Salinity effects on germination and seedlings biomass of *Pachycereus pecten-aboriginum*: an endangered species

Alfredo Beltrán-Morales[1], Miguel Víctor Córdoba-Matson[2], José Luis García-Hernández[3], Enrique Troyo-Diéguez[2], Hossein Azadi[4], Francisco Higinio Ruiz-Espinoza[1], Ricardo David Valdez-Cepeda[5], Bernardo Murillo-Amador[2,*]

[1] Universidad Autónoma de Baja California Sur. La Paz, B.C.S., Mexico.
[2] Centro de Investigaciones Biológicas del Noroeste, S.C. La Paz, BCS, Mexico.
[3] Universidad Juárez del Estado de Durango. Durango, Durango, Mexico.
[4] Department of Geography, Ghent University. Belgium.
[5] Universidad Autónoma Chapingo. Centro Universitario Centro Norte. El Orito, Zacatecas, Mexico.

[*]Corresponding Author: bmurillo04@cibnor.mx.

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ABSTRACT

Cacti are particularly sensitive to habitat disturbance since they have slow growth and a long life cycle. Their development and establishment depend on several factors, including environmental requirements for germination and establishment of seedlings. Two of the most influential factors are soil and water salinity. The objective of this study was to quantify seeds germination and seedling biomass production of the Aborigine's comb cactus (*Pachycerus pecten-aboriginum*) in salt stress using sodium chloride (NaCl) as the main salt source. The experiment was arranged in a completely randomized design using six salt levels (0.11, 2.03, 4.00, 6.03, 8.03 and 10.07 dS m^{-1}) with five replicates of 20 seeds each one. The seeds were collected in a wild population of *P. pecten-aboriginum* in Ejido Álvaro Obregón, Baja California Sur, Mexico in June 2011. The results showed that germination and seedling biomass were significantly affected by salt stress, which was higher at 2.03 and 0.11 dS m^{-1}, followed by 6.03 and 4.00 dS m^{-1}, lower at 8.03 dS m^{-1}, and no growth at 10.07 dS m^{-1}. It is concluded that saline levels using sodium chloride as the main source of salt, affect seed germination and seedling biomass production of *P. pecten-aboriginum* significantly.
Keywords: biomass, cacti, germination, salinity, species threatened.

RESUMEN

Las cactáceas son particularmente sensibles a los disturbios del hábitat; ya que tienen un crecimiento lento y un ciclo de vida largo. Su desarrollo y establecimiento dependen de varios factores, incluyendo requerimientos ambientales para la germinación y el establecimiento de plántulas. Uno de los factores que más influyen es el suelo y la salinidad en el agua. El objetivo de este estudio fue cuantificar la germinación de semillas y la producción de biomasa de plántulas de cardón barbón silvestre (*Pachycerus pecten-*

aboriginum) en estrés salino utilizando el cloruro de sodio (NaCl) como fuente principal de sal. El experimento se realizó en un diseño completamente al azar con seis niveles de salinidad (0.11, 2.03, 4.00, 6.03, 8.03 and 10.07 dS m^{-1}) con cinco repeticiones de 20 semillas cada una. Las semillas se colectaron en junio de 2011 en una población de cardón barbón silvestre, localizada en el Ejido Álvaro Obregón, Baja California Sur, México. Los resultados indicaron que la germinación y la producción de biomasa de plántulas fueron afectadas significativamente por el estrés salino, mostrando valores mayores en 2.03 y 0.11 dS m^{-1}, seguido de 6.03 y 4.00 dS m^{-1}, con valores menores en 8.03 dS m^{-1} y de cero en 10.07 dS m^{-1}. Se concluye que los niveles de salinidad utilizando el cloruro de sodio como fuente principal de sal, afectaron significativamente la germinación de las semillas y la producción de biomasa de plántulas de *Pachycereus pecten-aboriginum*.

Palabras clave: biomasa, cactáceas, especies amenazadas, germinación, salinidad.

INTRODUCTION

The cactus family has approximately 100 genera and from 1,500 to 2,000 species distributed from the northern Canada to Patagonia, Argentina (Barthlott and Hunt, 1993; Bravo-Hollis and Scheinvar, 1995). Mexico is the main center of cactus diversity, with approximately 48 genera and 570 species (about 38% of the total of the family), of which 78% are endemic (Hernandez and Gómez-Hinostrosa, 2002). Cacti have been exploited since pre-Columbian times (Luna and Aguirre, 2001); for example, the fruits of many columnar cacti, mainly from wild plants are traditionally used by some inhabitants of Mexico, Central America, and Northern South America. The pulp of the fruit of *P. pecten-aboriginum* is eaten fresh or cooked, and it is made in the form of preserves or syrup, previously used to make wine. The seed is ground and mixed with corn flour to make “tortillas”. The outside of the fruit was used to manufacture a hair comb; the thorns are removed around two thirds of the fruit and the remaining bones are clipped at 1 cm in length; hence the name of the species *pecten-aboriginum*, which translates as "*the aborigine´s comb*". The Tarahumara indians used the branches or "arms" of the plant to make a sacred drink that causes hallucinations. *Pachicereus pecten-aboriginum* has been used to heal wounds and stop bleeding.

In addition to the uses mentioned above, the Cactus family has always been subjected to intensive exploitation because of its great diversity and value, primarily as an ornamental plant. As a result, its populations have drastically been affected due to the loss and severe disturbance of its habitat. Therefore, 35% of Mexican cactus species are endangered; many of them are listed in the Appendix of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) including *P. pecten-aboriginum* (PNUMA-CMCM, 2011), which has a wide distribution throughout the Pacific, the northwestern coast of Mexico, Sonora, and Baja California to the Tehuantepec Isthmus (Photo 1).

It has been estimated that from one-third to one-half of Mexican cacti, *i.e.* 285 species are rare, threatened, or endangered (Hernández and Godinez, 1994; NOM-059-ECOL-2001, Official Journal of the Federation-Official Journal of the Government of Mexico- 06 March

2002). In fact, cacti are particularly sensitive to habitat disturbance since they have very slow growth and a long life cycle (Nobel, 1988). Their development and establishment depend on several factors, including environmental requirements for germination and establishment of seedlings. Two of the most influential factors are soil and water salinity.

Photograph 1. *Pachycerus pecten-aboriginum* adult plants growing in the study area of Baja California Sur, Mexico (photographed June 19, 2015).

Soil salinity is an important factor that limits productivity of most plants in general, affecting about 95 million hectares worldwide (Szabolcs, 1994). Saline soils contain salts soluble enough to stop plant growth through a series of factors that interact with each other, such as osmotic potential, ion toxicity, and nutrient antagonism, which induce nutritional imbalances in plants (Neumann, 1995). Saboora and Kiarostami (2006) warned about a dangerous trend of a 10% increase per year in salinity areas throughout the world. Salinity also affects morphological characteristics, physiological and biochemical processes of the plants, as well as nutrient and water absorption, growth, and seed germination.

Seed germination is a process that begins with water absorption by the seed and ends with spindle embryo elongation (Bewley and Black, 1994). This process is affected by adverse factors such as salinity, which is one of the major abiotic stresses affecting plant growth and development, especially in arid and semi-arid regions (Chinnusamy *et al*., 2005; Cony *et al*., 2006). This stress condition can affect seed germination when it creates a high osmotic potential, preventing water absorption (osmotic effect) or causing toxic effects of Na and Cl ions (Okcu *et al*., 2005).

On the other hand, several reports on cacti have shown that an increase in salt concentration decreases germination percentage (Romero-Schmidt *et al*., 1992; Nolasco *et al*., 1996; Vega-

Villasante *et al*., 1996). Due to the limited information on seed germination and biomass production of *P. pecten-aboriginum* in salt stress, the objective of this study was to quantify seeds germination and seedling biomass production of the Aborigine's comb cactus (*Pachycerus pecten-aboriginum*) in salt stress using sodium chloride (NaCl) as the main salt source.

MATERIALS AND METHODS

Ethics statement

The research conducted herein did not involve measurements with humans or animals. The study site is not considered a protected area. However, an endangered species was used *P. pecten-aboriginum* while performing this study, thus permissions were needed, which were granted by Leonel Valerio Castro Santana, Federal Officer of PROFEPA (Procuraduría Federal de Protección al Ambiente) in Baja California Sur. Because of the species used in this study is considered an endangered species and their its use therefore had negligible effects on broader ecosystem functioning, for future fruits collection of *P. pecten-aboriginum* and other endangered species in the study area will be sampled after attaining the appropriate permissions of PROFEPA.

Study area

The seeds of P. *pecten-aboriginum* were collected in June 2011, and the fruits were collected from established wild mature plants (Photo 2) in the Ejido Alvaro Obregon located at 23°52' 68'' N Latitude and 110°11' 04'' W Longitude, 360 masl., located 30 km South of the city of La Paz in Baja California Sur, Mexico.

The Ejido Alvaro Obregon has different climate characteristics from the rest of the area; it is less arid and presents a large variation of temperature and humidity. It has a BS climate (h') w (e), which is semi-arid warm with an annual average temperature of 22.7 °C taking Köppen climate classification modified by García (2004) as reference; the temperature of the coldest month is 18 °C, and the hottest one is 27.4 °C. The site shows summer rains and winter rain of 9.1% (CONANP, 2003). In accordance with Rzedowski's classification criteria, the vegetation of the area of study is composed by sarcocaulescent or scrublands (Photo 3) and to a lesser extent by deciduous tropical forest (Rzedowski, 1994).

Photograph 2. *Pachycerus pecten-aboriginum* adult plants established in Ejido Alvaro Obregon, Baja California Sur, Mexico where seeds were collected in June 2011 (photographed June 19, 2015).

Photograph 3. *Pachycerus pecten-aboriginum* adult plants in association with the vegetation of the area of study composed by two types of vegetation, sarcocaulescent scrub or matorral scrublands (photographed June 19, 2015).

Saline solutions preparation

The salinities proposed to determine seeds germination and seedling biomass production in *P. pecten-aboriginum* using NaCl as the main salt source were 0, 2, 4, 6, 8, and 10 dS m^{-1}.

However, before to apply these salinities, one experiment was developed with the objective to quantify the evaporated water of each Petri dish and if the salinities increased after replenish the evaporated water using similar saline solutions instead of distilled water. Three sterilized Petri dishes (55 mm in diameter and 15 mm height) for each saline solution were used, placing in the bottom of the dish two circles of sterilized filter paper (Whatman® code 20445120324). Then, 3.0 mL of each saline solution previously prepared using NaCl as main source of salt were added to each dish and their initial electrical conductivity was measured (0.02, 1.98, 3.98, 5.97, 8.00, and 10.00 dS m^{-1}). Later, the Petri dishes were covered and placed in a germination chamber (Seedburo® Equipment Company, Model 549, Chicago, Illinois, U.S.A.) under dark conditions. The temperature in the chamber was 25 ± 1°C. The relative humidity in the chamber was maintained at 80% ± 1%. Every week the quantity of evaporated water of each Petri dish was measured (0.5 mL) and 1.0 mL of similar saline solution was added to replace the evaporated water and to keep the moisture of the filter paper. After four weeks, the final electrical conductivity of each saline solution was measured and found to be 0.11, 2.03, 4.00, 6.03, 8.03 and 10.07 dS m^{-1}.

Seed preparation

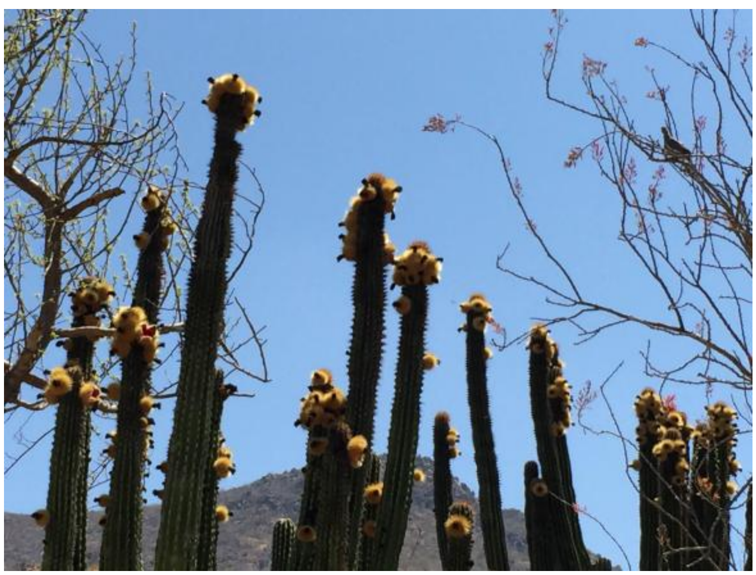
The seeds were obtained from fruits located in healthy and mature plants. After removing the pulp of the fruit, the seeds were disinfected by immersion during 5 min in calcium hypochlorite solution, containing 5% active chlorine. The seeds were washed three times with sterilized distilled water; then, they were dried at 25°C.

Germination conditions

The experiment was carried out in the laboratory of seeds of the Universidad Autónoma of Baja California Sur, which is located at 24° 08’ 32" N Latitude and 110° 18’ 32" W Longitude (INEGI, 2002). Twenty seeds of *P. pecten-aboriginum* were placed in Petri dishes (55 mm in diameter and 15 mm height), covered at the bottom with two disks of sterilized filter paper (Whatman® code 20445120324). Then, 3.0 mL of each saline solution previously prepared using NaCl as main source of salt were added to each dish. The Petri dishes were covered and placed in a germination chamber (Seedburo® Equipment Company, Model 549, Chicago, Illinois, U.S.A.) under dark conditions. The temperature in the chamber was 25 ± 1°C. The relative humidity in the chamber was maintained at 80% ± 1%. Taking into account the previous experiment on saline solutions, 1.0 mL of the appropriate saline solution were added every week to each dish to replace the evaporated water and to keep the moisture of the filter paper. The number of germinated seeds was recorded every two days during three months. Seeds were considered germinated when the radicle was at least 1-mm long (Photo 4). After germination, the fresh weight (biomass) of seedlings was registered (mg) using an analytical balance (Ohaus® model AS260D, Florham Park NJ, USA).

The experiment was arranged in a completely randomized design with six saline levels (0.11, 2.03, 4.00, 6.03, 8.03 and 10.07 dS m^{-1}) as treatments using NaCl as the main source of salt. Each of the five replicates consisted of just 20 seeds of *P. pecten-aboriginum* because of the limited number of seeds available from a limited number of mature fruits. PROFEPA authorized the collection of a restricted number of fruits of *P. pecten-aboriginum* based on the criteria considering resource conservation and management of this species (Photo 5).

Photograph 4. *Pachycerus pecten-aboriginum* germinated seeds under salinity (NaCl) treatments. The germinated seeds were recorded every two days for 3 months; seeds were considered germinated when the radicle was at least 1-mm long (photographed July, August and September, 2011).



Photograph 5. *Pachycerus pecten-aboriginum* plants and fruits established in Ejido Alvaro Obregon, Baja California Sur, Mexico where limited numbers of seeds were collected because of normativity restriction based on criteria considering conservation and management of resources of this species (photographed June 19, 2015).

Statistical analysis

Bartlett's test was performed on the data to test homogeneity of variance. Data were compared using univariate analysis of variance (ANOVA) according to an experimental one-way classification design, where saline solutions (0.11, 2.03, 4.00, 6.03, 8.03 and 10.07 dS m^{-1}) were modeled as fixed factor. The difference between the means was determined by Tukey's HSD multiple range test at $p = 0.05$ level. In all cases, mean values were considered significantly different when $p \leq 0.05$. All the analyses were done with Statistica® software program v. 10.0 for Windows® and SAS for Windows® v. 9.0. At the end of the experiment, the germination percentage was determined using the following formula: GP = ni/N*100. Germination expressed in percentage was arcsine transformed previously to ANOVA (Sokal and Rohlf, 1988).

RESULTS

Effect of salinity on germination

Seed germination of *P. pecten-aboriginum* was significantly affected by salinity levels (Table 1). The germination percentage was higher at 2.03 dSm^{-1} followed by 4.00 and 0.11 dS m^{-1}. A seed germination of 30% was recorded at 6.03 dS m^{-1} followed by the salinity level of 8.03 dS m^{-1}, while with a level of 10.07 dS m^{-1} seeds germination was zero (Table 2). The germination percentage of *P. pecten-aboriginum* seeds decreased as salinity levels increased ($r = -0.84$; $p=0.0001$; n=30).

Table 1. Analysis of variance of the effects of salinity (NaCl as main source of salt) on germination percentage and seedlings biomass production of *P. pecten-aboriginum*.

Effects	Degree of Freedom	Sum of Squares	Mean Squares	F	*P*
	Seedling biomass				
Salinity (NaCl)	5	49297.80	9859.56	25.47	<0.0001
Error	24	9288.78	387.03		
Total	29	58586.58			
Coefficient of variation (%)	35.62				
	Germination percentage				
Salinity (NaCl)	5	19046.53	3809.30	35.46	<0.0001
Error	24	2578.23	107.42		
Total	29	21624.77			
Coefficient of variation (%)	29.04				

Effect of salinity on the seedling biomass

The seedling biomass in fresh weight of *P. pecten-aboriginum* was significantly affected by salinity levels (Table 1). The seedling biomass was higher at 2.03 and 0.11 dS m^{-1}, followed by 6.03 and 4.00 dS m^{-1}. The seedling biomass at 8.03 dS m^{-1} was 11.18 mg, while at the salinity level of 10.07 dS m^{-1} was zero (Table 2). The seedlings biomass of *P. pecten-aboriginum* decreased as salinity levels increased (r = -0.86; *p*=0.0001; n=30) while the relationship between germination percentage and seedlings biomass was positive (r = 0.83; *p*=0.0001; n=30), which mean that as germination percentage of seeds increased, the seedlings biomass increased.

Table 2. Effects of salinity (NaCl as main source of salt) on germination percentage and seedlings biomass production of *P. pecten-aboriginum*.

Salinity (dSm^{-1})	Germination (%)	Biomass (mg)
0.11	69.00 ± 21.03 a	97.30 ± 14.21 a
2.03	75.00 ± 9.35 a	110.84 ± 28.50 a
4.00	72.00 ± 21.67 a	54.43 ± 10.26 b
6.03	30.00 ± 15.41 b	57.63 ± 24.03 b
8.03	4.00 ± 8.94 bc	11.18 ± 24.99 c
10.07	0.00 ± 0.0 c	0.00 ± 0.00 c
LSD	28.97	38.47

Values within the same column with same letters are not significantly different at p = 0.05 (Tukey´s HSD multiple range test). LSD= Least significant difference.

DISCUSSION

The results of this study showed that seed germination and consequently seedling biomass of *P. pecten-aboriginum* were affected by saline stress. Similar results have been reported in other cactus species, such as the fishhook barrel cactus (*Ferocactus peninsulae*) and Mexican giant cardon (*Pachycereus pringlei*), which were able to germinate in 25 and 50 mM NaCl. The decrease of germination percentage of *P. pecten-aboriginum* seeds as salinity levels increased agree with those results reported by Romero-Schmidt *et al*. (1992), Nolasco *et al*. (1996) and Vega-Villasante *et al*. (1996) who described that germination of *Pachycereus pringlei* seeds decreased at 100 and 200 mM and completely inhibited at 300 mM NaCl. Moreover, other cacti species such as *Opuntia* showed they can stand the strong droughts and the abrupt changes of temperature. Nevertheless, the majority are sensitive to salt concentration in the soil or in irrigation water (Nobel *et al*. 1984; Berry and Nobel, 1985; Silverman *et al*. 1988; Nerd *et al*. 1991; Le Houérou, 1996; Murillo-Amador *et al*. 2001; Nieto-Garibay *et al*. 2011).

The concentration of 1/5 seawater can decrease or inhibit root growth of agaves and cacti (Nobel, 1998). Species such as *Opuntia quimilo* from Argentina and *Cereus validus* are more tolerant to NaCl than other *Opuntia* species (Nobel, 1998). *Opuntia ficus-indica* reduces its growth by half when exposed continually to NaCl concentrations (1/10 of seawater) (Nobel, 1998).

In other species, the literature indicates that plants are particularly sensitive to salinity during the seedling stage and early vegetative growth compared with the germination stage. Some examples have been found in plants such as barley (Ayers *et al*. 1952); rice (Pearson and Ayers, 1966); cotton (Abul-Naas and Omran, 1974); corn (Maas *et al*. 1983); sorghum (Maas *et al*. 1986); wheat (Maas and Poss, 1989a); bean (Maas and Poss, 1989b); New Zealand spinach, and red orach (Wilson *et al*. 2000); tomato (Del Amor *et al*., 2001); and melon (Botia *et al*. 2005).

Seeds germination of *P. pecten-aboriginum* was affected by salt stress because the germination mechanism might be inhibited due to insufficient water absorption although it can also be attributed to toxic effects on the embryo (Azza *et al*. 2007); the increase in salt concentration in germination media often causes osmotic or specific toxicity which can reduce or delay germination percentage (Munns, 2002). The germination of *P. pecten-aboriginum* seeds decreased as concentrations of salinity increased.

These results agree with those reported by other authors (Abdul *et al*. 1992; Breen *et al*. 1997; Abbad *et al*. 2004; El-Tayeb, 2005) where they mentioned that the process of seed germination was reduced due to the increase of salinity levels. According to Waisel (1972), the presence of ions such as Na and Cl in cells can induce changes in proteins activity because ions affect the structure of the water surrounding the protein molecule. Thus, NaCl can inhibit the activity of some enzymes that could play important roles in seed germination (Flowers, 1972).

Likewise, the toxic effects of certain ions in the presence of higher salt concentrations can reduce water potential, making it difficult for water uptake by seeds and reducing germination (Rhoades *et al*. 1992). It appears related to disturbance of the metabolic process that leads to increased phenolic compounds from the seed and the salinity effects can induce a decrease in germination (Rehman *et al*. 1996).

On the other hand, the reduction of *P. pecten-aboriginum* seedling biomass as saline levels increased were similar to those reported by Al-Thabet *et al*. (2004) where they mention that plant growth inhibition is a usual response to salinity. Moreover, biomass reduction by increasing NaCl concentration has been reported by other authors (Gupta and Sharma, 1990; Läuchli and Epstein, 1990; Shannon, 1996; Ebert *et al*. 1999).

The results obtained with seedlings of *P. pecten-aboriginum* were similar to those reported by Gupta and Srivastava (1989) and Saboora and Kiarostami (2006) who established that when NaCl concentrations increase, the roots and shoots are affected significantly reducing biomass. In this respect, Munns (2002) highlights that after salinization, cells dehydrate and shrink but then recover their original volume. Despite this recovery, elongation and cell division are drastically reduced causing a decrease in growth rates. Meanwhile Miyamoto *et al*. (1985) and Esechie *et al*. (2002) considered that salinity delayed germination and seedling emergence since they may be more sensitive to injury in the hypocotyl and cotyledons affecting biomass development and production.

Because the biomass of *P. pecten-aboriginum* was minimum, the mineral analysis was not performed. However, as Yokoi *et al*. (2002) demonstrated, biomass reduction as saline concentrations increased can be due to ionic imbalances. They stated that ionic imbalances in plants were also triggered by excessive sodium and chloride absorption, which can generate side-effects such as toxicity or nutritional problems related to the absorption of ions essential for plant growth and development. In terms of nutritional effects and low biomass production of seeds subjected to salt stress, it might be due to high Na concentrations in the external solution, causing a decrease in K and Ca concentrations in plant tissues. These reductions may be due to the antagonism of the Na and K sites of absorption into the roots, the effect of Na transport to the xylem, or inhibition of the absorption processes (Hu and Schmidhalter, 2005).

Other study demonstrated that a high Na concentration not only inhibits nutrient absorption directly by interference with transporters in the plasma membrane of the root, such as selective K channels, but also by root growth inhibition and biomass decrease (Tester and Davenport, 2003).

The present study provides important aspects since *P. pecten-aboriginum* is considered an endangered species in the arid zones of México where saline water is often used in agriculture because fresh water is scarce, specifically in Baja California Sur, where underground water is used for crop irrigation and generally has a salinity range of 2.5 to 7.0 dS m^{-1} (Navejas, 1995).

One third of the irrigated lands around the world are affected by salinity, so it represents one of the most important problems affecting irrigated agriculture (El-Saidi, 1997). Since salinity is one of the most important factors that limits plant productivity and distribution, the studies of the effect of salinity has been conducted for a long time. However, the salinity resistance mechanism has not been totally understood (Mansour, 1997).

Navarro and Navarro (2013) reports that the average Cl content in the plants is 2-20 mg g^{-1} dry weight, although they also refer to optimal the plants most growth content must be in a range of 0.2-0.4 mg g^{-1} dry weight. Similarly, the Cl is part of photosystem II, which is important for photosynthesis, and is taking part in more than 130 organics compounds, for example 4-cloroindolacetic acid, which is a type of auxin (Salisbury and Ross, 2000). In this sense, this study is the first in determining the effects of salinity in *P. pecten-aboriginum* seed germination and biomass growth in seedlings. It provides the additional benefit of helping identify possible tolerant genetic materials to salinity, which could be a source of wild germplasm for the reforestation of this endangered species.

In Mexico, from ancestral times *P. pecten-aboriginum* is broadly utilized for ecological, nutritious, medicinal, and cultural purposes. Although its importance is recognized, no salt-tolerance studies have been previously carried out for this species. In this first study we conclude that salinity levels (0.11, 2.03, 4.00, 6.03, 8.03, and 10.07 dS m^{-1}) using NaCl as the main source of salt, affect the germination of *P. pecten-aboriginum* seeds and seedlings biomass production significantly.

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CONFLICT OF INTEREST STATEMENT

The authors have declared that no competing interests exist. The research was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: ABM, FHRE, ETD, BMA, JLGH. Performed the experiments: ABM, FHRE, JLGH. Analyzed the data: BMA, MVCM, HA, RDVC. Contributed reagents/materials/analysis tools/publication costs: ABM, HA, JLGH. Wrote, edited and revised the paper: BMA, HA, ETD, MVCM, RDVC. Approved the final version of the manuscript to be published: ABM, MVCM, JLGH, ETD, HA, FHRE, RDVC, BMA. All authors are in agreement that BMA be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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