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Water Relations of Individual Leaf Cells of *Mesembryanthemum crystallinum* Plants Grown at Low and High Salinity

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Summary. The effects of saline conditions on the water relations of cells in intact leaf tissue of the facultative CAM plant *Mesembryanthemum crystallinum* were studied using the pressure probe technique. During a 12-hr light/dark regime a maximum in turgor pressure was recorded for the mesophyll cells of salt-treated (CAM) plants at the beginning of the light period followed 6 hr later by a pressure maximum in the bladder cells of the upper epidermis. In contrast, the turgor pressure in the bladder cells of the lower epidermis remained constant during light/dark regime. Turgor pressure maxima were not observed in untreated (C_3) plants.

This finding strongly supports the assumption that water movement during malate accumulation and degradation in salt-treated plants occurs predominantly between the mesophyll cells and the bladder cells of the upper epidermis. The necessary calculations take differences in the compartment volumes and in the elastic moduli of the cell walls (ε) of the bladder cells of the lower and upper epidermis into account.

Measurements of the kinetics of water transport showed that the half-time of water exchange for the two sorts of bladder cells were nearly identical in CAM plants and in C_3 plants. The absolute values of the half-times increased by about 45% in salt-treated plants (about 113 sec) compared to the control plants (78 sec). Simultaneously, the half-time of water exchange of the mesophyll cells increased by about 60% from 14 sec (untreated plants) to 22 sec (salt-exposed plants). The leaves of this plant are apparently able to closely maintain the time of propagation of short-term osmotic pressure changes over a large salinity range.

A cumulative plot of the ε data measured on both C_3 and CAM plants showed that the differences between the values of the elastic moduli of bladder cells from the lower and from the upper epidermis are due to differences in volume and suggested that the intrinsic elastic properties of the differently located bladder cells of C_3 and CAM plants were identical.

A cumulative plot of the hydraulic conductivity of the membrane obtained both on mesophyll and on bladder cells of salt-treated and of untreated plants *vs.* the individual turgor pressure yielded a relationship well-known from giant algal cells and some higher plant cells: The hydraulic conductivity increased at very low pressure, indicating that the water permeability properties of the membrane of the various cell types of C_3 and CAM plants are pressure dependent, but otherwise identical.

The results suggest that a few fundamental physical relationships control the adaptation of the tissue cells to salinity.

Key Words crassulacean acid metabolism (CAM) · elastic modulus · hydraulic conductivity · *Mesembryanthemum crystallinum* · salt stress · turgor

Introduction

On a cellular basis, the overall response of a higher plant to salt or water stress in the environment is determined by several factors: geometric dimensions, turgor pressure, internal osmotic pressure, water permeability and reflection coefficients of the membranes as well as elastic properties of the cell wall of the various individual, specialized cells (Dainty, 1963, 1976; Zimmermann, 1977, 1978; Zimmermann & Steudle, 1978). Knowledge of all these parameters of the individual cells in a tissue and of their dependence on environmental changes is required in order to completely describe and to predict the water relations of a tissue under salt or water stress on the basis of the transport equations of the thermodynamics of irreversible processes.

Individual changes of these water-relation parameters are expected for different tissue cells if plants are grown under various conditions of salinity. It is well-known that many species can adapt to drought and to high salinity under maintenance of turgor (Zimmermann, 1978; Gutknecht, Hastings & Bisson, 1978). However, up to the present the data for a given tissue are not sufficient in order to formulate quantitative relationships for the short-term and long-term water exchange between the individual cells of a tissue under different saline conditions.

Considerable experimental information regarding the water relations between some individual cells in a tissue is available for the leaves of *Mesembryanthemum crystallinum* (Lüttge, Fischer &

Steudle, 1978; Rygol et al., 1986). The leaves of these species are covered on both sides by giant epidermal bladder cells, which are believed to supply the underlying mesophyll cells with water and thus protect the plant tissue against water deficiency. Young plants grown on nonsaline soil metabolize CO_2 via conventional C_3 photosynthesis, whereas several-month-old plants show weak CAM metabolism (Winter, 1973). The occurrence of CAM is associated with day/night variations in turgor pressure (Rygol et al., 1986). The pressure changes were measured by means of the pressure probe both in the mesophyll cells (where malate is accumulated during the night) and in the bladder cells of the upper epidermis (which are not involved in CAM).

These results suggest that the water-relation parameters in the individual bladder cells of both the upper and lower epidermis as well as of the individual mesophyll cells should be studied under controlled salt conditions. It is well known that plants of *M. crystallinum* exposed to high salinity during growth exhibit pronounced CAM (Winter, 1985).

The turgor and osmotic pressure measurements reported here are a first step in elucidating the fundamental physical processes and relationships that enable a tissue to adapt to salinity and to the associated changes in metabolism.

Materials and Methods

Plant Material

Plants of *Mesembryanthemum crystallinum L.* were grown in climatic chambers from seeds in potting soil under a light/dark regime of 12 hr. The light intensity was 400 μmol/m^2 · sec. The relative humidity was adjusted to 50 and 80% during the light and dark period, respectively. Daytime temperatures were about 25°C, nighttime temperatures about 18°C.

After four weeks plants were transplanted into aerated hydroculture solutions and were further grown under the same environmental conditions. Johnson's solution, modified after Winter (1973), was used for hydroculture. Every five days the medium was replaced by a new one. When the area of the second foliar leaf reached about 1 cm^2, part of the plants were exposed to increasing concentrations of NaCl. The sodium chloride was added stepwise to the nutrient solution (100 mM NaCl every day) until a final concentration of 400 mM was reached.

For pressure measurements, the control or salt-exposed plants were transferred into Erlenmeyer flasks that contained the corresponding medium. Leaves of an intact plant were carefully clamped to the microscope stage using metal clamps. Second foliar leaf pairs were used throughout the experiments because they exhibited strong CAM under salt stress. Insertion of the pressure probe into the leaf cells and the subsequent turgor pressure measurement required up to 15 min. Afterwards the plant was immediately transferred back to the climatic chamber, and a new one was taken for further experiments.

Turgor Pressure

The turgor pressure in the individual cells was measured by means of the pressure probe (Zimmermann, Raede & Steudle, 1969; Hüsken, Steudle & Zimmermann, 1978). The principle of this technique and the theoretical evaluation of the pressure relaxation have been described in detail elsewhere (Zimmermann, 1977, 1978; Zimmermann & Steudle, 1978; Rygol & Lüttge, 1983). Briefly, from the pressure relaxation curve the half-time, $T_{1/2}$, of the water exchange can be deduced. The hydraulic conductivity of the membrane barrier of a given cell can be calculated from the half-time according to

$$T_{1/2} = \frac{V}{A} \cdot \frac{\ln 2}{L_p(\varepsilon + \Pi^i)} \quad (1)$$

where V = initial cell volume, A = water exchange area, L_p = hydraulic conductivity, ε = the elastic modulus of the cell wall, and Π^i = the initial osmotic pressure within the cell.

For calculation of L_p the geometric dimensions, the elastic modulus of the cell wall, and the internal osmotic pressure must be determined in independent experiments. The elastic modulus of the cell wall is defined by

$$\varepsilon = \frac{\Delta P}{\Delta V} \cdot V. \quad (2)$$

Thus, ε can be measured by injection of incremental positive or negative volume changes (ΔV) of increasing amplitude into the cell and by simultaneous recording of the corresponding changes of turgor pressure using the pressure probe (for details, *see* Zimmermann & Steudle; 1978, Rygol et al., 1986). A plot of the volume changes *vs.* the corresponding changes in turgor pressure always revealed straight lines both for the large bladder cells and for the relative small mesophyll cells, indicating that leakages or water loss during the measurements of the elastic modulus could be definitely excluded.

Cell Dimensions

The volume and the area of water exchange of the bladder cells were determined as previously described (Rygol et al., 1986), i.e., the cell volumes were calculated from measurements of cell dimensions under a stereomicroscope (×160). Bladder cells were selected that were spheres or rotational ellipsoids. For calculation of the water exchange area, only the basal membrane of the bladder cell was taken into account (Steudle, Lüttge & Zimmermann, 1975). The average area and the cell volume of the smaller leaf mesophyll cells (which were considered to be spherical) were estimated from sections made from 50 cells of control and salt-treated plants, respectively.

Internal Osmotic Pressure

The osmotic pressure of the cells was cryoscopically determined after collection of the sap with the pressure probe. To this end the microcapillary of the pressure probe (tip diameter 3–5 μm) was inserted into the bladder or mesophyll cells and the cell sap was sucked into the capillary by appropriate displacement of the metal rod in the pressure probe. In the case of the mesophyll cells, the upper and the lower leaf epidermis were partly removed. After about 15 mm^3 cell sap had been collected it was

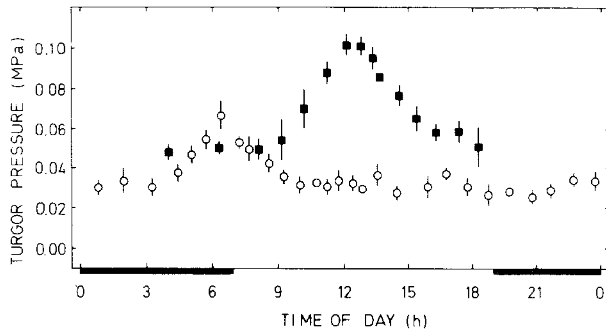

Fig. 1. Day/night changes of turgor pressure in individual leaf-bladder cells of the upper epidermis (filled squares) and leaf-mesophyll cells (circles) of intact plants of *Mesembryanthemum crystallinum* grown under high salinity conditions (400 mM NaCl). The data points measured on bladder cells of different leaves of CAM plants represent the mean of three to four measurements, the bars the SD

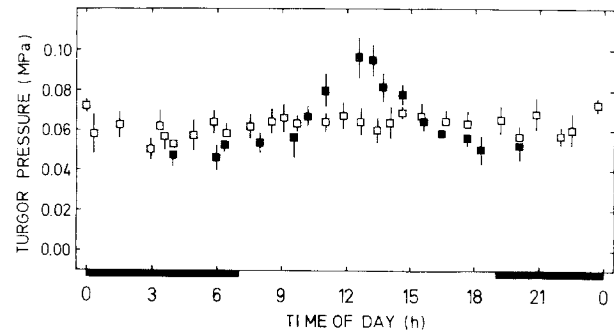

Fig. 2. Day/night changes of turgor pressure in bladder cells of the upper (filled squares) and the lower epidermis (open squares) of CAM plants (*see* Fig. 1). The corresponding turgor pressure in both bladder cell groups was measured on the same leaf. The data points represent the mean of four to five experiments, the bars the SD

centrifuged in closed plastic vials at 1000 × *g* for 1 min. The osmotic pressure in the supernatant was cryoscopically determined using a freezing-point osmometer (Osmomat 030, Gonotec, Berlin).

In addition, comparative measurements of osmotic pressure were also performed in leaf disks by using dew-point hygrometry (Wescor C-52 sample chamber connected to a Wescor HR-33 dew point microvoltmeter; for details, *see* Clipson et al., 1985). The values obtained by dew point hygrometry were always slightly lower than those obtained cryoscopically. The underestimation of the osmotic pressure in the method of dew point hygrometry is due to the processes of freezing and thawing of the cell sap and the associated contamination with the solution of the apoplasmic space (Jones & Turner, 1978). The data obtained by both methods were converted from mosmol · kg^{-1} to MPa by assuming that 1 MPa = 400 mosmol · kg^{-1} at 20°C (Wyn-Jones & Gorham, 1982).

Titratable Acidity

The nocturnal accumulation of titratable acidity was measured according to Winter et al. (1983) and Rygol, Winter and Zimmermann (1987).

Results

Diurnal Rhythm of Turgor Pressure and Osmotic Pressure

Bladder cells in the upper epidermis and mesophyll cells of leaves of salt-treated plants of *M. crystallinum* showed a day/night rhythm in turgor pressure similar to that which was measured recently on plants in which weak CAM was induced during aging of leaves (Fig. 1, *see also* Rygol et al., 1986). The maximum in turgor pressure observed for mesophyll cells at the beginning of the light period was followed 6 hr later by a pressure maximum in the bladder cells of the upper epidermis (Fig. 1). In these experiments, turgor pressure was also measured in the bladder cells of the lower epidermis as a function of the light/dark regime. Surprisingly, the turgor pressure remained almost constant during light/dark regime, except for some random fluctuations (Fig. 2). In this respect the bladder cells of the lower epidermis resembled mesophyll and bladder cells from leaves of plants grown in the absence of salinity (C_3 plants) (Rygol et al., 1986). Comparison of the time course of turgor pressure in the bladder cells of the upper epidermis in Figs. 1 and 2 indicates that turgor pressure measurements were reproducible, despite the fact that the data were obtained on bladder cells of different leaves and at different times of the year. Table 1 lists the data for the internal osmotic pressure in the two groups of bladder cells as well as in mesophyll cells measured at 7, 12 and 19 hr during the light period. The absolute values of this parameter showed somewhat larger fluctuations from leaf to leaf than the turgor pressure values although the values for the three cell types relative to each other were nearly constant. Therefore, the osmotic pressure data given in Table 1 for the two bladder cell groups were measured on the same leaf, whereas the values for the mesophyll cells had to be measured in different leaves for experimental reasons. It can be seen that the internal osmotic pressure in the mesophyll cells reached its maximum in the early morning (because of malate accumulation during the night) and declined during the light period. The osmotic pressures in both of the differently located sorts of bladder cells also assumed the highest values in the

Table 1. Diurnal changes in the osmotic, $\Delta\pi$, and turgor pressure, ΔP, differences between mesophyll cells and bladder cells of the upper and lower epidermis, respectively, of *Mesembryanthemum crystallinum* grown in culture solution containing 400 mM NaCl

Time of day (hr)	Kinds of cells	Osmotic pressure, π^i (MPa)	Osmotic pressure difference, $\Delta\pi^i$ (MPa)[a]	Turgor pressure difference, ΔP (MPa)[b]
07.00	Bladder upper side	2.23	+0.27	−0.015
07.00	Mesophyll	2.50	−0.10	+0.007
07.00	Bladder lower side	2.60		
12.00	Bladder upper side	2.07	+0.21	+0.079
12.00	Mesophyll	2.28	+0.10	+0.034
12.00	Bladder lower side	2.18		
19.00	Bladder upper side	2.28	−0.06	+0.021
19.00	Mesophyll	2.22	−0.05	+0.029
19.00	Bladder lower side	2.27		

[a] Values were calculated from the data in column 1; [b] Data were taken from Figs. 1 and 2; a positive sign indicates that water flow is driven from the epidermis to the mesophyll compartment, a negative sign the corresponding opposite direction.

morning. The absolute value of the osmotic pressure in the bladder cells of the lower epidermis was higher than those of the mesophyll and bladder cells in the upper epidermis. The osmotic pressure in both groups of bladder cells decreased during the following light period in order to reach a minimum value at noon. The values increased slightly again until 19 hr. The minimum in the values at noon is consistent with data measured previously for the bladder cells of the upper epidermis of leaves taken from plants grown in nonsaline soil (Rygol et al., 1986). The second and third columns of Table 1 show the differences in the osmotic pressure between the mesophyll cells and each of the two bladder groups as well as the corresponding turgor pressure differences (taken from Figs. 1 and 2).

The driving force for net water flow is generally given by

$$J_v = L_p(\Delta P - \sigma\Delta\Pi) \quad (3)$$

where ΔP is the turgor pressure difference between the mesophyll and the bladder cells of the upper or lower epidermis, $\Delta\Pi$ the corresponding osmotic pressure differences and σ the overall reflection coefficient for the membrane barriers between the mesophyll and the bladder cell compartments.

It is obvious that in the early morning the turgor pressure differences are nearly zero. The osmotic pressure differences between the mesophyll cells and the bladder cells of the lower epidermis are smaller than the osmotic pressure differences existing between the mesophyll cells and the bladder cells of the upper epidermis. At noon the turgor and osmotic pressure forces act in parallel, whereas in the evening they are antiparallel. It should be noted that the effective osmotic pressure differences (i.e., the product of the osmotic pressure and the reflection coefficient; Dainty, 1963; Zimmermann & Steudle, 1978) may be much smaller than the osmotic pressure differences. At present we do not know the reflection coefficients of the membrane barriers and their dependence on the diurnal varying salt concentration in the leaf. However, the reversal of the direction in the individual forces during the day/night rhythm suggests that the reflection coefficients of the membrane barriers must change during malate accumulation and degradation.

Kinetics of Water Transport

For determination of the kinetic parameters of water transport, data were used relating to the illuminated phase during which no maximum in turgor pressure occurred (9 to 16 hr for mesophyll cells, 6 to 11 hr and 14 to 16 hr for the bladder cells of the upper epidermis of salt-treated plants). From experimental and statistical standpoints this averaging process represents a reasonable approach. It allows preliminary indications of possible differences in the water-relation parameters of the two groups of bladder cells as well as of the mesophyll cells in their salinity dependence. Because of biological variations between plants, we tried to obtain as many data as possible for the different cell types on the same leaf at a given time of the day.

Bladder Cells

The various parameters that control the kinetics of water relations of the bladder cells located on either

Table 2. Kinetic parameters determining water transport through the bladder cell membranes of both epidermal sides of C_3 and CAM plants of *Mesembryanthemum crystallinum* L

Plant type	P (MPa)	V (mm^3)	A (mm^2)	V/A	ε (MPa)	$T_{1/2}$ (sec)	π (MPa)	$L_p \cdot 10^7$ ($m \cdot sec^{-1} \cdot MPa^{-1}$)
C_3 plants (0 mM NaCl)								
Upper side	0.02 – 0.07	0.02 – 0.06	0.11 – 0.21	0.17	1.1 – 14	58 – 116	1.90 – 1.96	0.6 – 3.9
	0.04 ± 0.02	0.03 ± 0.02	0.17 ± 0.03		7.0 ± 4.4	84 ± 19	1.93 ± 0.004	1.7 ± 0.9
	(17)	(17)	(17)		(12)	(10)	(14)	(10)
Lower side	0.05 – 0.09	0.12 – 0.25	0.52 – 0.92	0.24	9.0 – 36.6	53 – 103	1.96 – 2.04	0.6 – 1.8
	0.07 ± 0.02	0.16 ± 0.05	0.66 ± 0.16		23.1 ± 9.1	72 ± 16	1.98 ± 0.013	1.1 ± 0.4
	(16)	(16)	(16)		(10)	(10)	(15)	(10)
CAM plants (400 mM NaCl)								
Upper side	0.01 – 0.06	0.05 – 0.17	0.28 – 0.53	0.24	0.9 – 5.1	97 – 115	1.94 – 2.03	3.1 – 5.3
	0.04 ± 0.02	0.10 ± 0.04	0.42 ± 0.09		2.5 ± 1.2	103 ± 8	1.97 ± 0.020	3.8 ± 0.9
	(15)	(15)	(15)		(21)	(5)	(14)	(5)
Lower side	0.04 – 0.10	0.14 – 0.80	0.71 – 1.97	0.36	13.4 – 50	95 – 173	1.92 – 2.89	0.4 – 1.0
	0.06 ± 0.02	0.52 ± 0.19	1.46 ± 0.40		33.4 ± 10	129 ± 28	2.35 ± 0.520	0.6 ± 0.2
	(15)	(15)	(15)		(18)	(7)	(14)	(7)

The range of data and the mean value (with SD) are given for each parameter. Number of measurements are given in brackets. For further explanation *see* text.

the upper or lower leaf surface of *M. crystallinum* are listed in Table 2. Data are presented for growth under nonsaline conditions (C_3 metabolism) and at 400 mM NaCl (CAM).

The density of bladder cells was about 40% larger on the lower epidermis than on the upper one.

As indicated by Table 2 and Figs. 1 and 2, the stationary turgor pressure was in average a factor of about 1.4 to 1.6 higher in the bladder cells of the lower epidermis than in the cells of the upper one. This was the case for both C_3 and CAM plants even though the absolute values increased with salinity treatment. It is also evident that the average volume, V, of bladder cells from the lower epidermis was by a factor of about 5 larger than that of the upper epidermis. This volume ratio was apparently independent of the salt concentration, even though the absolute volumes of bladder cells on both leaf surfaces of the salt-treated plants increased by a factor of about 3 compared to the C_3 (control) plants. The ratio between the average value of the basal area, A, of the bladder cells of the lower epidermis to the basal area of the upper epidermis was about 4 and was also independent of salinity. Thus, V/A which controls—among the other parameters mentioned above—the half-time of water exchange, was a factor of about 1.5 larger for the bladder cells of the lower epidermis than for those of the upper one over the salinity range investigated. It could be argued that, because of the geometric dimensions, water exchange between bladder cells and the underlying tissue cells should proceed faster in the lower epidermis than in the upper one. However, measurements of the volume dependence of the elastic modulus of the walls of the two differently located bladder cells showed a relationship which counteracts the increase in the geometric dimensions of the bladder cells of the lower epidermis that occurs according to Eq. (1). The result is that the water exchange times of the two types of bladder cells are comparable.

The average values of the elastic modulus of the bladder cell walls showed similar differences between the two epidermal layers to the differences in the volume/area ratio. In C_3 plants the value of the elastic modulus of bladder cells of the lower epidermis was about three times higher, in CAM plants about 13 times higher, than that of the cells of the upper epidermis (Table 2). The dependence of the absolute values of the elastic moduli on salinity was not clear because of the large fluctuations of the ε values for a given bladder cell group. However, at least for the bladder cells of the lower epidermis, there seems to be a slight trend to higher values in response to increased salinity. The differences in the ε values of the bladder cells of both layers as well as the considerable variation of the magnitude of the elastic modulus for a given bladder cell group may be due to a pressure and/or volume dependence of the modulus as found for algal cells and cells of other higher plants (Zimmermann & Steudle, 1974, 1975; Steudle, Ziegler & Zimmermann, 1983).

No turgor-pressure dependence of ε could be detected within the turgor-pressure range given in

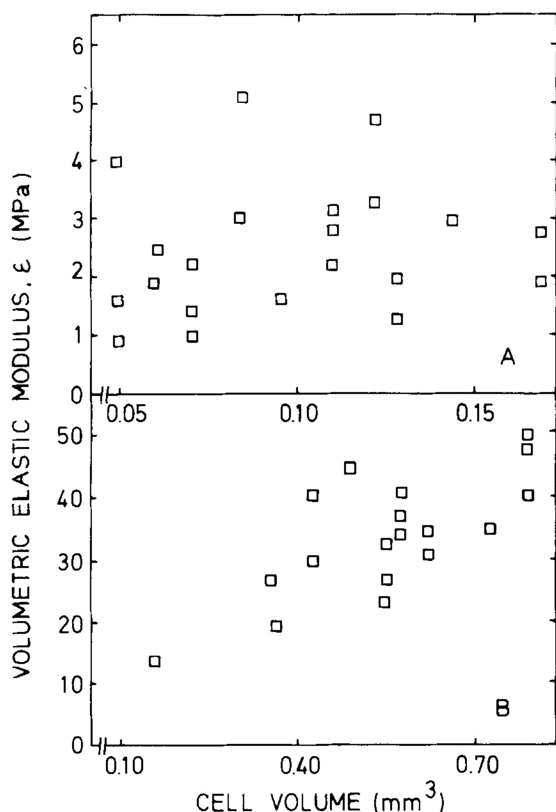

Fig. 3. The volumetric elastic modulus of the cell wall, ε, as a function of leaf-bladder cell volume for the upper (*A*) and lower (*B*) epidermis of CAM plants. The turgor pressure range in the bladder cells was between 0.01 and 0.04 MPa for the upper and 0.04 and 0.10 MPa for the lower epidermis. Note the different scale of the cell volume axis

Table 2. A volume dependence of the elastic modulus existed, at least for the bladder cells of the lower epidermis at high salinity (Fig. 3). Cumulative analysis of the ε- and the corresponding volume data of both bladder cell groups showed that the differences in the magnitude of the ε values could be explained by a volume-dependent relationship (Fig. 4).

Because of the opposing influences of the volume/area ratio and of the elastic modulus on the half-time of water exchange, the effect of the larger volume of the lower bladder cells on water exchange must be partly or almost completely compensated by the volume dependence of the elastic modulus.

According to Table 2 the values of the half-times for water exchange (as calculated from the experimentally determined pressure relaxation curves (Fig. 5*A*)) were indeed nearly identical for the two groups of bladder cells of C_3 plants. For CAM plants the absolute values seemed to be significantly higher, but still very similar compared to the corresponding values of the control plants.

Therefore, from a kinetic standpoint we can conclude that both bladder cell types behave very similarly.

From the parameters discussed above we can calculate the hydraulic conductivity of the membrane barriers of the two bladder cell groups provided that their internal osmotic pressure is known (*see* Eq. (1)). As shown by Table 2 the average osmotic pressure was practically identical for the two bladder groups of C_3 plants and for the upper bladder cells of CAM plants (if we exclude data taken at times when maximum turgor pressure occurred). The remarkable exception was the osmotic pressure in the lower bladder cells of salt-treated plants, which was always significantly higher (*see also* Table 1). However, this value was still small compared to the ε value in the bracket term of Eq. (1). Therefore, it is not surprising that the absolute values of the hydraulic conductivities of the upper and lower bladder cells were of the same order if the magnitude of the experimental error is considered. For C_3 plants the values seemed to be identical for the bladder cells of both epidermis, whereas the L_p value of the bladder cells of the upper epidermis of CAM plants was apparently higher, compared to the bladder cells of the lower epidermis.

Mesophyll Cells

The turgor pressure in the mesophyll cells was on average smaller than in the bladder cells. After salinity treatment, the mesophyll turgor pressure seemed to increase slightly (Table 3), if the maximum in turgor pressure at the beginning of the day was excluded from the averaged data. The volume of the mesophyll cells was three to four orders of magnitude smaller than that of the bladder cells and showed a similar dependence on salinity to that of the bladder cells (Table 3). At high salinity the average volume was about double that at zero salinity. In contrast to the bladder cells the volume/area ratio, however, remained constant.

The absolute values of the elastic moduli of mesophyll cells of C_3 and CAM plants were one to two orders of magnitude smaller than those of both groups of bladder cells. However, in contrast to the elastic moduli of the two bladder cell groups, the elastic properties of the mesophyll cell walls were strongly dependent on the salt treatment. The elastic modulus of the mesophyll cell walls increased by a factor of about 12 if CAM was induced by high salinity, despite the much smaller increase in volume and turgor pressure.

The half-time of water exchange of mesophyll cells (determined experimentally from curves such as in Fig. 5*B*) showed the same trend with salinity as

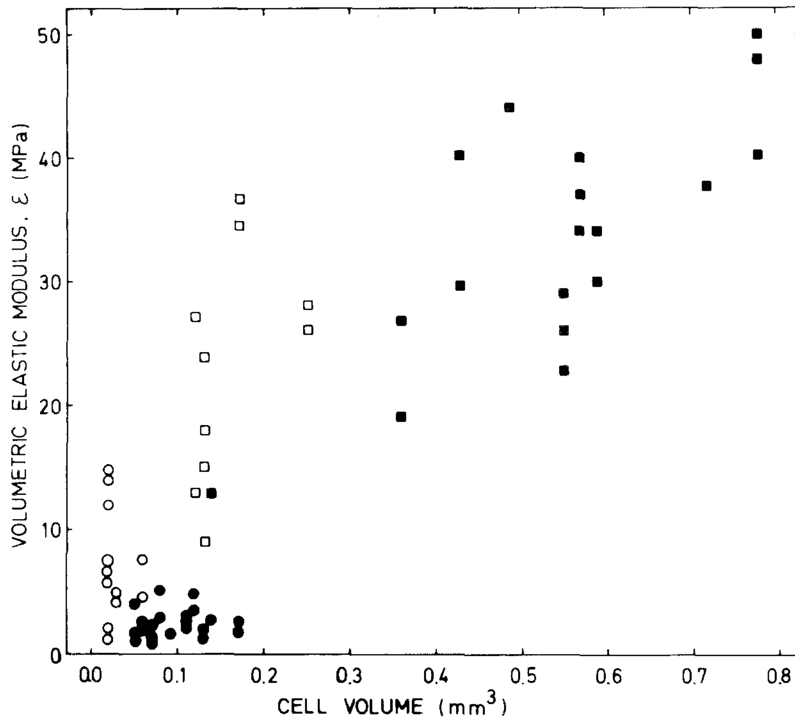

Fig. 4. Cumulative plot of ε as a function of cell volume of both C_3 (open symbols) and CAM (filled symbols) plants. Data of CAM plants were taken from Fig. 3*A* and *B*. The ε values of the bladder cells of the upper epidermis are denoted with circles, those of the lower epidermis with squares

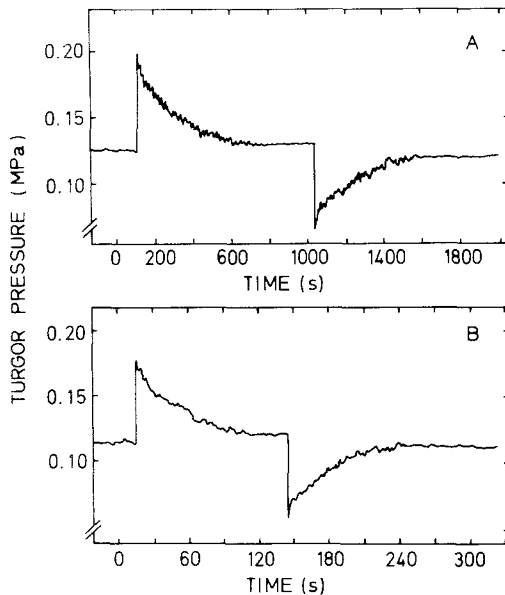

Fig. 5. Typical pressure relaxation curves measured in bladder cells of the lower epidermis (*A*) and in mesophyll cells (*B*) of intact leaves of CAM plants. The pressure relaxations were induced by injection of a pressure (volume) pulse into the cells by means of the pressure probe. Note the different time scales in *A* and *B*

the bladder cells. However, the half-times of 14 sec (0 mM NaCl) and 22 sec (400 mM NaCl) were considerably smaller than the corresponding values for the bladder cell groups (*see* Table 2).

From these values (and the internal osmotic pressure already discussed above) the hydraulic conductivities of the mesophyll cells of C_3 and CAM plants were calculated (Table 3). In contrast to the bladder cells the hydraulic conductivity of the mesophyll cells considerably decreased after salt treatment. If we plot the individual L_p values of mesophyll and bladder cells of C_3 and CAM plants *vs.* the corresponding turgor pressure values (which apparently increased with saline treatment, *see* Table 2), a similar relationship is obtained to that found before for giant algal cells (Zimmermann & Steudle, 1974, 1975; Wendler & Zimmermann, 1985*a*,*b*) and for leaf cells of *Elodea densa* (Steudle, Zimmermann & Zillikens, 1982) (Fig. 6). It is interesting to note that the L_p value measured in mesophyll cells of CAM plants (i.e., at higher turgor pressures) was comparable to those of the bladder cells, whereas the values measured in C_3 plants were about one magnitude higher (Fig. 6).

Discussion

The data presented here allow conclusions not only about the kinetics of, but also about the driving forces for water exchange between bladder and mesophyll cells in the leaves of C_3 and CAM plants of *M. crystallinum* involved in short- and long-term changes of osmotic pressure and of turgor pressure within the leaf. The half-times of water exchange of

Table 3. Kinetic parameters determining water transport through the membranes of mesophyll cells of C_3 and CAM plants of *Mesembryanthemum crystallinum*

Plant type	P (MPa)	V (mm^3)	A (mm^2)	V/A	ε (MPa)	$T_{1/2}$ (sec)	π (MPa)	$L_p \cdot 10^7$ ($m \cdot sec^{-1} \cdot MPa^{-1}$)
C_3 plants (0 mM NaCl)								
	0.01 – 0.04	$0.3 \cdot 10^{-3}$	0.017 ± 0.001	0.018	0.01 – 0.39	9 – 18	0.68 – 0.84	5.8 – 15.4
	0.03 ± 0.01	$\pm 0.17 \cdot 10^{-3}$			0.13 ± 0.11	14 ± 3	0.77 ± 0.05	10.6 ± 2.7
	(10)	(10)	(10)		(10)	(10)	(14)	(10)
CAM plants (400 mM NaCl)								
	0.02 – 0.07	$0.7 \cdot 10^{-3}$	0.040 ± 0.010	0.018	0.44 – 2.93	17 – 26	2.05 – 2.20	1.1 – 2.3
	0.04 ± 0.01	$\pm 0.20 \cdot 10^{-3}$			1.60 ± 1.27	22 ± 5	2.15 ± 0.09	1.6 ± 0.4
	(10)	(10)	(10)		(9)	(9)	(19)	(9)

The range of data and the mean value (with SD) are given for each parameter. Number of measurements are given in brackets. For further explanation *see* text.

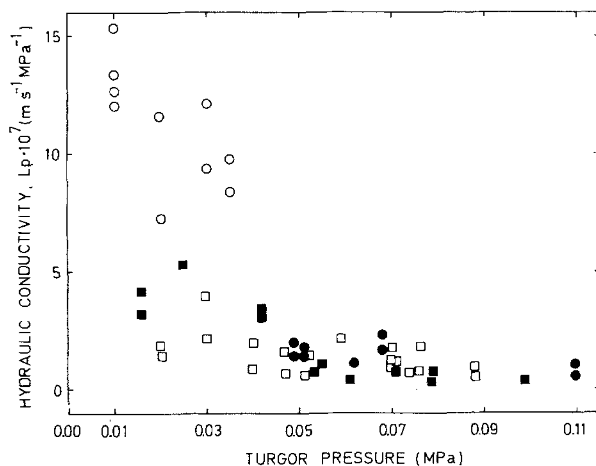

Fig. 6. Cumulative plot of the hydraulic conductivity of the membranes of bladder cells of both epidermis sides (squares) and of mesophyll cells (circles). Open symbols refer to measurements on C_3 plants, closed ones to measurements on CAM plants

the two differently located groups of bladder cells in both C_3 and CAM plants were very similar. If we compare the average values of untreated and salt-treated plants (i.e., 78 sec for C_3 plants and 113 sec for CAM plants, *see* Table 2), we can safely conclude that salt treatment extends the water exchange time through the basal membrane of the bladder cells by about 45%. However, this has no consequence for the water movement in the tissue (in response to short-term stress) because mesophyll cells showed a similar behavior. According to Table 3 the half-time of water exchange of the mesophyll cells also increased by about 60% in CAM plants. In addition, mesophyll cells of both untreated and salt-treated plants always responded much faster to internal and/or external changes in osmotic pressure than the bladder cells. This seems to be plausible in the light of our hypothesis (Steudle et al., 1975) that the bladder cells function as water reservoirs.

The ratio of the average half-time of water exchange of the bladder cells to that of the mesophyll cells was about 5 for C_3 and CAM plants. This leads to the remarkable conclusion that the leaves of this plant are able to closely maintain the time of propagation of osmotic pressure changes over a large salinity range.

The physical basis for this observation is that the influence of the volume/area ratio on the half-time was almost compensated by the volume dependence of the elastic modulus of the cell wall. The large bladder cells of the lower epidermis exhibited high values of the elastic modulus, whereas the cells of the upper epidermis showed correspondingly smaller ones independent of the salinity under which the plants were grown.

The differences in the ε values between the two groups of bladder cells can be explained by a volume dependence. This suggests that the intrinsic elastic (anisotropic) properties of the differently located bladder cells are identical.

Therefore, adaptation of cell volume (and exchange area) to salt treatment and the associated maintenance of the kinetics of water transport between the mesophyll and bladder cells is apparently dependent on the physical properties of the cell wall.

A further interesting result is that the hydraulic conductivities of the two groups of bladder cells were independent of turgor pressure, whereas the mesophyll cells of salt-treated and untreated plants showed an interdependence of these two parameters. The increase of L_p observed in giant algal cells at a pressure close to the plasmolytic point was explained by folding of (one of) the membranes (Zimmermann & Steudle, 1974, 1975; Wendler & Zim-

mermann, 1985*a*,*b*). The results reported here may be explained in a similar way, in particular if the very low turgor pressure in the mesophyll cells of C_3 plants is taken into account (*see above*). Comparison of the turgor pressure measured in the various cells of C_3 and CAM plants shows that the average turgor pressure in the bladder cells was never below that value which corresponded to the onset of the pressure-dependent increase of L_p in mesophyll cells. This may explain the apparent lack of a dependence of the hydraulic conductivities of the bladder cells on pressure.

If we accept this explanation we can trace (by analogy with the elastic modulus) the "differences" in magnitude and pressure dependence of the L_p values of the three cell types to common features of plant membranes. This assumption is supported by the L_p value of mesophyll cells, which was very similar to those measured for bladder cells in comparable turgor pressure ranges. The parameters discussed above control the kinetics of water transport between the different cells in the leaf in response to short-term water or salt stress.

The two groups of bladder cells can, in principle, function equally as water reservoirs in salt-treated and untreated plants as postulated by one of the authors more than 10 years ago (Steudle et al., 1975). However, this conclusion is not completely consistent with the differences in the day/night rhythm of turgor pressure observed for the upper and lower bladder cells of CAM plants (Fig. 2). The occurrence of a strong increase in turgor pressure in the upper bladder cells at noon suggests a preferential exchange of water between the mesophyll cells and upper bladder cell compartment (during long-term changes in the osmotic environment). It can be argued that the lack of a similar maximum in turgor pressure in the bladder cells of the lower epidermis results from the differences in size (and elasticity) of these two compartments. From the mean volume (*see* Table 2) and mean density of the bladder cells (49 and 68 bladder cells per 25 mm^2 for the upper and lower leaf surfaces, respectively), the mean total volumes of the upper and of the lower bladder cell compartments are calculated to be 907 mm^3 and 6542 mm^3 per leaf area (4625 mm^2) of a CAM plant. From the difference in turgor pressure between early morning and noon in the upper bladder cells (0.041 MPa, Fig. 2) and from the elastic modulus (2.5 MPa, Table 2) we can calculate the corresponding change in volume of the bladder cells, ΔV, according to Eq. (2) (ΔV = 14.9 mm^3). ΔV must be equal to the amount of water shifted from the mesophyll compartment to the upper bladder cells during the morning hours. Using this value and the appropriate values of the total volume and the elastic modulus (33.4 MPa, Table 2) of the lower bladder cells and assuming that the mesophyll compartment supplies water equally to the two bladder cell compartments, we can estimate the expected change in turgor pressure in the bladder cells of the lower epidermis. The calculated value of this theoretically expected pressure change is 0.076 MPa. If we use for the calculation ε values and values of volume and density measured on the same leaf, the expected pressure change for symmetric water exchange between the mesophyll and bladder cells would be 0.052 MPa. These values are at least equal to or even higher than those measured in the bladder cells of the upper epidermis. Because of the apparent lack of such a turgor pressure change in the bladder cells of the lower epidermis, we can conclude that the bladder cell compartment of the upper epidermis is predominantly involved in water exchange during malate accumulation and degradation in the mesophyll cells. In other words, water transport in the leaf of this inducible CAM plant is asymmetric even though the kinetics of water exchange are comparable for both compartments.

It is difficult to give a complete description of the water flows during the day/night rhythm in CAM plants because of the lack of reflection coefficient data on the membrane barriers and because it is unknown how much water is immobilized in the tissue cells. In any case, the comparison of the water-relation parameters of untreated and of salt-treated plants performed here has shown that more attention has to be paid to the physics of the system.

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