Effect of Sudden Salt Stress on Ion Fluxes in Intact Wheat Suspension Cells

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INTRODUCTION

Although salinity is one of the major problems limiting agricultural production around the world, the underlying mechanisms of salt stress perception are far from being well understood. Salt tolerance in non-halophytes is likely to be conferred by a large number of adaptive mechanisms. Among the most important of these is the ability of plants to maintain a low Na+/K+ ratio in the cytoplasm as well as to keep cytosolic Na+ content below some crucial value (Greenway and Munns, 1980; Maathuis and Amtmann, 1999; Tyerman and Skerrett, 1999).

To maintain cytosolic Na+ homeostasis, excess ions must be removed from the cytosol either to the vacuole (intracellular compartmentalization), or be exported from the cell (Tyerman and Skerrett, 1999). The higher the external Na+ concentration, the more pressure is on the cell ion transporters to maintain cytosolic Na+ content at the appropriate level. When the ability of the vacuole to compartmentalize excessive Na+ is limited, regulation of plasma membrane Na+ transport becomes crucial. Two major strategies are possible: reduction of Na+ uptake, and enhancement of Na+ efflux. Obviously these two mechanisms of protection can work simultaneously.

At high external Na+ concentrations, sodium uptake across the plasma membrane of plant cells is passive. The possible pathways for Na+ transport into plants were recently reviewed in detail by Tyerman and Skerrett (1999), Amtmann and Sanders (1999) and Maathuis and Amtmann (1999). Non-selective voltage-independent cation channels seem to play a substantial role in Na+ uptake by plants under salt stress conditions, and the study of the regulation of these channels is emphasized as a priority direction for studies on plant salt tolerance. Among other priority directions, a study of salinity effects on the membrane potential has been proposed by Amtmann and Sanders (1999). High external NaCl concentrations are known to cause depolarization of $E_m$ in various plant cells (Cakirlar and Bowling, 1981; Kalashnikova et al., 1987; Katsuhara and Tazawa, 1990; Kourie and Findlay, 1990; Yao and Bisson, 1993). This depolarization reduces the $E_m$-provided driving force for Na+ into the cell and so affects Na+ uptake (Amtmann and Sanders, 1999).

In contrast to Na+ uptake, sodium efflux through the plasma membrane occurs thermodynamically uphill. There is no molecular genetic information available for any cation efflux system in plants (Serrano et al., 1999). Some evidence exists that this system is dependent on cell metabolism, as application of metabolic inhibitors resulted in an increase in intracellular Na+, although the nature of active extrusion is still unknown (Prins, 1995, and references therein). Some authors have suggested that a plasma membrane Na+/H+ antiporter is a likely candidate for this role (Hassidim et al., 1990; Rausch et al., 1996; Apse et al., 1999). However, Staal et al. (1991) found no evidence for this antiport at the plasma membrane of Plantago maritima. Another mechanism for the Na+ efflux system is a plasma membrane Na+-ATPase, as was found for algae (Wada et al., 1989; Pick,
Clearly, more work is needed on Na$^+$ transport studies in plants. The transport must be ‘measured in physiological conditions’, and the ‘interactive behaviour of relevant permeant ions has to be studied’ (Amtmann and Sanders, 1999).

Among other non-invasive approaches that allow plants to be studied in physiological conditions, the MIFE technique (Shabala et al., 1997) provides an unique opportunity to measure simultaneously Na$^+$, K$^+$ and H$^+$ fluxes near plant tissue under salt stress conditions using intact plant cells. Net ion fluxes (the resultant sum of influx and efflux for the ions studied) could be measured with high temporal and spatial resolution, providing useful information on ion flux kinetics and interactions. A limitation of this technique, namely the inability to regulate plasma membrane potential, can be compensated by using different external solutions and compounds that can modify the membrane potential. A similar approach has been used by Reid and colleagues in studies on Ca$^{2+}$ uptake into Chara, where plant cells were depolarized by KCl solutions (Reid et al., 1997).

The aim of this study was to investigate the interdependence of Na$^+$ and K$^+$ fluxes into the salt-stressed intact cells of wheat suspension culture at different external NaCl concentrations and under modified $E_m$. The latter was achieved by cell pre-treatment with fusicoccin (FC), a known activator of plasma membrane H$^+$-ATPases (Marré, 1979). It is known that FC usually causes significant membrane hyperpolarization (Marré, 1979) which could therefore modify Na$^+$ and K$^+$ fluxes into the cell. Kinetics of intracellular Na$^+$ accumulation were also monitored; this allowed us to interpret measured net fluxes in terms of ion homeostasis in a saline environment.

MATERIALS AND METHODS

Plant material

The cell suspension was obtained from callus derived from immature embryos of *Triticum aestivum* ‘Macheta’ and was maintained in Murashige and Skoog (MS) medium (Sigma) containing 2 mg l$^{-1}$ 2,4-D, pH 5.5. The cells were cultivated with constant shaking, in darkness at 23°C. Subculturing was conducted at 14 d intervals. Cells in the exponential phase were used in experiments.

Na$^+$ and K$^+$ intracellular measurements

The cell suspension was filtered through a nylon cloth, and the required cell density (2 x 10$^4$ cells ml$^{-1}$) was established by adding fresh nutrient medium. During the experiment, the suspension was maintained in a water bath shaker at 22°C. After shaking for 3 h, NaCl solution was added to the flasks. At 3 min intervals of further shaking, aliquots of the suspension were sampled, placed on ash-free filters, and the medium components washed off with iso-osmotic mannitol plus 2 mM Ca(NO$_3$)$_2$ solution (Baburina and Leonova, 1994). Cell density was counted in a Fuchs–Rosenthal haemocytometer. Na$^+$ and K$^+$ concentrations were measured using a flame photometer (Karl Zeiss, Germany) after incinerating the cell-containing filters in a muffle furnace at 450°C and dissolving the ash in double-distilled water. Ion content was calculated per 10$^6$ cells. FC (Sigma) was added to the medium as a fresh aqueous solution 20 min before salt addition.

Ion flux measurements

Ion fluxes were measured non-invasively using the MIFE® system (University of Tasmania, Hobart, Australia) generally as described by Shabala et al. (1997). Both ion-selective and $E_m$ microelectrodes were fabricated essentially as described previously (Baburina et al., 1998). The ion-selective electrodes were backfilled by 0.15 mM NaCl + 0.4 mM KH$_2$PO$_4$ (adjusted to pH 6.0 using NaOH) for the hydrogen electrode, 0.5 M NaCl for sodium, and 0.5 M KCl for potassium. The electrode tips were filled with commercial ionophore cocktails (hydrogen 95297; sodium 71178; potassium 60031; Fluka). Net fluxes of these three ions were measured simultaneously. An exception is that H$^+$ fluxes could not be measured in MS medium due to its high buffering (Arif et al., 1995).

Experimental procedure

In FC and salt stress experiments, concentrated solutions of 100 µM FC or 1.2 M NaCl were added directly into the MIFE measurement chamber. Solutions were thoroughly mixed by sucking and expelling several times with a pipette. Addition, mixing, and establishing the diffusion gradients required approx. 2 min; this time was omitted from the analysis and appears as a gap in the figures. The media used were: MS (Murashige and Skoog, pH 5.5: 20 mM NH$_4$$_2$, 3 mM Ca$^{2+}$, 6 mM Cl$^-$, 0.1 mM EDTA, 2 mM Fe$^{2+}$, 20 mM K$^+$, 40 mM NO$_3$-, 0.2 mM Na$^+$, 1.25 mM H$_2$PO$_4$-, 3 mM SO$_4^{2-}$, 0.1 mM BO$_4^{2-}$, 0.1 µM Co$^{2+}$, 0.1 µM Cu$^{2+}$, 0.7 mM Mg$^{2+}$, 132 µM Mn$^{2+}$, 1 µM MoO$_3^{2-}$, 30 µM Zn$^{2+}$), BSM (1 mM KCl + 0.1 mM CaCl$_2$, pH 5.8) and KSM (10 mM KCl + 0.1 mM CaCl$_2$, pH 5.6).

To immobilize wheat suspension cells during ion flux measurements, cells were placed onto a thin layer of liquid 1% agar containing the corresponding medium (t = 28 ± 1°C). Once the agar had solidified, the chamber was flushed three times with fresh medium and left to settle for 1 h. Flux and membrane potential measurements were carried out at 23–25°C with normal room and microscope illumination.

Electrodes for membrane potential measurements had tip diameters <1 µm and were backfilled with 0.5 M KCl. In membrane potential studies, experimental conditions were close to those for the flux measurements. Membrane potentials were measured using standard techniques, with the MIFE electrocrometer. This measures the sum of plasma membrane and tonoplast potentials. Since it was very difficult to measure changes in membrane potential during the course of NaCl application under these conditions (bubbling and mixing), each point for the response time course was measured at least five times with different cells for a particular time after salt application. The membrane
potential value was calculated as the difference between the values in the solution initially and after impalement.

**RESULTS**

Membrane potential

There was a striking difference in $E_m$ values between wheat suspension cells incubated in enriched MS medium and media containing only KCl and CaCl$_2$, KSM and BSM (about $-30$, $-70$, $-80$ mV, respectively, Table 1). It should be noted that the cells were routinely grown in MS medium. In spite of such apparently low electrical gradients at the plasmalemma in the MS medium, cultivated cells were metabolically active, possessed normal division cycles and looked healthy. These small $E_m$ values of the cells grown in the MS medium were far less negative than found in cultivated plant cells, where measurements are made against bathing solutions with relatively low ion composition and containing Ca$^{2+}$ (Parsons and Sanders, 1989; Popham et al., 1995; Zimmerman et al., 1998). It has been shown that increased concentration of KCl in the bathing medium leads to depolarization of oat coleoptile cells (Higinbotham et al., 1964; Parsons and Sanders, 1989). As the MS medium contains high concentrations of K$^+$ (20 mM) and also includes EDTA (which binds Ca$^{2+}$), the low $E_m$ values measured are in good agreement with previous observations. Recovery of $E_m$ from $-30$ to almost $-70$ and $-80$ mV as result of cell transfer into KSM and BSM (lower K$^+$, no EDTA) is also consistent with these arguments. Assuming a K$^+$ concentration in the cytoplasm of between 60 and 80 mM (Walker et al., 1996), $E_K$ for MS media could range from $-28$ to $-38$ mV, for KSM from $-45$ to $-53$ mV, and for BSM from $-103$ to $-111$ mV. Therefore, $E_m$ was more positive than $E_K$ for cells exposed to BSM, less positive for KSM, and close to equilibrium in MS media. It appears unlikely that we were dealing with electrode leakage which Parsons and Sanders (1989) suggested might be a possible explanation for low $E_m$ values for suspension cells, although we cannot completely exclude this methodological error.

Addition of 120 mM NaCl led to $E_m$ depolarization in all media. This effect was most pronounced in KSM with $\Delta E_m$ about 50 mV; in BSM $\Delta E_m$ was about 43 mV, whereas in MS medium this value was about 10 mV.

Surprisingly, addition of 1–10 $\mu$m FC did not cause significant changes to $E_m$ of suspension culture cells in any medium, although changes were reported for other cell types in vivo (Marré, 1979; Felle, 1998; Amtmann et al., 1999). However, FC pre-treated cells did not demonstrate significant depolarization after NaCl addition in MS medium, and showed much smaller $\Delta E_m$ in all media studied (Table 1). Therefore, we concluded that the FC concentration range chosen in this study was suitable, and the lack of $E_m$ hyperpolarization by FC in non-stressed cells is likely to have a biological origin.

The observed $E_m$ depolarization in response to NaCl treatment in wheat suspension culture cells measured in our experiments (Table 1) is consistent with earlier studies on sunflower roots, Chlorella, Chara and Nitella (Cakirlar and Bowling, 1981; Kalashnikova et al., 1987; Katsuura and Tazawa, 1990; Kourie and Findlay, 1990; Yao and Bisson, 1993). Our results show that the intensity of the depolarization depends on initial $E_m$ values; cells with lower initial $E_m$ exhibited smaller changes in $E_m$ in the same salt stress conditions (Table 1).

Intracellular sodium kinetics

As expected, intracellular Na$^+$ concentrations of cells grown in the standard MS medium increased after they were treated with NaCl for 1 h (Fig. 1A). After rising from 3 to 6 $\mu$mol Na$^+$ per 10$^6$ cells in the low concentration range (below 60 mM NaCl), the dose-response curve plateaued to 130 mM external NaCl. Further NaCl increase up to 350 mM led to a proportional rise in internal Na$^+$.

Kinetic studies revealed that the process of Na$^+$ accumulation by the cells is multiphasic. After 120 mM NaCl was applied, a quick rise in internal Na$^+$ up to 8–3 $\mu$mol Na$^+$ per 10$^6$ cells occurred in the first 12 min, followed by a gradual decrease and plateauing at 6 $\mu$mol Na$^+$ per 10$^6$ cells (Fig. 1B). This level remained nearly constant during 60 min and was about four-times higher than in cells before NaCl addition. Similar multiphase changes in Na$^+$ internal content were observed in earlier studies on lucerne suspension cells (Babourina and Leonova, 1994).

FC pre-treatment (Fig. 1C) significantly changed the time course of Na$^+$ accumulation within the cell. After a transient peak at 8 $\mu$mol per 10$^6$ cells at the third min and a slight decrease for the following 5–6 min, the internal Na$^+$ concentration continued to rise until it stabilized at a level twice that of FC-untreated cells (Fig. 2A).

**Intracellular K$^+$ changes**

Internal K$^+$ content gradually decreased after medium salinization, although a statistically significant difference was not observed until 20 min (Fig. 2A).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>MS</th>
<th>KSM</th>
<th>BSM</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>$-31.1 \pm 2.0(10)$</td>
<td>$-69 \pm 1.8(6)$</td>
<td>$-78.0 \pm 2.6(9)$</td>
</tr>
<tr>
<td>20 min after addition of 120 mM NaCl to a medium</td>
<td>$-20.5 \pm 1.4(6)$</td>
<td>$-20.7 \pm 1.4(4)$</td>
<td>$-34.2 \pm 1.1(9)$</td>
</tr>
<tr>
<td>20 min after addition of 1 $\mu$m FC to a medium</td>
<td>$-30.7 \pm 2.3(4)$</td>
<td>$-68.1 \pm 0.9(4)$</td>
<td>$-82.10 \pm 2.7(8)$</td>
</tr>
<tr>
<td>20 min after addition of 120 mM NaCl to FC pre-treated cells</td>
<td>$-31.0 \pm 2.2(5)$</td>
<td>$-39.2 \pm 1.9(5)$</td>
<td>$-66.8 \pm 2.6(7)$</td>
</tr>
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</table>

Numbers in parentheses indicate $n$.
FC pre-treatment in MS medium led to a 50% higher internal K\(^+\) content (2.7 ± 0.3 µmol per 10\(^{6}\) cells compared with 1.8 ± 0.2 µmol per 10\(^{6}\) cells for untreated cells). Salt-stress caused a dramatic reduction in internal K\(^+\) concentration within the first 3–5 min. Subsequently, K\(^+\) content in FC-pre-treated NaCl-stressed cells (Fig. 2B) stabilized at 0.5 µmol per 10\(^{6}\) cells above the control (untreated, unstressed cells).

Addition of 120 mM NaCl to the MS medium led to similar changes in Na\(^+\)/K\(^+\) ratio for both FC treated and untreated cells, with a sharp increase in the first 3 min, followed by a slow rise to 7 and return to near 6 at the end of the first hour (Fig. 2C). FC pre-treatment for 20 min led to a higher content of both Na\(^+\) and K\(^+\) in suspension cells.

**Sodium fluxes outside the cell**

Time courses of representative experiments for 120 mM NaCl salt stress at different treatments where net ion fluxes were measured by the non-invasive MIFE system are presented in Figs 3–5; statistical data for experiments are summarized in Table 2.

Measurements of net Na\(^+\) fluxes revealed a massive Na\(^+\) influx to plant cells in the first minutes after 120 mM NaCl addition (Fig. 3). In full agreement with intracellular data, in the MS medium this net Na\(^+\) flux gradually reduced to zero during 20 min, at which time Na\(^+\) influx into the cell is balanced by Na\(^+\) efflux into the medium. After 20 min, Na\(^+\) fluxes shifted to negative values of about −2000 nmol m\(^{-2}\) s\(^{-1}\) (Fig. 3A, Table 2).

Both pre-treatments that lead to increased initial E\(_{m}\) (cell incubation in KSM and BSM and FC pre-treatment) caused a significant shift in Na\(^+\) fluxes towards positive values 30–60 min after 120 mM NaCl addition. As a result, in BSM and after FC pre-treatment in MS, the Na\(^+\) flux stabilized at a positive level (net influx) of 3200–3400 nmol m\(^{-2}\) s\(^{-1}\) (Fig. 3A, C and Table 2). In KSM, Na\(^+\) flux 20 min after 120 mM NaCl addition was still positive (about 3900 nmol m\(^{-2}\) s\(^{-1}\)), however, after 30 min its values became negative, although not so high as for measurements in MS medium (Fig. 3A, B).
NaCl addition followed by gradual decrease in net Na media studied (enormous increase in the first minutes after influx), some difference was observed. The return towards

**Fig. 3.** Net Na influx (influx positive) measured near suspension cell surface before and after addition of 120 mM NaCl for different bathing solutions: in MS medium and in MS medium with 20 min pre-treatment with 1 μM FC (A); in KSM and in KSM with 20 min pre-treatment with 1 μM FC (B); in BSM and in BSM with 20 min pre-treatment with 1 μM FC (C); in MS medium with 20 mM NaCl added (D). Results shown are from representative experiments for A–C (each point indicates mean of 12 data points measured during 1 min ± s.e.), and averages for six independent plants ± s.e. for D. Arrows and numbers indicate final concentration of NaCl in the chamber.

Although the behaviour of Na fluxes was similar in all media studied (enormous increase in the first minutes after NaCl addition followed by gradual decrease in net Na influx), some difference was observed. The return towards steady flux was faster with FC than without FC in all media studied (Fig. 3A–C). This observation was in agreement with changes in intracellular Na concentration, where the first peak in Na accumulation was observed earlier, in the first 3 min (Fig. 1C).

Less severe treatment (20 mM NaCl) applied to cells in the MS medium also resulted in gradual Na flux transition from net influx to efflux. The initial large Na influx quickly decreased to zero within 10 min (Fig. 3D). With a negative peak of about −1300 nmol m⁻² s⁻¹ at 18 min, it slowly returned to zero via overshoot to net influx, followed by return to balanced Na net flux around zero until the end of measurements at 60 min. Qualitatively, the multiphase Na flux kinetics were very similar to those measured for intracellular Na changes (Fig. 1B).

**Potassium fluxes**

Net K⁺ fluxes measured outside the cell (Fig. 4) were in good agreement with intracellular K⁺ data. Immediately after NaCl was applied, a significant net K⁺ efflux was observed (Fig. 4). These data probably indicate that under sudden salt stress conditions Na⁺ was the preferred ion for uptake via inward cation channels. Thus, during the first 10 min, we observed the efflux part of net K⁺ exchange for normal conditions. It takes 5–10 min for cells to adjust K⁺ transport to the initial flux values, probably by decreased activity of the K⁺ efflux system.

Initial K⁺ flux values were strongly dependent on the medium composition, with a significant net influx in MS around 1225 nmol m⁻² s⁻¹, less positive for KSM (59 nmol m⁻² s⁻¹), and about zero in BSM (Table 2).

Generally, the K⁺ flux response to NaCl in all media studied was similar: in 10 min the flux recovered from a large efflux to initial values (Fig. 4A–C). In MS medium, when the salt stress was milder (20 mM), the shift to K⁺ efflux in the first minutes was less apparent (Fig. 4D).

**Proton fluxes**

Because of the high buffer capacity, H⁺ flux measurements in the MS medium were not possible. When measured in KSM and BSM, the H⁺ flux response to 120 mM NaCl treatment was similar to that for K⁺ fluxes. Immediately after NaCl treatment, a large net H⁺ efflux was observed which gradually decreased within the next 15–20 min. FC pre-treatments did not alter H⁺ flux behaviour when cells were exposed to the salt stress (Fig. 5A, B; Table 2).

**DISCUSSION**

**Na⁺ efflux system**

Our results clearly demonstrated a functioning Na⁺ efflux system under short-term salt stress conditions. Generally, the decreased Na⁺ influx, which was observed in all media, could occur for two reasons: decreased uptake or activated efflux system. One of the two components in the
The electrochemical force driving Na\textsuperscript{+} into the cell is electrical. This component is dependent on $E_m$ of the plant cell, and decreased with decreased $E_m$. Therefore, even if channels that provide the major pathway for Na\textsuperscript{+} are voltage independent (VICs), $E_m$ depolarization of wheat cells in response to NaCl treatment (Table 1) would contribute to gradually reduced Na\textsuperscript{+} influx (Fig. 3A–D). However, the observed Na\textsuperscript{+} efflux in MS medium at both external concentrations of NaCl could be performed only by an Na\textsuperscript{+}-extrusion system.

There is no doubt that the system does exist, because in many dose-response studies investigators observed curves similar to ours (Fig. 1A), where plants could maintain steady internal Na\textsuperscript{+} with increased external NaCl concentration. It cannot be achieved by vacuolar Na\textsuperscript{+} accumulation, as Na\textsuperscript{+} content was measured for the whole cells, i.e. regulation of internal Na\textsuperscript{+} content in that case has to be due to regulation of Na\textsuperscript{+} transport at the plasma membrane level. In our studies, assuming a cell diameter 60 μm and a total Na\textsuperscript{+} content within the cell 30 min after NaCl treatment of 6 nmol per 10⁶ cells (Fig. 2B), the average Na\textsuperscript{+} concentration within the cell is calculated to be 53 mM. This is much lower than the 120 mM Na\textsuperscript{+} outside the cell. Therefore, a steep electrochemical gradient for Na\textsuperscript{+} under salt stress conditions favours Na\textsuperscript{+} influx into the cytosol. Hence, to maintain intracellular Na\textsuperscript{+} concentration at a certain level, cells must have an excreting transport system for Na\textsuperscript{+}, which in these conditions will be thermodynamically uphill. The identity of such an active Na\textsuperscript{+}-extrusion mechanism remains to be revealed.

In our studies we have identified some conditions under which the functioning Na\textsuperscript{+}-eflux system can be observed (MS media and relatively mild salt stress) and some time characteristics of the functioning of this system. At 20 mM NaCl, the time for Na\textsuperscript{+} flux recovery to zero values was 10 min shorter than at 120 mM NaCl (Fig. 3A, E), i.e. cells could overcome the milder salt stress faster.

As mentioned previously, an Na\textsuperscript{+}/H\textsuperscript{+} antiporter was proposed as a possible mechanism for an Na\textsuperscript{+} efflux system (Hassidim et al., 1990; Rausch et al., 1996); our data do not rule this out. We did observe a high negative correlation...
Table 2. Effect of 120 mM NaCl stress on ion fluxes of wheat cells

<table>
<thead>
<tr>
<th>Conditions</th>
<th>MS*</th>
<th>KSM</th>
<th>BSM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Na⁺ (nmol m⁻² s⁻¹)</td>
<td>K⁺ (nmol m⁻² s⁻¹)</td>
<td>Na⁺ (nmol m⁻² s⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>505 ± 49(11)</td>
<td>1255 ± 164(11)</td>
<td>45 ± 28(6)</td>
</tr>
<tr>
<td>20 min after addition of 120 mM NaCl to a medium</td>
<td>−1837 ± 1361(6)</td>
<td>305 ± 251(6)</td>
<td>3907 ± 571(3)</td>
</tr>
<tr>
<td>20 min after addition of 1 μM FC to a medium</td>
<td>384 ± 27(7)</td>
<td>1163 ± 112(7)</td>
<td>84 ± 48(3)</td>
</tr>
<tr>
<td>20 min after addition of 120 mM NaCl to FC pre-treated cells</td>
<td>3410 ± 426(7)</td>
<td>1062 ± 133(7)</td>
<td>2598 ± 827(3)</td>
</tr>
</tbody>
</table>

* Due to high buffer capacity of MS medium H⁺ fluxes could not be measured (Ariët et al., 1995). Numbers in parentheses indicate n.
between proton and sodium fluxes in the first 10 min after NaCl addition, when we observed a decreased influx (or increased efflux). However, this correlation, with stoichiometry very far from 1:1, need not be primary, but a consequence of other events, such as wall Donnan system exchange or changes in K⁺ and Cl⁻ transport.

Role of FC in plant salt tolerance

There are some indications that FC can change the plant response to salt or osmotic stress. FC alleviated inhibition of seed germination of *Allennorfeae occidentalis* by severe salt stress (Gul and Weber, 1998). It has been shown that rice biomass production in a saline environment increased after seed pre-treatment with FC (Braun and Khan, 1976; Gunavardana et al., 1990). The ‘silent’ presence of FC-binding sites that are activated under osmotic stress allowed Trofimova et al. (1999) to suggest that these sites could be involved in plant cell osmoregulation.

The salt stress-alleviating effect of FC is not obvious because of the dual nature of FC-controlled plasmalemma Na⁺ transporters. On the one hand, FC is known to cause plasma membrane hyperpolarization (Cleland et al., 1977; Marré, 1979; Bates and Goldsmith, 1983; Felle, 1998; Amtmann et al., 1999); in our studies it appeared as less $E_m$ depolarization. This should result in an increased driving force for Na⁺ and K⁺ influx via channels. On the other hand, it has been shown that FC increases H⁺ extrusion (Arif and Newman, 1993) and acidifies the external medium (Felle, 1998; Amtmann et al., 1999), which should increase the proton driving force for a putative Na⁺/H⁺ efflux system, and consequently enhance Na⁺ efflux. It is very likely that both these mechanisms might operate at the same time. There is also a possibility that FC might directly affect Na⁺ transport mechanisms.

In addition to its effect on Na⁺ during salt stress, FC could affect K⁺ transport. It is well known that salt stress results in loss of K⁺ by plants (Greenway and Munns, 1980). A plausible hypothesis is that FC prevents K⁺ loss from the cell that is normally observed under salt stress conditions. It has been repeatedly demonstrated that FC enhances K⁺ uptake into plant tissues (Marré et al., 1974). It is quite possible that enhanced K⁺ uptake can be also explained by the $E_m$ hyperpolarizing effect of FC that was found for plant cells, although increased proton motive force for H⁺/K⁺ transporters could also be considered. Thus the possibility that FC maintains the potassium balance in plant cells seems quite appropriate. From our results this FC effect on K⁺ uptake resulted in maintenance of the Na⁺/K⁺ ratio during 120 mm NaCl stress, despite a higher uptake of Na⁺ into the cells (Fig. 2C).

Another possible explanation for the alleviating effects of FC under high salinity is related to general metabolic changes that allow plants to cope better with highly saline conditions. It should be noted that although FC effects have been intensively studied in recent decades, the exact chain of events triggered by this fungal toxin is still obscure. Prolonged FC application leads to distortion in morphology and reduction in growth when considered at a whole plant level (Nadjimov et al., 1996). At a general metabolic level it has been found that FC stimulates oxygen consumption in *Egeria densa* leaves (Marré and Alberghini, 1998), and ethylene production in *Acer pseudoplatanus* cultured cells (Malerba et al., 1995). Hence, FC can cause other changes in plant metabolism in addition to direct activation of H⁺-ATPases that, in their turn, can be crucial for plants while adapting to high salinity conditions.

We did not observe enhancement of the Na⁺ efflux system after FC pre-treatment: there was not a bigger Na⁺ efflux. Moreover, in MS medium, Na⁺ flux was shifted towards influx (Fig. 3A; Table 2). However, it seems that FC pre-treatment affected the rate of activation of the Na⁺ efflux system: Na⁺ fluxes reached their minimum about 10 min earlier than without FC pre-treatment (Fig. 3A–C). In addition, the first peak in Na⁺ intracellular accumulation was reached sooner in MS medium with FC pre-treatment (Fig. 1). This FC effect on the Na⁺ kinetics during the first minutes of salt stress seems to be an important parameter to be tested in future experiments.

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