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Seed germination and seedling survival of six cacti species using natural zeolite as substrate

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KEYWORDS

Cacti propagation, endangered species, germination, plant growth,seedlings.

A B S T R A C T

Under natural conditions, cacti have low growth rate and high seedling mortality leading to difficulties in establishing wild populations. For alternative conservation strategy ofcacti species, were evaluatedgrowth and survival of*Astrophytum capricorne* (A. Dietr.) Britton & Rose, *A. myriostigma* Lem., *Echinocereus reichenbachii* (Terscheck ex Walp.) Haage, *Escobariadasyacantha* (Engelm) Britton & Rose, *Mammillaria prolifera* (Mill.) and *Sclerocactus scheeri* (Salm-Dyck) N.P. Taylor. Seeds collected in protected natural areas of northeastern Mexico were germinated in the laboratory and seedlings transferred to a greenhouse, where the stem diametersand number of surviving individuals were recorded after 4, 10 and 16 months in conventional substrate (perlite-peat moss 50/50)and zeolite-peat moss (50/50). Survival washigher in the substrate with zeolite(67.29%) than inconventional substrate (42.67%).*S. scheeri*, *M. prolifera*and *A. capricorne*had the best survival(85.39, 75 and 73.91%, respectively). Growth rate was also influenced by the kind of substrate. Seedling diameter was greater with zeolite substrate (9.97 mm) than with conventional substrate (7.72 mm). Thus, use of zeolite as a substratefor cacti cultivation promotes growth and decrease plant mortality, making it a good alternative for the establishment of successful conservation strategies in natural areas with vulnerable cacti populations.

Introduction

Species from the Cactaceae family are the most representative botanical life formin the Mexican landscape, particularly in arid and semi-arid areas (Alanís and Velazco, 2008). Those plants occur on approximately 50% of the area of Mexico, i.e. nearly 80 million hectares (Andrade, 1974; Lamas, 1984). The highest diversity of cacti is found in two main arid regions: the Chihuahua and the Sonora deserts, both located in the north of the country.

Factors such as slow growth and high mortality of seedlings due to overgrazing, illegal collection and land-use change, among others, have led to a decline in natural populations of cacti. That situation has caused a large number of cacti species to be included in international red lists for conservation of the diversity (Hunt, 1999; SEMARNAT, 2010; IUCN, 2012), leading too much effort to conserve this important group of plants. An alternative for the propagation of cacti is the development of new substrates with proper characteristics for optimum growth of seedlings. These substrates should contain substances that maintain an adequate level of humidity, have good texture, prevent fungi growth, have a mineralized substrate and buffer changes in pH. In fact, the final substrate is a balanced mix of elements which confer these properties.

Many substrates for cacti and other succulent plants are used and recommended by different authors and other people who cultivate succulents, which all have in common a porous matrix capable to absorb water without retaining any excess (Santos *et al*., 2004). A successful option to meet these needs is the use of natural zeolites, which are minerals of the group hydrous alumino silicates with a porous structure that have a high capacity for cation-exchange and moisture retention. The cation exchange surface of zeolites generally is occupied by Na^+, K^+, Ca^{+2} and Mg^{+2}, which, with the exception of Na^+ (a phytotoxic element for plants), may be used by cacti. However, zeolite can be treated with certain solution to exchange the Na^+either by K^+, Ca^{+2} or Mg^{+2}(Urbina *et al*., 2006).

The physical and chemical characteristics of zeolite make it an attractive substrate for use in agriculture (Urbina *et al*., 2006) and for cultivation of cacti and other plants. However, scientific studies on the use of this mineral as a substrate for cacti are scarce, (Izquierdo et al., 2002; Jankauskienė and Brazaitytė, 2007). Further, natural zeolites exist in large deposits in Mexico (Ostroumov et al., 2005),thus their cost in this country as substrate is not high. Thus, the purpose of this study was to evaluate the effect of zeolite as a natural substrate on the establishment, development and survival of six cactispecies that occur in natural protected areas in northeast Mexico.

Methods

Study site

Species in the study come from two Protected Natural Areas: "Sierra Corral de los Bandidos" and "Sierra El Fraile-San Miguel", located in the States of Coahuila and Nuevo Leon, Mexico respectively (Figure 1).

The “Sierra Corral de los Bandidos" is located between the coordinates 25 ° 40' 58" and 25 ° 39 ' 10" North latitude and 100° 46' 10" and 100° 43' 10" West longitude; covering 1175.01 ha with altitude range from 1000 to 1640 m and the climate is dry (BS0 to BW), according toGarcía (1994). The annual precipitation ranges from 200 to 400 mm. The dominant soils are lithosol, eutric-regosol. The vegetation type corresponds to a rosetophilous desert with a physiognomy of Crassulaceae and rosette-leaved spiny plants and nopalera (INEGI, 1986).

The “Sierra El Fraile- San Miguel” is located to the northwest of the city of Monterrey, Nuevo Leon between 25° 48´52’’ and 25° 58’ 03’’ North latitude and 100° 39’ 42’’ and 100° 23’ 52’’ West longitude and between 680 and 2300 m. It lays in part of the Sierra Madre Oriental over 23,506.36 hectares. Climate is semi-warm-subhumid (ACW1), with an annual average rainfall of 400 to 600 mm. The dominant soil type is lithosol. Vegetation associated to the study area may be described as microphyll scrub, rosetophilous desert, submontane scrub, and oak forest-pine (INEGI, 1986; Carmona *et al*., 2008).

Criteria for selection of species

The study species occur naturally withinboth "Sierra del Fraile y San Miguel" and "Sierra Corral de los Bandidos". These areas are important due their richness and diversity of cacti(Carmona, 2008). The species were selected to meet the following criteria: 1) with minimum nursery association, 2) established to rocky substrate, 3) high relative growth rate, 4) high ecological value and 5) high ornamental value.

Viability and germination

Seed viability was assessed using the tetrazolium chloride test (2,3,5, triphenyl tetrazolium-2H), according to the International Seed Testing Association (Delouche *et al*., 1962; ISTA, 2003). For each species 3 repetitions of 15 seeds each were treated in glass tubes with 0.5 ml of the tetrazolium solution(1%) and maintained during 5 days at 25 °C in darkness inside an incubator (Shel Lab Thermoly FB1525M). The test is considered positive when the indicator solution (tetrazolium) turns from clear to red.

For the germination test, seeds were disinfected prior to planting with a commercial solution of sodium hypochlorite (Cloralex®) at 20% (v/v) for 30 min for seeds of*A. myriostigma* and *A. capricorne* and for 20 min for the other species. Later the seeds were rinsed with distilled water and a natural fungicide was applied (5% Sedric 650 ®). Then the seeds were placed on filter paper moistened with distilled water in sterile Petri dishes inside a bioclimatic chamber (Biotronette Mark III®) at 14 hours light (fluorescent) and a temperature of 26 ± 1 °C. They were distributed in 4 replicates of 25 seeds for each species (ISTA, 2010). The number of germinated seeds was counted daily for 20 days. Germination was considered to occur after the radicle emerged from the seed.

Growth and survival of seedlings

Four-months-old seedlings, obtained from the germination experiment were transferred to soil for growth and survival evaluation. Seedlings were transferred to a greenhouse and placed in two types of substrate (treatments): 1) conventional, perlite and peat-moss (50/50) and 2) natural zeolite and peat-moss (50/50). The number of individuals per species and per treatment was variable. Diameter of each plant at 4, 10 and 16 months-age, as well as the total number of surviving individuals in each substrate were recorded.

Speed and index of germination

The response variables evaluated were: viability (percentage of viable seed), final percentage of germination, speed of germination (M) and germination index (GI).The latter two indices were calculated in accordance with Gonzalez and Orozco (1996), using the following equation:

$$M = \Sigma (n_i) /t \qquad GI = \Sigma (n_i t_i) /N$$

Where GI = germination index; ni = number of germinated seeds on the day i; ti = number of days after planting; N = total number of seeds sown; M = speed of germination; and t = time of germination from planting until germination of the last seed. According to the GI index, the greater the value the greater the speed of germination.

Natural zeolite

The zeolitic material used as an alternative substrate was a Mexican natural zeolite of clinoptilolite type, with a specific surface area of 23 m^2 g^{-1} and water adsorption capacity of 0,108 cm^3 g^{-1} (Hernández-Huesca *et al*., 1999).

Statistical analysis

The data were analyzed using the statistical software SPSS® (ver. 19.0). Analysis of variance(ANOVA) to detect significant differences between species and treatments in terms of germination and a Tukey test for multiple average comparisons were applied according to Zar (2010) in order to be able to identify groups of similar species and treatments. All comparisons were made with arcsine-transformed values of each percentage value. All statistical tests were conducted at the 0.05 significance level.

Results and Discussion

Viability and germination

Seed viability of the six species ranged from 31.1% (*E. reichenbachii*) to 100% (*A. capricorne, A. myriostigma* and *S. scheeri*). The percentage of germination varied from 48% (*E. reichenbachii*) to 99% (*S. scheeri*). Viability, germination percentage and index differed significantly (p<0.001) from germination rate, with a coefficient of variation of 2.20-3.73% (Table 1).

The highest values for viability (100%) were obtained for *A. myriostigma, A. capricorne* and *S. sheeri*,while *E. reichenbachii* (31%) and *M. prolifera* (33%) showed lower values, forming a statistically different group from the rest (Table 2). Both percentage of germination and germination rate showed the highest values inthe species *A. myriostigma*, *E. dasyacantha* and*S. scheeri*(83, 88 and 99% respectively; 3.31, 3.57 and 3.30 seeds day^{-1} respectively).

Germination ratefor the three groups differed statistically: the first with *S. scheeri* with a germination rate of 7.5 ± 0.53 seeds day^{-1}, followed by the group with *A. myriostigma*, *E. dasyacantha* and *M. prolifera* (5.25 ± 0.45, 5.51 ± 1.18, and 6.06 ± 1.16 seeds day^{-1} respectively), and the group with the lowest value with *A. capricorne* (4.00 ± 1.36seeds day^{-1}) and *E. reichenbachii* (4.57 ± 1.12seeds day^{-1}).

Germination curves for the six species were obtained (Figure 2), where it is possible to observe that the seeds of *S. scheeri* showed the best germination response in terms of the number of germinated seeds (99%), reaching its maximum value (stability) at day 12 after planting. On the contrary, *E. reichenbachii* had the lowest value (48%) at day 18 after seeding. In a general way, the maximum germination percentage was obtained at day 10, remaining relatively constant after this date with the exception of *M. prolifera* that showed a slight increase (14%) in day 15.

Growth and survival

There were highly significant differences (P<0.01) for growth rate and diameter at4, 10 and 16 month). The coefficient of variation ranged from 0.119 to0.246 for the age variable and 0.309 for the growth rate (Table 3).

Table 3. ANOVA for three time periods (4, 10 and 16 months age) and growth rate in six cacti species cultivated with zeolite. The six species had a higher growth in zeolite and peat moss (9.97 mm) than in perlite and peat-moss (7.72 mm). Before the 4th month of the experiment the seedlings in perlite were slightly higher (3.48 mm) than the seedlings transferred to zeolite (3.43 mm) but a clear increase in the growth of all seedlings in zeolite occurred subsequently (Table 4).

Table 5 shows the data with respect to growth by species, where, at the time of the second (10 months) and third measurements (16 months), *E. reicheinbachii* and *M. prolifera* had the highest growth(15.50 and 13.07 mm, respectively) even when they had a lower initial height (at month 4th). Likewise, the growth rate was greatest for *E. reicheinbachii* (13.17 mm) followed by *M. prolifera* (11.46 mm). On the contrary, *A. myriostigma* and *A. capricorne* had the lowest growth rate (6.56 and 7.15 mm, respectively).

Seedling survival was higher in zeolite and peat-moss (67.29%) than in perlite and peat moss (42.67%) (Figure 3). Thus, the use of zeolite and peat-moss may be preferred as substrate for conservation of endangered species. However, despite the higher numerical in zeolite and peat-moss, the t-test showed that the survival rate was independent on the species and treatments with a probability equal to or greater than 5% ($t=5.911$, $df=5$, $p\leq 0.002$) (Table 6).

For most species, there was a clear increase in the percentage of survival with the use of zeolite and peat moss as substrate, with overall values between 15.15 and 85.39%. An exception was *E. reichenbachii*, whose survival percentage was the same on both substrates (27.27%).*S. scherii* was the species with the highest number of live plants at the end of the experiment (see Table 6 and Figure 4).

When using perlite and peat moss as a substrate, the highest percentages of survival were obtained with *S. scheeri* (60%) and *A. capricorne* (54.55%), whereas when using zeolite and peat moss the percentage increased substantially, with the highest value for *S. scheeri*(85.39%) followed by *M. prolifera* (75%). For this evaluation, the lowest percentage (15.15%) was also for*E. dasyacantha* in perlite and peat mosssubstrate. In general,*S. scheeri*had the highest survival in both treatments (Table 6).

Viability and germination

The percentage and rate of germination was highest for *A. myriostigma*, *E. dasyacantha* and *S. scheeri*. These results are similar to thoseof Sanchez *et al*., (2006), who reporteda germination percentage of 74.7% in *A. myriostigma* without anygermination promoter. Similarly, Navarro and Demenegui (2007) reported 95% germination for *Mammillaria pectinifera*in light. Sanchez *et al*., (2006) obtained a rate of germination of 3.8 seeds per day for *A. myriostigma*, a value similar to the obtained in the present study (3.31 ± 0.37mm). Most Cactaceae have a maximum germination at 25°C (Sanchez *et al*., 2010), indicating that the temperature used in this study (26±1°C) could be considered as optimal.

In the present study seed viability was 100% in *A. myriostigma, A. capricorne* and *S. sheeri* and 31% in *E. Reicheinbachii*. Trejo and Garza (1993) reported that for some species of Cactaceae the viability is increased as the seed ages.

Figure.1 Geographical location of the Protected Natural Areas: "Sierra Corral de los Bandidos" and "Sierra El Fraile-San Miguel"

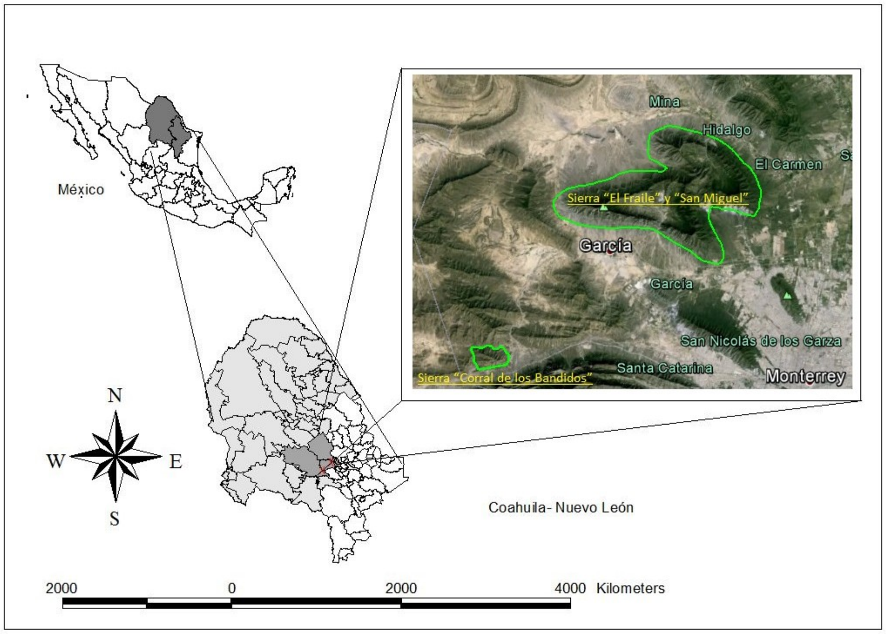

Table.1 ANOVAfor viability, germination percentage, rate and index of six species of Cactaceae under laboratory conditions

Variables	F calculated	Probability	VC[+]
Viability	20.631**	<0.001	0.2202
Germination percentage	10.660**	<0.001	0.3347
Germination rate	14.114*	0.002	0.2643
Germination index	5.788**	<0.001	0.3735

*Significant values P < 0.01, **highly significant values P < 0.001.

[+]VC= Variation coefficient.

Table.2 Viability, germination percentage, rate and index for six species of Cactaceae under laboratory conditions

Species	Viability(%)	Germination percentage	Germination rate (seeds day^{-1})	Index
A. myriostigma	100.00 ± 0.00 a	83.00 ± 8.25 abc	3.31 ± 0.37 ab	5.25 ± 0.45 ab
A. capricorne	100.00 ± 0.00 a	61.00 ± 19.97 bcd	2.35 ± 0.73 bc	4.00 ± 1.36 b
S. scheeri	100.00 ± 0.00 a	99.00 ± 2.00 a	3.30 ± 0.18 ab	7.53 ± 0.53 a
E. dasyacantha	71.11 ± 13.88 a	88.00 ± 16.33 ab	3.57 ± 0.62 a	5.51 ± 1.18 ab
M. prolifera	33.33 ± 24.04 b	57.00 ± 3.83 cd	1.47 ± 0.10 c	6.06 ± 1.16 ab
E. reicheinbachii	31.11 ± 13.88 b	48.00 ± 12.65 d	1.55 ± 0.63 c	4.57 ± 1.12 b
Average	72.59 ± 32.97	72.67 ± 21.71	2.59 ± 0.98	5.49 ± 1.47

Different letters within columnsindicate statistically differences (p ≤0.05)

date with the exception of *M. prolifera* that showed a slight increase (14%) in day 15.

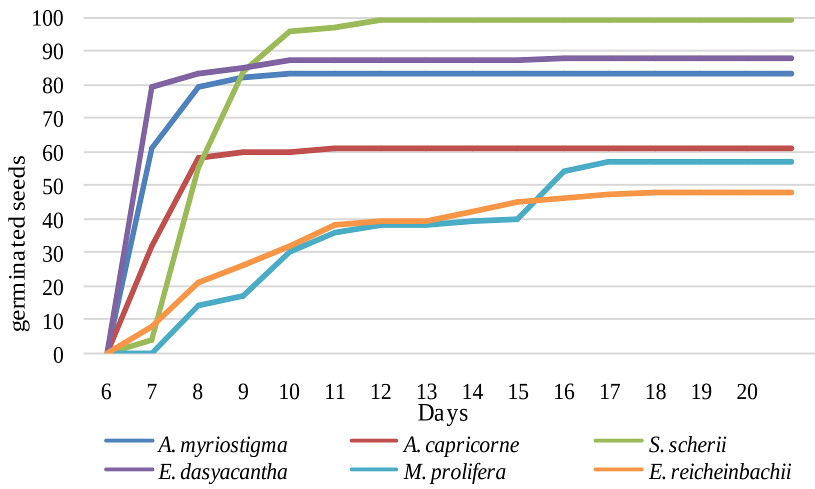

Figure.2 Germination percentage for six Cactaceae species during 20 days of assessment

Table.3 ANOVA for three time periods (4, 10 and 16 months age) and growth rate in six cacti species cultivated with zeolite

Variables	F_{value}	Probability	VC*
4 months age	18.242	<0.001	0.1193
10 months age	31.798	<0.001	0.2463
16 months age	23.243	<0.001	0.2180
Growth rate	25.744	<0.001	0.3089

* VC: Variation coefficient.

Table.4 Growth rate (mm/16 months) and diameter mean values (mm) ± standard deviation for six cacti species cultivated in perlite or zeolite substrate

Treatment	Growth rate	4 months	10 months	16 months
Perlite – peat moss (n=67)	7.72 ± 2.10	3.48 ± 0.40 c	9.91± 2.03 b	11.21 ± 2.14 a
Zeolite – peat moss (n=107)	9.97 ± 2.86	3.43 ± 0.42 c	12.02 ± 2.85 b	13.40 ± 2.74 a
Total (n= 174)	9.11 ± 2.81	3.45 ± 0.41	11.21 ± 2.76	12.56 ± 2.74

Different letters within rows indicate statistically differences ($p \leq 0.05$)

Table.5 Average diameter values (mm) ± standard deviation for six species of Cactaceae depending on dates of measurement and final rate of growth (mm/16 months)

Species	4 months	10 months	16 months	Growth rate
A. myriostigma (n=54)	3.37 ± 0.41	8.42 ± 1.36	9.93 ± 1.53	6.56 ± 1.51
A. capricorme (n=45)	3.71 ± 0.31	9.10 ± 1.45	10.86 ± 1.90	7.15 ± 1.87
S. scheeri (n=5981)	3.65 ± 0.35	12.20 ± 2.09	13.44 ± 2.25	9.79 ± 2.28
E. dasyacantha (n=66)	3.29 ± 0.37	11.71 ± 2.15	12.62 ± 2.06	9.33 ± 2.01
M. prolifera (n=48)	3.07 ± 0.22	13.07 ± 2.24	14.54 ± 2.22	11.46 ± 2.29
E. reichenbachii (n=22)	3.00 ± 0.00	15.50 ± 4.04	16.17 ± 4.12	13.17 ± 4.12
Total (n=174)	3.45 ± 0.41	11.21 ± 2.76	12.56 ± 2.74	9.11 ± 2.81

n= number of individuals for each specie.

Figure.3 Survival percentage for six cacti species grown in two types of substrates ± standard deviation

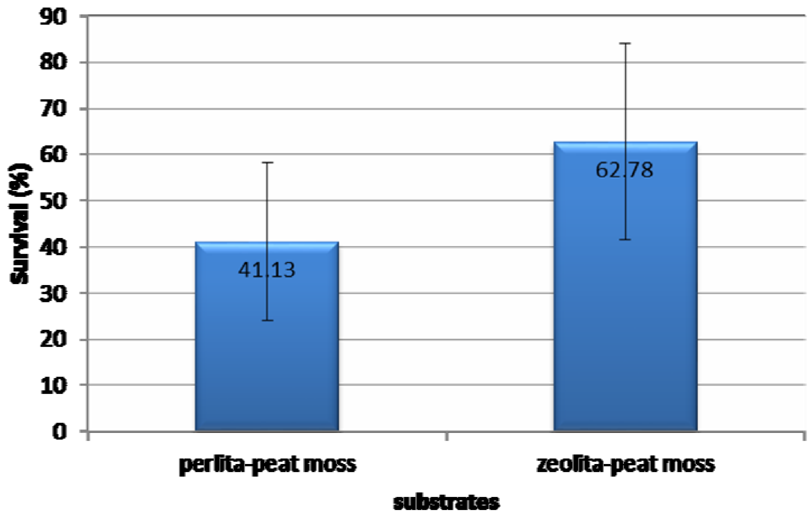

Figure.4 Survival curves for six cactispecies during 16 months of assessment

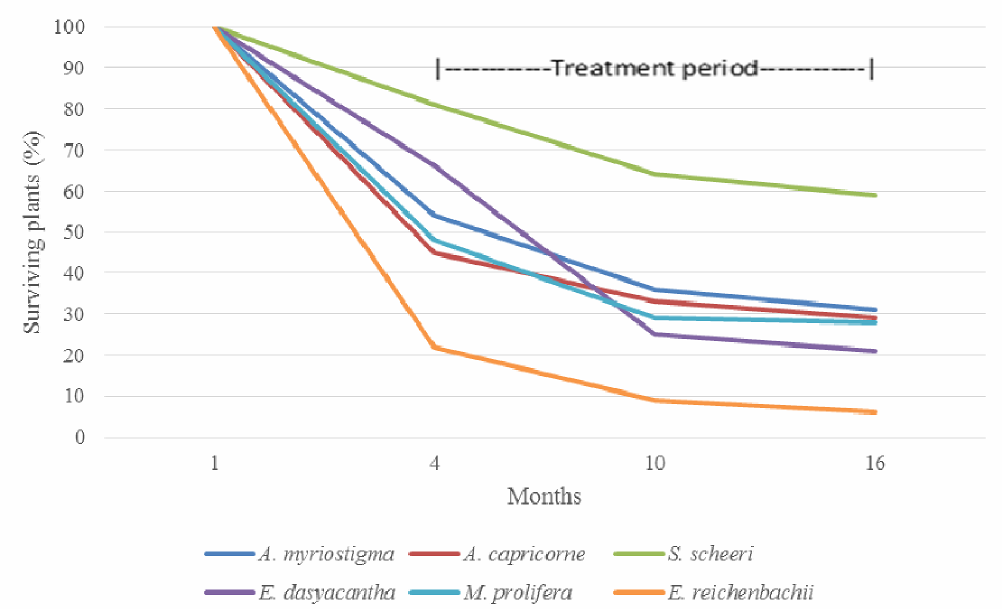

Table.6 Observed frequencies in six cacti species of two treatments

Species/treatment	Peat moss – perlite	Peat moss – zeolite	Average ± S.D.
A. myriostigma	48.15	66.67	57.41 ± 13.81
A. capricorne	54.55	73.91	64.23 ± 13.69
S. scheeri	60.00	85.39	72.68 ± 17.94
E. dasyacantha	15.15	48.48	31.81 ± 23.57
M. prolifera	41.67	75.00	58.35 ± 23.57
E. reicheinbachii	27.27	27.27	27.27 ± 0.00
P[a]	0.001	0.001	-

*Chi-square = 10.71; $p< 0.05$,

[a]Probability value for t student test, pairwise comparison (peatmoss perlite – peatmoss zeolite)

Those authors reported that zero years old seeds of *M. heyderi* have a lower percentage of germination than seeds two and three years old. Similar results were found by Simao *et al.* (2007, 2010) for *Hylocereus setaceus*. Germinability and germination rate for these species were higher in stored seeds than in newly collected ones. On the other hand, Ayala-Cordero *et al.*, (2004) reported that the germination od Cactaceae seeds is better in newly collected seeds than in those stored ones. Thus, it is possible that in the present investigation the storage period was a factor that influenced the viability and germination for seeds of *E. reicheinbachii* and *M. prolifera*at the time of the test since their collection was carried out 3 months before planting.

Growth and survival

The seedlings in zeolite substrate reached a higher growth rate and survival percentage than seedlings in conventional substrate. These findings demonstrate that zeolite provides greater availability of nutrients and greater ease of absorption of water, thus favoring the growth, as mentioned by Left *et al*., (2002) and Manolov*et al*., (2005). On the contrary, Castro *et al*., (2006) indicated that the type of soil is not significant in the establishment of the seedlings since cacti have evolved in soils of arid areas,which usuallyarenutrient poor. It should be noted that for this assessment seedlings were 4 months old, an age at which the absorption of nutrients is limited due to the early development of the root system. In thisstudy roots were well developed (up to 16 months of age in the six species) thus allowing further assimilation of nutrients from the soil enriched with zeolite.

Alvarez *et al*., (2004) reported low survival in the field for*Strombocactus disciformis* and *Turbinicarpus pseudomacrochele* due to the fact that after 20 days there were noseedlings alive. The authors relate this result to the reduced ability of the seedlings of cacti to store water because its surface is greater than its volume, favoring the water loss by transpiration (Nobel, 1994). Castro *et al*., (2006) found that seedlings of *Melocactus peruvianus* and *Haageocereus pseudomelanostele* subsp. *aureispinus*were healthier in shady conditions than under direct sun, which reinforces the hypothesis that the recruitment observed in the field is favored by the beneficial action of the nurse-plants in the early years of development(Collantes, 2011). Due to the absence of shrub plants in this study, occurring nurse vegetation were cacti themselves; thus, we inferred that the conditions of both the shade supplied by acrylic sheets of translucent material and the humidity in the greenhouse were enough to maintain the water loss caused by transpiration and the proper uptake of nutrients by seedlings.

Conclusions

The use of zeolite as substrate for cacti propagation is a good alternative for*ex situ* conservation of endangered and overexploited species, in particular for *A. capricorne* and *A. myriostigma*, since plants are maintained in optimum growth conditions. Thus, as the demand for cacti individuals is satisfied, vulnerability of their population in natural areas decreases.

Germination of the six evaluated species of Cactaceae increased. Thus, propagation by seeds is a good alternative for obtaining a high number of seedlings for the six species since viability was 100% for three of them (*A. capricorne*, *A. myriostigma* and *S. scheeri*), with high germination rate and index values.

Growth and survival after germination under controlled conditions for the six species of Cactaceae was noticeably favored by the use of natural zeolites, which promoted growth and reduced mortality rate, in comparison with the conventional substrate. The species that had the highest survival were *S. scheeri* and *A. capricorne*, while *E. reichenbachii* and *M. prolifera*had the highest growth rate.

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