Water Relations of Individual Leaf Cells of *Mesembrinimum 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 *Mesembrinimum 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 (C₃) 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 (e) 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 C₃ 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 e data measured on both C₃ 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 C₃ 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 as 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 C₃ 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 • *Mesembrinimum 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 *Mesembrinimum crystallinum* (Lütting, Fischer &
Seehler, 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 CO₂ via conventional C₃ 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).

The results suggest that the water-relations 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/m²·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. For every five days the medium was replaced by a new one. When the area of the second foliar leaf reached about 1 cm², 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, Rueda & 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, T½, 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

\[
T_{1/2} = \frac{V}{A} \cdot \ln \frac{2}{L_p(e + \Pi_i)}
\]

where V = initial cell volume, A = water exchange area, \(L_p\) = hydraulic conductivity, \(e\) = the elastic modulus of the cell wall, and \(\Pi_i\) = 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

\[
e = \frac{\Delta P}{\Delta V} \cdot V.
\]

Thus, \(e\) 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 (X160). 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 mm³ cell sap had been collected it was