The K⁺/Na⁺ Selectivity of a Cation Channel in the Plasma Membrane of Root Cells Does Not Differ in Salt-Tolerant and Salt-Sensitive Wheat Species

Daniel P. Schachtman, Stephen D. Tyerman, and Bernard R. Terry

Commonwealth Scientific and Industrial Research Organization Plant Industry and The Australian National University Botany Department, GPO Box 1600, Canberra ACT 2601 Australia (D.P.S.) and Flinders University of South Australia, Biological Sciences Department, Bedford Park, SA 5042 Australia (S.D.T., B.R.T.)

ABSTRACT

The characteristics of cation outward rectifier channels were studied in protoplasts from wheat root (Triticum aestivum L. and Triticum turgidum L.) cells using the patch clamp technique. The cation outward rectifier channels were voltage-dependent with a single channel conductance of 32 ± 1 picosiemens in 100 millimolar KCl. Whole-cell currents were dominated by the activity of the cation outward rectifiers. The time- and voltage-dependence of these currents was accounted for by the summed behavior of individual channels recorded from outside-out detached patches. The K⁺/Na⁺ permeability ratio of these channels was measured in a salt-sensitive and salt-tolerant genotype of wheat that differ in rates of Na⁺ accumulation, using a voltage ramp protocol on protoplasts in the whole-cell configuration. Permeability ratios were calculated from shifts in reversal potentials following ion substitutions. There were no significant differences in the K⁺/Na⁺ permeability ratios of these channels in root cells from either of the two genotypes tested. The permeability ratio for K⁺/Cl⁻ was greater than 50:1. The K⁺/Na⁺ permeability ratio averaged 30:1, which is two to four times more selective than the same type of channel in guard cells and suspension culture cells. Lowering the Ca²⁺ concentration in the bath solution to 0.1 millimolar in the presence of 100 millimolar Na⁺ had no significant effect on the K⁺/Na⁺ permeability ratios of the channel. It seems unlikely that the mechanism of salt tolerance in wheat is based on differences in the K⁺/Na⁺ selectivity of these channels.

In saline conditions, most salt-tolerant monocotyledonous plants accumulate lower amounts of sodium in their leaves than salt-sensitive monocotyledonous plants (1). Salt-tolerant genotypes of wheat have been shown to transport less sodium from roots to shoots than do salt-sensitive genotypes, both in the long term (24) and in the short term (5). There is only indirect evidence for a sodium-specific effect on growth in the long term; wheat plants grown in high sodium chloride solutions have lower growth rates than those grown in high macronutrient solutions (16). As yet, it is not known how sodium inhibits plant growth.

The plasma membrane of root cells is the most likely site for selective uptake of ions into the plant, so the specific characteristics of transport processes across this membrane should be important in salt tolerance. At high external salinity, Na⁺ may enter the cytoplasm passively across the plasma membrane because the cytoplasm is electrically negative with respect to the apoplast and the concentration of Na⁺ is probably higher outside the cytoplasm. In plants, a Na⁺-selective channel has not been identified in the plasma membrane; therefore, entry of Na⁺ into the cytoplasm may be through a K⁺ channel with some permeability to Na⁺. Na⁺ may cross the plasma membrane via the cation-selective outward rectifier, which has been observed in the plasma membranes of guard cells (27), corn-root suspension culture cells (15), cells from cotyledons of Amaranthus (28), and algae (29). The cation outward rectifier channel is characterized by its strong voltage dependence of opening, causing it to dominate the membrane conductance when the membrane potential goes more positive than the reversal potential of the channel, which is usually very close to the E₉ (3). In glycophytes, the membrane depolarizes in response to external NaCl, generally to membrane potentials at or more positive than E₉ (3, 17). Depolarization will open the cation outward rectifier channels and Na⁺ could move into the cell at a rate governed by the electrochemical gradient for Na⁺ and the effective permeability of the channels to Na⁺.

In this study, we have used the patch-clamp technique on protoplasts to compare the characteristics of the cation outward rectifying channels in wheat root cells from a salt-tolerant and -sensitive species. Little is known about the diversity of properties of particular ion channels in the plasma membranes of plants, and in particular in plant roots. Studies on Drosophila and mammals have shown that the DNA coding for K⁺ and Na⁺ channels contain highly conserved regions among different organisms (11); yet within a class of ion channel (e.g., voltage-activated K⁺ channels), differences occur both in DNA sequences and in electrophysiological parameters (23). It is possible that diversity occurs in ion channels found in plant plasma membranes, sufficient per-
haps to account for variational differences in salt tolerance. This paper reports on the K⁺ and Na⁺ permeability ratio of the cation outward rectifier channels in the plasma membrane of cells from roots of a salt-tolerant and a salt-sensitive species of wheat. The comparison of these genotypes, divergent in salt tolerance, should reveal how sodium is more efficiently excluded from shoots by salt-tolerant wheat.

MATERIALS AND METHODS

Plant Material

A salt-tolerant wheat (Triticum aestivum) land race, Khar­chia (22), and a salt-sensitive Triticum turgidum cv Modoc (22) were used. Seeds of T. aestivum cv Kharchia or T. turgidum cv Modoc were imbibed in water at 4°C and germinated in Petri dishes. Seedlings were transferred to continuously aerated nutrient solution containing half concentration of Hoagland solution (10) with full strength iron. They were grown at 25°C during the day and 21°C at night in a growth chamber (12 h light/d) for most experiments; in some experiments, they were grown in the greenhouse under similar conditions.

Protoplast Isolation

About 3 g of roots from 5 to 7 d old seedlings were chopped finely in a small volume of solution A (500 mM sorbitol, 5 mM Mes, 1 mM CaCl₂, 0.5% [w/v] PVP, 0.5% BSA, pH 5.5) and washed thoroughly in the same solution. The chopped roots were suspended in 10 mL of solution A containing 0.8% cellulase (Onozuka RS; Yakult Honsha Co. Ltd., Tokyo) and 0.08% pectolyase (Sigma Chemical Co.), pH 5.5; the tissue was then exposed to partial vacuum to infiltrate the enzyme solution into air spaces. The tissue was agitated at 30°C in the dark for 2 h, followed by a further 1 h after the addition of another 10 mL of solution A containing 0.8% cellulase, at pH 5.8. The digest was filtered through fine muslin, centrifuged at 60g for 5 min, and the pellet resuspended in 5 mL of ice-cold 500 mM sucrose, 5 mM Mes, 1 mM CaCl₂, pH 6.0. On top of this was layered 2 mL of 400 mM sucrose, 100 mM sorbitol, 5 mM Mes, 1 mM CaCl₂, pH 6.0, followed by a 1 mL layer of 500 mM sorbitol, 5 mM Mes, 1 mM CaCl₂, pH 6.0. After centrifugation for 5 min at 200g, clean protoplasts were collected from the interface between the top two layers. The protoplasts were mixed with 5 mL of the top gradient solution for a final wash, centrifuged at 60g for 5 min, and resuspended in 2 mL of the top gradient solution. Throughout the purification, the protoplasts were kept at 4°C. If stored at 4°C, protoplasts remain patchable for more than 4 h.

Patch Clamp Recording

Data for this paper were obtained from the whole-cell configuration and outside-out patches (8) of streaming and phase bright protoplasts. The same criteria were applied in selection of protoplasts for all experiments such that they should have derived from cortical tissue rather than stele. The protoplasts selected for patch clamping had an average surface area of 2.9 × 10⁻⁹ m² (n = 20) with streaming cytoplasm. The specific capacitance was not significantly different in the two genotypes, averaging 0.97 ± 0.06 μF cm⁻² (n = 14). All recordings were made at 22 to 25°C, unless specified. All whole-cell recordings were made with series resistance compensation at 50%. Patching of protoplasts was done in a flow chamber of less than 0.3 mL volume, in which solutions could be exchanged rapidly without disturbance to whole-cell or cell-attached preparations. The chamber had a thin glass base to which the protoplasts without cell walls adhered firmly. Patch pipettes were pulled from borosilicate glass blanks (Clark Electromedical, Reading, UK), coated with SylgardR (Dow-Corning) and fire-polished immediately before use. Pipettes for detached patches were usually polished to between 15 to 20 MΩ (measured in 100 mM KCl); pipettes with lower resistance were used for whole-cell recordings, polished to about 5 to 10 MΩ. Solution in the flow chamber was earthed via an agar salt-bridge connected to a silver/silver chloride bath electrode. Patch and bath electrodes were filled with the same, or similar, solutions to minimize offset potentials. Unbalanced tip potentials, when present, were corrected for as described in Tyerman and Findlay (30). The potential difference across a patch is expressed in terms of conventional Vm, the resting membrane potential, pipette potential, and tip potential (pipette with respect to bath) are included (where applicable) in calculations of Vm.

The unfiltered output from a List EPC-7 (List Electronic, Darmstadt, FRG) was fed through a modified pulse code modulator (PCM 701ES; Sony) and stored on VHS video tape. The amplifier was also connected via a 12 bit A/D converter to a control computer that could be used to monitor the pipette resistance, initiate saw tooth or triangular waveforms (ramps) of the pipette potential from any chosen holding potential, generate sequences of voltage command pulses from a given holding potential of from 2 to 12 s duration, and record the current and voltage data resulting from the procedures described, at sampling rates of between 10 to 2 kHz.

Solutions

All solutions were osmotically balanced with sorbitol, to a total osmotic concentration of 700 and 720 mM for intracellular solution. All solutions were filtered (0.22 μm Millipore) before use. Details of particular external solutions are listed in the legends to the appropriate figures and tables. The standard intracellular solution contained (mm): 100 KCl, 2 MgCl₂, 2 EGTA, 2 Na₂ATP + 2 MgCl₂, 490 sorbitol, 10 Hepes, 13 KOH, pH 7.2.

RESULTS

Identification of the Cation Outward Rectifier Channel

A time-dependent outward current was observed in 33 out of 42 wheat root cells when the plasma membrane was depolarized to potentials more positive than the EK. The time-dependent current in whole cell recordings was identified on the basis of reversal potential shifts and the voltage- and time-dependence of single channel data. Figure 1 shows the time-dependent increase in current to a steady-state in the whole cell (see inset) for a sequence of pulses during which the Vm was progressively stepped to more positive values. When Vm

Downloaded from on July 19, 2017 - Published by www.plantphysiol.org
Copyright © 1991 American Society of Plant Biologists. All rights reserved.
was held positive of the reversal potential of the conductance and pulsed to more negative values (closing the channels), most of the inward current decayed exponentially (Fig. 2).

Single channel recordings of the cation outward rectifier confirmed that the voltage- and time-dependent currents observed in the whole cell were due to the cation outward rectifier. Single channels were recorded in the outside-out patch configuration (see inset, Fig. 3). When $V_m$ was held more positive than $E_K$, the cation outward rectifier remained open most of the time. Upon hyperpolarization of $V_m$, which caused inward current to flow through the channels, the kinetics of the channels changed markedly, with many rapid and regular flickers to a closed state (Fig. 3). After a variable time, depending on how negative $V_m$ was from $E_K$, the channel would enter a longer-lived closed state from which it would rarely open. Such behavior is typical of the cation outward rectifier channel (28). The summation of 30 sequential single channel current records each similar to that seen in Figure 3 was used to generate the exponentially decaying current versus time curve shown in Figure 4 (27). Similarity between the current decay generated from single channel data (Fig. 4) and whole cell data (Fig. 2) indicates that whole cell currents originate from the activity of the single channels observed in isolated patches.

**Single Channel I/V Curves Generated with Voltage Ramps**

$I/V$ curves could be generated from pulse sequences to different values of $V_m$. This method is time consuming and prone to errors when dealing with single channel recordings because a different channel type may appear at a particular voltage and be mistaken for the channel in question. A more efficient way to extract $I/V$ curves is to apply fast ramps of voltage. The voltage dependence of the cation outward rectifier can be used to advantage by ramping from a positive holding $V_m$ to more negative values. The channel closes during the ramp sequence, because when the voltage of the sequence of ramps is averaged over time, the mean voltage is more negative than $E_K$. With this method, both the open channel ramps and the closed ramp are obtained, the difference yielding the single channel $I/V$ curve. Figure 5 shows a single channel $I/V$ curve for the cation outward rectifier in two external solutions. The short closures that have been captured at negative $V_m$ during the ramp in 100 mM KCl illustrate the rapid change in kinetics that occurs when inward current flows through the channel. With 100 mM external KCl and 100 mM KCl in the pipette, single channel $I/V$ curves yielded an average single channel conductance of 32 picoSiemens (SE ± 1, n = 5) for the cation outward rectifier.
Whole Cell I/V Curves Generated with Voltage Ramps

Voltage ramps were also applied to whole cell preparations. The voltage- and time-dependence of the cation outward rectifier was used to obtain I/V curves. The membrane potential was held positive of E_K, (usually at +50 mV) so that the channels were open and the outward current was activated. A series of ramps was then given to some membrane potential negative of E_K. During a series of ramps, the cation channels began to turn off. The proportion that closed by the end of the series depended on the amplitude and frequency of the voltage ramps. Subtraction of the last ramp from the first in a series yielded the I/V curve for the proportion of time-dependent current that turned off during the sequence. The non-time-dependent component, common to all the ramps in a sequence, is removed in the subtraction. Figure 6 shows whole-cell I/V curves for the time-dependent current (non-time-dependent component subtracted) in different external solutions. Although this method does not always reveal the I/V curve for the total time-dependent current, it does provide an accurate measure of the reversal potential for that current. The reversal potential of the time-dependent component could also be obtained by subtraction of any two ramps in a sequence as the channels are deactivating. This was done and gave the same reversal potential. Others (15, 27) have used a step protocol, extracting the time-dependent portion of the current to generate the current/voltage relationship. A comparison of the two techniques yielded similar reversal potentials. In 100 mM NaCl, a reversal potential of −82 mV (SE ± 5 mV, n = 16) was measured with the ramping technique, as compared to a reversal potential of −83 mV (SE ± 9 mV, n = 4) with the step protocol. In both single channel (Fig. 5) and whole cell (Fig. 6) I/V curves, inward current was observed with Na⁺ as the only external monovalent cation.

The reversal potentials for the time-dependent outward current obtained in the whole cell mode were the same as those obtained from single channel I/V curves for the cation

Figure 4. Summation of 30 sequential pulses that capture the closure of cation outward rectifier channels in an outside-out patch from the plasma membrane of wheat root cells. Voltage pulse protocol as in Figure 3. Bath solution was 100 mM KCl, 10 mM CaCl₂, 10 mM Mes, 460 mM sorbitol at pH 6.0, and standard intracellular solution. Summation shows that whole cell behavior can be reconstructed from single channel data.

Figure 5. Single channel current/voltage relationship for the cation outward rectifier in an outside-out patch from root cell protoplasts of Kharcha with (a) 100 mM KCl, 10 mM CaCl₂, 10 mM Mes, 460 mM sorbitol, pH 6.0, in bath, and (b) 100 mM NaCl, 10 mM CaCl₂, 10 mM Mes, 460 mM sorbitol, pH 6.0, in bath. Standard intracellular solution was used. The same relationships were obtained with root cells from Modoc. The holding potential was +50 mV, at which the channels were open most of the time. The negative going voltage ramps tended to close the channels. The data were sampled at 10 kHz and the duration of each ramp was approximately 50 ms.

Figure 3. Typical single channel recording of the cation outward rectifier channel in an outside-out patch (see inset) from the plasma membrane of wheat root cells. Two channels are closing in this figure. At time zero, the voltage was stepped from a holding potential of +50 mV, at which channels were open, to −50 mV, which led to the closure of the channels over an interval of 2 s. Bath solution was 100 mM KCl, 10 mM CaCl₂, 10 mM Mes, 460 mM sorbitol at pH 6.0, and standard intracellular solution.
outward rectifier. This further substantiates that whole cell recordings represent the collective behavior of single cation outward rectifier channels. This result also indicates that no other time-dependent current in the whole cell recordings is confounding the ramping results. In later experiments, voltage ramps were used instead of the step protocol.

Selectivity of the Cation Outward Rectifier

The selectivity of the cation outward rectifier channels in root cells from two genotypes of wheat (Kharchia and Modoc) was characterized in the whole cell configuration. In this configuration, sufficient replication could be obtained to test for differences in ion selectivity of the cation outward rectifier. The I/V curves and the reversal potentials in Figure 6 are typical of those generated for both genotypes. A negative shift in reversal potential was measured when the bath solution of 100 mM KCl was replaced with 10 mM KCl and a more negative shift with 100 mM NaCl in the bath (standard intracellular in pipette). Average reversal potential shifts are shown in Table I.

A K⁺/Na⁺ permeability ratio was calculated using changes in reversal potential between solutions (Eq. 10-16 in Ref. 9). Although it is generally assumed that the pipette solution thoroughly perfuses the cell, concentration gradients may form between the tip of the pipette and the inside of the cell (29). Calculation of K⁺/Na⁺ permeability ratios from shifts in reversal potential avoided the need to know the actual ion concentrations in the cytoplasm, although these were assumed to remain constant throughout an experiment. The shifts in reversal potentials (Table I) measured in root cells from Modoc and Kharchia were compared by a one-way analysis of variance. No statistical difference was found for reversal potential shifts for the cation outward rectifier between Kharchia and Modoc. Because the reversal potential shifts were not significantly different, the K⁺/Na⁺ and K⁺/Cl⁻ permeability ratios were calculated from the mean reversal potential shifts of the two genotypes. A change in bath KCl concentration from 100 mM to 10 mM produced shifts in reversal potential that ranged from −39 mV for Modoc, to −48 mV for Kharchia. These shifts were not significantly different. The channel is at least 50 times more selective to K⁺ over Cl⁻ (Table I). An average shift of −88 mV was measured for the cation outward rectifier in Kharchia and Modoc when the KCl concentration of 100 mM was changed to 100 mM NaCl with 10 mM CaCl₂. A similar shift of −83 mV was observed when the ionic composition of the bath was changed from 100 mM KCl to 100 mM NaCl with 0.1 mM CaCl₂. No statistically significant difference was found between the permeability of the cation outward rectifier in 10 mM Ca and in 0.1 mM Ca. The reversal potential shifts indicate that the

Table I. Reversal Potential Shifts (±SE) and Permeability Ratios for T. aestivum cv Kharchia, a Salt-Tolerant Wheat, and T. turgidum cv Modoc, a Salt-Sensitive Wheat

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Reversal Potential Shifts (mV)</th>
<th>Solution change from 100 mM KCl to 10 mM KCl (10 mM CaCl₂)</th>
<th>Solution change from 100 mM KCl to 100 mM NaCl (10 mM CaCl₂)</th>
<th>Solution change from 100 mM KCl to 100 mM NaCl (0.1 mM CaCl₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kharchia</td>
<td>−48 ± 2 (7)</td>
<td>−87 ± 8 (7)</td>
<td>−77 ± 9 (4)</td>
<td></td>
</tr>
<tr>
<td>Modoc</td>
<td>−39 ± 5 (5)</td>
<td>−90 ± 7 (9)</td>
<td>−89 ± 6 (7)</td>
<td></td>
</tr>
<tr>
<td>Mean for Kharchia and Modoc</td>
<td>P_K/P_C &gt; 50</td>
<td>P_K/P_Na = 32</td>
<td>P_K/P_Na = 28</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. Current/voltage relationships for the time-dependent outward current in a whole cell of Modoc with (a) 100 mM KCl, 10 mM CaCl₂, 10 mM Mes, 460 mM sorbitol, pH 6.0, in bath (holding potential = +50 mV); (b) 10 mM KCl, 10 mM CaCl₂, 10 mM Mes, 460 mM sorbitol, pH 6.0, in bath (holding potential = +30 mV); (c) 100 mM NaCl, 10 mM CaCl₂, 10 mM Mes, 460 mM sorbitol, pH 6.0, in bath (holding potential = +10 mV). Standard intracellular solution was used. The same relationships were obtained with root cells from Kharchia. The data were sampled at 2 kHz with each ramp taking approximately 250 ms. The negative going ramps tended to deactivate the current with time.
permeability of the cation outward rectifier channel for $K^+$ over $Na^+$ is between 28 to 32 (Table I). Several outside-out patches were obtained that contained the cation outward rectifier channel. Current voltage curves from outside-out patches (Fig. 5) in different bath solutions showed similar shifts in reversal potentials to those from the whole cell (Fig. 6). The average shift in reversal potential for both genotypes was $-48 \text{ mV (se \pm 3)}$ for the single channel recording and $-44 \text{ mV (se \pm 2)}$ for the whole cell recordings when the KCl in the bath solution was changed from 100 to 10 mm. When 100 mm KCl was substituted with 100 mm NaCl, the average shift in reversal potential for both genotypes was $-84 \text{ mV (se \pm 1)}$ for single channel recordings and $-88 \text{ mV (se \pm 5)}$ for whole cell records.

Non-time-Dependent Currents

In about 20% of the cells that were successfully patch clamped, we observed no apparent time dependence in the outward or inward currents (Fig. 7). A sigmoid I/V curve was observed from both ramp and pulse protocols with a region of very low slope conductance between 0 and $-75 \text{ mV}$. In some cells, the time-dependent outward current changed to a non-time-dependent current. In some other cells, this instantly activated current was gradually replaced by the more usual time-dependent type.

**DISCUSSION**

At high external NaCl concentrations, the influx of Na$^+$ into root cells may be via a cation channel. Inward rectifier cation channels may not be the pathway for Na$^+$ transport. NaCl causes an increase in the concentration of cytosolic calcium of maize root protoplasts (19). If inward rectifier channels in wheat roots behave like the same channels in guard cells (26), the elevated level of cytosolic $Ca^{2+}$ will cause the inward rectifiers to close. We suggest that a possible route for Na$^+$ entry into the cell may be via the cation outward rectifier. For Na$^+$ to move into the cell through the cation outward rectifier, the channel must be permeable to Na$^+$, although its permeability may be modified by the concentration of $K^+$, other ions, and by voltage. Secondly, the channel must open. Cation outward rectifiers open when the membrane potential depolarizes. Na$^+$ has been shown to depolarize the membrane potential of plant root cells (3) and giant algal cells (17); thus, the cation outward rectifier will probably be open under these conditions. In saline soils, the salt concentrations around the root can be two times higher than the bulk soil solution when plants are rapidly transpiring or the soil begins to dry out (20). In 100 mm external NaCl, cytoplasmic concentrations of sodium may reach a maximum of about 100 mm (2), whereas the concentration of Na$^+$ in the apoplast may be twice as high. If the cation outward rectifier is open under saline conditions, the electrochemical gradient for Na$^+$ could result in the passive movement of Na$^+$ ions through open cation channels into the cell.

Although we have suggested that Na$^+$ could enter root cells via the cation outward rectifier, we recognize that there are four principal objections to our data that prevent the suggestion from becoming proven fact. (a) The permeability calculated from the channel's reversal potential does not necessarily reflect the relative flux ratio of ions through the channel (18). (b) There is the possibility that Na$^+$ may block the cation outward rectifier channels (29). (c) Even though we observed inward current through single channels (e.g. Fig. 5) when Na$^+$ was the only external monovalent cation in the bath, there is the possibility that Cl$^-$ may be moving out or $Ca^{2+}$ moving in through the channel. In this context, it is interesting to note that the reversal potentials obtained from whole cell and single channel I/V curves are very similar. This adds weight to the argument that the whole cell currents are carried by the type of channel recorded in Figure 5. The possibility that the inward current was due to a leak conductance or carrier is excluded because the leak was subtracted in both cases. (d) We have not considered the voltage-dependent kinetics of the channel, which may prevent the channel from opening under saline conditions. The kinetics are such that, normally, the channel closes when there is an inwardly directed $K^+$ current. However, under saline conditions, the membrane potential may depolarize to a value more positive than the reversal potential of the channel. Notwithstanding these objections, it is interesting to compare $K^+$ and Na$^+$ permeabilities from our data with those in the literature. In the absence of Na$^+$, the mean conductance accounted for by the cation outward rectifier in these whole cell experiments on both varieties was 1.0 siemens $m^{-2}$ with 10 mm KCl in the bath solution. Using the Goldman-Hodgkin-Katz current equation, the permeability to $K^+$ can be calculated as $1 \times 10^{-8} m s^{-1}$. From the average $P_{Na}/P_{Na}$ ratio of 30 for the cation outward rectifier (measured in these experiments), a $P_{Na}$ of $3.0 \times 10^{-10} m s^{-1}$ is calculated. These values are within the range obtained for other plant roots ($P_{K} = 2.2$ to $4.8 \times 10^{-9} m s^{-1}$, $P_{Na} = 0.03$ to $1.0 \times 10^{-9} m s^{-1}$) and are sufficient to account for the fluxes required to support rapid expansion

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Current/voltage relationship for root cells from Modoc in the whole cell configuration. This type of current/voltage curve (non-time-dependent) was occasionally measured in root cells of both genotypes. The bath solution was 100 mm KCl, 0.1 mm CaCl$_2$, 10 mm Mes, 460 mm sorbitol, pH 6.0. Standard intracellular solution was used. The holding potential was $+50 \text{ mV}$ with ramps in a negative direction. Data sampled as in Figure 6.
growth if Na⁺ uptake was required to generate a vacuolar concentration of 500 mM (7). If the objections listed above can be satisfied, these calculations demonstrate that the cation outward rectifier may account for the measured fluxes of Na⁺ into cortical cells of wheat roots in conditions of high external NaCl.

The high K⁺/Na⁺ selectivity of between 28 to 32 measured in wheat root cells indicates that there may be diversity within the cation outward rectifier channels found in the plant kingdom. The K⁺/Na⁺ selectivity of the cation outward rectifier in wheat root cells was higher than that measured for the cation outward rectifier in guard cells of bean (Pₖ/Pₙa = 8 [27]) and in suspension cells from butterfly weed (Pₖ/Pₙa = 15 [25]). In *Amaranthus* hypocotyl/cotyledonic parenchyma, in which the same experimental protocols were used, the cation outward rectifier was also less selective to K⁺/Na⁺ (Pₖ/Pₙa = 6 [28]). The K⁺/Na⁺ selectivity of the cation outward rectifier also varies within the animal kingdom. A K⁺/Na⁺ permeability ratio of greater than 100 for the cation outward rectifier was measured from frog node and only 14.5 for snail neurons (9).

It is not clear why apparently nontime-dependent currents appeared in whole cell preparations from both Kharchia and Modoc. These currents may originate from voltage- and time-dependent channels whose activation and deactivation behavior differs from that of the cation outward rectifier. A piece of evidence against this is that, in at least five cells, the whole cell currents changed from the normal time-dependent type to the nontime-dependent type, and vice versa. Recent work with *Amaranthus* has indicated that the deactivation and activation kinetics of the cation outward rectifier can change sufficiently so that the same channels can generate currents that appear to activate and deactivate apparently instantly, or at some later point, in a time-dependent fashion (B.R. Terry, personal communication).

Calcium is known to ameliorate the effects of high Na⁺ on plant growth. The mechanism behind this response may be due to a decrease in membrane permeability with high concentrations of calcium. High concentrations of sodium, in the presence of low concentrations of calcium, have been shown to displace calcium from the plasma membrane in cotton epidermal cells (4). Increases in membrane permeability have been observed in *Nitelopsis* when cells are bathed in high sodium and low calcium (13). However, the K⁺/Na⁺ permeability of the cation outward rectifier channel was not affected by very low concentrations of Ca²⁺ (0.1 mM) and high concentrations of Na⁺ (100 mM) in the bath solution.

These experiments aimed at measuring the possible differences in K⁺/Na⁺ selectivities of the outwardly rectifying cation channels for a salt-tolerant and a salt-sensitive variety of wheat. There appear to be no differences in this property of the channels in the contrasting genotypes. The genotypic differences in Na⁺ uptake and transport may depend instead on the regulation of plasma membrane channels in response to NaCl stress. The techniques used in these experiments did not measure the *in vivo* response of root cells to salinity, because the intracellular concentrations of K⁺, Ca²⁺, and ATP were held constant due to perfusion by the contents from the patch pipette. Salt stress leads to an increase in the cytosolic concentration of free calcium in maize root protoplasts (19), and there is evidence that cytosolic calcium plays a part in regulation of the cation outward rectifiers in maize suspension culture protoplasts (14). Cytosolic ATP concentration may also regulate cation channels in the plasma membrane of root cells, as was demonstrated in *Nitelopsis* (12). In salinized roots of barley, vacuolar concentrations of ATP were lower in a salt-sensitive genotype than in the salt-tolerant genotype (6). Measurements of intracellular Ca²⁺, Na⁺, K⁺, and ATP in salinized root cells of salt-tolerant and -sensitive genotypes are needed for a more complete understanding of the role the cation outward rectifier, or any other plasma membrane channel, may play in the processes that lead to Na⁺ uptake or exclusion from plants under NaCl stress.

**ACKNOWLEDGMENTS**

We thank R. Munns for catalyzing the collaboration and for critical comments on the manuscript, G. P. Findlay for comments on the manuscript, M. I. Whitecross for his support, and M. Boult for technical assistance.

**LITERATURE CITED**