

Longevity of *Mammillaria supertexta* Seeds under Ultra-dry Long-term Storage



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Summary

The purpose of this study was to evaluate the *ex situ* conservation potential of *Mammillaria supertexta* seeds by quantifying their response to desiccation with silica gel, and cold storage, and by defining optimal conditions for seed germination. Seeds were dried at about $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$ using silica gel (g)/seeds (g) ratios of 10:1, 100:1 and 150:1. Drying from 16.8 % to 2.3% moisture content and subsequent storage for 6 years at -20°C had no negative effect on germination percentage. Light conditions (24 h d^{-1}) promoted seed germination at temperatures of 19°C , 25°C and 35°C , both before and after drying and storage. In contrast, germination under nominal dark conditions was lower before drying and increased after drying and storage, suggesting the alleviation of dormancy. It is concluded that mature seeds of *M. supertexta* are tolerant of desiccation to very low moisture contents and of storage at conventional seed bank temperature.

Introduction

Mammillaria supertexta Mart. ex Pfeiff. is a member of the *Cactaceae* family. The plant is subglobose, occasionally caespitose, with a stem that is 5 to 16 cm high and 5 to 8 cm wide. The fruit is a berry, which ranges in size from 1 to 1.5 cm long and 2 to 3.6 cm wide. The seeds are clavate and range in size from 0.7 to 0.8 mm long. The seed-coat is foveolate and brownish (Bravo-Hollis and Sánchez-Mejorada, 1991; Arias *et al.*, 1997; Corner, 1976).

The species is endemic to the arid region of the Biosphere Reserve of Tehuacán-Cuicatlán. Its populations are scarce and its distribution pattern is restricted only to three localities in the state of Oaxaca and one in Puebla. The Reserve represents one of the most important distribution areas of columnar and globose cacti of Mexico (Dávila *et al.*, 1993, 1995). In addition, Mexico is the main center of diversification of the genus *Mammillaria*, with approximately 200 species, nine of which are endemic to the Valley of Tehuacan-Cuicatlan (Arias *et al.*, 1997; Arias, 1997; Guzmán, 1997).

The plant is used as an ornamental and the fruits are extracted for local consumption or for seed propagation (Reyes-Santiago, 1997; Valiente-Banuet and del Coro-Arizmendi, 1997). In addition, local people sell both plants and seeds to collectors, naturalists, horticulturists and nurseries in Mexico and Europe. Such actions may impact ultimately on the capacity of the species for natural regeneration, decreasing seed germination, seedling establishment and plant recruitment in the field. Consequently, there is an urgent need to implement conservation actions, both *in situ* and *ex situ*.

Ex situ conservation of *Cactaceae* may be achieved through seed storage. For example, seeds of *Ferocactus* and *Opuntia* have been maintained adequately, under long-term storage conditions, in the Seed Bank of the Royal Botanic Gardens, Kew (Hong *et al.*, 1998). However, information on the seed storage potential of other species of *Cactaceae* is sparse. Often, seed banks have drying rooms operating at low relative humidity. However, lower cost solutions are available and FAO/IPGRI (1994) and Cromarty *et al.* (1982) have suggested the use of silica gel as a seed desiccant. The aim of this study was to assess the *ex situ* conservation potential of *M. supertexta* seeds by quantifying their response to desiccation with silica gel, and cold storage, and by defining optimal conditions for seed germination.

Materials and Methods

The species was sampled on 24th September 1995 in the Tehuacán-Cuicatlán Biosphere Reserve. Mature fruits (berries) were collected from 35 plants, kept in an open paper box and carried to the laboratory of UNAM's Seed Bank within 3 d. Seed processing started 1 d after receipt of the fruit.

Seeds were manually extracted and the fleshy red pulp removed by rubbing the seeds carefully on a sieve under running water. Then the seeds were dried for 3 d on a flat surface at an ambient temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $24\% \pm 3\%$ relative humidity (RH). Seed moisture content (% fresh weight basis) was then determined by drying five replicates, each of 10 seeds, in an oven at 130°C for 2.5 h (ISTA, 1999).

For the drying experiment, the seeds were placed (soon after processing) in three glass desiccators with a silica gel weight/seed weight (g) ratio of 10:1, 100:1 and 150:1, respectively. Also, a control set up without silica gel was included. Silica gel was regenerated regularly by heating at 130°C . The experiment was run in a room at $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and *c.* 20% RH. Seeds reached equilibrium by four weeks, as assessed by the attainment of constant weight. Dried samples were sealed in foil laminate bags and, after equilibration at 18°C for 2 d, maintained at -20°C for 1 month and 6 years. Moisture contents after drying were determined gravimetrically by determining the difference between weights before and after oven drying (described above). To evaluate changes in viability after both drying and storage treatments, samples from each moisture content were germinated.

The seeds were sown for germination (replicates of 3×25 seeds) on 1% agar-water in 9 cm diameter Petri dishes. Three incubation temperatures were used (19°C , 25°C and 35°C) with light applied for 24 h d^{-1} , using white fluorescent tubes. Alternatively, a nominal dark treatment was used, whereby dishes were

completely covered in aluminium foil and only unwrapped briefly for daily germination assessment under laboratory conditions (Pritchard and Miller, 1995). Positive germination was scored when the emerging radicle reached *c.* 2 mm outside the seed. Germination was recorded daily, for 30 d, and the data were subjected to analysis of variance after arc-sine transformation.

Results

After processing, seeds had an initial moisture content of $16.8 \pm 1.4\%$ and a maximum germination level of 97%. Initial germination was affected by both constant temperature and light (Table 43.1). In the light, germination reached 97% and 92% at 19°C and 25°C respectively, compared to only 62% at 35°C. In contrast, germination levels under nominal dark conditions were <11%, irrespective of the temperature.

Table 43.1 The effect of light and temperature on the germination of *Mammillaria supertexta* seeds before silica gel drying. Data represent mean values with the standard deviation in parentheses.

Temperature (°C)	Germination (%)	
	Light	Nominal dark
19	97.3 (1.9)	9.3 (1.9)
25	92.0 (5.7)	10.7 (6.8)
35	62.0 (14.7)	4.0 (3.3)

The final level of seed moisture was dependent on the ratio of silica gel to seeds (Table 43.2). Ratios of 10:1, 100:1 and 150:1 resulted in seed moisture contents of 5.8%, 4.5% and 2.3% respectively (Table 43.2). Such drying, and subsequent short-term (1 month) storage at -20°C, did not adversely affect seed germination in the light; under all temperature regimes, germination ranged from 85.3% to 98.7% (Table 43.2). In contrast, such treatment enhanced germination under nominal dark conditions compared to undried seeds. This effect was greatest for germination at 19°C, reaching more than 68% irrespective of the moisture content reached. At 25°C and 35°C, however, nominal dark germination was still just under 40% for the 2.3% moisture content seeds (Table 43.2).

Table 43.2 The effect of moisture content (MC) and storage time at -20°C on germination at different temperatures and under light and nominal dark conditions. Data represent mean values \pm standard deviation in parentheses

Drying treatments	Lighting	Germination (%)											
		0 month storage				1 month storage				6 years storage			
		19°C	25°C	35°C		19°C	25°C	35°C		19°C	25°C	35°C	
Ratio silica gel / seeds (%MC \pm s.d.)	Light	97.3 (1.9)	89.3 (5.0)	92.0 (5.7)		94.7 (1.9)	96.0 (3.3)	96.0 (3.3)		90.7 (5.0)	88.0 (5.7)	97.3 (3.8)	
	Dark	74.7 (5.0)	58.7 (1.9)	50.7 (1.9)		98.7 (1.9)	62.7 (1.9)	54.7 (8.2)		56.0 (22.6)	77.3 (8.2)	62.7 (5.0)	
10:1 (5.8 \pm 0.6)	Light	98.7 (1.9)	85.3 (5.0)	90.7 (1.9)		93.3 (1.9)	88.0 (3.3)	90.7 (1.9)		96.0 (0.0)	86.7 (5.0)	92.0 (3.3)	
	Dark	72.0 (3.3)	69.3 (15.4)	62.7 (21.3)		78.7 (5.0)	73.3 (19.7)	65.3 (24.5)		37.3 (1.9)	57.3 (3.8)	60.0 (5.7)	
100:1 (4.5 \pm 0.4)	Light	93.3 (3.8)	90.7 (6.8)	96.0 (3.3)		93.3 (3.8)	97.3 (3.8)	94.7 (1.9)		93.3 (6.8)	89.3 (1.9)	93.3 (1.9)	
	Dark	68.0 (11.3)	52.0 (11.8)	56.0 (8.6)		94.7 (10.5)	37.3 (8.2)	37.3 (9.4)		48.0 (8.6)	57.3 (8.2)	57.3 (8.2)	
150:1 (2.3 \pm 0.4)	Light	93.3 (3.8)	90.7 (6.8)	96.0 (3.3)		93.3 (3.8)	97.3 (3.8)	94.7 (1.9)		93.3 (6.8)	89.3 (1.9)	93.3 (1.9)	
	Dark	68.0 (11.3)	52.0 (11.8)	56.0 (8.6)		94.7 (10.5)	37.3 (8.2)	37.3 (9.4)		48.0 (8.6)	57.3 (8.2)	57.3 (8.2)	

The effect of long-term (6 years) storage on seed germination was also evaluated. The maximum germination value for stored seeds previously dried to 5.8% moisture content was 97.3% at 35°C in the light (Table 43.2). For 4.5% and 2.3% moisture content stored seeds, the highest germination in the light was 96% at 19°C and 93% at both 19°C and 35°C. The results indicate that seeds of *M. supertexta* maintained high viability after long-term storage. By comparison, maximum germination levels for each moisture content treatment after long-term storage were lower in nominal dark conditions, although still in the range 37% to 77% (Table 43.2). Generally, germination in nominal darkness was higher at warmer temperatures (25°C and 35°C) for long-term stored seeds, irrespective of moisture content (Table 43.2).

Analysis of variance revealed that there was no significant interaction between the effects of light and temperature on germination; germination was always high in the light, irrespective of temperature (Table 43.3). Also, drying was seen to have no significant overall effect on germination (Table 43.3).

Table 43.3 Analysis of variance for dried and stored seeds in relation to germination treatments. Parameters = Light, Temperature (Temp), Storage time (Storage-T) and Drying.

Source	Sum of Squares	D. F.	Mean Square	F-ratio	P
Light	2.552	1	2.552	247.28	0.0001
Temp	0.131	2	0.065	6.33	0.006
Storage-T	0.632	2	0.316	30.59	0.0001
Light*Temp	0.000	2	0.000	0.018	0.992 ns
Light*Storage-T	0.393	2	0.196	19.03	0.0001
Temp*Storage-T	0.230	4	0.057	5.57	0.003
Light*Temp*Storage-T	0.290	4	0.073	7.03	0.001
Drying	0.037	1	0.037	3.63	0.069 ns
Error	0.237	23	0.010		

ns = not significant, $P > 0.05$.

Discussion

There are some concerns about the use of silica gel as a desiccant due to its potential ability to overdry the seeds (Walters, 1998). For example, seeds of three orchids tolerated silica gel desiccation, but subsequent longevity in storage was marginally lower than at slightly higher moisture contents (Pritchard *et al.*, 1999). In contrast, silica gel drying, and 6 years subsequent storage at -20°C , had no discernable effect on the quality of *M. supertexta* seeds. A similar response was observed in dried seeds of *Echinocactus platyacanthus* f. *grandis* after 5 years storage at the same temperature (Ocampo-López, 2002, in press). Silica gel thus remains a practical, low cost drying technique for some seeds when controlled humidity rooms are not available.

When seeds were exposed to light, the total germination level at 19°C , 25°C and 35°C was significantly higher than in nominal dark conditions, indicating that the seeds are light sensitive (Tables 43.1, 43.2 and 43.3). Indeed, it appears that undried seeds (17% moisture content) have an obligate requirement for light to germinate (Table 43.1). The photon dose to stimulate germination was not determined, but clearly it was not saturated by the short exposures associated with the nominal dark treatment. However, desiccation to $< 6\%$ moisture content increased germination in nominal darkness possibly as a result of an increased sensitivity of the seeds to light (Baskin and Baskin, 1998). Similar germination responses to temperature and light have been observed in seeds of five other cacti distributed in the Tehuacán-Cuicatlán Biosphere: *Opuntia* sp., *Echinocactus platyacanthus* f. *grandis*, *Mammillaria* sp., *Neobuxbaumia tetetzo*, and *Pachycereus hollianus* (Flores and Briones, 2001; Hong *et al.*, 1998; Ocampo-López, 2002, in press).

The results suggest that at dispersal, *M. supertexta* seeds are capable of entering dormancy when imbibed in the dark. This type of response may serve to delay germination until sibling and parental competition in the natural environment is reduced/removed (Baskin and Baskin, 1998). Lowered levels of dormancy (better germination in nominal dark) after drying (and short-term storage) indicate that the seeds are capable of dry after-ripening, which has adaptive value for seeds in a dryland environment.

Conclusion

Undried seeds of *M. supertexta* require light for germination, but have improved capacity to germinate in nominal darkness after dehydration using silica gel, possibly as a result of lowered seed dormancy through after-ripening. The optimal temperature for germination is 19°C. Dried seeds tolerate long-term storage at conventional seed bank temperature, suggesting orthodox seed storage behaviour. The results support the notion that the *ex situ* conservation of arid and semi-arid plants is possible by means of seed storage.

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