Mechanisms of solute efflux from seed coats: whole-cell K$^+$ currents in transfer cell protoplasts derived from coats of developing seeds of *Vicia faba* L.

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Abstract

In developing seed of *Vicia faba* L., solutes imported through the phloem of the coats move symplastically from the sieve elements to a specialized set of cells (the thin-walled parenchyma transfer cells) for release to the seed apoplast. Potassium (K$^+$) is the predominant cation released from the seed coats. To elucidate the mechanisms of K$^+$ efflux from seed coat to seed apoplast, whole-cell currents across the plasma membranes of protoplasts of thin-walled parenchyma transfer cells were measured using the whole-cell patch-clamp technique.

Membrane depolarization elicited a time-dependent and an instantaneous outward current. The reversal potential ($E_R$) of the time-dependent outward current was close to the potassium equilibrium potential ($E_K$) and it shifted in the same direction as $E_K$ upon changing the external K$^+$ concentration, indicating that this current was largely carried by an efflux of K$^+$. The activation of the time-dependent outward K$^+$ current could be well fitted by two exponential components plus a constant. The instantaneous outward current could also be carried by K$^+$ efflux as suggested by ion substitution experiments. These K$^+$ outward rectifier currents elicited by membrane depolarization are probably too small to represent the mechanism for the normal K$^+$ efflux from seed coat cells.

Membrane hyperpolarization more negative than $-80$ mV activated a time-dependent inward current. K$^+$ influx was responsible for the inward current as the current reversed at membrane voltage close to $E_K$ and shifted in the same direction as $E_K$ when external [K$^+$] was varied. Activation of this K$^+$ inward rectifier current was well fitted with two exponential components plus a constant. A regulating function for this current is suggested.

Key words: Potassium outward rectifier, potassium inward rectifier, transfer cell protoplast, seed coat, *Vicia faba* L.

Introduction

There is a discontinuity between the symplasts of the maternal coats and of the embryos of developing grain legume seeds. This provides a unique experimental system to study post-phloem transport in sinks (Wolswinkel, 1992; Patrick and Offler, 1995). In the case of developing seeds of *Vicia faba* L., solutes imported through the phloem of the coats move symplastically from the sieve elements to the thin-walled parenchyma transfer cells that line the inner surface of the coats. From these cells solutes are released to the seed apoplast (Offler and Patrick, 1993; Patrick *et al.*, 1995; Wang *et al.*, 1995). The solutes released from the seed coat are subsequently retrieved from the seed apoplast by the abutting epidermal transfer cells of the cotyledons and accumulated in their storage parenchyma cells (McDonald *et al.*, 1995). Sucrose,
potassium and chloride are among the major chemical species of solutes effluxed to the seed apoplastic (Patrick, 1984; Walker et al., 1995). A proton-sucrose antipporter has been proposed to mediate sucrose efflux (Fieuw and Patrick, 1993; Walker et al., 1995). The mechanisms for ion efflux from the seed coat are unknown, but could include movement through ion channels located in the plasma membrane of the efflux cells (Walker et al., 1995).

Two classes of ubiquitous K⁺ rectifiers have been found in higher plant cells so far studied (Tester, 1990). A K⁺ outward rectifier (KOR) is activated when membrane potential difference (PD) becomes more positive than the equilibrium potential for potassium (E钾). It carries current with a sigmoidal (van Duijn, 1993) or exponential time-course (Garrill et al., 1994; Fairley-Grenot and Assman, 1993). Membrane hyperpolarization activates a K⁺ inward rectifier (KIR) current with an exponential time-course (Findlay et al., 1994; White and Lenton-Chlieh, 1995). The KOR is probably responsible for the large K⁺ efflux during stomatal closure (Schroeder, 1988), rapid leaf movement in Samanea saman (Moran et al., 1988) and osmoregulation in seagrass leaf cells (Garrill et al., 1994). Recent studies on root xylem parenchyma cells of barley (Wegner and Raschke, 1994) and stelar cells of maize (Roberts and Tester, 1995) suggest that the KOR could be involved in unloading K⁺ into the xylem vessels. The KIR probably provides a mechanism for low affinity K⁺ uptake (Maathuis and Sanders, 1996).

To elucidate the mechanism of K⁺ efflux from the coats of developing V. faba seeds, protoplasts of the coat cells responsible for solute efflux were isolated. Whole-cell currents across these protoplasts were characterized using the whole-cell patch-clamp technique.

**Materials and methods**

**Plant material**

Broad bean plants (Vicia faba L. cv. Coles Dwarf Prolific) were raised under glasshouse conditions as described previously (Fieuw and Patrick, 1993). Seeds used to isolate protoplasts were at the developmental stage of mid- to late Phase II (Briarty et al., 1969).

**Preparation of protoplasts**

Pods used in these experiments were removed from the plant and kept at 4°C for 1-4 weeks before use for protoplasts. This differs from the use in an earlier paper (Walker et al., 1995) of pods freshly picked from the plant. K⁺ efflux is similar in fresh and stored pods (Patrick, unpublished results). Six uniform seed coats were cut longitudinally around the integumentary fusion line and carefully removed from the embryo. Each resulting coat half was filled with an enzyme solution containing (mM): 95 KCl, 65 MgCl₂, 0.1 CaCl₂, 30 2[N-morpholinol]ethanesulphonate (MES), 0.1% bovine serum albumin (BSA), 0.5% polyvinylpyrrolidone (PVP), 0.1% pectinase (Pectolyase Y23, Seishin Pharmaceutical, Tokyo, Japan), 1% Rohament CT cellulase (a kind gift from Röhm, Darmstadt, Germany), 1% cellulase TC (Seroa, Heidelberg, Germany). The solution pH was adjusted to 5.5 with KOH and osmolality to 550 mOsmol kg⁻¹ with sorbitol. After 4 h digestion at 20°C in a water-saturated atmosphere, the coat halves were cut longitudinally into small pieces and agitation in a 20 ml volume of ‘wash solution’ composed of 500 mM sorbitol, 0.1% BSA and 0.5% PVP, and 30 mM MES (pH 6.0). The wash solution was then filtered through 90 μm nylon mesh and centrifuged at 60 g for 5 min. The supernatant was discarded and the pellet resuspended in 3 ml of ice-cold 500 mM sucrose, 1 mM CaCl₂ and 5 mM MES (pH 6.0). The protoplast suspension was layered with 2 ml of a solution containing 400 mM sucrose, 100 mM sorbitol, 1 mM CaCl₂, and 5 mM MES (pH 6.0), followed by a 1 ml layer composed of 300 mM sorbitol, 1 mM CaCl₂ and 5 mM MES (pH 6.0). All solutions were chilled and filtered (0.2 μm Millipore) before use. After centrifugation of the density gradient for 5 min at 200 g, a clean population of protoplasts of thin-walled parenchyma transfer cell protoplasts was collected from the interface between the top two layers. The protoplasts were washed with 5 ml of the top gradient solution and centrifuged for 5 min at 60 g. The final pellet was resuspended in 1 ml of the top gradient solution. Throughout the purification procedure, the protoplasts were kept at 4°C.

**Electrophysiology**

Protoplasts were transferred to a small flow chamber for patching. The chamber had a thin glass base to which the clean protoplasts adhered firmly and a cover slip was placed on top of the chamber. Patch pipettes were pulled from borosilicate glass blanks (Clark Electromedical, Reading, UK), coated with Silgard® (Dow Corning, Midland, Mich., USA), and fire polished to a bubble number between 5.5 and 6.0 measured with a 10 ml syringe (Corey and Stevens, 1983). The seal-success rate was 15%. Experiments were conducted using the whole-cell mode of the patch clamp technique (Hammill et al., 1981). The whole-cell configuration was obtained by forming a gigaseal (resistance ≥2 GΩ) in the cell-attached mode and then applying a brief suction to rupture the plasma membrane. The attainment of the whole-cell mode was judged by a substantial increase in capacitance.

The voltage across the patch was controlled and current measured using a List EPC-7 (List Electronic, Darmstadt, Germany). Series resistance was compensated to about 50% and capacitance compensation was used. Whole-cell data were collected and analysed using the pCLAMP programs. Whole-cell current-voltage curves were fitted with third-order polynomials. Junction PDs were calculated and corrected for, using the program JPcalc (PH Barry, University of New South Wales, Sydney, Australia). All experiments were carried out at 22-25°C.

**Solutions**

Pipette solution contained (mM): 10 KCl, 90 KGlutamate, 2 MgCl₂, 2 CaCl₂, 2 Na₃ATP, 10 HEPEs, and 10 EGTA, pH 7.2 adjusted with 40 KOH and osmolality of 720 mOsm kg⁻¹ with sorbitol. The free calcium concentration, calculated with the program Buffa (Dr R.G. Ryall, Flinders Medical Centre, South Australia), was 50 nM. Unless otherwise stated, bath solutions included (in mM): 10 CaCl₂, 5 MES, pH 6.0 adjusted with 3 KOH and osmolality of 700 mOsm kg⁻¹ with sorbitol. All solutions were filtered with a 0.22 μm filter immediately before use.
Equilibrium potentials for $K^+$ and $Cl^-$ were calculated using ionic activities according to Robinson and Stokes (1959). All the results in this paper are given as mean $\pm$ SE of the number of protoplasts measured.

**Results**

**Identification of protoplasts responsible for solute efflux**

It has been shown that efflux of solute from the coats to the seed apoplast of developing *Vicia faba* L. seeds is likely to occur from the thin-walled parenchyma transfer cells (Offler and Patrick, 1993; Wang *et al.*, 1995). The membrane impermeant sulphydryl fluorochrome, bromobimane, has been shown to bind selectively to the thin-walled parenchyma transfer cells of *Vicia faba* seed coat halves exposed to the fluorochrome (Wang *et al.*, 1995). This phenomenon was used to identify the protoplasts of thin-walled parenchyma transfer cells by tagging these *in situ* with bromobimane, treating the seed coats with wall degrading enzymes and observing the cellular characteristics of the released protoplasts labelled with the fluorescent tag (Fieuw and Patrick, unpublished results). The distinguishing characteristics exhibited by the protoplasts of the thin-walled parenchyma transfer cells are their relatively large nucleus, granulated cytoplasm, off-centre vacuole and absence of chloroplasts (Fig. 1). The mean diameter of the transfer cell protoplasts used for patch-clamping was $41.3 \pm 2.7 \mu m$ ($n = 21$).

**Resting membrane PD**

Upon the pipette gaining access to the whole-cell (protoplast), the membrane potential of the protoplast was measured immediately under current-clamp mode. The membrane PDs of protoplasts bathed in 100 mM KCl showed a large variation, falling in the range of 5 to $-90$ mV. Some 70% of the protoplasts had a membrane PD more positive than $E_K$ ($E_K = -7$ mV), whereas the remainder had a membrane PD more negative than $E_K$. The addition of DCCD, a proton pump inhibitor (cf. Findlay *et al.*, 1994), to the latter hyperpolarized protoplasts rapidly depolarized their membrane PD to close to $E_K$ (data not shown). These results indicate that the protoplasts were either dominated by the $K^+$ (K-state) or by the proton pump (P-state) conductance. Similar observations have been made in protoplasts derived from wheat roots (Findlay *et al.*, 1994) and roots of *Plantago media* L. (Vogelzang and Prins, 1994).

**Outward current**

In most protoplasts a time-dependent outward current was activated when membrane PD was clamped more positive than $E_K$ (Fig. 2A). The conductance increased as membrane PD became more positive (Fig. 2B), so this can be regarded as a rectifier current. The reversal potential of this outward current followed $E_K$ when external KCl concentrations were changed from 100 mM to 10 mM (Fig. 2B). This type of $K^+$ outward rectifying (KOR) current was observed in about 60% of the protoplasts patch-clamped ($n = 28$). The KOR current did not show inactivation, even during prolonged depolarization pulses lasting for several minutes (data not shown).

In contrast to most outward rectifying currents found in various plant cells (cf. van Duijn, 1993), the time-
course of activation of the KOR current was best fitted (using the least-squares method) by the sum of two exponential components of the form:

\[ I = I_0 + I_1 \exp(-t/\tau_1) + I_2 \exp(-t/\tau_2) \]

where \( \tau_1 \) and \( \tau_2 \) are the two time constants of the current activation (Fig. 2C) and where \( I_0 \) is a component of current not dependent on time (see below). The fast activation time constant \( \tau_1 \) was relatively independent of the membrane PD (Fig. 2D), while the slow activation time constant \( \tau_2 \) decreased from 2.1 s to 1.0 s as the membrane PD increased from 17 mV to 77 mV (Fig. 2D).

Tail current' protocols were used to determine the reversal potential of the KOR current and hence the ions responsible for carrying it. KOR current was first fully activated by a depolarizing pre-pulse, then followed by step negative-going pulses to deactivate the current (Fig. 3A). The reversal potential \( (E_R) \) of the KOR current determined from the 'tail-current' measurements was more positive than \( E_K \) (Fig. 3B). As external K+ concentration was varied, \( E_R \) of the time-dependent outward current changed by a similar amount to \( E_K \), but remained about 15 mV more positive than \( E_K \). With a [K+] of 140 mM in the pipette, \( E_R \) of the KOR current was 4.3 \pm 3.3 mV \( (n=3) \) in 100 mM KCl external solution and -38.0 \pm 4.8 mV \( (n=4) \) in 10 mM KCl external solution, where \( E_K \) was -7 mV and -53 mV, respectively. This result shows that the observed KOR current was mainly carried by K+ efflux. The deviation of \( E_R \) from \( E_K \) suggests that other permeant ions with positive equilibrium PDs also contribute to the current. With the pipette and bathing solutions, the equilibrium PDs for Mg2+, Na+ and Cl- were all such that these ions could not contribute to the positive value of \( E_R \). Hence the observation that \( E_R \) was more positive than \( E_K \) is likely to be due to permeability to Ca2+ ions \( (E_{Ca}=150 \, mV) \).

A similar argument has been used to account for the discrepancy between \( E_K \) and the \( E_R \) of KOR currents observed in maize root stelar cells (Roberts and Tester, 1995) and cultured tobacco cells (van Duijn et al., 1993). The KOR current in the protoplasts averaged 150 pA at 50 mV in 10 mM K+, a 7 times lower current density than found in stelar cell protoplasts by Roberts and Tester (1995) who show (their Fig. 3) a current of 500 pA under the same conditions in a protoplast of about half the surface area. More remarkably, Wegner and Raschke (1994) report currents of 1.5 nA through their KORC at 50 mV and 30 mM K+ in a protoplast of one-quarter the surface area of that in the present experiment (their Fig. 5A), a current density some 40 times greater than that measured here.

In addition to the time-dependent outward current, an instantaneous, time-independent current was observed in almost all the protoplasts (Fig. 4A). This instantaneous current occurred at membrane PDs both negative and positive of \( E_K \) (Figs 4A, 5A). The conductance increased as the membrane PD became more positive (Fig. 4B), so this is referred to as a rectifier current. In most of the protoplasts time-dependent outward and inward currents
also appeared as the membrane was depolarized or hyperpolarized further (Figs 2B, 5B). However, in about 25% of the protoplasts it was found that outward current was dominated by the instantaneous outward rectifier (IOR) current alone, even when the membrane PD was as positive as 77 mV (Fig. 4A). The reversal potential of a protoplast, showing IOR current only, followed \( E_K \) when external KC1 was changed from 100 mM to 10 mM (Fig. 4B), indicating that the IOR current is likely to be carried by \( K^+ \). This is supported by the finding that replacement of bath Cl~ with glutamate had little influence on the outward current. With a mean of 200 pA at 50 mV in 10 mM \( K^+ \), the IOR current observed is comparable to a value from Roberts and Tester (1995, Fig. 2A) of about 200 pA at 60 mV in 30 mM \( K^+ \) for a wheat root cortical cell. The IOR current is likely to be responsible for most of the constant component used in curve-fitting to time-courses of KOR and KIR (see below) currents.

**Inward current**

Membrane hyperpolarization more negative than about \(-80\) mV induced a slowly activating inward current which was observed in about 75% of protoplasts (Fig. 5A). An increase in inward current, with increasing external \( K^+ \) concentration, is shown in the current-voltage curves of the steady-state current (Fig. 5B). The inward current became very noisy as the membrane PDs became more hyperpolarized (Fig. 5C).

Like the outward current, the inward current could be best fitted with a constant component plus two exponential components (Fig. 5C). The fast activation time constant was relatively independent of the membrane PD (Fig. 5D), while the slow activation time constant increased from 0.99 s at PD of \(-133\) mV to 1.96 s at PD of \(-173\) mV (Fig. 5D).

The \( E_R \) of the inward current determined from ‘tail current’ measurement showed that the inward current reversed at PDs more positive than either \( E_K \) or \( E_A \), and \( E_R \) followed \( E_K \) rather than \( E_A \) when external KC1 concentration was varied (Fig. 6). This behaviour indicates that this inward rectifier (KIR) current was predominantly carried by \( K^+ \) influx. Note that \( E_R \) was always more positive than \( E_K \) by about 20 mV (Fig. 6B). This demonstrates that other permeant ions with positive equilibrium PDs also contribute to the observed KIR current. As already argued in the case of the KOR current, the fact that \( E_R \) is more positive than \( E_K \) could be due to permeation by Ca\(^{2+}\) ions.

**Discussion**

Previous studies have revealed that the thin-walled parenchyma transfer cells in coats of developing *Vicia faba* seeds are the principal site(s) for photosynthate efflux from the seed coat to the seed apoplast (Offler and Patrick, 1993; Wang *et al.*, 1995; Patrick *et al.*, 1995). \( K^+ \) ions are the predominant mineral ions released from
the coat to the seed in *V. faba* (Wolswinkel et al., 1992) and *Phaseolus vulgaris* L. (Patrick, 1984; Walker et al., 1995) and are assumed to be released from the same transfer cells. In this study, protoplasts of the seed coat thin-walled parenchyma transfer cells were isolated and the whole-cell patch-clamp technique was applied to determine the current across their plasma membranes. It was demonstrated that three types of current dominated the conductance of the thin-walled parenchyma transfer cell protoplasts: (i) a time-dependent K⁺ outward rectifier (KOR) which was activated when the membrane PD was more positive than *Eₓ*, (ii) an instantaneous outward rectifier current (IOR) which was seen, alone or together with the KOR current, when membrane PDs became more positive than *Eₓ*, and (iii) a time-dependent K⁺ inward rectifier (KIR) current seen at membrane PDs more negative than −80 mV.

**Outward current**

Time-dependent KOR current, activated by membrane depolarization, has been observed in almost all higher plant cells patch-clamped to date (Tester, 1990; Tyerman, 1992). Activation of KOR current often follows a sigmoidal time-course which can be fitted by the Hodgkin–Huxley model (Hodgkin and Huxley, 1952), in tissues including guard cells of *Zea mays* protoplasts (Fairley-Grenot and Assman, 1993), tobacco suspension cells (van Duijin, 1993), and maize root cells (Roberts and Tester, 1995). It was found that the time-course of the KOR current observed in the present study was best fitted with a constant plus two exponential components (cf. Fig. 3), suggesting that the channel has two closed states or that two populations of channels contribute to the current. Similar kinetics have been demonstrated for KOR in *V. faba* guard cells (Fairley-Grenot and Assman, 1993) and in seagrass leaf cells (Garrill et al., 1994). The occurrence of this current in 60% of seed coat transfer cell protoplasts corresponds with its observation in 80% of maize root stelar cell protoplasts (Roberts and Tester, 1995) and in 50% of barley root stelar cell protoplasts (Wegner and Raschke, 1994).

A time-independent IOR current was seen when the membrane PD was more positive than *Eₓ* (Fig. 5). This IOR current is likely to be the result of efflux of K⁺ rather than influx of an anion since the reversal potential of a protoplast showing IOR alone followed *Eₓ* rather than *Eₒ* when external [KCl] was changed (Fig. 5B). IOR current driven by membrane depolarization has been shown to be present in various plant cells, including *Amaranthus* cotedylon cells (Terry et al., 1992), wheat (Schachtman et al., 1991) and maize root cells (Roberts and Tester, 1995). In most cases, the IOR current coexists with KOR current: IOR current is dominant when membrane PD is less positive and as the membrane PD becomes more positive KOR current dominates the outward conductance (cf. Ketchum et al., 1989; White and Lemtiri-Chlieh, 1995). However, in the seed coat cells of *V. faba* it was found that in about 25% of the protoplasts the IOR current appeared alone even when membrane voltage was 80 mV more positive than *Eₓ* (Fig. 5A).

The thin-walled parenchyma transfer cells in seed coat, which appear to be specialized for delivery of solutes from seed coat to seed apoplast, are known to deliver sucrose (Patrick, 1993) and are expected to deliver part or all of the potassium. Thus the two K⁺ outward rectifiers found in this study might play a role in the K⁺ efflux. The K⁺ efflux from the (stored or fresh) seed coat of *V. faba* is about 120 nmol m⁻² s⁻¹ under both normal and zero turgor, without added K⁺ in the bathing solution (Patrick, 1994; and unpublished results), if the efflux is restricted to the transfer cells. It is about 60 nmol m⁻² s⁻¹ if there is equal K⁺ efflux from all the seed coat cells in the symplastic pathway between phloem and transfer cell. Thus the efflux corresponds to a current of 60 pA across the plasma membrane of a protoplast with a diameter of 40 μm. A nominal current requirement of 50 pA will be used, and the question, what membrane PD would be needed to give such a current will be asked, and whether this required PD is plausible. This can be judged only by a comparison with values measured in seed coat cells of *Phaseolus vulgaris* at about 1.4 mM K⁺ (Walker et al., 1995).

There are no current–voltage curves for 1 mM K⁺, but there are mean current–voltage curves for 10 and 100 mM K⁺ (Fig. 7). For the 60% of protoplasts which show KOR current, Fig. 7A shows that changing [K⁺] from 100 mM to 10 mM shifts the current–voltage curve 16 mV to the left, from 24 mV to 8 mV at 50 pA. Roberts and Tester (1995) show current–voltage curves for their KOR at 1 mM to 35 mM K⁺. Their Fig. 6 shows a little shift in the curve between 1 mM and (an interpolated) 10 mM, perhaps 30 mV at low current and zero at high current. We hazard the opinion that at 1 mM K⁺ the curve will be shifted about 15 mV leftwards, to say −7 mV at 50 pA. For the 25% of protoplasts showing IOR current alone, Fig. 7B shows that changing [K⁺] from 100 mM to 10 mM shifts the current–voltage curve markedly to the left, from 25 mV to −9 mV at 50 pA. Applying a similar guess, it is supposed that at 1 mM K⁺ the curve would be shifted to −43 mV. This suggests that the IOR would carry much more current than the KOR at moderate depolarizations. However, the measured value of the membrane PD (in *Phaseolus vulgaris* L.) at no added K⁺ is −95 mV (Walker et al., 1995), a value about 50 mV more negative than that needed to produce a K⁺ outward current of 50 pA through the IOR. It is not likely that the concentration of K⁺ in the cytoplasm would be significantly higher than the 140 mM determined in these experiments by diffusion from the...
pipette solution, so this is unlikely to remove the 50 mV discrepancy.

Thus the K$^+$ outward rectifiers of the transfer cell have not been shown to be an adequate mechanism for the observed K$^+$ efflux at zero to normal turgor under the experimental conditions of a few mM K$^+$. This casts doubt on their being the mechanism in vivo at about 80 mM K$^+$. However, the possibility that in vivo either the K$^+$ rectifiers described here may show larger outward currents or other K$^+$ outward rectifiers may be activated, has not been ruled out. This may be resolved by future studies of cell-attached patches. In a subsequent paper, the question of the outward rectifiers described here providing the increase in K$^+$ efflux under elevated turgor is examined.

Inward current

The KIR current, activated by membrane hyperpolarization in the seed coat protoplasts, is comparable with KIR currents demonstrated in various plant tissues (cf. Findlay et al., 1994). It was observed in 75% of the protoplasts used in this study, in 70% of those of xylem parenchyma (Wegner and Raschke, 1994), but in 23% of those of wheat root cortical cells (Findlay et al., 1994) and in only 20% of those of stelar cells (Roberts and Tester, 1995), which similarly are dedicated to unloading K$^+$. In xylem parenchyma cells of barley roots the KIR has been suggested to mediate the re-absorption of ions from the xylem apoplast (Wegner and Raschke, 1994), and it may be that the KIR in seed coat cells also functions to take up K$^+$ under some conditions. It may be significant that in this study it was found that some protoplasts, that are hyperpolarized with respect to $E_K$, at PDs that would cause K$^+$ influx. It is suggested that the KIR may have a role to play in regulating the transport activity of efflux cells or tissues.

Conclusion

The present study has demonstrated that current flow through the plasma membrane of the thin-walled transfer cell protoplasts derived from developing V. faba seed coats is dominated by K$^+$ conductances. The two outward rectifiers (KOR and IOR) characterized in the present study have not been shown to carry enough current to mediate the normal K$^+$ efflux from coats to the seed apoplast, but might play a role under conditions of elevated turgor, and so to contribute to osmoregulation in this tissue. It is suggested that the normal efflux of K$^+$, which happens at PDs close to $E_K$, may well be mediated by K$^+/H^+$ antiport, by analogy with sucrose (Walker et al., 1995), but have not completely ruled out a channel mechanism.

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