RESEARCH PAPER

Potassium activities in cell compartments of salt-grown barley leaves

Tracey Ann Cuin¹, Anthony J. Miller², Sophie A. Laurie³ and Roger A. Leigh¹,⁴

¹ Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK
² Agriculture and Environment Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
³ Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS18 9AF, UK

Received 6 June 2002; Accepted 3 October 2002

Abstract

Triple-barrelled microelectrodes measuring K⁺ activity (aK⁺), pH and membrane potential were used to make quantitative measurements of vacuolar and cytosolic aK⁺ in epidermal and mesophyll cells of barley plants grown in nutrient solution with 0 or 200 mM added NaCl. Measurements of aK⁺ were assigned to the cytosol or vacuole based on the pH measured. In epidermal cells, the salt treatment decreased aK⁺ in the vacuole from 224 to 47 mM and in the cytosol from 68 to 15 mM. In contrast, the equivalent changes in the mesophyll were from 235 to 150 mM (vacuole) and 79 to 64 mM (cytosol). Thus mechanisms exist to ameliorate the effects of salt on aK⁺ in compartments of mesophyll cells, presumably to minimize any deleterious consequences for photosynthesis. Thermodynamic calculations showed that K⁺ is actively transported into the vacuole of both epidermal and mesophyll cells of salinized and non-salinized plants. Comparison of the values of aK⁺ in K⁺-replete, non-salinized leaf cells with those previously measured in root cells of plants grown under comparable conditions indicates that cytosolic aK⁺ is similar in cells of both organs, but vacuolar aK⁺ in leaf cells is approximately twice that in roots. This suggests differences in the regulation of vacuolar aK⁺, but not cytosolic aK⁺, in leaf and root cells.

Key words: Barley, cytosol, potassium, salinity, vacuole.

Introduction

Soil salinity is estimated to affect between 340 and 950 million hectares of land worldwide and is a serious limitation to crop productivity because the majority of crop plants are not particularly salt tolerant (Flowers and Yeo, 1995). Therefore, much effort is being directed towards identifying the physiological and biochemical processes that are affected by NaCl with the aim of modifying these to increase salt tolerance (Apse and Blumwald, 2002). Compartmentation of Na⁺ and Cl⁻ in the vacuole is considered to be important in ameliorating the effects of salt on plants, and increasing Na⁺ transport into the vacuole has been shown to increase salt tolerance (Apse et al., 1999). In addition, the maintenance of a high cytosolic K⁺:Na⁺ ratio is also considered important (Maathuis and Amtmann, 1999). Thus, the capacity of plants to counteract salinity stress may strongly depend on their ability to compartment ions in the vacuole and to minimize ionic changes in the cytosol.

Although many studies have confirmed the sequestration of Na⁺ and Cl⁻ in the vacuole (Storey et al., 1983; Fricke et al., 1996), the behaviour of ions in the cytosol has been more difficult to determine because the small volume of this compartment makes quantitative measurements technically difficult. Those approaches that have been used, such as X-ray microanalysis (Storey et al., 1983; Flowers and Hajibagheri, 2001) and compartmental tracer efflux analysis (Yeo, 1981), generally support the hypothesis that maintenance of the cytosolic K⁺:Na⁺ ratio is important in salt tolerance. However, none of these techniques is fully quantitative and so accurate estimates of cytosolic ion concentrations in salinized plant cells are still lacking. In particular, measurements are needed in leaf cells because adverse effects of salinity in these are likely to have significant consequences for plant productivity. The lack of such measurements represents a significant impediment to testing hypotheses about responses of plants to salt and hence to identifying targets for manipulation.

4 To whom correspondence should be addressed. Fax: +44 (0)1223 333953. E-mail: RL225@cam.ac.uk

Downloaded from http://jxb.oxfordjournals.org/ by guest on May 3, 2016
Another confounding aspect is that different cell types in leaves can be quite distinct in the ionic composition of their vacuoles (Leigh and Tomos, 1993; Karley et al., 2000), raising the possibility that cytosolic ion concentrations may also vary between cells, or may respond differently to salinity in different cell types. Differential responses of cytosolic K⁺ in epidermal and cortical cells of K⁺-deficient barley roots show that this is a real possibility (Walker et al., 1996).

Triple-barrelled microelectrodes measuring K⁺ activity (aK), pH, and membrane potential (Em) (Walker et al., 1995, 1996) can be used to make quantitative intracellular measurements of aK in the vacuole and cytosol of cells in situ. The inclusion of the pH-sensing barrel allows assignment of values to either the vacuole or the cytosol (Walker et al., 1995). In the study described here, such electrodes were used to measure quantitatively aK in the vacuole and cytosol of leaf cells of barley plants grown in nutrient solutions with or without added NaCl. The results show that there is regulation of cytosolic aK in response to salinity, but this is restricted to mesophyll cells; cytosolic aK declines to very low levels in epidermal cells, although these remain viable. Comparison of the results with published data (Walker et al., 1996) indicates important similarities and differences in the regulation of cytosolic and vacuolar aK in K⁺-replete leaf and root cells.

Materials and methods
Plant material and whole-tissue measurements
Barley (Hordeum vulgare L. cv. Gerbel) seeds were germinated and grown in a modified Hoagland’s nutrient solution containing 5 mM K⁺ (Walker et al., 1996). Plants were grown at a constant temperature of 20 °C with a photoperiod of 16 h, a photon flux density of approximately 300 μmol m⁻² s⁻¹ at plant height, and a relative humidity of 75%. Plants were grown in unamended nutrient solution for the first 7 d after germination and then the NaCl concentration in the nutrient solution of some of the plants was increased by 50 mM d⁻¹ to a final concentration of 200 mM. When treated in this way, the cultivar used, Gerbel, is relatively salt resistant (Flowers and Hajibagheri, 2001). All measurements were made on the third leaf of intact plants. Leaves were mounted on a Perspex holder with their lower edge touching a reservoir of dilute nutrient solution that also contained the reference bath electrode (Miller et al., 2001). This arrangement ensured that an electrical circuit was completed when the triple-barrelled microelectrode was inserted into the leaf. Leaves were placed on the holder at least 30 min before the measurements were made, and stomates remained open throughout the experiment. Impalements were made in cells of the ‘trough’ region of the upper epidermis (Fricke et al., 1995; Fricke, 1997) and on the first layer of mesophyll cells immediately beneath these. Mesophyll cells were accessed by pushing the electrode through an epidermal cell. Successful penetration was monitored by following the Em, which became more negative when the electrode was within a cell. Values of aK are expressed as mean and 95% confidence limits (Fry et al., 1990).

Electrophysiology
Triple-barrelled microelectrodes measuring aK, pH and Em were made and calibrated as described previously (Walker et al., 1995) except that the K⁺ sensor contained dibutyl sebacate as the plasticizer (Cuin et al., 1999). Potassium concentrations in calibration solutions were converted to aK using activity coefficients of between 0.75 and 0.78 (Robinson and Stokes, 1959; Walker et al., 1995). Electrodes were inserted into cells half-way along the length of the third leaf of intact plants. Leaves were mounted on a Perspex holder with their lower edge touching a reservoir of dilute nutrient solution that also contained the reference bath electrode (Miller et al., 2001). This arrangement ensured that an electrical circuit was completed when the triple-barrelled microelectrode was inserted into the leaf. Leaves were placed on the holder at least 30 min before the measurements were made, and stomates remained open throughout the experiment. Impalements were made in cells of the ‘trough’ region of the upper epidermis (Fricke et al., 1995; Fricke, 1997) and on the first layer of mesophyll cells immediately beneath these. Mesophyll cells were accessed by pushing the electrode through an epidermal cell. Successful penetration was monitored by following the Em, which became more negative when the electrode was within a cell. Values of aK are expressed as mean and 95% confidence limits (Fry et al., 1990).

Results
Treatment of the barley plants with NaCl adversely affected growth and significantly changed leaf ion concentrations. Compared with control (non-salinized) plants, the fresh weights of the whole shoot and third leaf decreased by 47% and 35%, respectively, in plants grown in 200 mM NaCl (Fig. 1A). This change was accompanied by a decrease in leaf K⁺ concentration and an increase in leaf Na⁺ concentration (Fig. 1B). These results are comparable with those reported by Flowers and Hajibagheri (2001) for the same barley variety grown under similar conditions.

Measurements of aK were assigned to the vacuole or the cytosol on the basis of the accompanying pH measurements. In both epidermal and mesophyll cells, impalements yielded two populations of pH measurements with mean values of 7.1 and 5.1 (Table 1), which were taken to indicate insertions in the cytosol and vacuole, respectively (Walker et al., 1995, 1996). Compartmental pH values were not statistically different between epidermal and mesophyll cells, and were also unaffected by salinity. The mean values of Em ranged from −83 to −91 mV in cells of non-salinized plants, but were 16 to 28 mV less negative in salinized plants, with the greatest effect on epidermal cell Em (Table 1). There were no statistically-significant differences between Em for impalements into the cytosol and the vacuole, suggesting that the Em across the tonoplast was close to zero. This contrasts with Arabidopsis where Miller et al. (2001) measured a trans-tonoplast Em of about −30 mV (cytosol with respect to the vacuole; Berti et al., 1992).

In non-salinized plants, mean vacuolar aK was 224 and 235 mM in epidermal and mesophyll cells, respectively, while the corresponding values in the cytosol were 68 and 79 mM (Table 2). Following growth in 200 mM NaCl, the mean vacuolar aK in epidermal cells declined to 47 mM and aK in the cytosol of these cells to 15 mM. In contrast, vacuolar aK in mesophyll cells decreased only to 150 mM, while, in the cytosol, aK declined to 64 mM.
Discussion

As far as is known, this paper reports the first quantitative measurements of cytosolic $a_K$ in leaf cell compartments of plants subjected to salt stress. Overall, the results indicate that both vacuolar and cytosolic $a_K$ in the mesophyll are maintained at the expense of the supply to the epidermis. The results are consistent with the observations by Fricke et al. (1996) who found, using single-cell sampling, that decreases in $K^+$ concentrations and increases in $Na^+$ concentrations were less in vacuoles of mesophyll cells than in those of epidermal cells when plants were grown at NaCl concentrations of 100 mM or more. This indicates that mechanisms exist to ameliorate ionic changes in leaf mesophyll cells, but not in epidermal cells. This may be a device to protect and maintain the photosynthetic activity of the mesophyll cells. However, since the fresh weight of the third leaf declined by 35% in the salt-treated plants, other changes must have affected growth detrimentally. For instance, it is possible that the decreases in $a_K$ in epidermal cell compartments may ultimately be affecting some fundamental growth process. Testing the importance of changes in subcellular ion activities in response to salinity will require investigation to relate the ion activities to the rates of key physiological processes, as done for cytosolic $a_K$ and protein synthesis in $K^+$-deficient barley roots (Walker et al., 1998).

The differential behaviour of mesophyll and epidermal cells is similar to that seen in $K^+$-deficient barley roots where cytosolic $a_K$ declined to a much greater extent in epidermal cells than in cortical cells (Walker et al., 1996). These collective observations indicate that ion supplies to key cell types in both leaves and roots are protected in order to maintain their function in response to deficiencies or excesses of ions. How this is achieved in leaves is unclear, but the observation is consistent with work showing that epidermal and mesophyll cells have quite distinct ionic compositions (Leigh and Tomos, 1993; Karley et al., 2000). It seems clear that supply and/or retention of ions in different cell types is important in leaf function and in responses to ionic stresses.

Leigh and Wyn Jones (1984) proposed that cytosolic $K^+$ concentrations are buffered by the mobilization of vacuolar $K^+$. Measurements of cytosolic and vacuolar $a_K$ in barley roots grown under a range of external $K^+$ concentrations supported this contention, with cytosolic $a_K$ declining only when the vacuolar $a_K$ fell below about 25 mM (Walker et al., 1996). This does not seem to occur

Table 1. Vacuolar and cytosolic pH and membrane potentials ($E_m$) in ‘trough’ cells of the upper epidermis and in mesophyll cells of the third leaf of 21-d-old barley plants grown in nutrient solution containing 0 mM or 200 mM added NaCl

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Epidermis Vacuole</th>
<th>Cytosol</th>
<th>Mesophyll Vacuole</th>
<th>Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>pH: 5.1±0.4(19) E_m (mV): -86±10(21)</td>
<td>pH: 7.2±0.2(10) E_m (mV): -83±9(10)</td>
<td>pH: 5.0±0.3(9) E_m (mV): -87±15(10)</td>
<td>pH: 7.0±0.1(11) E_m (mV): -91±15(12)</td>
</tr>
<tr>
<td>200</td>
<td>pH: 5.1±0.2(12) E_m (mV): -58±10(13)</td>
<td>pH: 7.0±0.2(9) E_m (mV): -57±8(12)</td>
<td>pH: 5.0±0.4(7) E_m (mV): -71±19(7)</td>
<td>pH: 7.2±0.1(8) E_m (mV): -64±16(10)</td>
</tr>
</tbody>
</table>

Fig. 1. Fresh weights of the whole shoot and third leaf (A) and ion concentrations in the third leaf (B) of 21-d-old barley plants grown in nutrient solution containing 0 mM or 200 mM added NaCl. Results are mean ±SD ($n=9$).
in leaf epidermal cells of salt-grown barley where cytosolic $a_k$ declined to 15 mM despite the vacuole still containing about 50 mM K$^+$ (Table 2). Why the vacuolar pool is not fully mobilized to maintain cytosolic $a_k$ is unclear. It is unlikely to be due to the existence of a minimum K$^+$ concentration below which the vacuolar pool is not fully mobilized to maintain cytosolic $a_k$ as proposed by Leigh and Wyn Jones, 1984 because very low K$^+$ concentrations can be measured in sap extracted from epidermal vacuoles of leaves of barley plants grown in 150 mM NaCl (Fricke et al., 1996).

Therefore, it seems more likely that the maintenance of a high cytosolic $a_k$ is not important in these cells, and so it declines, despite the availability of vacuolar K$^+$. Fricke et al. (1996) observed large differences in K$^+$ concentrations in vacuolar sap extracts from different epidermal cells of salt-grown leaves, so it is important that other cells are investigated in order to determine whether the behaviour of vacuolar and cytosolic $a_k$ observed in upper epidermal 'trough' cells is typical of all epidermal cells.

At 15 mM, the cytosolic $a_k$ in the ‘trough’ epidermal cells from the salt-grown plants was at levels considered inadequate to support key K$^+$-dependent functions such as protein synthesis (Lubin and Ennis, 1964; Leigh and Wyn Jones, 1984; Walker et al., 1998). It therefore seems unlikely that these cells are particularly metabolically active. Nonetheless, they appear to retain their viability, as evident by (1) a relatively negative $E_m$, indicating that their plasma membrane is intact and ion transporters functioning; (2) a near-neutral cytosolic pH, which, at the measured values of $E_m$ and a typical leaf apoplastic pH of about 5.5 (Mühling and Läuchli, 2000), could only be maintained by active H$^+$ extrusion; and (3) a turgor of approximately 0.9 MPa with correspondingly high sap osmotic pressure and ion concentrations (Fricke, 1997; Fricke et al., 1996). Thus, cytosolic $a_k$ values below those normally considered optimal for protein synthesis do not necessarily lead to a loss of viability of epidermal cells.

The compartment-specific values of $E_m$, pH and $a_k$ obtained with the triple-barrelled microelectrodes provide sufficient information to make thermodynamic calculations of the direction of active K$^+$ transport at the tonoplast, and the feasibility of different active transport mechanisms (see Walker et al., 1996, for equations). These calculations (results not presented) show that for both epidermal and mesophyll cells, under both sets of growth conditions, K$^+$ is always actively transported into the vacuole. Two possible mechanisms for active transport into the vacuole have been proposed, a 1:1 K$^+$-H$^+$ antiport (Hassidim et al., 1990) and the vacuolar K$^+$-dependent, H$^+$-transporting inorganic pyrophosphatase (Davies et al., 1992). Calculations of the thermodynamic feasibility of each of these indicates that both are possible mechanisms in barley leaf cells (results not shown). Therefore, it seems that salinization of barley leaf cells does not have any obvious effect on the direction or mechanism of active K$^+$ transport at the tonoplast. This contrasts with K$^+$-deprivation in barley roots which changes the direction of active K$^+$ transport from into the vacuole in K$^+$-replete conditions to into the cytosol in K$^+$-deficiency (Walker et al., 1996). It was not possible to make similar calculations for active K$^+$ transport at the plasma membrane of leaf cells because $a_k$ in the apoplast of control and salinized leaves was not measured.

Walker et al. (1996) showed that cytosolic and vacuolar $a_k$ in K$^+$-replete barley root cells are about 80 mM and 120 mM, respectively, with little difference between epidermal and cortical cells. Comparison of these values with those for leaf cells from K$^+$-replete, non-salinized plants (Table 2) shows that the $a_k$ in the vacuoles of leaf cells is approximately twice that in the root cells, but the cytosolic values are similar. This indicates that cytosolic $a_k$ is regulated to similar values in different cell types of barley while vacuolar $a_k$ has different set points in root and leaf cells (see also Leigh, 2001). It would now be interesting to determine what regulatory processes operate on K$^+$ transport at the plasma membrane and tonoplast to bring about this uniformity of cytosolic $a_k$, but differences in the vacuolar values. In particular, tonoplast transport must be under quite different control in root cells compared to leaf cells.

Finally, the work reported here will assist in attempts to use Na$^+$-selective microelectrodes to measure the effect of salinity on cytosolic and vacuolar Na$^+$ activities (Carden et al., 2001). Currently-available Na$^+$ sensors show some sensitivity to K$^+$, especially at Na$^+$ activities between 1 and

---

**Table 2.** Compartmental $a_k$ in ‘trough’ cells of the upper epidermis and in mesophyll cells of the third leaf of 21-d-old barley plants grown in nutrient solution containing 0 or 200 mM added NaCl

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Compartmental $a_k$ (mM)</th>
<th>Mesophyll</th>
<th>Epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vacuole</td>
<td>Cytosol</td>
<td>Vacuole</td>
</tr>
</tbody>
</table>
10 mM (Carden et al., 2001). Therefore, knowledge of the values and behaviour of subcellular $a_K$ in salinized plant cells is an important prerequisite for such studies. An approach combining the use of Na$^+$- and K$^+$-selective microelectrodes would allow the effects of salinity on cytosolic activities of these ions to be quantified and so allow hypotheses about the importance of cytosolic K$^+$:Na$^+$ ratio in salinity tolerance to be tested (Maathuis and Amtmann, 1999).

Acknowledgements

The research was supported by grant B104CT960775 from the EU Biotechnology Programme 1994–1998. Rothamsted Research and Long Ashton Research Station are grant aided by the Biotechnology and Biological Sciences Research Council of the UK.

References


