The effects of ABA on channel-mediated $K^+$ transport across higher plant roots

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Abstract

The transport and accumulation of $K^+$ in higher plant roots is regulated by ABA. Molecular and electrophysiological techniques have identified a number of discrete transporters which are involved in the translocation of $K^+$ from the soil solution to the shoots of higher plants. Furthermore, recent reports have shown that ABA regulates $K^+$ channel activity in maize and Arabidopsis roots which suggests that ABA regulation of $K^+$ transport in roots is, at least in part, ion channel-mediated. The signalling processes which underlie the ABA regulation of $K^+$ channels have been investigated. The effects of ABA on the membrane potential of intact maize root cells were also studied. It was found that ABA regulated the membrane potential of root cells and that this regulation is consistent with the hypothesis that ABA-induced $K^+$ accumulation in roots is mediated by $K^+$ channels.

Key words: ABA, $K^+$ channels, roots, maize.

Introduction

The roots of higher plants are responsible for the transport of ions from the soil solution to the xylem vessels and, via the transpiration stream, to the shoot. Although there are many specialized root cell types, with respect to ion transport they can be generalised to those that mediate the uptake of ions from the soil solution (i.e. epidermal and cortical cells) and those that mediate ion release into the transpiration stream (i.e. stelar cells). Separating the cortex from the stele is a single layer of endodermal cells. Associated with these cells is a unique structure which prevents ions from the soil solution being transported directly to the xylem vessels via the extracellular space of the root (i.e. apoplastic transport; see Fig. 1). The structure, called the Casparian strip, is located between the walls of the endodermal cells and forms a barrier to apoplastic movement of ions. In its mature form, the Casparian strip is composed of lignin and suberin which render it impermeant to ions (Robards et al., 1973). Thus the presence of the Casparian strip ensures that ions must enter the root symplasm via the plasma membrane of the cortical and epidermal cells or the outer face of the endodermis. Likewise, efflux from the root symplasm to the transpiration stream must cross the plasma membrane of cells within the stele of the root (Fig. 1).

$K^+$ transport across the root has been widely studied. $K^+$ is a major plant nutrient and is important in many

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cellular functions including turgor regulation, charge balance, regulation of the membrane potential, and the activity of cytosolic enzymes (Evans and Sorger, 1966). An overview of the transporters that facilitate the radial movement of K\(^+\) across the root is presented. Also discussed is recent evidence showing that some of these transporters are regulated by ABA and that this regulation probably reflects a mechanism to induce K\(^+\) accumulation in roots during water stress.

### K\(^+\) transport across higher plant roots

**K\(^+\) uptake from the soil solution**

Tracer flux studies investigating K\(^+\) uptake by roots identified two distinct systems (Epstein et al., 1963); a high affinity pathway operating at extracellular K\(^+\) concentration less than 1 mM (System 1) and a low affinity pathway predominant at concentrations greater than approximately 1 mM (System 2). Subsequent to these findings two types of mechanism have been identified at the molecular level in plant cells which mediate K\(^+\) uptake across the plasma membrane: (1) ion channel proteins which form pores in the plasma membrane (Doyl et al., 1998) and allow passive K\(^+\) transport down its electrochemical gradient, and (2) carrier proteins (Maathuis and Sanders, 1994) which couple the transport of K\(^+\) to the transport of another ion with an electrochemical gradient favouring its uptake across the membrane. The electrochemical gradient for an ion (e.g. K\(^+\) is determined by the electric potential difference across the membrane (\(V_m\)), the cytosolic K\(^+\) activity (K\(^+\)\(_{cyt}\)) and the extracellular K\(^+\) activity (K\(^+\)\(_{ext}\)). The Nernst equation indicates whether passive K\(^+\) uptake can be mediated by ion channels or if there is a need for carrier-mediated transport. If the membrane potential is more negative than the Nernst potential for K\(^+\) (\(E_K = \frac{RT}{F} \ln \frac{K_{ext}}{K_{cyt}}\)) then net K\(^+\) uptake can proceed via ion channels. When the membrane potential is positive of \(E_K\) then net passive K\(^+\) efflux is favoured and K\(^+\) uptake must occur via a carrier-type mechanism.

**High affinity transporters:** Using a yeast complementation method, a wheat cDNA (HKT1) was isolated which was (after heterologous expression in Xenopus oocytes) identified as a high affinity K\(^+\) symporter (Schachtman and Schroeder, 1994). Initially it was reported to be a K:\(\text{H}^+\) symporter (1:1 ratio), however, later studies concluded that HKT1 acted as a Na:\(\text{K}^+\) symporter in the presence of low concentrations of extracellular Na\(^+\) and as a Na:\(\text{Na}^+\) transporter (i.e. a low affinity Na\(^+\) transporter) when high (mM) Na\(^+\) was present (Rubio et al., 1995). Although in situ hybridization located expression to the cortex of the root, the activity of HKT1 in planta has yet to be confirmed either as a high affinity K\(^+\) transporter or as a low affinity Na\(^+\) transporter (Walker et al., 1996).

More recently, other genes coding for high affinity K\(^+\) transporters have been identified. HvHAK1 was identified in barley by homology to HAK1 and the KUP family from *S. occidentalis* and *E. coli*, respectively (Santa-Maria et al., 1997). Heterologous expression studies using a yeast mutant (trak1\(\Delta trk2\ Delta\), which is defective in K\(^+\) uptake and requires high (mM) extracellular K\(^+\) to grow, revealed that HvHAK1 mediated K\(^+\) uptake (with a \(K_m\) of 27 \(\mu\)M) independently of (but was inhibited by) extracellular Na\(^+\). HvHAK1 is expressed in the roots and its expression is enhanced in K\(^+\)-depleted conditions. In *Arabidopsis*, AtKUP genes were identified independently by three groups (Quintero and Blatt, 1997; Fu and Luan, 1998; Kim et al., 1998). They are highly homologous to the HvHAK1 and KUP genes in *E. coli* and are expressed in the roots and shoots. Heterologous expression in *trak1\(\Delta trk2\Delta yeast* revealed that they mediate both high affinity (\(K_m\) approximately 20 \(\mu\)M) and low affinity K\(^+\) uptake and are also inhibited by extracellular Na\(^+\). The mechanism of K\(^+\) transport for the HAK and KUP proteins is not known.

**Low affinity transporters:** Channel-mediated K\(^+\)-dependent inward currents have been characterized in many plant cell types including those from the cortex of maize roots (Roberts and Tester, 1995) and the epidermis of wheat roots (Gassmann and Schroeder, 1994). The inward-rectifying (\(K_m\)) channels which underlie these currents were proposed to represent the pathway for low affinity K\(^+\) uptake (Kochian and Lucas, 1988). This type of channel only activates at potentials negative of \(E_K\) (in a voltage-dependent manner) and is K\(^+\) selective, showing lesser permeability for Rb\(^+\) and Na\(^+\) and channel blockage by extracellular Cs\(^+\). Complementation studies of the K\(^+\) uptake-defective yeast mutant identified two cDNAs (KAT1 and AKT1) coding for K\(^+\) channels from *Arabidopsis* (Sentenac et al., 1992; Anderson et al., 1992). Since their identification several homologues to AKT1 and KAT1 have been reported (Coa et al., 1995). However, the focus will exclusively be on AKT1 because it was found to be expressed predominantly in the cortex and epidermis of roots. Unlike expression of the AtKUP and HvHAK1, AKT1 expression is not regulated by K\(^+\) availability. Patch clamp analysis of AKT1 activity after heterologous expression in yeast (Bertl et al., 1997) revealed \(K_m\) channels which displayed similar biophysical properties (i.e. pharmacology, selectivity and activation kinetics) to the \(K_m\) channels observed in maize and wheat roots. However, AKT1 expression also conferred the ability of the K\(^+\) uptake-defective yeast mutant to grow at submillimolar K\(^+\) concentrations. These results suggested that the \(V_m\) of the yeast plasma membrane was either very negative (i.e. greater than -300 mV) or that AKT1 possessed a dual functionality that allowed K\(^+\) uptake which was thermodynamically uphill. Recently,
an Arabidopsis T-DNA insertion AKT1-knockout mutant (akt1–1) has been identified (Hirsch et al., 1998). The absence of $K_m$ channels in the cortex was confirmed using the patch clamp technique. The phenotype of akt1–1 was defective $K^+$ uptake and growth in solutions as dilute as 10 $\mu$M $K^+$; this suggested that AKT1 mediated $K^+$ uptake at concentrations previously thought to be beyond the realms of $K^+$ channels (i.e. high affinity $K^+$ uptake). However, electrode impalement experiments with akt1–1 revealed that the $V_m$ of the plasma membrane was more negative than $-230$ mV, indicating that channel-mediated $K^+$ uptake from 10 $\mu$M $K^+$-containing solutions was energetically possible. The akt1–1 phenotype was only apparent in the presence of extracellular $NH_4^+$, in the absence of $NH_4^+$ the mutant and wild-type Arabidopsis grew similarly. The most likely explanation for this is that $NH_4^+$ inhibits high affinity $K^+$ transporters and maintains a sufficiently negative membrane potential to enable high affinity $K^+$ uptake via $K_m$ channels. Consistent with this, HvHAK1 is inhibited by $NH_4^+$ (Santa-Maria et al., 1997). In summary, it appears that channels can mediate high affinity $K^+$ uptake in some conditions. However, in most conditions, when high affinity $K^+$ transporters are functional, channels are unlikely to contribute to $K^+$ uptake from solutions which contain less than 100–200 $\mu$M $K^+$ (Maathuis and Sanders, 1993)

**$K^+$ transport in the stele**

After entering the root symplasm, $K^+$ can diffuse (via plasmodesmata) to the cells within the stele. Radial diffusion across the root probably results from a $K^+$ gradient and/or potential difference between the cytosol of the cortical and stelar cells. It was originally proposed that oxygen deficiency in the stele prompted a passive ‘leak’ of $K^+$ into the stelar apoplast (Crafts and Broyer, 1938). However, it is now clear that ion release is highly regulated and likely to involve ion channels. Early studies in support of this notion showed that fusccococcin (which enhances $H^+$ ATPase activity and hyperpolarizes the membrane potential) inhibited $K^+$ release from the stele of Plantago (DeBoer and Prins, 1985) and onion (Clarkson and Hanson, 1986) roots, whereas depolarization of the membrane potential of stelar cells increased the release of $K^+$ into the apoplast (Clarkson and Hanson, 1986). Recent technological advances have allowed access to the plasma membrane of the root stelar cells and several ion channels have now been identified using the patch clamp technique. Isolated root stelar from barley and maize roots have been treated with cell wall-digesting enzymes to release protoplasts from the barley xylem parenchyma cells (XPCs: Wegner and Raschke, 1994) and maize stelar cells (Roberts and Tester, 1995). Also fluorescent microscopy has been used to identify stelar protoplasts isolated from transgenic Arabidopsis plants which have GFP expression restricted to the root stele (Maathuis et al., 1998).

**$K_{out}$ channels:** Outwardly-rectifying $K^+$-dependent currents displaying sigmoidal activation kinetics have been identified in stelar cells from Arabidopsis, maize and barley roots. The $K_{out}$ channels that underlie these currents activate at potentials positive of $E_K$ and are highly selective for $K^+$ amongst the monovalent cations (Wegner and Raschke, 1994; Roberts and Tester, 1995). It is noteworthy that the absolute permeability of the $K_{out}$ channel for Rb$^+$ is a fraction of that for $K^+$, indicating that flux studies, in which Rb$^+$ is used as a tracer for $K^+$, will yield an under-estimation of $K^+$ transport through these channels (Roberts and Tester, 1997). $K_{out}$ channels are also permeable to Ca$^{2+}$ such that under physiological conditions, channel activation will mediate both a $K^+$ efflux and a Ca$^{2+}$ influx (Roberts and Tester, 1997). A new gene (SKOR) has been recently identified and shown to be expressed exclusively in the stele of Arabidopsis roots (Gaymard et al., 1998). Although it has high homology to AKT1 and KAT1, heterologous expression of SKOR in Xenopus oocytes showed that this gene coded for a $K_{out}$ channel and that it shared many properties with $K_{out}$ channels observed in maize and barley roots (e.g. similar activation kinetics, activation potentials and a capability to mediate Ca$^{2+}$ influx). SKOR-knockout Arabidopsis mutants had a $50\%$ reduction in the shoot $K^+$ content, illustrating that $K_{out}$ channels mediate $K^+$ release to the xylem. Moreover, the mutants also exhibited increased shoot Ca$^{2+}$ content indicating that SKOR-type channels are involved in Ca$^{2+}$ re-absorption from the xylem.

**NORC:** The XPCs from barley roots also possess a unique outward current with exponential activation kinetics (Wegner and Raschke, 1994). It appears to be non-selective amongst monovalent anions and cations and is activated at high cytosolic Ca$^{2+}$ concentrations (see below for regulation of $K_{out}$ channels). The physiological role of NORC is unknown, though it is likely that the activation of this channel would clamp the membrane to 0 mV and induce a net efflux of ions into the xylem (since the chemical gradient for most monovalent ions is outwardly-directed). It has been suggested (Wegner and De Boer, 1997a) that the NORC-mediated efflux of ions will drive water influx and turgor pressure changes in the xylem vessels which may be involved in long-distance signalling during wounding responses.

**$K_m$ channels:** $K_m$ channels have been observed in root stelar cells (Wegner and Raschke, 1994; Roberts and Tester, 1995; Maathuis et al., 1998) but are most extensively characterized in XPCs from barley (Wegner et al., 1994). $K_m$ channels in the root stele are proposed to
mediate $K^+$ uptake from the xylem apoplastic and hence may play a role in $K^+$ circulation between the root and shoot. Although the $K_{in}$ channels from XPCs share many properties with their counterparts in the root cortex and epidermis, they possess two distinguishing features. First, their activation potential is fixed at $-110$ mV and second, $K_{in}$ channels from XPCs are not blocked by Cs$^+$. The physiological relevance of these differences is unclear. Also, it remains to be seen if these characteristics are specific to barley or if the $K_{in}$ channels of maize and *Arabidopsis* root stelar cells have similar properties.

**Regulation of $K^+$ transport by ABA**

Cram and Pitman were the first to report ABA regulation of ion transport in higher plant roots (Cram and Pitman, 1972). In this study, radiotracers were used to show that ABA applied to excised barley roots decreased the net efflux of $K^+$ (and Cl$^-$) from the stelar cells to the xylem vessels, but was without effect on the net uptake of these ions in the root cortex. Since this study, there have been many concurring and contradicting reports of the effects of ABA on $K^+$ transport across higher plant roots. Most reports on barley and maize roots show that ABA reduces $K^+$ efflux into the xylem vessels (Cram and Pitman, 1972; Schaefer et al., 1975; Pitman and Wellfare, 1978; Behl and Jenschke, 1981; Bassirirad and Radin, 1992); the exception is a report that showed that ABA increased $K^+$ efflux into the xylem vessels of maize roots (Collins and Kerrigan, 1974). However, it seems likely that this discrepancy results from the fact that the effects of ABA on ion transport in the root are sensitive to many factors including nutrient status of the roots, temperature, the availability of extracellular glucose, and aeration (Pitman et al., 1974; Fournier et al., 1987; Quintero et al., 1998). Also, it appears that the response to ABA is species dependent (e.g. only an ABA-induced promotion of $K^+$ efflux into the xylem vessels has been reported for sunflower using a variety of experimental and growth condition; Fournier et al., 1987).

The molecular mechanisms which underlie ABA-regulated ion transport in root cells will be discussed. However, ABA regulation of ion transport has been extensively studied in guard cells—thus it will be useful to present a brief overview of current understanding of ABA regulation of ion transport in this cell type. For more detail on the regulation of ion transport in guard cells by ABA, the reader is referred to other reviews (Grabov and Blatt, 1998; Pei et al., 1998).

**Ion channel regulation**

**Guard cells:** ABA causes a rapid depolarization of the guard cell plasma membrane (Thiel et al., 1992). This is thought to result from anion efflux following activation of S-type (for slow-activating) anion channels (Pei et al., 1997). However, inhibition of the plasma membrane H$^+$-ATPase may also contribute to the ABA-induced depolarization (Goh et al., 1996). There are two dominant $K^+$ channels in the plasma membrane of guard cells; $K_{in}$ channels which are inhibited by ABA and $K_{out}$ channels which are activated by ABA. ABA-induced membrane depolarization (to potentials positive of $E_K$) coupled with the up-regulation of $K_{out}$ channel activity induces net $K^+$ efflux from guard cells; the consequence of which is loss of cell turgor and stomatal pore closure.

There is evidence indicating that ABA regulation of ion channels in guard cells involves both Ca$^{2+}$-dependent and Ca$^{2+}$-independent pathways. ABA induces elevations of cytosolic Ca$^{2+}$ in guard cells which may result from either a release from intracellular stores (Gilroy et al., 1990; Lee et al., 1996) or from an influx of Ca$^{2+}$ across the plasma membrane (Schroeder and Hagiwara, 1989). Moreover, experimental elevation of cytosolic Ca$^{2+}$ mimics some of the effects of ABA on ion channels (i.e. activation of anion channels and inhibition of the $K_{in}$ channels). However, there are numerous reports which indicate that Ca$^{2+}$-independent ABA signalling also occurs. For example, ABA-induced stomatal pore closure is not always associated with measurable elevations in cytosolic Ca$^{2+}$ (McAinsh et al., 1992; though this could reflect technical problems which prevent detection of very small localized elevation of cytosolic Ca$^{2+}$; Roberts et al., 1994). In addition, $K_{out}$ channel activity is up-regulated by ABA, but insensitive to cytosolic Ca$^{2+}$ (Lemtiri-Chlieh and MacRobbie, 1994). Several second messengers have been proposed to form part of the Ca$^{2+}$-independent ABA signalling pathway. ABA increases cytosolic pH which may account for the up-regulation of $K_{out}$ channels (Blatt and Armstrong, 1993) and there are reports which indicate a role for G-proteins in the regulation of ion channels, both as part of a Ca$^{2+}$-dependent pathway (Fairley-Grenot and Assmann, 1991) and a Ca$^{2+}$-independent membrane-delimited pathway (Wu and Assmann, 1994; Armstrong and Blatt, 1995). Also, there is good evidence showing that protein phosphorylation is a central component in ABA signal transduction. Stomatal closure is enhanced by phosphatase PP1 and PP2A inhibitors and inhibited by kinase inhibitors (Schmidt et al., 1995). Moreover, an ABA-insensitive *Arabidopsis* mutant (abi-1; which displays a number of ABA-related phenotypes including aberrant control of stomatal aperture) has an impaired response of the S-type anion channel to ABA which can be partially restored by the application of kinase inhibitor K-252a (Pei et al., 1997). The ABI1 gene has been shown to encode a protein phosphatase 2C homologue (Leung et al., 1994). Although a number of signalling components have now been identified in ABA signalling in guard cells, it remains to
be seen how these different signalling components are integrated in vivo to bring about stomatal closure.

Roots: It was demonstrated, for the first time (Roberts, 1998), that $K^+$ channels in roots are also subject to regulation by ABA. In this study, the patch clamp technique was applied to protoplasts isolated from the cortex and stele of maize roots which were either non-stressed (control), water-stressed or pretreated with ABA. It was found that water stress and ABA had no effect on the $K^+$ channel activity in the root cortex, but both treatments significantly reduced the activity of the $K_{out}$ channel in stelar cells. Also, in contrast to water-stressed roots, ABA pretreatment significantly activated $K_{on}$ activity in the stele (Fig. 2). It was concluded that both ABA and water stress modify the permeability of the plasma membrane of root stelar cells in favour of reduced $K^+$ transport to the xylem vessels and enhanced $K^+$ accumulation in the root. This is entirely consistent with the $K^+$ flux studies in whole root and suggests that ABA regulation of $K^+$ transport in higher plant roots is mediated by modulation of $K^+$ channel activity.

The similarities between the regulation of $K^+$ channel activity in the root stele by water stress and ABA suggested that ABA regulated $K^+$ channels as part of the drought response. It is well known that ABA accumulates in plants during drought (Davies et al., 1986) and that ABA mimics a variety of responses during water stress (e.g. stomatal closure: MacRobbie, 1992; and accumulation of solutes in the root: Sharp and Davies, 1979).

However, the effects of water stress on the $K^+$ channel activity in the root stele were not faithfully mimicked by ABA treatment. Two likely explanations for this observation have been suggested. First, the ABA content of roots from water-stressed plants may differ from that in ABA-treated roots and that $K^+$ channel activity is dependent on ABA concentrations. Second, water stress modulates the levels of phytohormones other than that of ABA (e.g. jasmonates, auxin and cytokines; Xin et al., 1997) and that these phytohormones regulate $K^+$ channel activity.

Consistent with this, is the observation that kinetin inhibits $K^+$ efflux from maize root stele (Collins and Kerrigan, 1974) and that auxin regulates $K^+$ channel activity in guard cells (Blatt and Thiel, 1994). Clearly, accurate correlation of the activity of $K^+$ channels with the levels of various phytohormones will be necessary to investigate further the regulation of $K^+$ channels by water stress.

How does $K^+$ accumulation in roots help plants to survive drying soils? An early response of plants to water stress is an inhibition of shoot growth while root growth is maintained (Sharp and Davies, 1979; Saab et al., 1990). $K^+$ is an essential cytosolic component and thus it would be essential for the maintenance of root growth, whereas ions transported to the shoot would accumulate in the apoplasts of leaves, increasing the extracellular osmotic potential and increasing the water deficit of leaf cells. Water stress also induces a net accumulation of solutes in the roots which is thought to maintain a water potential favouring the uptake of water and cell turgor pressure necessary for root growth. Although organic compounds (i.e. sugars and amino acids) are the major constituents involved in osmoregulation in plant cells during water stress (Morgan, 1984), inorganic ions may also play a role, for example, as an osmolyte (as is observed in guard cells during stomatal closure; see above) or as charge, balancing negative charges associated with the accumulation of amino acids (Jones et al., 1980).

It is noteworthy that the regulation of $K^+$ channels in roots is opposite to that in guard cells suggesting that alternative mechanisms underlie the ABA regulation of $K^+$ channels in roots. There appear to be at least two distinct mechanisms involved in the regulation of $K^+$ channel activity in roots: a ‘long-term’ mechanism via the regulation of transcription and a more rapid ‘short-term’ post-translational mechanism. Similarities have been noted between the effects of ABA and inhibitors of protein synthesis on $K^+$ efflux from the root stele (Schaefer et al., 1975) and it was inferred that ABA may be acting via regulation of transcription of ion channels in the stelar cells. Definite proof for this idea has been provided (Gaymard et al., 1998) which showed that the abundance of SKOR mRNA was dramatically reduced.

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**Fig. 2.** Whole cell current densities resulting from $K_{out}$ and $K_{on}$ channel activity in protoplasts from the cortex and stele of maize roots that were either well-watered (control and high $Ca^{2+}_{in}$), water-stressed (WS) or treated with 20 $\mu$M ABA approximately 16 h prior to protoplast isolation. Water stress was recorded as a 1.5% reduction in the relative water content of the shoots compared to that recorded in control plants. Detailed methods for plant growth and protoplast isolation have been given elsewhere (Roberts, 1998). Patch clamp conditions protein synthesis on $K^+$ or treated with 20 $\mu$M ABA. $CaCl_2$, 3 mM MgCl$_2$, 4 mM EGTA, 3 mM $K_2$ATP, 10 mM HEPES adjusted to pH 7.2 for all experiments except for high $Ca^{2+}_{in}$, in which 3.94 mM $CaCl_2$ was added to give a final $Ca^{2+}$ activity of 10 $\mu$M. Methods for whole-cell patch clamp recordings have been given elsewhere (Roberts, 1998).
3 h after the application of ABA to *Arabidopsis* roots. Furthermore, AKT1 expression in the root cortex was unaffected by ABA (which is also consistent with the findings of Roberts, 1998). The gene(s) encoding the $K_{\text{in}}$ channels in root stelar cells have not yet been identified and thus it remains to be seen whether transcriptional regulation by ABA also modulates $K_{\text{in}}$ channel expression. Post-translational control of root cell ion channels is also evident. Application of ABA directly to maize root stelar protoplasts during patch clamp experiments reduced the magnitude of the outward current (Roberts 1998). The mechanisms responsible for this post-translational control are unknown. However, although the effects of ABA on $K^+$ channel activity in root cells are opposite to that in guard cells, several observations indicate that components of the ABA signalling pathway may be common to both cell types. In maize root stelar cells, increasing cytosolic Ca$^{2+}$ to 10 μM (from less than 100 nM) modulated $K^+$ channel activity in a similar way as was observed in ABA-treated stelar cells (i.e. increased $K_{\text{in}}$ channel activity and decreased $K_{\text{out}}$ channel activity; Fig. 2). The Ca$^{2+}$ sensitivity of the $K_{\text{out}}$ channel in maize roots can be further demonstrated using the calcium ionophore A23187. In such experiments whole cell $K_{\text{out}}$ currents were completely abolished by the addition of extracellular A23187 (Fig. 3), presumably as a result of an A23187-induced elevation of cytosolic Ca$^{2+}$. Down-regulation of $K_{\text{out}}$ channels by cytosolic Ca$^{2+}$ is also reported in barley root XPCs (Wegner and De Boer, 1997a). However, two crucial experiments are required before a role for cytosolic Ca$^{2+}$ in ABA signalling in root stelar cells can be assigned. First, it remains to be seen if ABA elevates cytosolic Ca$^{2+}$ in stelar cells and second, whether experimental modulation of cytosolic Ca$^{2+}$ inhibits ABA-induced regulation of $K^+$ channel activity. The activity of $K^+$ channels in the root stele can also be modulated independently of cytosolic Ca$^{2+}$. Figure 4 shows that the open probability of $K_{\text{out}}$ channels in maize root stelar cells is increased with increasing cytosolic pH and there is evidence for G protein modulation of $K_{\text{in}}$ channels in barley XPCs (Wegner and De Boer, 1997b). However, it is not known if cytosolic pH or G proteins are regulated by ABA in root cells.

It is noteworthy that stelar cells from ABA-pretreated maize roots had increased $K_{\text{in}}$ channel activity (Fig. 2), whereas the addition of ABA directly to stelar protoplasts during patch clamp experiments had no effect on the inward current (see Fig. 4, Roberts, 1998). Also, using a pipette (cytosolic) solution containing 10 μM Ca$^{2+}$ to record whole cell currents increased $K_{\text{in}}$ channel activity (Fig. 2), but elevation of cytosolic Ca$^{2+}$ (using A23187) after achieving the whole cell configuration of the patch clamp technique did not increase $K_{\text{in}}$ activity (data not shown). The most likely explanation for these observations is that during patch clamp experiments, the pipette solution replaces the cytosol and washes out cytosolic factors which may play an integral role in the ABA signalling pathway. By analogy to guard cells, the cytosolic factors may be phosphatases—indeed, it would be interesting to determine whether *abi-1* mutants have aberrant ABA regulation of $K^+$ channel regulation in root cells.

**Membrane potential regulation**

Regulation of channel-mediated $K^+$ transport across roots is dependent on the resting membrane potential of
root cells; both as a regulatory factor of channel activity and as part of the driving force for passive K⁺ transport. Previously, technical difficulties have prevented accurate measurement of stelar cell membrane potentials. The authors’ best estimates came from studies using microelectrodes forced through the cortex and into the stele of maize roots (Dunlop, 1982) and from measurements of the potential difference between the cortical and stelar apoplasts of Plantago roots (DeBoer and Prins, 1985). These studies showed that the membrane potential of stelar cells is more depolarized than that recorded for cortical cells.

In the present study, impalement microelectrodes were used to record membrane potentials from intact cortex and stelar tissue derived from control and ABA-treated maize roots. To gain free access to the stelar cells, the cortex and endodermis were removed by hand (Gronwald and Leonard, 1982; Roberts and Tester, 1995). Furthermore, isolated steles were allowed to recover for three hours prior to electrode impalement (see Fig. 5 legend), from which stable membrane potentials could be recorded. This circumvented problems associated with forcing the microelectrode through several layers of cortical cells (i.e. soiling and possibly blocking the electrode tip with cell debris) and greatly increased the likelihood of recording accurate membrane potentials. It was confirmed that the membrane potentials of cortical cells were more hyperpolarized than those observed for stelar cells in control roots (Table 1). The addition of ABA to maize roots had no effect on the membrane potentials of cortical cells, at least in 1 mM extracellular K⁺. In contrast, the membrane potential of stelar cells from ABA-treated roots was significantly more negative than those from control roots. Figure 5 shows typical recordings from the root stelar in varying extracellular K⁺ concentrations. In 10 mM K⁺ the average membrane potential in control roots (i.e. not treated with ABA) was −71 ± 4 mV (n = 5), reducing extracellular K⁺ to 10 μM hyperpolarized the cell to −110 ± 3 mV (n = 5). In ABA-treated roots, the stelar cells possessed membrane potentials ranging from 104 ± 6 (n = 5) and −145 ± 2 (n = 4) mV in 10 mM
and 10 μM K⁺, respectively. These results are summarized in Table 1. The dependence of the membrane potential on extracellular K⁺ indicated that the major conductance across the plasma membrane results from K⁺ transport.

The physiological significance of the ABA regulation of the membrane potential is to favour the activation of K⁺ channels and inhibit K⁺ channel activity in root stelar cells. Moreover, the more negative membrane potential in ABA-treated cells increases the driving force of their regulation will provide more detailed insights into net K⁺ transport. Further investigation of at least five separate experiments. Plant growth and root stelar isolation was as for Fig. 5. Extracellular recording solution was as described in Fig. 5 and contained 1, 5 or 10 mM K⁺.

from the soil solution to the shoots. Further investigation of their regulation will provide more detailed insights into the role of these ion channels in both nutrient acquisition and in adaptation responses to abiotic stresses. It is clear that fundamental differences exist in the regulation of K⁺ channels (and membrane potential) in guard cells and root cells. The different regulation appears physiologically appropriate for the specialized function of each cell type and highlights the limitations of making generalizations and extrapolations from a single (model) cell type.

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