Stimulation and Promotion of Germination in *Opuntia ficus-indica* Seeds♦

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ABSTRACT

Opuntia ficus-indica seeds, as many other *Opuntia* species, show low germination capacity due mainly to their hard lignified integuments, the most inward of these is the funiculus that envelops the embryo, obstructing radicle protrusion. The purpose of this study was to accelerate the initiation of the germination process and to shorten their completion time by the action of physical and chemical agents. Germination assays were performed in Petri dishes and under controlled conditions at constant temperature (25°C) and photoperiodic regime of 14 hours light per day, continuous light, and continuous darkness. Aside from these environmental factors during the incubation period, we tested the effects of agents able to break different types of dormancy or inhibitory effects. All assays followed a completely randomized design with three replications of 25 seeds for each treatment. We found that chemical scarification of the seeds with concentrated H_2S0_4 or with Schweizer reagent, followed by incubation of the treated seeds in solutions of H_20_2, under photoperiodic conditions gave the highest germination percentage in the shortest time. None of the assays carried out to establish if different agents could remove the inhibitory effect of continuous darkness on seed germination during the incubation period were able to eliminate the mentioned effect.

Keywords: *Opuntia* seeds; germination; dormancy; scarification; stimulant and promoter germination agents

INTRODUCTION

Cactus pear cultivation currently relies on vegetative propagation because this technique is uncomplicated and guarantees the cultivar's traits. Notwithstanding, clonal multiplication may involve a serious risk of disease spreading owing to reduced genetic variability. In contrast, propagation by seeds is an important method that allows the genetic diversity of populations and species to be maintained (Rojas-Aréchiga and Vázquez-Yanes, 2000). Variability facilitates researchers to select genotypes for desirable traits such as tolerance to different stress factors, biomass production, fruit quality, etc., which may be utilized for breeding purposes.

Particularly, in the subfamily Opuntioideae, seeds show low germination capacity and extended time to complete the process (Bregman and Bouman, 1983). This is due, mainly, to the particular structure of the ovule formed by three integuments, the inner of which envelops the ovule completely (Eames, 1961). The third integument of the hard-coated shell of the seed, derives from the funiculus, which becomes strongly lignified, acting in consequence, as a restricting envelop to radicle protrusion (Werker, 1997).

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To reduce the hard-lignified seed coat, several methods have been attempted, including mechanical breaking, immersion in hot water, chemical scarification with sulphuric acid, and exposure to dry heat, among others (Mondragón-Jacobo and Pimienta-Barrios, 1995). These techniques have been entirely or partially successful, but there are species and even cultivar differences which modify the response as has been shown by a series of studies on the germination capabilities of seeds of diverse *Opuntia* species subjected to the action of physical, chemical, or hormonal agents (Potter et al., 1984; Mandujano et al., 1997; Godínez-Alvarez and Valiente-Banuel, 1998; Olvera-Carrillo et al., 2003; Monteiro et al., 2005).

Notwithstanding, there are several requirements for germination that appear to be common for the majority of cacti and particularly for the genera *Opuntia.* These are: optimal temperature, which is frequently around 25°C (Nobel, 1988), or light supply as a photoperiodic regime. In addition, it must be taken into account that some characteristics of cacti seeds such as form, size, weight, structure, number per fruit, and viability are important traits that, in many instances, are related to the dynamics of germination. For example, when the number of seeds per fruit increases, the possibility of physiological variation, such as degree of differentiation, maturation, dormancy, etc., also increases, which may lead to differences in initiation time, duration, and velocity of germination.

Our hypothesis was that if the lignified triple seed coat was scarified by chemical means and then the treated seeds were incubated in an oxidative solution, under appropriate conditions of temperature and light, the germination process could be triggered and completed in a short time. The purpose of this work was to accelerate the initiation of the germination process and to shorten the time to complete it by the action of physical and chemical agents.

MATERIALS AND METHODS

The seeds used in this experiment came from *Opuntia ficus-indica* L. f. *inermis* (Web.) Le Houér. ("Cuenca" accession with green-colored fruit pulp) whose origin and photographs of fruit and cladodes was described in a previous publication (Guevara et al., 2006).

Previous to germination assays, seeds were examined for several features, e.g., number per fruit, percentage of normal and aborted, weight of 1,000, viability, and imbibition capacity. All assays were performed with normal seeds. To examine viability, split seeds were soaked in 1% tetrazolium solution for 24 hours at 30°C. Embryos that did not acquire a deep red color, but only pink, were scored as viable but were considered to have low vigor. The imbibition capacity, a measure of permeability to water, was determined by the seed weight increase when seeds were immersed in water at 25°C during 48 hours. Intact, scarified mechanically with abrasive powder, and split seeds were used for determining the latter feature.

Before incubation, seed surface was sterilized during 10 minutes with sodium hypochlorite (55% active chlorine) that was diluted to 15% and then seeds were washed 3 or 4 times with sterilized distilled water. After this, seeds were arranged in Petri dishes (10-cm diameter), prepared with a cotton layer over which a disk of filter paper was disposed (Figure 1). This system was saturated with distilled water or solutions of different substances, according to assays. Germination was conducted in a growth chamber (Precision Scientific, Mod. 818, G.C.A. Corporation, Chicago, Ill.) in which temperature was set, except when specified, at constant 25°C. When light was used, it was supplied as a photoperiodic regime of 14 hours day^{1}. Intensity of white light was 16 µmol m^{-2} s^{-1}. Germination occurred when radicle and hypocotyls emerged.

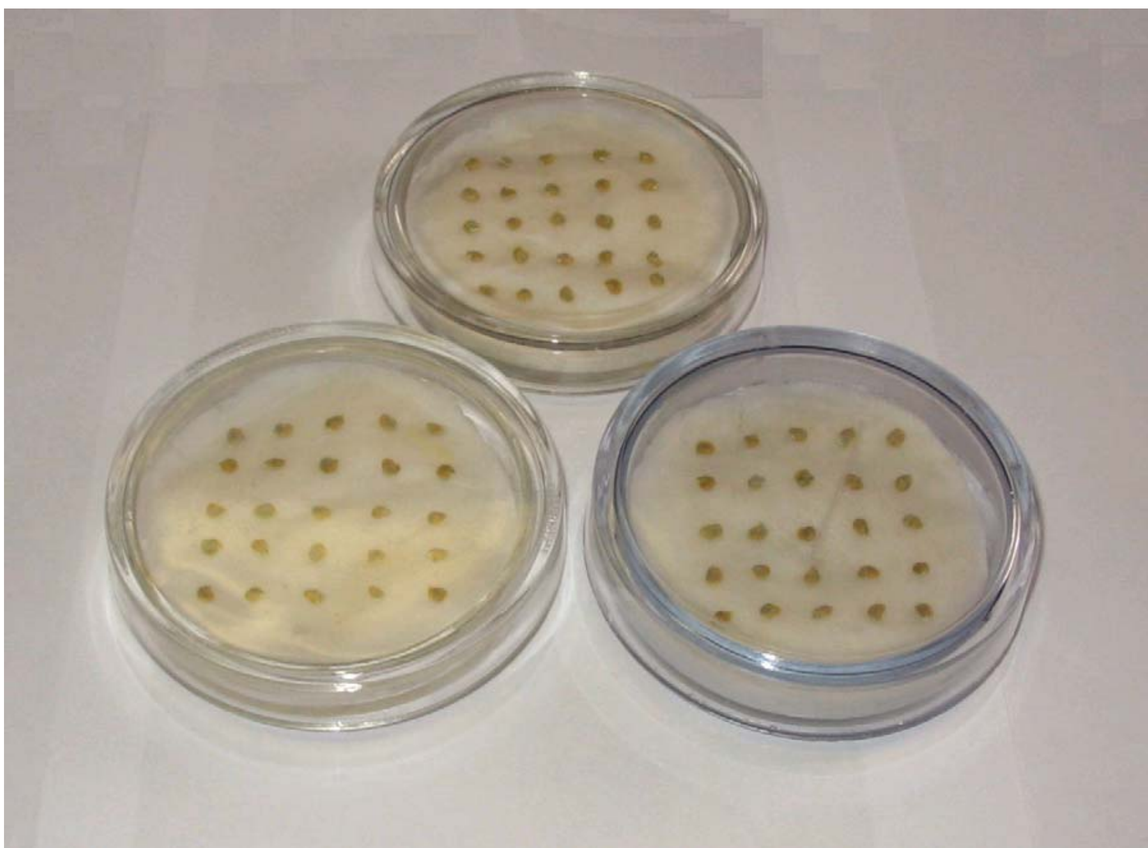

Figure 1. Seeds of *Opuntia ficus-indica* L. f. inermis (Web.) Le Houér. arranged in Petri dishes (10-cm diameter) with a cotton layer over which a disk of filter paper was disposed

The following assays were carried out:

1. Incubation of seeds in distilled water under the following conditions:

   a) daily photoperiodic regime; b) continuous light; and c) continuous darkness.

The following series of assays were performed under the photoperiodic regime:

1.1 Incubation in hydrogen peroxide solutions of 5, 15, and 30% H_2O_2.

1.2 Chemical scarification with concentratedH_2SO_4 during 0, 5, 10, 30, and 90 minutes. After washing with 0.1% KOH and then with tap water, seeds were incubated in distilled water.

1.3 Chemical scarification with Schweizer Reagent (dissolvent of cellulose) during 0, 30, 60, and 90 minutes. After washing, seeds were incubated in distilled water.

1.4 Scarification with concentrate H_2SO_4 during 5 minutes, followed by incubation in H_2O_2 solutions: 0, 1.0, 2.5, and 5.0%.

1.5 Scarification with Schweizer Reagent during 30, 60 and 90 minutes, followed by incubation in H_2O_2 solution (5%).

2. The following series of assays were conducted under continuous darkness:

2.1 Lixiviation of seeds with running tap water during 1 to 7 days and then incubated in distilled water.

2.2 Seed wet-chilled at 4°C during 0 to 6 weeks and then incubated in distilled water.

2.3 Direct incubation in solutions of thiourea: 0, 0.15, 0.60 and 1.2%.

2.4 Direct incubation in KNO_3 solutions from 0 to 1.5%.

2.5 Treatments of hydration-dehydration cycles (12/12 hours) carried out at 25°C, given 0, 2, 4,and 8 cycles, and then incubated in distilled water.

2.6 Priming the seeds using manitol solution of osmotic potentials –1.5 and –3.0 MPa during 10 and 20 days at 1°C, and then incubated in gibberellic acid solution (500 mg kg^{-1}).

All the assays followed a completely randomized design with three replications of 25 seeds for each treatment. Data were expressed as percentage of seeds germinated during the course of the incubation period or at the maximum time taken to stop germination. Percentages were arc sine transformed and subjected to ANOVA. Means were separated according to Tukey's test ($p < 0.05$). The 95% confidence intervals of the means were also calculated. In some instances, the number of seeds used per replication was indicated by N.

RESULTS

Table 1 shows that fruits of about 150 g fresh weight contain an average of approximately 440 seeds of which about 56% were aborted. One thousand normal seeds weighted 14 grams. All normal seeds were viable, having only 3% of low vigor. The imbibition capacity was similar for intact, scarified and split seeds.

Table 1. Quality of *Opuntia ficus-indica* seeds used in the assays, which were obtained from fruits with 149.8 ± 2.5 g fresh weight (95% CI is shown between parenthesis)

Seeds per Fruit N=35			**Weight of 1,000 Seeds (g) N = 100**	**Viability (%) N = 50**		**Imbibition (g H_2O 100 g^{-1}) N = 100**		
				Vigor				
Number	**Normal (%)**	**Aborted (%)**		High	Low	**Intact**	**Scarified**	**Split**
437 (47.4)	44.2	55.8	14.3 (0.9)	97.2 (2.6)	2.8 (2.6)	46.5 (6.2)	48.9 (4.5)	50.3 (9.1)

The germination responses of seeds to the action of different physical agents (Table 2) showed that seeds of this species require light, given in photoperiodic regime during 40 or 60 days, to stimulate germination, while continuous light or darkness did not induce germination.

Table 2. Effect of light and darkness on the time course of germination

of *Opuntia ficus-indica* seeds

Treatment	Days of Incubation	Germination Percentage Mean ±95% CI	
Photoperiod 14/10 h (light/darkness)	20	1.3 ± 5.7	a
	40	8.0 ± 9.9	b
	60	9.3 ± 5.7	b
Continuous light	20	0.0	a
	40	0.0	a
	60	1.3 ± 5.7	a
Continuous darkness	20	0.0	a
	40	0.0	a
	60	1.3 ± 5.7	a

Different letters indicate significant differences at $p < 0.05$.

The series of assays conducted under photoperiodic conditions indicate that H_2O_2 5% also had a stimulant effect on germination (Table 3). Chemical scarification using sulphuric acid produced germination percentages generally higher than those for seeds incubated in H_2O_2 5% (Table 4). In contrast, chemical scarification using Shweizer Reagent produced germination percentages generally lower than those obtained with H_2O_2 5%. The combination of scarified seeds (H_2SO_4 for 5 minutes) and incubation in H_2O_2 from 1 to 5% (Table 5) had a synergistic effect on the process, achieving a high germination rate in a short time. Although at germination percentages lower than those obtained with the combination H_2SO_4 - H_2O_2, Schweizer Reagent – H_2O_2 (Table 6) had also a synergistic action on seed germination.

None of the six assays carried out to establish if different agents could remove the inhibitory effect of continuous darkness on seed germination during the incubation period were able to eliminate the mentioned effect (data not shown).

Table 3. Effect of different concentration of H_2O_2 on the time course of germination of *O. ficus-indica* seeds under photoperiodic conditions

Treatment	Days of Incubation	Germination Percentage Mean ±95% CI
H_2O_2 5%	20	8.0 ± 9.9 a
	30	17.3 ± 15.2 b
	35	24.0 ± 17.2 b
	50	30.7 ± 5.7 b c
	60	32.0 c
H_2O_2 15%	60	0.0 e
H_2O_2 30%	60	0.0 e
H_2O_2 0% (control)	35	2.7 ± 5.7 a
	50	4.0 a
	60	5.3 ± 5.7 a

Different letters indicate significant differences at $p < 0.05$.

Table 4. Effect of chemical scarification with H_2SO_4 or Schweizer Reagent during different times on the germination of *O. ficus-indica* seeds incubated under photoperiodic conditions

Treatment and Time (minutes)		Days of Incubation	Germination Percentage Mean ±95% CI
H_2SO_4	5	40	58.6 ± 11.5 a
	10		40.0 ± 9.9 b
	30		40.0 ± 9.9 b
	90		32.0 ± 9.9 c
	0		5.3 ± 5.7 d
Schweizer Reagent	30	30	16.0 ± 26.3 a
	60		12.0 ± 9.9 a
	90		9.3 ± 5.7 a b
	0		5.3 ± 15.2 b

Different letters for each chemical product indicate significant differences at $p < 0.05$.

Table 5. Effect of different concentrations of H_2O_2 on the germination of *O. ficus-indica* seeds previously scarified with H_2SO_4 during 5 minutes and incubation under photoperiodic conditions

Treatment			**Germination Percentage (Mean ±95% CI) at day 30th (completed process)**	
		1.0%	80.0 ± 34.4	a
H_2SO_4	H_2O_2	2.5 %	86.7 ± 20.7	a
		5.0 %	85.3 ± 31.9	a
H_2SO_4 for 5 min. (control)			52.0 ± 45.5	b
		1.0% (control)	28.0 ± 19.9	c
H_2O_2		2.5% (control)	26.7 ± 15.2	c
		5.0% (control)	29.3 ± 15.2	c

Different letters indicate significant differences at $p < 0.05$.

Table 6. Effect of H_2O_2 2.5% on the germination of *O. ficus-indica* seeds previously scarified with Schweizer Reagent during different times and incubation under photoperiodic conditions

Treatment and Time (minutes)			**Germination Percentage (Mean ±95% CI) at day 30th (completed process)**	
		30	60.0 ± 9.9	a
H_2O_2	Schweizer	60	54.7 ± 25.0	a
		90	52.0 ± 19.9	a
Control from Table 5: H_2O_2 2.5%			26.7 ± 15.2	b
		30	16.0 ± 26.3	b
Schweizer		60	12.0 ± 9.9	b c
		90	9.3 ± 5.7	c

Different letters indicate significant differences at $p < 0.05$.

DISCUSSION

The size of the fruits depends on the percentage of normal and aborted seeds, the age and size of the plant, and the number of flowers per plant (Barbera et al., 1994; Rojas-Aréchiga and Vázquez-Yanes, 2000). Fruit weight in *O. ficus-indica* is also affected by the order of production of the flower bud (Reyes-Agüero et al., 2006). Thus, floral buds that sprout early usually become heavier fruits.

Weight of 1,000 seeds was similar to that reported by Olvera-Carrillo et al. (2003) for *O. tomentosa*. According to the review carried out by Reyes-Agüero et al. (2006), the average of seeds per fruit varies considerably, from 55 in *O. brunneogemmia* (F. Ritter) C. Schlindwein to 208 in *O. rastrera* F.C.A. Weber. These authors also reported that in *Opuntia* fruit cultivars with different degrees of domestication and wild plants growing in the meridional highlands of Mexico had from 16 to 518 seeds per fruit. The number of normal seeds per fruit in our study was slightly lower than those reported for Israeli and Mexican *O. ficus-indica* cultivars (Reyes Agüero et al., 2006). This fact may be attributed to cultivar genetic characteristics and/or environmental conditions.

The imbibition capacity would be largely a function of seed dry mass and the structure of seed coat, whose mechanism involves the walls of dead cells. Notwithstanding, the similar percentage of water imbibed by intact, scarified, and split seeds indicates that the hard seed coat, in this species, may function for mechanical protection of the embryo rather than for impermeability (Werker, 1997).

Our data showed that there exists an embryo dormancy mainly caused by a mechanical barrier to radicle protrusion that can be removed by chemical scarification of seeds with sulphuric acid or Schweizer Reagent. These agents can loosen the cellulose fabric and the incased lignin of the hard-coated shell that envelops the embryo. Evidently, chemical agents rather than an abrasive powder must carry out scarification, because the inner third integument (lignified funiculus) might not be pierced by this mechanical procedure. The use of sulphuric acid has been regularly employed to increase seed germination in other *Opuntia* species (Potter et al., 1984; Olvera-Carrillo et al., 2003). However, embryo envelopes not only act as a mechanical barrier but also may influence germination in an indirect manner. For example, embryo envelopes, acting as filters, can change light qualitatively or quantitatively (Ballard, 1973).

Another class of dormancy showed by this species has a physiological origin that was partially removed by two agents: light as a photoperiodic regime and hydrogen peroxide solution. Enforced dormancy is regulated by environmental conditions, such as light, and seeds are ready to germinate immediately upon the removal of the environmental limitation (Alcorn and Kurtz, 1959; McDonough, 1964; Rojas-Aréchiga and Vázquez-Yanez, 2000). Positive photoblastism is common among cacti and in the genera *Opuntia* (Reyes-Aréchiga and Vázquez-Yanez, 2000). Light requirement for germination found in our study is a feature shared by other *Opuntia* species. The studies of Olvera-Carrillo et al. (2003) and Monteiro et al. (2005) demonstrated the importance of light regime in the germination of *O. stricta* Haw. and *O. tomentosa* S.D. seeds, respectively.

Hydrogen peroxide solution, acting as oxidant agent, regulates primary or innate dormancy, taking into account that this class of dormancy that prevents seed germination on the mother plant and for a time after dispersal, could be caused by asphyxia of the embryo by the hard integuments. The oxygen supplied by H_2O_2 dissolved in water and normally available for the embryo, should serve for the oxidation of phenolic compounds, as occur in sugar-beet seeds (Coumans, 1974).

In short, light has a stimulant effect on germination and H_2O_2 promoted germination of the *O. ficus-indica* seeds used in this study. Because scarification with H_2SO_4 followed by incubation of seeds in a solution of

H_2O_2 under photoperiodic conditions gave the maximum germination percentage in the shortest time, the main objective of this work was fulfilled.

The series of assays conducted under continuous darkness to determine if some agents could remove the inhibitory effect imposed by darkness showed that the tested substances that have been cited to be effective on the germination of seeds of different plant species, such as thiourea as promoter, nitrate as stimulant agent (Salisbury and Ross, 1994), discontinuous hydration (Dubrovsky, 1996; Ren and Tao, 2003), cold stratification, priming treatments, etc., were unable to induce germination under the darkness condition during the incubation period. Consistent with our findings, Olvera-Carrillo et al. (2003) reported that darkness inhibited germination of *Opuntia tomentosa* D.C. after 130 days of incubation at constant (24°C) or alternating temperature (20-35°C, 18/6 hours day^{-1}) and Monteiro et al. (2005) found that no germination of *O. stricta* Haw. seeds occurred in dark conditions when seed germination was evaluated under three constant temperature regimes (25, 30, and 35°C).

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