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RESEARCH PAPER

Morpho-anatomical changes and photosynthetic metabolism of *Stenocereus beneckei* seedlings under soil water deficit

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Abstract

Characteristics developed by Cactaceae for adaptation to climates where water is limited include crassulacean acid metabolism (CAM), a thick cuticle, and spines and trichomes that intercept a proportion of solar radiation. A few studies consider morpho-anatomical and physiological characteristics of Cactaceae seedlings, which may help understand their establishment, growth, and eventual reproduction. In this study, photosynthetic metabolism (titratable protons) and morpho-anatomical features of *Stenocereus beneckei* seedlings were examined under limiting water conditions. Soil moisture treatments consisted of −0.03, −0.5, −1.5, and −3.0 MPa, and seedling samples were taken at 3 h intervals on one day at 7 and 9 months of age with three replicates per treatment. The results show irregular fluctuations in acidity concentrations during the first 6 and 7 months of age; at 9 months, an increase in titratable proton values was observed during the night, and it seems that soil moisture does not determine CAM expression. Seedlings from smaller seeds are less tolerant to water stress, they had poor growth in all treatments, and at −3.0 MPa after 3 months of drought none survived. Anatomical observations show collapsed cells associated with a high accumulation of calcium oxalate crystals and starch grains, as a response to water deficit. Titratable acidity concentration increased with seedling age, and CAM expression did not accelerate with soil water deficit.

Key words: CAM, collapsible parenchyma cells, development, drought, oxalate crystals.

Introduction

Cactaceae in arid and semi-arid regions display unique morphological, physiological, and anatomical characteristics that allow them to tolerate extreme weather conditions and to complete their life cycle in these regions (Nobel, 1988; Bravo-Hollis and Scheinvar, 1995). Among the most important of these characteristics are crassulacean acid metabolism (CAM), a type of CO_2 fixation characterized by fixing atmospheric CO_2 during the night and keeping stomata closed during the day, a fast rate of water absorption by the roots, and spines or refractive trichomes covering some stems to reduce the incidence of solar radiation on the plant's surface (Gibson and Nobel, 1986; Vázquez-Yanes, 1997; Pimienta-Barrios *et al.*, 1998; Dodd *et al.*, 2002). Cactaceae can tolerate drought, displaying changes that reduce water loss from internal tissues to the surface of the root (North and Nobel, 1992), as well as epicuticular wax, a thick cuticle, and multiple epidermis with sunken stomata (Terrazas and Mauseth, 2002).

In addition, drought tolerance involves an element that is defined by the amount of water stored in the tissues during development in the first year of growth (Jordan and Nobel, 1981), which makes water availability during the seedling establishment phase a decisive factor (Ruedas *et al.*, 2000). Seedling germination, establishment, and survival have also been associated with nurse plants and rocks that provide shade (Valiente-Banuet *et al.*, 1991; Reyes-Olivas *et al.*, 2002). These elements, i.e. plants and rocks, create moist microclimates and provide protection against excessive radiation during the initial stages of growth, both of which are considered basic requirements for seedling survival (Godinez-Álvarez and Valiente-Banuet, 1998; Rojas-Aréchiga and Vázquez-Yanes, 2000).

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Only a few studies of this family of plants describe seedling anatomical (De Fraine, 1910; Bernard, 1967; Freeman, 1969; Loza-Cornejo *et al.*, 2003) and physiological characteristics (Altesor *et al.*, 1992; Loza-Cornejo *et al.*, 2003; Hernández and Briones, 2004) that may help to understand their establishment, growth, and eventual reproduction patterns (Loza-Cornejo *et al.*, 2003). Moreover, Loza-Cornejo *et al.* (2003) mention the need to test if water deficit is related to the onset of CAM in Cactaceae seedlings. This study evaluated the accumulation of biomass, the anatomical characteristics, and the photosynthetic metabolism (titratable protons) of *Stenocereus beneckei* (Ehrenb.) Buxb. seedlings growing under different soil moisture conditions. *Stenocereus beneckei* is an endemic from the Balsas Depression and Tehuacan-Cuicatlán Valley, Mexico that never grows more than a 1.5 m tall and has distinctive decumbent stems. The few individuals of *S. beneckei* per population reproduce mostly vegetatively, although seeds are produced each year at the end of the winter. The working hypotheses were that soil moisture deficit accelerates the presence of CAM, inhibits biomass accumulation affecting the tissues, and that seedlings from small seeds are less tolerant to water stress.

Materials and methods

Seedlings from five weight categories of 1-month-old seeds (Ayala-Cordero *et al.*, 2004) were transplanted to pots of 3.0 cm in diameter and depth. The pots contained a mixture of 10.5 g of soil taken from the harvest site and 2.5 g of tezontle (volcanic rock) with a diameter of <0.5 cm. The pots were placed in plastic trays under laboratory conditions and allowed to grow for 6 months. Seedlings of 6 months of age were selected to start the water deficit treatments based on Loza-Cornejo *et al.* (2003) who studied the congeneric *S. queretaroensis*, a candelabriform plant, whose seedlings growing under laboratory conditions show acid fluctuations up to the age of 56 weeks. Seedlings were watered once a week to maintain growth; temperature was recorded every 2 h with a data logger. During the 9 months of the experiment, the temperature ranged between 19 °C and 22 °C. During the same period, photosynthetic photon flux density (PPFD) was measured with a Li-Cor (LI 185A) photometer and was, on average, 50 $\mu mol\ m^{-2}\ s^{-1}$; periods of light and darkness each lasted for 12 h. Seedlings from category 1 (seeds <7 mg) did not survive; most of them died and those that survived were insufficent in number to continue with the treatments. From 6 months of age onwards, soil moisture was maintained at different soil water potentials (Ψ_{soil}).

Soil moisture conditions

In order to establish the different soil moisture treatments, the amount of water required per experimental unit (one pot) at field capacity was first determined. Evaporation was then allowed and soil samples were taken at different times to determine Ψ_{soil}. This was determined by incubating soil samples for 3 h in C-52 (Wescor, Inc.) psychrometric chambers which were connected to a microvoltmeter HR-33 (Wescor, Inc.) using the dew point method. Simultaneously, soil samples were taken to determine fresh weight, and were subsequently dried in an oven at 105 °C until constant weight was reached. Percentage moisture was determined using the equation:

$$h(\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100$$

In this manner, a curve of percentage of moisture in the soil versus Ψ_{soil} was obtained. This curve was used to determine soil water potential in the treatment pots. Based on this information, four moisture treatments were selected: −0.03 MPa (field capacity), −0.5, −1.5, and −3.0 MPa. To maintain Ψ_{soil} or moisture percentages, the pots were constantly weighed and water added with a micropipette (Gilson) to maintain a constant weight of 20.7 g for −0.03 MPa, 19.8 g for −0.5 MPa, 19.2 g for −1.5 MPa, and 18.6 g for −3.0 MPa.

Two samplings were performed throughout the experiment. The first one was conducted 1 month after starting the moisture treatments (7 months of age) and the second 3 months after starting the treatments (9 months of age). Ninety-six seedlings were taken at each sampling and were used to evaluate malic acid concentration, water accumulation, and size and anatomy of each of the seedling's parts.

Photosynthetic metabolism (titratable protons)

Twenty-four seedlings were taken from each weight category [2 (7.3–10.8 mg), 3 (10.9–13.2 mg), 4 (13.3–16.8 mg), and 5 (16.9–21.0 mg)] at 6 months of age in order to evaluate their acidity at this stage of development. Titratable acidity was evaluated at 3 h intervals for a period of 24 h at 6, 7, and 9 months of seedling age. Three seedlings were taken per treatment; each seedling was weighed and measured with a digital vernier Mitutoyo Digimatic SR44 and immediately stored in liquid N_2. Subsequently, each seedling was placed in a mortar and soaked with 3 ml of deionized water. The extract was filtered, several drops of phenolphthalein indicator were added, and the mixture was titrated with 0.01 N NaOH solution to calculate the concentration of acid per unit of fresh weight according to Osmond's technique (Osmond *et al.*, 1989). To detect whether there was a significant interaction effect between seed weight, soil moisture treatment, and seedling age variables with titratable acidity, an analysis of variance was conducted by means of a factorial design including a factor for interaction among treatment, seedling age, and seed weight ($Ac = T \times E \times P$) where Ac=is the titratable acidity, T=is the treatment, E=is the age, and P=is the seed weight.

Tissue water accumulation

The amount of water in seedlings was calculated based on the fresh weight of three seedlings per treatment. Once weighed, each seedling

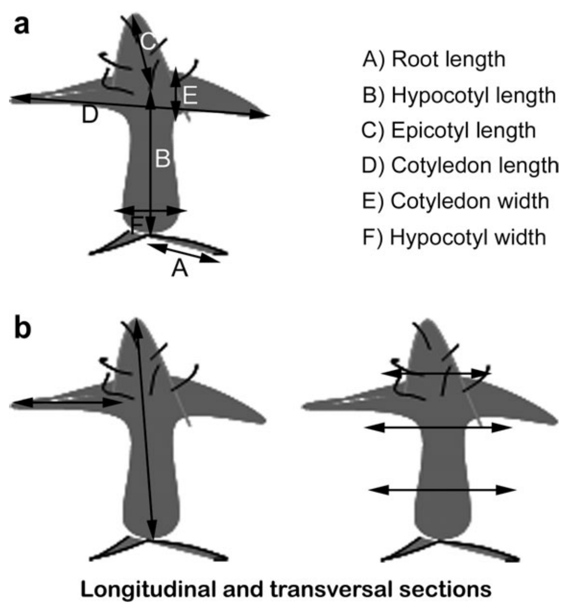

Fig. 1. Seedling diagram showing the sampling method. (a) Lengths measured. (b) Section for anatomy.

was placed in a paper bag in an oven at 80 °C for 72 h. The seedlings were weighed and the percentage moisture was calculated based on the difference between the dry weight and the wet weight. Percentage moisture was again obtained by the formula:

$$h(\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100$$

Due to the percentage moisture being similar among treatments and seed categories, no statistical analysis was performed. However, a Kruskal–Wallis analysis (SAS, 1989) was used to test for seedling dry weight differences among treatments and seed categories.

Size

Seedlings used for assessing titratable acidity, tissue water accumulation, and anatomy were used to characterize the size. Root length, and length and width of hypocotyl, cotyledons, and epicotyl were measured in seedlings from each weight category with a digital vernier (Mitutoyo Digimatic SR44) to establish the size of each of the parts (Fig. 1a). An analysis of variance and Tukey's comparison of means (SAS, 1989) were performed to evaluate whether there were significant statistical differences in total size among the different treatments groups for the different seed categories.

Anatomy

Two seedlings were used per seed category per soil moisture treatment at 1 and 3 months after beginning treatments to describe the anatomy of the dermal, fundamental, and vascular tissue. The seedlings were cut into cross- and longitudinal sections (Fig. 1b) and fixed in 5 ml of 50% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0–7.2 for 48 h. They were then washed with the buffer solution and embedded in paraffin (Berlyn and Miksche, 1976). Sections 10–12-mm thick were cut with a rotary microtome, stained with safranin-fast green, and mounted in synthetic resin (Berlyn and Miksche, 1976). Anatomical characteristics were described for each seedling part. The number of crystals per mm^2 in cells from hypocotyl was assessed, and photographs were taken with an image analyser Media-Cybernetics (1997) adapted to an Olympus Bx-50 microscope.

Results

Photosynthetic metabolism: titratable protons

Irregular fluctuations in acid concentrations were observed in 6-month-old seedlings, with the highest concentrations, 0.66 and 0.71 μmol H^+ g^{-1} fresh weight, occurring at 12 h and 18 h, respectively (Fig. 2). Figure 3 shows that after 1 month of different soil moisture treatments, 7-month-old seedlings exhibited slight fluctuations in titratable acidity concentration values during the 24 h of acidity determination. Acidity had a mean value of 0.58 μmol H^+ g^{-1} fresh weight during the 24 h of sampling and did not display any identifiable pattern or soil moisture treatment effect (Fig. 3a, c, e, g). At 9 months of age and after having remained in the different Ψ_{soil} for 3 months, titratable acidity displayed a clearly defined pattern during the 24 h of sampling. In all moisture treatments, maximum acidity was observed at 24 h (midnight). Acidity gradually decreased until reaching the lowest values, with small fluctuations, between 6 h and 21 h, and again increased to maximum values at 24 h. This same pattern was observed in all Ψ_{soil} treatments, which suggests that plant ontogeny is the decisive factor in the expression of this physiological characteristic. However, it is also clear that, as Ψ_{soil} decreased, the absolute values of the observed maximum acidity increased, reaching a maximum fresh weight of 1.97±0.58 in −1.5 MPa Ψ_{soil}. Later, in the treatment with less Ψ_{soil} (−3.0 MPa), these maximum values decreased to values of 1.20±0.14 μmol H^+ g^{-1} fresh weight (Fig. 3b, d, f, h), similar to those

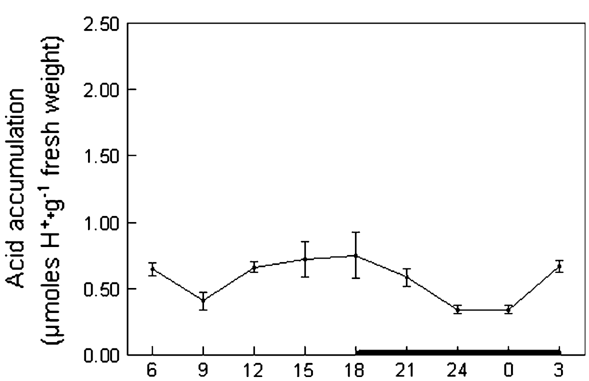

Fig. 2. Acid accumulation at intervals of 3 h during 24 h in 6-month-old seedlings of *Stenocereus beneckei*. The points represent the mean ±SE. On the *x*-axis, the black line indicates the period of darkness.

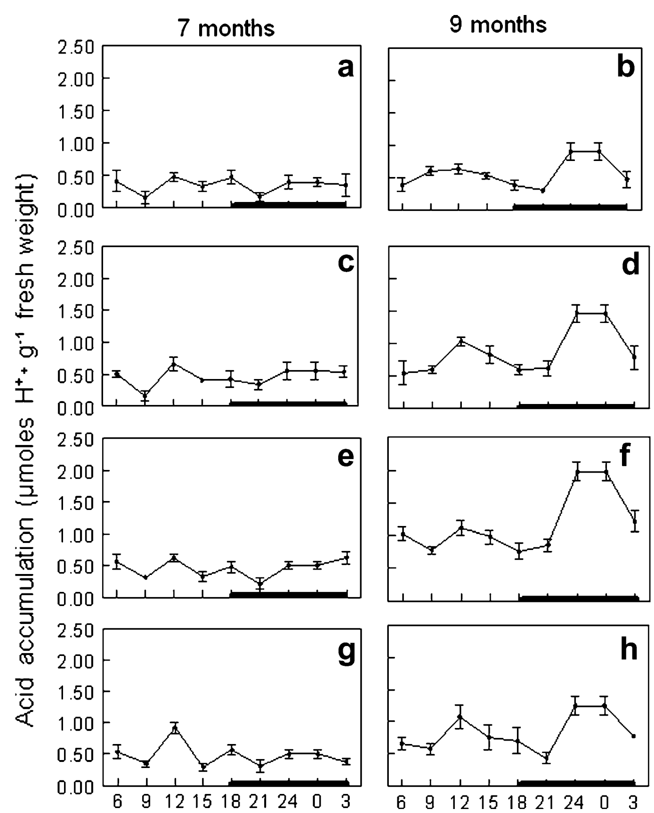

Fig. 3. Acid accumulation at intervals of 3 h during 24 h in 7- and 9-month-old seedlings of *Stenocereus beneckei* growing at: (a, b) −0.03 MPa, (c, d) −0.5 MPa, (e, f) −1.5 MPa Ψ_{soil}, (g, h) −3.0 MPa. The points represent the mean ±SE. On the *x*-axis, the black line indicates the period of darkness.

recorded in the −0.03 and −0.5 MPa Ψ_{soil} treatments. The analyses of variance showed no significant differences (P <0.55) in titratable acidity within each seed weight category in the different treatments. However, significant differences were detected (Table 1) in titratable acidity accumulation between treatment and seedling age.

Tissue water accumulation

Differences in dry weight were found among the four treatments for all seed categories (Table 2). The analyses showed significant differences (P <0.05) in category 2 among all treatments at the age of 7 months. At 9 months, no significant differences were observed among treatments or among categories. No significant differences were detected at 7 months of age in mean dry weight (P <0.36) of seedlings from small or medium seeds (categories 2 and 3), although there was a significant difference (P <0.005) in seedlings from large seeds (categories 4 and 5). Nine-month-old seedlings showed significant differences (P <0.03) in dry weight among seedlings from small and medium seeds (categories 2 and 3), but no significant difference was detected in seedlings from larger seeds (P <0.49). In general, when comparing mean dry weights of all treatments in all seed categories, significant differences were observed at 7 (P <0.0001) and at 9 (P <0.001) months of age.

Size

The results indicate that the size of each seedling part is different among the different treatments (Fig. 4). Category 2, at 7 months of age, in the −0.03 MPa control (Fig. 4a), showed an increase compared with the size at 6 months; while a decrease of up to 50% was observed in the −3.0 MPa treatment group. Nine-month-old seedlings in the −0.03 MPa control showed an increase of 71%, although a slight decrease up to 60% was observed in the −0.5 and −1.5 MPa treatments.

Seven-month-old seedlings in category 3 showed a size increase of 12% compared with the size at 6 months (Fig. 4b). Treatments of −0.5 and −1.5 MPa did not present differences in relation to the control; however, the −3.0 MPa treatment was observed to be 71% of that of the control. At 9 months of age, the control (−0.03 MPa) and the −0.5 and −1.5 MPa treatments had similar sizes, while the −3.0 MPa treatment was 63% of the control size (Fig. 4b). In 7-month-old seedlings belonging to seed weight category 4, the control (−0.03 MPa) seedlings were 97%, decreasing slightly down to 79%. At 9 months of age, control seedlings displayed the highest size values along with the −1.5 MP a treatment, the latter with 90%; the lowest values were observed in the −0.5 and −3.0 MPa (Fig. 4c) treatment groups.

Seedlings from seed weight category 5 showed the highest values of seedling size; however, at 7 and 9 months of age, their size was reduced in the −3.0 MPa treatment by nearly 74% and 64%, respectively (Fig. 4d). In general, soil moisture conditions (−0.05 to −3.0 MPa) tended to reduce seedling size in all four seed weight categories. The statistical analyses showed significant differences (P <0.0001) in total size in each of the treatment groups at the different seedling ages.

Table 1. *Significant difference in titrated protein accumulation between treatment, seedling age weight, and interactions*

Dependent variable (titratable acidity)	df	$P > F$
Seed weight	3	0.095
Treatments	3	<0.0001
Seedling age	1	<0.0001
Seed weight×treatments	9	0.368
Seed weight×age	3	0.2347
Treatments×age	3	<0.0001
Seed weight×treatments×age	7	0.5343

Table 2. *Fresh and dry weight and percentage of tissue water accumulation of* Stenocereus benecke*i seedlings growing under different soil water potentials*

Different letters indicate significant difference, lower case letters for 7-month-old seedlings and upper case letters for 9-month-old seedlings. *P <0.0001 **P <0.001.

Seed category	Age	Water treatment (MPa)	Fresh weight (mg)	Dry weight (mg)	Tissue water accumulation (%)
2	6	Water	163.5	6.7^{A}	96
	7^{a}*	−0.03	160	6.4^{B}**	96
	7^{a}*	-0.5	113.2	6.4^{B}**	94
	7^{a}*	−1.5	116.6	5.9^{B}**	95
	7^{a}*	−3.0	52.5	3.8^{C}**	93
	9^{K}*	−0.03	126.3	6.7^{D}	95
	9^{K}*	−0.5	80.1	7.0^{D}	91
	9^{K}*	−1.5	44.3	6.4^{D}	86
3	6	Water	213.3	8.2^{A}	96
	7^{b}*	−0.03	179.1	6.7^{E}	96
	7^{b}*	−0.5	139.6	6.5^{E}	95
	7^{b}*	−1.5	103.2	6.4^{E}	94
	7^{b}*	−3.0	132.9	5.8^{E}	96
	9^{K}*	−0.03	163.8	7.9^{F}	95
	9^{K}*	−0.5	9.28	8.0^{F}	91
	9^{K}*	−1.5	77.0	6.2^{F}	92
	9^{K}*	−3.0	114.9	6.9^{F}	94
4	6	Water	291.1	12.3^{A}	96
	7^{c}*	−0.03	274.5	8.2^{G}	97
	7^{c}*	−0.5	216.5	8.7^{G}	96
	7^{c}*	−1.5	209.0	9.4^{G}	96
	7^{c}*	−3.0	233.4	8.3^{G}	96
	9^{L}*	−0.03	233.3	11.4^{H}	95
	9^{L}*	−0.5	141.0	11.8^{H}	92
	9^{L}*	−1.5	170.8	12.0^{H}	93
	9^{L}*	−3.0	198.9	7.8^{H}	96
5	6	Water	341.6	13.6^{A}	96
	7^{c}*	−0.03	197.2	9.3^{I}	95
	7^{c}*	−0.5	142.5	8.3^{I}	94
	7^{c}*	−1.5	194.8	10.2^{I}	95
	7^{c}*	−3.0	225.9	9.3^{I}	96
	9^{L}*	−0.03	277.4	12.7^{J}	95
	9^{L}*	−0.5	225.6	11.6^{J}	95
	9^{L}*	−1.5	212.8	12.4^{J}	94
	9^{L}*	−3.0	197.3	9.3^{J}	95

Anatomy

The hypocotyl cross-sections in 6-month-old seedlings from the four seed weight categories displayed simple epidermis with imperceptible cuticle and stomata at the level of the other epidermal cells (Fig. 5a). Subjacent to the epidermis is the cortex, comprised of isodiametric cells with abundant chloroplasts that are less evident near the vascular cylinder. The vascular tissue, primary xylem and phloem, is arranged in two arcs (Fig. 5b). The pith is also exclusively composed of isodiametric cells with no visible contents (Fig. 5b).

Once the different Ψ_{soil} treatments were initiated, the following modifications could be observed at 7 and 9 months of age: seedlings from the four seed weight categories in the −0.03 MPa treatment group showed hypertrophy in cortical cells and accumulated crystals in the few collapsible cells (Figs 6a, 7a). Seedlings from seed weight category 2 presented the highest number of crystals of all categories at 7 months of age. At 9 months, an increase in crystal density was observed in three of the four seedling categories, category 3 being the exception (Table 3). However, within this treatment (−0.03 MPa), a larger proportion of collapsed cells was observed in category 3.

In the −0.5 MPa treatment group (Figs 6b, 7b), seedlings from the four seed weight categories showed hypertrophied and swollen cells, and a larger proportion of collapsed cells with crystals. Category 5 showed a smaller number of crystals at 7 months of age, while at 9 months this category had a larger number of crystals, similar to category 2 (Table 3). Category 3 had more collapsed cells and very few swollen ones. Seedlings in the −1.5 MPa treatment group showed collapsing cortex cells in all four categories. In these cells, a larger number of crystals (Table 3) and only a few swollen cells were observed, with no vascular tissue modifications (Figs 6c, 7c). The number of crystals was

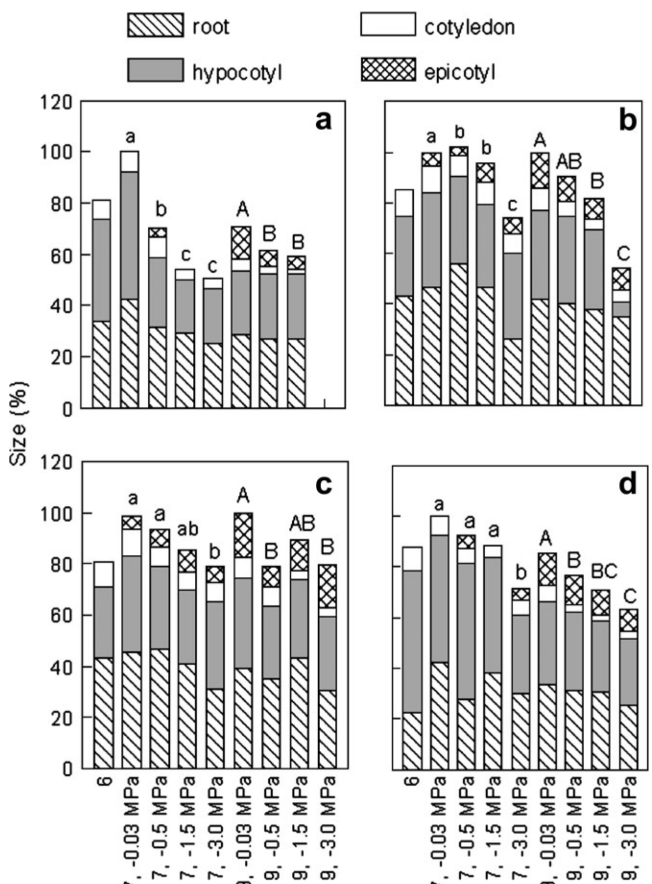

Fig. 4. Root, hypocotyl, cotyledon, and epicotyl size in *Stenocereus beneckei* seedlings growing under different water potentials in the soil. Seed categories: (a) 2, (b) 3, (c) 4, and (d) 5. A significant difference is indicated by different lower case letters for the 7-month-old seedlings and by different upper case letters for the 9-month-old seedlings.

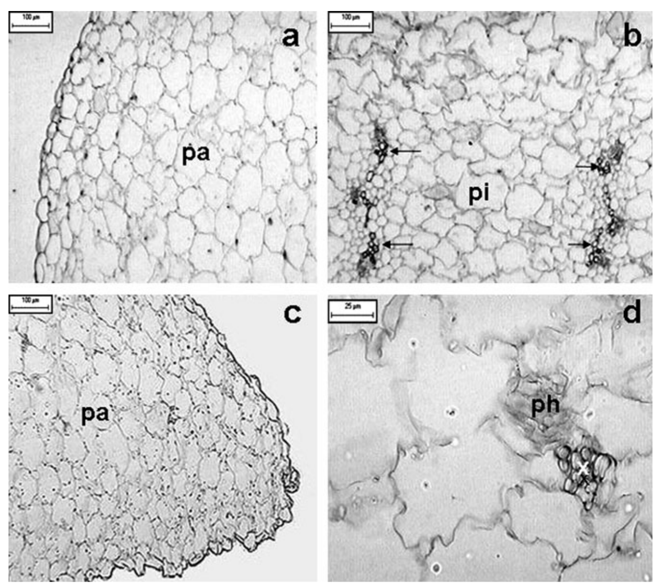

Fig. 5. Transverse sections of seedling hypocotyl and cotyledon of 6-months old *Stenocereus beneckei*. (a) Detail of epidermal cells and cortex in hypocotyl, category 2. (b) Pith and vascular tissue in hypocotyl, category 5. (c) Epidermal cells and mesophyll in cotyledon, category 2. (d) Detail of the vascular bundle in cotyledon (arrows=xylem elements), category 5. Scale=10 μm, pa, parenchyma; ph, phloem; pi, pith; X, xylem elements.

Table 3. *Crystal density in the cortical tissue of* Stenocereus beneckei *hypocotyl of 7- and 9-month-old seedlings growing under different water potentials in the soil*

Age	Treatment	Crystals mm^{-2}			
		Category 2	Category 3	Category 4	Category 5
7 months	−0.03 MPa	1.5	0.5	0.4	0.4
	−0.5 MPa	2.2	1.0	2.8	0.3
	−1.5 MPa	2.8	3.9	3	3.2
	−3.0 MPa	2.5	7.1	2.5	6.8
9 months	−0.03 MPa	2.6	0.5	2	1.4
	−0.5 MPa	4.7	1.5	2.4	3.6
	−1.5 MPa	6.5	1	3.5	5
	−3.0 MPa		1.7	7.6	11.1

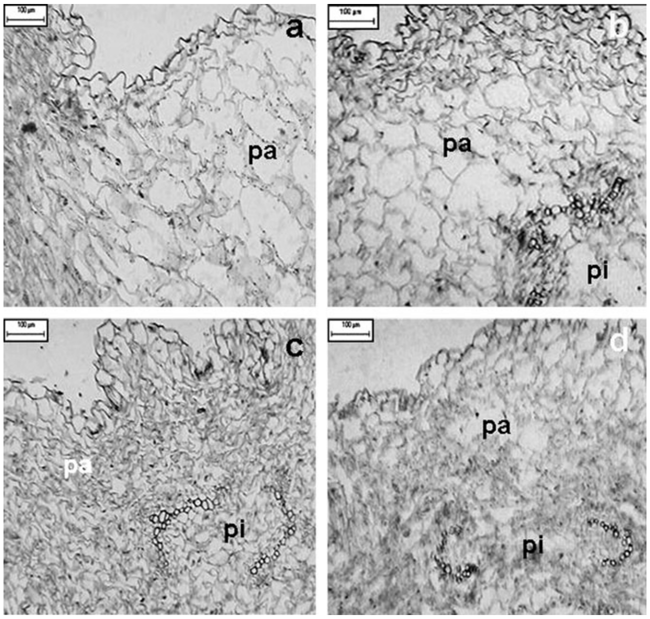

Fig. 6. Transverse sections of hypocotyl of category 2 seedlings of 7-month-old *Stenocereus beneckei* growing under different water potentials in the soil. (a) A few collapsed cells, −0.03 MPa. (b) Collapsed cells subjacent to the epidermis, −0.5 MPa. (c) Most cortical parenchyma cells are collapsed, −1.5 MPa. (d) All parenchyma cells are collapsed in cortex and pith, −3.0 MPa Ψ_{soil}. Scale=10 μm, pa, parenchyma; pi, pith.

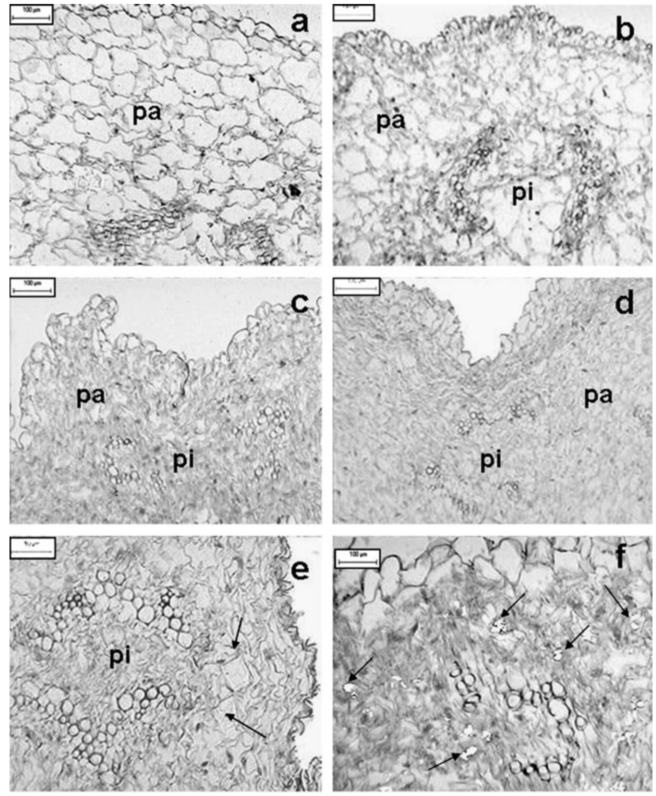

Fig. 7. Transverse sections of seedling hypocotyl of 9-month-old *Stenocereus beneckei* by seed categories growing under different water potentials in the soil. (a) Detail of collapsible cells with abundant intercellular spaces, category 4, −0.03 MPa. (b) Cortical cells collapsed subjacent to the epidermis, category 2, −0.5 MPa. (c) All parenchyma cells collapsed, category 3, −1.5 MPa. (d) All parenchyma cells collapsed, category 3, −3.0 MPa. (e) Detail of vascular tissue with secondary growth and large crystals (arrow), category 3, −1.5 MPa. (f) Crystals in parenchyma collapsed cells (arrows), category 5, −3.0 MPa Ψ_{soil}. Scale=10 μm, pa, parenchyma; pi, pith.

greater at 9 months of age, except in category 3 (Table 3). For the −3.0 MPa treatment, collapsed cells with crystals and some swollen cells were observed (Figs 6d, 7d). At 7 months, categories 3 and 5 showed the highest crystals mm^{-2} values and, at 9 months, categories 4 and 5 were the highest (Table 3; Fig. 7e, f).

In the cotyledon cross-sections, at 6 months of age, a simple epidermis was observed with rectangular to square-shaped cells and an imperceptible cuticle (Fig. 5c). Subjacent to the epidermis, mesophyll cells had an isodiametric shape and contained abundant chloroplasts (Fig. 5c). Small collateral bundles were also present (Fig. 5d). For different soil moisture values, 7- and 9-month-old seedlings displayed the following changes: cotyledons of seedlings from all seed weight categories in the −0.03 MPa (Figs 8a, 9a) and control showed hypertrophied mesophyll cells with a few normal sized cells, generally toward the epidermis. In the −0.5 MPa treatment, the mesophyll cells also showed hypertrophy, although the central part of the mesophyll also displayed some collapsed cells with few crystals (Figs 8b, 9b). The −1.5 MPa treatment group seedlings (Figs 8c, 9c, e) had collapsed mesophyll cells containing crystals, although a few cells remained swollen and hypertrophied. In the −3.0 MPa treatment group, collapsible mesophyll cells were observed to contain crystals and starch grains, especially in cotyledons from category 5 at 6 months of age and category 3 at 9 months of age (Figs 8d, 9d, f).

Discussion

Seed weight is associated with embryo and endosperm resources, which are distributed during germination to give rise to the seedling and later help to establish them. This distribution also depends on the environmental conditions surrounding the seedlings when they are established (Foster, 1986). Cactaceae seeds do not have endosperm, their perisperm is scarce (Nuñez, 2004), and the embryo supplies all reserve material. Nevertheless, viability or percentage survival depends strongly on seed size, meaning the amount of reserves accumulated for the seedling's development (Kigel, 2001). *Stenocereus beneckei* seedlings grown from different seed weight categories displayed significant growth differences at different ages in each soil-water treatment. In general, there was a tendencey for all the parts of seedlings from small seeds to be a smaller size than those of seedling from large seeds. A possible explanation is that large seeds hold greater metabolic reserves for their seedling than smaller seeds (Leishman and

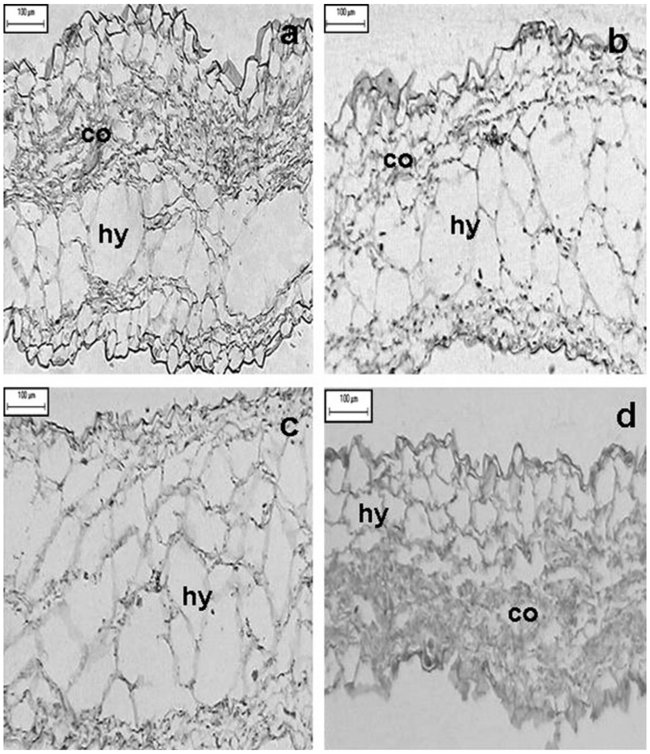

Fig. 8. Transverse sections of seedling cotyledon of 7-month-old *Stenocereus beneckei* by seed categories growing under different water potentials in the soil. All have hypertrophied and collapsed mesophyll cells. (a) Category 2, −0.03 MPa. (b) Category 2, −0.5 MPa. (c) Category 5, −1.5 MPa. (d) Category 5, −3.0 MPa Ψ_{soil}. Scale=10 μm, co, collapsed mesophyll parenchyma cells; hy, hypertrophied mesophyll parenchyma cells.

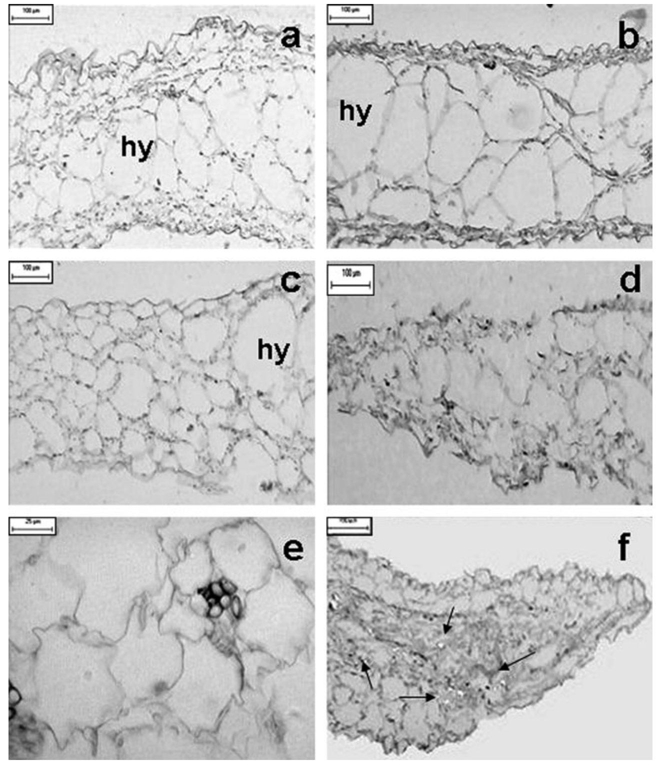

Fig. 9. Transverse sections of seedling cotyledon of 9-month-old *Stenocereus beneckei* by seed categories growing under different water potentials in the soil. All have hypertrophied and collapsed mesophyll cells. (a) Category 2, −0.03 MPa. (b) Category 2, −0.5 MPa. (c) Category 5, −1.5 MPa. (d) Category 5, −3.0 MPa. (e) Detail of vascular bundle, category 3, −1.5 MPa. (f) Crystals in collapsed mesophyll cells (arrows), category 3, −3.0 MPa Ψ_{soil}. Scale=10 μm, hy, hypertrophied mesophyll parenchyma cells.

Westoby, 1994). *Stenocereus beneckei* has the largest seeds (3.99 mm×2.75 mm) of *Stenocereus*, while *S. queretaroensis* has intermediate seed (2.37 mm×1.64 mm) size (Arroyo-Cosultchi *et al*., 2006). Notably, *S. beneckei* seedlings coming from the largest seeds had slower development than *S. queretaroensis* seedlings (Loza-Cornejo *et al*., 2003) growing under similar laboratory conditions. It has been reported that plants growing in environments exposed to drought during their establishment tend to have larger seeds which are capable of assigning a greater proportion of energy to the root than to the stem during the first stages of growth (Jurado and Westoby, 1992). However, *S. beneckei* roots did not show differences and were found on the upper soil layer, probably because the subsurface root system helped them to absorb water more quickly since soil moisture is located at the surface (Dubrovsky and North, 2002). In addition, this response in root growth coincides with the manner in which water was supplied.

Shade and water are important for seedling germination and establishment. *Stenocereus beneckei* seedlings under constant moisture conditions were larger than seedlings receiving less water. Nolasco *et al.* (1997) showed that *S. thurberi* seedlings receiving more irrigation and shade were larger than seedlings under environmental conditions of less water and shade. The loss of water under different soil moisture conditions—specifically −1.5 and −3.0 MPa—produced smaller *S. beneckei* seedlings (in all seed weight categories) with flaccid hypocotyls and cotyledons during the 3 months under different levels of soil water deficit. *Cereus* seedlings growing under limited water conditions and with alterations in abscisic acid (ABA) levels showed flaccid hypocotyls and cotyledons. Merida and Arias (1979) attribute such changes to alterations in the slow metabolism that characterizes Cactaceae, correlated with protein synthesis inhibition. This may also occur in *S. beneckei* seedlings under limited water conditions.

Stenocereus beneckei seedlings displayed irregular fluctuations in concentrations of protonable ions at 6 and 7 months of age; however, at 9 months, peaks of titratable acidity typical of CAM were observed. Altesor *et al*. (1992) state that the absence of concentration peaks at night, typical of CAM plants, is due to Cactaceae maintaining a C_3 ancestral photosynthetic metabolism in their first ontogenetic stages, interpreting it as an adaptive response. However, Loza-Cornejo *et al*. (2003) suggest that irregular fluctuations may result from an immaturity of the photosynthetic system; therefore, as seedlings get older, their typical CAM pattern is defined as observed with *S. beneckei*.

It is known that water stress may induce CAM in some plant species (Fioretto and Alfani, 1988). However, under different soil water deficit treatments in the present experiment, *S. beneckei* showed that water availability was not a decisive factor in the induction of CAM. With respect to *Polaskia* and *Echinocactus* seedlings, Rosas (2002) mentions that water availability was not decisive in the induction of CAM, although typical C_3 metabolism was not seen either. In *S. queretaroensis*, the increase in concentration of titratable protons at increasing ages showed that seedling age defines the typical CAM pattern (Loza-Cornejo *et al*., 2003). The presence of CAM in *S. beneckei* seedlings is a response to age rather than to a lack of water. A similar response is observed in *Agave* during the first stages of the life cycle when there are still cotyledons that display CAM activity (Wen *et al*., 1997).

After germination, one of the main factors that determine the seedling's establishment is water availability (Gonzalez-Zertuche *et al*., 2000). Adult organisms can cope with conditions of water stress and high temperatures, but young plants are more susceptible to these extremes. Nurse plants are a possible solution for one of the most critical processes in the Cactaceae life cycle; these plants change the microenvironmental conditions under their crowns, protecting the seedlings against predation (Franco and Nobel, 1989; Valiente-Banuet *et al*., 1991) and shading them against high and low thermal extremes (Nobel, 1988). However, during their first months of life, the seedlings must develop characteristics that will help them survive water stress during drought and high temperatures. Six-month-old *S. beneckei* seedlings, under appropriate moisture conditions, showed anatomical structures that are typical of other Cactaceae at that age, although their epicotyls showed a slower elongation than *S. queretaroensis*. The characteristics observed, namely simple epidermis, cortex formed by isodiametric cells with abundant chloroplasts, vascular tissue—primary xylem and phloem—in two arcs, and a parenchymatous pith with no contents, are similar to what has been described for *S. queretaroensis* (Loza-Cornejo *et al*., 2003).

At 7 and 9 months of age, seedlings that have been growing under different soil water potentials for 1 and 3 months displayed significant changes in the parenchyma cells. Regardless of the seed weight category from which the seedlings developed, −0.03 MPa provoked, in general, a response of hypertrophy in the fundamental tissue cells in the seedling hypocotyl and cotyledons. This indicates that *S. beneckei* seedlings surely developed appropriately with soil water potentials below field capacity, a response which, along with their photosynthetic metabolism, favoured their establishment. In the −1.5 and −3.0 MPa treatment groups, the number of collapsed cells was greater in the hypocotyl and cotyledons. A similar response is observed in *Polaskia* and *Echinocactus* seedlings, with abundant collapsed parenchyma cells under water stress (Rosas, 2002). Mauseth (1995) points out that adult cacti show collapsible parenchyma cells, specialized in water storage due to thin walls that are composed of a distinctive chemical structure that allows them flexibility. Mauseth also suggests that the cells release water when the soil is dry, and absorb water, expanding enormously, when water is available in the soil, which may also be the case for hypertrophied cells. The parenchyma cells that form most of the hypocotyl and cotyledon tissues were interpreted as collapsible due to the abundance of intercellular spaces and slightly wavy walls that are observed at 6 months of age. These cells in *S. beneckei*, as Mauseth (1995) states, facilitate wall flexibility during the loss of turgidity. The presence of collapsible parenchyma cells in *S. beneckei* seedlings is always associated with the accumulation of crystals and starch grains, which may possibly stop the cytoplasm from collapsing by condensing in these solutes as an adaptive response to water loss.

In general, calcium oxalate crystals are found in many plant species and in several different organs and tissues, but they are common in Cactaceae (Gibson and Nobel, 1986). The role of calcium oxalate deposits in plants is controversial; these deposits have been seen to be involved in several roles—from Ca^{2+} ions and pH intracellular regulation and mechanical support, to defence against predators (Franceschi and Horner, 1980; Webb, 1999). As mentioned earlier, in Cactaceae they are distributed in several tissues, and it has been speculated that calcium oxalate precipitation in stem tissues may be related to particular physiological aspects. Monje and Baran (2002) point out that there is a greater accumulation of calcium oxalate in tissue near the stomata, aiding stomatal closure during the day. Ruiz and Mansfield (1994) show evidence that high concentrations of calcium in the xylem are associated with a reduction in stomatal aperture in *Commelina*. The presence of calcium oxalate crystals in *S. beneckei* hypocotyls and cotyledons in the −1.5 and −3.0 MPa, treatments is an adaptive response to water deficit, related to their variable photosynthetic metabolism.

Stenocereus beneckei seedlings displayed differential growth responses depending on the seed weight category. Seedlings under constant moisture conditions were larger than seedlings under water stress conditions. Significant differences in growth were observed in each of the treatments at different seedling ages, suggesting that an embryo's reserves are important for seed germination and seedling establishment. Seedlings from smaller seeds showed the least growth and none of them survived the 3 months of drought. Different Ψ_{soil} treatments did not favour CAM switching in *S. beneckei* seedlings. The anatomical modification of *S. beneckei* seedlings in the −0.03 and −0.5 MPa treatment groups was cell hypertrophy, possibly associated with excessive water. By contrast, in the −1.5

and −3.0 MPa treatment groups, collapsed cells with abundant solutes (calcium oxalate crystals and starch grains), along with changes in the photosynthetic metabolism and reduced growth serve to favour seedling survival under conditions of extreme drought.

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