIONS

The germination in *Polaskia chichipe* was generally low and the scarification treatments diminished the percentage of germination. The chemical scarification with strong acids favored the speed and synchrony of the germination in *Echinocactus platyacanthus*, which was high in general. The medium *MS* to 50% with agar to 1% was the best one for the germination and development of the seedlings. It is proposed that for *in vitro* cultures the medium *MS* to 50% is sufficient to promote the growth of both species.

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