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María Elena Monroy Vázquez, Cecilia Beatriz Peña-Valdivia, José Rodolfo García, Eloy Solano, Huitziméngari Campos, and Eduardo García

*Postgrado en Botánica, Colegio de Postgraduados, Montecillo, 56230 México; †Universidad Nacional Autónoma de México, Herbario FEZA, Ciudad de México, 09230 México; ‡Instituto Politécnico Nacional, Jiquilpan, Michoacán, 59510 México; †Postgrado en Recursos Genéticos y Productividad, Colegio de Postgraduados, Montecillo, 56230 México

**ABSTRACT**

Protocols for seed germination in the *Opuntia* genus are different and unsuitable for all their species. Dormancy of *Opuntia* seeds can be modified by the combination of scarification and an oxidizing agent such as O$_3$, which could induce antioxidant and DNA-repair mechanisms or dormancy-breaking effects in hydrated seeds. The objective of this study was to evaluate the effects that the combination of mechanical and chemical scarification with exposition to sublethal O$_3$ doses have on seed germination and seedling growth of *O. streptacantha* Lem., *O. megacantha* Salm-Dyck, and *O. ficus-indica* (L.) Mill. Our hypothesis was that O$_3$ favors germination on scarified seeds and that the magnitude of the effect is species-dependent. Water uptake and germination were quantified in seeds every 48 h, until their roots reached a 5-mm length. The results were analyzed with an analysis of variance and multiple means comparisons with the Tukey test. Accelerated water uptake was observed during the first 48 h; the maximum average was 33.5% in all species. The combination of acid scarification and imbition in the presence of O$_3$ increased (P ≤ 0.05) germination (between 17.8 and 44.4%), mainly in *O. streptacantha*. O$_3$ increased germination, regardless of the species. O$_3$ can be used in sublethal doses to increase seed germination and seedling development in *Opuntia* genus.

**Introduction**

*Opuntia* is one of the most diverse and widely distributed genera in the Cactaceae family. It comprises 188 species, 78 of them native to México (Anderson, 2001; Reyes-Agüero, Aguirre-Rivera, and Flores-Flores, 2005). Archaeobotanical evidence shows that *Opuntia* spp. have been used by humans for more than 9000 years (Callen, 1966). In México *Opuntia* sp. have evolved from wild “nopaleras” (wild *Opuntia* communities) followed by the establishment of plants in gardens next to dwellings. *Opuntia* then became a formal crop in specialized commercial plantations; and finally, managed as a formal culture in commercial specialized plantations for the production of nopal vegetable (cladode) or “nopalito” (young tender cladodes), prickly pear (fruit), fodder nopal, or as a host of *Dactylopius coccus*, for carminic acid-dye-production, also known as cochineal (López-Palacios et al., 2015).

At the same time, during the continuous process of domestication, wild plants have differ from the domesticated types in morpho-physiological, genetic and chemical characters through the cultivation and selection of outstanding characters (Pickersgill, 2007). Those differences between domesticated species and their wild counterparts are known as domestication syndrome, which in some cases may decrease the ability of the domesticated types to survive in natural environments (Zohary, 2004). The modifications in the attributes that make up the domestication syndrome are diverse, and include seed size increase (when that plant structure is of anthropocentric interest), decrease or loss of seed dormancy, reduced seed dispersal and loss of chemical or mechanical protection against herbivores (McKey et al., 2010; Peña-Valdivia, Aguirre, and Arroyo-Peña, 2012; Pickersgill, 2007).

Reyes-Agüero, Aguirre-Rivera, and Flores-Flores (2005) recognized a domestication gradient in *Opuntia* after comparing 42 morphological characteristics in nopal plants, cactus pads, and prickly pears of 483 accessions. The authors identified *O. streptacantha*...
Lem. as the most wild, followed by *O. hyptiacantha* F.A. C. Weber and *O. megacantha* Salm-Dyck with an intermediate degree of domestication, and *O. albicarpa* Sheinvar and *O. ficus-indica* (L.) Mill. with the highest degree of domestication. Among the many changes related to *Opuntia* domestication, Reyes-Agüero, Aguirre-Rivera, and Flores-Flores (2005) defined that in *Opuntia* a decreasing amount and hardness of seeds as well a higher proportion of the aborted seeds per fruit occur.

Changes in the morphological characteristics of the seeds related to domestication have also been documented (Aguilar-Estrada, Reyes-Agüero, and Aguirre 2003). In a study on the effect of domestication in fruit biomass and physical seed characteristics, López-Palacios et al. (2015) observed that the number of seeds per fruit was higher (342 seeds) in species with a higher degree of domestication, while wild species had up to 50% fewer seeds (174 seeds). In addition, seed size and hardness were lower in normal seeds of wild species among the five species of the study (*O. albicarpa*, *O. ficus-indica*, *O. hyptiacantha* F.A.C. Weber, and *O. megacantha*). The seed coat of this genus is hard, requiring as low as 0.2 and as high as 4.6 kN for fracturing (Aguilar-Estrada, Reyes-Agüero, and Aguirre 2003; López-Palacios et al. 2015; Reyes-Agüero, Aguirre-Rivera, and Flores-Flores 2005).

Seeds of *Opuntia* spp. need time after ripening to break physiological dormancy (i.e. the embryo has low growth potential). This can be removed by scarification (Orozco-Segovia et al. 2007). The potential of *Opuntia* seed germination has been evaluated with different procedures in order to reduce or eliminate dormancy including chemical, mechanical and biological scarification, after-ripening as long as several years at room temperature, stratification, phytohormone application, exposure to photoperiod, to thermo-period and combinations of them (Beltrán 1984; Delgado-Sánchez et al. 2011, 2013; Mandujano, Montaña, and Rojas-Arèchiga 2005; Mandujano, Golubov and Rojas-Arèchiga 2007; Olvera-Carrillo et al. 2009a; Potter, Petersen, and Uckert 1984; Romo-Campos et al. 2010; Sánchez-Coronado et al. 2011; Sánchez-Venegas 1997). However, intra- and inter-specific results are significantly variable. Studies published generally include only one or two species, and no more than 23 of the 188 *Opuntia* species have been evaluated for dormancy (Anderson 2001).

Ozone (O$_3$) is a gas present in the troposphere. Yet, is the most phytotoxic air pollutant because of its higher oxidizing capacity, higher than that of O$_2$. Exposure in plants at for short periods in concentrations greater than 160 µg O$_3$ m$^{-3}$, causes chlorosis and necrosis on one or both sides of the leaves (Olszyk et al. 1990) and quick tissue dead (Vazquez-Ybarra et al. 2015). Frequent, regular and intermittent exposure adversely affects growth, productivity and quality of crops (Biswas et al. 2008). The decrease in the global production for this pollutant amounted from 72 to 121 Mt and 11 to 18 trillion dollars (Avnery et al. 2011). Still, recently interest has been focused on the study of positive effects on plant growth and seed germination (Abeli et al. 2016; Landesmann et al. 2013; Sudhakar et al. 2011; Vazquez-Ybarra et al. 2015).

Sudhakar et al. (2011) observed that in low doses O$_3$ in tomato (*Lycopersicon esculentum*) seeds resulted in faster germination rate and seedlings development with longer roots compared to a control without O$_3$. According to these authors the oxidation of the seed coat compounds by oxidizing agents like O$_3$ can promote cell signaling that triggers germination, such as peroxides and dioxides triggered the increase of ethylene synthesis and the abscisic acid (ABA) decreases in seedlings.

Landesmann et al. (2013) suggested that long time O$_3$ exposure probably acted as natural selection factor over weedy species. They evaluate the consequences of prolonged (4 years) O$_3$ (90 and 120 ppb) exposure on phenotypic traits linked to persistence in *Spergula arvensis*. Then they evaluated seed viability and dormancy in seeds produced by plants growing under O$_3$ exposition, and seed longevity in seeds coming from ambient and with added-O$_3$. They found that seeds in prolonged 90 ppb O$_3$ exposure had the highest germination level when stored at 75% RH and 25 °C and the lowest dormancy when subjected to several combinations of RH, low temperatures and store. These authors after proving that exposure of plants to O$_3$ increased seed persistence in the soil through maternal effect concluded that O$_3$ exposure could increase weed persistence in fields.

Abeli et al. (2016) investigated the effects of anomalous concentrations of O$_3$ mediated by summer heat waves on seed germination in alpine plants. They observed that few cases, among nine species, chronic exposure to O$_3$ (125 for 10 days’ treatment) enhanced seed germination compared to the control, suggesting that O$_3$ may induce antioxidant and DNA-repair mechanisms or dormancy-breaking effects in hydrated seeds.

Vazquez-Ybarra et al. (2015) evaluated the effects of 0.53 and 59.40 mg L$^{-1}$ O$_3$ applied weekly on the growth of lettuce (*Lactuca sativa* L.) plants in a hydroponic float system and compared to a control without O$_3$. These researchers observed that plants with 2.66 and 3.96 mg L$^{-1}$ O$_3$ doses completed growth and development with a significant increase in the root length, stem
diameter and shoot and root biomass compared to the control, after 10 weeks of growth. The authors suggested that the responses of lettuce plants to O₃ may result from the adaptive response that increased plant resistance to stress of certain doses of O₃, describing a behavior related with the hormesis concept.

The aim of this study was to evaluate the effects that the combination mechanical and chemical scarification with exposition to sub-lethal O₃ doses have on seed germination of O. streptacantha, O. megacantha and O. ficus-indica. Our hypothesis was that O₃ favors germination on the scarified seed and that the magnitude of the effect is species dependent.

Materials and methods

Plant material

Seeds of O. streptacantha, O. megacantha and O. ficus-indica (sample No. 1) were obtained from ripe fruit, collected in 2012, from plants growing in the Germplasm Bank of the Centro Regional Universitario Centro-Norte at the Universidad Autónoma Chapingo, located 4 km southwest of the city of Zacatecas, México, in the town El Orito (22°44'49.6"N and 102°46'28.2"W), with climate BS₁kw(w), 382 mm annual rainfall, rainy season in summer and cold winters (García 2004). Also seeds of O. ficus-indica (sample No. 2) were collected in a family’s backyard in San Salvador Atenco municipality, State of México (19°33’30"N and 98°54’45”W), with climate BS₁Kw(W)(i) semidry, 604 mm annual rainfall, rainy season in summer and cold winters (García 2004). Seeds were stored (around one year) in paper envelopes at room temperature (25 ± 2 °C) until used in the study, since Opuntia seeds could need an 1–3 years after-ripening period to germinate (Mandujano, Montaña, and Rojas-Aréchiga 2005). All evaluations were carried out with normal seeds (identifying visually and eliminating aborted and damaged seeds).

Evaluation of seed water uptake

Fifty seeds were individually weighed, identified and maintained in batches of 10 seeds in Petri dishes, with cotton and filter paper moistened with distilled water. Dishes were kept at constant temperature (30 ± 1 °C) and in dark (in a controlled environment chamber, Riossa digital E-71, México). Thereafter, seeds were weighted every 48 h for 10 days, until constant weight, on an analytical balance (± 0.0001 g accuracy; Scientech SA 120, USA). Seed water uptake was expressed as the mean individual seed weight increase (percentage with respect to the original dry mass) resulting from water absorption.

The experimental design for seed water uptake test was completely randomized, with four treatments (one sample of O. streptacantha, one of O. megacantha and two of O. ficus-indica) of unscarified seeds and five replicates with 10 seed each.

Evaluation of embryo viability

Embryo viability was quantified with the tetrazolium (TZ) (2,3,5 triphenyl tetrazolium chloride) test according to International Seed Testing Association (2010) guidelines for the use of TZ. Embryos were extracted from seeds after 18 days of seed imbibition, placed in a 1% TZ solution and maintained at a constant temperature (30 ± 1 °C) in the dark. After 24 h, embryos were removed from the tetrazolium solution, rinsed with distilled water and were observed in a stereoscopic microscope (Leica Microsystems EZ4, Switzerland). The embryos were considered alive when they were completely stained from an intense red color. Viability was expressed as the percentage of living embryos of total seeds evaluated by repetition.

The experimental design for TZ test was completely randomized, with four treatments (one sample of O. streptacantha, one of O. megacantha and two of O. ficus-indica) of unscarified imbibed seeds and five replicates with 10 seeds each.

Seed ozonation

Four lots per Opuntia sample, including 50 seeds and five replicates per sample were ozonized. Seeds were placed in 1 L of distilled water and a stream of O₃ (0.58 mg L⁻¹) was bubbled for 1 min (Ozonator Aqua–Equipos de México®, México), as a sub-lethal ozone doses according with Vazquez-Ybarra et al. (2015). During germination, these seeds were kept moist with ozonized water.

Evaluation of seed germination

Three assays, according to International Seed Testing Association (2010) methods, with some modifications, were conducted in sequence. The effects of seed scarification were examined in the first test. Batches of 100 unscarified seeds, or mechanically (by nicking the seed coat of individual seeds with abrasive paper) and chemically (with concentrated H₂SO₄ for 5 min and 10 min) scarified, were kept in Petri dishes (with filter paper and cotton) and moistened during the test with distilled water.
Dishes were placed in translucent plastic bags and maintained in a greenhouse under natural thermo-period (on average 30:15 °C day:night, and natural light:dark, 12:12 h; photosynthetically active radiation between 115 and 615 μmoles m⁻² s⁻¹).

The experimental design for the first seed germination test was completely randomized, with four seed samples (one of O. streptacantha, one of O. megacantha and two of O. ficus-indica) and four scarification conditions (unscarified, mechanically scarified, short time chemical scarification and long-time chemical scarification, 5 and 10 min). This resulted in 16 treatments. Each included four replicates with 25 seeds. The data were analyzed with analysis of variance (ANOVA), and Tukey’s multiple comparison tests.

In the second test seeds were placed in Petri dishes with cotton, filter paper and kept moist with either distilled water or ozonized water. Captam 50 fungicide [(cis-N-[(trichloromethyl)thio]-4-cyclohexane1,2-dicarboxiamida)] 0.2% (v:v) aqueous solution was added to each Petri dish (20 mL). Petri dishes were placed in translucent plastic bags and maintained in an environmentally controlled chamber (Thermoscientific, Inc. Massachusetts, USA) with alternating temperature (35:28 ± 1 °C day:night for 8:16 h) and alternating photoperiod (12:12 h; 25.2 μmol m⁻² s⁻¹). Germination was assessed daily for 90 d.

The experimental design for the second test was completely randomized, with four seed samples (one of O. streptacantha, one of O. megacantha and two of O. ficus-indica); two scarification conditions (uncarified and scarified), and two ozonized conditions (without or with ozonation). This resulted in 16 treatments; each included four replicates with 50 seeds. The data were analyzed with ANOVA, and Tukey’s multiple comparison tests.

In the third test seeds were obtained as in the second one but germination was carried out under constant temperature (32 ± 1 °C) and alternating photoperiod (12:12 h; 25.2 μmol m⁻² s⁻¹). Germination was assessed daily for 90 d.

The experimental design for the third test was completely randomized, with four chemically scarified seed samples (one of O. streptacantha, one of O. megacantha and two of O. ficus-indica), and two ozonized conditions (without or with ozonation). This resulted in eight treatments; each included four replicates with 50 seeds. The data were analyzed with ANOVA, and Tukey’s multiple comparison tests.

In all tests seeds were considered germinated when 0.5 mm of the radicle had emerged from the seed coat; and percentage of seed germination was calculated with the data obtained.

**Evaluation of polyembryony and polyclotyledon**

After germination polyembryony and polyclotyledon seedling of all treatments were quantified according with description of Johri (1984), and the percentage of each was calculated.

**Results**

**Seed water uptake and viability**

Accelerated water uptake was observed during the first 48 h. At that time, differences (P < 0.05) in imbibition were observed among Opuntia species. O. streptacantha had imbibed the smallest amount of water, O. megacantha an intermediate amount, and O. ficus-indica the greatest amount. Seven days later seeds of the three species had a similar (33.5%) water uptake respect to their initial weight (Figure 1). During the next 60 days water uptake remained without any change (data no shown) and at that time between 1% and 10% of seeds of O. ficus-indica and O. streptacantha had germinated; but specifically, these seeds had 47 and 55% fresh weight increase.

Tetrazolium test showed a 98% average of seed viability in O. streptacantha, O. megacantha and O. ficus-indica No. 1 samples and 96% of O. ficus-indica No. 2 with no significant differences between them (P > 0.05). These results indicate that seeds of Opuntia may not be dormant.

**Seed germination**

Mechanical or chemical scarification for 5 and 10 min, and 30:15 °C day:night temperature had little effect on increasing seed germination, except for O. streptacantha,

![Figure 1. Water uptake by seeds of three species of Opuntia from México. EZ: Seeds from Zacatecas, México; EM: seeds from Estado de México, México.](image-url)
which had a 5–12 times germination increase. Furthermore, *O. megacantha* showed a lower response to both types of seed scarification compared to *O. streptacantha*, but was higher than that the domesticated species *O. ficus-indica* (Table 1).

Germination timing of unscarified seeds was significantly different (*P* ≤ 0.05) among species. *O. streptacantha* seeds were the earliest to germinate starting 27 days before the other two species. In addition, the maximum cumulative germination of these seeds was also significantly different among them. Germination of *O. streptacantha* was on average four times higher than in *O. ficus-indica* No. 1, which had the lowest value. The seeds of the three species germinated asynchronously (Figure 2). Chemical scarification accelerated the germination onset 1 to 27 days, except for *O. ficus-indica* No. 1. Furthermore, chemical scarification significantly increased seed germination of the three species in varying proportions. On average scarified seeds of *O. streptacantha* had a 62% increase in germination compared to unscarified seeds. In contrast, to the rise of more than three times in *O. ficus-indica* No. 1 germination, chemical scarification decreased seed germination in *O. ficus-indica* No. 2 (Figure 2).

Ozone did not modify germination of unscarified seeds of *O. megacantha* and *O. ficus-indica* (Figure 2B–D). In contrast, O₃ decreased (*P* ≤ 0.05) seed germination of unscarified seeds of *O. streptacantha* by 13% (Figure 2A).

Ozone accelerated the germination onset (3 up to 18 days) of scarified seeds of the three species and significantly increased (*P* ≤ 0.05) the maximum cumulative seed germination, up to three times, of *O. megacantha* and *O. ficus-indica*, in comparison with those unscarified and unozonized seeds (Figure 2).

During the seed germination fungi recurrently developed in the treatments containing Captan and O₃. This was controlled by rising seeds, changing Petri dishes weekly, and moistening with ozonized water. Similar handling was applied to the other treatments but they were kept moist with distilled water.

Cumulative seed germination curves showed that scarified-unozonized seeds of *O. streptacantha* started germination between 1 and 11 days before the other samples and reached the maximum percentage of germination between 1 and 22 d before (Figure 3). In contrast, germination of scarified-ozonized seeds decreased between 4 and 9 days in *O. streptacantha* and *O. megacantha*, respectively. The most outstanding effect of O₃ and constant temperature on seed germination was the significant increase (*P* ≤ 0.003) of cumulative maximum germination in the three species, between 33% in *O. megacantha* and up to threefold in *O. streptacantha* compared to scarified-unozonized seeds (Figure 3).

### Polyembryonic seeds and polycotyledons in seeds

Three species exhibited seedling development in polyembryonic seeds (Figure 4A), and in seeds with more than two cotyledons (Figure 4B). They also had atypical seedlings with an initial emergence of the cotyledons instead of the radicle during germination (Figure 4C). Seedlings from polyembryonic seeds were observed in 31 of 32 treatments in the study, but were more abundant in *O. streptacantha* and *O. megacantha* (between 3 and 4% on average by treatment) (Table 2).

Seedlings from seeds with polycotyledons were observed in 11 of 32 treatments in the three assays. This less frequent phenomenon occurred between 0 and 3.5% seedlings per treatment. This result showed that there is no direct relationship between the amounts of seedlings coming from seeds with polycotyledons (Table 2).

### Discussion

Seed ability to uptake water and its viability have not been documented in most of the 188 *Opuntia* species and neither comparison has been done among wild and domesticated species. A water uptake gradient was observed 48 h after seed imbibition among all evaluated species. The wild seeds of *O. streptacantha* imbibed a small amount of water. The seeds of the semi-domesticated *O. megacantha* imbibed an intermediate amount of water and those of the domesticated *O. ficus-indica* showed the greatest water uptake, amounting a third

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**Table 1.** Germination of unscarified and mechanically and chemically scarified *Opuntia* seeds in Petri dishes and under greenhouse conditions.†

<table>
<thead>
<tr>
<th>Seed condition</th>
<th><em>O. streptacantha</em></th>
<th><em>O. megacantha</em></th>
<th><em>O. ficus-indica</em> 1§</th>
<th><em>O. ficus-indica</em> 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unscarified</td>
<td>3.00 c</td>
<td>0.00 c</td>
<td>0.00 a</td>
<td>0.00 a</td>
</tr>
<tr>
<td>Mechanically scarified</td>
<td>16.00 b</td>
<td>9.00 a</td>
<td>2.00 a</td>
<td>1.00 a</td>
</tr>
<tr>
<td>Acid scarified (5 min)</td>
<td>19.00 b</td>
<td>2.88 b</td>
<td>0.96 a</td>
<td>0.00 a</td>
</tr>
<tr>
<td>Acid scarified (10 min)</td>
<td>38.00 a</td>
<td>3.00 b</td>
<td>0.00 a</td>
<td>0.00 a</td>
</tr>
</tbody>
</table>

† On average 30:15 °C day:night temperature and 12 h light (115–615 μmoles m⁻² s⁻¹ of photosynthetically active radiation):12 h dark. § *Opuntia ficus-indica* 1: seeds from Zacatecas, México; and *O. ficus-indica* 2: seeds from Estado de México. Values followed by the same letter in a column are not significantly different (*P* ≤ 0.05); *n* = 100.
more regard the wild seeds. Seven days later, seeds of the three species had imbibed similar amounts and this did not change during the next 60–70 d, when some seeds start germination (Figure 2). Our results indicated that testae of the seeds of these Opuntia species are permeable to water, and that the water needed for germination is less than a half of the seed mass. Results confirmed that seeds of some Opuntia species do not show physical seed dormancy as stated by Orozco-Segovia et al. (2007), and the lack of seed germination at greenhouse conditions and several environmental conditions, as well in growth chamber, suggested that the three species are showing physiological dormancy.

Seed imbibition is largely a function of seed dry mass (Artare et al. 2006). In the present study seed biomass was significantly different among species, O. streptacantha (12.27 mg; n = 250) had the lower seed biomass among the evaluated species (P ≤ 0.05), with 20 to 30% less than of O. ficus-indica (17.50 mg; n = 500) and O. megacantha (17.51 mg; n = 250). But, these differences do not match uniformity in maximum seed imbibition of the three species.

The high embryo viability of the three species in this study confirmed that seeds of the whole samples could germinate, and the seed sample which did not germinate is dormant (Baskin and Baskin 1977). Seeds of all species failed to germinate under standard laboratory and greenhouse conditions. Chemical scarification increased seed germination in different proportions (20 to 30%) among the evaluated species, under adequate environmental conditions, at 30 °C of daytime temperature, as well as 30 and 32 °C constant temperature. This

Figure 2. Cumulative seed germination of (A) Opuntia streptacantha, (B) O. megacantha, and (C) O. ficus-indica collected in Zacatecas, México, and (D) O. ficus-indica collected in State of México. (○) Unscarified seeds soaked with distilled water, (●) chemically scarified (using concentrate H$_2$SO$_4$) seeds soaked with distilled water, (△) unscarified ozonized seeds soaked with ozonized water, and (▲) chemically scarified ozonized seeds soaked with ozonized water. Germination conditions: 35:25 °C (8:16 h) and 12 h light (25.2 µmol m$^{-2}$ s$^{-1}$):12 h dark; n = 50.
indicated that accelerate imbibition by scarification probably improves the embryo physiological processes related with germination, in some Opuntia species, under certain environmental conditions.

Wild seeds reached a significantly high cumulative germination compared to that of the semi-domesticated and domesticated seeds. The increase in germination

**Figure 3.** Cumulative seed germination of *Opuntia streptacantha* (A), *O. megacantha* (B), *O. ficus-indica* from Zacatecas, México (C) and *O. ficus-indica* from Estado de México (D) with constant temperature (32 ± 1 °C) and 12 h: 12 h of light: darkness photoperiod. Acid scarified seeds (soaking of seed in concentrate H$_2$SO$_4$) (○) and acid scarified and ozonated seeds (●); *n* = 200.

**Figure 4.** Seedlings of polyembryonic Opuntia seed (A), seed with four cotyledons (B), and with no root emergence (C).

**Table 2.** Percentage of seedling developed from polyembryonic seeds (Pe), and from seeds with polycotyledons (Pc) in *Opuntia* species.

<table>
<thead>
<tr>
<th>Seed condition</th>
<th><em>O. streptacantha</em> Pe</th>
<th><em>O. megacantha</em> Pe</th>
<th><em>O. ficus-indica</em> 1 Pc</th>
<th><em>O. ficus-indica</em> 2 Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4 2.5 3.5 0.5 0.5 0</td>
<td>2.5 3.5</td>
<td>0.5 0 3 1.5</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2 2 2 0 0 0.5 3</td>
<td>2 2.5 0 3 2 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C‡</td>
<td>3.5 2 2.5 0 3 2</td>
<td>6 5 2.5 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D§</td>
<td>2.5 0 7 0 6 5 2.5</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† A: Unscarified seeds soaked with distilled water during germination, B: unscarified-ozonized seeds soaked with ozonized water during germination, ‡ (C): chemically scarified seeds (using concentrate H$_2$SO$_4$) and soaked with distilled water during germination, § (D) chemically scarified seeds soaked with ozonized water. † *Opuntia ficus-indica* 1: seeds from Zacatecas, México; and *O. ficus-indica* 2: seeds from Estado de México; *n* = 200.
after seed scarification is coincident with the results obtained by Altare et al. (2006) in seeds of O. ficus-indica. These authors reported low germination and long time to complete this process in Opuntia seeds because, according with Werker (1997), the integument of the hard seed coat is lignified and prevents the protrusion of the radicle from the seed during germination. However, scarification with concentrated acid did not increase seed germination, nor did so in low proportion. It exceptionally increased up to 85% in O. lindheimeri, O. edwardsii (Potter, Petersen, and Ueckrt 1984), O. imbricata, O. robusta (Beltrán 1984), O. pure-rula (Godínez and Valiente-Banuet 1998), O. tumetosa (Olvera-Carrillo et al. 2003), and O. rastrera (Mandujano, Montaña, and Rojas-Aréchiga 2005).

Curves of cumulative germination after the elimination the seed coat barrier, by chemical abrasion showed the similarities and differences in germination among wild, semi-domesticate and domesticate species. The seeds of domesticate species reached maximum cumulative germination later than the other two species, under the second test (more than 7 to 22 days) and (more than 5 to 20 days) in the third test conditions. Altare et al. (2006) proposed that 5-min acid scarification was adequate to increase seed germination of O. ficus-indica about 50%; Olvera-Carrillo et al. (2003) and Mandujano, Montaña, and Rojas-Aréchiga (2005) maintained acid seed immersion up to 60 and 140 min to get similar results in other Opuntia species. Our results (20 and 38% germination) are partially similar to those in the literature. Still they all indicate that the optimal time for chemical scarification seems is quite different among species and probably among samples of Opuntia.

Results also indicated that the seeds of the three species had a heterogeneous response to the thermostatic and photoperiod combination during germination. Similarly, it has been documented that some factors, such as humidity, temperature and light affect seed germination of different cacti (Delgado-Sánchez et al. 2013; Olvera-Carrillo et al. 2009b; Potter, Petersen, and Ueckrt 1984; Rojas-Aréchiga and Vázquez-Ybarra 2000). On average, night temperature under the first test conditions was 17 ºC lower than in the second and the third tests. In general, total seed germination was the lowest under low night temperature in the three species. In this condition mechanical and chemical scarification of the seed coat did not promoted seed germination, except for the wild species O. streptacantha. Warm night conditions, like in the second and the third test, promoted seed germination and increased two or three times when the seed coat was eroded by scarification. In general, wild O. streptacantha seed were the earliest to germinate and domesticated O. ficus-indica were later, with a difference among them of 11–43 days.

Seed germination rate of Opuntia can be increased with a combination of pretreatments including chemical scarification and thermoperiod (Baskin and Baskin 1977); chemical scarification and temperature of 20 to 35 ºC (Potter, Petersen, and Ueckrt 1984), mechanical scarification and gibberellic acid (Sánchez-Venegas 1997) and chemical scarification and an oxidizing agent (Altare et al. 2006). But in general, poor increases in germination have been obtained. However, all these factors can be used to evaluate morphological and physiological differences and similarities among Opuntia species, with different degrees of domestica-
tion, which are currently unknown.

Ozone significantly increased the seed germination of the three species, the greatest O₃ effect was observed in the wild O. streptacantha, besides the time to onset germination decreased (up to 10 days). A similar syner-
gistic action of acid scarification and hydrogen peroxide, as oxidizing agent, was documented by Altare et al. (2006) to increase and accelerate O. ficus-indica seed germination. It has been stressed that the oxidation of the seed coat compounds can promote the cell signaling that triggers germination (El-Maarouf-Bouteau and Bailly 2008; El-Maarouf-Bouteau et al. 2015; Oracz et al. 2007). Similar effects can be attributed to O₃ in the present study. Yet, further research on the response to O₃ is required to demonstrate this effect.

It should be noted that the applied O₃ dose to the seeds was nonlethal, and in general for plant tissues (Vázquez-Ybarra et al. 2015). Also, it seems that O₃ did not affect seed viability, since they maintained germination for more than 70 days. Oxidizing agents, such as peroxides and dioxides, trigger an increase of ethylene synthesis and ABA decrease in seedlings (Sudhakar et al. 2011). These agents increase reactive oxygen species, which probably act as a positive signal in metabolism of dormant seeds by the oxidation of compounds that act as such cellular signaling (Bykova et al. 2011; El-Maarouf-Bouteau and Bailly 2008; El-Maarouf-Bouteau et al. 2007) of seed imbibition (Dębska et al. 2013; Oracz et al. 2009; Zhang et al. 2014) to seedling emergence and root elongation (Kranner et al. 2010).

Evidence also suggests that O₃ may trigger antioxidant and DNA-repair mechanisms or dormancy-breaking effects in hydrated seeds (Abeli et al. 2016). The results showed that seeds of the three species reacted different to combination of scarification, O₃ and germination temperature. The heterogeneity of responses may be due to environmental factors
species, contrasted with those previously classified as intermediate and highly domesticated species. To improve seed germination and increase seedling growth of the *Opuntia* genus, a combination of chemical scarification, and seed ozonization and constant temperature of 32 °C during germination is recommended. Ozone should be used in sublethal doses to increase the germination potential and seedling development.

**ORCID**

Cecilia Beatriz Peña-Valdivia [http://orcid.org/0000-0003-4245-0547]

**References**


