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Enhancement of Na^+ Uptake Currents, Time-Dependent Inward-Rectifying K^+ Channel Currents, and K^+ Channel Transcripts by K^+ Starvation in Wheat Root Cells[1]

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Excessive low-affinity Na^+ uptake is toxic to the growth of glycophytic plants. Recently, several reports have suggested that the interaction between K^+ and Na^+ uptake might represent a key factor in determining the Na^+ tolerance of plants. We investigated the effects of K^+ starvation on Na^+ and K^+ uptake mechanisms in the plasma membrane of wheat (*Triticum aestivum* L.) root cortex cells using the patch-clamp technique. Unexpectedly, K^+ starvation of wheat seedlings was found to enhance the magnitude and frequency of occurrence of time-dependent inward-rectifying K^+ channel currents ($I_{K^+_{in}}$). We examined whether the transcription of a wheat root K^+_{in} channel gene is induced by K^+ starvation. A cDNA coding for a wheat root K^+ channel homolog, *TaAKT1* (accession no. AF207745), was isolated. *TaAKT1* mRNA levels were up-regulated in roots in response to withdrawal of K^+ from the growth medium. Furthermore, K^+ starvation caused an enhancement of instantaneous Na^+ currents (I_{Na^+}). Electrophysiological analyses suggested that $I_{K^+_{in}}$ and I_{Na^+} are not mediated by the same transport protein based on: (a) different activation curves, (b) different time dependencies, (c) different sensitivities to external Ca^{2+}, and (d) different cation selectivities. These data implicate a role for I_{Na^+} in Na^+ uptake and stress during K^+ starvation, and indicate that K^+_{in} channels may contribute to K^+-starvation-induced K^+ uptake in wheat roots.

The sensitivity of crop plants toward salinity is one of the major factors causing agricultural losses in arid regions (Greenway and Munns, 1980; Maas 1986). Studies analyzing the mechanisms of salinity have shown specific toxic effects of Na^+ ions (Kingsbury and Epstein, 1986). Under saline conditions, the large electrochemical Na^+ gradient results in passive Na^+ uptake into root cells (Smith and Walker, 1989; Allen et al., 1995; Tyerman and Skerrett, 1999). Long-term Na^+ influx by Na^+-permeable channels/transporters can elevate the cytoplasmic Na^+ concentration to toxic levels and trigger a variety of detrimental cellular effects (for review, see Volkmar et al., 1999). Kinetic analyses of low-affinity Na^+ uptake show multiple phases with distinguishable low-affinity components, leading to the suggestion that multiple low-affinity Na^+ uptake pathways exist in roots (Epstein and Rains, 1965).

Several ion transport and channel currents have been described in wheat (*Triticum aestivum* L.) root cells, including an outward-rectifying K^+ channel current ($I_{K^+_{out}}$) (Schachtman et al., 1991), inward-rectifying K^+ channel currents ($I_{K^+_{in}}$) (Findlay et al., 1994; Gassmann and Schroeder, 1994), a spiky inward K^+ channel (Findlay et al., 1994), an aluminum-activated anion channel current (Ryan et al., 1997), and a Na^+-dependent instantaneous current (Tyerman et al., 1997). Only the spiky K^+ current and the Na^+-dependent current were shown to carry Na^+ uptake currents (Tyerman et al., 1997). In contrast to animal cells, no specific Na^+ channel has been isolated in plants so far.

Inward Na^+ channel in root cells have also been reported from other species (for review, see Amtmann and Sanders, 1998; Tyerman and Skerrett, 1999). Several non-selective cation channels from rye root plasma membrane have been characterized in lipid bilayers, and one of these might be responsible for instantaneous currents measured in protoplasts of epidermal rye root cells (White and Lemtiri-Chlieh, 1995; White 1997). A Na^+-permeable channel was characterized in protoplasts of maize root cortical cells (Roberts and Tester, 1997). In barley suspension-cultured cells, instantaneously activating inward-rectifying currents that were permeable to Na^+ and K^+ were identified (Amtmann et al., 1997). From guard cells of two *Aster* species, non-rectifying cation channels that possess similar characteristics as the Na^+-dependent inward currents from roots have been reported (Véry et al., 1998).

Several earlier studies reported that K^+ starvation induces increased low-affinity Na^+ uptake (Pitman, 1967; Pitman et al., 1968; Ding and Zhu, 1997) and low-affinity K^+ uptake (Pitman et al., 1968; Benlloch et al., 1989; Maathuis and Sanders, 1995). In addition, K^+ starvation has been shown to reduce the selectivity of root membranes for K^+ over Na^+ (Pitman et al., 1968; Kochian et al., 1985). Enhancement of mRNA levels by K^+ starvation was also found for the high-affinity K^+ transporters *HKT1* in barley, wheat, and rice (Golldack et al., 1997; Wang et al., 1998), *HvHAK1* in barley (Santa-Maria et al., 1997), and *AtKUP3* in Arabidopsis (Kim et al., 1998). Interestingly, these K^+ transporters have been shown to mediate low-

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affinity Na^+ uptake under saline conditions (Rubio et al., 1995; Gassmann et al., 1996; Santa-Maria et al., 1997; Fu and Luan, 1998).

The characterization of K^+ channels proposed to contribute to low-affinity K^+ uptake is far more advanced than for the pathways of low-affinity Na^+ uptake. Plant inward-rectifying K^+ channels have been cloned from Arabidopsis and potato (Sentenac et al., 1992; for review, see Schroeder et al., 1994; Fox and Guerinot, 1998; Czempinski et al., 1999), but so far no inward-rectifying K^+ channel genes have been characterized from wheat.

In the present study we addressed the question of whether Na^+ and K^+ uptake currents in wheat root cells are affected by external K^+ supply during growth. We found that instantaneous Na^+ currents and time-dependent $I_{K^+_{in}}$ are enhanced by K^+ starvation. In addition, we observed a correlation between induced $I_{K^+_{in}}$ and the induction of an isolated wheat root K^+ channel gene.

MATERIALS AND METHODS

Isolation of the TaAKT1 cDNA

A wheat (*Triticum aestivum* L.) root-specific cDNA library constructed from wheat plants grown under K^+ starvation conditions (Schachtman and Schroeder, 1994) was used as template DNA for PCR using the following primers: 5′-TG GAA TTC ATG YTI MGI YTI TGG MG-3′ as the forward primer and 5′-ATR ACC WSI TAI TGI TG CCT AGG AA-3′ as the reverse primer where M represents A or C; R: A or G; S: C or G; W: A or T; Y: C or T; and I: inosine triphosphate. The resulting 270-bp PCR fragment, which was cloned and sequenced, shared homology to plant K^+ channels. With this PCR product as a probe, the wheat root cDNA library was screened to identify 16 putative clones. The cDNA with the largest insert size, named *TaAKT1*, was used for further studies. The *TaAKT1* cDNA was sequenced on both strands with an automatic sequencer (ABI, Sunnyvale, CA). Sequence analysis was performed with Lasergene 99 software from DNASTAR (Madison, WI). Homologous sequences were identified by searching within the GenBank database using BLAST (Altschul et al., 1990). The amino acid sequence was analyzed with the SMART program (Schultz et al., 1998). Putative transmembrane spans were predicted by the TopPred2 program (von Heijne, 1992).

RNA Isolation and Reverse Transcriptase (RT)-PCR Analysis of TaAKT1 Expression

Total RNA was isolated from wheat roots grown hydroponically in either 1 mM $CaCl_2$ and 0 mM KCl or 1 mM $CaCl_2$ and 5 mM KCl, as described below. Total RNA was quantitated spectrophotometrically. Dilutions of the RNA were electrophoresed on an RNA formaldehyde gel, and the intensity of the rRNA bands was compared to confirm that equal quantities of RNA were taken for first-strand cDNA syntheses. First-strand cDNA was synthesized from 5 μg of total RNA with a first-strand cDNA synthesis kit (Amersham-Pharmacia Biotech, Uppsala), diluted 1:10, and used as the template for the PCR reaction. For competitive PCR analyses, a PCR fragment was amplified from wheat genomic DNA using the same primer pair as for the RT-PCR. Due to the presence of introns, this fragment was 900 bp longer than the fragment amplified from the cDNA. Ten to 600 fg of competitive DNA was added to the PCR reactions, and 30 cycles of 94°C, 30 s; 60°C, 30 s; and 72°C, 1 min were performed. PCR products were analyzed by agarose gel electrophoresis. To confirm the results, total RNA was isolated from another set of plants grown under the same conditions and the RT-PCR experiment was repeated.

Growth of Plants and Protoplast Preparation

Wheat (cv Atlas 66) seeds were surface-sterilized with 0.5% (w/v) NaOCl, germinated on wetted filter paper for 4 d in the dark, and then transferred to hydroponic solutions containing either 1 mM $CaCl_2$ and 0 mM KCl or 1 mM $CaCl_2$ and 5 mM KCl. After 5 to 7 d, protoplasts were isolated as described by Schachtman et al. (1991). About 1 g of root tissue was chopped, washed, and then enzymatically digested in 0.8% (w/v) cellulase (Onozuka RS, Yakult Honsha, Tokyo) and 0.08% (w/v) pectolyase (Sigma Chemical Co., St. Louis) for 3 to 4 h. Root protoplasts were collected by a Suc density gradient. Large protoplasts with visible cytoplasmic streaming were selected for patch clamping. Previous studies have shown that protoplasts greater than 30 μm in diameter originate predominantly from the root cortex (Tyerman et al., 1997).

Electrophysiology

Patch-clamp pipettes were pulled from glass capillaries (Kimax 51, Kimble, Toledo, OH) on a multistage puller (P-87, Sutter Instrument, Novato, CA) and fire-polished to a tip resistance of 5 to 10 MΩ. Whole-cell currents across wheat root protoplasts were measured using the patch-clamp technique (Hamill et al., 1981) with an amplifier (Axopatch-1D, Axon Instruments, Foster City, CA). pClamp software (Axon Instruments) was used for the generation of voltage pulses, data recording, data storage, and data analysis. Patching of protoplasts was performed in a flow chamber of about 0.2-mL volume. To minimize offset potentials, agar salt bridges were used for the reference electrode. Ionic activities were calculated and corrected using GEOCHEM software (D.R. Parker, University of California, Riverside). Liquid junction potentials were calculated and corrected using JPCalc software (Barry, 1994). Data are presented as means ± SE, and the statistical significance of differences between currents was determined by the Student's *t* test.

Experimental Solutions

The standard intracellular solution used in patch-clamp experiments was composed of: 30 mM KCl, 70 mM K-Glu, 2 mM $MgCl_2$, 6.7 mM EGTA, 3.35 mM $CaCl_2$, 5 mM MgATP, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/Tris, pH 7.1. The standard bath solutions

contained: 100 mM KCl, 10 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM 2-(N-morpholino)-ethanesulfonic acid (MES)/Tris, pH 5.6 (standard K^+ solution), or 50 mM Na_2SO_4, 2 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 5 mM MES/Tris, pH 5.6 (standard Na^+ solution).

In addition, low-Cl^- intracellular and extracellular solutions were used in some experiments to exclude possible Cl^- efflux currents. These solutions included: (a) low-Cl^- intracellular solution: 100 mM K-Glu, 2 mM $MgCl_2$, 2 mM EGTA, 5 mM MgATP, and 5 mM HEPES/BTP, pH 7.1; (b) K-Glu extracellular solution: 100 mM K-Glu, 10 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM MES/Tris, pH 5.8; and (c) Na-Glu extracellular solution: 100 mM Na-Glu, 2 mM $MgCl_2$, 0.05 mM $CaCl_2$, 10 mM MES/Tris, pH 5.8. For selectivity examinations, the following solutions were used: 100 mM LiCl, 100 mM RbCl, or 100 mM CsCl, plus 2 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 10 mM MES/Tris, pH 5.8. Sorbitol was used to adjust the osmolality of the bath solutions to 625 mOsmol/kg and the pipette solutions to 650 mOsmol/kg.

RESULTS

Enhancement of $I_{K^+_{in}}$ by K^+ Starvation

In previous studies on wheat root cortex protoplasts from plants grown with regular K^+ supply (Schachtman et al., 1991; Findlay et al., 1994; Tyerman et al., 1997), $I_{K^+_{out}}$ were more frequently observed (79%) than $I_{K^+_{in}}$ (23%). We examined K^+ currents in wheat roots grown under K^+ starvation conditions (hydroponics with 1 mM $CaCl_2$ and 0 mM KCl) and found $I_{K^+_{in}}$ in 91% of the protoplasts (Fig. 1A) and $I_{K^+_{out}}$ in only 45% (n = 101 total protoplasts). Patch-clamp experiments in which the extracellular K^+ concentration was shifted from 100 to 30 mM followed by current reversal ("tail") analyses showed that $I_{K^+_{in}}$ were carried by K^+ (data not shown) and had similar properties to the $I_{K^+_{in}}$ previously described by Findlay et al. (1994). The increased frequency of $I_{K^+_{in}}$ under K^+-starved conditions suggested an effect of the growth conditions on the K^+ channel activity. We tested different K^+-supplemented growth solutions with regard to their effect on $I_{K^+_{in}}$. In general, we found a reduction of $I_{K^+_{in}}$ occurrences and magnitudes for wheat root protoplasts grown with K^+. $I_{K^+_{out}}$ were observed in 79% of the protoplasts and $I_{K^+_{in}}$ in only 54% of the measured root protoplasts grown with 1 to 5 mM K^+ (n = 56 total protoplasts). The average magnitude of $I_{K^+_{in}}$ from K^+-starved root protoplasts was higher (-19.96 ± 1.60 pA/pF at -148 mV, n = 18) than the average magnitude of $I_{K^+_{in}}$ from K^+-pretreated root protoplasts (-9.85 ± 1.39 pA/pF at -148 mV, n = 11; only protoplasts with $I_{K^+_{in}}$ were included for this analysis). The effect of adding 5 mM K^+ to the growth medium, enzyme solution, and protoplast storage solution is shown in Figure 1, A through C (Fig. 1C, $P < 0.001$ at -148 mV). Interestingly, for 6-d-old wheat seedlings grown in 1 mM $CaCl_2$ and 5 mM KCl, the root/shoot length ratio was about one-half the value of seedlings grown in 1 mM $CaCl_2$ and 0 mM KCl.

Further control experiments were performed with a low-Cl^- pipette solution (see "Material and Methods") to ex-

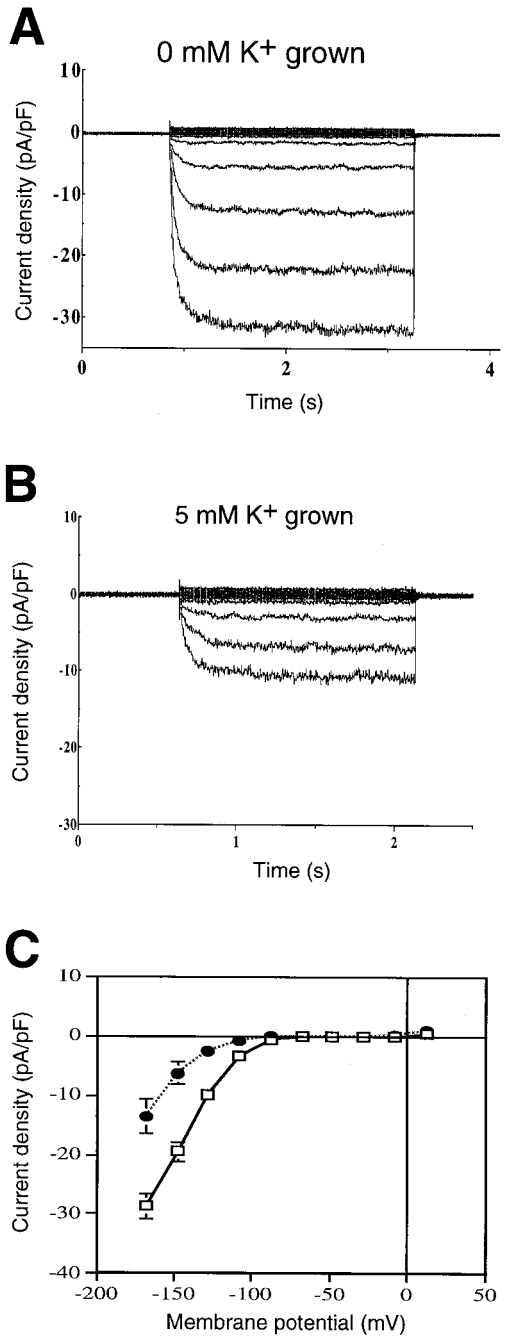

Figure 1. Whole-cell K^+_{in} currents in the plasma membrane of wheat root cortex protoplasts are enhanced by K^+ starvation during growth. A, Whole-cell currents from a wheat root cortical cell grown 6 d in 1 mM $CaCl_2$ and 0 mM KCl (capacitance = 30 pF). B, Whole-cell currents from a wheat root cortical cell grown for 6 d in 1 mM $CaCl_2$ and 5 mM KCl (capacitance = 34 pF). C, Average current-voltage relationships of whole-cell currents from wheat root cortical cells grown for 6 d in 1 mM $CaCl_2$ and 0 mM KCl (□, n = 11) or in 1 mM $CaCl_2$ and 5 mM KCl (●, n = 8). Means ± SE of time-dependent currents obtained by voltage pulses from +60 to −160 mV in 20 mV steps are shown in C. The holding potential was −30 mV. A standard K^+ bath solution and a standard pipette solution were used.

clude the possibility that inward-rectifying anion currents (Ryan et al., 1997) contributed to I_{K^+in}. We could not observe a significant difference in the kinetics (e.g. Fig. 6A) or current density of I_{K^+in} in response to the changed pipette solution (standard pipette solution: −19.4 ± 1.6 pA/pF at −148 mV, n = 11; low-Cl^- pipette solution: −19.6 ± 2.8 pA/pF at −148 mV, n = 8).

Cloning of the TaAKT1 Gene and K^+-Dependent mRNA Levels

The inward-rectifying K^+ channel AKT1 plays a role in K^+ uptake from roots, as evidenced by its preferential localization in roots (Lagarde et al., 1996) and reduced root growth in Arabidopsis mutants disrupted in the *AKT1* gene under low-K^+ and high-NH_4^+ conditions (Hirsch et al., 1998). However, there was no induction of *AKT1* RNA levels by low-K^+ conditions in *Brassica napus* roots (Lagarde et al., 1996). To examine whether a K^+ uptake channel gene is induced by K^+ starvation in wheat, we first cloned a wheat homolog of the Arabidopsis AKT1 channel gene. Degenerate PCR primers were based on the sequence MLRLWR in the S4 domain for the forward primer and on the sequence YWSITT in the pore domain of K^+_{in} channels for the reverse primer. These primers were used to amplify a fragment from a K^+-starved wheat root cDNA library (Schachtman and Schroeder, 1994) that showed homology to the corresponding region of *AKT1*. The PCR product was used as a probe to screen the wheat root cDNA library. A full-length cDNA of 3,300 bp (accession no. AF207745), encoding a deduced polypeptide of 897 amino acids, having a predicted molecular mass of 100.9 kD, and showing 76% similarity to the Arabidopsis AKT1 sequence (Sentenac et al., 1992), 84% similarity to a K^+ channel cDNA from maize (accession no. Y07632), and 77% to SKT1 from potato (Zimmermann et al., 1998) at the amino acid level, was isolated. The deduced polypeptide exhibited all of the structural features that are shared by the plant inward-rectifying K^+ channels: six transmembrane domains (S1–S6) (Uozumi et al., 1998), a K^+-selective pore-forming domain (P) between S5 and S6, a putative cyclic nucleotide-binding domain, and five ankyrin repeat sequences that are present only in the AKT subfamily of plant inward-rectifying K^+ channels (Sentenac et al., 1992). We therefore designated this gene *TaAKT1* (*T. aestivum AKT1*-like) (Fig. 2).

Since standard northern-blot experiments did not give us a clear signal with the *TaAKT1* probe, we performed RT-PCR experiments with TaAKT1-specific primers to determine whether the TaAKT1 gene was induced by K^+ starvation in wheat roots. Fragments of the expected size were detected in both K^+-starved (Fig. 3B) and 5 mM K^+-grown roots (Fig. 3A). Figure 3C shows the amount of RNA used for the first-strand cDNA synthesis. To analyze the level of induction of the *TaAKT1* transcript, we used the wheat cDNA together with different amounts of competitor DNA in PCR reactions. The comparison of the band intensity indicates a 3- to 10-fold induction in the *TaAKT1* message in replicate experiments in wheat roots grown at K^+-starved conditions (Fig. 3).

In our studies, K^+ starvation induced an increased expression of *TaAKT1*, and patch-clamp studies also showed an increased K^+_{in} current in K^+-starved wheat roots. AKT1 has been shown to function as a K^+_{in} channel in yeast (Bertl et al., 1997) and in insect cells (Gaymard et al., 1996).

Figure 2. Predicted amino acid sequence of TaAKT1. Transmembrane segments S1 to S6, pore region P, and the putative cyclic nucleotide binding site cNMP are underlined and labeled below the respective sequence; ankyrin-like repeats A1 to A5 are boxed. The protein sequence on which degenerate PCRs were based is boxed.

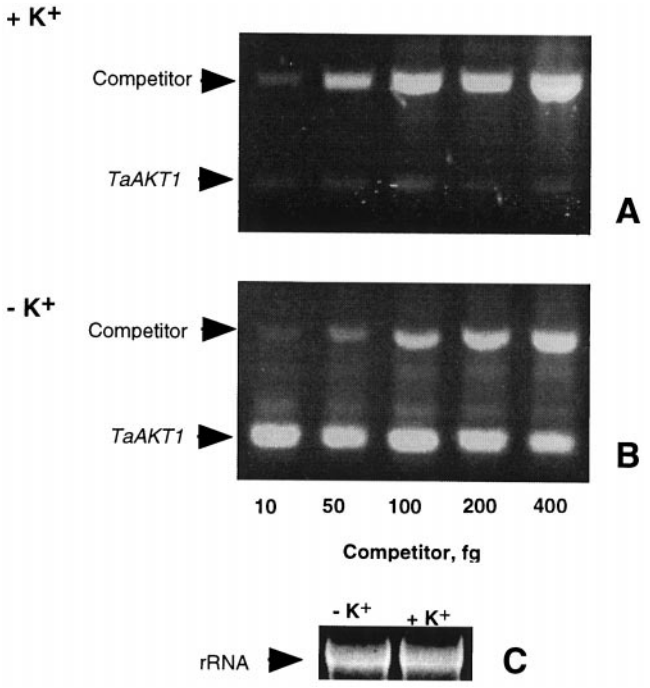

Figure 3. Enhancement of *TaAKT1* mRNA levels in response to K^+ deprivation. Total RNA extracted from wheat roots grown in 1 mM $CaCl_2$ and 5 mM KCl ($+K^+$ in A) or in 1 mM $CaCl_2$ and 0 mM KCl ($-K^+$ in B) was used for first-strand cDNA synthesis and subsequent PCR reactions. C shows the quantity of total RNA from K^+-starved ($-K^+$) and K^+-grown ($+K^+$) wheat roots used for first-strand cDNA synthesis. Total RNA electrophoresed on a formaldehyde gel was stained by ethidium bromide. For the competitor DNA, a PCR-amplified fragment isolated from the genomic DNA was used. This fragment was approximately 900 bp longer than the cDNA-amplified fragment because of introns. The indicated amounts of competitor DNA were added to the PCR reactions. Aliquots were analyzed by agarose gel electrophoresis.

Therefore, we propose that the TaAKT1 channel might contribute to induced $I_{K^+_{in}}$ in wheat root protoplasts.

Enhancement of I_{Na^+} by K^+ Starvation

After exchanging the K^+ bath solution with a Na^+-containing bath solution (100 mM Na^+), we found at low extracellular Ca^{2+} concentrations (0.05 mM) instantaneous inward-directed currents in root cortex protoplasts from K^+-starved wheat seedlings (Fig. 4; $n = 46$ protoplasts). When roots and root protoplasts were pretreated with K^+, only reduced instantaneous currents were observed ($n = 21$ protoplasts). Adding 5 mM KCl to the growth medium, enzyme solution, and protoplast storage solution caused about a 3-fold decrease of the instantaneous currents (Fig. 4C; $P < 0.01$ at -147 mV). On the other hand, increasing the bath Na^+ concentration from 100 to 180 mM Na^+ increased the current and shifted the whole-cell reversal potential to more positive values (Fig. 4, C and D, $P < 0.05$ at -147 mV). Control measurements with low-Cl^- pipette solutions were performed to exclude effects of inward-rectifying anion currents ($n = 9$). The time dependence of activation of the Na^+-dependent currents (e.g. Fig. 6B) and the current density were not affected by Cl^- ions (standard pipette solution: -4.6 ± 0.6 pA/pF at -147 mV, $n = 11$; low-Cl^- pipette solution: -4.3 ± 1.2 pA/pF at -147 mV, $n = 9$). We examined the selectivity of the instantaneous currents with respect to monovalent cations. For Li^+ and Cs^+ ions (100 mM), we found only small background currents at hyperpolarized membrane potentials (Fig. 5, A and C). When protoplasts were extracellularly exposed to 100 mM Rb^+, we observed small time-dependent currents that might have been mainly mediated by $I_{K^+_{in}}$ (data not shown). The positive shifts in reversal potentials upon increasing extracellular Na^+ concentration, the Cl^- independence, and the reduction of clearly resolved currents in the presence of Li^+ or Cs^+ show that the observed instantaneous currents were mainly carried by Na^+ ions which were named I_{Na^+}.

We pursued experiments to determine whether I_{Na^+} was related to $I_{K^+_{in}}$. The time dependencies of activation and deactivation of $I_{K^+_{in}}$ and I_{Na^+} were found to be clearly different. $I_{K^+_{in}}$ showed a time-dependent activation characteristic for K^+_{in} channels, whereas I_{Na^+} activated with a major rapid component (Fig. 6, B and C). In addition, the activation curve of $I_{K^+_{in}}$ could be well described by a Boltzmann curve, as reported for other $I_{K^+_{in}}$ in roots (e.g. White, 1997). In contrast, the conductance-voltage relationship for I_{Na^+} was nearly linear (Fig. 6D). A similar conductance-voltage relationship for Na^+-dependent currents has been reported in maize roots (Roberts and Tester, 1997). In addition, we found, in agreement with other studies (Tyerman et al., 1997), that recordings of I_{Na^+} were possible in protoplasts without $I_{K^+_{in}}$ ($n = 8$). Furthermore, $I_{K^+_{in}}$, but not I_{Na^+}, could be blocked by addition of 10 mM CsCl to the bath solution. Thus, I_{Na^+} shared several biophysical properties that could be clearly distinguished from $I_{K^+_{in}}$, showing that different proteins account for the two currents.

Ca^{2+} is known to have ameliorative effects on plants under high-salinity conditions (LaHaye and Epstein, 1969) and to reduce low-affinity Na^+ uptake (Rengel, 1992). Therefore, we tested the effect of increased external Ca^{2+} on I_{Na^+}. Protoplasts recorded in the presence of 4 mM Ca^{2+} (Fig. 7A) showed reduced I_{Na^+} in comparison with I_{Na^+} recorded in the presence of 0.05 mM Ca^{2+} (Fig. 7, B and C, $P < 0.02$ at -147 mV). Reversibility of Ca^{2+} reduction in I_{Na^+} was tested next. When the Na^+ bath solution with 4 mM Ca^{2+} (Fig. 7A) was exchanged by a Na^+ bath solution with 0.05 mM Ca^{2+} (same protoplast), the magnitude of I_{Na^+} increased (Fig. 7B, $n = 2$).

Protein kinases and phosphatases are involved in the response to salt stress in yeast and plant cells (Lee et al., 1999; Serrano et al., 1999). We tested the effect of externally applied deltamethrin (2 nM), an inhibitor of type 2B protein phosphatases, and okadaic acid (100 nM), an inhibitor of type 2A protein phosphatases. In both cases we observed only small changes in the current density of $I_{K^+_{in}}$ (not shown) or I_{Na^+} (standard Na^+ bath solution: -4.6 ± 0.6 pA/pF at -147 mV, $n = 11$; standard Na^+ bath solution with 2 nM deltamethrin: -5.9 ± 1.1 pA/pF at -147 mV,

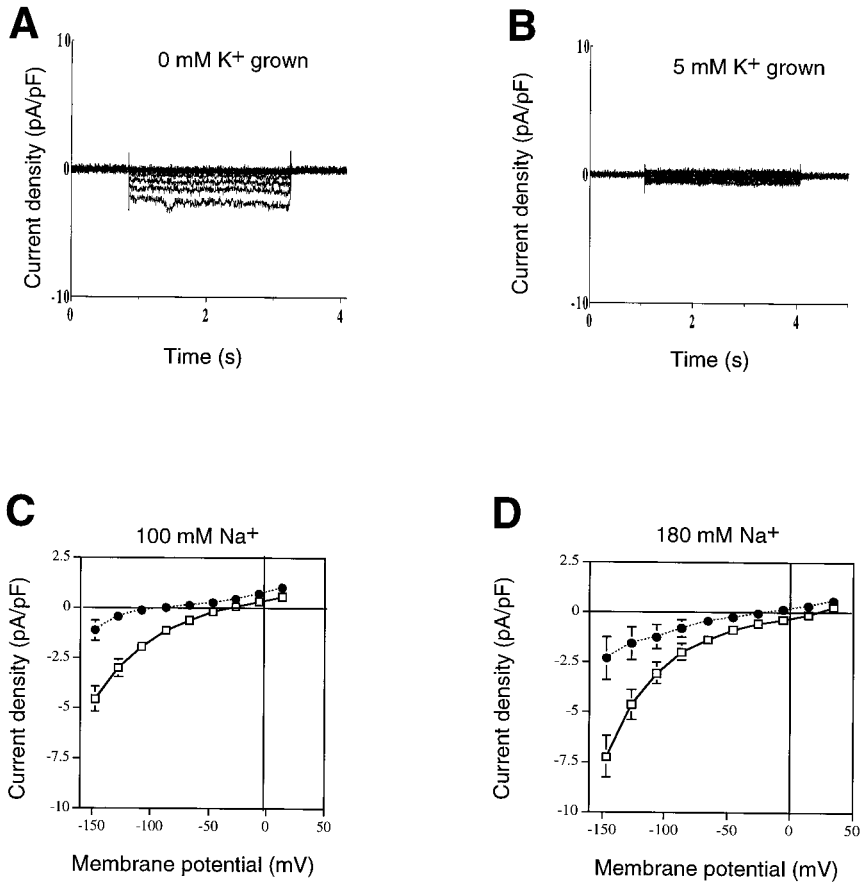

Figure 4. K^+ deprivation induces instantaneous Na^+ currents measured across the plasma membrane of protoplasts isolated from the cortex of wheat roots. A, Whole-cell currents from a K^+-starved wheat root cortex cell measured in a bath solution with 50 mM Na_2SO_4, 2 mM $MgCl_2$, and 0.05 $CaCl_2$ (capacitance = 36pF). Wheat seedlings were grown 6 d in 1 mM $CaCl_2$. B, Whole-cell currents from a 5 mM K^+-grown wheat root cortex cell measured in a bath solution with 50 mM Na_2SO_4, 2 mM $MgCl_2$, and 0.05 $CaCl_2$ (capacitance = 52pF). Wheat seedlings were grown 6 d in 1 mM $CaCl_2$ and 5 mM KCl. C, Average current-voltage relationships of whole-cell currents from wheat root cortex cells measured as in A and B. Wheat seedlings were grown for 6 d in 1 mM $CaCl_2$ and 0 mM KCl (□, n = 11) or in 1 mM $CaCl_2$ and 5 mM KCl (●, n = 11). D, Average current-voltage relationships of whole-cell currents from wheat root cortex cells measured in bath solution with 90 mM Na_2SO_4, 2 mM $MgCl_2$, and 0.05 $CaCl_2$. Wheat seedlings were grown for 6 d in 1 mM $CaCl_2$ and 0 mM KCl (□, n = 9) or in 1 mM $CaCl_2$ and 5 mM KCl (●, n = 6). Holding potential was −30 mV. Voltage pulses from +40 to −140 mV in 20 mV steps were applied.

n = 7; standard Na^+ bath solution with 100 nM okadaic acid: −5.3 ± 0.8 pA/pF at −147 mV, n = 5).

DISCUSSION

Induction of K^+ Currents and of TaAKT1 mRNA

Inward-rectifying K^+ channels provide a pathway for K^+ uptake in root cells (see the introduction). In an earlier study on wheat roots grown with regular K^+ supply, $I_{K^+_{in}}$ could be found in only 23% (n = 184) of the protoplasts (Findlay et al., 1994). Several explanations have been discussed, and it has been suggested that the occurrence of $I_{K^+_{in}}$ is limited to specific locations in the root cortex. On the other hand, the outward-rectifying K^+ channel could be observed in 66% to 79% of the wheat root protoplasts (Schachtman et al., 1991; Findlay et al., 1994). Here we show that $I_{K^+_{in}}$ are induced by K^+ starvation. We found $I_{K^+_{in}}$ in 91% and $I_{K^+_{out}}$ in 45% of the K^+-starved wheat root protoplasts (n = 101 protoplasts). However, when protoplasts were pretreated with 5 mM K^+ we observed $I_{K^+_{in}}$ in 54% and $I_{K^+_{out}}$ in 79% of the measured root protoplasts (n = 56 protoplasts). In addition, the K^+-pretreated root protoplasts were found to have a decreased magnitude of $I_{K^+_{in}}$. Interestingly, in a study on Arabidopsis root protoplasts low external K^+ supply also caused an enhanced activity of $I_{K^+_{in}}$ (Maathuis and Sanders, 1995).

We examined the possibility that a K^+ uptake channel gene is induced by K^+ starvation in wheat roots. We isolated *TaAKT1*, a complementary DNA from wheat that shows high sequence homology to the Arabidopsis inward-rectifying K^+ channel gene *AKT*1 (Sentenac et al., 1992). The deduced polypeptide sequence of TaAKT1 shows the typical features of the plant K^+_{in} channels and animal shaker K^+ channels. Each of the four subunits of a functional K^+ channel consists of six transmembrane domains and a short hairpin segment called the P loop that is located between S5 and S6 and determines the selectivity of the pore. The AKT family of plant K^+ uptake channels contain five ankyrin repeats in their C-terminal halves, which might represent potential domains for interactions of membrane proteins with the cytoskeleton, as has been shown in animal cells (Sentenac et al., 1992; for review, see Czempinsky et al., 1999).

Earlier models suggested that K^+ absorption is mediated by cotransporters at micromolar K^+ concentrations and by channels at higher concentrations. The level of the *AKT1* gene in *B. napus* roots was unaffected by external K^+ concentrations, suggesting that AKT1 is a constitutive component of K^+ uptake (Lagarde et al., 1996). On the other hand, models have been proposed in which K^+_{in} channels could contribute to both low- and high-affinity K^+ uptake (Hedrich and Schroeder, 1989; Gassmann et al., 1993; Schroeder et al., 1994). AKT1 expression in yeast (Sente-

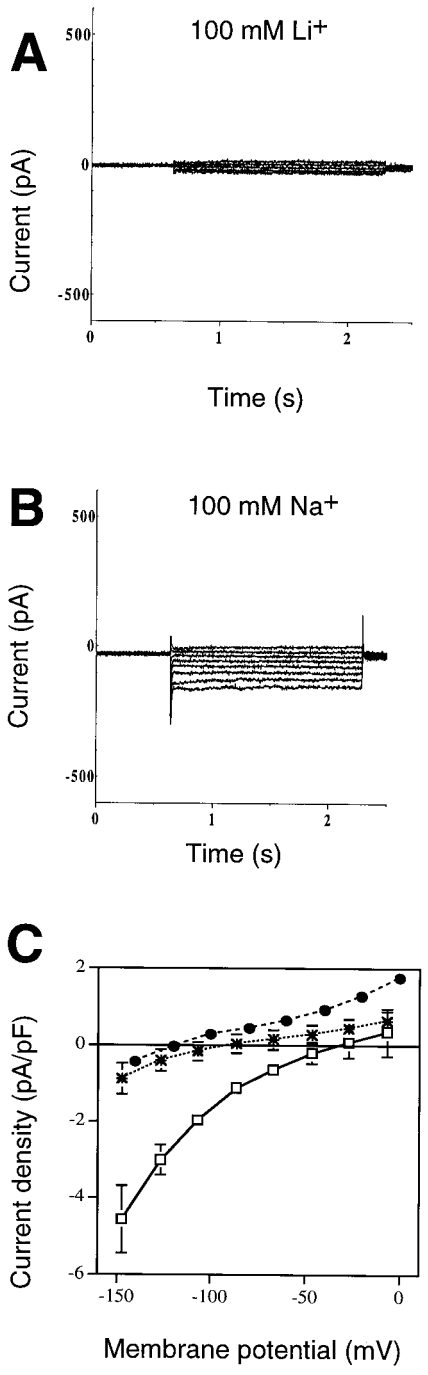

Figure 5. Alkali cation selectivity of instantaneous whole-cell inward currents from wheat root protoplasts under low external Ca^{2+} conditions (0.05 mM $CaCl_2$). A, Whole-cell currents from wheat root protoplasts measured with 100 mM LiCl and 0.05 mM $CaCl_2$ in the bath solution and using standard pipette solution (C = 65pF). B, Whole-cell currents from wheat root protoplasts measured with 100 mM Na-Glu and 0.05 mM $CaCl_2$ in the bath solution and standard pipette solution (same protoplast as in 5A). C, Average steady-state current-voltage relationships of whole-cell currents from wheat root cortex cells measured in bath solutions with 100 mM Na^+ (□, n = 11), Cs^+ (×, n = 6), and Li^+ (●, n = 5). Holding potential was −30 mV. Voltage pulses from 0 to −140 mV in 20 mV steps were applied. Wheat seedlings were grown for 6 d in 1 mM $CaCl_2$.

nac et al., 1992) and native expression in Arabidopsis roots (Hirsch et al., 1998; Spalding et al., 1999) have demonstrated that AKT1 can mediate high-affinity K^+ uptake at micromolar K^+. An Arabidopsis mutant disrupted in the *AKT1* channel gene showed reduced growth at 100 μM external K^+ when ammonium was added to the growth medium (Hirsch et al., 1998). These results, together with the induction of the *TaAKT1* mRNA and K^+_{in} in wheat roots by K^+ starvation, suggest that K^+_{in} channels might be involved not only in constitutive, but also in inducible, K^+ uptake in wheat roots.

I_{Na}^+ in Wheat Root Cells

We studied a Na^+-dependent inward current found in a bath solution with high Na^+ and low Ca^{2+} concentrations. I_{Na}^+ have been reported in previous studies. In wheat roots, Na^+ currents that show a fast-activating and a slow-activating component have been described (Tyerman et al., 1997). It is likely that the instantaneous currents reported here are related to these currents, although a comparison is complicated by the use of different varieties of wheat, different growth conditions, and the fact that in the previous study all Na^+ bath solutions contained 10 mM KCl. Under the conditions reported here, we found that the magnitude of I_{Na}^+ from K^+-starved wheat root cortex protoplasts was about 3-fold enhanced compared with protoplasts from K^+-supplemented roots. This increase in I_{Na}^+ could be mediated by transcriptional regulation and/or by post-translational modification.

Previous studies reported that Na^+-dependent inward currents from different root plasma membranes are insensitive to the K^+ channel blockers Cs^+ (up to 10 mM), TEA^+ (up to 20 mM), and TTX^+ (up to 50 μM) (Roberts and Tester, 1997; Tyerman and Skerrett, 1999). Tyerman et al. (1997) determined permeability ratios of Na^+-dependent currents in wheat root protoplasts showing a relatively low whole-cell P_{K+}/P_{Na+} (approximately 1.7:1). In addition, Cs^+ ions were found to be more permeable than Na^+ ions. As pointed out previously (Tyerman et al., 1997), whole-cell reversal potentials of relatively small I_{Na}^+ provide only approximate permeability ratio values, because proton ATPases hyperpolarize root cells and other background conductances are included. Under the K^+ starvation conditions used here, we found a K^+/Na^+ permeability ratio of about 2.6 and a Cs^+/Na^+ permeability ratio of about 0.12 based on shifts in the whole-cell reversal potential. It is possible that the previously described Na^+-dependent currents (Tyerman et al., 1997) and K^+-starvation-induced I_{Na}^+ are mediated by different transport proteins, or that under these different conditions several channels/transporters make different relative contributions to an observed whole-cell I_{Na}^+.

Ca^{2+} is known to have ameliorative effects on plants under high-salinity conditions (LaHaye and Epstein, 1969; Greenway and Munns, 1980; Rengel, 1992). In agreement with previous Na^+ flux measurements (Allen et al., 1995; Davenport et al., 1997) and patch-clamp studies (White and Lemtiri-Chlieh, 1995; Roberts and Tester, 1997; Tyerman et al., 1997), we found a suppression of I_{Na}^+ by high external

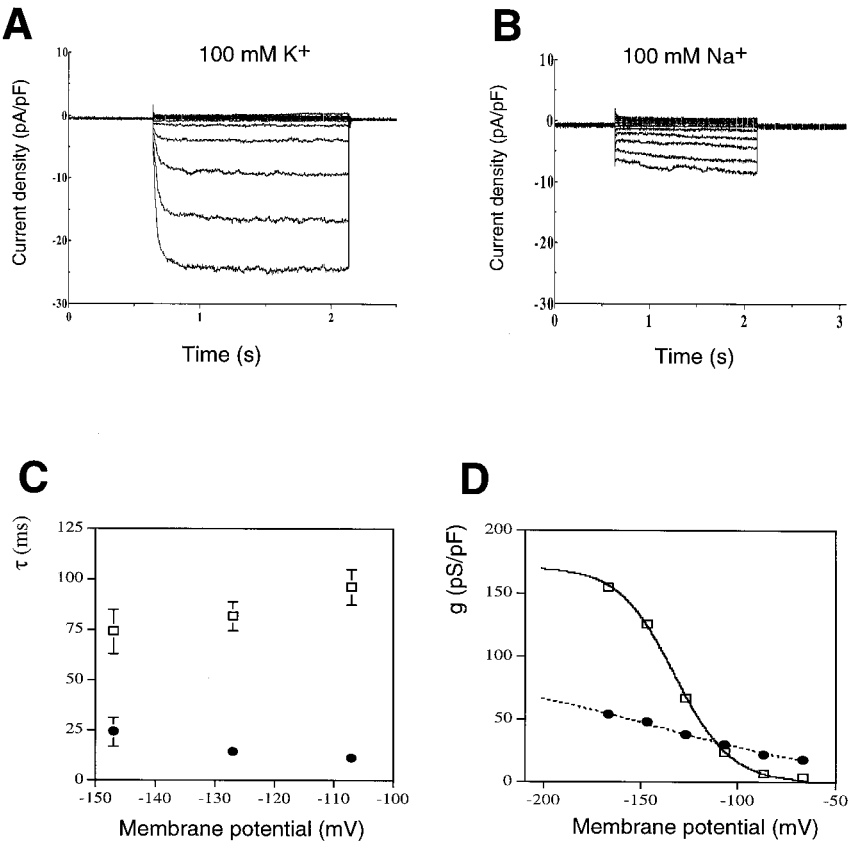

Figure 6. Comparison of whole-cell K^+- and Na^+-dependent inward currents from wheat root cortex cells. A, Whole-cell currents from a wheat root protoplast measured with 100 mM K^+ bath solution low-Cl^- pipette solution (C = 65 pF). Voltage pulses from +20 to −160 mV in 20 mV steps were applied. B, Whole-cell currents from a wheat root protoplasts measured with Na-Glu bath solution and low-Cl^- pipette solution (C = 31 pF). Voltage pulses from +40 to −140 mV in 20 mV steps were applied. C, Average fast time constants of activation for $I_K{}^+{}_{in}$ (□, $n = 6$) and $I_{Na}{}^+$ (●, $n = 5$). D, Average steady-state chord conductance as a function of membrane potential. The chord conductance density was calculated as $g = I/(E_M - E_X)$, where I is the steady-state inward current density, E_M the clamped membrane potential, and E_X the reversal potential of the inward currents. A Boltzmann equation of the form $g = g_{max}/(1+\exp[\{E_M - E_{1/2}\}/\{RT/z_gF\}])$ was fitted to the data, where g_{max} is the maximal chord-conductance density, $E_{1/2}$ is the voltage at which the chord conductance density is half-maximal, R, T, and F have their usual thermodynamic meanings, and z_g is the valency of the gating charge. Fitting was done by a simplex method. For $I_K{}^+{}_{in}$ we obtained g_{max} = 171.1 pS/pF, $E_{1/2}$ = −132.5 mV, RT/z_gF = 14.4 mV (z_g = 1.75). Holding potential was −30 mV. Wheat seedlings were grown for 6 d in 1 mM $CaCl_2$.

Ca^{2+} concentrations. The correlation between the protective effect of external Ca^{2+} under high-salinity conditions for the whole plant and the suppression of Na^+ uptake by Ca^{2+} indicates a physiological role for these Na^+-dependent currents in salt stress. Note that the Na^+ uptake rate into the roots of a salt-sensitive wheat species and a salt-tolerant wheat species did not differ significantly (Davenport et al., 1997), suggesting that Na^+ tolerance is mediated by other mechanisms.

Protein phosphorylation and dephosphorylation constitute a general mechanism by which plant cells regulate cellular mechanisms in response to changes in the extracellular environment (Stone and Walker, 1995; Luan, 1998). Several protein kinases and phosphatases are involved in the response to salt stress in yeast and plant cells (Lee et al., 1999; Serrano et al., 1999). In Arabidopsis, the *SOS3* gene was found to exhibit sequence similarity to the Ca^{2+}-binding domain of a 2B-type protein phosphatase. Recessive *sos3* mutants show a Ca^{2+}-dependent reduction in high-affinity K^+ uptake and hypersensitivity to NaCl stress (Liu and Zhu, 1998). We found small effects of externally applied phosphatase inhibitors deltamethrin (2 nM) and okadaic acid (100 nM) on $I_{Na}{}^+$ under the imposed conditions, and further studies will be pursued.

Candidate Molecular Mechanisms and Physiological Roles of Na^+ Currents

Thus far, the Na^+-dependent currents in plant root cells have not been directly linked to specific genes or proteins, but several candidate genes have been characterized. It is possible that channels/transporters, which under non-saline conditions mediate the transport of other ions, become Na^+ permeable under saline conditions (Khakh and Lester, 1999). This possibility has also been examined for $K^+{}_{in}$ channels. In several studies it was found that $I_K{}^+{}_{in}$ are highly selective for K^+ over Na^+, and therefore are not likely to represent Na^+ uptake pathways (Gassmann and Schroeder, 1994; Tyerman et al., 1997; Amtmann and Sanders, 1998).

However, some of the recently isolated cation uptake transporters could be involved in Na^+ uptake (for review, see Schachtman and Liu, 1999). The K^+ transporter HKT1 is rapidly induced at the transcriptional level in wheat and barley roots by K^+ starvation (Wang et al., 1998) and mediates low-affinity Na^+ uptake in oocytes and in yeast (Rubio et al., 1995). In addition, the voltage dependence, time dependence, and cation selectivity of $I_{Na}{}^+$ show correlations to HKT1-mediated low-affinity Na^+ currents. However, HKT1-mediated low-affinity Na^+ currents were reported to be insensitive to external Ca^{2+} in *Xenopus* oocytes (Tyerman and Skerrett, 1999). The absence of HKT1 modulation by Ca^{2+} in oocytes does not exclude the possibility of Ca^{2+} regulation of HKT1 in plants via post-translational mechanisms, as recent research suggests that Ca^{2+} regulation is mediated by phosphorylation/dephosphorylation (Liu and Zhu, 1998).

Other channels/transporters may also contribute to $I_{Na}{}^+$. Recently, a non-specific cation channel NSC1 that is regulated by Ca^{2+} was found in yeast (Bihler et al., 1998),

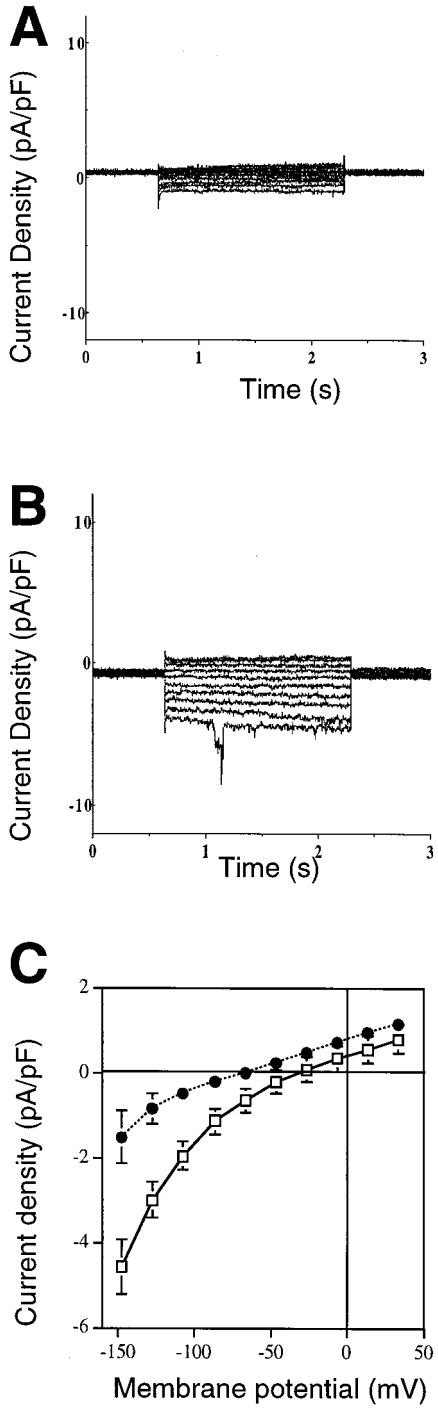

Figure 7. Reduction of I_{Na}^+ from wheat root protoplasts by increased external Ca^{2+} concentration. A, Whole-cell currents from a wheat root cortical cell measured with 100 mM Na-Glu and 4 mM $CaCl_2$ bath solution and standard pipette solution (C = 50 pF). B, Whole-cell currents from the same protoplasts as in Figure 7A measured with 100 mM Na-Glu and 0.05 mM $CaCl_2$ bath solution and standard pipette solution. C, Average current-voltage relationships of whole-cell currents from wheat roots cortex cells measured with 100 mM Na-Glu and 4 mM $CaCl_2$ (●, $n = 5$) or 0.05 mM $CaCl_2$ (□, $n = 6$) bath solution and standard pipette solution. Holding potential was −30 mV. Voltage pulses from +40 to −140 mV in 20 mV steps were applied. Wheat seedlings were grown for 6 d in 1 mM $CaCl_2$.

but the underlying gene remains unknown. LCT1 (Schachtman et al., 1997), AtKUP1 (Fu and Luan, 1998; Kim et al., 1998), and HvHAK1 (Santa-Maria et al., 1997) have also been shown to mediate low-affinity Na^+ uptake in yeast. The low-affinity Na^+ transport capabilities of these transporters seemed to be lower than HKT1 under the imposed conditions in yeast. Electrophysiological recordings of I_{Na}^+ via LCT1, AtKUP1, and HvHAK1 have not yet been reported, and therefore a comparison to I_{Na}^+ in root cells is not yet possible.

Single-channel currents that can account for the Na^+-dependent whole-cell currents have been reported (Roberts and Tester, 1997; Tyerman et al., 1997; White, 1997). In animal systems it was discovered in recent years that transporters also can exhibit channel behavior, and that the classical distinction between transporters and channels does not always hold at the molecular level (Fairman et al., 1995; Cammack and Schwartz, 1996; Lin et al., 1996; Sonders and Amara, 1996; Su et al., 1996). As discussed previously, it seems likely that plant transporters such as AtKUPs and HKT1 could show channel conductance states (Gassmann et al., 1996; Fu and Luan, 1998; Chrispeels et al., 1999, Durell et al., 1999). Further research is needed to directly identify the different membrane channel/transporter genes contributing to inward-directed Na^+ currents in root cells.

The physiological role of Na^+ uptake has been the subject of discussion, because Na^+ is not essential for most plants and excessive low-affinity Na^+ uptake can result in toxic cytoplasmic Na^+ concentrations. Our results, together with other studies (Pitman et al., 1968; Zhu et al., 1998), suggest that the availability of K^+ in the growth medium is a crucial parameter in inducing Na^+ uptake. The recent findings that high-affinity K^+ uptake transporters are induced by K^+ starvation, and that these transporters also mediate low-affinity Na^+ uptake provide a possible model for the inducibility of I_{Na}^+ reported here. Another non-exclusive explanation would be that low-affinity Na^+ uptake pathways are activated in K^+-starved plants, because the plants can to a certain degree compensate K^+ deficiency by Na^+ uptake (Mengel and Kirkby, 1982; Flowers and Läuchli, 1983; Rodriguez-Navarro, 2000). Further research should allow the establishment of relative contributions of individual genes responsible for Na^+ uptake and I_{Na}^+ induced by K^+ starvation. Furthermore, the finding that both I_{K^+in} and *TaAKT1* mRNA are induced by K^+ starvation indicates that K^+_{in} channels may contribute to both constitutive and inducible K^+ uptake in wheat roots.

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LITERATURE CITED

Allen GJ, Jones RGW, Leigh RA (1995) Sodium transport measured in plasma membrane vesicles isolated from wheat geno-

types with differing K^+/Na^+ discrimination traits. Plant Cell Environ **18:** 105–115

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol **215:** 403–410

Amtmann A, Laurie S, Leigh R, Sanders D (1997) Multiple inward channels provide flexibility in Na^+-K^+ discrimination at the plasma membrane of barley suspension culture cells. J Exp Bot Special Issue **48:** 481–497

Amtmann A, Sanders D (1998) Mechanisms of Na^+ uptake by plant cells. Adv Bot Res **29:** 75–112

Barry PH (1994) JPCalc, a software package for calculating liquid junction potential corrections in patch-clamp, intracellular, epithelial and bilayer measurements and for correction junction potential measurements. J Neurosci Methods **51:** 107–116

Benlloch M, Moreno I, Rodriguez-Navarro A (1989) Two modes of rubidium uptake in sunflower plants. Plant Physiol **90:** 939–942

Bertl A, Reid JD, Sentenac H, Slayman CL (1997) Functional comparison of plant inward-rectifier channels expressed in yeast. J Exp Bot Special Issue **48:** 405–413

Bihler H, Slayman CL, Bertl A (1998) NSC1: a novel high-current inward rectifier for cations in the plasma membrane of *Saccharomyces cerevisiae.* FEBS Lett **432:** 59–64

Cammack JN, Schwartz EA (1996) Channel behavior in a gamma-aminobutyrate transporter. Proc Natl Acad Sci USA **93:** 723–727

Chrispeels MJ, Crawford NM, Schroeder JI (1999) Proteins for transport of water and mineral nutrients across the membranes of plant cells. Plant Cell **11:** 661–675

Czempinski K, Gaedeke N, Zimmermann S, Müller-Röber B (1999) Molecular mechanisms and regulation of plant ion channels. J Exp Bot Special Issue **50:** 955–966

Davenport RJ, Reid RJ, Smith FA (1997) Sodium-calcium interactions in two wheat species differing in salinity tolerance. Physiol Plant **99:** 323–327

Ding L, Zhu J-K (1997) Reduced Na^+ uptake in the NaCl-hypersensitive *sos1* mutant of *Arabidopsis thaliana*. Plant Physiol **113:** 795–799

Durell SR, Hao Y, Nakamura T, Bakker EP, Guy HR (1999) Evolutionary relationship between K^+ channels and symporters. Biophys J **77:** 775–788

Epstein E, Rains DW (1965) Carrier-mediated cation transport in barley roots: kinetic evidence for a spectrum of active sites. Proc Natl Acad Sci USA **53:** 1320–1324

Fairman WA, Vandenberg RJ, Arriza JL, Kavanaugh MP, Amara SG (1995) An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. Nature **375:** 599–603

Findlay GP, Tyerman SD, Garrill A, Skerrett M (1994) Pump and K^+ inward rectifiers in the plasmalemma of wheat root protoplasts. J Membr Biol **139:** 103–116

Flowers TJ, Läuchli A (1983) *In* A Läuchli, RL Bieleski, eds, Inorganic Plant Nutrition, Vol 15B. Springer-Verlag, Berlin, p 651

Fox TC, Guerinot ML (1998) Molecular biology of cation transport in plants. Annu Rev Plant Physiol Plant Mol Biol **49:** 669–696

Fu HH, Luan S (1998) AtKUP1: a dual-affinity K^+ transporter from *Arabidopsis*. Plant Cell **10:** 63–73

Gassmann W, Rubio F, Schroeder JI (1996) Alkali cation selectivity of the wheat root high-affinity potassium transporter HKT1. Plant J **10:** 869–882

Gassmann W, Schroeder JI (1994) Inward-rectifying K^+ channels in root hairs of wheat: a mechanism for aluminum-sensitive low-affinity K^+ uptake and membrane potential control. Plant Physiol **105:** 1399–1408

Gassmann W, Ward JM, Schroeder JI (1993) Physiological roles of inward-rectifying K^+ channels. Plant Cell **5:** 1491–1493

Gaymard F, Cerutti M, Horeau C, Lemaillet G, Urbach S, Ravallec M, Devauchelle G, Sentenac H, Thibaud J-B (1996) The baculovirus-insect cell system as an alternative to *Xenopus* oocytes: first characterization of the AKT1 K^+ channel from *Arabidopsis thaliana*. J Biol Chem **271:** 22863–22870

Golldack D, Kamasani UR, Quigley F, Bennett J, Bohnert HJ (1997) Salt-stress-dependent expression of a HKT1-type high affinity potassium transporter in rice. Plant Physiol **114:** 118

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol **31:** 149–190

Hamill OP, Marty A, Neher E, Sakman B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch **391:** 85–100

Hedrich R, Schroeder JI (1989) The physiology of ion channels and electrogenic pumps in higher plants. Annu Rev Plant Physiol Plant Mol Biol **40:** 539–570

Hirsch RE, Lewis BD, Spalding EP, Sussman MR (1998) A role for the AKT1 potassium channel in plant nutrition. Science **280:** 918–921

Khakh BS, Lester HA (1999) Dynamic selectivity filters in ion channels. Neuron **23:** 653–658

Kim EJ, Kwak JM, Uozumi N, Schroeder JI (1998) *AtKUP1*: an arabidopsis gene encoding high-affinity potassium transport activity. Plant Cell **10:** 51–62

Kingsbury RW, Epstein E (1986) Salt sensitivity in wheat. Plant Physiol **80:** 651–654

Kochian LV, Xin-Zhi J, Lucas WJ (1985) Potassium transport in corn roots. Plant Physiol **79:** 771–776

Lagarde D, Basset M, Lepetit M, Conejero G, Gaymard F, Astruc S, Grignon C (1996) Tissue-specific expression of Arabidopsis *AKT1* gene is consistent with a role in K^+ nutrition. Plant J **9:** 195–203

LaHaye PA, Epstein E (1969) Salt toleration by plants: enhancement with calcium. Science **165:** 395–396

Lee JH, Van Montagu M, Verbruggen N (1999) A highly conserved kinase is an essential component for stress tolerance in yeast and plant cells. Proc Natl Acad Sci USA **96:** 5873–5877

Lin F, Lester HA, Mager S (1996) Single-channel currents produced by the serotonin transporter and analysis of a mutation affecting ion permeation. Biophys J **71:** 3126–3135

Liu J, Zhu J-K (1998) A calcium sensor homolog required for plant salt tolerance. Science **280:** 1943–1945

Luan S (1998) Protein phosphatases and signaling cascades in higher plants. Trends Plant Sci **3:** 271–275

Maas EV (1986) Salt tolerance of plants. Appl Agric Res **1:** 12–26

Maathuis FJM, Sanders D (1995) Contrasting roles in ion transport of two K^+-channel types in root cells of *Arabidopsis thaliana*. Planta **197:** 456–464

Mengel K, Kirkby EA (1982) Principles of Plant Nutrition, Ed 3. International Potash Institute, Worblaufen-Bern, Switzerland, pp 425–426

Pitman MG (1967) Conflicting measurements of sodium and potassium uptake by barley roots. Nature **216:** 1343–1344

Pitman MG, Courtice AC, Lee B (1968) Comparison of potassium and sodium uptake by barley roots at high and low salt status. Aust J Biol Sci **21:** 871–881

Rengel Z (1992) The role of calcium in salt toxicity. Plant Cell Environ **15:** 625–632

Roberts SK, Tester M (1997) A patch clamp study of Na^+ transport in maize roots. J Exp Bot Special Issue **48:** 431–440

Rodriguez-Navarro A (2000) Potassium transport in fungi and plants. Biochim Biophys Acta **1469:** 1–30

Rubio F, Gassmann W, Schroeder JI (1995) Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. Science **270:** 1660–1663

Ryan PR, Skerrett M, Findlay GP, Delhaize E, Tyerman SD (1997) Aluminum activates an anion channel in the apical cells of wheat roots. Proc Natl Acad Sci USA **94:** 6547–6552

Santa-Maria GE, Rubio F, Dubcovsky J, Rodriguez-Navarro A (1997) The *HAK1* gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. Plant Cell **9:** 2281–2289

Schachtman DP, Kumar R, Schroeder JI, Marsh EL (1997) Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. Proc Natl Acad Sci USA **94:** 11079–11084

Schachtman DP, Liu W (1999) Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. Trends Plant Sci **4:** 281–286

Schachtman DP, Schroeder JI (1994) Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. Nature **370:** 655–658

Schachtman DP, Tyerman SD, Terry BR (1991) The potassium, sodium selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. Plant Physiol **97:** 598–605

Schroeder JI, Ward JM, Gassmann W (1994) Perspectives on the physiology and structure of inward-rectifying K^+ channels in higher plants: biophysical implications for K^+ uptake. Annu Rev Biophys Biomol Struct **23:** 441–471

Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: Identification of signaling domains. Proc Natl Acad Sci USA **95:** 5857–5864

Sentenac H, Bonneaud N, Minet M, Lacroute F, Salmon J-M, Gaymard F, Grignon C (1992) Cloning and expression in yeast of a plant potassium ion transport system. Science **256:** 663–665

Serrano R, Mulet JM, Rios G, Marquez JA, de Larrinoa IF, Leube MP, Mendizabal I, Pascual-Ahuir A, Proft M, Ros R, Montesinos C (1999) A glimpse of the mechanisms of ion homeostasis during salt stress. J Exp Bot Special Issue **50:** 1023–1036

Smith FA, Walker NA (1989) Transport of potassium in *Chara australis*: I. A symport with sodium. J Membr Biol **108:** 125–138

Sonders MS, Amara SG (1996) Channels in transporters. Curr Opin Neurobiol **6:** 294–302

Spalding EP, Hirsch RE, Lewis DR, Qi Z, Sussman MR, Lewis BD (1999) Potassium uptake supporting plant growth in the absence of AKT1 channel activity: inhibition by ammonium and stimulation by sodium. J Gen Physiol **113:** 909–918

Stone JM, Walker JC (1995) Plant protein kinase families and signal transduction. Plant Physiol **108:** 451–457

Su A, Mager S, Mayo SL, Lester HA (1996) A multi-substrate single-file model for ion-coupled transporters. Biophys J **70:** 762–777

Tyerman SD, Skerrett IM (1999) Root ion channels and salinity. Sci Hortic **78**: 175–235

Tyerman SD, Skerrett M, Garrill A, Findlay GP, Leigh RA (1997) Pathways for the permeation of Na^+ and Cl^- into protoplasts derived from the cortex of wheat roots. J Exp Bot Special Issue **48:** 459–480

Uozumi N, Nakamura T, Schroeder JI, Muto S (1998) Determination of transmembrane topology of an inward-rectifying potassium channel from *Arabidopsis thaliana* based on functional expression in *Escherichia coli*. Proc Natl Acad Sci USA **18:** 9773–9778

Véry A-A, Robinson MF, Mansfield TA, Sanders D (1998) Guard cell cation channels are involved in Na^+-induced stomatal closure in a halophyte. Plant J **14:** 509–521

Volkmar KM, Hu Y, Steppuhn H (1999) Physiological responses of plants to salinity: a review. Can J Plant Sci **78**: 19–27

von Heijne G (1992) Membrane protein structure prediction: hydrophobicity analysis and the positive-inside rule. J Mol Biol **20:** 487–494

Wang T-B, Gassmann W, Rubio F, Schroder JI, Glass ADM (1998) Rapid up-regulation of *HKT1*, a high-affinity potassium transporter gene, in roots of barley and wheat following withdrawal of potassium. Plant Physiol **118:** 651–659

White PJ (1997) Cation channels in the plasma membrane of rye roots. J Exp Bot Special Issue **48:** 499–514

White PJ, Lemtiri-Chlieh F (1995) Potassium currents across the plasma membrane of protoplasts derived from rye roots: a patch-clamp study. J Exp Bot **46:** 497–511

Zhu J-K, Liu J, Xiong L (1998) Genetic analysis of salt tolerance in *Arabidopsis*: evidence for a critical role of potassium nutrition. Plant Cell **10:** 1181–1191

Zimmermann S, Talke I, Ehrhardt T, Nast G, Müller-Röber B (1998) Characterization of SKT1, an inwardly rectifying potassium channel from potato, by heterologous expression in insect cells. Plant Physiol **116:** 879–890