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Water Relations and Low-Temperature Acclimation for Cactus Species Varying in Freezing Tolerance[1]

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Opuntia ficus-indica **and** ***Opuntia streptacantha*** **are widely cultivated cacti that can tolerate temperatures no lower than −10°C, whereas** ***Opuntia humifusa*****, which is native to southern Canada and the eastern United States, can tolerate −24°C. As day/night air temperatures were decreased from 30/20 to 10/0°C, the osmotic pressure increased 0.10 MPa for** ***O. ficus-indica*** **and** ***O. streptacantha*** **but 0.38 MPa for** ***O. humifusa*****. The increases in osmotic pressures were due mostly to the synthesis of fructose, glucose, and sucrose. In addition,** ***O. humifusa*** **produced a substantial amount of mannitol during exposure to low temperatures. Substantial accumulation of sugars and mannitol in cells of** ***O. humifusa*** **may help prevent intracellular freeze dehydration and ice formation as well as provide noncolligative protection to its membranes. Mucilage was slightly higher in all three species at the lower temperatures. Extracellular nucleation of ice occurred closer to the equilibrium freezing temperature for plants at 10/0°C compared with 30/20°C, which could make the cellular dehydration more gradual and, thus, less damaging. Results from nuclear magnetic resonance indicated a restricted mobility of intracellular water at the lower temperatures, especially for** ***O. humifusa*****, which is consistent with its lower water content and higher levels of low molecular weight solutes.**

Most species of cacti are native to seasonally hot, arid regions where freezing seldom occurs, but some grow at northern latitudes or high elevations where subzero temperatures are common during the winter (Benson, 1982; Gibson and Nobel, 1986). For instance, *Opuntia humifusa*, one of the species considered in the present study, occurs in regions in southern Canada and eastern United States that experience average nighttime temperatures of −4°C from December through February (Ruffner and Bair, 1981). Most cacti increase their tolerance to subzero temperatures after exposure to low day/night air temperatures; the average acclimation in the low temperature tolerated for 14 species is 0.7°C when the growth temperature is reduced by 10°C (Nobel, 1982, 1988). Species with the greatest low-temperature tolerance, such as *Coryphantha vivipara* and *O. humifusa*, exhibit substantial acclimation of 2 to 4°C per 10°C decrease in growth temperature (Nobel, 1981; Nobel and Loik, 1990).

Freezing of cactus stems does not occur at the equilibrium freezing temperature of their contents, because some supercooling is observed (Steenbergh and Lowe, 1976; Nobel, 1981). Ice nucleation leading to freezing apparently occurs extracellularly (Uphof, 1916; Nobel, 1981). Intracellular water is distilled into the apoplastic spaces where ice crystals are formed, leading to a progressive intracellular dehydration and eventually irreversible damage. Mucilage, a highly branched polysaccharide that is prevalent extracellularly in many cacti, may be involved in ice nucleation (Goldstein and Nobel, 1991). In particular, mucilage may cause nucleation to occur closer to the equilibrium freezing temperature, leading to a more gradual net water efflux from the cells to the extracellular ice crystals, which may be less damaging to the cells. Increases in Π also accompany low-temperature acclimation for cacti (Koch and Kennedy, 1980; Littlejohn and Williams, 1983). For *Opuntia ficus-indica*, another species considered in the present study (although a different cultivar was used), the increases in Π at low growth temperatures are accompanied by increases of low-mol-wt sugars (Goldstein and Nobel, 1991). Even though the total water content decreases during acclimation for this species, the relative amount of apoplastic water, where ice nucleation first occurs, increases. NMR studies suggest restricted mobility of intracellular water, an increased mobility of extracellular water, and apparently no changes in membrane permeability for *O. ficus-indica* during low-temperature acclimation (Goldstein and Nobel, 1991). Soon after the onset of freezing, less than 10% of the water is retained intracellularly and in a liquid form, consistent with the relatively low tolerance of *O. ficus-indica* to subzero temperatures.

Three species were chosen for this study: *Opuntia ficus-indica* and *Opuntia streptacantha*, which are widely cultivated for fruits, fodder, and even vegetables (Russell and Felker, 1987) and can tolerate temperatures down to only about −10°C (Nobel, 1990), and *Opuntia humifusa*, which can tolerate −24°C when properly acclimated (Nobel and Loik, 1990). Opuntias of differing cold hardiness were selected to help determine the cellular properties associated with low-temperature tolerance. The solutes responsible for the increase in Π during low-temperature acclimation were identified in both the photosynthetic chlorenchyma and the water-storage parenchyma for these three species of cactus.

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Abbreviations: FID, free-induction decay; Π, osmotic pressure; RWC, relative water content; T_1, spin-lattice relaxation time; T_2, spin-spin relaxation time.

Such solutes should help retain liquid water within the cells and may act as noncolligative cryoprotectants. Another objective was to determine whether the ice nucleation temperature increased during acclimation, and, if so, whether this was correlated with an increase in the extracellular mucilage. In addition, NMR studies were performed to help understand any changes in water compartmentation during low-temperature acclimation.

MATERIALS AND METHODS

Plant Material

Three species of platyopuntia were studied: *Opuntia ficus-indica* (L.) Miller, *Opuntia humifusa* Rafinesque, and *Opuntia streptacantha* Lemaire. Single mature cladodes of *O. ficus-indica* and *O. streptacantha* averaging 30 cm in length were obtained from a nursery at the University of California, Riverside, and planted in 4-L pots of sandy soil from Agave Hill, near Palm Desert, CA (Nobel, 1976), with one-quarter of the cladode area below the soil surface. Mature plants of *O. humifusa* about 10 cm tall with two sequential cladodes were collected from Bald Knob near Rocky Mount, VA, and also were planted in soil from Agave Hill. The plants were maintained in a glasshouse at the University of California, Los Angeles, with day/night air temperatures averaging 27/16°C and day/night RHs averaging 40/60% from February 1990 to April 1991. During this period, the plants developed new cladodes, which were used for the measurements. The air inside the glasshouse was charcoal filtered, and the CO_2 level was about 360 μmol mol^{-1}. The plants were irrigated twice per week with distilled water and once every 2 weeks with one-quarter-strength Hoagland solution No. 1 supplemented with micronutrients (Nobel, 1988). Soil water potential in the root zone was maintained above −0.3 MPa, as determined with Wescor PCT 55-05 soil thermocouple psychrometers.

In early April 1991, four plants of each species were transferred to growth chambers with day/night air temperatures of 30/20 or 10/0°C. The PPFD on a horizontal surface at the top of the plants was 800 μmol m^{-2} s^{-1} for 12 h d^{-1}, leading to a similar total daily PPFD on the cladode surfaces as in the glasshouse. The plants were maintained under the new growth conditions for 3 to 4 weeks before measurements were obtained (acclimation to new temperatures occurs within 2 weeks; Nobel, 1988; Goldstein and Nobel, 1991). Samples for all measurements were taken in the early afternoon.

Water Relations Parameters

To obtain tissue samples, cylindrical cores through the cladodes were removed with a cork borer 13 mm in diameter. The cores were immediately wrapped in Parafilm and sealed in plastic bags to prevent changes in water content before measurements. The chlorenchyma occupied the outermost 2 to 6 mm of stem tissue internal to the epidermis and contained virtually all of the Chl; the remaining tissue, 10 to 16 mm in thickness for *O. ficus-indica* and *O. streptacantha* but only 2 to 4 mm for *O. humifusa*, was the water-storage parenchyma. After the epidermis was removed, the chlorenchyma and the water-storage parenchyma were separated from each core with a razor blade and blotted lightly to remove sap from cut cells. Mucilage canals and vascular bundles occurred primarily between the chlorenchyma and the water-storage parenchyma and were not included in the samples examined.

RWC was determined from (fresh weight − dry weight)/(turgid weight − dry weight). Dry weight was measured after samples were dried in a forced-draft oven at 60°C for 48 h; turgid weight was determined after exposing samples to 100% RH at 25°C for 6 h. The water:dry matter ratio was expressed as weight of liquid water (tissue fresh weight − tissue dry weight) per weight of the dry sample. Π was determined for cell sap extruded from samples squeezed between plastic plates using a vise; sap osmolality was determined with a Wescor 5500 vapor pressure osmometer and converted to Π at 20°C using the Van't Hoff relation (Nobel, 1991).

Carbohydrates

Low-mol-wt carbohydrates were analyzed chromatographically. Two grams of fresh tissue (obtained with the cork borer) were frozen at −80°C until used. The frozen material was homogenized in a mortar, mixed with 1 mL of distilled water and 7 mL of isopropanol (to precipitate mucilage and to minimize enzymatic activity), and vacuum filtered using glass-fiber paper to remove precipitates (these steps required less than 3 min). The residue was immediately oven dried at 60°C for 2 d (residue appeared dry within 10 min), redissolved in 1 mL of distilled water for 2 h, and centrifuged at 70,000*g* for 15 min. Compounds in the supernatant were separated by HPLC using a Waters Chromatography Sugar-pak II column with 25 mM EDTA and a Rainin Microsorb-MV column with 9:1 acetonitrile:water and were quantified using specific standards. Identification was checked using TLC with silica gel plates and 6:3:1 isopropanol:acetone:0.2 M lactic acid (Kremer, 1978).

Mucilage was chemically extracted from the tissues (Goldstein and Nobel, 1991). Four grams of tissue were removed with the cork borer, frozen at −80°C, homogenized in a mortar, and then transferred to boiling ethanol for 5 min to arrest enzymic activity. After vacuum filtration through Whatman No. 1 paper, the residue was dried at 50°C for 24 h, weighed, solubilized at 50°C for 1 h in 30 mL of distilled water, and then vacuum filtered to remove cell wall material and other large debris using a metal sieve (pore size of 70 μm). Mucilage was precipitated with 250 mL of isopropanol, and the precipitate was washed three times in isopropanol, followed by air drying until the weight was constant.

Ice Nucleation Temperatures

To investigate the patterns of air and tissue temperatures during freezing, 0.2 g of tissue was placed in sealed glass tubes (50 mm long and 5 mm in diameter). The tubes were placed in a Styrofoam box between heat exchange coils through which was circulated 55% ethylene glycol/45% water. Temperature was adjusted with a refrigerated bath and was monitored at 20-s intervals with copper-constantan thermocouples (0.26 mm in diameter) placed in contact with

Table I. *Water contents of the chlorenchyma and the water-storage parenchyma for cladodes of platyopuntias at day/night air temperatures of 30/20 or 10/0°C*

Data are means ± SE (n = four plants).

Measurement	Species	Chlorenchyma		Water-Storage Parenchyma	
		30/20°C	10/0°C	30/20°C	10/0°C
RWC (%)	*O. ficus-indica*	80.7 ± 2.3	72.1 ± 1.1	78.7 ± 1.3	80.2 ± 2.8
	O. humifusa	58.4 ± 1.3	55.7 ± 3.2	62.9 ± 1.0	55.4 ± 1.8
	O. streptacantha	82.2 ± 1.2	77.4 ± 2.3	93.9 ± 1.3	90.7 ± 1.5
Water:dry matter ratio	*O. ficus-indica*	10.1 ± 0.2	8.4 ± 0.5	21.1 ± 0.4	15.8 ± 0.4
	O. humifusa	7.6 ± 0.3	6.9 ± 0.3	6.1 ± 0.3	6.6 ± 0.2
	O. streptacantha	10.0 ± 0.5	8.5 ± 0.2	21.0 ± 0.7	15.7 ± 0.2

the tissue. The temperature of the tissue samples was lowered from 20 to −28°C at about 8°C h^{-1}. The ice nucleation temperature was indicated by the rapid increase in temperature, representing the release of the heat of fusion of water during freezing.

The possible presence of ice-nucleating bacteria in the samples was checked using serial dilutions of homogenized tissue incubated on *Pseudomonas* Difco F agar (Anderson and Ashworth, 1985). After the samples were resuspended in a sterile solution, colonies were detected using the plate-harvesting technique. Very few bacterial colonies were observed, none were identified as *Pseudomonas syringae*, and no ice nucleation was observed until the temperature of the suspensions of the homogenized tissue was reduced below −9°C.

NMR Studies

Approximately 0.2 g of tissue was lightly blotted and then placed at the bottom of a 5-mm-diameter tube. The tube was immediately sealed to prevent water loss from the tissue and inserted into a Bruker AM360 pulsed NMR spectrometer with an 89-mm bore and a superconducting magnet producing a magnetic flux density of 8.5 tesla (Goldstein and Nobel, 1991). A fluorine probe was tuned to ^1H with a tissue sample in the tube before each experiment. Relaxation times, reflecting interactions between water and various cellular components, were measured at 0°C to determine the water mobility in various compartments.

The longitudinal T_1 was measured using a 180-τ-90 pulse sequence (τ is the time between the radio-frequency pulses in the sequence, and the indicated angles are between the average direction of the original proton spins and that induced by the radio-frequency pulse; Farrar and Becker, 1971; Burke et al., 1974). Fourier transforms were used, and T_1 was determined from the slope of $\ln(A_\infty - A_\tau)$ versus τ, where A_τ is the amplitude of the FID of the water proton signal following the 90° pulse at τ, and A_∞ is the limiting value of A_τ. The transverse T_2 was measured by the Carr-Purcell-Meiboom-Gill technique from the slope of $\ln A_{echo}$ versus t, where A_{echo} is the magnetization amplitude of the water proton signal occurring at time t ($t = 2n\tau$, where n is the number of refocussing pulses and τ was 2 ms) after the initial 90° pulse in the 90-τ-180-2τ-180-2τ . . . pulse sequence (Farrar and Becker, 1971).

The liquid water content of the tissue sample was obtained from the Fourier transform of the FID after applying a short (6.5–8.0 μs) 90° pulse at different tissue temperatures. The FIDs were obtained in 3°C steps from 25 to −20°C after thermal equilibrium was achieved for each step (up to 20 min). After a Boltzmann factor correction was applied (Farrar and Becker, 1971; Nobel, 1991), the amplitude of the transformed signal was assumed to be proportional to the liquid water content.

RESULTS

Tissue water content measured both as RWC and as water:dry weight ratio was generally lower for cladodes of plants maintained at 10/0°C than at 30/20°C, both for the chlorenchyma and the water-storage parenchyma (Table I). The water contents were similar for *O. ficus-indica* and *O. streptacantha*, whereas tissues of *O. humifusa* had a substantially lower water content. Specifically, RWC and the water:dry weight ratio were 25 to 70% lower for tissues from *O. humifusa* compared with the other two species (Table I).

The tissue Π was higher for plants acclimated to the low

Table II. Π *for the chlorenchyma and the water-storage parenchyma for cladodes of platyopuntias at day/night air temperatures of 30/20 or 10/0°C*

Data are means ± SE (n = four plants).

Species	Π (MPa)			
	Chlorenchyma		Water-storage parenchyma	
	30/20°C	10/0°C	30/20°C	10/0°C
O. ficus-indica	0.70 ± 0.02	0.80 ± 0.01	0.63 ± 0.02	0.69 ± 0.03
O. humifusa	0.88 ± 0.05	1.23 ± 0.06	0.89 ± 0.03	1.29 ± 0.04
O. streptacantha	0.71 ± 0.02	0.83 ± 0.03	0.59 ± 0.02	0.71 ± 0.02

Table III. *Sugar contents in the chlorenchyma and the water-storage parenchyma for cladodes of platyopuntias at day/night air temperatures of 30/20 or 10/0°C*

Data are means ± SE (*n* = four plants).

Sugar	Species	Chlorenchyma		Water-Storage Parenchyma	
		30/20°C	10/0°C	30/20°C	10/0°C
Fru (mmol kg^{-1} fresh weight)	*O. ficus-indica*	5.1 ± 1.2	27.9 ± 4.8	13.6 ± 0.5	23.4 ± 0.3
	O. humifusa	15.3 ± 1.5	69.0 ± 4.2	22.1 ± 1.6	71.3 ± 5.0
	O. streptacantha	2.4 ± 1.2	38.2 ± 3.3	4.5 ± 0.8	29.4 ± 2.4
Glc (mmol kg^{-1} fresh weight)	*O. ficus-indica*	4.4 ± 1.4	28.2 ± 6.0	12.5 ± 8.8	30.0 ± 0.8
	O. humifusa	18.9 ± 0.7	54.1 ± 6.3	20.1 ± 3.5	46.3 ± 3.2
	O. streptacantha	3.4 ± 1.5	39.8 ± 6.1	6.7 ± 1.3	40.9 ± 1.6
Suc (mmol kg^{-1} fresh weight)	*O. ficus-indica*	6.2 ± 1.9	9.4 ± 0.0	1.2 ± 0.1	1.3 ± 0.0
	O. humifusa	8.4 ± 2.2	19.7 ± 1.3	7.9 ± 2.4	17.8 ± 0.8
	O. streptacantha	3.4 ± 0.0	10.7 ± 0.9	2.4 ± 0.8	4.6 ± 0.3

temperatures (Table II). The average Π for the chlorenchyma and the water-storage parenchyma of *O. ficus-indica* and *O. streptacantha* increased 0.10 MPa during acclimation to low temperatures, whereas the average Π increased 0.38 MPa for *O. humifusa*. Not only did Π increase more for *O. humifusa* during acclimation to low temperatures, but also Π was higher at 30/20°C. As a consequence, Π of cold-acclimated *O. humifusa* was 56% higher than the average Π for the other two species (Table II).

Consistent with the above differences in Π for plants at 30/20°C versus 10/0°C, the amounts of Fru, Glc, and Suc per unit fresh weight were severalfold higher at the lower temperatures (Table III). The observed changes were greater in the chlorenchyma than in the water-storage parenchyma for all three species. For example, on a fresh weight basis, Glc for *O. streptacantha* increased 12-fold in the chlorenchyma and 6-fold in the water-storage parenchyma as the day/night air temperature was lowered (Table III). For *O. humifusa*, the amounts per unit fresh weight of Fru, Glc, and Suc were higher than for the other two species, consistent with its higher Π. Furthermore, *O. humifusa* also had substantial amounts of mannitol: 10.2 and 9.4 mmol kg^{-1} fresh weight for the chlorenchyma and the water-storage parenchyma, respectively, at 30/20°C and 17.8 and 27.9 mmol kg^{-1}, respectively, at 10/0°C. For the other two species, mannitol was less than 0.5 mmol kg^{-1} fresh weight. As the day/night air temperatures were changed from 30/20 to 10/0°C, the total amount of sugars and mannitol in the chlorenchyma increased from 15.7 ± 2.6 to 65.5 ± 7.7 mmol kg^{-1} fresh weight for *O. ficus-indica*, 52.8 ± 2.7 to 160.6 ± 7.7 for *O. humifusa*, and 9.2 ± 1.9 to 88.7 ± 7.0 for *O. streptacantha*; the comparable increases for the water-storage parenchyma were 27.3 ± 8.8 to 54.7 ± 0.9, 59.5 ± 4.5 to 163.3 ± 6.0, and 13.6 ± 1.7 to 74.9 ± 2.9 mmol kg^{-1} fresh weight, respectively.

The mucilage content of the chlorenchyma and the water-storage parenchyma increased slightly for all three species during low-temperature acclimation (Table IV). Mucilage averaged 29% of the dry weight of the chlorenchyma and 30% for the water-storage parenchyma for plants at 30/20°C; these contents increased 4 to 5% at 10/0°C. In all cases, mucilage was highest for *O. humifusa* (Table IV).

As the air temperatures were lowered below 0°C, cooling below the equilibrium freezing temperature occurred without freezing (supercooling; Fig. 1). Ice nucleation eventually caused the tissue temperatures to increase to nearly the equilibrium freezing temperature, which was about −0.5°C based on the Π and the Van't Hoff relation. For *O. ficus-indica* at 10/0°C compared with 30/20°C, the ice nucleation temperature was higher, both for the chlorenchyma and the water-storage parenchyma (Fig. 1). For the three species, the ice nucleation temperature averaged approximately 1.8°C higher at 10/0°C than at 30/20°C for both the chlorenchyma and the water-storage parenchyma (Table V).

The relative amount of liquid water, as inferred from the maximum amount determined by NMR, decreased below −3°C for both tissues of all three species (Fig. 2). The amount of water that remained unfrozen at −6°C, soon after ice formation began, was less for tissues from plants at 30/20°C than at 10/0°C. At −10°C, less than 10% of the water

Table IV. *Mucilage content in the chlorenchyma and the water-storage parenchyma for cladodes of platyopuntias at day/night air temperatures of 30/20 or 10/0°C*

Data are means ± SE (*n* = four plants).

Species	Mucilage (% of dry wt)			
	Chlorenchyma		Water-storage parenchyma	
	30/20°C	10/0°C	30/20°C	10/0°C
O. ficus-indica	23.5 ± 3.4	26.7 ± 4.8	25.6 ± 4.8	31.4 ± 3.7
O. humifusa	39.7 ± 3.4	44.7 ± 4.2	36.3 ± 4.3	40.6 ± 3.1
O. streptacantha	24.8 ± 2.1	28.1 ± 4.9	28.2 ± 2.4	32.5 ± 2.7

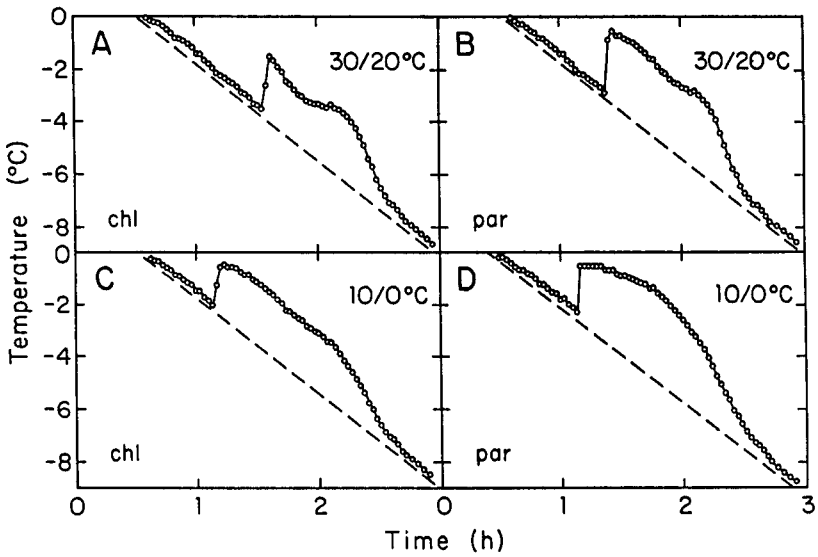

Figure 1. Temperatures during cooling for chlorenchyma (chl) from plants at day/night air temperatures of 30/20°C (A), water-storage parenchyma (par) from plants at 30/20°C (B), chlorenchyma at 10/0°C (C), and water-storage parenchyma at 10/0°C (D) for *O. ficus-indica*. Dashed lines indicate air temperatures. Data are representative of measurements from four plants.

remained unfrozen for *O. ficus-indica* and *O. streptacantha*, whereas more than twice as much water remained unfrozen for *O. humifusa* (Fig. 2). Even at −15°C, generally at least 10% of the water was unfrozen for the chlorenchyma and the water-storage parenchyma of *O. humifusa*.

T_1 and T_2 were estimated for the slower changing fraction (a) and the faster changing fraction (b) for the chlorenchyma and the water-storage parenchyma of plants at 30/20 and 10/0°C (Table I). T_1 of the two water fractions was larger in the water-storage parenchyma than in the chlorenchyma for all three species. For *O. humifusa* at 30/20°C, T_{1a} was 0.73 s for the chlorenchyma and 1.10 s for the water-storage parenchyma (Table VI). T_{1a} was higher for plants at 30/20°C than at 10/0°C,and T_{1b} was lower. T_{2a} was also higher for both tissues and all three species at 30/20°C compared with 10/0°C, but the pattern for T_{2b} was inconsistent (Table VI). Both relaxation times for both tissues and both day/night air temperatures were lower for *O. humifusa* than for *O. ficus-indica* and *O. streptacantha*.

DISCUSSION

The Π in the chlorenchyma and the water-storage parenchyma increased by 9 to 31% for *O. ficus-indica*, *O. humifusa*, and *O. streptacantha* as the day/night air temperatures were lowered by 20°C. Even though the water content tended to decrease during low-temperature acclimation, the increases in the overall Π of the tissues resulted mainly from an increase in the overall amount of sugars and mannitol. In particular, accumulation of Fru, Glc, and Suc is consistent with the observed Π increases for both tissues of *O. ficus-indica* and *O. streptacantha* at 10/0°C compared with 30/20°C. For *O. humifusa*, these three sugars accounted for 71% of the Π increases at the lower temperatures in the chlorenchyma and 52% in the water-storage parenchyma; mannitol accounted for an additional 10%. The accumulation of organic solutes, particularly sugars, during low-temperature acclimation also occurs for other plant species (Perras and Sarham, 1984; Koster and Lynch, 1992).

The production of low-mol-wt solutes may increase tolerance to subzero temperatures by helping retain liquid water inside the cells osmotically and thus ensuring metabolic activity for a longer period after extracellular freezing has been initiated. These organic solutes may also act as noncolligative cryoprotectants for membranes and proteins (Santarius, 1982; Franks, 1985). Both of these low-temperature acclimation mechanisms have been observed for citrus and spinach (Yelenosky and Guy, 1989). Because the increase in sugars and mannitol accounted for only about 72% of the tissue increases for *O. humifusa*, other osmotically active compounds, such as glycine betaine (Hitz and Hanson, 1980; Koster and Lynch,

Table V. *Ice nucleation temperatures for the chlorenchyma and the water-storage parenchyma for cladodes of platyopuntias at day/night air temperatures of 30/20 or 10/0°C*

Data are means ± SE (n = four plants).

Species	Ice Nucleation Temperature (°C)			
	Chlorenchyma		Water-storage parenchyma	
	30/20°C	10/0°C	30/20°C	10/0°C
O. ficus-indica	−4.9 ± 2.0	−2.1 ± 0.3	−4.2 ± 1.0	−2.2 ± 0.3
O. humifusa	−3.6 ± 0.3	−2.8 ± 0.4	−3.9 ± 0.2	−2.8 ± 0.5
O. streptacantha	−4.8 ± 0.4	−2.9 ± 0.4	−4.8 ± 1.4	−2.6 ± 0.2

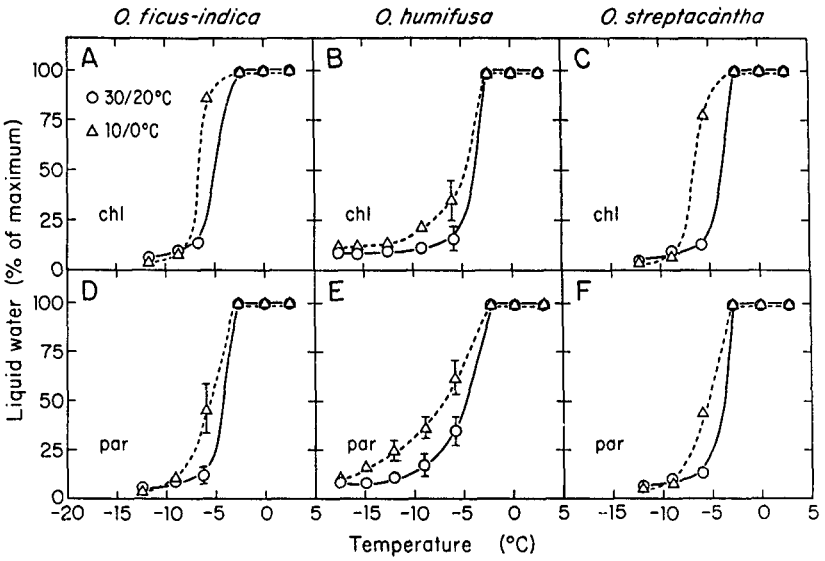

Figure 2. Percentage of the maximum amount of liquid water inferred from NMR measurements as a function of the temperature of the chlorenchyma (chl; A, B, and C) and the water-storage parenchyma (par; D, E, and F) of *O. ficus-indica* (A and D), *O. humifusa* (B and E), and *O. streptacantha* (C and F) at day/night air temperatures of 30/20°C (○) or 10/0°C (△). Data are means ± 1 SE (n = 4 plants). Absence of a bar indicates that the SE is smaller than the symbol.

1992), apparently accumulated during its low-temperature acclimation.

Ice nucleation consistently occurred at temperatures 1 to 2°C higher for cacti at 10/0°C compared to 30/20°C. Ice nucleation at higher temperatures would result in a more gradual cellular dehydration as tissue temperature decreases and should be less damaging than the sudden freezing that occurs after substantial supercooling. In this regard, ice formation commonly occurs closer to the equilibrium freezing temperature for species that tolerate substantial intracellular freezing dehydration compared to those that avoid freezing injury by substantial supercooling (Franks, 1985; Nobel and Loik, 1990). Ice formation also occurs closer to the equilibrium freezing temperature, and therefore supercooling is less, for tissues with a larger apoplastic water fraction (Levitt, 1980; Goldstein et al., 1985) and with a larger amount of mucilage (Loik and Nobel, 1991). When acclimated to the lower temperature, the three cactus species had more mucilage, which can act as an ice-nucleating agent (Goldstein and Nobel, 1991). Water from such mucilage could also supply molecules to the growing extracellular ice crystals (Nobel et al., 1992); this would tend to release heat and thus delay cellular water loss, which depends on the difference in chemical potential between the ice and the intracellular solution.

NMR studies have shown that intracellular water relaxes more slowly (longer T_1 and T_2) than does extracellular water (Gusta et al., 1975; Stout et al., 1978). In cacti, the water with the shorter relaxation time is thus assumed to be predominantly extracellular and to be bound to cell walls or mucilage (Goldstein and Nobel, 1991). The relaxation times of the intracellular water tended to be shorter for plants at 10/0°C compared to 30/20°C, suggesting greater restriction in the motion of the intracellular water during low-temperature acclimation. The restricted mobility of the intracellular water is consistent with the increase of low-mol-wt solutes in the three cactus species during acclimation. The extracellular

Table VI. *T_1 and T_2 for the chlorenchyma and the water-storage parenchyma from cladodes of platyopuntias at day/night air temperatures of 30/20 or 10/0°C*

Data are means ± SE (n = three to four plants). The water fraction with the longer relaxation time was denoted by a and the other by b. NMR measurements were performed at 0°C.

Relaxation Time(s)	Species	Chlorenchyma		Water-Storage Parenchyma	
		30/20°C	10/0°C	30/20°C	10/0°C
T_{1a}	*O. ficus-indica*	1.091 ± 0.011	1.006 ± 0.009	1.190 ± 0.007	1.180 ± 0.010
	O. humifusa	0.730 ± 0.030	0.610 ± 0.017	1.122 ± 0.010	1.038 ± 0.012
	O. streptacantha	1.102 ± 0.016	0.958 ± 0.006	1.568 ± 0.007	1.180 ± 0.009
T_{1b}	*O. ficus-indica*	0.588 ± 0.009	0.588 ± 0.004	1.005 ± 0.002	1.113 ± 0.006
	O. humifusa	0.208 ± 0.006	0.239 ± 0.005	0.670 ± 0.010	0.720 ± 0.002
	O. streptacantha	0.447 ± 0.003	0.571 ± 0.007	0.900 ± 0.007	0.926 ± 0.004
T_{2a}	*O. ficus-indica*	0.112 ± 0.003	0.110 ± 0.002	0.235 ± 0.010	0.131 ± 0.003
	O. humifusa	0.095 ± 0.001	0.080 ± 0.006	0.139 ± 0.000	0.069 ± 0.009
	O. streptacantha	0.099 ± 0.002	0.081 ± 0.002	0.289 ± 0.003	0.130 ± 0.004
T_{2b}	*O. ficus-indica*	0.044 ± 0.007	0.091 ± 0.010	0.117 ± 0.005	0.123 ± 0.002
	O. humifusa	0.038 ± 0.001	0.035 ± 0.001	0.114 ± 0.000	0.094 ± 0.001
	O. streptacantha	0.063 ± 0.003	0.052 ± 0.001	0.215 ± 0.006	0.134 ± 0.002

water motion characterized by T_1, on the other hand, increased during low-temperature acclimation. Similar responses of extracellular and intracellular water occur for dogwood during acclimation to low temperatures (Burke et al., 1974).

The winter-hardy *O. humifusa* can tolerate −24°C compared with −10°C for the other two species (Nobel, 1990; Nobel and Loik, 1990). The Π in its chlorenchyma and water-storage parenchyma at 10/0°C averaged 41% higher than for *O. ficus-indica* and *O. streptacantha*. Consistent with the higher Π, the amounts of sugars and mannitol were substantially greater in *O. humifusa* compared with the other two species. The larger amounts of these osmotically active solutes may help prevent substantial intracellular freeze dehydration and/or provide noncolligative protection to cell membranes. The water content, measured either as RWC or water content: dry matter ratio, was also substantially lower in *O. humifusa*. Mucilage content was highest for *O. humifusa*. The proportion of water that remained unfrozen below −10°C was more than 2-fold greater for *O. humifusa* than for the other two species, which is probably crucial for its tolerance of such low temperatures. Relaxation times of extracellular and intracellular water were lowest in *O. humifusa* for both tissues and both day/night air temperatures. Its reduced mobility of water may relate to the increase in solutes in its cells and its lower water content than for the other two species considered. Thus, *O. humifusa*, which is native to southern Canada and eastern United States, exhibits a greater low-temperature tolerance and acclimation than do the cultivated *O. ficus-indica* and *O. streptacantha* because of various cellular responses that tend to keep water in a liquid state within its cells.

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