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Salinity-induced changes in the structure and ultrastructure of bean root cells

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Abstract

The effect of 80 mM NaCl on the structure and ultrastructure of root cells of *Phaseolus vulgaris* plants has been investigated. Roots of plants treated with NaCl were shorter and had less secondary roots than control plants. In control plants, epidermal cells were isodiametric and uniformly placed forming a thin layer, whereas in stressed plants, the shape and disposition of epidermal cells was less regular. The cortical cells of control plant were round-shaped and distributed allowing large and well defined intercellular spaces, whereas stressed plants presented irregular cells, which were interdigitated, thus decreasing the volume of the intercellular spaces. Presence of 80 mM NaCl did not result in significant differences in the number, shape or distribution of the cell organelles. Membrane vesiculation was often observed in cells of NaCl treated plants. Addition of 80 mM NaCl to the growth medium considerably increased the leakage of solutes from intact plant roots back to the solution especially K^+ and Ca^{2+}.

Key words: cortical cells, epidermal cells, intercellular spaces, leakage, membrane vesiculation, NaCl, *Phaseolus vulgaris*.

Introduction

Plant responses to excess NaCl in the root media include physiological, anatomical and morphological changes. A number of salinity-induced morphological alterations of the whole plant as well as changes in the ultrastructure of plant cells have been found in several species, including changes in the size and development of wheat plant roots (Udovenko *et al.* 1970), vesiculation of cortical cells of barley and beans

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(Nassery and Jones 1976), damage to epidermal mitochondria of wheat plants (Udovenko *et al.* 1970) or nuclear chromatin condensation of barley cells (Werker *et al.* 1983).

Despite the fact that salinity affects the submicroscopical structure of plant cells (Meiri and Poljakoff-Mayber 1967, Meiri *et al.* 1971, Poljakoff-Mayber 1975), very few studies have related ultrastructural changes of plant cells to their physiological significance. In fact, it is often not clear whether the changes observed are a consequence of the damaging effect of salinity or an adaptive response of the plant cells to overcome the excess ions in the root media (Nazarenko and Serebryakova 1990, Werker *et al.* 1983).

Most physiological and morphological studies on the effect of salinity have been devoted to shoots; however it is the root which is directly exposed to the soil salinity and this should be the plant organ where the effects of this stress should be more pronounced (Hagibagheri *et al.* 1985).

In the present work we have investigated the morphological and ultrastructural changes occurring in cortical root cells of *Phaseolus vulgaris* as a consequence of NaCl stress. As well, leakage of ions from the roots to the solution has been correlated with morphological alterations of the cell membranes.

Materials and methods

Plant growth: Bean seeds (*Phaseolus vulgaris* L., cv. Contender) were germinated on filter paper saturated with 1 mM $CaSO_4$ in the dark at 28 °C. After 2 d, 30 seedlings of uniform size were transferred to hydroponic culture in buckets containing 10 dm^3 of aerated Hoagland nutrient solution. The pH of the solution was kept between 5.5 and 6.0. The solutions were fully renewed every 3 d. Plants were held in a controlled environment chamber under relative humidity of 70 %, photoperiod of 16 h irradiance of 380 - 400 µmol m^{-2} s^{-1} and temperatures of 25 and 20 °C, respectively. After 3 d 80 mM NaCl was added to initiate the saline treatment. Plants grown in the presence of 5 mM NaCl, added as a nutrient, were considered as control.

Plants were collected after 1, 2 and 12 d of treatment and separated into roots and shoots. Root samples (2 mm length) were cut from a zone 2 cm from the root tip. Each treatment had three replicates.

Electron microscopy: Samples were fixed for 2.5 h at 4 °C in a 0.1 M Na-cacodylate buffered (pH 7.2) mixture of 2.5 % glutaraldehyde and 4 % paraformaldehyde. In order to avoid artifacts due to osmotic effects, 5 mM NaCl (control), 80 mM NaCl (salinized) and 2 mM $CaCl_2$ (to all samples), were added to the fixation solutions. Tissue was postfixed with 1 % osmium tetroxide for 2 h, dehydrated in a graded alcohol series and embedded in Spurr's resin. Blocks were sectioned in a *Reichert Ultramicrotome*. Thin sections for transmission electron microscopy were picked up on copper grids and stained with uranyl acetate followed by lead citrate. Ultrastructure of the cortical root cells was observed in a *Zeiss EM 109* electron microscope.

For morphometric analysis a minimum of 19 micrographs were studied for each treatment. Sections were photographed at regular intervals to avoid overlapping. The morphometric parameters were measured for both control and salt stressed cells according to the methodology described by Steer (1992).

Solute leakage: Solute leakage was determined in intact 15-d-old *Phaseolus vulgaris* seedlings grown in the presence of different concentrations of NaCl for 12 d. The plants were first washed in running deionized water and dried, and then 2 plants from each treatment were transferred to 250 cm^3 of leakage solution, continuously aerated, for 7 h. The leakage solutions contained 10 mM PEG 6000 for control and 27 mM PEG 6000 for salinized plants (corresponding to equivalent osmotic potentials to those of the nutrient solutions), in order to avoid osmotic effects. 0.5 mM $CaCl_2$ was also added to these solutions since the presence of Ca^{2+} is essential for membrane structure and function. After the leakage experiment was finished, 50 cm^3 of each solution were dialyzed against 100 cm^3 of deionized water for 24 h at room temperature and under continues stirring, to remove PEG 6000 which might interfere with subsequent ion determinations. Each treatment had three replicates. The leakage solutions were analyzed for NO_3^-, $H_2PO_4^-$ and SO_4^{2-} by ion chromatography and for K^+, Ca^{2+} and Mg^{2+} by atomic absorption spectro-photometry.

Results

General root anatomy: The roots of *Phaseolus vulgaris* plants treated with 80 mM NaCl were shorter and had less secondary roots than control plants. As well, secondary roots appeared delayed in stressed plants with respect to the control.

The epidermis was formed by 1 or 2 layers of small cells, irrespective of the treatment. In control plants (Fig. 1A) epidermal cells were isodiametric and uniformly placed forming a thin layer, whereas in stressed plants, the shape and disposition of epidermal cells was irregular (Fig. 1B). The cortex occupied approximately 50 % of the measured section diameter and was formed by 5 or 6 layers of large cells (Fig. 1). There were no changes either in the number of cell layers or in the width of the cortical zone. However, the shape and distribution of the cortical cells was clearly affected by NaCl. Control plants had round-shaped cells and these were distributed allowing large and well defined intercellular spaces (Fig. 1A); however, stressed plants showed irregular cells, which were interdigitated, thus decreasing the volume of intercellular spaces (Fig. 1B). Morphometric data (Table 1) quantitatively supported these observations. The ratio of the relative area of intercellular spaces/relative area of cortex was reduced by half under salinity treatment. These thin sections (Fig. 1) also showed a very well defined stele within the endodermis.

Ultrastructure of the cortex: The cortex was formed by large cells, containing a large central vacuole with cytoplasm lining the cell perimeter, which was just a narrow band between the tonoplast and the plasmalemma (Fig. 2). The cytoplasm contained numerous round or enlarged mitochondria, a well developed Golgi apparatus and the

endoplasmic reticulum had a normal appearance. Occasionally some plastids were also observed. The nucleus was of irregular shape and euchromatic in most cells, presenting only one nucleolus per cell. The addition of 80 mM NaCl to the growth medium did not result in significant differences in the number, shape or distribution of cell organelles. Also the width of the cell wall was not modified by NaCl (Table 1).

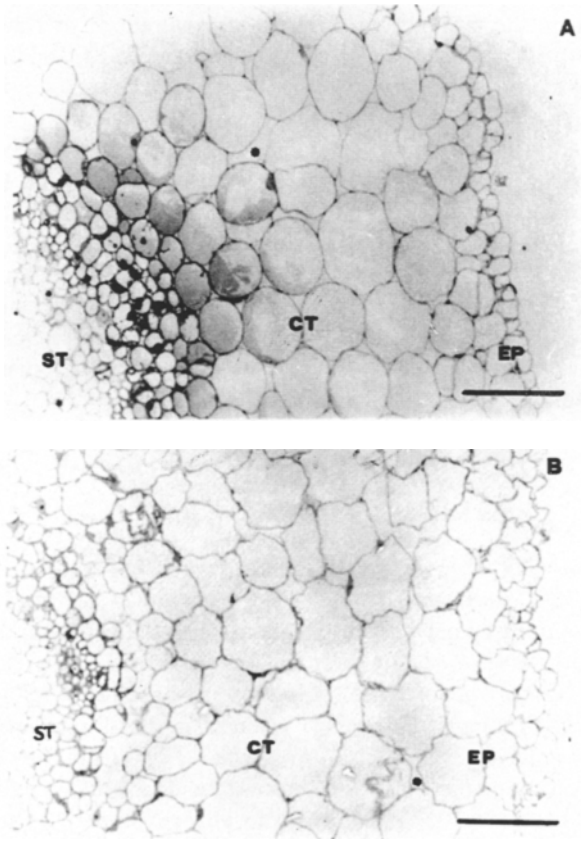

Fig. 1. Ligth microscopy of thin sections of 15-d-old *Phaseolus vulgaris* roots grown under control conditions (*A*) or in the presence of 80 mM NaCl for 12 d (*B*). CT - cortex; EP - epidermal layer; ST - stele; * - intercellular space. Bar corresponds to 25 μm.

Table 1. Effect of NaCl on the morphometric data (mean ± S.E.) for *Phaseolus vulgaris* root cortical cells.

	5 mM NaCl	80 mM NaCl
Plasma membrane surface/cell volume [$\mu m^2\ \mu m^{-3}$]	0.12 ± 0.04	0.29 ± 0.09*
Intercellular space area/cortex area [%]	7.66 ± 2.14	3.88 ± 1.81*
Cell wall width [μm]	0.32 ± 0.08	0.32 ± 0.07

Membrane vesiculation was often observed in cells of stressed plants (Fig. 3B, C and D). The morphology of the vesicles varied as a function of the time exposed to NaCl, and changed from small and empty vesicles (Fig. 3B) to large vesicles

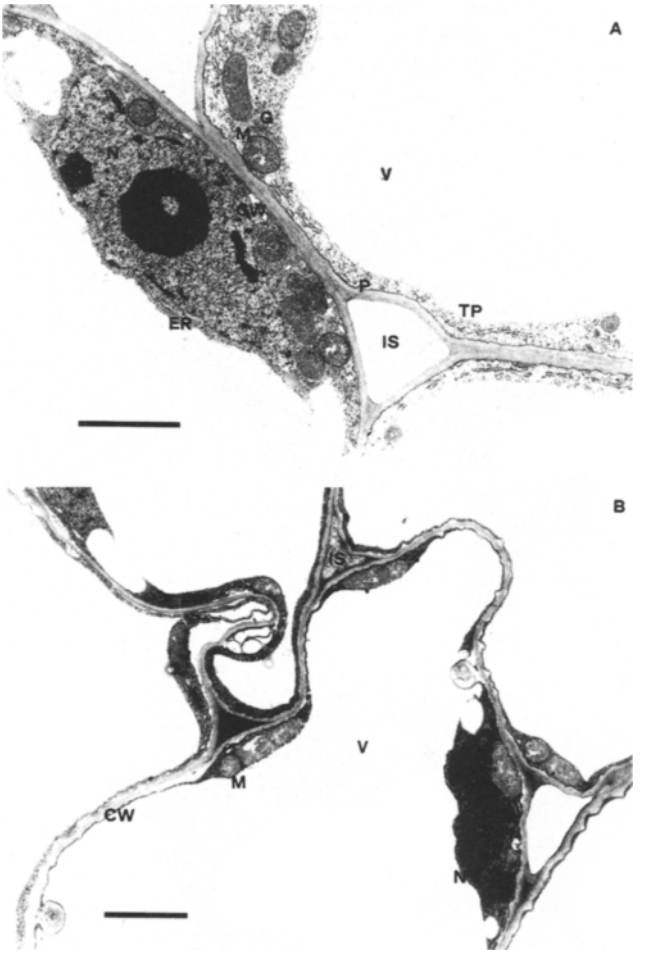

Fig. 2. Electron microscopy of ultrathin sections of 15-d-old *Phaseolus vulgaris* roots grown under control conditions (*A*) or in the presence of 80 mM NaCl for 12 d (*B*). CW - cell wall; ER - endoplasmic reticulum; G - Golgi; IS - intercellular space; M - mitochondrion; N - nucleus; P - plasmalemma; TP - tonoplast; V - vacuole. Bar corresponds to 2 μm.

containing smaller vesicles inside, which appeared at different growth stages of the roots (Fig. 3C and D). The frequency of appearance of these vesicles was also changing, showing a relationship between the number of vesicles and the duration of the saline treatment. It seems clear that, specially in younger plants, these vesicles originate from the plasmalemma. In control plants, these vesicles were not observed in cortical cells at any time up to 15 d (Fig. 3A).

The cells of plants grown with 80 mM NaCl showed an alteration in the shape and disposition of the plasma membrane. The length of the cell plasma membrane in relation to the cell surface was higher in salinized than in control plants (Table 1). This is related to the irregular shape and the ripples of the cell surface observed in Fig. 4B. In stressed plants, the plasmalemma was clearly separated from the cell wall at different positions (Fig. 4B), which was not found in control plants (Fig. 4A).

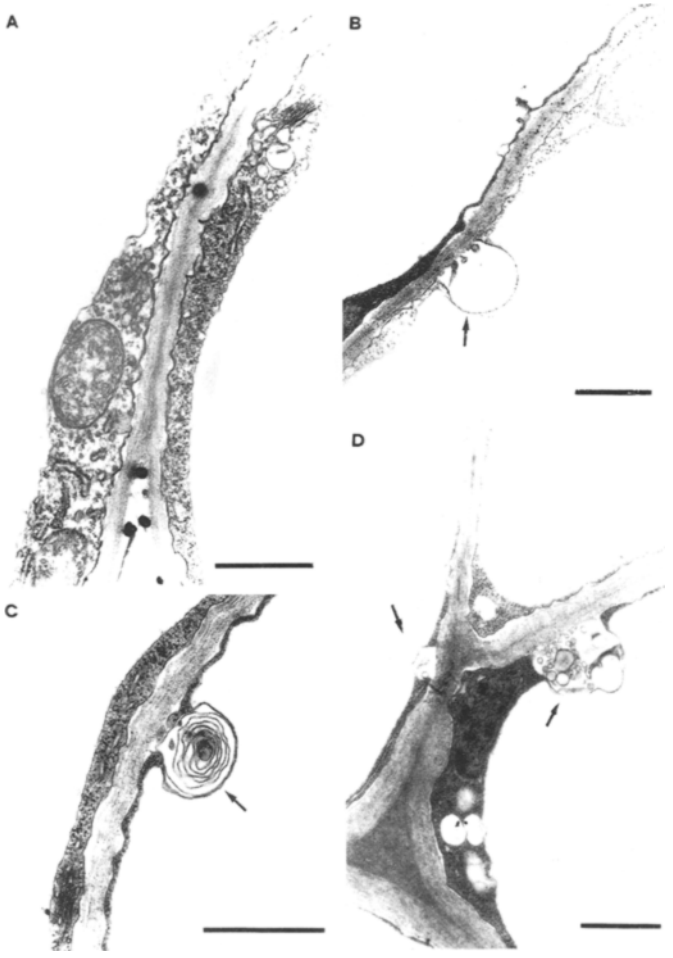

Fig. 3. Electron microscopy of ultrathin sections of 15-day-old *Phaseolus vulgaris* roots grown under control conditions (*A*) or in the presence of 80 mM NaCl for 1 d (*B*) or 12 d (*C* and *D*). Arrows indicate different forms of vesiculation observed. Bar corresponds to 1 μm.

In general, it was also observed that the intercellular spaces of the cortex of salinized plants (Fig. 4D) were smaller than in control plants (Fig. 4C), confirming light microscopy results. This might be a consequence of the general alteration of the cell shape and it gives rise to a considerable decrease in the intercellular volume.

Solute leakage: The addition of 80 mM NaCl to the growth medium considerably increased the leakage of solutes from the roots of intact plants back to the solution.

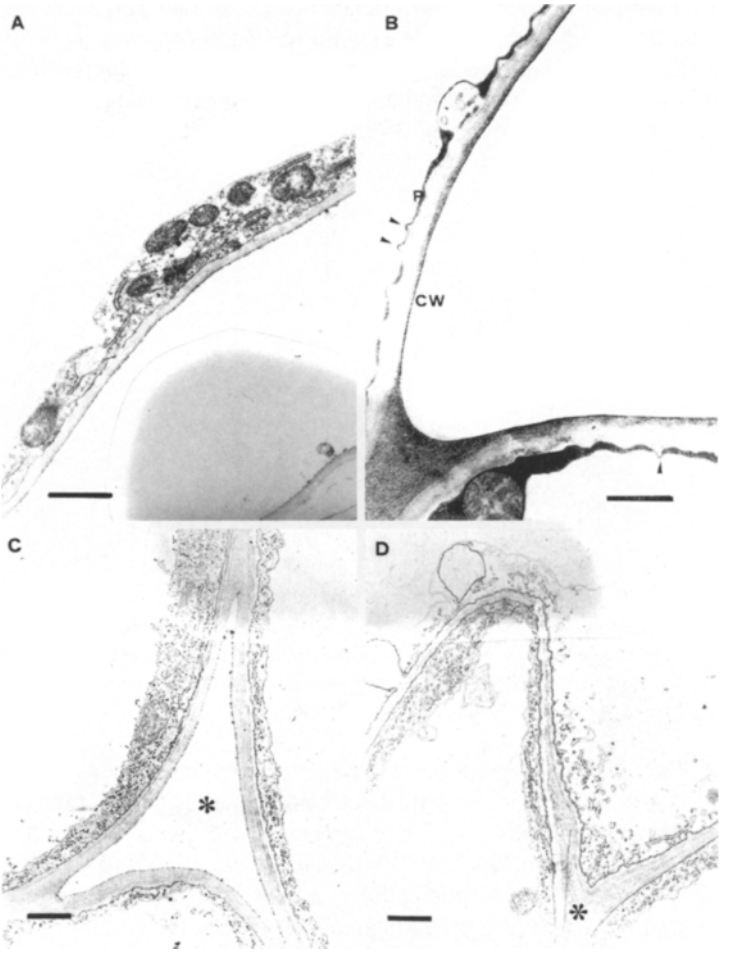

Fig. 4. Electron microscopy of ultrathin sections of 15-d-old *Phaseolus vulgaris* roots grown under control conditions (*A* and *C*) or in the presence of 80 mM NaCl for 12 d (*B* and *D*). CW - cell wall; P - plasmalemma; * - intercellular spaces. Arrowheads indicate the places were ripples of the cell surface are formed. Bar corresponds to 1 μm.

The effect was greatest for K^+, for which there was an increase from 0.228 to 0.500 nmol g^{-1}(root f.m.) s^{-1} and also important for Ca^{2+}, which increased from 0.297 to 0.461 nmol g^{-1}(root f.m.) s^{-1}, as a consequence of the saline treatment (Fig. 5A). The leakage of other ions such us $H_2PO_4^-$, SO_4^{2-} and Mg^{2+} was also slightly affected by NaCl. Leakage of solutes from roots of higher plants can be used as an indication of the integrity of the cell membranes. In fact, we observe a correlation between solute leakage and the alteration of the microscopical morphology of the plasma membrane of cortical cells of stressed plants (Fig. 4B).

Discussion

Among the responses of glycophytes to excess NaCl in the growth medium, one of the most rapid is the inhibition of root growth. In *Phaseolus vulgaris* we have found both a reduction in growth rate as well as a delay in the production of secondary roots. This is in agreement with results reported for other species (Udovenko *et al.* 1970, Kurth *et al.* 1986, Cramer *et al.* 1986, Cachorro *et al.* 1993a).

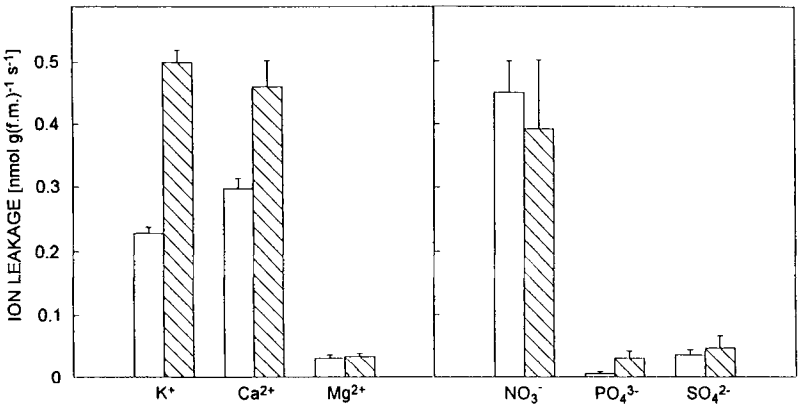

Fig 5. Ion leakage from roots of 15-d-old *Phaseolus vulgaris* intact plants back to the solution. Plants were grown for 12 d in nutrient solution in the presence of the different concentrations of NaCl (5 mM NaCl - *empty columns*; 80 mM NaCl - *hatched columns*). Mean of three different experiments. Bars indicate the standard errors.

The inhibition of root growth that we have observed in bean plants is not related to changes in the rate of division of cortical cells since there is no variation in either the number or the size of the cells (Fig. 1), in agreement with previous results (Meiri and Poljakoff-Mayber 1967, Kurth *et al.* 1986). However, we found alterations in the morphology and size of the intercellular spaces. Control cells (Fig. 1A) are isodiametric and present well defined intercellular spaces (Nir *et al.* 1969), whereas stressed plants (Fig. 1B) showed irregular shapes and reduced intercellular spaces. It has been reported that also the number of cells of bean leaves did not decrease due to the addition of NaCl to the growth medium, but the leaves were smaller and more compact (Meiri and Poljakoff-Mayber 1967).

The decrease of the relative area of intercellular spaces due to the saline treatment (Table 1) might be a consequence of the pernicious effect of salinity on the cell shape or, more probably, may reflect a change in the plant in order to avoid toxic ions, like Na^+, passing through the cortex to the stele. It has been shown that the NaCl-induced growth reduction is not due only to the accumulation of ions in metabolic cell compartments but also in the apoplast (Speer and Kaiser 1991). Our results could indicate that, in beans, the passage of ions is mechanically avoided by a decrease in the size of the cortical intercellular space.

We did not find important changes in the number and distribution of some cell organelles (Fig. 2) which is in agreement with other authors with respect to the nucleus and cytoplasm (Huang and Steveninck 1990) or the endoplasmic reticulum (Werker *et al.* 1983). This lack of effect is probably due to the short duration of the saline treatment, to the relatively low NaCl concentration or to both. In this respect it has been reported (Poljakoff-Mayber 1975) that in maize a minimum concentration of NaCl of 240 mM is necessary in order to observe ultrastructural changes. Lower levels, although affecting growth, did not produce any effect on the ultrastructure.

The appearance of vesiculation in cells exposed to 80 mM NaCl (Fig. 3B,C,D) is in agreement with results reported for other species (Nassery and Jones 1976). Appearance of cell organelles having transport functions has been shown by means of ultrastructural studies (Yeo *et al.* 1977), suggesting for them a merely circumstantial function. This could be the case for the vesicles described in this work. These vesicles might act as a place of storage of toxic ions, thus avoiding their injurious action in the cytoplasm. In fact it has been suggested for other plants that their capacity to stand NaCl salinity is in relation to the degree of compartmentation of toxic ions within the cell (Nassery and Jones 1976). On the other hand, it is well known that bean plants reduce the transport of Na^+ to the shoots by increasing its accumulation in the roots (Lessani and Marschner 1978, Bhivare and Nimbalkar 1984, Cachorro *et al.* 1993b). It is therefore possible that the vesiculation of the root cells observed in our experiment (Fig. 2B,C,D) has the function of accumulating toxic ions, particularly Na^+, thus decreasing its transport to the shoot.

We have found the plasmalemma to be separated from the cell wall at different points of the cell periphery (Fig. 4B) (Nir *et al.* 1969), and to increase in length as a consequence of NaCl salinity (Table 1). Both the increase of the length of the plasma membrane and the appearance of membrane vesicles are in accordance with the increase in the total phospholipid content reported for bean plants grown under saline conditions (Cachorro *et al.* 1993a). Deterioration of the cell plasma membrane has been reported as a consequence of changes in the physico-chemical characteristics of the plant growth medium or to cell aging (Yao *et al.* 1991).

The alterations of the plasma membrane reported above, namely increase of surface, vesiculation and separation from the cell wall, are clearly related to the increase in solute leakage due to NaCl treatment (Fig. 5). In fact, the massive leakage of ions back to the solution indicates a loss of membrane integrity, and we have described some of the possible structural changes causing this alteration. It is interesting to mention that Ca^{2+}, which is considered to be implicated in membrane stabilization (Van Steveninck 1965, Hepler and Wayne 1985, Poovaiah and Reddy 1987, Rengel 1992), is able to diminish the leakage of solutes to the medium caused by excess NaCl (Cachorro *et al.* 1994).

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