



Germination of the hard seed coated *Opuntia tomentosa* S.D., a cacti from the México valley

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Received 4 December 2001; received in revised form 25 June 2002; accepted 28 August 2002

Abstract

Germination of *Opuntia tomentosa* seeds collected during 1998 was evaluated using treatments of scarification (H_2SO_4), gibberellic acid (GA_3), dry heat, soaking, light, after-ripening and stratification at constant and alternating temperatures. Seeds were positive photoblastic, germinated best at constant temperatures, and showed a combined dormancy (physiological and physical). Acid scarification, GA_3 , and heating induced germination, soaking and stratification did not. After 15 and 18 months, GA_3 inhibited germination. Dormancy of seeds collected during 2000 was overcome with a short scarification. Differences between baths could be due to 1998 was very dry due to “El Niño”. It is important to identify maternal effects.

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Keywords: Acid scarification; After-ripening; Dry heat; Physical dormancy; Physiological dormancy; Seed age; Stratification

1. Introduction

Seed germination in arid and semi-arid regions has been studied mainly in annual species (Gutterman, 1993; Kigel, 1995, pp. 645–699) but their germination patterns differ widely from those of perennial species. Many perennial species present a combination of endogenous (morphological and/or physiological) and exogenous

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(physical and/or mechanical) dormancy (Morpeth and Hall, 2000). Seeds with water impermeable coverings are common among perennial species, such as members of the Fabaceae. They have a physical dormancy (Baskin and Baskin, 1998) and the process of seed coat breakdown distributes germination of seeds over time to increase chances of successful establishment (Egley, 1993, pp. 207–222).

The Cactaceae is one of the perennial plant families, which germination has been poorly studied (Rojas-Aréchiga et al., 1997). In the subfamily Opuntioideae a strongly lignified funicular tissue surrounds the seed (Bregman and Bouman, 1983). Due to that viable seeds of *Opuntia* spp. present low germination (Baskin and Baskin, 1977). Immersion in concentrated sulphuric acid increases germination in some species of *Opuntia* (Potter et al., 1984), while mechanical scarification gives better results for *Opuntia joconostle* F.A.C. Weber (Sánchez-Venegas, 1997). Temperature requirements differ among species; constant temperatures enhance germination of *O. phaeacantha* var. *discata* (Griffiths) L.D. Benson and Walk, and *O. lindheimeri* Engelm. (Potter et al., 1984), while, temperature fluctuation improve germination of *O. puberula* Pfeif. (Godínez-Alvarez and Valiente-Banuet, 1998). Also in the seed coat of two former have been detected the presence of inhibitory compounds (Pilcher, 1970; Potter et al., 1984), as well as in *O. joconostle* (Sánchez-Venegas, 1997). Stratification and after-ripening are germination requirements for *O. compressa* (Salisb.) T. Macbr., and *O. rastrera* F.A.C. Weber, respectively (Baskin and Baskin, 1977; Mandujano et al., 1997).

The prickly pear, *O. tomentosa* S.D. (Cactaceae, subfamily Opuntioideae) is distributed in the Valley of Mexico and the arid zone of the Central Plateau (Bravo-Hollis, 1978). In the Valley of Mexico it grows in a volcanic area (Pedregal), at a xerophilous shrubland on a shallow soil (Rzedowski, 1994, pp. 9–65). Climate in the area is semi-arid and modified by altitude (2240 m above sea level), however, water availability is highly restricted due to a great percolation caused by the rock structure and to low water retention from the shallow accumulated soil and also a daily wide temperature fluctuation could occur (as wide as $\approx 34^{\circ}\text{C}$). Seasonal rain distribution, altitude and the rock and soil distribution turn the area into a heterogeneous and discontinuous arid environment.

To understand, from an ecophysiological point of view, the effect of the main environmental factors at the Pedregal on *Opuntia* seed dormancy breaking, we studied on *O. tomentosa* seed germination the effect of acid scarification, temperature, light quality, after-ripening, stratification, exogenous gibberellins, soaking, and extreme temperatures during dry season. Also it was considered the variation in seed dormancy between two seed production years (1998 and 2000).

2. Material and methods

2.1. Study area

The Ecological Reserve “El Pedregal de San Angel” ($19^{\circ}19'N$, $99^{\circ}11'W$) is located at the southern part of the Valley of Mexico. Mean annual precipitation is 870 mm

and mean annual temperature is 15.5°C (Valiente-Banuet and de Luna, 1990). During the dry winter extreme temperatures can reach more than 50°C on the soil surface (Olvera-Carrillo, 2001).

2.2. Study plant and seed collection

At the study site, *O. tomentosa* blooming starts in April and ends in June. Ripe fruits are found from August to October, they are purplish red coloured and contain on average 80 ± 9 seeds per fruit, seed weight is 0.016 ± 0.001 g (Olvera-Carrillo, 2001). Seeds of *O. tomentosa* produced during 1998 have hard coat dormant seeds, and immediately after collection do not germinate after scarification with sulphuric acid during 1–45 min (Olvera-Carrillo, 2001). Seeds were collected during the shedding season of years 1998 and 2000. At the laboratory, seeds were manually removed from the fleshy pulp, washed with tap water, and air-dried in darkness. Seeds were stored in paper bags at laboratory temperature (23–25°C, 20–50% RH).

2.3. General procedures

Germination trials were performed sowing seeds in Petri dishes with 1% agar (Bioxon, Mexico) and incubated in growth chambers (Lab-Line Instruments, Inc., 844, IL, USA) at constant (24°C) or alternating temperature (20–35°C, 18/6 h day⁻¹). Temperature fluctuation was determined in base to mean daily temperature fluctuation during the rainy season in the area. Before sowing, seeds were disinfected with a 15% sodium hypochlorite and a 0.2% fungicide solution (Captan 50 [*cis*-*N*-[(trichloromethyl) thio]-4-ciclohexene-1, 2-dicarboximide], AGM, Mexico). Three replicates of 30 seeds per Petri dish per treatment were used. Germination was recorded every other day until 130 days of incubation. Germination occurred when radicle and hypocotyls emerged.

Growth chambers were equipped with fluorescent cool white (F20T12/CW, Sylvania, 20 W) and incandescent (B9, General Electric, 25 W) lamps. Photoperiod was 12 h day⁻¹, red:far red ratio (R:FR) was 1.73, photon flux density (PFD) was $33.21 \mu\text{mol m}^{-2} \text{s}^{-1}$. All light measurements were made with a portable spectroradiometer (LI-COR, Inc., LI 185 B, Nebraska, USA). Red and Far red (FR) light was measured between 654–666 and 724–736 nm, respectively. White light photon flux was measured from 400 to 750 nm.

2.4. Seeds collected in 1998

2.4.1. Effect of light, scarification, GA₃ and temperature

Two-month-old seeds were scarified with concentrated H₂SO₄ for 0, 45, 60 and 90 min with the addition of 0, 1 and 2 mg ml⁻¹ GA₃ (G-7645 Sigma Chemical Co, USA) and placed in growth chambers at both temperatures with three light conditions: white light, FR light and darkness. To provide the light condition required for FR treatments, Petri dishes were placed inside Plexiglass boxes (34 × 44 × 10 cm) made with red and blue acrylic (Series No. 2424 and 2423,

respectively, Rohm and Hass, Mexico; R:FR = 0.05, PFD = $9.742 \mu\text{mol m}^{-2} \text{s}^{-1}$). Darkness was obtained by wrapping Petri dishes with two layers of aluminium foil.

2.4.2. Effect of seed age

Seeds stored in the laboratory were scarified with concentrated H_2SO_4 for 0 and 90 min with the addition of 0, 1 and 2 mg ml^{-1} GA_3 , incubated with white light at constant temperature. Germination trials were carried out when seeds were 2, 7, 11, 15 and 18 months old.

2.4.3. Effect of stratification

Seven-month-old seeds stored in the laboratory were scarified with concentrated H_2SO_4 for 0 and 90 min, sown on 1% agar and exposed to a 21 days stratification period at 4°C and 12 h of fluorescent light in a refrigerator (American, Mexico, D.F.). Seeds were then incubated at constant and alternating temperatures, using non stratified seeds as controls, with and without scarification. Duration of stratification was calculated from temperatures recorded at 4°C (or less) during winter of 1998. Temperatures were recorded with a data logger (Campbell Scientific, 21X, Logan, UT, USA) provided with an electronic sensor (Vaisala, HMP 35C, Helsinki, Finland), and placed at a height of 1.5 m from the soil at the study site.

2.4.4. Effect of dry heat and soaking in water

Nine-month-old seeds received two separate treatments: (a) dry heat at 60°C and (b) soaking in water for 0, 24, 48 and 72 h. After receiving the complete treatment, seeds were sown in agar at constant temperature in all cases.

In the dry heat experiment seeds received an average thermoperiod of 4 h day^{-1} until 90 and 180 h of cumulative time (23 and 45 days, respectively) were achieved. Time of exposure to 60°C was calculated from soil temperature at the study site during dry season. In order to determine adequately the number of hours of exposition to 60°C , soil temperature were recorded each hour in periods of 24 h in 21 sites in November 1998 and March 1999 with data loggers Hobo Temp (H01-001-01, Onset Computer Co., Pocasset, MA, USA), and these data were correlated with the records of the data logger placed at 1.5 m from the soil, which recorded air temperature during all the dry season (November 1998–April 1999).

2.5. Seeds collected in 2000

Two months after collection seeds were scarified with concentrated H_2SO_4 during 0, 5, 10, 30, 45, 60, 75, 90 and 120 min and placed in a growth chamber with constant temperature and white light. Germination was recorded after 90 days in this case.

2.6. Statistical analyses

Cumulative germination percentages of each replicate were arcsine transformed (Zar, 1984) and fitted to an exponential sigmoid model ($Y = a / \{1 + b \exp[-cx]\}$) to obtain maximum germination rates (MGR, first maximal derivative) and lag time

(LT). First derivative along the exponential sigmoid curve was fitted to a Gaussian model ($Y = a + b \exp(-0.5[\{x-c\}/d]^2)$) to obtain mean germination time (MGT) and synchrony (S) (Boas, 1983; Finkelstein and Carson, 1986). Models were fitted using Table Curve 2D, v. 3 (AISN Software, Chicago, IL, USA). Final germination percentages, MGR, LT, MGT and S of the replicates were compared by ANOVA (Sokal and Rohlf, 1981). Means were compared by the least significance difference (LSD) interval method at $p < 0.05$, using Statgraphics (v. 5.0, Statistical Graphics Corporation, Englewood Cliffs, NJ, USA).

3. Results

3.1. Seeds collected in 1998

3.1.1. Effect of light, scarification, GA_3 and temperature

In 2-month-old seeds, FR and darkness treatments inhibit germination after 130 days of incubation at both temperatures; therefore, these results were excluded from analysis. On the contrary, white light treatments induced germination at constant (Fig. 1) and alternating temperatures (Fig. 2), but germination percentages were always higher at constant. Temperature and scarification had a significant effect on germination percentage ($F_{(1,59)} = 19.56$ and $F_{(4,59)} = 11.33$, respectively, both with $p < 0.0001$). Gibberellic acid and the interaction between the three factors were not significant ($F_{(1,59)} = 0.35$, $p = 0.56$ and $F_{(4,59)} = 2.08$, $p = 0.09$). Best germination percentage was obtained with 90 min of scarification and the addition of GA_3 in 2 mg ml^{-1} at constant temperature (Fig. 1i) without significant differences to 60 min of scarification and 1 mg ml^{-1} GA_3 (Fig. 1f). MGR, MGT and S had significant effects due to scarification ($F_{(4,59)} = 3.61$, $p = 0.012$; $F_{(4,59)} = 3.11$, $p = 0.023$ and $F_{(4,59)} = 5.73$, $p = 0.0007$, respectively).

When each temperature was analysed separately, the favourable effect of scarification remained significant in germination percentage at both temperatures ($F_{(4,29)} = 8.97$, $p = 0.0001$ and $F_{(4,29)} = 5.26$, $p = 0.0035$, respectively). The effect on MGR, MGT and S was significantly lower at alternating temperature ($F_{(4,29)} = 3.52$, $p = 0.025$; $F_{(4,29)} = 4.03$, $p = 0.015$ and $F_{(4,29)} = 11.06$, $p = 0.0001$, respectively).

3.2. Effect of seed age

Seeds stored in the laboratory for 2, 7, 11, 15 and 18 months showed a tendency to reduce their germination capacity with time (Fig. 3). Germination percentages of control (Fig. 3a) and seeds scarified 90 min (Fig. 3b) were fitted to a linear model with negative slope ($Y = a + bx$, Table 1). The treatments of 90 min scarification with addition of GA_3 in 1 and 2 mg ml^{-1} and those with only the application of GA_3 in 1 and 2 mg ml^{-1} (Figs. 3c–f), were fitted to an exponential model with negative slope ($Y = a + be^x$, Table 1). Even though the negative slope was significant, in the case of untreated seeds, there were significant differences only between seeds of 15 and 18 months old with those of 2 months old ($F_{(4,14)} = 3.79$, $p = 0.04$).

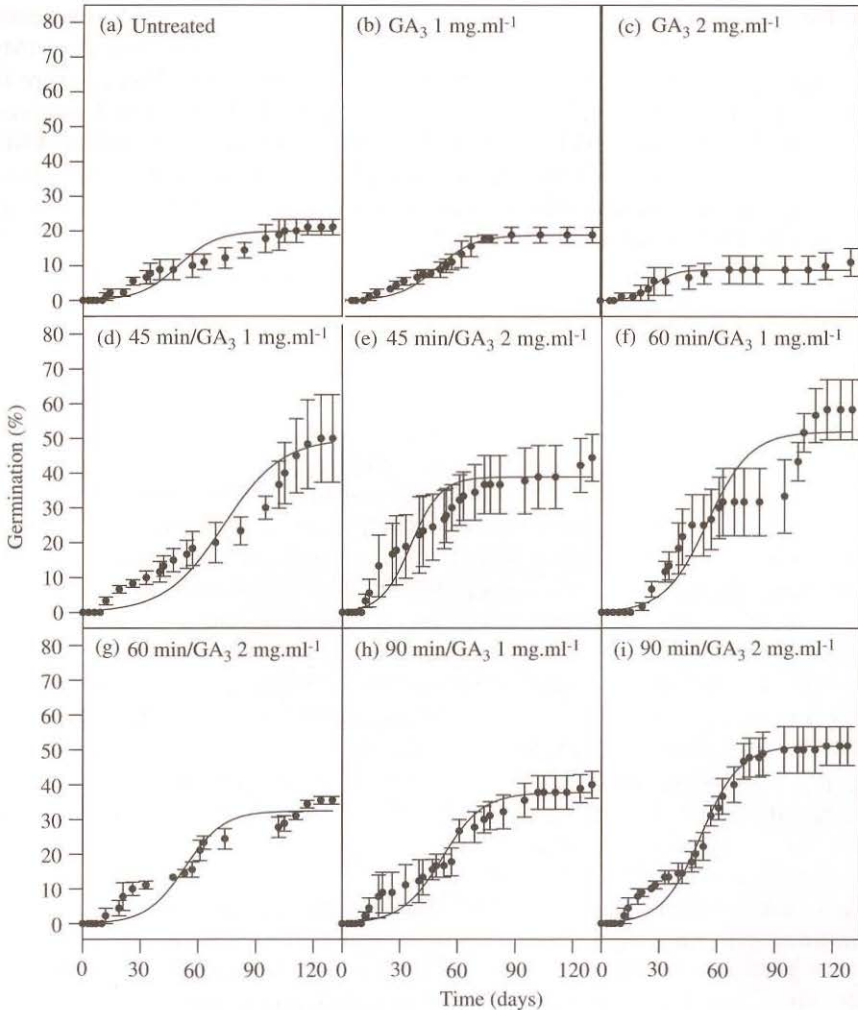


Fig. 1. Effect of GA₃ and acid scarification on cumulative germination percentages of 2-month-old *O. tomentosa* seeds collected in 1998. Seeds were incubated in white light at 24°C. Determination coefficients of the exponential sigmoid curves were 0.88–0.98 ($p < 0.00001$ in all cases). Means are shown ± 1 S.D.

3.3. Effect of stratification

Germination percentages were significantly affected by scarification and incubation temperatures ($F_{(1,23)} = 90.25$, $p < 0.0001$ and $F_{(1,23)} = 5.68$, $p = 0.03$, respectively, Fig. 4). Stratification significantly reduced germination percentages (Figs. 4b and f). The highest germination percentages occurred at constant temperature, in the scarification treatment (Fig. 4c) and when seeds were exposed to scarification and stratification simultaneously (Fig. 4d). Accordingly, scarification significantly

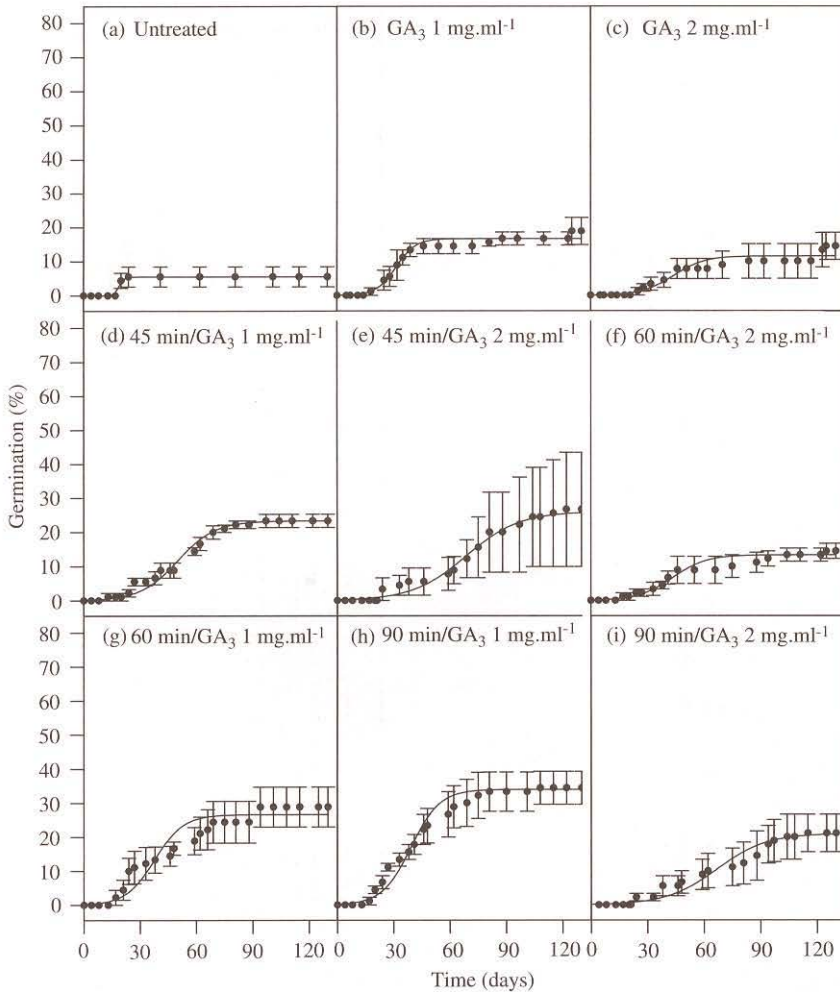


Fig. 2. Effect of GA_3 and acid scarification on cumulative germination percentages of 2-month-old *O. tomentosa* seeds collected in 1998. Seeds were incubated in white light at 20–35°C. Determination coefficients of the exponential sigmoid curves were 0.88–0.98 ($p < 0.00001$ in all the cases). Means are shown ± 1 S.E.

increased MGR ($F_{(1,23)} = 13.95$, $p = 0.0018$) while stratification significantly reduced it ($F_{(1,23)} = 16.43$, $p = 0.0009$).

3.4. Effect of soaking in water and dry heat

Dry heat-induced significant effects in germination percentage and LT ($F_{(3,7)} = 16.21$, $p = 0.02$ and $F_{(3,7)} = 4.56$, $p = 0.045$, respectively). Exposure to 60°C for a cumulative time of 180 h (Fig. 5c) increased significantly germination

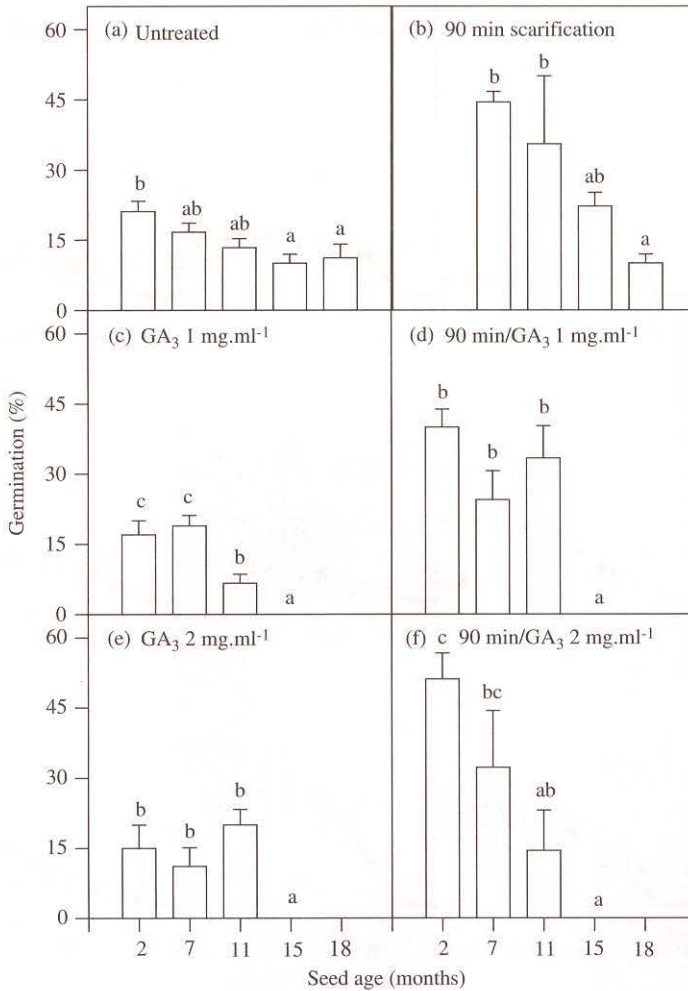


Fig. 3. Effect of GA₃ and acid scarification on final germination percentages of different ages *O. tomentosa* seeds collected in 1998. Seeds were incubated in white light at 24°C. Letters above error bars indicate homogeneous groups inside each treatment ($p < 0.05$). Means are shown ± 1 S.E.

percentages. Soaking in water did not induce significant effects in any of the analysed parameters ($p > 0.05$ in all cases, Figs. 5d–e).

3.5. Seeds collected in 2000

Two-month-old seeds germinated in average 20% (Fig. 6a) or higher when scarified (Figs. 6b–i). When 2-month-old seeds collected in 1998 were compared with those collected in 2000, untreated seeds showed similar germination percentages

Table 1

Statistical parameters of adjustments to linear ($Y = a + bx$) and exponential ($Y = a + be^x$) models relating percentage germination (arcsine transformed) as a function of seed age

Treatment	r^2	F	p
Untreated ^a	0.54	14.96	0.0019
90 min scarification ^a	0.61	15.42	0.0028
GA ₃ 1 mg ml ^{-1b}	0.92	85.66	0.00004
GA ₃ 2 mg ml ^{-1b}	0.64	12.65	0.0093
90 min scarification/GA ₃ 1 mg ml ^{-1b}	0.81	42.25	0.00007
90 min scarification/GA ₃ 2 mg ml ^{-1b}	0.72	25.73	0.0005

^aFitted to $Y = a + bx$.

^bFitted to $Y = a + be^x$.

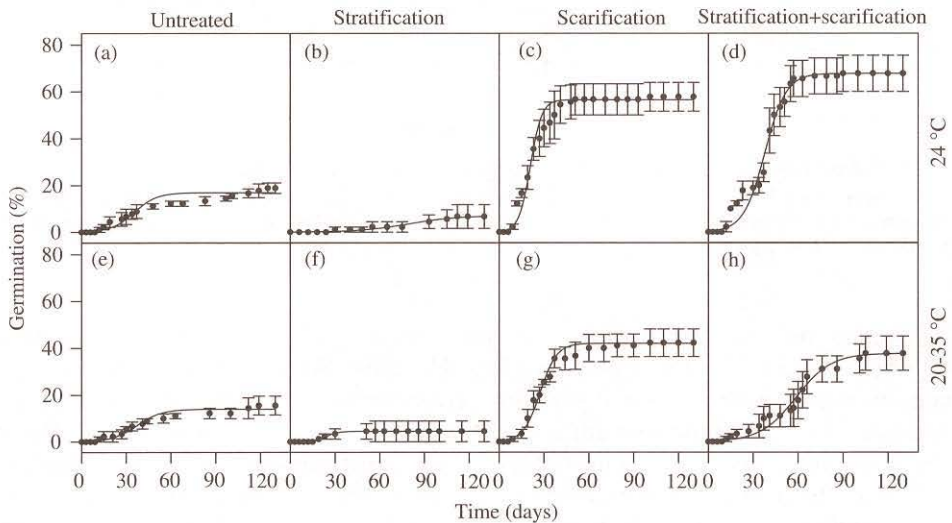


Fig. 4. Effect of stratification (21 days at 4°C) and/or acid scarification (0, 90 min) on cumulative germination percentages of 7-month-old *O. tomentosa* seeds collected in 1998. Seeds were incubated in light at 24°C (a)–(d) or 20–35°C (e)–(h). Determination coefficients of the exponential sigmoid curves were 0.9–0.99 ($p < 0.00001$ in all cases). Means are shown ± 1 S.E.

(Figs. 1a and 6a), but the scarified seeds collected in 2000 germinated to higher percentages than seeds collected in 1998 ($F_{(17,53)} = 9.69$, $p < 0.0001$).

4. Discussion

O. tomentosa seeds produced during 1998 were positive photoblastic and had a deep dormancy, because in addition to their physical dormancy, they also showed physiological dormancy probably caused by embryo immaturity. The initial requirement of long scarification times and high concentrations of GA₃ to achieve

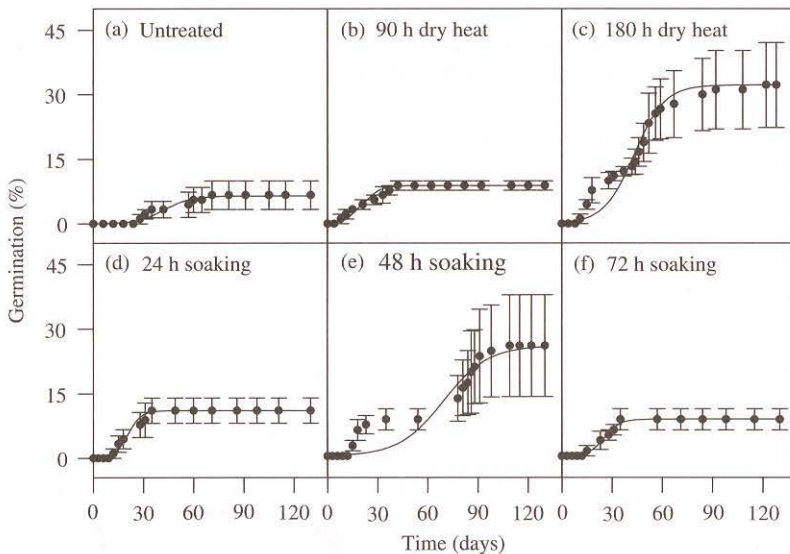


Fig. 5. Effect of dry heat (0, 90 and 180 h at 60°C) or soaking in water (0–72 h) on cumulative germination percentages of 9-month-old *O. tomentosa* seeds collected in 1998. Seeds were incubated in light at 24°C. Determination coefficients of the exponential sigmoid curves were 0.92–0.99 ($p < 0.00001$ in all cases). Means are shown ± 1 S.E.

an average of 50% germination, contrasts markedly with the complete inhibition induced by GA₃ in 15- and 18-month-old seeds. At that time, GA₃ in high concentrations could be turned toxic for permeable seeds. Nevertheless, the embryo had not yet acquired the strength to break seed coat and needed scarification. Other *Opuntia* seeds require exogenous GA₃, previous scarification to germinate (Sánchez-Venegas, 1997) or an after-ripening period of 1 year at least (Potter et al., 1984; Mandujano et al., 1997). The high concentrations of GA₃ used here, are commonly used with species from temperate regions (Gómez-Campo, 1985, pp. 237–247) and the study site is a temperate region due to its altitude (Rzedowski, 1994). However, along time, changes in seed response to GA₃ occur (Karssen et al., 1989).

Positive photoblastism is common among cacti and in the genera *Opuntia* (Rojas-Aréchiga and Vázquez-Yanes, 2000). Darkness or FR inhibited germination of *O. tomentosa*, this seed response has been related to detection of the proximity of the soil surface, because it is difficult for little seedlings emerge from deep layers of the soil (Rojas-Aréchiga et al., 1997; Leishman et al., 2000, pp. 31–57). On the other hand, cacti seedlings ought to reach fast the surface because the short establishment period in seasonal habitats (Altesor et al., 1992). In the Pedregal, a mechanism of light detection could be necessary to detect the presence of light on the soil accumulated on the lava, opposite to the dark sites in the crevices, as occur in other species of the area (Vázquez-Yanes and Orozco-Segovia, 1990).

The favourable effect of dry heat on germination (180 h at 60°C) indicates that the high temperature at the soil surface and on the rocks during the dry season of the

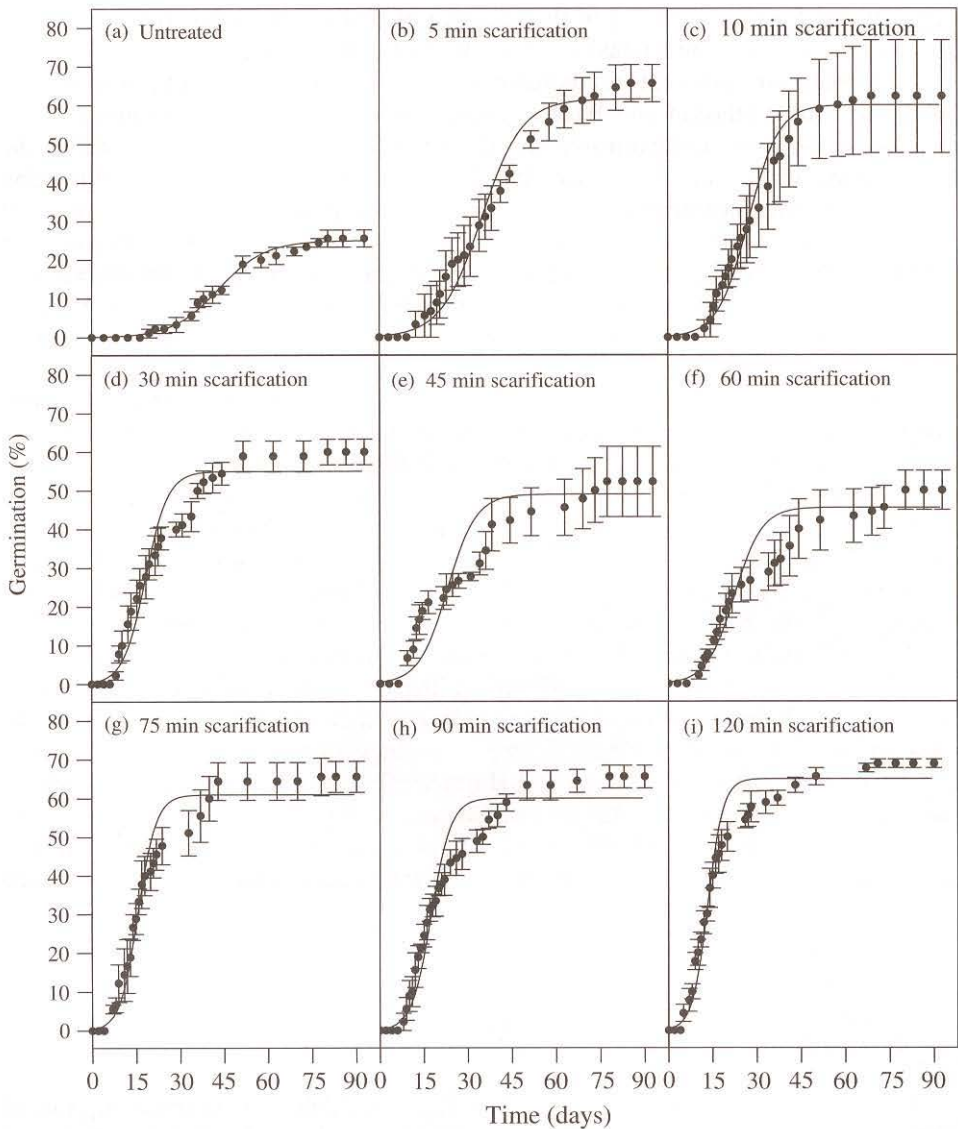


Fig. 6. Effect of acid scarification on cumulative germination percentages of 2-month-old *O. tomentosa* seeds collected in 2000. Seed were incubated in white light at 24°C. Determination coefficients of the exponential sigmoid curves were 0.85–0.99 ($p < 0.00001$ in all cases). Means are shown ± 1 S.E.

year could be one of the major causes to break the hard seed coat. During dry storage in the laboratory, seeds were exposed to stable conditions, which did not improve the germination percentage of untreated seeds over time. Temperature fluctuations are necessary to improve seed germination of some species in nature

(Baskin and Baskin, 1998), or in the presence of biotic factors that turn permeable the seed coats, as in the studied species (Olvera-Carrillo, 2001).

In the field, low temperatures increase the levels of endogenous GA₃ or sensitivity of the seed to it (Baskin and Baskin, 1998). The stratification period given to the studied species was unfavourable for the proportion of permeable seeds in the population, while for the seeds that ought to be scarified for germination stratification was meaningless. Other *Opuntia* species growing in regions with lower temperatures than in the Pedregal, show an increase in germination percentages when exposed to prolonged time of stratification, nevertheless, it is not an essential feature to break dormancy of all the seed population (Baskin and Baskin, 1977).

The presence of inhibitors in the seed coat is overcome by soaking in water in *O. phaeacantha* var. *discata* and *O. lindheimeri* (Pilcher, 1970) but in *O. tomentosa* it did not increase germination percentages. This could be due to a lack of inhibitors in this species, their removal during seed handling after collection, or simply because the hard seed coat did not become permeable with these treatments.

Between-years differences in seed germination behaviour were observed in *O. tomentosa*. The dormancy of seeds collected in 1998 was more complex than that of seeds collected in 2000. The latter only needed a short scarification (5 min) to germinate in higher percentages, while seeds of the same age collected in 1998 required 90 min in sulphuric acid to reach similar germination percentages. This could be related to the fact that 1998 was an unusually dry year due to the climatic phenomena known as “El Niño”, providing stressful conditions during seed development. Environmental conditions during seed development have shown to be very important in determining the degree of hardness of seed coats in several species (Marbach and Mayer, 1974; Argel and Humphreys, 1983). Maternal effects ought to be explored to understand the morphological and physical seed polymorphism (Guterman, 2000, pp. 59–84), which could be relevant for germination and establishment of species that grow in heterogeneous and changing stressing environments, as arid zones.

Acknowledgements

We would like to thank M.Sc. Mariana Rojas Aréchiga for technical support as well as B.Sc. José Antonio Hernández Gómez for his help in laboratory and fieldwork. This work was supported by CONACyT (The Mexican Council of Science and Technology) research Grant G0011N.

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